Effect of some weak acids and *Moringa oleifera* leaf extract powder on the physicochemical and storage properties of dried Granny Smith apple

by

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DECLARATION

I, Washiela Arendse, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

This study aimed to find alternatives to sulphite as a preservative for dried fruits which provided a quality product with a reasonable shelf life. Granny Smith apples were sanitised in a 200 ppm sodium chlorite solution, de-cored, peeled and cut into slices. The sliced apples were pre-treated/dipped in a water solution containing the three weak acids namely; ascorbic acid, citric acid and potassium sorbate as well as *Moringa oleifera* leaf extract powder (MOLEP). A screening fractional factorial experiment consisting of five independent variables (ascorbic acid, citric acid and potassium sorbate, time and temperature and MOLEP) constrained at their upper and lower levels (ascorbic acid: 0.5% to 2.0%, citric acid: 0.3 to 2.0%, MOLEP: 0.1% to o.2%, time: 7 h to 16 h and temperature: 57°C to 70°C) were evaluated for their effect on the colour of the dried sliced apples. Ascorbic acid and potassium sorbate did not have an impact on the lightness of the dried sliced apples. A dipping solution of citric acid at 2.0%, *Moringa oleifera* leaf extract powder at 0.1% (CMO) and drying time of 7 h at 70°C reduced the browning of the dried sliced apples. Storage stability of the dried sliced apples were done using the optimum dipping solution with citric acid at 2.0%, MOLEP at 0.1% and another dipping solution with citric acid at 2.0%, MOLEP at 0.1% and potassium sorbate at 0.2% (CMOP). Microbiological testing (osmophilic yeast, *Escherichia coli* and yeast and moulds) and total acidity were done as well as physical tests such as moisture analysis, water activity, texture analysis and colour at day 0, day 60 and day 120. The optimum combination of 2% citric acid and 0.1% *Moringa oleifera* extract powder (CMO) effectively minimized the increase in the redness and the yellowness of the dried apple slices over the storage period. The osmophilic yeasts, *E.coli,* yeasts and moulds results of the dried apple slices pr-treated with the water solution of 2.0% citric acid and 0.1% *Moringa oleifera* leaf extract powder (CMO) were within the limits as prescribed by Codex Alimentarius Commission (2003) at the start and end of the shelf life study. The water activity of the dried apple slices pre-treated with CMO was below the limit (<0.6%) conducive to microbial growth during the storage time. Moisture increased over the shelf life period which affected the extensibility of the pre-treated dried sliced apples negatively. A 3 month (120 days) shelf life was accomplished, using the optimal combination of 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder. A consumer acceptability test was done using the 9-point hedonic scale. The dried apple slices pre-treated with the optimal combination of 2% citric acid and 0.1% *Moringa oleifera leaf* extract powder water solution were acceptable to consumers with regards to colour, texture and taste. The CMO pre-treatment was effective in reducing browning and inhibiting microbial growth of the dried apple slices.

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DEDICATION

To my husband, Dion and daughters, Shihaam and Mishka for their unwavering love and support during this journey.

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Language and style used in this research proposal are in accordance with the requirements of the *International Journal of Food Science and Technology*.

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GLOSSARY

NCD: Non-communicable diseases

SO_2: Sulphur dioxide

AHR: Airway hyper responsiveness

US: United States of America

GRAS: Generally recognised as safe

PPO: Polyphenoloxidase

FDA: Food and drug administration

QDA: Quantitative descriptive analysis

MOLEP: *Moringa oleifera* leaf extract powder

CA: Citric acid

A_w: Water activity

CHAPTER 1

MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Apples play an important role in fruit production and are eaten in their original form, processed into fruit drinks, preserves (jams and marmalades) or as dehydrated fruit (Doymaz, 2010). Growers of fruit and vegetables are currently faced with a serious challenge of how to stop their products from deteriorating and becoming unsuitable for consumption. Dehydrating fruit and vegetables is one of the most appropriate ways of conserving these products (Doymaz, 2010; Mercer, 2012; Shrestha *et al.*, 2020). It is also one of the most long-standing means to preserve food for future use (Harrison, 1993; Sethi, 2007; Miranda *et al.*, 2009; Shrestha *et al.*, 2020).

Fruit deterioration is a result of microorganisms such as bacteria, yeasts and moulds that spoils fruit and shortening the shelf life. Drying removes the water content from the fruit and these organisms cannot proliferate and cause the fruit to rot. The actions of enzymes, which occur naturally in fruit are slowed down by drying but are not deactivated by the process (Harrison 1993; Mercer 2012).

Some fruit dry better if pre-treated. Pre-treatment decreases oxidation, giving a better appearance, reduces vitamin loss and increase shelf life (LaBorde, 2009). It also inhibits the browning that occurs on light coloured fruit when it is cut and exposed to oxygen. Sulphuring or sulphite dips are recommended to extend the shelf life of dried fruit (Harrison,1993; LaBorde, 2009; Gunduz & Akman, 2015). Sulphurous acid has long been acknowledged as a relatively cost-effective and easily obtainable preservative for various products. Its preservation value for colour maintenance and its prevention of microbial growth activity have long been used for the control of unwanted colour and flavour changes during processing (Joslyn & Braverman1954; Green 1976; Pleskovich *et al.,* 2014). Nonetheless, the negative effects of sulphites have been queried as a result of their part in causing asthmatic reactions in sensitive persons (Li *et al.*, 2014).

There is growing evidence, which suggests that synthetic antioxidants are toxic and as consumer pressure on the food industry intensifies to move away from chemical preservatives; there is an increased interest in natural antioxidants (Siddiq *et al.*, 2005; Singh *et al.*, 2009). Weak acids such as ascorbic and citric acid are found naturally and have antioxidant and antimicrobial properties. Sorbic acid is known to be safe and is widely employed as a preservative (Anyasi *et al.*, 2015).

Sorbic acid and its salts have GRAS (generally recognised as safe) status (Silveira *et al.*, 2007). Commonly used in an extensive range of foods as a preservative sorbic acid slows the growth of yeast and moulds. It is generally put into foods as salt (Wood *et al.*, 2004). The

water-soluble salt of sorbic acid, potassium sorbate is used considerably in fruit with high moisture (Alagöz *et al.*, 2015). Potassium sorbate is commonly used as a broad-spectrum food preservative (Parra *et al.*, 2014).

The most significant natural acid which is manufactured by the ton through the fermentation process is citric acid (Soccol *et al.*, 2006). Various reports show that it is commonly used as an anti-browning agent (Son *et al.*, 2001; Jiang *et al.*, 2004). It is recognized worldwide as having GRAS status and as a result, is extensively used by the food and pharmaceutical industries (Soccol *et al.*, 2006). According to Cárcel *et al.* (2010) is a noteworthy alternative to sulphites.

The most used alternative to sulphite is ascorbic acid (Li *et al.*, 2015); it is a safe (GRAS) antioxidant and allowed by the U.S. Food and Drug Administration (FDA) for use on fruits and vegetables to inhibit browning (Javdani *et al.*, 2013; Krasnova *et al.*, 2013). According to Krasnova *et al.* (2013), ascorbic acid is to a great extend acknowledged as a vital nutrient for human health and is an inherent element in fresh fruits and vegetables.

Studies by Siddiq *et al.* (2005) and Verma *et al.* (2009) stated that phytochemicals are found in abundance in plants and their products have a range of biological activities which include antioxidant potential. One such plant, *Moringa oleifera Lamarack* that belongs to the Moringaceae family, and grows in many parts of the tropics and sub-tropics of Asia and Africa leaves have an antioxidant effect (Ghasi *et al.*, 2000; Singh *et al.*, 2009). A study by Dahot (1998) showed noteworthy antimicrobial activity against Gram-positive and negative fungal species by the liquid extract of *Moringa oleifera* leaves. The many biological activities of the leaves include hypolipidaemic, anti-atherosclerotic and antioxidant activities (Singh *et al.*, 2009; Das *et al.*, 2012; Vongsak *et al.*, 2013a).

1.2 Problem Statement

One of the major quality attributes of dried fruit is colour. The first impression of quality for consumers is the appearance and the colour of the food (Chong *et al.*, 2013). To safeguard dried fruit from chemical and microbiological deterioration, one of the most frequently used compounds is sulphur dioxide applied as sodium or potassium metabisulphite. Nevertheless, health concerns have been raised around the use of sulphites especially with regards to the part it plays in causing asthmatic reactions in sensitive people (Cárcel *et al.*, 2010).

Various studies have been done on weak acids such as the one done by Alagoz *et al*., (2015) on the effects of different sorbic acid and moisture levels on chemical and microbiological qualities of sun-dried apricots during storage. Mrada *et al*., (2012) reported the influence of air-drying temperature on kinetics, physicochemical properties, total phenolic content and ascorbic acid of pears. Doymaz (2010) conducted a study on the effect of citric acid and blanching pre-treatments on drying and rehydration of Amasya red apples. However, nothing is reported on weak acids (ascorbic acid, citric acid and potassium sorbate) in

combination with *Moringa oleifera* leaf powder on apples. Thus, it is of interest to investigate the suitability of using some weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera* leaf extract powder in combination as an effective pre-treatment that will produce dried fruit (apples) which are microbiologically safe with an acceptable colour, texture, taste and shelf life.

1.3 Broad Objective

The broad objective is to study the effect of some weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera extract* leaf powder on the quality and storage stability of dried Granny Smith apples to find an alternative to sulphites as a preservative for dried fruit.

1.3.1 Specific objectives

The specific objectives are the following:

1. Identify the best weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera* leaf extract powder combination that will reduce the discolouration of the dried apple slices.
2. Establish whether the optimal combination of weak acids and *Moringa oleifera* leaf extract powder will give the required physicochemical properties (colour, texture, moisture, water activity and total acidity) as well as microbial safety to the dried fruit (apples).
3. Determine the storage stability of the dried fruit (apples) using the optimum combination of the weak acids and *Moringa oleifera* leaf extract powder.
4. Establish consumer acceptability with regards to colour (appearance), texture and taste using the 9-point hedonic scale.

1.4 Hypothesis

The following hypothesis will be tested in the study:

1. The optimum combination of weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera* leaf powder will preserve the colour and give the required physicochemical properties and microbial safety of the dried apples.
2. The most effective combination of weak acids and *Moringa oleifera* leaf extract powder as pre-treatment will produce dried apples (Granny Smith) with reasonable storage stability.
3. Consumers will find the dried Granny Smith apple slices colour (appearance), texture and taste acceptable.

1.5 Delineations

Only one cultivar apple will be used namely, Granny Smith apple.

1.6 Importance of the Study

The harvests of fruit and vegetables have been significantly improved by modern technology; regrettably, a quarter of fresh food produce harvested is never eaten due to decay during its storage, transportation and processing (Verma & Joshi, 2000). Dehydrating these perishables helps to store them for longer periods without the risk of decomposition. It moreover avoids post-harvest losses and thus reduces wastage in the agricultural sector which can assist with food security (Sethi, 2007).

The purpose of this study is to find alternatives to sulphites that will provide a quality product with a reasonable shelf life. This can lead to the provision of a safe and healthy dried fruit snack to the population; and especially to vulnerable individuals such as children and pregnant women as well as people who are sensitive to sulphites. It will also give an added benefit which is the availability of off-season fruit without the negative effects of sulphur dioxide (Sethi, 2007; Mercer, 2012). This study will also promote food safety as well as improve sensitive consumer's quality of life. By reducing the quantity of sulphites released into the air it will further provide a safer environment. In addition, if this study proves successful and is approved for commercial use it can lead to the generation of employment.

1.7 Thesis Overview

The thesis is written in article format. It comprises of six chapters with each chapter having its references. Figure 1.1 illustrates the thesis overview.

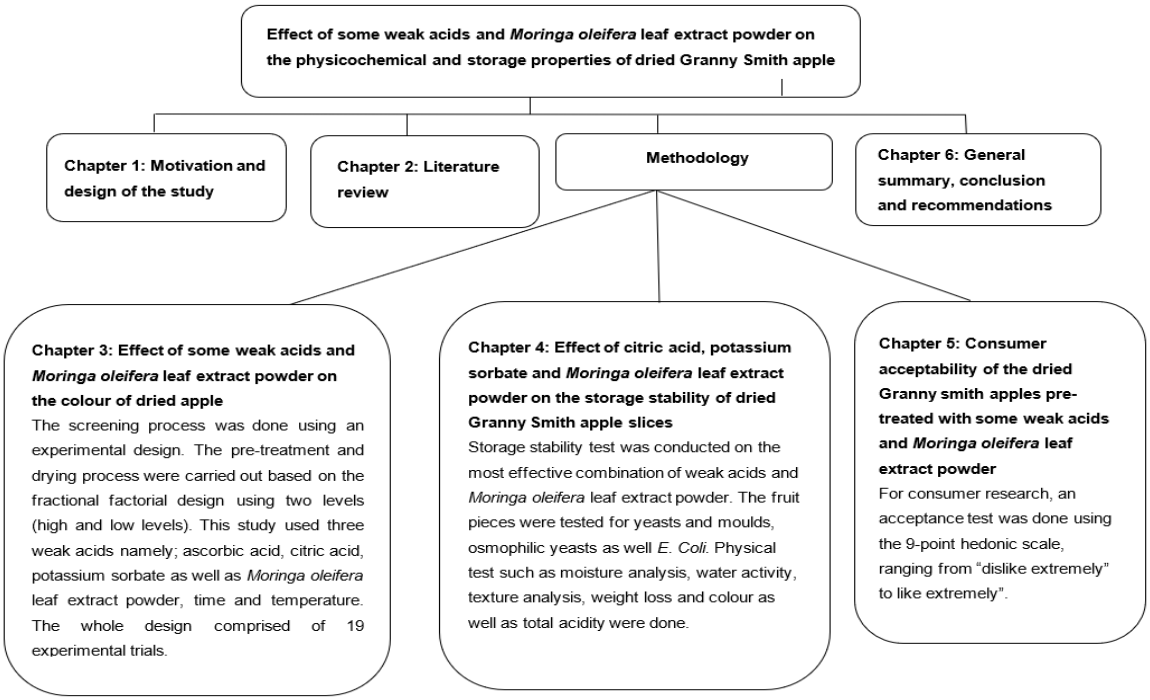

Figure 1.1 Diagram of thesis overview

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CHAPTER 2
LITERATURE REVIEW

2.1 Overview of Fruits and Vegetables in Human Nutrition

One of the key nutritional recommendations to avoid non-communicable diseases (NCDs) is to eat 3-5 servings of fruits and vegetables daily. Reports published revealed that except for basic nourishment, fruits as a result of their supply of vital vitamins, minerals, as well as a range of phytochemicals, offer noteworthy health benefits to individuals (Alasalvar and Shahidi, 2013; Chang *et al.*, 2016; Shrestha *et al.*, 2020). Fruits are customarily eaten in their natural form and because they easily succumb to microbial attacks are often dried to extend their shelf life (Chong *et al.*, 2013; Megías-Pérez *et al.*, 2014). Studies have shown that fruits play a bigger part in the prevention of NCDs than vegetables (Alasalvar & Shahidi, 2013; Chang *et al.*, 2016).

The seasonality of fruits and vegetable often results in their oversupply and by dehydrating these easily degradable products its storage time can be prolonged in a minimally controlled facility (Kutyła-Olesiuk *et al.*, 2013; Chang *et al.*, 2016). Fruits have a high sugar and acid content which makes them safe for sun drying (Harrison 1993). Losses after the harvesting process fluctuate from an expected 5% to over 20% in first and second world nations and as high as 50% in third world nations. In storage, more than 70% of these losses in fruits and vegetables is a consequence of moulds (Frankova *et al.*, 2016). Microbiological, chemical (enzymatic) and physical (bruising) are the key reasons for the wastage of fresh produce (Sethi, 2007; Chen *et al.*, 2016; Koushesh Saba & Sogvar, 2016a).

Nearly all the varieties of fresh fruit can be found in dehydrated form. Generally raisins, figs, dates and apricot are amongst the dried fruits normally found in stores Alasalvar & Shahidi (2013), but health shops and local markets provide a wide selection which includes dried apple, mango and several other variants (Alasalvar & Shahidi, 2013; Kundu & Chun, 2014). It is also worth mentioning that dried fruits with high dietary fibre content assists with meeting our dietary recommendation of 14 g of fibre per 1000 calories of food consumed daily (Alasalvar & Shahidi, 2013). Chang *et al.* (2016) reported that an extensive variety of phytochemicals is supplied by dehydrated fruit.

The phytochemicals present in dried fruit include phenolic acids, flavonoids, phytoestrogens, and carotenoids. Health benefits seem to be linked with regular consumption of fruits and vegetables partly because of these phenolic substances Alasalvar & Shahidi (2013) and Chang *et al.* (2016) and include the removal of free radicals, the protection and production of other dietary antioxidants and the chelation of metals that can act as a catalyst for oxidation (Pace *et al.*, 2014). Studies have shown that the antioxidant and anti-cancer properties of fruits are a result of the polyphenols contained in them according to Kundu &

Chun (2014) and Pace *et al.* (2014) and that these health-beneficial phytochemicals are available even after processing (Alasalvar & Shahidi, 2013; Chang *et al.*, 2016).

Phenolic acids are natural antioxidants and present in nearly all plants as reported by Alasalvar & Shahidi (2013); Pace *et al.* (2014) and Viuda-Martos *et al.* (2014), and in several instances, they play a role in providing their hue and flavour (Viuda-Martos, *et al*., 2014). The majority of the phenolic compounds found in the cell walls are insoluble and provide strength, control and safeguard plant growth and tissue integrity, while the soluble phenolics are found inside the cell (Diamanti *et al.*, 2017). Phenolic constituents can be categorised as flavonoids and non-flavonoids and possess a minimum of one aromatic ring with single or multiplehydroxyl groups. In addition to their role as an antioxidant, flavonoids have other properties such as anti-mutagenic activity, atherosclerosis, hair tonic and antibacterial properties (Pace *et al.*, 2014). It has also been reported that enzymes such as 5-lipoxygenase, cyclooxygenase, monooxygenase, or xanthine oxidase involved in oxidation systems are inhibited by flavonoids (Viuda-Martos *et al.*, 2014). Tannins, flavonoids and phenolic acids are the phenolics normally eaten by individuals (Pace *et al.*, 2014). Figure 2.1 shows the chemical structures of some phenolic compounds that are found in dried fruits.

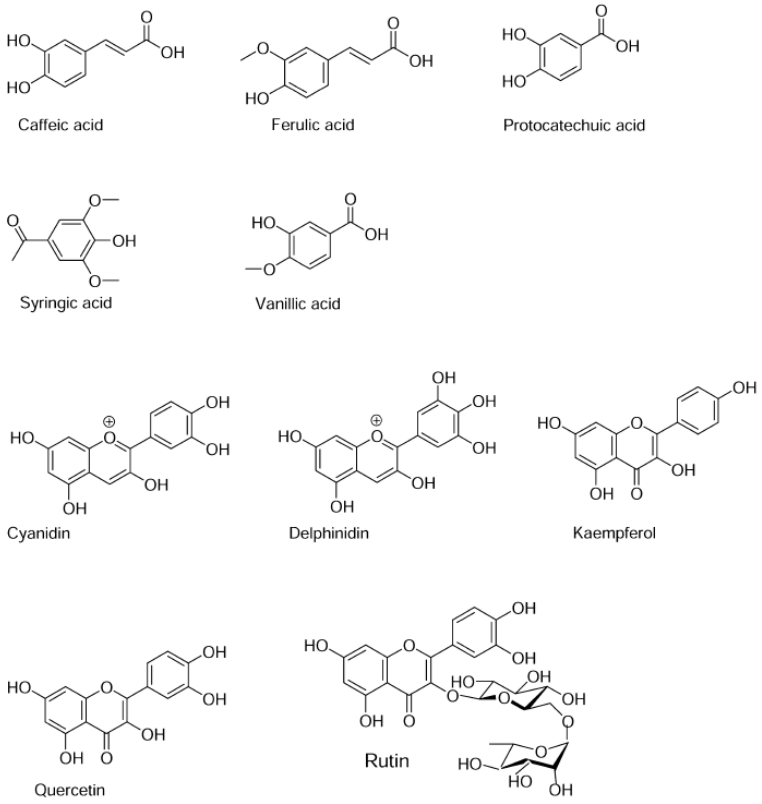


Figure 2.1 Chemical structures of representative phenolic compounds (phenolic acids, flavanols, and anthocyanidins) reported in dried fruits (Chang *et al.*, 2016)

Consumers are becoming more aware of dried foods as a result of our current lifestyle Megías-Pérez *et al. (*2014) and they value fresh produce of exceptional quality not only for their nutritive worth and pleasurable tastes but because it also aids their well-being as a result of its vitamin and antioxidant content (Djendoubi Mrad *et al.*, 2012; Chong *et al.*, 2013; Megías-Pérez *et al.*, 2014; Udomkun *et al.*, 2015).

A need has been identified for the commercialisation of chemical-free fruit since statements on the harmful effects of artificial chemicals on the surroundings and the well-being of the people have been published (Parra *et al.*, 2014). It has become an established procedure in the food industry to add antioxidants to increase the storage period of foods. Consumers nowadays believe that natural compounds are non-toxic and favour natural antioxidants over synthetic ones (Siddiq *et al.*, 2005). The next section will discuss apples in the human diet.

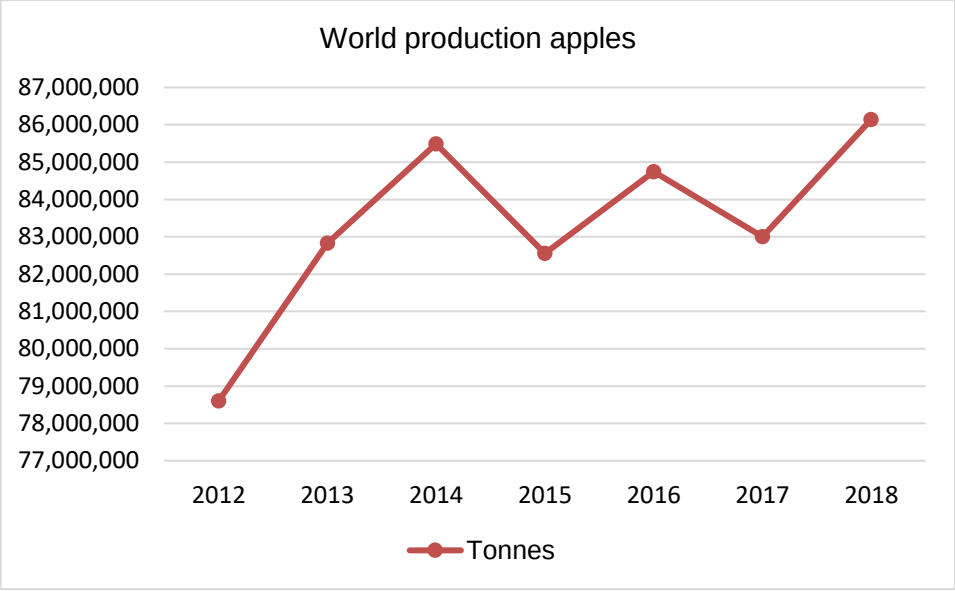
2.1.1 Apples in the human diet

Globally apples are one of the most extensively cultivated and eaten fruit. It is part of the genus *Malus* and the family *Rosaceae* (Candrawinata *et al.*, 2012). Worldwide apples, oranges, bananas, grapefruits and grapes are the five most consumed fruit as stated by Duda-Chodak *et al. (*2010) with apples placed fourth in this group (Candrawinata *et al.*, 2012). Following the banana, it is the second most-consumed fruit in America according to Candrawinata *et al.* (2012) and its total production was valued at almost 3.1 million dollars in 2012 (Leemans & Kleynen, 2008). It was reported that in 2007 the quantity of apples harvested internationally was 64.3 million tonnes which make it a major player in fruit production worldwide (Doymaz, 2010). Figure 2.2 shows images of whole and halved Granny Smith apples.

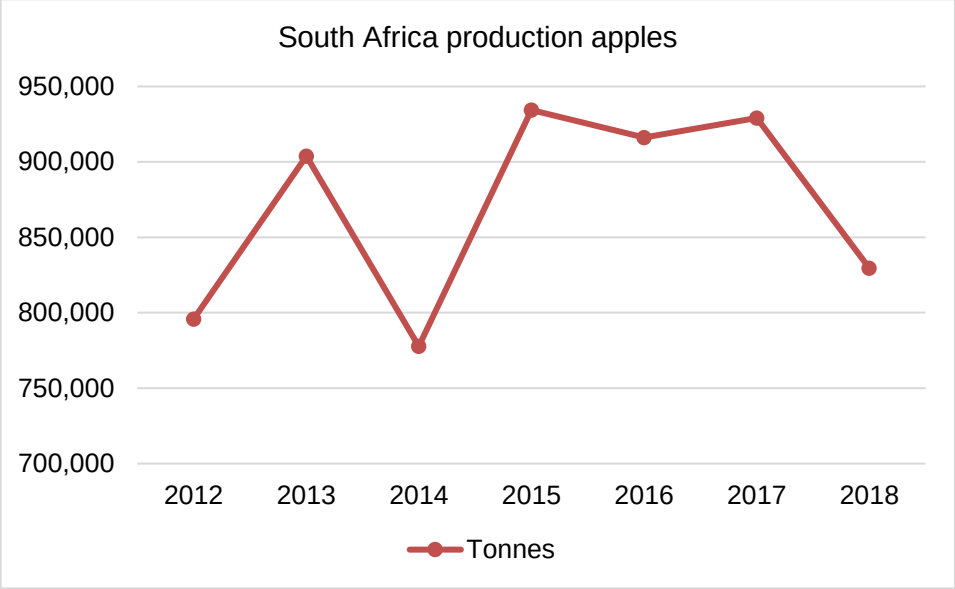
Figure 2.2 Images of whole and halved Granny Smith apples

China is the top producer of apples with approximately 40 million metric tonnes recorded in 2013 followed by the United States, Italy, Poland, Turkey and Russia (Leemans & Kleynen, 2008; Doymaz, 2010). The newly picked apples are placed in storage straight after harvesting so that it can be available throughout the year (Li *et al.*, 2011). Figure 2.3 illustrates production quantities of apples in the world and South Africa. Figure 2.3a clearly shows that worldwide production of apples has substantially increased from approximately 78,600,228 tonnes in 2012 to approximately 86,142,197 tonnes in 2018. Figure 2.3b shows that the

productions of apples in South Africa have grown noticeably from approximately 795,758 tonnes in 2012 to approximately 829,636 tonnes in 2018.



a



b

Figure 2.3 Production of apples, **a**: quantities produced in the world and **b**: quantities produced in South Africa 2012-2018 (FAOSTAT, 2020)

Of the many types of apples, Granny Smith and Red Gala are but a few that differ in sweetness, pH, firmness, shape and size. It is a source of fibre which is beneficial for reducing cholesterol. They also assist in maintaining a healthy digestive system since they consist of enzymes that help with the breakdown of foodstuff. Also, apples are fat, sodium and cholesterol-free (Candrawinata *et al.*, 2012). Apples are eaten fresh or can be used to produce various other products such as jams, jelly, pulps, dried apple, canned and juices (Doymaz,

2010; Duda-Chodak *et al.*, 2010). It is an excellent resource of antioxidants especially the apple peels and is consumed by many ethnic groups (Boyer & Liu, 2004a). Figure 2.4 illustrates the chemical structures of several antioxidants found in apples.

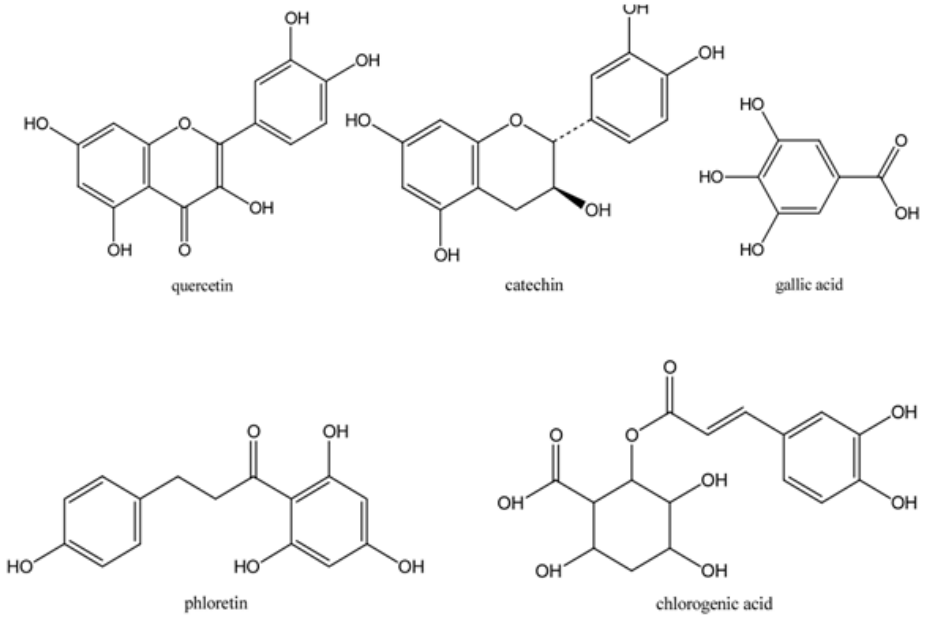


Figure 2.4 Chemical structures of certain apple antioxidants (Boyer & Liu, 2004a)

Fungal diseases after harvesting are the main reason for the short shelf life of fresh apples. When in storage there are over 90 fungal species that can be responsible for the spoilage of apples with grey mould and blue rot namely *Botrytis cinerea* Pers.:Fr. and *Penicillium expansum Link* respectively being the main diseases (Leibinger *et al.*, 1997; Li *et al.*, 2011). At present apples have almost no resistance to fungal disease as breeders rarely check when they combine different variants to create a new cultivar whether the new variant is resistant to fungal diseases (Janisiewicz *et al.*, 2016).

Polyphenols and flavonoids are only a few of the various phytochemicals found in apples as well as many vitamins and minerals (Lozowicka, 2015). As a result of its accessibility and cost-effectiveness according to Duda-Chodak *et al.* (2010) apples are an essential dietary resource of phenolic constituents (Wang *et al.*, 2015). The six main classes of polyphenols present in apples are; flavanol glycosides, catechins and epicatechins, anthocyanins, dihydrochalcones, phenolic acids and procyanidins (Candrawinata *et al.*, 2012). Figure 2.5 displays a few chemical structures of polyphenols present in apple and apple products (Health & Hyson, 2011). These numerous nutritional compounds (vitamins and phenolic compounds) found in apples assist in enhancing public health (Health & Hyson, 2011; Lozowicka, 2015; Liu *et al.*, 2016).

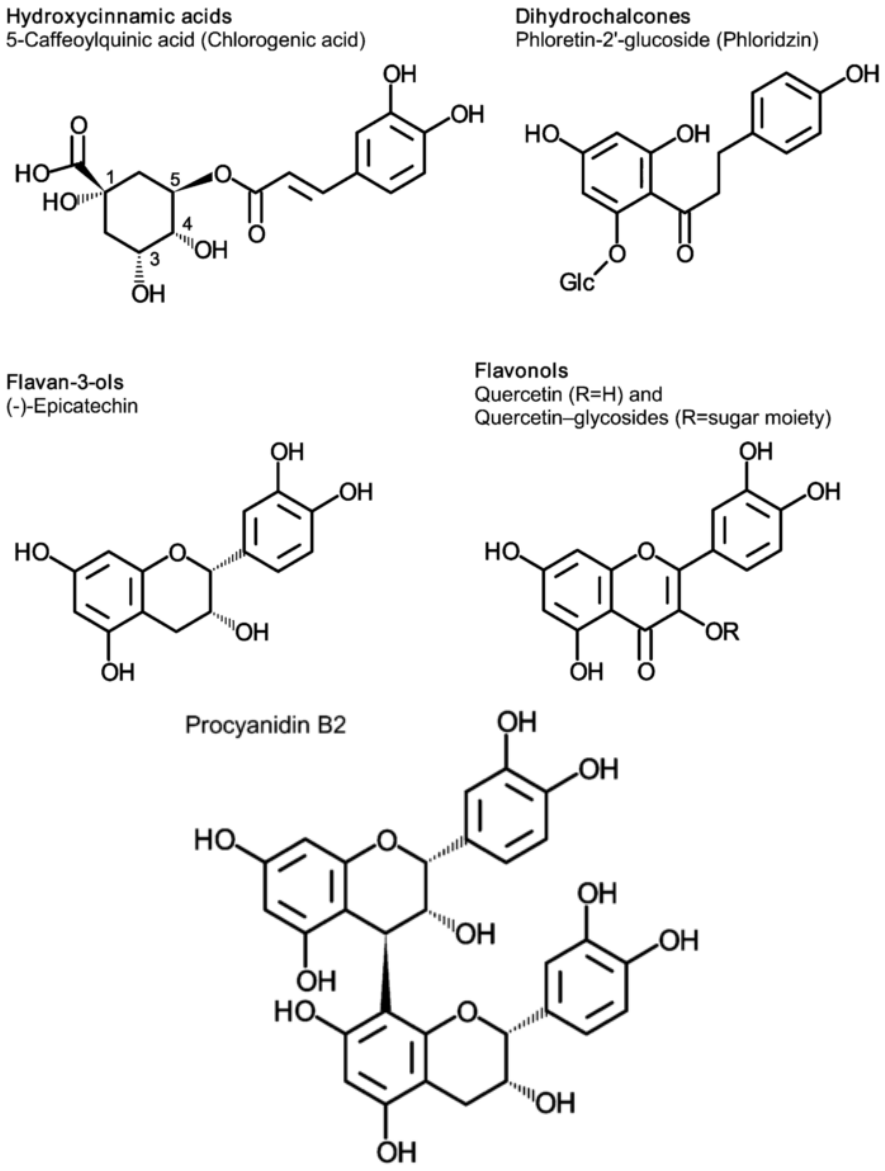


Figure 2.5 Chemical structures of some polyphenols present in apple and apple products (Health & Hyson, 2011)

Noteworthy is the fact that apples contain the highest quantity of unbound phenolic compounds when evaluated with other fruits which imply that their phenolics is maybe more accessible for absorption into the bloodstream (Boyer & Liu, 2004a). Several studies have found a correlation between the mitigation of risk for some cancers, cardiovascular disease, asthma and diabetes and the consumption of apples (Boyer & Liu, 2004b; Duda-Chodak *et al.*, 2010; Health & Hyson, 2011). A decreased risk of lung cancer was also observed in women who consume a minimum of one serving of apples and pears a day. Furthermore, the flavonoids in apples were found to be one of the major dietary sources which showed a link with the reduction in human fatalities (Boyer & Liu, 2004a).

Nowadays customers are insisting on superior quality, uniform apples with the right taste and texture. Saei *et al.* (2011) and Mendoza *et al.* (2014) reported that the main eating

quality features that consumer values nowadays are firmness, crispness and juiciness. To guarantee that apples of consistently superior quality can be provided to customers for an extended period, numerous markets currently expect fruit firmness and solid concentration as a minimum quality standard for apples (Saei *et al.*, 2011).

2.2 Pre-treatment of Fruits before Drying

2.2.1 Overview of sulphur dioxide (SO_2) and fruit dehydration

Sulphur dioxide is frequently employed as a pre-treatment when dehydration is used to preserve the quality of products (Miranda *et al.*, 2009). Sulphur dioxide is a gas devoid of colour with a strong, repulsive odour (Rotter, 2008; Vale, 2012). Its molecular formula is SO_2 and its molecular weight is 64.07. In nature, sulphur dioxide is created by active volcanoes and forest fires (Rotter, 2008). It is non-explosive and heat stable up to 2000°C (IARC, 1992). It was used by the Greeks and Egyptians to kill pests, disinfect and refresh homes and the boats they used to ferry food. It was employed for the first time in 1803 as an agricultural fungicide, utilizing lime sulphur as a liquid suspension (Moore & Payne, 2004). This chemical is highly soluble in water and is one of the key substances which produce acid rain (Rotter, 2008). It is also widely used in the production of sulphuric acid (Rotter, 2008; Vale, 2012).

These days sulphur dioxide is used in numerous food industries particularly in the production of low acid food products (Guerrero & Cantos-Villar, 2015). Sulphur dioxide has been used extensively in the winemaking industry for quite some time and is an essential part of the industry (Salaha *et al.*, 2008; Guerrero & Cantos-Villar, 2015). It is present in its unbound form as SO_2 which is the active form, or as bound known as combined and together are calculated as total SO_2 (Assessment, 2012; Guerrero & Cantos-Villar, 2015). Sulphur dioxide has various functions in the food industry which include preventing enzymic and non-enzymic browning, antimicrobial properties and also operate as an antioxidant (Salaha *et al.*, 2008; Thomas & Forbes, 2010; Assessment, 2012).

Legally only certain quantities of SO_2 are allowed in foods and 2000 ppm is the maximum limit for dried fruits (Duan *et al.*, 2016). In dried fruit, it functions as an antimicrobial agent and assists with maintaining the colour and taste. The moisture content of dried fruit is normally thirty percent or less, as previously mentioned SO_2 is very soluble in water, thus this remaining water allows the sulphur dioxide to dissolve into solution. Examples of fruits that are normally pre-treated with sulphur dioxide are sultanas, dried apples, peaches and mango (Assessment, 2012).

When sulphur dioxide comes in contact with the skin it causes numerous negative reactions such as blisters. Compressed air or its aqueous form can cause frostbite and eye contact in severe cases can result in blindness (Rotter, 2008). Sulphur dioxide is a strong irritant and could initiate inflammation through epithelial injury, airway hyper responsiveness

(AHR) and neurogenic inflammation (Li *et al.*, 2014). Proper labelling (Directive 2003/89/EC) of sulphited products is also required (Cárcel *et al.*, 2010).

There are severe risks to well-being and ecological effects of traditional chemical treatments. In the US the use of sulphur dioxide is only allowed in grapes as a fungicide and is prohibited in all other foodstuffs. It is considered a health hazard for individuals hypersensitive to sulphur (Ducamp-Collin *et al.*, 2008). Further evidence shows that low quantities of sulphur dioxide can result in bronchoconstriction in people with asthma and that high quantities of this air pollutant can produce the same effect in individuals that are in good physical shape (Cetinkaya *et al.*, 2006). Consequently, the safety of food processed with sulphites has been queried as a result of its negative effect on health, particularly on asthmatic people (Cárcel *et al.*, 2010; Alagöz *et al.*, 2015). In this study, the weak acids, ascorbic, citric acid, potassium sorbate and *Moringa oleifera* leaf extract powder will be reviewed as a pre-treatment of fruits.

2.2.2 Ascorbic acid as a pre-treatment for fruits

Ascorbic acid is white or almost white crystals or crystalline powder and almost odourless. Its molecular formula is $C_6H_8O_6$ and its molecular weight is 176.13 (Rowe *et al.*, 2006; EFSA, 2013). Its structural formula is shown in Figure 2.6. Naturally found in fruits and vegetables it is a significant antioxidant which in addition to food fortification is also used as a food component or food additive (Comunian *et al.*, 2013; De'Nobili *et al.*, 2016; Sartori & Menegalli, 2016). It is a weak acid that dissolves easily in water and is used as a supplement as well as a preservative for pharmaceutical products (De'Nobili *et al.*, 2016). An additional function that ascorbic acid performs is that of an oxygen scavenger by removing molecular oxygen in polyphenol oxidase reactions (Rojas-Graü *et al.*, 2007; Krasnova *et al.*, 2013).

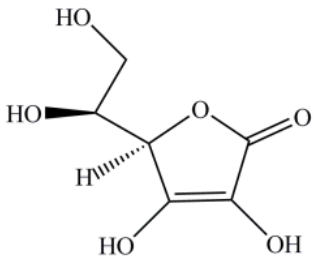


Figure 2.6 Structural formula of ascorbic acid (Rowe *et al.*, 2006; Efsa, 2013)

Ascorbic acid can be produced artificially or extracted from a variety of fruit and vegetable sources such as rose hips, blackcurrants, the juice of citrus fruits, and the ripe fruit of *Capsicum annuum L* in which it is found naturally. A widespread method to produce ascorbic acid synthetically requires the hydrogenation of D-glucose to D-sorbitol, after which oxidation using *Acetobacter suboxydans* to form L-sorbose takes place. A carboxyl group is then added at C1 by air oxidation of the diacetone derivative of L-sorbose and the resulting diacetone-2-

keto-L-gulonic acid is converted to L-ascorbic acid by heating with hydrochloric acid (Rowe *et al.*, 2006).

Temperature, pH, concentrations of polyphenol oxidase (PPO), phenolic substances and free oxygen in the tissue are five of the key factors that influence the rate of enzymatic browning in agricultural products (Limbo & Piergiovanni, 2006). Ascorbic acid is currently used the most as a substitute for sulphites to prevent browning and oxidation reactions in the minimally processed fruit industry (Rojas-Graü *et al.*, 2007; Krasnova *et al.*, 2013). The majority of polyphenols are contained in the vacuoles and cell walls of fresh produce whereas the chromoplast, cytoplasm and mitochondria hold minimal quantities of these substances (Li-Qin *et al.*, 2009). Most of the processing of fruit and vegetables are automated and it is this type of operation that results in the breakage of the cellular compartments causing the phenolic compounds and polyphenol oxidase to make contact (Limbo & Piergiovanni, 2006; Rojas-Graü *et al.*, 2008; Li-Qin *et al.*, 2009; Skinner, 2010). This brings about the oxidation of the phenolic constituents, usually as a result of the action of the enzymes polyphenol oxidase and peroxidase on the polyphenols and consequently polymerization of the quinones which are responsible for the brown or black look of the fresh produce (Li-Qin *et al.*, 2009).

It was reported by various studies that ascorbic acid reduces the quinones produced back to dihydroxy polyphenols and thus surface browning (Rocculi *et al.*, 2007; Rojas-Graü *et al.*, 2008; Javdani *et al.*, 2013). At 0.2% dosage ascorbic acid, polyphenol oxidase activity decreased by 10-23% and at 0.5% by 15-21%, at its maximum concentration of 1% PPO activity declined by 17-25% (Barbagallo *et al.*, 2012). Despite this, the effect of ascorbic acid does not last as it is permanently oxidised over time to dehydro-ascorbic acid, either under enzymatic or chemical oxidation conditions and polymerization of o-quinones take place after it is depleted completely which leads to browning (Limbo & Piergiovanni, 2006; Rojas-Graü *et al.*, 2008; Salaha *et al.*, 2008; Chow *et al.*, 2011; Javdani *et al.*, 2013; Li *et al.*, 2015; Koushesh Saba & Sogvar, 2016). The breakdown of ascorbic acid is accelerated by enzymes containing iron or copper in their prosthetic groups. This ability of ascorbic acid to function as a free radical initiator as well as an antioxidant is called the crossover effect (Comuzzo *et al.*, 2015). Figure 2.7 illustrates the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid.

The polyphenol oxidase action in fruit can be minimized by various compounds, but ascorbic acid is the most widely used and is vastly successful when it comes to decreasing browning in fresh produce (Javdani *et al.*, 2013; Li *et al.*, 2015; Koushesh Saba & Sogvar, 2016). It has been used as a dip for fresh-cut fruit for quite some time (Li *et al.*, 2015). Arroyo-López *et al.* (2008) stated that the results of ascorbic acid success against surface browning in apples and peas are well supported. It has GRAS (generally recognised as safe) status, is economical and is not frowned upon by consumers (Javdani *et al.*, 2013).

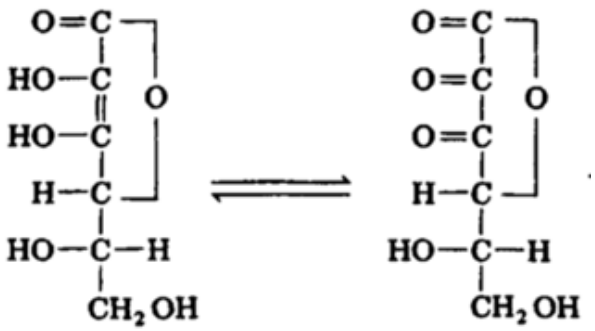


Figure 2.7 Oxidation of L-ascorbic acid to dehydro-L-ascorbic acid (Skinner, 2010)

2.2.3 Citric acid as a pre-treatment for fruits

The method to produce citric acid through fermentation using microorganisms was started in 1893 by Wehmer. He discovered that *Penicillium glaucum* used sugar to produce citric acid by fermentation. However, the winning combination for industrial manufacture of citric acid was achieved many years later in 1916 by Currie who found that large quantities of citric acid can be produced by various strains of *Aspergillus niger* (Soccol *et al.*, 2006). Although it can be extracted from lemon or pineapple waste, citric acids manufactured by fermentation exists for many years (Rowe *et al.*, 2006; Kutyła-Olesiuk *et al.*, 2014).

Nowadays the industrial manufacture of citric acid is achieved using molasses and a variety of *Aspergillus niger* strains (Soccol *et al.*, 2006; Kutyła-Olesiuk *et al.*, 2014; Adeoye *et al.*, 2015). To obtain higher yields and assist fungal growth inorganic salts are added (Kutyła-Olesiuk *et al.*, 2014). Purification of citric acid is obtained by re-crystallisation; the monohydrate structure is achieved from a cold concentrated liquid solution whereas the anhydrous form from a hot concentrated liquid (Rowe *et al.*, 2006).

Citric acid is a white or transparent, odourless powder with a sharp sour taste (Rowe *et al.*, 2006; Soccol *et al.*, 2006; Adeoye *et al.*, 2015). Its molecular formula is $C_6H_8O_7$ and its molecular weight is 192.12 (Brittain, 2014). The structural formula for citric acid is illustrated in Figure 2.8. It is also identified as 2-hydroxyl-1, 2, 3-propane tricarboxylic acid (Xie *et al.*, 2010; Betiku & Adesina, 2013; De'Nobili *et al.*, 2016).

It is a weak organic acid with significant quantities spread throughout nature (Betiku & Adesina, 2013; Adeoye *et al.*, 2015; De'Nobili *et al.*, 2016). It is found in plants (lemon. pineapple, orange, raspberry and grapefruit) and animals (bones, muscle and blood) (Francis, 2000; List *et al.*, 2016).

It is also highly soluble in water. Worldwide, approximately 1.4 million tons of citric acids are produced on an annual basis (Soccol *et al.*, 2006; Adeoye *et al.*, 2015). Records show that China is responsible for 35-40% of the global manufacture of citric acid (Soccol *et al.*, 2006). Citric acid is worldwide generally recognized as safe and as a result is used widely by the food and pharmaceutical industries (Rowe *et al.*, 2006; Soccol *et al.*, 2006; Betiku & Adesina, 2013).

Furthermore citric acid is a vital constituent in the Krebs cycle connecting the metabolic reactions of carbohydrates, protein and lipids (Murray *et al.*, 2009; Brittain, 2014).

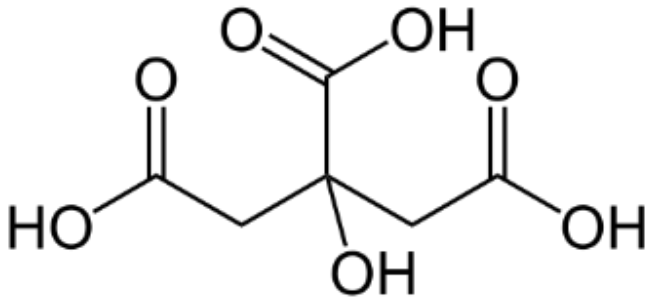


Figure 2.8 Structural formula of citric acid (Brittain, 2014)

Citric acid has many applications and is used for flavour enhancement (0.3-2.0%) for its tart and acidic taste, as a sequestering agent (0.3-2.0%), buffer (0.1-2.0%) as well as an antioxidant synergist (Rowe *et al.*, 2006). The cosmetic and toiletries industry use it as a buffer and in the cleaning of metals (Betiku & Adesina, 2013). Various methods have been assessed to decrease the browning of fresh-cut produce of which the most frequently used is dipping the product in an anti-browning agent.

Citric acid is an example of such a dipping agent and is a copper chelating agent and lowers the pH as well (Limbo & Piergiovanni, 2006; Goyeneche *et al.*, 2014). A decrease of 21-27% PPO activity was reported at 1% concentration of citric acid by (Barbagallo *et al.*, 2012). The binding of the copper at the active enzyme site become looser at pH values below 4, allowing the citric acid to remove copper (Limbo & Piergiovanni, 2006). This chelating action is responsible for the inhibition of the enzyme polyphenol oxidase (Jiang *et al.*, 2004; Limbo & Piergiovanni, 2006; Ansorena *et al.*, 2014; Goyeneche *et al.*, 2014). PPO needs copper ions to function (Ioannou and Ghoul, 2013). Citric acid thus prevents browning by decreasing the pH and by its direct action on the copper present on the active site of PPO enzyme (Rocculi *et al.*, 2007; Barbagallo *et al.*, 2012; Abd-Elhady, 2014). Quite a few studies reported that pre-treatment of sliced apple with citric inhibits the browning of sliced apple and resulted in a longer storage period for foodstuff (Jiang *et al.*, 2004; Chen *et al.*, 2016).

Citric acid is also frequently utilized to contain microbial contamination of fresh produce. Some studies revealed that citric acid inhibits the growth of several microorganisms which includes *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* (Kim & Rhee, 2015; Chen *et al.*, 2016). When the antibacterial activity of citric acid was studied against *L. monocytogenes*, *S. aureus* and *B. cereus* at different concentrations (0.3, 0.5, 1, 1.5 and 2%), using the inhibition zone method, the results showed an increase in the inhibition zone with an increase in the citric acid concentration (Barzegari *et al.*, 2014).

2.2.4 Potassium sorbate as a pre-treatment for fruits

Sorbic acid and its salts have become the foremost preservative across the globe in the food industry as a result of their physiological inertness, their high success rate even in the weakest acid pH range and neutral taste. Sorbic acid and potassium sorbate are the most frequently used products with sorbic acid only slightly soluble in water, whilst potassium sorbate is freely soluble in water (Lück, 1990). Reports show that sorbic acids and its salts are favoured by the food industry because it has no significant effect on the public's health, no antimicrobial activity at high pH and an allowable everyday consumption of 25 mg/kg body weight (Rowe *et al.*, 2006; Alagöz *et al.*, 2015). Sorbic acid's metabolism is similar to fatty acids in human beings and animals and thus has a low order of toxicity (Turantaş & Göksungur, 1999).

Sorbic acid is a white, odourless powder or crystals with a weak acidic, astringent taste and a barely noticeable smell. In 1859 sorbic acid was extracted for the first time from the berry of the mountain ash tree (Dharmadhikari, 1974; Eastman, 1998). It can also be extracted as parasorbic acid. Sorbic acid can also be produced artificially by the condensation of crotonaldehyde and ketene in the presence of boron trifluoride, which is just one of the many ways it can be produced synthetically. It can also be produced in a culture medium with bacteria by the fermentation of sorbitol (Rowe *et al.*, 2006). Its molecular formula is $C_6H_8O_2$ and its molecular weight is 112.126 g/mol (Dharmadhikari, 1974; Rowe *et al.*, 2006). The structural formulas of sorbic acid and potassium sorbate are shown in Figure 2.9.

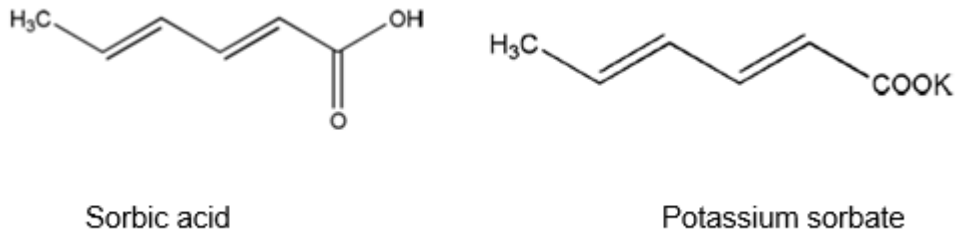


Figure 2.9 Structural formulas of sorbic acid and potassium sorbate (Rowe *et al.*, 2006)

Potassium sorbate's molecular formula is $C_6H_7O_2K$ and its molecular weight is 150.22 g/mol. It is a white crystalline powder with a faint, distinctive odour and is produced from sorbic acid and potassium hydroxide (Rowe *et al.*, 2006). When potassium sorbate is mixed with water, hydrolysis occurs and sorbic acid is produced which is the most active antimicrobial form (Mendonca, 1992).

It is a valuable food preservative as a result of it being highly soluble in water (Mendonca, 1992; Rowe *et al.*, 2006; Montesinos-Herrero *et al.*, 2009). This makes it highly suitable for liquid applications (Montesinos-Herrero *et al.*, 2009). The general dosage of sorbate's, when used as a food preservative, is 0.1%-0.3% (Mendonca, 1992; Stanojevic *et al.*, 2009). Potassium sorbate is generally used as an antimicrobial preservative (Turantaş &

Göksungur, 1999; Hsu & Sun, 2006; Rowe *et al.*, 2006; Mohammad, 2009). Despite having anti-bacterial properties it is mainly used as an anti-fungal preservative (Rowe *et al.*, 2006).

The preservation effect of potassium sorbate is greatly improved with an increase in temperature and concentration (Wild, 1987, Rowe *et al.,* 2006, Montesinos-Herrero *et al.,* 2009). When used with other anti-microbial preservatives the preservation effect of potassium sorbate is enhanced (Rowe *et al.*, 2006; Stanojevic *et al.*, 2009). Parra *et al.* (2014) reported that potassium sorbate solutions are at present extensively employed in Spain and some citrus producing countries that export the fruit as an alternative method for controlling decay. The United States Environmental Protection Agency has exempted potassium sorbate from residue tolerance and categorised it as a low-risk active compound (Montesinos-Herrero *et al.*, 2009).

Potassium sorbate is generally used as a preservative in a vast number of foods (Lück, 1990; Guillard *et al.*, 2009; Montesinos-Herrero *et al.*, 2009; Parra *et al.*, 2014). The water-soluble salts of sorbic acid, particularly potassium sorbate are extensively used in high moisture dried fruit (Alagöz *et al.*, 2015). Sorbates have an added advantage that it does not influence the odour or taste of foods (Jaster & Moore, 1992).

2.2.5 *Moringa oleifera* for human nutrition

Plants are inherently practical resources of antioxidants according to Siddiq *et al.* (2005) and phytochemicals (Singh *et al.*, 2009; Verma *et al.*, 2009). According to Verma *et al.* (2009), the oxidative damage of tissue/cells could be alleviated by these antioxidants indirectly by augmenting the natural defences of cells or directly by scavenging free radicals

The *Moringa oleifera* tree is one such plant and is the most prevalent of the 13 known species of *Moringa* (Doerr, 2005; Salem *et al.*, 2013; Padayachee & Baijnath, 2020). It is part of the *Moringaceae* family and spread throughout the tropics and sub-tropics of Asia and Africa (Siddiq *et al.*, 2005; Singh *et al.*, 2009; Jayawardana *et al.*, 2015). Currently, India is the biggest producer of *M. oleifera,* generating up to 80% of the world's requirements (Falowo *et al.*, 2018). This medium size, evergreen tree is 2.5-10m in height according to Chuang *et al.* (2007) and is generally recognised as horseradish tree, drumstick tree, *Moringa* tree and ma-rum tree (Singh *et al.*, 2009; Verma *et al.*, 2009; Das *et al.*, 2012; Vongsak *et al.*, 2013a). According to Chuang *et al.* (2007), a fully developed tree's fruit is brown with 10-15 seeds inside. The tree has more than ninety nutritional chemical compounds which include carbohydrates, dietary fibres, lipids, and proteins (Brilhante *et al.*, 2017).

Virtually the whole tree namely the leaves, roots, bark, flowers and seeds are customarily used in tropical and sub-tropical countries (India, Pakistan, Philippines, Thailand and Africa) as a remedy for several illnesses (Chuang *et al.*, 2007; Singh *et al.*, 2009; Verma *et al.*, 2009; Vongsak *et al.*, 2013a,b). These illnesses include treatments for inflammation and anti-tumour as stated by Chuang *et al.* (2007) and Verma *et al.* (2009), and according to Chuang *et al.* (2007) and Jayawardana *et al.* (2015) for paralysis and hypertension also. Of

the numerous nutrients found in the different parts of the tree, protein accounts for almost 25% of its dry weight. In the tropics, it is used as a food source to overcome malnutrition particularly in children and babies (Brilhante *et al.*, 2017). Phytochemicals of certain *Moringa* species are shown in Figure 2.10.

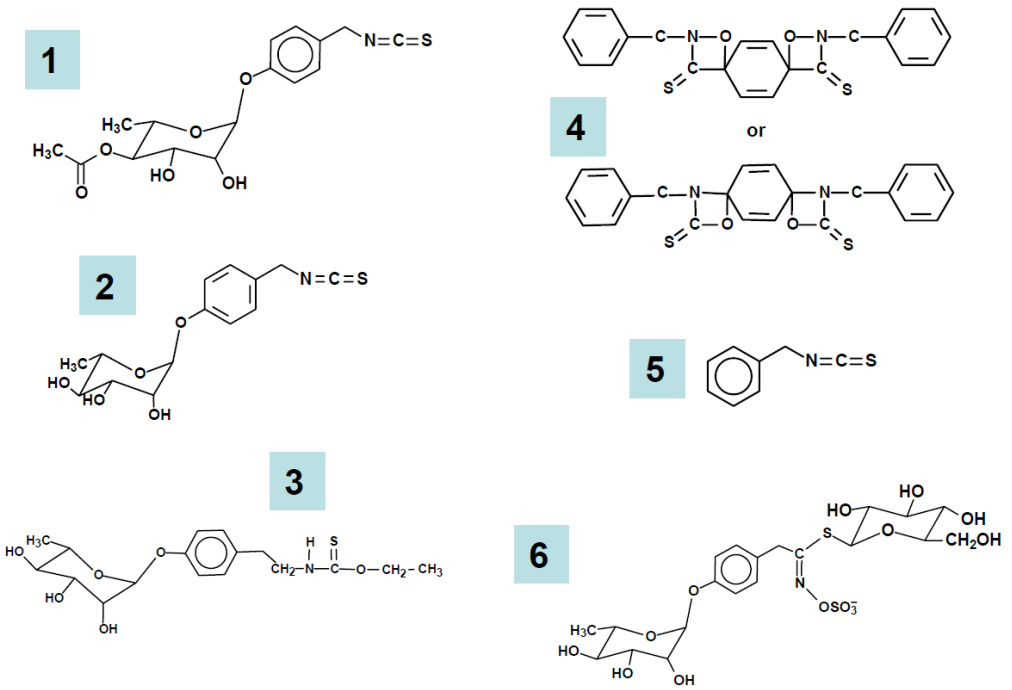


Figure 2.10 Molecular structures of selected phytochemicals from Moringa spp.: **1**: 4-(4'-O-acetyl-α-Lrhamnopyranosyloxy) benzyl isothiocyanate, **2**: 4-(-L-rhamnopyranosyloxy) benzyl isothiocyanate, **3**: niazimicin, **4**: pterygospermin, **5**: benzyl isothiocyanate, and **6**: 4-(α-L-rhamnopyranosyloxy)benzyl glucosinolate) (Fahey, 2005)

It was reported that the pips of the *M. oleifera* tree are an excellent source of natural antioxidants and that the oil of the seed is exceptionally stable under specific storage conditions (Siddiq *et al.*, 2005). The seeds sheltered in long slim pods are circular or triangular with a semi-permeable brown seed hull (Falowo *et al.*, 2018). The seed oil also has significant quantities of tocopherols and possess physical and chemical qualities comparable to that of olive oil (Chuang *et al.*, 2007). Studies have been done using the seeds oil as a solidifying agent in solid and semi-solid fat spreads instead of the hydrogenation process. An average of 400 ml of cooking oil is produced using one kilogram of seeds (Falowo *et al.*, 2018).

In addition to its medicinal properties, the flowers of the tree also contain a few phytochemicals namely quercetin, kaempferol, isoquercitrin and kaempferitrin (Siddiq *et al.*, 2005). The pale to white petals of the flowers are 1.0-1.5 cm in length and 2.0-2.5 cm in width (Falowo *et al.*, 2018). The pods were reported to have hypotensive and chemo-modulatory effects whereas the roots showed antimicrobial and anti-inflammatory activity (Verma *et al.*,

2009). The alkaloids, moriginine and moringine are found in the stem bark of the *M. 0leifera* tree (Das *et al.*, 2012). Ayurveda one of the world's oldest healing systems affirmed that *M. oleifera* can supply the nutrients and therapeutic ingredients to prevent, relieve or deal with many ailments (Singh *et al.*, 2009; Vongsak *et al.*, 2013b; Padayachee & Baijnath, 2020).

As a result of its many medicinal benefits numerous pharmaceutical products of the tree are manufactured and sold locally and internationally (Vongsak *et al.*, 2013a). In Thailand, herbal teas made from the leaves and seeds are for sale as well as leaf powder and plant extracts in capsules form (Sahakitpichan *et al.*, 2011). Known as the miracle tree *M. oleifera* has been endorsed by the World Health organisation as an option to treat malnutrition (Salem *et al.*, 2013). In this study, the *Moringa oleifera* leaf extract powder will be reviewed as a pre-treatment of fruits.

2.2.6 *Moringa oleifera* leaves

The *Moringa oleifera* leaves are very good for the health (Doerr, 2005; Verma *et al.*, 2009). *Moringa* leaves can be collected at any time once the tree is matured. To give the tree enough time to recover, leaves should be harvested during the rainy season; this will give the tree enough time to recover before summer starts. To ensure no vitamins are lost, they should be dried away from light (Doerr, 2005). The *Moringa oleifera* leaf is rich in β-carotene, vitamin C, protein and iron (Verma *et al.*, 2009; Jayawardana *et al.*, 2015). Also, it is a significant source of essential amino acids of which methionine, cystine, tryptophan and lysine are examples (Jayawardana *et al.*, 2015). It was noted the quantities of iron, magnesium, calcium and potassium found in *M. oleifera* leaves are higher compared to other plant sources. Studies also showed that significant quantities of vitamins A, B, C and E are found in *M.oleifera* leaves (Falowo *et al.*, 2018; Padayachee & Baijnath, 2020). It can be consumed fresh or cooked and the powdered leaves can be kept for many months without refrigeration. Figure 2.11 is an illustration of fresh *Moringa oleifera* leaves.

Figure 2.11 Image of *Moringa oleifera* leaves (Lin *et al.*, 2018)

M*oringa oleifera* leaves has been described as radioprotective, able to regulate thyroid status as stated by Verma *et al.* (2009) and reported to display anti-tumour activities (Verma

et al., 2009; Lin *et al.*, 2018). As a result of the numerous types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids, *M. oleifera* leaves is an outstanding source of natural antioxidants and thus can extend the storage period of foods that contain fat (Siddiq *et al.*, 2005). Phenolic compounds and flavonoids are the main phytochemical components in the leaves of *Moringa oleifera* (Verma *et al.*, 2009; Vongsak *et al.*, 2013a; Lin *et al.*, 2018; Padayachee & Baijnath, 2020). Das *et al.* (2012) reported the sum of the phenolic acid constituents in *Moringa oleifera* leaf extract as 45.81 mg g^{-1} and the flavonoids in terms of catechin content to be 22.4 mg g^{-1}. The presence of polyphenols may, therefore, be responsible for their overall antioxidant potential.

Secondary metabolites identified in *M. oleifera* leaf tissue which includes glucosinolates (glucomoringin), polyphenolics (3-pCoumaroyl quinic acid, 4-Feruloyl quinic acid) and flavonoids (Kaempferol hexose, Quercetinhexosse) are shown in Figure 2.12 (Hamany Djande *et al.*, 2018). The flavonoids kaempferol and quercetin have a significant role in reducing oxidative stress. The *M. oleifera* leaves can be used to fortify food such as cereals porridges, bread, yoghurt and cheese and their inclusion in these products has shown an improvement in their sensory properties and shelf life. The leaves also play a significant role in combating malnutrition among children and pregnant women and in addition boosts milk production in lactating mothers (Falowo *et al.*, 2018). Small protein peptides were isolated from the *Moringa oleifera* leaves which showed fungicidal and antibacterial attributes (Dahot, 1998). The study by Jayawardana *et al.* (2015) also shows that concentrations of 0.5% *M. oleifera* leaves significantly slow down lipid oxidation and decreases the presence of microbes with no changes to the appearance and other sensory characteristics of chicken sausages.

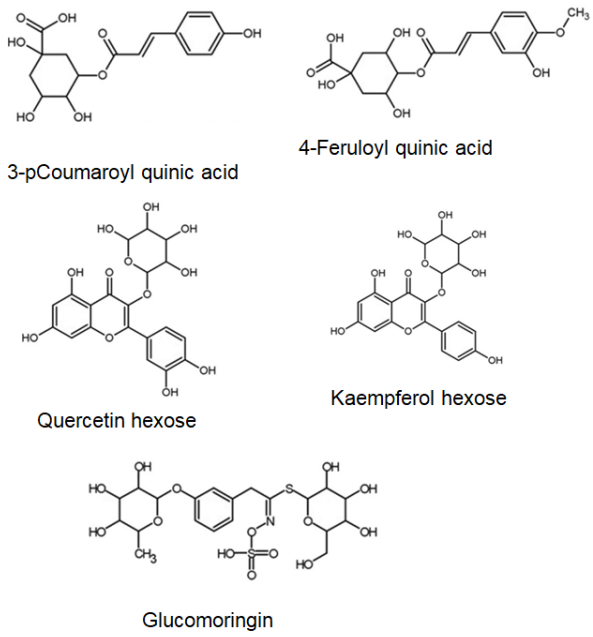


Figure 2.12 Structures of selected secondary metabolites identified in *M.oleifera* leaf tissue (Hamany Djande *et al.*, 2018)

The fresh leaf juice demonstrated antibacterial activity against Gram-negative and positive bacteria (Rahman *et al.*, 2009). *Moringa oleifera* leaves thus have a recognised history as a bio-preservative in food applications and nutraceuticals according to Jayawardana *et al.* (2015) and is, therefore, the perfect compound to use as a functional food (Singh *et al.,* 2009). According to Stohs & Hartman (2015), in research done with humans and animals that different preparations of *M. oleifera* leaves which include liquid extracts are very safe at the quantities generally in use.

2.3 History and Background of Food Drying

In ancient times food processing consists of simple methods such as cooking, sun drying, fermentation, steaming and salt preservation (Sethi, 2007; Lazaridesa, 2011; Dudeja & Singh, 2017). In ancient Greece beer, wine, cheese and bread were common products produced by fermentation (Lazaridesa, 2011). These basic food processing methods stayed unchanged until the industrial revolution when vacuum bottling was developed to supply the French troops which paved the way for canning (Sethi, 2007; Dudeja & Singh, 2017). This has led to an increase in the accessibility of food in the national and international markets (Augustin *et al.*, 2016). With the race for space at beginning of the 20th century, people became more aware of food quality and their eating habits changed. This led to further innovation in food processing such as spray drying, freeze-drying, concentrates as well as food additives and preservatives (Sethi, 2007; Augustin *et al.*, 2016; Dudeja & Singh, 2017). Peoples eating habits changed from fresh, unprocessed, no name food products to processed, packaged and branded products (Dudeja & Singh, 2017).

Processing aims are to stop or prevents and significantly reduce or destroy spoilage microorganisms, increase the shelf life of foods while preserving nutrients, texture and taste (Sethi, 2007; Valdramidis & Koutsoumanis, 2016). Some of the benefits of processing are; increasing off-season availability of various foods, produce food that is safe to eat, improve quality of life by developing allergen-free foods and save time by creating ready to eat food (Sethi, 2007). The less harsh the process, the less it impacts the nutritional composition of the foodstuff.

There are various technologies for preservation in food manufacturing namely; chemical (e.g. chlorine and organic acids), biological (e.g. plant extracts and physical (e.g. high pressure and pulse electric field) (Valdramidis & Koutsoumanis, 2016). The past century has seen conventional food preparation and preservation methods being industrialised. This caused a rise in food availability in the domestic and export markets, for instance, the spray drying of milk made it accessible in countries without a sufficient supply of milk (Augustin *et al.*, 2016).

Even though conventional processing still has a central role in food preservation innovative food manufacturing technologies such as high pressure processing, pulsed electric

field and ultrasonic processing has been extensively researched (Augustin *et al.*, 2016; Knoerzer *et al.*, 2016; Muntean *et al.*, 2016; Valdramidis and Koutsoumanis, 2016). High-pressure processing shows potential since no heat or chemicals are employed and the result is a stable and preservative-free food. Other benefits of this process include colour, vitamins, minerals and taste retention (Denoya *et al.*, 2015a, 2016; Knoerzer *et al.*, 2016; Muntean *et al.*, 2016).

Even though there are numerous benefits linked to these technologies there are many disadvantages such as low yields, consistency of the method, and the need for lot processing as stated by Knoerzer *et al.* (2016), as well as the inability to destroy bacterial spores and the inactivation of for instance the enzyme, polyphenol oxidase which is known for causing enzymatic browning (Denoya *et al.*, 2015b).

Drying of fruits goes back to archaic times when preservation with salt was in practice and basic processing methods consist of various kinds of cooking and sun drying. (Sethi, 2007; Lazaridesa, 2011; Dudeja and Singh, 2017). Dehydrating fruits assist with their preservation, management of post-harvest losses, out of season availability, food security, the extension of their storage life, creates income and a reduction in transportation and storage cost (Sethi, 2007).

2.3.1 Drying of fruits

Drying is the most widespread method to conserve food (Hatamipour *et al.*, 2007; Doymaz, 2010; Blanco-Cano *et al.*, 2016). Drying significantly decreases the water, enzymatic and microbiological activity by vaporization or sublimation and thus an increase in food stability which results in reduced physicochemical changes when stored (Hatamipour *et al.*, 2007; Doymaz, 2010). This results in decreased losses throughout storage, increase storage life and consequently savings in distribution expenses (Sethi, 2007; Doymaz, 2010; Djendoubi Mrad *et al.*, 2012; Kurozawa *et al.*, 2014).

Factors that influence the speed and dehydration time are the size of the fruit pieces, how and where the fruit is placed relative to the heating elements, and the characteristics of the drying equipment (Purohit *et al.*, 2006; Sethi, 2007). Another key factor is the moisture present in the air used when drying the fruit. The way drying works is, the increase in temperature of the fruit allows the moisture in the fruit to evaporate and the air moving over the fruit carries the moisture away. However, if the air is too moist it will be unable to carry the moisture away and the drying of the fruit will be unsuccessful (LaBorde, 2009; Mercer, 2012). There are several methods used to dry fruit namely; sun drying, oven drying, food dehydrator or mechanical drying, freeze-drying according to and drum drying, spray drying and osmotic dehydration to name but a few (Harrison, 1993; Sethi, 2007; Mercer, 2012). Several of these drying methods will be discussed in this section**.**

2.3.2 Sun and solar drying of fruits

This type of drying is the most cost effective but it is also the most time-consuming method (Karabulut *et al.*, 2007; Sethi, 2007). With sun drying, halved or sliced fruit are placed on trays constructed of a screen or wooden dowels. The fruit is pre-treated, placed in an enclosed area to be gassed with sulphur and then placed in the sun for drying. Space is left between the ground and the trays for better air circulation around the fruit (Harrison 1993). Disadvantages of open sun drying especially in the rural areas of third world countries are low yields as a result of fungal growth, interference of insects, birds and rodents (Purohit *et al.*, 2006). Figure 2.13 shows an example of an outdoor drying rack.

Figure 2.13 Example of an outdoor drying rack (Harrison 1993)

2.3.3 Mechanical (cabinet) drying

Cabinet dryers are an example of a mechanical dehydrator. Temperature, humidity and airflow can be controlled when using this drying method (Sethi, 2007). Food dehydrators operate by using the correct sequence of warm temperature, low humidity and air currents. The warm air results in the evaporation of the moisture and the low humidity allow for quick movement of the moisture into the air. The air flow improves the rate of drying by moving the humid air away from the product (Harrison 1993; Mercer 2012). Compared to sun drying mechanical drying provide several benefits such as quick drying time, saves space, is not weather dependent, a sanitary environment, higher yields, better quality and is visually more appealing (Sethi, 2007). Figure 2.14 shows a diagram of a characteristic cabinet dryer.

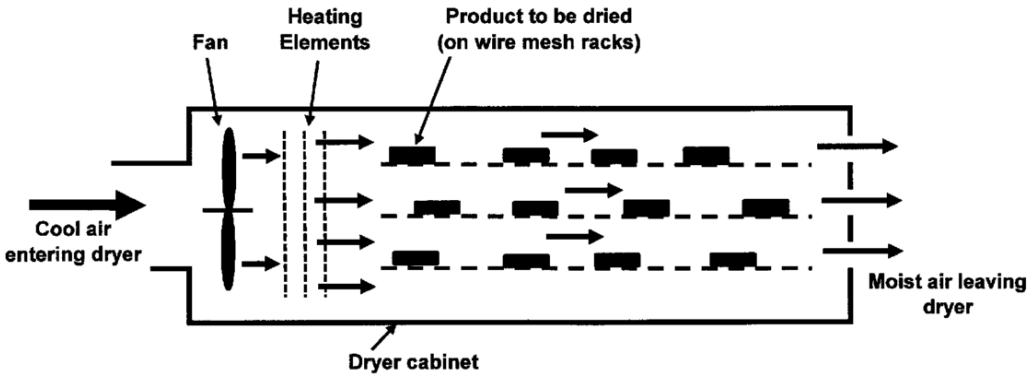


Figure 2.14 Diagram of a typical forced-air cabinet dryer (Mercer, 2012)

Solar dryers are entirely dependent on the heat from the sun to heat the air. The heat collector becomes very warm which cause the air inside the pipe to significantly increase in temperature. The air is circulated as a result of the heated air that raises from the base of the dryer through the drying chamber. The air collects the moisture that evaporates from the fruit on its way up and the moist air departs from the chamber through an opening at the top of the chamber. An illustration of a basic solar dryer can be seen in Figure 2.15 (Mercer, 2012).

2.3.4 Oven drying of fruits

Not all ovens have built-in fans for air movement and are thus more time consuming than dehydrators. With oven drying the fruit is placed in a single layer on a tray (sprayed with non-stick spray) or in a shallow pan and placed in an oven preheated to 60˚C (Harrison, 1993; LaBorde, 2009). The fruit is turned after 1-2 hours (Harrison, 1993). To allow air circulation the oven door can be left slightly open (Harrison, 1993; LaBorde, 2009). One of the disadvantages of oven drying compared to dehydrators is that food takes double the amount of time to dry food in an oven (Harrison 1993).

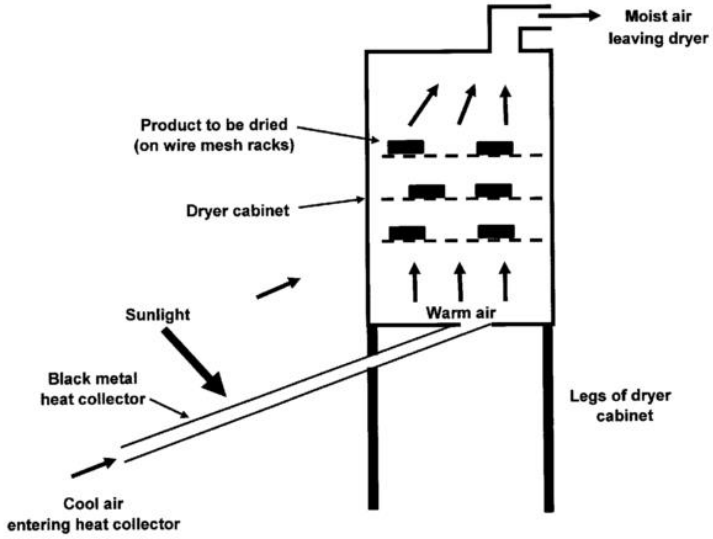


Figure 2.15 A simple illustration of a solar dryer (Mercer, 2012)

2.3.5 Freeze Drying

Freeze drying make use of the principle that under pressure, ice crystals can be removed from food without going through a water stage (Sethi, 2007; Harnkarnsujarit *et al.*, 2015). In this method the material is spread on trays in a freeze drier, frozen and then dried under pressure. The ice crystals transition directly to the gas phase without passing through the intermediate liquid phase (Sethi, 2007; Sette *et al.*, 2016).

One of the key characteristics of freeze-dried food is the capability to rehydrate well after adding a liquid (milk or water). Freeze-drying also maintains the high quality of foods and

bio-products including the colour, nutritional components, taste and the ability to rehydrate properly (Harnkarnsujarit *et al.*, 2015). According to Sethi (2007), freeze-drying is costly and is mainly used for products that can either be sold at a premium price or which can withstand only small amounts of sensory deterioration.

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CHAPTER 3

Effect of some weak acids and *Moringa oleifera* leaf extract powder on the colour of dried apple

3.1 Introduction

Fruits have become a vital part of our everyday life as a result of them being an excellent source of vitamins and minerals (Golisz *et al.,* 2013). The contemporary lifestyle of today hampers the intake of fresh fruit and vegetables (Denoya *et al.*, 2015). Giving rise to a need for innovative products that can act in response to the change in lifestyle and working pace. An advantage of this is that consumers are now paying attention to eating nourishing, organic and flavoursome foods. Dehydrated fruits can fulfil these requirements as they are delicious, healthy food because of their nutritional value and high fibre content (Rizzolo *et al.*, 2014). Processing fruits provide a method to make them accessible throughout the year, adds value and increase their shelf life (Campbell & Padilla-Zakour, 2013). Furthermore, dehydration also allows for market surplus management (Golisz *et al*., 2013).

Dehydrated apples used primarily as a snack food, have sparked renewed interest in recent times (Rizzolo *et al.*, 2011). Dried apples are used on their own and in quite a few ready to eat food such as breakfast foods and snack mixes (Lewicki & Jakubczyk, 2004). According to Rojas-Graü *et al.* (2008) apples are eaten in all parts of the world for their health benefits which are attributed to their numerous nutritional components such as vitamins and phenolic constituents.

Injury to light flesh fruits (peach, apples, and bananas) will start the oxidation of the phenolic compounds by polyphenoloxidase to quinines to form brown pigments. Polyphenoloxidase is an enzyme and therefore, it is possible to inactivate it by treatments that can denature proteins such as heat, extremes of pH and high ionic strength solutions. Hence, blanching, acidification, salt dips and sugar packs will drastically reduce the problem (Brewer, 2013). Huque *et al.* (2013) reported that dips containing ascorbic acid as a reducing agent and citric acid as an acidulant, on their own or as a mixture had been broadly described as anti-browning agents for fresh produce. According to Lunadei *et al.* (2011), anti-browning agents based on citric acid or ascorbic acid in combination with atmospheric packaging and low temperature increased the storage life of sliced fresh fruit.

Phenolic compounds, as well as flavonoids, are vital phytochemicals found in the leaves of *Moringa oleifera* as reported by Verma *et al.* (2009) and known for a variety of activities which include antioxidant, anti-hypertension and anti-inflammatory actions (Vongsak *et al.*, 2013a,b). Also, the aqueous extract of *Moringa oleifera* leaves are antimicrobial (Dahot, 1998).

At the correct levels, sorbates prevent the proliferation of spoilage bacteria such as *Escherichia coli*. They also successfully stop the growth of moulds, including mycotoxin producing types, and at 0.01-0.2% can inhibit most yeasts (Mendonca, 1992). However, nothing is known about the effect of weak acids in combination with *Moringa oleifera* leaf extract powder (MOLEP) on dried apples. Therefore, this research aimed to determine the main effects of the weak acids and *Moringa oleifera* leaf extract powder and to identify the acids and *Moringa oleifera* leaf extract powder combination that will reduce discolouration of the dried apple slices.

3.2 Materials and Methods

3.2.1 Source of materials and equipment

Granny Smith apples were bought at a retailer in Cape Town, South Africa. The apples were refrigerated at 4°C $\pm$1 °C until needed for the experiment (Deng & Zhao, 2008; Rojas-Graü *et al.*, 2008; Li-Qin *et al.*, 2009; Doymaz, 2010). MOLEP was purchased from Dohler, South Africa Pty (Ltd) (Paarl), ascorbic acid from Lake Foods (Johannesburg, South Africa), citric acid from Savanna Fine Chemicals (Pty) Ltd (Cape Town, South Africa) and potassium sorbate from Bragan Chemicals CC (Johannesburg, South Africa).

The sliced apples were dried using a cabinet dehydrator (Excalibur, model no: EXC 10). The cabinet dryer consists of a fan (horizontal airflow), 10 trays, a heating element and a thermostat which are at the back of the dehydrator. Cool air is drawn in heated and distributed evenly over each tray (Excalibur, 2012).

3.2.2 Experimental design for drying of the apples

The apple slices were pre-treated based on an augmented 2^{6-3} fractional factorial design using two levels (low and high) of each variable as shown in Table 3.1.

Table 3.1: Factors in augmented 2^{6-3} fractional factorial design*

		Factor level (x_i)	
Factor	Notation	Low (-1)	High (+1)
Ascorbic acid (%)	X_1	0.5	2.0
Citric acid (%)	X_2	0.3	2.0
Potassium sorbate (%)	X_3	0.1	0.3
Moringa oleifera (%)	X_4	0.1	0.2
Time (hours)	X_5	7	16
Temperature (°C)	X_6	57	70

*Transformation of the coded variables (x_i) to uncoded (X_i) is given by $X_1 = 0.75x_1 + 1.25$; $X_2 = 0.85x_2 + 1.15$; $X_3 = 0.1x_3 + 0.2$; $X4 = 0.05x_4 + 0.15$; $X_5 = 4.5x_5 + 11.5$; $X_6 = 6.5x_6 + 63.5$

The apples were manually washed and sanitised in 200 ppm sodium chlorite solution (Rojas-Graü*et al.*, 2008), peeled, de-cored and sliced to approximately 4 mm in thickness (Rojas-Graü *et al.*, 2008; Doymaz, 2010; Anyasi *et al.*, 2015; Hazervazifeh *et al.*, 2016).

The sliced apples were dipped into water containing the weak acids and MOLEP in dosages based on the experimental design in Table 3.2. The entire design comprised of 19 experimental trials, including triplicates of the midpoint point, as shown in Table 3.2. Each of the 16 runs was carried out in duplicate. The sliced apples were submerged for 5 minutes (Deng & Zhao, 2008). The excess water was gently dabbed off with a paper towel (Rojas-Graü *et al.*, 2008; Cárcel *et al.*, 2010). The independent factors were ascorbic acid (AA), citric acid (CA), potassium sorbate (PS), *Moringa oleifera* leaf extract powder (MOLEP), time and temperature. The dehydrator was set as per the drying time and temperature set in Table 3.2.

Table 3.2: Experimental runs based on the augmented 2^{6-3} fractional factorial design

	Independent variables (%)					
Run	X_1	X_2	X_3	X_4	X_5	X_6
1	2.00	0.30	0.30	0.10	15	57
2	1.25	1.15	0.20	0.15	11	64
3	0.50	2.00	0.30	0.10	7	70
4	0.50	0.30	0.30	0.20	7	57
5	0.50	0.30	0.30	0.20	7	57
6	1.25	1.15	0.20	0.15	11	64
7	2.00	2.00	0.30	0.20	15	70
8	2.00	0.30	0.10	0.10	7	70
9	2.00	0.30	0.30	0.10	15	57
10	0.50	2.00	0.10	0.10	15	57
11	2.00	2.00	0.30	0.20	15	70
12	2.00	2.00	0.10	0.20	7	57
13	0.50	0.30	0.10	0.20	15	70
14	1.25	1.15	0.20	0.15	11	64
15	0.50	0.30	0.10	0.20	15	70
16	2.00	0.30	0.10	0.10	7	70
17	2.00	2.00	0.10	0.20	7	57
18	0.50	2.00	0.10	0.10	15	57
19	0.50	2.00	0.30	0.10	7	70

X_1 = Ascorbic acid, X_2 = Citric acid, X_3 = Potassium sorbate,

X_4 = *Moringa oleifera* leaf extract powder, X_5 = Time, X_6 = Temperature

The pre-treated sliced apples were placed evenly as a single layer on the dehydrator trays. The dried sliced apples were packed in aluminium foil pouches, heat sealed and kept at room temperature before analysis. The dried sliced apples were analysed for weight loss and colour characteristics.

3.2.3 Weight loss

The pre-treated/dipped apple slices were weighed on a scale (UWE UPS 600E, Mettler PE 3000) before and after the drying process. The apple slices prior to and subsequent to the drying were weighed and the change in the weight was calculated as weight loss (Zhang *et al.*, 2013). The weight loss was expressed as a percentage of the weight before drying.

3.2.4 Colour measurement

The surface colour of the pre-treated and untreated dried apples was measured using a spectrophotometer (Spectrocolorimeter Datacolor 600). This method allowed the determination of the trichromatic coordinates CIELAB (L* a* b*) using a spectrophotometer with measurement geometry d/8 (diffuse illumination, directional observation at 8° $\pm$ 2°) and D65 illuminant.

The spectrophotometer was calibrated using a black trap and a white tile before the readings. The sample was placed directly on the instrument in such a way that the reading surface is perfectly flat. The colour was recorded as L* a* b* values. L* represents lightness, ranging from 0-100, 0 being black (no light) and 100 white (maximum illumination). The a* is green (negative) to red (positive). The b* is blue (negative) to yellow (positive) (Acevedo *et al.*, 2008; Huang *et al.*, 2015).

3.2.5 Numerical optimisation

Data were modelled using the two-factor interaction factorial model to establish the variables that have a significant effect on the colour of the dried sliced apples. Analysis of variance (ANOVA) was used to determine the statistical significance of the independent variables (AA, CA, MOLEP, PS, time and temperature) on the lightness, redness and yellowness of the dehydrated sliced apples.

The goodness of the fit was determined by the lack of fit, F-value and the adequate precision ratio. Numerical optimisation was applied to obtain the optimum combination of significant independent variables that will minimise redness (+a*) and yellowness (+b*).

3.2.6 Profiling of the *Moringa oleifera* leaf extract powder (MOLEP)

The MOLEP components were identified ad quantified by liquid chromatography-mass spectrometry. Two grams of the MOLEP was accurately weighed into a 50 ml centrifuge

tube with a screw-cap. A 15 ml of 50% methanol/1% formic acid was added and the tubes were tightly capped. Thereafter the samples were vortexed for 1 minute, followed by extraction in an ultrasonic bath for 1 hour. Two millilitres of the sample was then withdrawn and centrifuged at 14,000 rpm for 5 minutes. The clear supernatant was then transferred into 1.5 ml glass vials for analysis.

A Waters Synapt G2 quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatography (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. 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 ionization was used 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 optimized for best resolution and sensitivity.

Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, one at low collision energy (4 V) and the second using a collision energy ramp (40−100 V) to obtain fragmentation data as well. Leucine enkaphalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate. Separation was achieved 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 minute and changed to 28% B over 22 minutes in a linear way. It then went to 40% B over 50 s and a wash step of 1.5 minutes at 100% B, followed by re-equilibration to initial conditions for 4 minutes.

The flow rate was 0.3 mL/min and the column temperature were maintained at 55°C. Compounds were quantified relatively against a calibration curve established by injecting a range of catechin standards from 0.5 to 100 mg/L catechin. Data were processed using MSDIAL and MSFINDER (RIKEN Centre for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan)

3.2.7 Statistical analysis

Statistical analysis was performed by testing significant differences ($p \leq 0.05$) between treatments using multivariate analysis of variance and Duncan's multiple range test was used to separate means where differences existed.

3.3 Results and Discussion

3.3.1 Model adequacy

The summary of the linear model parameters for the effect of the independent variables on the colour (L*, a*, b*) of the dried apple slices are shown in Table 3.3. The linear model for L* (lightness) was not significant (P = 0.0836). The negative predicted R-squared suggests that the overall mean may be a better predictor for the response than this model, therefore this model will not be used to predict the effects of the independent variables (AA, CA, MOLEP, time and temperature) on the colour of the apple slices.

The effect of the AA, CA, MOLEP, time and temperature on a* (redness) was sufficiently explained by the linear model (p = 0.0112) as indicated in Table 3.3. The model F-value of 4.72 suggests only a 1.12% likelihood that an F-value this big could occur because of noise. The model lack-of-fit of 0.11 suggests that the lack of fit is not significant, indicating a model with adequate goodness of fit. This was validated by the adequacy precision ratio of 6.136, which indicated that this model could be used to navigate the design space.

The impact of the independent variables (AA, PS, MOLEP, time and temperature) on b* (yellowness) was significant (p = 0.0106). The model F-value of 4.78 suggests that the model is significant with only a 1.06% possibility that an F-value this big could occur because of noise. The lack-of-fit of 0.02 implies the lack of fit is not significant, indicating a model with acceptable goodness of fit. This is supported by the adequate precision ratio of 6.56, therefore this model can be used to navigate the design space.

Table 3.3: Model parameters for dried apple slices

	Colour		
Model parameter	Lightness (L*)	Redness (+a*)	Yellowness (+b*)
p-value	0.0836	0.0112[#]	0.0106[#]
F-value	2.52	4.72	4.78
R2	0.4918	0.6448	0.6478
Predicted R2	-0.2213	0.1779	0.1882
Adeq precision	4.566	6.136	6.555

[#]Significant $p \leq 0.05$

The linear model for lightness (L*) was not adequate to explain the variation in the colour of the apple slices, however, the linear models for redness (a*) and yellowness (b*) adequately described the colour variation of the dried apple slices. Hence in searching for the optimal combination of the independent variables for the optimum colour of the apple slices the models for the redness and yellowness will be used for numerical optimisation.

3.3.2 Effect of the ascorbic acid, citric acid, potassium sorbate, *Moringa oleifera* leaf extract powder, time and temperature on the weight loss of the dried apple slices

The weight loss of the dehydrated sliced apples is presented in Table 3.4 and ranged from 85 to 90% with a mean of 87.7%. Studies done by Lewicki & Jakubczyk (2004) recorded the moisture content of fresh apples as approximately 86.2% and Kutyła-Olesiuk *et al.* (2013) reported it as 85.8%. Hot air drying is a process in which moisture is removed, this action reduces the weight of foodstuff (Orikasa *et al.*, 2014). The weight loss is thus a result of the hot air removing the moisture from the apple slices during the drying process.

The temperature range in this study was 57 - 70°C, a study by Lewicki and Jakubczyk, (2004) showed that in the temperature range 50 - 70°C the difference in moisture content of dehydrated apples were not significant. This might be the reason why the independent variables did not significantly affect the weight loss of the dehydrated sliced apples ($p = 0.8855$).

Table 3.4: Weight loss of the dried apple slices

	Independent variables (%)						
Run	X_1	X_2	X_3	X_4	X_5	X_6	Weight loss (%)
1	2.00	0.30	0.30	0.10	15	57	85.4 ± 0.4
2	1.25	1.15	0.20	0.15	11	64	87.8 ± 5.3
3	0.50	2.00	0.30	0.10	7	70	86.6 ± 4.3
4	0.50	0.30	0.30	0.20	7	57	90.2 ± 3.8
5	2.00	2.00	0.30	0.20	15	70	88.3 ± 2.0
6	2.00	0.30	0.10	0.10	7	70	88.2 ± 6.1
7	0.50	2.00	0.10	0.10	15	57	87.8 ± 3.8
8	2.00	2.00	0.10	0.20	7	57	85.4 ± 3.4
9	0.50	0.30	0.10	0.20	15	70	90.3 ± 6.5

Values are mean ± standard deviation. X_1 = Ascorbic acid, X_2 = Citric acid, X_3 = Potassium sorbate, X_4 = *Moringa oleifera* leaf extract powder, X_5 = Time, X_6 = Temperature

3.3.3 Effect of the ascorbic acid, citric acid, potassium sorbate, *Moringa oleifera* leaf extract powder, time and temperature on the colour of dried apples

Table 3.5 details the lightness (L*), redness (a*) and yellowness (b*) of the different combinations of the weak acids with *Moringa oleifera* leaf extract powder (MOLEP). The results indicate that run 3 with citric acid at 2.00% and the MOLEP at 0.10% dosage had the greatest effect on the redness (2.1) and yellowness (21.3) of the dehydrated sliced apples. Images of the dehydrated sliced apples for the different runs are illustrated in Figure 3.1.

Table 3.6 indicates that the effect of ascorbic acid, time and temperature on the lightness, redness and yellowness were not significant.

Table 3.5: Colour parameters of dried apples pre-treated with various combinations of weak acids and *Moringa oleifera* leaf extract powder (MOLEP)

Run	X_1 (%)	X_2 (%)	X_3 (%)	X_4 (%)	X_5 (h)	X_6 (°C)	Colour parameters		
							L*	a*	b*
1	2.00	0.30	0.30	0.10	15	57	78.9 ± 2.6	4.0 ± 2.5	22.8 ± 4.3
2	1.25	1.15	0.20	0.15	11	64	77.8 ± 1.4	3.7 ± 1.1	25.3 ± 1.9
3	0.50	2.00	0.30	0.10	7	70	81.90 ± 1.2	2.1 ± 0.3	21.3 ± 1.9
4	0.50	0.30	0.30	0.20	7	57	75.0 ± 4.4	6.7 ± 2.6	23.9 ± 4.7
5	2.00	2.00	0.30	0.20	15	70	82.0 ± 1.2	3.4 ± 0.3	28.0 ± 0.8
6	2.00	0.30	0.10	0.10	7	70	78.2 ± 0.7	4.3 ± 0.9	24.5 ± 2.0
7	0.50	2.00	0.10	0.10	15	57	81.4 ± 0.5	2.4 ± 0.5	23.5 ± 0.5
8	2.00	2.00	0.10	0.20	7	57	77.7 ± 3.7	3.7 ± 1.8	27.0 ± 0.8
9	0.50	0.30	0.10	0.20	15	70	76.0 ± 5.7	6.5 ± 1.2	29.2 ± 0.8

Values are mean ± standard deviation. L*, lightness; a*, red (+a); b*, yellow (+b).
X_1 = Ascorbic acid, X_2 = Citric acid, X_3 = Potassium sorbate, X_4 = *Moringa oleifera* leaf extract powder, X_5 = Time, X_6 = Temperature.

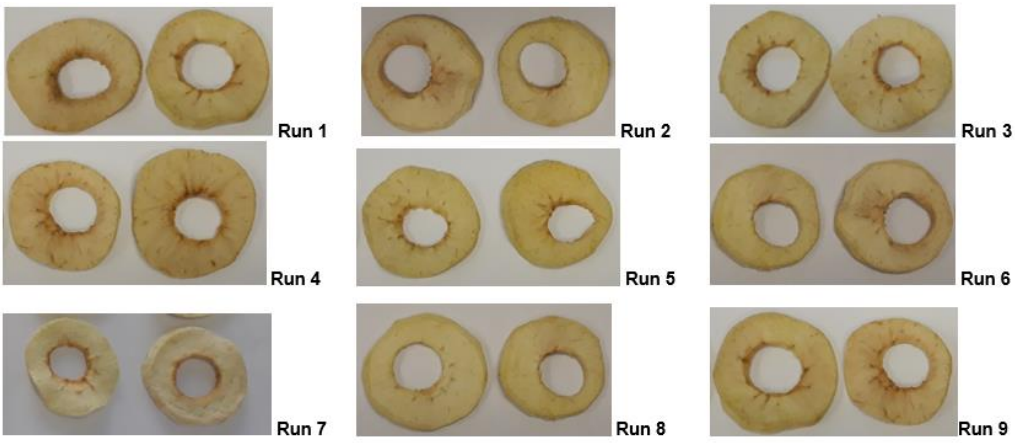


Figure 3.1 Images of the dried apple slices

Similarly, potassium sorbate did not significantly affect the yellowness of the dehydrated sliced apples. The effect of the citric acid on the lightness and redness of the dehydrated sliced apples were significant. The impact of MOLEP on the lightness, redness and yellowness of the dried apple slices was significant. The response surface for the effect of ascorbic and citric acid as well as that of ascorbic acid and MOLEP on the lightness of the dehydrated sliced apples are illustrated in Figure 3.2.

Table 3.6: Effect of the independent variables on the dried apple slices

	p-value		
Model parameter	Lightness (L*)	Redness (+a*)	Yellowness (+b)
Ascorbic acid	0.7034	0.3646	0.3151
Citric Acid	0.0539[#]	0.0022[#]	-
Potassium sorbate	-	-	0.0714
MOLEP	0.0149[#]	0.0136[#]	0.0022[#]
Time	0.8403	0.8169	0.1307
Temperature	0.8849	0.8698	0.1817

[#]Significant p ≤ 0.05, MOLEP = *Moringa oleifera* leaf extract powder

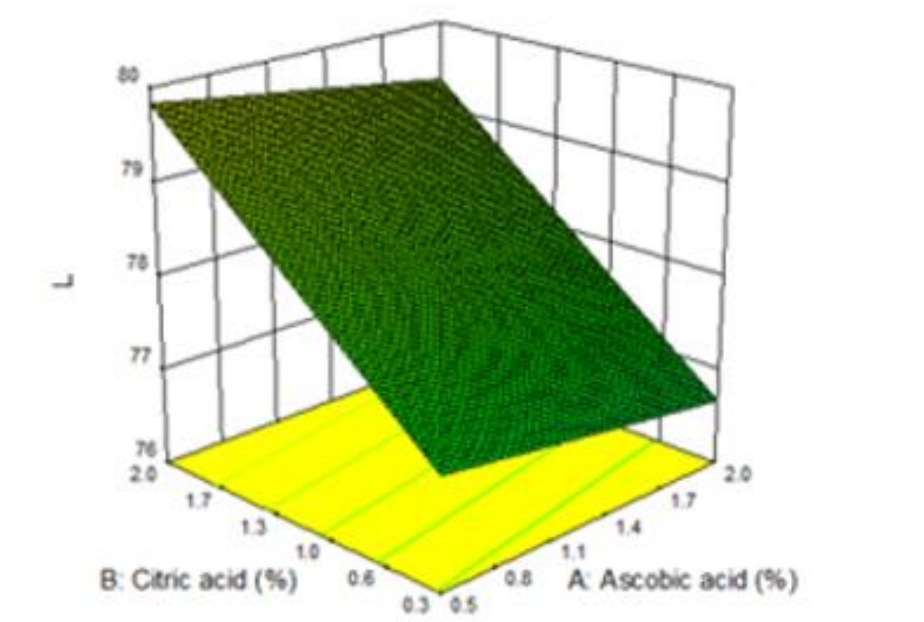
Figure 3.2a indicates that an increase in the concentration of citric acid significantly (p = 0.05) increased the lightness (L*) of the dehydrated sliced apples. Figure 3.2b illustrates that an increase in the concentration of MOLEP significantly (p = 0.01) reduced the lightness (L*) of the dried apple slices.

The response surface for ascorbic and citric acid as well as for MOLEP and ascorbic on the redness (a*) of the dried apple slices are illustrated in Figure 3.3. Figure 3.3a indicates that an increase in the concentration of citric acid significantly (p = 0.0022) decreased the redness (a*) of the dried apple slices. A unit increase in citric acid decreased the redness of the apple slices by -1.24.

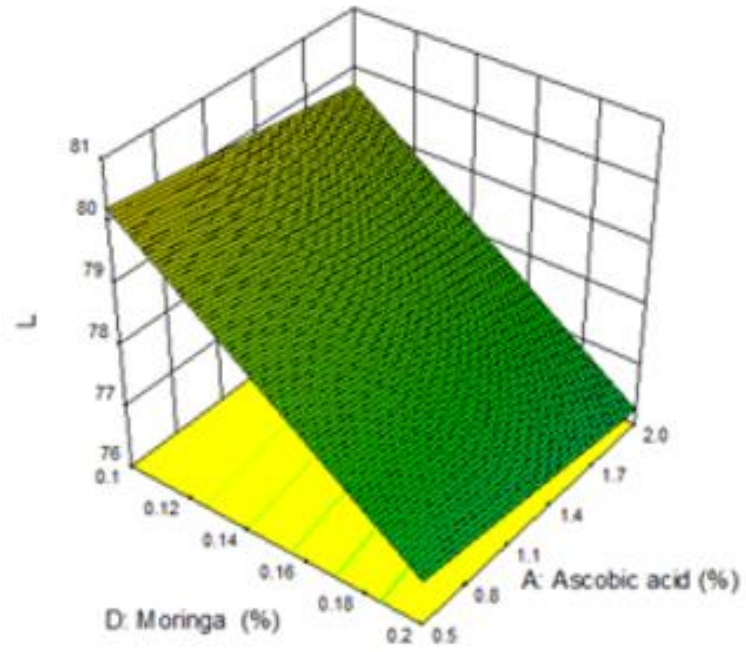
The three-dimensional response surface plot (Figure 3.3b) for MOLEP and ascorbic on the redness (a*) of the dried apple slices indicated that an increase in the concentration of MOLEP increased the redness (a*) of the dehydrated sliced apples significantly (p = 0.0136). The equation of coded factors (Eq 1) indicated that the impact of MOLEP is positive (+0.93) on the redness of the dehydrated sliced apples.

$$a = +4.06 - 0.31\,(AA) - 1.24\,(CA) + 0.93\,(MOLEP) - 0.08\,(time) - 0.05\,(temperature) \quad \text{(Eq 1)}$$

The response surface for citric acid and ascorbic as well as MOLEP and ascorbic acid on the yellowness (b*) of the dried apple slices are illustrated in Figure 3.4. The three-dimensional response surface plot (Figure 3.4a) indicates that an increase in the concentration of ascorbic acid increased the yellowness (b*) of the dehydrated sliced apples, however not significantly (p = 0.3151). The impact of ascorbic acid on the yellowness of the dehydrated sliced apples was positive (+0.55). The plot (Figure 3.4a) indicated that yellowness decreased with an increase in citric acid.

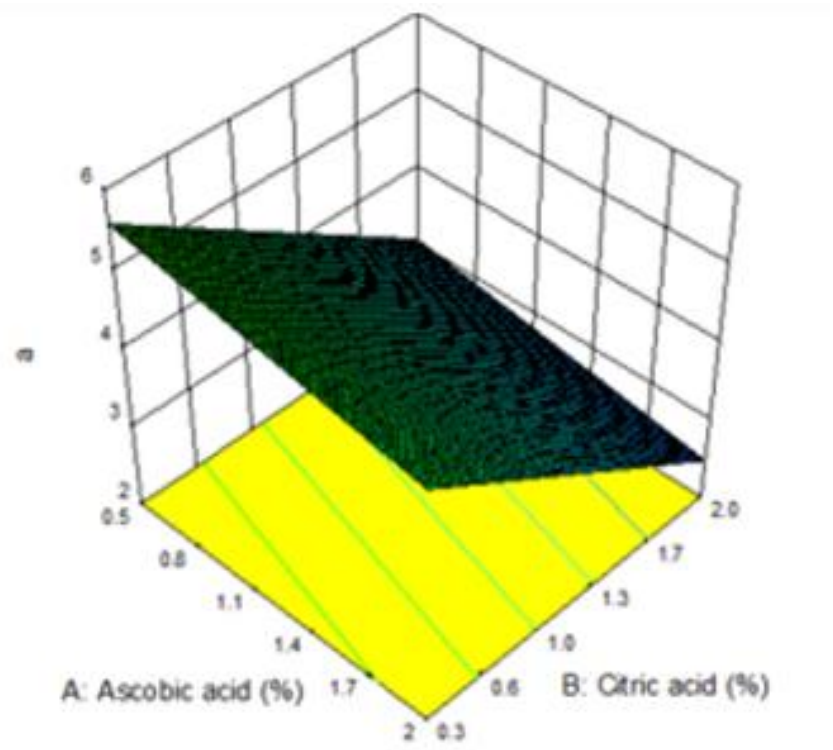


a

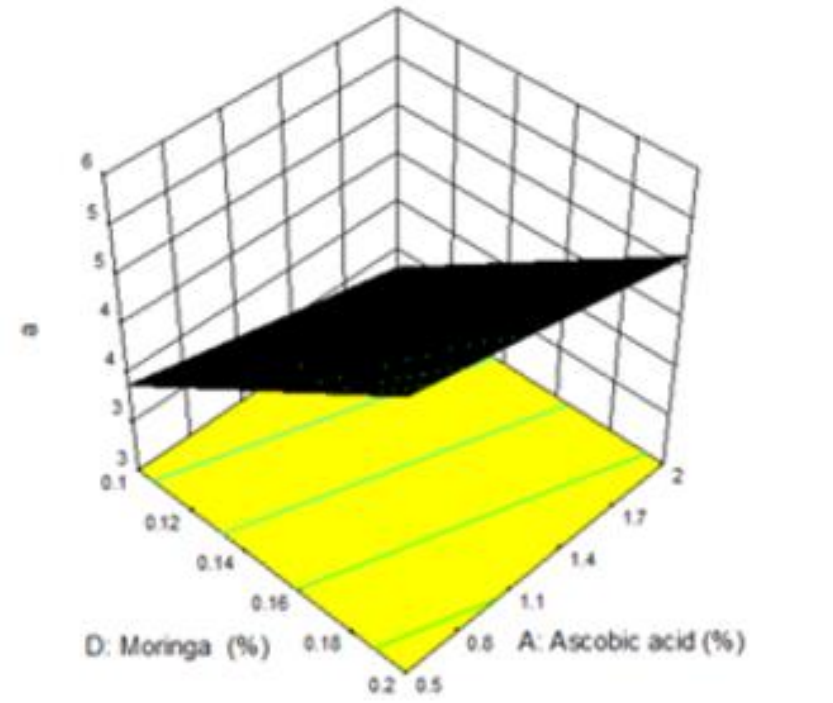


b

Figure 3.2 Response surface plot for the effect of; **a**: citric acid and ascorbic acid and **b**: *Moringa oleifera* leaf extract powder and ascorbic acid on the lightness (L*) of dried apple slices

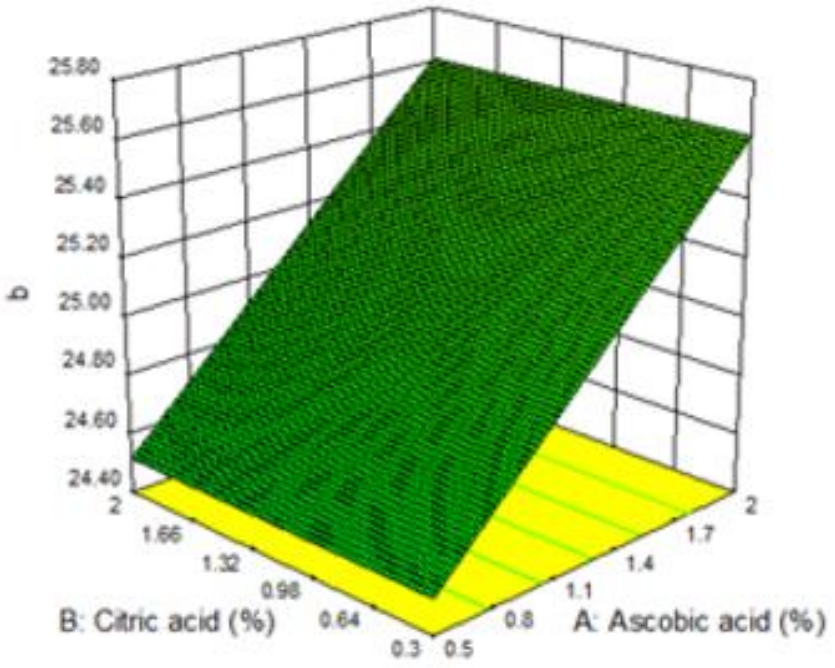


a

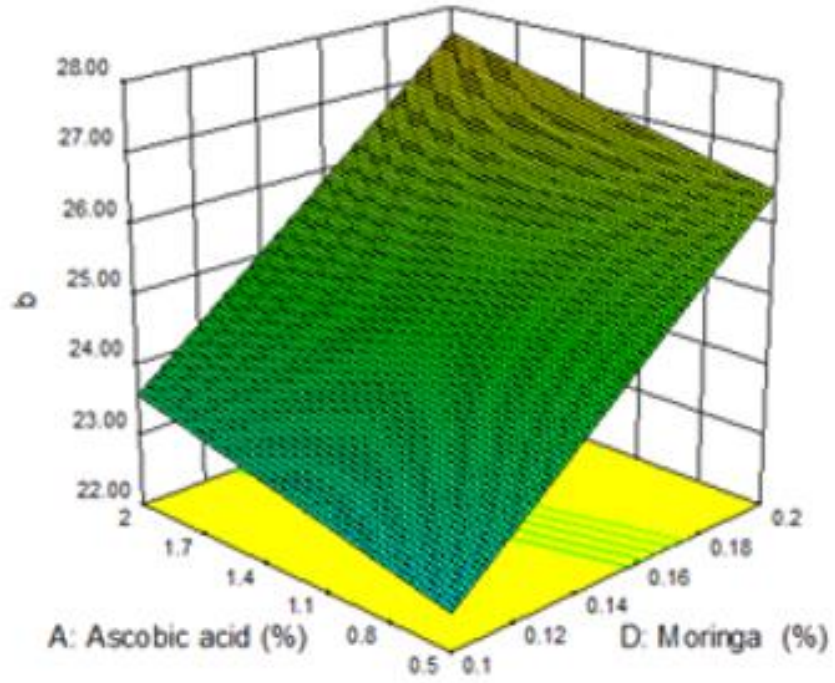


b

Figure 3.3 Response surface plot for the effect of **a**: ascorbic acid and citric acid and **b**: *Moringa oleifera* leaf extract powder and ascorbic acid on the redness (a*) of dried apple slices



a



b

Figure 3.4 Response surface plot for the effect of **a**: citric acid and ascorbic acid and **b**: ascorbic acid and *Moringa oleifera* leaf extract powder on the yellowness (b*) of dried apple slices

The response surface plot (Figure 3.4b) indicated that an increase in the concentration of MOLEP increased the yellowness (b*) of the dried apple slices significantly (p = 0.0022). The impact of MOLEP was positive (+1.99) on the yellowness (b*) of the dehydrated sliced apples indicated by the equation of coded factors (Eq 2).

$$b = +25.07 + 0.55\,(ascorbic\ acid) - 1.03\,(potassium\ sorbate) + 1.99\,(Moringa\ oleifera) + 0.85\,(time) + 0.74\,(temperature) \quad \text{(Eq. 2)}$$

The positive effect of citric acid on the redness and yellowness of the dried apple slices can be explained by its pH lowering effect and the fact that it is a copper chelating agent (Limbo & Piergiovanni, 2006; Goyeneche *et al.*, 2014). The enzyme, polyphenol oxidase needs copper to function as reported by Ioannou & Ghoul (2013). According to Limbo & Piergiovanni (2006), the binding of the copper at the active site of the enzyme becomes looser at pH values below 4, hence allowing the copper ions to be removed by the citric acid. The pH of the dipping solutions were all < 4 (2.36 - 3.68) allowing the copper ions to be removed by the citric acid at the active site of the PPO enzyme and thus prevent browning (Rocculi *et al.*, 2007; Barbagallo *et al.*, 2012; Abd-Elhady, 2014).

It was reported by various studies that ascorbic acid reduces the quinones which are responsible for the brown or black pigments and therefore surface browning, however, the effect does not last as it is permanently oxidized over time to dehydro-ascorbic acid (Rojas-Graü *et al.*, 2008; Javdani *et al.*, 2013). Polymerization of o-quinones takes place after it is depleted and thus darkening of the product occurs (Limbo & Piergiovanni, 2006; Rojas-Graü *et al.*, 2008; Salaha *et al.*, 2008; Chow *et al.*, 2011; Javdani *et al.*, 2013; Li *et al.*, 2015; Koushesh Saba and Sogvar, 2016). Thus, the effect of ascorbic acid on the lightness (L*), redness (a*) and yellowness (b*) of dehydrated sliced apples was not significant.

The response surface plots (Figure 3.1b, 3.2b and 3.4b) illustrated that decreasing MOLEP caused a decrease in redness and yellowness of the dried sliced apples. According to Siddiq *et al.* (2005), *Moringa oleifera* leaves are an excellent source of natural antioxidants and can extend the storage time of food containing fat. Phenolic compounds and flavonoids are the main phytochemical components in the leaves of *Moringa oleifera*, (Verma *et al.*, 2009; Vongsak *et al.*, 2013a). Das *et al.* (2012) also reported that *Moringa oleifera* leaves are an excellent source of phenolic constituents and have effective antioxidant activity. He reported that a 0.1% extract (100 mg/100 g) were sufficient to prevent lipid oxidation in goat meat patties stored at refrigeration temperatures. This is in agreement with the study by Siddiq *et al.* (2005) which reported that a lower dosage of *Moringa* leaves indicated a slightly higher antioxidant activity over time. The presence of phytochemicals (polyphenols and flavonoids) may, therefore, be responsible for the overall antioxidant effect.

3.3.4 Optimum combination of some weak acids (ascorbic acid, citric acid and potassium sorbate), *Moringa oleifera* leaf extract powder, temperature and drying time for the apple slices

Redness and yellowness due to good model fit were used to search for an optimal combination of the weak acids and *Moringa oleifera* leaf extract powder. Ascorbic acid and potassium sorbate did not have a significant impact on the colours and were constrained to zero, the other variables were in the range. The goal for optimization was to minimize the redness (a*) and yellowness (b*) of the dehydrated sliced apples. The optimal solution with the desirability of 0.721 was to pre-treat the apple slices in a solution with 2.0% citric acid and 0.1% *Moringa oleifera* leaf extract powder, drained and dried for 7 h at 70°C.

3.3.5 Phytochemical constituents of the *Moringa oleifera* leaf extract powder

Most of the phytochemicals identified in the *Moringa oleifera* leaf extract powder used in this study were o-glycosyl compounds and phenolic glycosides as illustrated by Figure 3.5. The diagram also indicates the link between the different components. The majority of the phytocompounds present in the *Moringa oleifera* leaf extract powder are flavonoids and alkaloids and are presented in Table 3.7. This agrees with the various studies regarding the phytoconstituents present in the *Moringa oleifera* leaves (Rani *et al.*, 2018; Padayachee & Baijnath, 2020; Zhu *et al*., 2020).

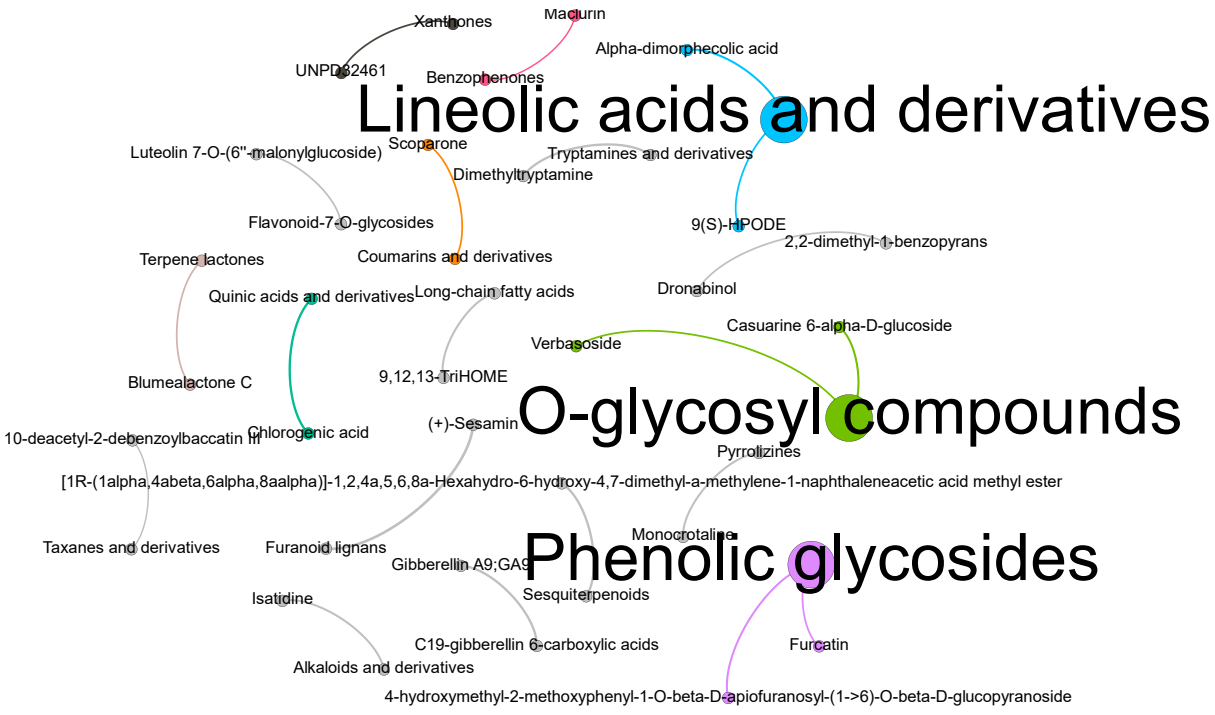


Figure 3.5 Phytochemicals identified in the *Moringa oleifera* extract powder

Table 3.7: Phytocompounds identified in the Moringa oleifera extract used in this study

Average Rt (min)	Average Mz	Structure rank 1	Formula	Total score	Ontology	Classification
7.097	570.093	UNPD32461	$C_30H_{21}NO_{11}$	5.902	Xanthones	Alkaloid
7.186	205.063	Scoparone	$C_{11}H_{10}O_4$	5.961	Coumarins and derivatives	Flavonoid
7.490	261.026	Maclurin	$C_{13}H_{10}O_6$	5.438	Benzophenones	Flavonoid
7.602	353.102	Chlorogenic acid	$C_{16}H_{18}O_9$	7.943	Quinic acids and derivatives	Flavonoid
7.707	323.145	Blumealactone C	$C_{17}H_{24}O_6$	5.829	Terpene lactones	Flavonoid
7.824	315.129	Gibberellin A9; GA9	$C_{19}H_{24}O_4$	7.719	C19-gibberellin 6-carboxylic acids	Phytohormone
8.115	353.108	(+)-Sesamin	$C_{20}H_{18}O_6$	8.836	Furanoid lignans	Flavonoid
8.404	447.160	4-hydroxymethyl-2-methoxyphenyl-1-O-beta-D-apiofuranosyl-(1->6)-O-beta-D-glucopyranoside	$C_{19}H_{28}O_{12}$	6.893	Phenolic glycosides	Flavonoid
8.718	427.185	Furcatin	$C_{20}H28O10$	6.067	Phenolic glycosides	Flavonoid
9.006	461.175	Verbasoside	$C20H_{30}O_{12}$	5.839	O-glycosyl compounds	Flavonoid
9.126	439.192	10-deacetyl-2-debenzoylbaccatin III	$C_{22}H_{32}O_9$	5.483	Taxanes and derivatives	Alkaloid
9.295	324.129	Monocrotaline	$C_{16}H_{23}NO_6$	7.420	Pyrrolizines	Alkaloid
9.987	261.157	[1R-(1alpha,4abeta,6alpha,8aalpha)]-1,2,4a,5,6,8a-Hexahydro-6-hydroxy-4,7-dimethyl-a-methylene-1-naphthaleneacetic acid methyl ester	$C_{16}H_{22}O_3$	7.599	Sesquiterpenoids	Flavonoid
10.227	533.098	Luteolin 7-O-(6''-malonylglucoside)	$C_{24}H_{22}O_{14}$	5.766	Flavonoid-7-O-glycosides	Flavonoid

10.478	187.126	Dimethyltryptamine	$C_{12}H_{16}N_2$	7.863	Tryptamines and derivatives	Alkaloid
10.628	366.138	Isatidine	$C_{18}H_{25}NO_7$	6.541	Alkaloids and derivatives	Alkaloid
10.954	366.137	Casuarine 6-alpha-D-glucoside	$C_{14}H_{25}NO_{10}$	7.134	O-glycosyl compounds	Alkaloid
12.919	329.249	9,12,13-TriHOME	$C_{18}H_{34}O_5$	6.984	Long-chain fatty acids	Fatty Acid
13.938	313.261	Dronabinol	$C_{21}H_{30}O_2$	6.485	2,2-dimethyl-1-benzopyrans	Alkaloid
14.123	311.242	9(S)-HPODE	$C_{18}H_{32}O_4$	5.954	Lineolic acids and derivatives	Fatty acid
14.351	295.244	Alpha-dimorphecolic acid	$C_{18}H_{32}O_3$	6.983	Lineolic acids and derivatives	Fatty acid

Rt = retention time, Mz = molecular weight

. The phenolic compounds capability to scavenge free radicals is associated with their capacity to donate their phenolic hydrogen atom to a free radical (Biela *et al.*, 2020; Messaadia *et al.*, 2020). Studies by De Marino *et al.* (2012) and Dong *et al.* (2021) stated that flavonoids can stop auto-oxidation by chelating the free radical producing metal ions. Also, according to Ahmad *et al.* (2014) flavonoids are oxygen scavengers and inhibits peroxidation. Several studies also mentioned that phenolic compounds such as flavonoids also exhibits antiviral, antibacterial and anti-inflammatory activity (Ahmad *et al.*, 2014; Hichri *et al.*, 2018; Biela *et al.*, 2020). The ability of flavonoids to chelate with metal ions as well as being oxygen scavengers may be the reason why the *Moringa oleifera* leaf extract contributed to reducing the browning and microbial count of the dried apple slices.

3.4 Conclusion

The hypothesis, whether the optimum combination of weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera* leaf powder will preserve the colour of the dried apples was tested in this study. The results of this work indicated that ascorbic acid and potassium sorbate did not impact the colour of the dehydrated sliced apples. Citric acid had a positive effect on the colour of dried sliced apples whereas that of *Moringa oleifera* at the high dosage was negative. This study showed that a dipping solution with citric acid at 2.0%, *Moringa oleifera* leaf extract at 0.1% and a drying time of 7 h at 70°C will minimize the discolouration of the dried apple slices. The objective to identify the best weak acid and *Moringa oleifera* leaf extract powder combination to minimize the discolouration of the dried apple slices was thus achieved.

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Chapter 4

Effect of citric acid, potassium sorbate and *Moringa oleifera* leaf extract powder on the storage stability of dried Granny Smith apple slices

4.1 Introduction

The consumer has become more conscious of the value of good eating habits as a result of the escalation in the number of chronic illnesses which include heart diseases and cancer, particularly in developed countries. Research has revealed growing substantiation that individuals who do not consume or who eat insufficient quantities of fruits and vegetables are at greater risk of these illnesses. Plant polyphenols, chlorophylls, carotenoids, betalains, flavonoids, and various vitamins are some of the main cancer preventers studied. Increasing the intake of fruits and vegetables in the diet will boost the antioxidant levels in blood and body tissue and the capability of the cells and tissue to defend itself against oxidative destruction (Rosa *et al.*, 2010; Kundu and Chun, 2014; Alasalvar *et al.*, 2020; Mostafidi *et al.*, 2020).

Several technologies are used to increase the storage period of fresh fruit of which drying is one. This is achieved by traditional sun drying or contemporary drying of which cabinet and freeze-drying are examples. Dehydrated fruits provide a healthier option to sweetened and salted snacks, are accessible all through the year (Guiné *et al.*, 2011; Alasalvar & Shahidi, 2013; Chang *et al.*, 2016). The rare mixture of the phytochemicals, fibre, vital nutrients and sensory characteristics of dehydrated fruits are not only convenient but is healthier and assist with the recommended daily allowance proposed for fruits consumption. Even though the serving sizes, 30 to 43 g of dried fruit are lower, depending on the fruit, its nutritional value is comparable to that of fresh fruit (Alasalvar & Shahidi, 2013; Chang *et al.*, 2016). It was reported by Chang *et al.* (2016), atherogenic cholesterol was considerably reduced with the consumption of at least one serving of dried apples (approximately 2 standard-sized apples) per week.

To fully comprehend the shelf life of foods, it is imperative to recognise that foods are dissimilar, a multifaceted living system where physicochemical, microbiological and enzymatic reactions are taking place simultaneously. The storage period, taste and texture of foods are greatly influenced by these reactions (Singh & Cadwallader, 2004). A product shelf life is described as a period between its production date and the point where it cannot maintain the required safety and quality criteria anymore (Singh & Cadwallader, 2004; Robertson, 2011). The ingredients, production process and storage environment of a product plays an important role in its shelf life as well as the water activity (A_w), temperature and the environment affect the shelf life of foods. All foods contain moisture, from minute quantities in dehydrated products to an excess in cold or hot drinks. There is a direct link between the speed at which food spoils

and its water content which makes it vital in the stability and shelf-life of foods (Singh & Cadwallader, 2004). The required water activity can be obtained by the removal of moisture (drying) or by adding a component (salt or sugar). The majority of spoilage bacteria and moulds do not proliferate at A_w levels below 0.91 and 0.80 (Robertson, 2011). Xerophylic moulds and osmophilic yeasts do not grow at water activities below 0.60 (Corrêa *et al.*, 2011). According to Robertson (2011) and Singh & Cadwallader (2004) water activities below 0.6 are required to avoid microbial growth.

Oxidation is one of the main spoilage mechanisms responsible for the spoilage in foods sensory characteristics. It frequently results in inferior texture, colour and flavour of the food products (Brewer, 2013). Physical, chemical and microbial changes are used as indicators of the quality of the foodstuff over the storage period. The absorption of moisture by dry products leading to mushiness and sugar crystallization in dried fruit are some examples of physical changes in foods. Studies show that enzymatic, non-enzymatic browning and oxidation are the main chemical changes responsible for food spoilage and the reduction of shelf life. The result of microbial activity and growth in foods can lead to gas production, changes in pH, discolouration, undesirable flavours and odours (Singh & Cadwallader, 2004; Robertson, 2011).

Various methods which include consumer research, sensory trained panels and instrumental testing are used to assess the changes in sensory characteristics of foods and beverages over time (de Bouillé & Beeren, 2016). Instruments can be used to examine the texture and sensory changes such as rancidity or pH shift in the place of consumer research (Robertson, 2011). According to Miranda *et al.* (2009) & Gunduz & Akman (2015), when drying is used as a preservation method sulphur dioxide is normally used as a preservative to maintain the quality of foodstuff. However, the safety of food processed with sulphites has been questioned as a result of its harmful effect on health, particularly asthmatic individuals (Cárcel *et al.*, 2010; Alagöz *et al.*, 2015).

Chapter 3 of this study showed that a dipping solution with citric acid at 2.0%, *Moringa oleifera* leaf extract at 0.1% and a drying time of 7 h at 70°C minimized the discolouration of the dried apple slices. Therefore, this research aimed to establish whether the 2% citric acid (CA) and 0.1% *Moringa oleifera* leaf extract powder (MOLEP) was effective as a pre-treatment to give the required microbiological safety as well as the physicochemical properties to the dried apple slices during storage. Even though according to Chapter 3 the citric acid was more effective than the potassium sorbate, it was thought it may assist to increase the storage period of the dried apple slices. Hence, the 2.0% CA, 0.1% MOLEP and 0.2% potassium sorbate pre-treatment (CMOP) was included in the shelf life study to compare the two pre-treatments to determine whether it might assist with extending the shelf life of the dried apple slices.

4.2 Materials and Methods

4.2.1 Source of materials and equipment

Granny Smith apples were bought at a retailer in Cape Town, South Africa. The apples were refrigerated at 4°C ±1 °C until needed for the experiment (Deng & Zhao, 2008; Rojas-Graü *et al.*, 2008; Li-Qin *et al.*, 2009; Doymaz, 2010). MOLEP was purchased from Dohler, South Africa Pty (Ltd) (Paarl), ascorbic acid from Lake Foods (Johannesburg, South Africa), citric acid (CA) from Savanna Fine Chemicals (Pty) Ltd (Cape Town, South Africa) and potassium sorbate from Bragan Chemicals CC (Johannesburg, South Africa).

The sliced apples were dried using a cabinet dehydrator (Excalibur, model no: EXC 10). The cabinet dryer consists of a fan (horizontal airflow), 10 trays, a heating element and a thermostat which are at the back of the dehydrator. Cool air is drawn in heated and distributed evenly over each tray (Excalibur, 2012).

4.2.2 Preparation and drying procedure of the apples

Before drying the apples, they were physically washed and sanitised in a 200 ppm sodium chlorite solution (Rojas-Graü *et al.*, 2008). The apples were then peeled, de-cored and sliced to approximately 4 mm in thickness (Rojas-Graü *et al.*, 2008; Doymaz, 2010; Anyasi *et al.*, 2015; Hazervazifeh *et al.*, 2016).

The prepared apple slices were pre-treated with a water solution of 2.0% CA and 0.1% MOLEP (CMO) and another solution of 2.0% CA, 0.1% MOLEP and 0.2% potassium sorbate (CMOP). The apple slices were submerged for 5 minutes in the CMO and CMOP solutions (Deng and Zhao, 2008). After the pre-treatment, the excess solution was gently dabbed off with a paper towel (Rojas-Graü *et al.*, 2008; Cárcel *et al.*, 2010). The untreated (control) and pre-treated apple slices were placed evenly and as a single layer on the trays and dried for 7 h at 70°C.

4.2.3 Shelf life stability assessment of the dried apple slices

The untreated and pre-treated dried apple slices were packed in pouches (polyester, foil and low-density polyethylene), heat-sealed and were stored under ambient/room temperature (Torrieri & Federico, 2016; Bian *et al.*, 2018). The dried apple slices were stored for 120 days (Henríquez *et al.*, 2013; Li *et al.*, 2018). The dried apple slices were tested for colour (L*, a*, b*), water activity, moisture, yeasts and moulds, osmophilic yeasts, *Escherichia coli*, total acidity and texture on the day of the production (day 0), day 60 and day 120.

4.3.4 Colour measurement

The surface colour of the pre-treated and untreated dried apples was measured using a spectrophotometer (Spectrocolorimeter Datacolor 600).

This method allows the determination of the trichromatic coordinates CIELAB (L* a* b*) using a spectrophotometer with measurement geometry d/8 (diffuse illumination, directional observation at 8° ± 2°) and D65 illuminant.

The spectrophotometer was calibrated using a black trap and a white tile before the readings. The sample was placed directly on the instrument in such a way that the reading surface is perfectly flat. The analysis was performed on 10 randomly selected dried apple slice pieces.

The colour was recorded as L* a* b* values. L* represents lightness, ranging from 0-00, 0 being black (no light) and 100 white (maximum illumination). The a* is green (negative) to red (positive). The b* is blue (negative) to yellow (positive) (Acevedo *et al.*, 2008; Huang *et al.*, 2015). The *L**, *a** and *b** values were used to calculate the whiteness index (WI) using equation1 (Eq. 1) (Rocculi *et al.*, 2007; Krasnova *et al.*, 2013). The browning index (BI) were calculated using L*, a* and b* values with equation 2 (Eq. 2) (Guerreiro *et al.*, 2017).

$$WI = 100 - \sqrt{\left(100 - L^*\right)^2 + a^{*2} + b^{*2}} \qquad \text{Eq. 1}$$

where:

L* (lightness), a* (redness) and b* (yellowness) are colour measurements of the dried apple slices.

$$BI = \frac{[100\ (x - 0.31)]}{0{,}172} \qquad \text{Eq. 2}$$

where:

$$x = \frac{(a^* + 1.75L^*)}{5.646L^* + a^* - 3.012b^*}$$

4.3.5 Total acidity determination

The total acidity of the dried apple slices was determined using an automatic titrator (METROHM). The analysis was performed on 10 randomly selected dried apple slice pieces. The apple slices were ground, and a 4 g was weighed and dissolved in boiled distilled water and titrated with NaOH 0.1 N at room temperature using an automatic titrator. Total acidity was expressed as grams of citric acid monohydrate per 100 g.

4.3.6 Water activity determination

ISO 21807:200 method for the determination of water activity (a_w) in foodstuffs by measuring dew point was used to determine the water activity in the dried apple slices at 25°C. The instrument was verified using salt solution standards with known water activity. A representative sample was placed in a cup and placed in the instrument (Aqualab) and sealed.

The final a_w reading was displayed on the screen of the instrument. The analysis was based on the correlation between the air dew point in equilibrium with the sample and its free water content.

4.3.7 Moisture determination

Moisture was determined using the Karl Fischer titration method. The analysis was performed on 10 randomly selected dried apple slice pieces which were ground, and a 4 g sub-sample was weighed and dissolved in 40 g of methanol formamide mixture and titrated with Karl Fischer reagent at room temperature. The final titration mark was determined electronically.

4.3.8 Microbiological analysis

The dried apple slices were tested for yeast and mould, osmophilic yeast and *Escherichia coli* (Codex Alimentarius Commission, 2003). A 10 g representative sample was added to 90 g of buffered peptone water (10^{-1} dilution).

One gram of the inoculum was plated onto the respective agars stated in Table 4.1. Thereafter the plates were incubated for the periods and temperatures as specified for the respective microorganisms as indicated in Table 4.1. Results were reported as cfu/g (colony-forming unit per gram).

Table 4.1: Microbiological test methods information

Microorganisms	Method	Plating technique	Growth media	Incubation temperature	Incubation period
Osmophilic yeast	ISO 21527-2:2008	Spread	DG 18	25°C	5 days
Escherichia coli	ISO 16649-2:2001	Pour	TBX	44°C	24 hours
Yeast and moulds	NFV 08-059:200	Pour	YGC	25°C	5 days

DG 18 = dychloran 18% concentration glycero agar, TBX = Tryptone Bile-X glucuronide agar, YGC = yeast extract glucose chloramphenicol agar.

4.3.9 Texture analysis

The texture analysis was done using a texture analyser (TA. XT plus Texture analyser - Stable Micro System). A metallic cylinder probe 4 mm in diameter, a force of 5 g and a test speed of 1 mm/s were used to test the extensibility of the dried apple slices. The analysis was performed on 10 randomly selected dried apple slice pieces.

4.4 Statistical analysis

Significant differences between treatments were evaluated by multivariate analysis of variance (MANOVA). Duncan's multiple range test was used to separate the means where differences existed at $p \leq 0.05$.

4.5 Results and Discussions

4.5.1 Effect of the pre-treatments on the colour and storage time of the dried apple slices

Table 4.2 indicates the effect of the pre-treatment on the colour of the dehydrated sliced apples. The lightness of the dehydrated sliced apples pre-treated with CMO (2% citric acid and 0.1% *Moringa oleifera* leaf extract powder), L* = 85.6 was significantly (p = 0.000) higher than the CMOP (2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate), L* = 80.8 and the control (L* = 79.6) at day 0. Similarly, the lightness of the CMO (L* = 80.1) dried apple slices was significantly (p = 0.022) higher than CMOP (L* = 78.4) and the control (L* = 75.0) at day 120. The redness of the CMO (a* = 1.5) dried apple slices was significantly (p = 0.000) lower than the CMOP (a* = 4.0) and the control (a* = 4.8) at day 0. Likewise, the redness of the CMO (a* = 3.9) dried apple slices was significantly (p = 0.018) lower than the CMOP (a* = 6.2) and the control (a* = 7.0) at day 120. Yellowness of the CMO (b* = 17.9) dried apple slices was significantly (p = 0.000) lower than the CMOP (b* = 20.5) and the control (b = 33.3) at day 0. At day 120 yellowness of the CMO (b* = 28.4) and the CMOP (b* = 25.1) dehydrated sliced apples were significantly (p = 0.000) lower than the control (b = 33.8). Thus, CMO dried apple slices was significantly lighter, and less red and yellow than the control over the storage time. Also, the CMO dried apple slices was significantly lighter and less red over the storage period than the CMOP.

Table 4.3 details the effect of storage time on the lightness (L*), redness (a*) and yellowness (b*) of the pre-treated and untreated dehydrated sliced apples. The lightness of the dehydrated sliced apples pre-treated with CMO was at day 0 (L* = 85.6) and day 60 (L* = 85.1) significantly (p = 0.000) higher than at day 120 (L* = 80.1). Similarly, the redness of the dried apple slices pre-treated with CMO at day 0 (a* = 1.5) and day 60 (a* = 0.9) was significantly (p = 0.000) lower than at day 120 (a* = 3.9). It was observed that the yellowness of the CMO apple slices at storage day 0 (b* = 17.9), day 60 (b* = 21.7) and day 120 (b* = 28.4) was significantly (p = 0.00) different. The yellowness of the CMO dried apple slices at day 0 was significantly lower than at day 120. Therefore, the CMO dried apple slices were significantly lighter and less red at day 0 and day 60 of storage than at the end (day 120), whereas the yellowness significantly increased over the storage time.

The lightness of the dried apple slices pre-treated with CMOP at day 0 (L* = 80.8), day 60 (L* = 80.1) and day 120 (L* = 78.4) was not significantly different (p = 0.139). However, the redness of the CMOP dehydrated sliced apples was significantly higher (p = 0.007) at day 120

(a* = 6.2) than at 60 (a* = 3.9) and day 0 (a* = 4.0). Similarly, the yellowness of the CMOP apple slices was significantly (p = 0.000) higher at day 120 (b* = 25.1) than at day 60 (b* = 21.5) and day 0 (b* = 20.5). Therefore, the CMOP dried apple slices were more red and yellow at the end of the storage time while no significant change in the lightness over the storage period was observed. Figure 4.1 illustrates the untreated and pre-treated dried apple slices at storage days 0, 60 and 120.

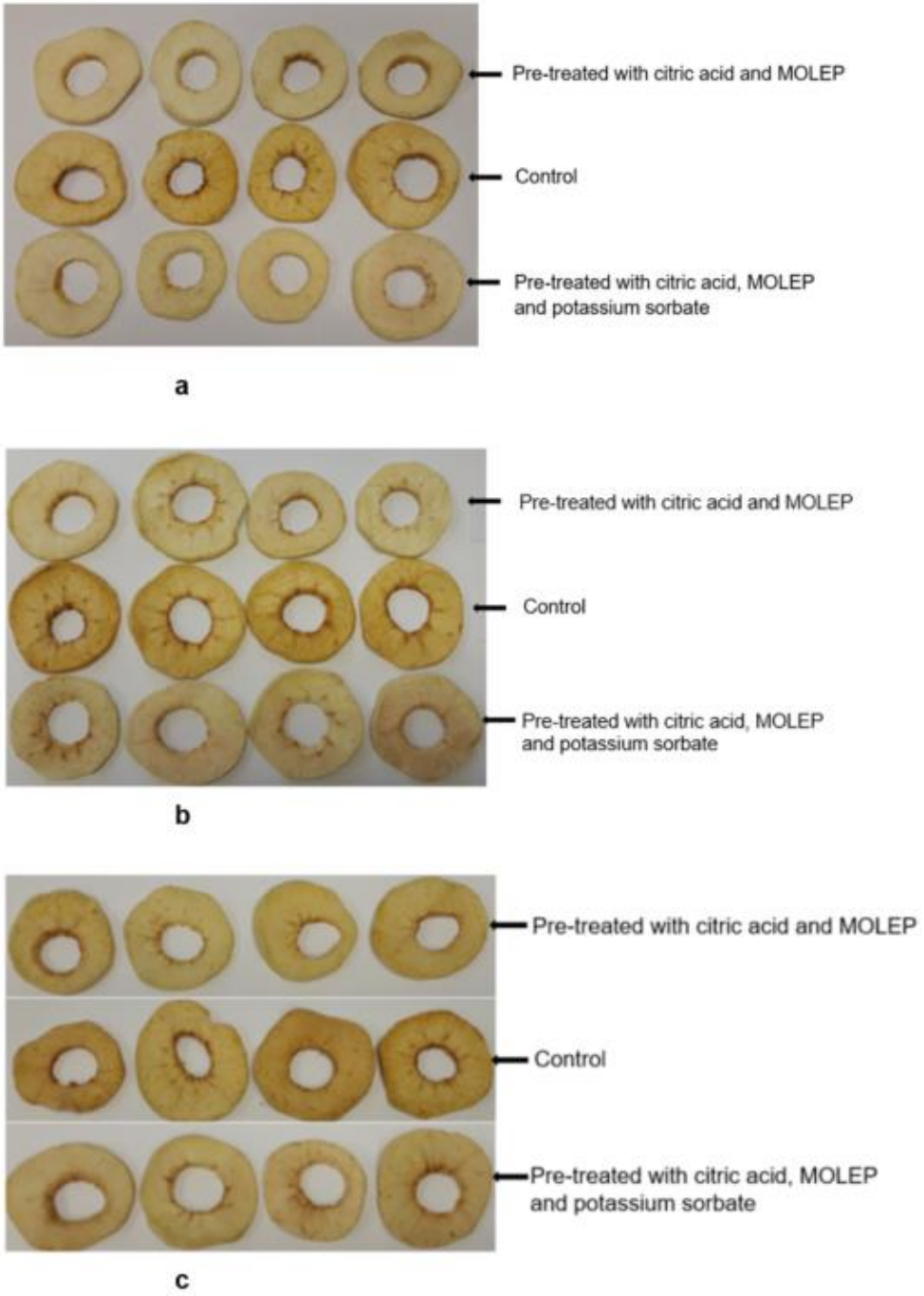

Figure 4.1 Image of untreated and pre-treated dried apple slices at storage time (**a)** 0 day; (**b)** 60 days and (**c)** 120 days

Table 4.2: Effect of pre-treatment on the colour parameters of the dried apple slices[1]

	Colour parameters								
	Lightness (L*)			Redness (a*)			Yellowness (b*)		
	Storage time (day)			Storage time (day)			Storage time (day)		
Pre-treatment	0	60	120	0	60	120	0	60	120
Control	79.6 ± 3.5[a]	77.2 ± 2.6[a]	75.0 ± 5.6[a]	4.8 ± 2.3[a]	4.8 ± 2.0[a]	7.0 ± 3.3[a]	33.3 ± 4.4[a]	34.2 ± 2.7[a]	33.8 ± 3.6[a]
CMO	85.6 ± 1.5[b]	85.1 ± 2.6[b]	80.1 ± 2.6[b]	1.5 ± 0.7[b]	0.9 ± 0.9[b]	3.9 ± 2.0[b]	17.9 ± 1.4[b]	21.7 ± 3.2[b]	28.4 ± 4.4[b]
CMOP	80.8 ± 2.8[a]	80.1 ± 2.9[c]	78.4 ± 2.5[a]	4.0 ± 1.6[a]	3.9 ± 2.0[a]	6.2 ± 1.3[a]	20.5 ± 2.3[b]	21.5 ± 1.9[b]	25.1 ± 2.7[b]

[1]Values are mean ± standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different. Control = untreated, CMO = 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder (MOLEP), CMOP = 2% citric acid, 0.1% *Moringa oleifera* leaf extract leaf powder and 0.2% potassium sorbate.

Table 4.3: Effect of storage time on the colour parameters of dried apple slices[1]

Storage time (days)	Colour parameters								
	Lightness (L*)			Redness (a*)			Yellowness (b*)		
	Pre-treatment			Pre-treatment			Pre-treatment		
	Control	CMO	CMOP	Control	CMO	CMOP	Control	CMO	CMOP
0	79.6 ± 3.5^{a}	85.6 ± 1.5^{a}	80.8 ± 2.8^{a}	4.8 ± 2.3^{a}	1.5 ± 0.7^{a}	4.0 ± 1.6^{a}	33.3 ± 4.4^{a}	17.9 ± 1.4^{a}	20.5 ± 2.3^{a}
60	77.2 ± 2.6^{b}	85.1 ± 2.6^{a}	80.1 ± 2.9^{a}	4.8 ± 2.0^{a}	0.9 ± 0.9^{a}	3.9 ± 2.0^{a}	34.2 ± 2.7^{a}	21.7 ± 3.2^{b}	21.5 ± 1.9^{a}
120	75.0 ± 5.6^{b}	80.1 ± 2.6^{b}	78.4 ± 2.5^{a}	7.0 ± 3.3^{a}	3.9 ± 2.0^{b}	6.2 ± 1.3^{b}	33.8 ± 3.6^{a}	28.4 ± 4.4^{c}	25.1 ± 2.7^{b}

[1]Values are mean ± standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different. Control = untreated, CMO = 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder (MOLEP), CMOP 2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate.

4.5.2 Effect of the pre-treatments on the browning index, whitening index and storage time of the dried apple slices

The effect of the pre-treatment on the browning and whitening index of the dried apple slices is presented in Table 4.4. The browning index (BI) of the dried sliced apples pre-treated with 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder (CMO) was significantly ($p = 0.000$) different over the storage time. The BI of the CMO dried apple slices was 23.9 at storage day 0, 29.4 at day 60 and 46.4 at day 120. However, BI for the CMO dehydrated sliced apples was significantly ($p = 0.000$) lower than the BI of CMOP (2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate) which was 32.0 at day 0 and 34.0 at day 60 while at day 120 (BI = 43.6) there was no significant difference between CMO and CMOP. The CMO dehydrated sliced apples BI were significantly ($p = 0.000$) lower than the control, which was 57.5 at day 0, 61.0 at day 60 and 66.1 at day 120 ($p = 0.001$). Even though the browning index of the CMO and CMOP dried apple slices increased overtime it was significantly less brown than the control at the beginning ($p = 0.000$) and end ($p = 0.001$) of the storage time.

The whitening index (WI) of the CMO dried apple slices was significantly ($p = 0.000$) different over the storage period. The BI for CMO was 76.9 at day 0, 73.6 at day 60 and 65.0 at day 120. Though the CMO dried apple slices WI was significantly higher than CMOP at day 0 and 60 which were 71.6 at day 0 and 70.4 at day 60 there was no significant difference observed at day 120 (WI = 66.2). The WI of the CMO dried apple slices was significantly higher than the control which were 60.6 at day 0, 58.6 at day 60 and 57.2 at day 120. Thus, the WI of the CMO and CMOP dried apple slices decreased over the storage time but was more evident at day 120. Although the CMO and CMOP dried apple slices whitening index decreased overtime it was significantly whiter than the control at the start ($p = 0.000$) and the end ($p = 0.001$) of the storage time.

The effect of storage time on the BI and WI of the dehydrated sliced apples is indicated in Table 4.5. The BI of the dehydrated sliced apples pre-treated with CMO at day 0 (BI = 23.9) and day 60 (BI = 29.4) was significantly ($p = 0.000$) lower than day 120 (BI = 46.4 ± 11.0). Similarly, the BI of CMOP at day 0 (BI = 32.0) and day 60 (BI = 34.0) was significantly ($p = 0.000$) lower than day 120 (BI = 43.6 ± 7.5). The BI of the control was not significantly ($p = 0.421$) different over the storage time. The dried apple slices pre-treated with CMO at day 0 (23.9) and 60 (29.4) BI was lower than CMOP (day 0 = 32.0 and day 60 = 34.0). However, at day 120 the dried apple slices pre-treated with CMO (BI = 46.4) was higher than CMOP (BI = 43.6) though not significantly ($p = 0.001$). It was observed that the BI of the CMO dehydrated sliced apples was lower than that of the control at day 0 (57.5), day 60 (61.0) and 120 (66.1). Thus, dried apple sliced pre-treated with the 2% citric acid and 0.1% *Moringa oleifera* (CMO) were less brown at days 0, 60 and 120 compared to the control over the same period. Furthermore, CMO dried apple slices were less brown than the CMOP (2% citric acid, 0.1%

Moringa oleifera leaf extract powder and 0.2% potassium sorbate) at days 0 and 60 with no significant difference noted at day 120.

The whitening index (WI) of the dried apple slices pre-treated with CMO (2% citric acid and 0.1% *Moringa oleifera* leaf extract powder*)* at day 0 (WI = 76.9) and day 60 (WI = 73.6) was significantly (p = 0.000) higher than day 120 (WI = 65.0). Likewise, the WI of CMOP at day 0 (WI = 71.6) and day 60 (WI = 70.4) was significantly (p = 0.002) higher than day 120 (WI = 66.2), whereas that of the control was not significantly (p = 0.371) different over the storage period. An observation was made that the WI of the dehydrated sliced apples pre-treated with CMO at days 0 and 60 was higher than CMOP. However, the WI of the dried apple slices at day 120 pre-treated with CMOP was higher, though not significantly (p = 0.001). Thus, dried apple sliced pre-treated with the 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder (CMO) were whiter at days 0, 60 and 120 compared to the control over the same storage time. Additionally, CMO dried apple slices were whiter than the CMOP (2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate) at days 0 and 60 with no significant difference at day 120 observed.

The CMO dried apple slices showed a significantly higher value in lightness, and a lower value in redness and yellowness during the storage period compared to the control. The browning index (BI) of the CMO dried apple slices increased significantly while that of the whitening index (WI) declined significantly over the storage period. But, the BI of the CMO dried apple slices were significantly lower and the WI significantly higher than the control.

As mentioned in chapter 3, the positive effect of citric acid on the redness and yellowness of the dried apple slices can be explained by its pH lowering effect and the fact that it is a copper chelating agent (Limbo & Piergiovanni, 2006; Goyeneche *et al.*, 2014). The enzyme, polyphenol oxidase needs copper to function as reported by Ioannou & Ghoul (2013). According to Limbo & Piergiovanni (2006), the binding of the copper at the active site of the enzyme becomes looser at pH values below 4, hence allowing copper ions to be removed by the citric acid. The pH of the dipping solutions were all < 4 (2.36 - 3.68) allowing the copper ions to be removed by the citric acid at the active site of the PPO enzyme and thus prevent browning (Rocculi *et al.*, 2007; Barbagallo *et al.*, 2012; Abd-Elhady, 2014). According to Siddiq *et al.* (2005), *Moringa oleifera* leaves are an excellent source of natural antioxidants and can increase the storage period of foods containing fat (Siddiq *et al.*, 2005). Phenolic compounds and flavonoids are the main phytochemical components in the leaves of *Moringa oleifera*, (Verma *et al.*, 2009; Vongsak *et al.*, 2013a). Das *et al.* (2012) also stated that *Moringa oleifera* leaves are an excellent source of phenolic constituents and have effective antioxidant activity. He reported that a 0.1% extract (100 mg/100 g) were sufficient to prevent lipid oxidation in goat meat patties stored at refrigerated temperatures. This is in agreement with the study by Siddiq *et al.* (2005) which reported that a lower dosage of *Moringa* leaves indicated a slightly higher antioxidant activity overtime. The presence of phytochemicals (polyphenols and flavonoids)

may, therefore, be responsible for the overall antioxidant effect of the MOLEP. The CMOP pre-treatment indicated better lightness and yellowness compared to the control for the storage period, however, CMO dried apple slices showed significantly higher lightness and lower redness when compared to the CMOP dried apple slices. The difference in yellowness between the CMO and CMOP dried apple slices was not significant during the storage time. Initially, the BI (day 0) and WI (day 0 and 60) of the CMO dried apple slices was superior to that of CMOP, however, at the end of the storage the difference was not noticeable.

According to Mendonca (1992), sorbic acid is susceptible to oxidation and degrade by first-order reaction kinetics in aqueous solutions. Furthermore, the breakdown of sorbates occurs with prolonged heating, since the apple slices were dried for 7 h at 70°C (prolonged heat treatment) which might be another reason why CMOP pre-treatment was not as effective as the CMO pre-treatment in reducing the discolouration (L*, a*) of the dried apple slices during storage.

Table 4.4: Effect of pre-treatment on the colour browning and whitening index of the dried apple slices[1]

	Browning Index			Whitening Index		
	Storage time (day)			Storage time (day)		
Pre-treatment	0	60	120	0	60	120
Control	57.5 ± 14.0^{a}	61.0 ± 9.3^{a}	66.1 ± 18.5^{a}	60.6 ± 5.6^{a}	58.6 ± 3.5^{a}	57.2 ± 6.3^{a}
CMO	23.9 ± 2.2^{b}	29.4 ± 6.3^{b}	46.4 ± 11.0^{b}	76.9 ± 1.4^{b}	73.6 ± 3.9^{b}	65.0 ± 5.0^{b}
CMOP	32.0 ± 5.0^{c}	34.0 ± 4.7^{b}	43.6 ± 7.5^{b}	71.6 ± 2.9^{c}	70.4 ± 2.9^{c}	66.2 ± 3.7^{b}

[1]Values are means ± standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different. Control = untreated, CMO = pre-treated with 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder, CMOP = pre-treated with 2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate.

Table 4.5: Effect of storage time on the browning and whitening index of the dried apple slices[1]

	Browning Index			Whitening Index		
	Pre-treatment			Pre-treatment		
Storage time (days)	Control	CMO	CMOP	Control	CMO	CMOP
0	57.5 ± 14.0[a]	23.9 ± 2.2[a]	32.0 ± 5.0[a]	60.6 ± 5.6[a]	76.9 ± 1.4[a]	71.6 ± 2.9[a]
60	61.0 ± 9.3[a]	29.4 ± 6.3[a]	34.0 ± 4.7[a]	58.6 ± 3.5[a]	73.6 ± 3.9[a]	70.4 ± 2.9[a]
120	66.1 ± 18.5[a]	46.4 ± 11.0[b]	43.6 ± 7.5[b]	57.2 ± 6.3[a]	65.0 ± 5.0[b]	66.2 ± 3.7[b]

[1]Values are means ± standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different. Control = untreated, CMO = pre-treated with 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder, CMOP = pre-treated with 2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate.

4.5.3 Effect of the pre-treatments on the microbial count of the dried apple slices during storage

The microbial count for osmophilic yeasts, yeasts and moulds and *E. Coli* at storage days 0, 60 and 120 are presented in Table 4.6. The osmophilic yeasts count for the control, CMO and CMOP was <100 cfu/g and the *E. Coli* count for the control, CMO (2% citric acid and 0.1% MOLEP) and CMOP (2% citric acid, 0.1% MOLEP and 0.2% potassium sorbate) dried apple slice samples were <10 cfu/g, over the storage period.

The initial (day 0) yeasts count for the CMO (<100 cfu/g) dried apple slices showed a one log reduction and CMOP pre-treated dried apple slices showed a 2 log (<10 cfu/g) reduction compared to the control (<1000 cfu/g). The mould count of the CMO (140 cfu/g) dried apple slices at storage day 0 showed a 2-log reduction and the CMOP (40 cfu/g) pre-treated sample showed an even further reduction when compared to the control (14000 cfu/g) at the same storage time. At 120 days, the yeasts and moulds count for all three samples (control, CMO and CMOP) were <10 cfu/g.

Yeasts and moulds are the leading cause of microbial spoilage in dried fruit (Alagöz *et al.*, 2015; Akharume *et al.*, 2018). This research showed that the CMO and CMOP pre-treatment reduced the initial (day 0) yeasts and moulds count of the dried apple slices. The effect of the CMO and CMOP pre-treatments on the yeast and moulds count of the dried apple slices showed better results than the untreated (control); however, the impact of the CMOP pre-treatment on the yeasts and moulds count was more effective than that of the CMO pre-treatment.

Table 4.6: Microbial count of untreated and pre-treated dried apple slices overtime[#]

	Osmophilic yeast (cfu/g)			Yeast (cfu/g)			Moulds (cfu/g)			*E. coli* (cfu/g)		
	Storage time (day)			Storage time (day)			Storage time (day)			Storage time (day)		
Pre-treatment	0	60	120	0	60	120	0	60	120	0	60	120
Control	<100	<100	<100	<1000	130	<10	14000	<10	<10	<10	<10	<10
CMO	<100	<100	<100	<100	<10	<10	140	<10	<10	<10	<10	<10
CMOP	<100	<100	<100	<10	<10	<10	40	<10	<10	<10	<10	<10

[#]cfu/g = colony-forming unit per gram. Control = untreated, CMO = pre-treated with 2% citric acid and 0.1% Moringa oleifera extract powder, CMOP = pre-treated with 2% citric acid, 0.1% Moringa oleifera extract powder and 0.2% potassium sorbate.

4.5.4 Effect of the pre-treatments on the total acidity, water activity and moisture of the dried apple slices during storage

The summary of the mean values for the effect on the total acidity, A_w and the moisture of the dried apple slices overtime are presented in Table 4.7. Total acidity (TA) of the dried apple slices ranged for the control from 2.1 - 1.5, for CMO from 3.8 - 2.9 and CMOP from 3.0 - 4.1 over the storage period. The dried apple slices initial TA of the CMO (3.8) and CMOP (3.0) was higher than the control (2.1) with CMO showing the highest result. The total acidity of the control decreased by 29% and CMO by 9.6%, whereas the CMOP dried apple slices increased by 39.1% over the storage period.

The A_w of the dehydrated sliced apples ranged for CMO from 0.3 - 0.5, for CMOP from 0.3 - 0.4, the control remained unchanged at 0.4 over the storage period. The initial (day 0) water activity of the CMO (A_w = 0.3) and CMOP (A_w = 0.3) dried apple slices was lower compared to the control (A_w = 0.4). A substantial increase of 32.4% in the a_w of the CMO dried apple slices was noted while that of CMOP increased by 9.1% and that the of control decreased by 2.7% over the storage period. The moisture of the dried apple slices for the control over the storage period ranged from 7.3 - 7.8%, for CMO from 6.0 - 10.4% and for CMOP from 6.1 - 7.7%. A decrease in the initial moisture of the CMO (6.0 %) and CMOP (6.1%) dried apple slices was observed when compared to the control (7.3%) with CMO showing the highest increase overtime. The moisture of the CMO dried apple slices showed a considerable increase of 73.6% while CMOP increased by 26.9% and the control increased by 6.5% over the storage period.

According to Doymaz (2010) apples pre-treated with citric acid has a higher moisture diffusivity rate than untreated samples. Since all the dried apple slices were dried at the same temperature and time, the initial lower water activity (a_w) and moisture observed in the apple slices pre-treated with CMO and CMOP can be due to the citric acid present in the pre-treatment solutions. According to Tapia *et al.* (2007) & Chirife *et al.* (1994), no spoilage bacterial growth occurs at water activity levels <0.95 and generally no proliferation of yeast and mould takes place at water activity levels <0.61. The a_w for the CMO sample ranged from 0.34 – 0.45 and for the CMOP sample from 0.33 – 0.36, which is well within the water activity levels not conducive to bacterial, yeasts and mould growth.

As expected, the total acidity of the dried apple slices pre-treated with 2% citric acid, 0.1% *Moringa oleifera* (CMO) and 2% citric acid, 0.1% *Moringa oleifera* and 0.2% potassium sorbate (CMOP) increased, this can be ascribed to the presence of citric acid and potassium sorbate in the pre-treatment solutions. According to Xu *et al.* (2012), fruit acidity plays an important role in determining fruit flavour. It was observed in this study that the total acidity of the CMO (9.6%) dried apple slices changed the least over the storage period, whereas the CMOP (39.1%) showed the highest increase. This suggests that the CMO dried apple slices might have the least change in its flavour profile.

Table 4.7: Effect of the pre-treatment and storage time on the total acidity, water activity and moisture of the untreated and pre-treated dried apple slices

	Total acidity			Water Activity (A_W)			Moisture (%)		
	Storage time (day)			Storage time (day)			Storage time (day)		
Pre-treatment	0	60	120	0	60	120	0	60	120
Control	2.1 ± 0.2	2.9 ± 0.3	1.5 ± 0.2	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	7.3 ± 0.7	6.5 ± 0.6	7.8 ± 0.7
CMO	3.8 ± 0.4	4.2 ± 0.5	2.9 ± 0.3	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	6.0 ± 0.5	6.2 ± 0.6	10.4 ± 0.9
CMOP	3.0 ± 0.3	4.1 ± 0.5	4.1 ± 0.5	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	6.1 ± 0.5	7.9 ± 0.7	7.7 ± 0.7

Control = untreated, CMO = pre-treated with 2% citric acid and 0.1% Moringa oleifera extract powder, CMOP = pre-treated with 2% citric acid, 0.1% Moringa oleifera extract powder and 0.2% potassium sorbate.

The moisture of the control, CMO and CMOP samples increased over the storage period, this can be as a result of samples absorbing moisture from the environment. Even though the moisture of the CMO dried apple slices increased considerably over the storage period it was still within the moisture limit of ≤ 27% as prescribed by regulation 653, relating to the quality, packing and marking of dried fruit intended for sale in the Republic of South Africa.

4.5.5 Effect of the pre-treatments on the extensibility and storage time of the dried apple slices

The effect of the pre-treatment on the extensibility of the dehydrated sliced apples is presented in Table 4.8. The extensibility of the CMO (4.1 mm), CMOP (4.7 mm) and control (4,7 mm) was not significantly ($p = 0.099$) different at day 0. It was noted that the CMO (5.1mm), CMOP (6.3 mm) and control (4.5 mm) dried apple slices extensibility was significantly ($p = 0.000$) different at day 60 with the CMO and CMOP showing higher results than the control. However, the CMO dried apple slices were significantly lower than CMOP at the same time. Though this changed at day 120 with the CMO (8.0 mm) dehydrated sliced apples showing a significantly ($p = 0.000$) higher extensibility than the CMOP (7.0 mm) and the control (6.9 mm). Thus, the dried apples pre-treated with 2% citric acid and 0.1% MOLEP (CMO) and 2% citric acid, 0.1% MOLEP and 0.2% potassium sorbate (CMOP) became less crispy and chewier during the storage period.

Table 4.8: Effect of treatment on the extensibility of the dried apple slices*

	Extensibility (mm)		
	Storage time (day)		
Pre-treatment	0	60	120
Control	4.7 ± 0.6^{a}	4.5 ± 0.6^{a}	6.9 ± 0.6^{a}
CMO	4.1 ± 0.7^{a}	5.1 ± 0.4^{b}	8.0 ± 0.5^{b}
CMOP	4.7 ± 0.7^{a}	6.3 ± 0.3^{c}	7.0 ± 0.5^{a}

*Values are mean ± standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different. Control = untreated, CMO = pre-treated with 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder, CMOP = pre-treated with 2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate.

The effect of storage time on the extensibility of the dehydrated sliced apples is indicated in Table 4.9. The overall effect overtime on the extensibility of the dehydrated sliced apples was significant ($p = 0.000$). It was noted that the CMO dehydrated sliced apples extensibility significantly ($p = 0.000$) increased over the storage time (day 0 = 4.1, day 60 = 5.1 and day 120 = 8.0). Likewise, the CMOP dried apple slices also significantly ($p = 0.000$) increased over

the storage period (day 0 = 4.7, day 60 = 6.3 and day 120 = 7.0). Therefore, the CMO and CMOP dried apple slices are less crunchy and leatherier overtime.

Table 4.9: Effect of storage time on the extensibility of dried apple slices*

Extensibility (mm)			
	Pre-treatment		
Storage time (day)	Control	CMO	CMOP
0	4.7 ± 0.6^{a}	4.1 ± 0.7^{a}	4.7 ± 0.7^{a}
60	4.5 ± 0.6^{a}	5.1 ± 0.4^{b}	6.3 ± 0.3^{b}
120	6.9 ± 0.6^{b}	8.0 ± 0.5^{c}	7.0 ± 0.5^{c}

*Values are mean $\pm$ standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different. CMO = pre-treated with 2% citric acid and 0.1% *Moringa oleifera* leaf extract powder, CMOP = pre-treated with 2% citric acid, 0.1% *Moringa oleifera* leaf extract powder and 0.2% potassium sorbate.

Research by Soni *et al.* (2014) stated that moisture content affects the texture of dried fruit during storage. A substantial increase in moisture of the dried apple slices pre-treated with CMO (moisture increase = 73.6%) and CMOP (moisture increase = 26.9%) was noticed over the storage period. This increased moisture can cause a reduction of the crispness in the dried apple slices and can thus be the reason for the increase in the extensibility (texture) over the storage period (Acevedo *et al.*, 2008; Kutyła-Olesiuk *et al.*, 2013).

A report by Doymaz (2010) indicated that pre-treatments with citric acid reduces the drying time of foods and according to Xiao *et al.* (2018) the increased texture is an effect of the higher drying temperature which accelerates the removal of moisture from the tissue and thus cause case hardening. An increase in extensibility was observed in the dried apple slice samples pre-treated with CMO and CMOP. The increased extensibility of the pre-treated samples (CMO, CMOP) can thus also be linked to the presence of citric acid and the method used to dry the apple slices.

4.6 Conclusion

The optimum combination of 2% citric acid and 0.1% *Moringa oleifera* extract powder (CMO) effectively minimized the redness and yellowness of the dehydrated sliced apples over the storage period. Even though the CMOP dried apple slices showed some positive results in minimizing the colour over the storage time, the CMO dried apple slices demonstrated better results. The CMO and CMOP pre-treatments effectively decreased the yeasts and mould count

of the dried apple slices. The increase in moisture during the storage period affected the extensibility of the pre-treated dried apple slices negatively.

The osmophilic yeasts, moulds and *E.coli* of the CMO and CMOP were within the limits as prescribed by Codex Alimentarius Commission (2003) at the start and end of the storage period and therefore safe for consumption. The A_w of the dehydrated sliced apples pre-treated with CMO was below the limit conducive to microbial growth and thus is an additional guarantee of food safety for the dried sliced apples pre-treated with 2% citric acid and 0.1% *Moringa oleifera* extract powder (CMO).

Furthermore, the shelf life of 3 months (120 days) was accomplished, therefore the objectives to determine whether the optimal combination of 2% citric acid and 0.1% *Moringa oleifera* leaf extract will give the required physicochemical properties, microbial safety and storage stability was thus achieved.

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Chapter 5

Consumer acceptability of the dried Granny smith apples pre-treated with some weak acids and *Moringa oleifera* leaf extract powder

5.1 Introduction

Food producers use sensory studies as quality criteria, to determine product attributes and shelf life and to measure the acceptability of a product when developing new products. The sensory characteristics usually evaluated are appearance, odour, taste and texture (Brewer, 2013). Sensory studies as practised presently are still in its infancy. At the beginning of the 1940's with the growth of hedonic food acceptability methodologies the use of the 9-point hedonic scale developed by the US Army Corps of Engineers became standard practice for several US food companies. Sensory analysis, however, has been practised since the 1800's with the advancement of research into human psychology to calculate and foresee an individual's reactions to outside stimuli (Drake, 2007; Schiano *et al.*, 2017).

Sensory evaluation is defined as a scientific approach whereby the reactions of individuals to foodstuff are awakened, calculated, translated and evaluated by their sense of sight, taste, touch and hearing (Drake & Carolina, 2011; de Bouillé & Beeren, 2016). Its fundamental goal whether using trained panels for quality purposes or acceptance using consumers is to gain insight into a human's view on how they experience the consumption of food using their senses (sight, taste, touch and hearing). In addition to taste and odour, humans also experience trigeminal sensations or pain responses produced by chemical irritants in products such as capsaicin in chillies, menthol, alcohol and ginger. Together with taste, odour and trigeminal sensations are referred to as flavour. In the food industry, sensory statistics or information are used to determine quality standards, consumer acceptability, for product development and shelf life studies. Sensory science allows food manufacturers to consistently produce and develop good quality products (Drake & Carolina, 2011; Brewer, 2013; de Bouillé & Beeren, 2016; Schiano *et al.*, 2017). Sensory studies provide data on which sound decisions can be made (de Bouillé & Beeren, 2016).

A sensory evaluation has two major classifications namely, analytical and hedonic or affective test. Analytical tests are used to determine whether an overall difference exists between two or more products, where no particular characteristic can be recognised as been affected. The most well-known analytical sensory test is the difference or discrimination test (Drake, 2007; Drake & Carolina, 2011; de Bouillé & Beeren, 2016). The triangle, paired comparison, tetrad and difference from control tests are examples of discrimination tests (Drake & Carolina, 2011; de Bouillé & Beeren, 2016). The different tests which are used most extensively are the triangle and duo-trio tests (Drake, 2007; Drake & Carolina, 2011). The

principle of the triangle test is; three samples are presented at the same time to the panellist. The panellist is instructed that 2 samples are identical, and one is different and is asked to select the odd sample. With the duo-trio test, the panellist is presented with 3 samples, one is the reference sample and the other two is coded of which one is identical to the reference sample. The panellist is asked to indicate which sample is the same as the reference sample.

With the paired comparison test the panellists are given 2 samples and asked whether the samples are identical or different. In the case of the tetrad test, the panellists are presented with four samples, two samples of one product and two samples of another product, the panellists are asked to place the products which are the same into groups of two. In the difference from the control test, the panellists are presented with a known control and an assortment of coded samples. The panellists are asked to rate the samples using an appropriate scale with fixed points ranging from “not different from control” to very different from control” (Drake & Carolina, 2011). Depending on the objective of the sensory evaluation the quantity of panellists proposed for analytical discrimination testing is 25-50 (Drake, 2007). Quantitative descriptive analysis (QDA) tests are some of the most regularly used profiling procedures and use trained panellists to carry out these methods. With QDA the sensory characteristics are defined for a product. The trained panellist must fully comprehend the developed terminology of the approved characteristics and their scales (Drake & Carolina, 2011; de Bouillé & Beeren, 2016). For the QDA based procedures, a minimum of eight screened, selected and trained assessors are recommended (de Bouillé & Beeren, 2016).

Hedonic tests have two classifications namely, preference and acceptability tests. As the names indicate these tests merely measure the degree of liking and preference. If the objective of your research is to set one product against another then the preference test should be used, two or more samples may be used. Consumers are asked to rank their preference if a sample size greater than two are used (Drake, 2007; de Bouillé & Beeren, 2016). A disadvantage of this test is that the degree of liking is not established (Drake, 2007). When the objective is to assess how much a product is liked by a consumer then the acceptance test should be used to indicate the degrees of like or dislike, of which the most widely used, is the 9-point hedonic scale. It shows an acceptance on a 9-point numerical scale labelled from “dislike extremely” to “like extremely”.

A minimum of 50 consumers is suggested for acceptance testing. Consumers used for this test should be untrained and regular end users of the product tested (Drake, 2007; de Bouillé & Beeren, 2016). Chapter 4 of this study demonstrated that the dried apple slices pre-treated with a water solution containing 2% citric acid (CA) and 0.2% *Moringa oleifera* leaf extract powder (MOLEP) are safe for human consumption over a 3 months shelf life period. Thus, the purpose of this study is to establish consumer acceptability of the dried apple slices pre-treated with the optimal combination of 2% citric acid (CA) and 0.2% *Moringa oleifera* leaf extract powder (MOLEP) using the 9-point hedonic scale.

5.2 Materials and Methods

5.2.1 Source of materials and equipment

Granny Smith apples were bought at a retailer in Cape Town, South Africa. The apples were refrigerated at 4°C ±1 °C until needed for the experiment (Deng & Zhao, 2008; Rojas-Graü *et al.*, 2008; Li-Qin *et al.*, 2009; Doymaz, 2010). MOLEP was purchased from Dohler, South Africa Pty (Ltd) (Paarl), ascorbic acid from Lake Foods (Johannesburg, South Africa), citric acid from Savanna Fine Chemicals (Pty) Ltd (Cape Town, South Africa) and potassium sorbate from Bragan Chemicals CC (Johannesburg, South Africa).

The sliced apples were dried using a cabinet dehydrator (Excalibur, model no: EXC 10). The cabinet dryer consists of a fan (horizontal airflow), 10 trays, a heating element and a thermostat which are at the back of the dehydrator. Cool air is drawn in heated and distributed evenly over each tray (Excalibur, 2012).

5.2.2 Preparation and drying procedure of the apples

Before drying the apples were physically washed and sanitised in a 200 ppm sodium chlorite solution (Rojas-Graü *et al.*, 2008). It was then peeled, de-cored and sliced to approximately 4 mm in thickness (Rojas-Graü *et al.*, 2008; Doymaz, 2010; Anyasi *et al.*, 2015; Hazervazifeh *et al.*, 2016).

The prepared apples slices were pre-treated with a water solution of 0.2% citric acid and 0.1% MOLEP (CMO) and another solution of 2.0% citric acid, 0.1% MOLEP and 0.2% potassium sorbate (CMOP). The apple slices were submerged for 5 minutes in the CMO and CMOP solutions (Deng and Zhao, 2008). After the pre-treatment, the excess solution was gently dabbed off with a paper towel (Rojas-Graü *et al.*, 2008; Cárcel *et al.*, 2010). The untreated (control) and pre-treated apple slices were placed evenly and as a single layer on the trays and dried for 7 h at 70°C.

The dried apple slices pre-treated with CMO and CMOP and the control were weighed (20 g/sample) and packed in aluminium foil pouches and heat sealed. The samples were labelled with random number codes.

5.2.3 Consumer acceptability testing

An acceptability test was done using the 9-point hedonic scale. Fifty-six consumers participated in the study. The participant's pool consisted of Cape Peninsula University of Technology (CPUT) staff and students as well as external consumers. The sensory test was conducted in CPUT's sensory facility. The sensory test room is regulated with an air-conditioner, run at 18ºC, with white light. The consumers each received on a tray three (control, CMO and CMOP); 20 g randomly coded dried apple sliced samples, packed in aluminium foil pouches as indicated in Figure 5.1. A cup with water, to clean the palate between tastings was provided. The consumers were asked to complete the questionnaire and to indicate the

acceptability of the samples in terms of colour, texture and taste on the 9-point hedonic scale, where 1 indicates "dislike extremely" and 9 indicates "like extremely". An example of the questionnaire is attached (Addendum A).

Figure 5.1 Randomly coded aluminium foil pouches containing the dried apple slices

5.2.4 Statistical analysis

Sensory ratings were expressed as mean ± standard deviation. The data were tested for normality. Where normality assumption was violated sensory differences between samples was estimated using the non-parametric analysis of variance equivalent, Kruskal-Wallis test.

5.3 Results and Discussion

Fifty-six consumers completed the questionnaire of which 51.8% were female, 48.2% were male, 16.1% were in the age group 18-25, 8.9% were 26-30, 10.7% were 31-35 and 64.3% were > 36. All the panellists indicated their frequency of consumption as once a month.

The mean values of the consumer's acceptability scores for colour, texture and taste are shown in Figure 5.2. The mean colour scores for CMO was 6.8, whereas CMOP was 5.3 and the control was 5.1. The texture scores indicate mean values of 6.1 for CMO, 5.2 for CMOP and 5.6 for the control. The mean value for taste was 6.5 for CMO, while CMOP was 5.9 and the control was 6.1. The acceptability of the CMO dried apple slices for colour was significantly ($p = 0.00$) higher than the CMOP and the control. Similarly, for texture, the CMO acceptability was significantly ($p = 0.029$) higher than CMOP and the control dried apple slices. The study showed that the pre-treatment did not impact the taste ($p = 0.161$) of the dehydrated sliced apples negatively.

The pairwise comparison of the dehydrated sliced apples indicated no significant difference ($p = 0.663$) in colour for CMOP compared to the control, however, colour between

the control and CMO there were significantly different (p = 0.000). The data analysis showed that the acceptability of the dried apple slices colour between the CMO and CMOP is significantly (p = 0.000) different. A statistically significant (p = 0.000) difference in colour between the pre-treated (CMO, CMOP) and untreated (control) dried apple slices were observed with the mean colour ranking score for CMO being the highest at 109.52, followed by CMOP at 73.97 and the control at 70.01 (Figure 5.3a).

The pairwise comparison results for texture illustrated no significant (p = 0.203) difference for CMOP compared to the control as well as no significant (p = 0.164) difference between the CMO and the control sample. A significant (p = 0.008)) difference was noted between the CMO and CMOP dried apple slices for texture. The texture between the pre-treated and untreated dried apple slice samples were also significant (p = 0.029) with a mean texture ranking score for CMO being the highest at 96.75 followed by CMOP 84.14 and the control at 72.61 (Figure 5.3b). The rankings indicated that the panellist perceived the colour and texture of the CMO dried apple slices as better than the CMOP and control.

Multiple comparisons for taste was not performed because the overall test did not show significant (p = 0.161) differences across the control, CMO and CMOP dried apple slices, thus no difference in taste was observed by the panellist.

The CMO dried apple slices colour was liked moderately (6.8) and texture liked slightly (6.1), whereas the colour of the control was neither liked nor disliked (5.1) though the texture was liked slightly (5.6) by the consumers. The colour (5.3) and the texture (5.2) of the CMOP dried apple slices were neither liked nor disliked. The taste of the CMO dried apple slices was liked moderately (6.5) while both CMOP (5.9) and control (6.1 $\pm$ 1.8) were liked slightly by the consumers.

The CMO dried apple slices mean rank was the highest for colour (109.52) and texture (96.75). The CMOP dried apple slices ranked second highest for colour (73.97) whereas the control (84.14) was second highest for texture. The colour (p = 0.000) and texture (p = 0.029) across samples (CMO, CMOP and control) were significantly different, whereas for taste it was not significantly different (p = 0.161). Therefore, the CMO dried apple slices was liked more than the CMOP and control.

Hot air drying of plant material often results in a decline in the quality of dried foodstuff, presenting unwanted differences in sensorial properties such as appearance, texture and taste (Djekic *et al.*, 2018). The consequence of dehydration using hot air inevitably leads to the worsening of surface browning and affect the sensorial properties of dried apple products. According to Li *et al.* (2019) & Shrestha *et al.* (2020), browning also influences consumer liking, buying behaviour and consumption of the products. Consequently, dehydration of fruit might produce unacceptable changes in physicochemical attributes such as colour changes, differences in form and volume that could cause reduced consumer acceptance and value perception. (Shrestha *et al.*, 2020).

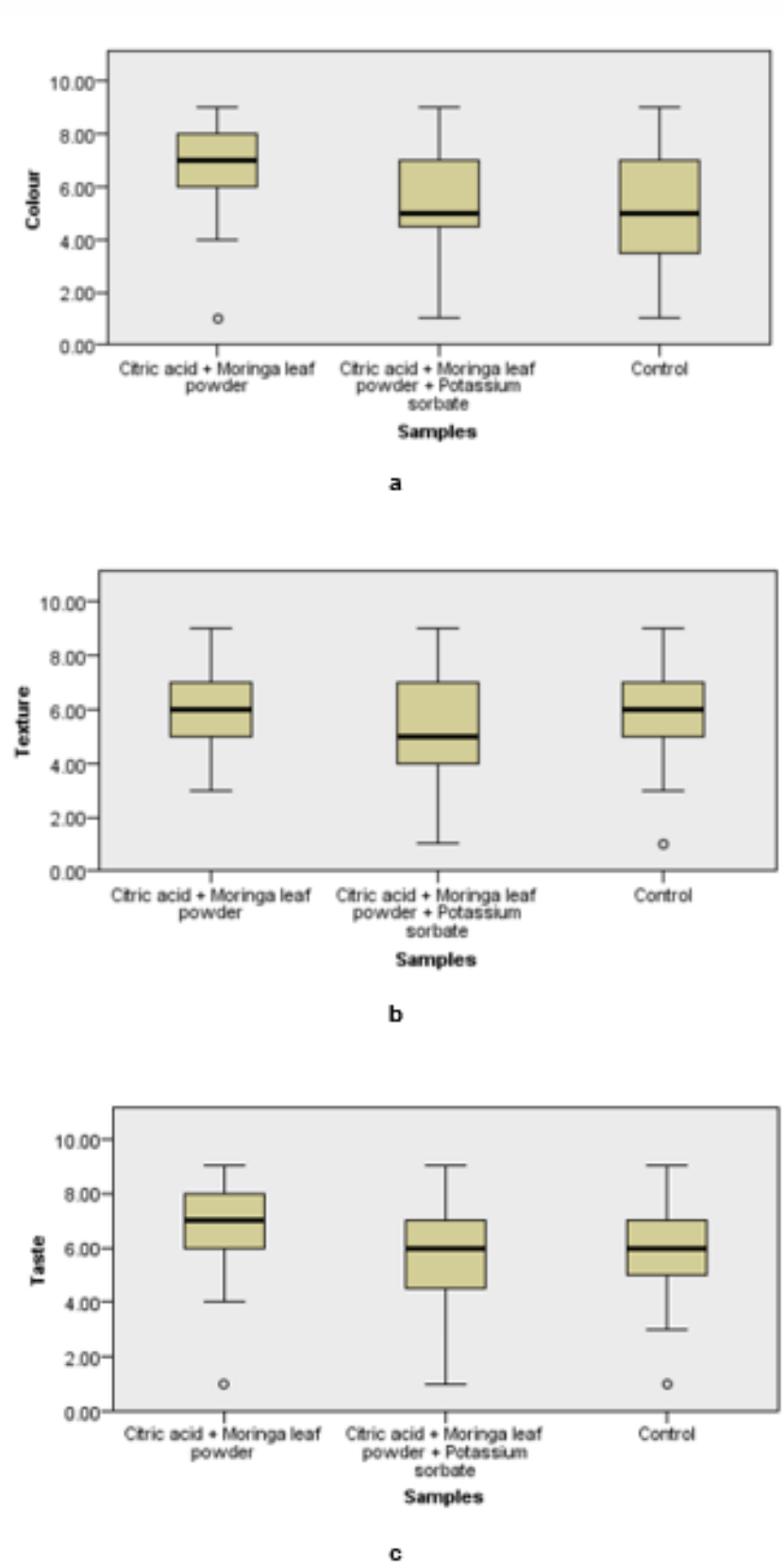
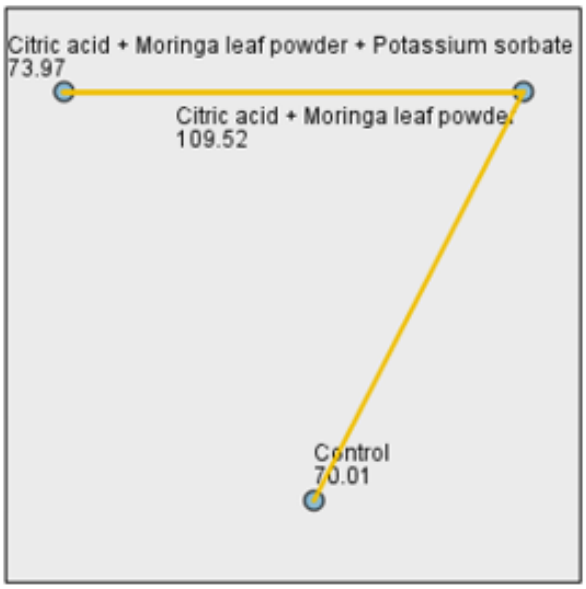
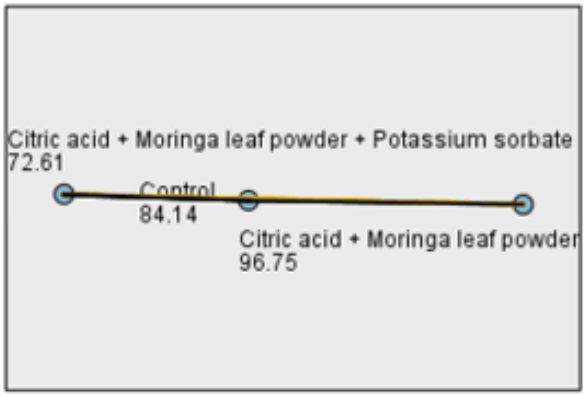


Figure 5.2 Mean value scores of acceptability for, **a**: colour, **b**: texture and **c**: taste scores of the CMO, CMOP and control dried apple slices



a



b

Figure 5.3 The average ranking of the dried apple slices for, **a**: colour and **b**: the texture of the CMO, CMOP and the control

The key physical properties of dried fruit are physical appearance and colour changes and the initial assumptions of food quality by consumers are physical appearance and colour (Chong *et al.*, 2013). According to Shrestha *et al.* (2020), these physical properties have become important quality characteristics in consumers perception of the quality of dehydrated apples. The sensory parameter most commonly used to substantiate consumer acceptance of dried fruit is colour (Chong *et al.*, 2013; Kutyła-Olesiuk *et al.*, 2013; Shrestha *et al.*, 2020). Therefore, colour becomes one of the main quality aspects in consumer acceptability of dried fruit.

The dried sliced apples pre-treated with CMO obtained the highest acceptability score (6.8 = liked moderately) for colour. *Moringa* is a rich source of polyphenols (flavonoids, phenolic acid and tannins) of which the highest concentration is found in the leaves (Ma *et al.*, 2020). Thus, the *Moringa oleifera leaf* extract powder's antioxidant properties can be attributed

to the polyphenols present in the leaves. Citric acid's pH lowering effect and the fact that it is a copper chelating agent is responsible for minimizing the discolouration of the dried apple slices (Limbo & Piergiovanni, 2006; Goyeneche *et al.*, 2014). Chapter 4 of this study also showed that the CMO dried apple slices colour (lightness, redness and yellowness) and whiteness was better than CMOP and the control. Since colour is one of the main aspects of consumers perception of quality, might be the reason why the CMO dried apple slices was liked more by the consumers. Figure 5.4 illustrates some of the dried apple slices used for the consumer study.

a

b

c

Figure 5.4 Images of dried apple slices, **a**: pre-treated with CMO; **b**: control and **c**: pre-treated with CMOP

According to Ntila *et al.* (2020), *Moringa oleifera* leaf powder can result in poor consumer acceptability due to its unfamiliar sensory attributes. The same study indicated that an increase in *Moringa oleifera* leaf powder resulted in a decrease in acceptability in maize porridge, but at a 1% dosage of *Moringa oleifera* leaf powder, the soft porridge was acceptable by consumers. A research article by Das *et al.* (2012) showed that a 0.1% *Moringa oleifera*

leaf extract addition to goat patties was sensorially acceptable. Also, according to Chen *et al.* (2016) citric acid is one of the organic acids naturally present in fruit and does not affect the flavour of plant products negatively. This consumer acceptability study agrees with these research articles since the taste of the dried apple slices was not significantly ($p = 1.61$) affected at the 2% citric acid and 0.1% *Moringa oleifera* extract powder contained in the pre-treatment (CMO and CMOP) water solutions.

5.4 Conclusion

The consumer acceptability test established that the dried apple slices pre-treated with the optimal combination of 2% citric acid and 0.1% *Moringa oleifera leaf* extract powder (CMO) water solution, colour and taste were liked moderately, and the texture liked slightly and therefore acceptable to consumers. The objective to establish consumer acceptability of the dried apples pre-treated with the optimal combination of the weak acid and *Moringa oleifera* leaf extract powder was thus achieved.

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Chapter 6

General Summary, Conclusions and Recommendations

6.3 General Summary

The effect of some weak acids and *Moringa oleifera* leaf extract powder (MOLEP) on the physicochemical and storage properties of dried Granny Smith apple were investigated in this study. There have been concerns around the usage of sulphites particularly regarding the role it plays in causing asthmatic reactions in vulnerable individuals (Cárcel *et al.*, 2010). Thus, this study aimed to analyse the effect of some weak acids (ascorbic acid, citric acid and potassium sorbate) and MOLEP on the physicochemical properties and storage stability of dried Granny Smith apples to find an alternative to sulphites as a preservative for dried fruit. Four specific objectives were identified to achieve this goal.

The first objective was to identify the best weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera* leaf extract powder combination using response surface methodology. Granny Smith apples were purchased at a local retailer in Cape Town, South Africa. The apples were refrigerated at 4°C $\pm$1 °C until needed for the experiment. The Granny Smith apples were sanitised in a 200 ppm sodium chlorite solution, de-cored, peeled and cut into slices. The sliced apples were pre-treated/dipped in a water solution containing the three weak acids namely; ascorbic acid, citric acid and potassium sorbate as well as MOLEP. Numerous studies indicated that ascorbic acid reduces the quinones produced back to dihydroxy polyphenols and thus surface browning (Rocculi *et al.*, 2007; Rojas-Graü *et al.*, 2008; Javdani *et al.*, 2013). Citric acid is a copper chelating agent, this chelating action is responsible for the inhibition of the enzyme polyphenol oxidase, which needs copper to function (Jiang *et al.*, 2004; Limbo & Piergiovanni, 2006; Ansorena *et al.*, 2014; Goyeneche *et al.*, 2014). Reports show that sorbic acids and its salts are favoured by the food industry because it has no significant effect on the public's health, no antimicrobial activity at high pH values and acceptable daily intake which is 25mg/kg body weight (Rowe *et al.*, 2006; Alagöz *et al.*, 2015). The water-soluble salts of sorbic acid, particularly potassium sorbate are extensively used in high moisture dried fruit (Alagöz *et al.*, 2015).

The second objective was to establish whether the optimal combination of weak acids and *Moringa oleifera* leaf extract powder will give the required physicochemical properties (colour, texture, moisture, water activity and total acidity) as well as microbial safety to the dried fruit (apples). The pre-treatment and drying process was based on the augmented 2^{6-3} fractional factorial design. The entire design comprised of 19 experimental trials, including triplicates of the midpoint. This experimental design was used to screen the main effects of the 5 variables (ascorbic acid, citric acid, potassium sorbate, time and temperature) and the

MOLEP as well as to identify the weak acids and MOLEP combination that will reduce discolouration of the dried apple slices.

The sliced apples were dried using a cabinet dehydrator (Excalibur, model no: EXC 10). A screening process was done using an experimental design with the view to identify the best weak acids and *Moringa oleifera* leaf extract powder combination that will give the strongest colour (lightness) effect. Weight loss was calculated by weighing the pre-treated apples before and after drying. The study indicated that ascorbic acid and potassium sorbate did not impact the colour of the dehydrated sliced apples. Citric acid had a positive effect on the colour of the dried sliced apples whereas that of *Moringa oleifera* was negative. This study showed that a dipping solution with citric acid at 2.0%, *Moringa oleifera* leaf extract at 0.1% and drying time of 7 h at 70°C reduced the browning of the dried apple slices. The objective to identify the best weak acids (ascorbic acid, citric acid and potassium sorbate) and Moringa oleifera leaf extract powder combination using response surface methodology were thus achieved, confirming the hypothesis that the optimum combination of weak acids (ascorbic acid, citric acid and potassium sorbate) and *Moringa oleifera* leaf extract powder will preserve the colour of the dried apples.

The third objective was to determine the storage stability of the dried fruit (apples) using the optimum combination of the weak acids and *Moringa oleifera* leaf extract powder. Storage stability was done on dried apple slices pre-treated with a water solution of 2% citric acid and 0.1% MOLEP (CMO) and another solution of 2.0% citric acid, 0.1% MOLEP and 0.2% potassium sorbate (CMOP). Even though this study showed that citric acid was more effective than potassium sorbate, it was included in the shelf life study to determine whether it will assist with the extension of the shelf life of the dried apple slices because of its known antimicrobial properties. The pre-treated and untreated (control) dried apple slices were kept at ambient/room temperature for up to 120 days. The dried apple slices were tested on days 0, 60 and 120 for osmophilic yeasts, *Escherichia coli,* yeasts and moulds. Also tested at the same intervals were total acidity, moisture analysis, water activity, texture and colour. The 2% citric acid and 0.1% *Moringa oleifera* extract powder (CMO) effectively minimized the redness and yellowness of the dehydrated sliced apples over the storage period. Even though the CMOP dried apple slices showed some positive results in reducing the browning, the CMO dried apple slices demonstrated better results over the storage period.

The moisture of the CMO dried apple slices increased significantly over the storage period which had a negative effect on the extensibility. Thus, the CMO dried apple slices were less crisp than that of the control at the end of the shelf life. Predictably the total acidity of the dried apple slices increased, this can be due to the citric acid and potassium sorbate present in the pre-treatment water solution. The CMO and CMOP pre-treatments effectively decreased the yeasts and moulds count of the dried apple slices compared to the standard. The osmophilic yeasts, moulds and *E.coli* colony-forming units per gram (cfu/g) of the CMO and

CMOP dried apple slices were within the limits as prescribed by Codex Alimentarius Commission (2003) at the start and end of the storage period, while the water activity (<0.61) was below the limit conducive to microbial growth and therefore safe for consumption. The objective to establish whether the optimum combination (2% citric acid and 0.1% *Moringa oleifera* leaf extract powder) will give the required physicochemical properties (colour, texture, moisture, water activity and total acidity) and microbial safety to the dried apples was thus achieved, confirming the hypothesis that the optimum combination will give the required physicochemical properties and microbial safety to the dried apple slices.

Also, shelf life of 3 months was achieved, thus confirming the hypothesis that the most effective combination (2% citric acid and 0.1% *Moringa oleifera* leaf extract powder) will produce dried apple slices with reasonable storage stability.

The fourth objective was to establish consumer acceptability with regards to colour (appearance), texture and taste using the 9-point hedonic scale. Consumer acceptability test was done with the pre-treated (CMO and CMOP) dried apple slices and the control (untreated). The consumers had to complete a questionnaire and were asked to indicate the acceptability of the samples by using the 9-point hedonic scale, where 1 indicates "dislike extremely" and 9 indicates "like extremely" in terms of colour, texture and taste. Fifty-six consumers participated in the study. The dried apple slices treated with 2% citric acid and 0.1% *Moringa oleifera* extract powder (CMO) obtained the best scores for taste and texture, the taste was liked moderately, and texture was liked slightly. No difference in taste across the dried apple slices (control, CMO and CMOP) were observed. The CMO dried apple slices also ranked highest for colour and texture. The objective to establish consumer acceptability with regards to colour, texture and taste using the 9-point hedonic scale was thus achieved, confirming the hypothesis that the consumers will find the dried Granny Smith apple colour, texture and taste acceptable.

6.2 Conclusion

The following conclusions can be made from this study:

1. A dipping solution with citric acid at 2.0%, *Moringa oleifera* leaf extract powder at 0.1% and drying time of 7 h at 70°C minimized the discolouration of the dried apple slices.
2. The optimum combination of 2% citric acid and 0.1% *Moringa oleifera* extract powder (CMO) effectively minimized the redness and yellowness of the dehydrated sliced apples over the storage period. The CMO pre-treatment gave the required microbial safety to the dried apple slices. As moisture content increased during storage, the extensibility of the dried apple slices increased, therefore the dried apple slices became less crisp overtime.
3. A shelf life of 3 months (120 days) was achieved using CMO as pre-treatment for dried apple slices.

4. The addition of 0.1% Moringa oleifera leaf extract powder does not affect the sensorial attributes (colour, texture and taste) of the dried apple slices negatively. The CMO dried apple slices colour and taste were liked moderately, and the texture was liked slightly and therefore acceptable to consumers.

Thus, the CMO pre-treatment provides antioxidant and antimicrobial benefits without altering the sensorial attributes of the dried apple slices and could be used instead of sulphur dioxide (SO_2) to arrest the discolouration and to increase the storage period of dried fruit. This would beneficial to individuals sensitive to sulphites and the environment since sulphur dioxide is not only a health hazard but an air pollutant as well.

6.3 Recommendations

Future studies should explore which of the components (D-glycosyl compounds, and phenolic compounds) identified in the *Moringa oleifera* leaf extract powder used in this study are responsible for the observed antioxidant and antibacterial activity.

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Addendum A

Questionnaire for Consumer Study

Product: Dried Apples

Sex

Male	Female

Age

18-25	26-30	31-35	>36

How often do you eat dried apples?

Every day	Once a week	Once a month

Acceptability Test

Name _____ **Date** _____

You are provided with three samples of dried apples. Please take a sip of water in between tasting the different samples. Please indicate, the degree of acceptability of the samples by using the 9-point scale, where 1 indicates "dislike extremely" and 9 indicates "like extremely in terms of colour, hardness/texture and taste.

Sample code	Colour	Texture / Hardness	Taste
838	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely

Sample code	Colour	Texture / Hardness	Taste
491	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely

Sample code	Colour	Texture / Hardness	Taste
749	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely	1 2 3 4 5 6 7 8 9 Dislike extremely Like extremely