QUALITY CHARACTERISTICS OF A FUNCTIONAL BEVERAGE DEVELOPED FROM *MORINGA OLEIFERA* AND *ALOE VERA*

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ABSTRACT

The lifestyle of most populations has changed over the past few decades. Usually, the lack of time is perceived as the major barrier to practicing healthy habits such as eating healthy food. As a result, there seems to be a greater incidence of chronic or degenerative disorders such as cancer in most countries. In this study, a functional beverage was developed by blending Moringa oleifera and Aloe vera at different proportions (0M: 100A; 30M: 70A; 50M: 50A; 70M: 30A; 100M: 0A, *M. oleifera*: *A. vera*, respectively). The nutritional and phytochemical composition of the developed beverages was determined. Further, consumer acceptability and storage stability of the beverage blends were determined.

The pH of the beverages ranged from 3.66 - 4.15 with the least and highest pH obtained with 100 % Aloe (100A) and 100 % Moringa (100M) beverages, respectively. The titratable acidity (TA) of the beverages ranged from 0.14 - 0.12 %, while total soluble solids ranged from 0.5-0.8 °Brix. Beverage blends with high Aloe concentration contained high levels of vitamin C possibly due to the high concentration of the vitamin in Aloe. The highest protein content (343.60 µg/ml) was observed with 100M beverage and the least (74.60 µg/ml) with 100A beverage. The concentration of Ca, K, Mg in the beverage blends increased with an increase in the concentration of Moringa. Also, the total phenolic content and total flavonoid content of the beverage blends improved with increase in Moringa concentration and so did the antioxidant activity when determined with DPPH assay. However, the beverage blends showed similar antioxidant activity with ABTS.

Consumer acceptability of Moringa-aloe beverage blends was investigated using the 9-point hedonic scale method. The results showed that 100A beverage was the most preferred beverage by the taste panel as it was scored highest for taste (7.08), flavour (6.76) and overall acceptability (7.04). The least preferred beverage was 100M followed by the beverage blend with high Moringa concentration (70M: 30A). With colour, the highest score (6.48) was observed with 70M: 30A beverage blend and all the other beverage blends were scored high for colour compared to control samples. With storage stability, a slight increase in pH accompanied by a decrease in TA occurred on all beverages after 12 weeks. However, most beverage blends still maintained a pH of less than 4.2 at the end of the storage period. There was no microbial growth observed for total aerobic bacteria and yeast and mold after 12 weeks of storage, possibly due to added preservatives and low pH of the products. Overall, the current study confirms that beverage blends with Moringa

have high nutritional quality and possess health-promoting properties. However, an increase in Moringa concentration impacts negatively on sensory quality. Therefore, the 30M: 70A beverage blend appears to be the best concerning consumer acceptability and storage stability.

DECLARATION

I, **Maikemisetso Thobakgale** hereby declare that this dissertation, which I hereby submit for the award of MSc Food Technology degree at the University of Johannesburg, represents my work and has not been previously submitted by me to the University of Johannesburg or any other institution in the application for a degree or diploma or any other qualification.

Signature. ..... Date 30 January 2020

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PLAGIARISM DECLARATION

- I hereby declare that I know what plagiarism entails, namely to use another's work and to present it as my own without attributing the sources in the correct way.
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LIST OF ABBREVIATIONS

ABTS	2, 2-azionobiz 3-ethylbenzothiazoline– 6-sulfonic acid
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Ca	Calcium
DPPH	2, 2 -diphenyl-1-picrylhydrazyl
FAO	Food and Agriculture Organization
Fe	Iron
GAE	Gallic Acid Equivalent
K	Potassium
kDa	Kilodaltons
Mg	Magnesium
Na	Sodium
p	Probability
pH	Hydrogen-ion-concentration
QE	Quercetin Equivalent
SD	Standard Deviation
TA	Titratable Acidity
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TSS	Total Soluble Solids
WHO	World Health Organization

LIST OF UNITS AND SYMBOLS

&	And
%	Percent
+	Plus
±	Plus-minus
=	Equal to
/	Per
<	Less than
>	Greater than
°C	Degree Celcius
Δa*	Difference in red and green
Δb*	Difference in yellow and blue
ΔL*	Difference in lightness and darkness
µg/ml	Microgram per millilitre
µmol	Micromole
CFU	Colony Forming Unit
mg/L	Milligram per Litre

DISSERTATION OUTLINE

This section presents a brief overview of the chapters found in the dissertation below:

Chapter One: General introduction

This chapter introduces the plants (*Moringa oleifera* and *Aloe vera*), the problem statement of the research, the hypotheses, as well as aims and objectives.

Chapter Two: Literature review

In this chapter, functional beverages and their benefits are discussed. An overview of the plants' (*Moringa oleifera* and *Aloe vera*) background, their nutritional and medicinal value, and their uses is given. Phytochemicals found in these plants are also discussed, including the importance of phytochemicals and antioxidants in food. This chapter concludes by highlighting the missing gap knowledge on medicinal beverages.

Chapter Three: Product characteristics of Moringa-aloe beverage blends

Chapter three discusses the Moringa-aloe beverage blends' in terms of pH, TA, °Brix, and colour. Nutritional characteristics such as protein, vitamin C and minerals are also quantified. TPC, TFC, DPPH and ABTS results are discussed and beverage blends with higher amounts phenolics and antioxidant activities are reported.

Chapter Four: Consumer acceptability and shelf-life studies of Moringa-aloe beverage blends

In this chapter, the consumer acceptability of the beverage blends is determined using sensory evaluation and the 9-point hedonic scale method. Results are discussed and the most preferred and least preferred beverage blends are reported. This chapter also reports on the shelf-life of beverage blends, where blends stored at 4 °C for 12 weeks were evaluated for pH, TA, Brix, colour and total aerobic count, yeast and moulds at two-week intervals.

Chapter Five: General discussion and conclusions

Chapter five summarizes the findings of the study, in addition it gives recommendations for future researchers who would like to carry out or continue a similar study.

CHAPTER 1

1.0 GENERAL INTRODUCTION

Beverages are excellent nutrient carriers and can be used to transport essential vitamins and minerals. They are the most preferred nutrient-carriers due to their convenience as well as shelf-life stability (Wootton-Beard and Ryan, 2011). The current food trends have inspired the development of new beverages that besides being organoleptically acceptable, are also highly nutritious as well as contain functional activity. As a result, plant-based beverages have gained popularity because they contain bioactive compounds such as phenolic acids, alkaloids, flavonoids, terpenoids, coumarins, saponins and polyacetylenes, among others (Chandrasekara and Shahidi, 2017). These compounds have been associated with a number of health benefits to humans. Plants are also rich in vitamins and minerals which are essential for growth and maintenance of the human body (WHO, 2005).

Moringa oleifera (*M. oleifera*) appears to be one of the most studied and cultivated species in the world (Pachava et al. 2018). This species originated in India, Bangladesh, Pakistan and Afghanistan and has now spread to tropical and subtropical regions (Gopalakrishnan et al., 2016). It has high nutritional value and all parts of the plant, including leaves, seeds, pods, barks can be utilized as food or commercial purposes (Dinesha et al., 2018). Due to the fact that it is easily cultivable and fast growing, *M. oleifera* is used to combat malnutrition in some parts of the world (Dhakar et al., 2011). *M. oleifera* has been reported to provide 25 times more iron than spinach, 9 times more protein than yogurt, 7 times more vitamin C than oranges, 17 times more calcium than milk and 10 times more vitamin A than carrots (Rockwood et al., 2013). In addition, Moringa leaves are rich in other minerals such as potassium, zinc, magnesium and copper (Kasolo et al., 2010), as well as vitamin A (beta-carotene), vitamin B (folic acid), vitamin C, vitamin D, vitamin E, nicotinic acid and pyridoxine (Mbikay, 2012). Moringa leaves are also rich in antioxidants and polyphenols which are responsible for the treatment of cardiovascular diseases, nervous disorder, regulation of thyroid status (Shanmugavel et al., 2018), antitumor and anticancer activity (Kumar et al., 2010), diuretic activity on blood pressure (Faizi et al., 1995), wound healing activity (Pramanik and Islam, 1998), anticancer activity (Mishra et al., 2011) and antidiabetic activity (Divi et al., 2012).

Commercial uses of Moringa plant includes fortification of food and as an ingredient in functional beverages. Moringa has been used in several beverages and the addition of this plant was reported to increase the overall quality of the product. The addition of 7.5% Moringa leaf extract to whey-guava increased the total phenolic content, protein content, ash and minerals such as manganese, potassium, iron and magnesium (Ali et al., 2015). A similar trend was reported by Aderinola (2018), where the addition of 4.5% Moringa leaf extract increased the smoothies' protein content, vitamins C and E content, as well as minerals such as calcium and iron.

Aloe vera is a plant belonging to the Liliaceae family, species Barbadensis Mill (Boudreau and Beland, 2006). It is a perennial plant with large basal leaves that has been used for decades for its medicinal and therapeutic properties (Nazir and Ahsan, 2017). Factors such as age of the plant, region, climate, growing conditions and processing methods affect the chemical composition of the plant (Boudreau and Beland, 2006). *Aloe vera* contains potentially active constituents including amino acids, minerals, vitamins, saponins, salicylic acids, enzymes anthraquinones and saccarides (Atherton, 1998). The vitamins in Aloe include beta-carotene of vitamin A, vitamin C and E. These vitamins are antioxidants which scavenge free radicals (Sevindik et al. (2017). *Aloe vera* contains a variety of minerals including copper, calcium, magnesium, potassium, chromium, sodium, selemium, zinc and manganese (Surjushe et al., 2008). It contains anthraquinones (emodin and aloin) that act as analgesics, antivirals and antibacterials (Surjushe et al., 2008).

Aloe contains 20 of the 22 amino acids required by humans as well as 7 of the 8 essential amino acids that humans cannot synthesize (Surjushe et al., 2008). 2017). Commercially, *Aloe vera* is used for the preparation of functional foods, health drinks, tea (Surjushe et al., 2008), aloe sports drinks with electrolytes, soft drinks and diet drinks with soluble fiber (Eshun and He 2004; Grindlay and Reynolds, 1986; Hamman, 2008). Aloe gel can also be used as edible coating for various fruits and vegetables such as grapes (Christaki et al., 2010) and tomatoes (Chrysagyris et al., 2016).

1.1 PROBLEM STATEMENT

The lifestyle of most populations has changed over the past few decades. Usually, the lack of time is perceived as the major barrier to practicing healthy habits such as exercising and eating healthy

foods. As a result, there seem to be a greater incidence of chronic or degenerative disorders such as cancer in most countries (Steyn et al., 2006). In South Africa, statistics indicate that 1 in 27 women are at risk of developing breast cancer, while 1 in 19 males are reported to be at risk of developing prostate cancer in their lifetime (NCR, 2014). Consequently, society is gradually adopting healthy living life-styles that are characterised by an increased consumption of plant-based products believed to reduce or prevent certain life-style induced diseases (Steyn et al., 2006). This new trend has forced the food industry to reformulate some food products to meet the new demand. The developed Moringa-aloe beverage in the current study is expected to provide protection against some human diseases and thus improve the health of the consumer. At commercial scale, production of the beverage will create employment opportunities for both food technology graduates and other skills. The commercial production of the product will encourage production of Moringa and aloe plants by farmers and this will result in more income for farmers and employment opportunities for the community.

1.2 HYPOTHESES

Blending two medicinal plants (*Moringa oleifera* and *aloe vera*) will produce a functional beverage that is better than when the plants are used individually. This has been observed with other beverage blends such as aloe gel-papaya beverage blend (Ramachandran and Nagarajan, 2014) and whey-guava beverage blended with Moringa leaf extract (Ali et al., 2015).

Beverage blends with high concentration of *M. oleifera* will be least preferred by consumers because of the bitter taste of Moringa leaves (Aderinola, 2018).

Moringa-aloe beverage blends will have acceptable shelf-life at 4°C because of their low pH. Low pH inhibits the growth of most microorganisms (Out et al., 2013b; USDA, 2012).

1.3 OBJECTIVES

- To develop a functional beverage using *Moringa oleifera* extract and *Aloe vera* juice at different proportions.

- To determine quality characteristics of Moringa-aloe beverage blends (pH, titratable acidity, °Brix, colour).

- To determine the nutritional composition of Moringa-aloe beverage blends (vitamin C, protein, and mineral content).

- To determine the total phenolic content (TPC), total flavonoid content (TFC) and determining antioxidant activity of the beverage blends using 2, 2 -diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-azionobiz 3-ethylbenzothiazoline– 6-sulfonic acid (ABTS) method.

- To determine the consumer acceptance of Moringa-aloe beverage blends using a sensory panel.

- To determine storage stability of the Moringa-aloe beverage blends stored at 4°C for 12 weeks monitoring: pH, TA, TSS, Colour, total aerobic count and yeast and moulds.

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CHAPTER 2

LITERATURE REVIEW

This chapter gives a brief introduction to functional foods, plant-based beverages as well as a background of Moringa oleifera and aloe vera plants, concerning their history, nutritional value, medicinal value and applications in food. Phytochemicals found in these plants are also discussed, including the importance of phytochemicals and antioxidants in food. Later, consumer acceptability and storage of plant-based beverages are discussed.

2.1 FUNCTIONAL FOODS

Functional foods are whole, enriched, fortified or enhanced foods that provide health benefits (Hasler et al., 2009). There are several important aspects that are recognized as key factors that lead to the diffusion of functional foods. These include deterioration of health as a result of a busy lifestyle, insufficient exercise, a highly competitive food market, high incidents of self-medication, awareness of the link between health and diet from information by health authorities and media on nutrition (Granato et al., 2010).

There are various ranges of functional foods, according to Ofori and Hsieh (2013) these include confectionary, baked goods and cereals, baby foods, snacks, spreads, meat products, ready meals and beverages. The active functional foods category are beverages, this is due to (i) their convenience, ability to meet customer demands for packaging content, size and shape (ii) they are easy to distribute and provide better storage for shelf-life stable and refrigerated products (Sanguansri and Augustin, 2009; Wootton-Beard and Ryan, 2011).

There are several plant-based functional beverages that have been developed in order to improve the health of consumers. These beverages usually include at least one medicinal plant which significantly increases the beverages' nutritional and medicinal value. Beverages that have been developed include; Aloe gel and ginger juice (Singh et al., 2017), *Moringa oleifera* beverage (Otu et al., 2013a), fermented Moringa leaves-based beetroot beverage (Vanajakshi et al., 2015) and Aloe gel and papaya beverage (Ramachandran and Nagarajan, 2014). Previous studies have reported that using more than one plant type in the development of a beverage increases the phytochemical content, which implies an increase in the health benefit of the product (Badejo and Ojuade, 2014). *Aloe vera* and *Moringa oleifera* were selected for the current study. These plants

were selected based on their acclaimed nutritional benefits, health benefits and wide applications in food.

2.2 *MORINGA OLEIFERA*

2.2.1 History of *Moringa oleifera*

Moringa oleifera plant (Figure 2.1) belongs to the family "*Moringaceae*" with genus "Moringa Adans" and species "*Moringa oleifera* Lam". This species originates in India but has now spread to the tropics and subtropics all over the world (Enwa et al., 2013). *Moringa oleifera* in some parts of the world is also known as the Drumstick tree, Mlonge, Marango, Moonga, Saijhan or Ben oil tree (Prabhu et al., 2011). It is a perennial softwood tree and according to Dinesha et al. (2018) almost all the parts of Moringa plant (flowers, leaves, roots, seeds and bark) can be utilised for therapeutic and medicinal purposes as well as food. Moringa has gained popularity due to its amazing health benefits and variety of potential uses.

Figure 2.1. Moringa oleifera plant (Raja et al., 2016)

South African farmers indicate that they utilize Moringa in several ways, such as; a high nutritional supplement for good health, immune booster, energy booster, livestock feeding, food security, water purification and a source of income (Mabapa, et al., 2017). *Moringa oleifera* is an important and fast-growing tree which is recognized for its industrial, medicinal, human and livestock nutritional values. In the Limpopo province of South Africa, Moringa is mainly grown by a limited number of farmers in the backyards and spaces around homesteads. More farmers started planting Moringa after realizing its nutritional and health benefits as well as possibilities of income

generation from its products (Mabapa, et al., 2017). The widespread of production of Moringa in the Limpopo province is still at the development stage.

2.2.2 Nutritional value of *Moringa oleifera*

Moringa seeds contain essential bioactive compounds that have been used for anti-inflammatory, anti-genotoxic, antimicrobial and anti-tumour promoting activities (Dinesha et al, 2018; Prabhu et al., 2011). Moringa seed oil is an excellent source of tocopherols which enhances cardiac function and purifies blood (Middleton et al., 2000).

The Moringa tree is reported to be high in nutrients and phytochemicals (Ashfaq et al., 2012). According to several researchers, dried leaves of Moringa provide four times the amount of carotene as carrots, while fresh leaves contain seven times the amount of vitamin C as oranges (Huber et al 2017; Dhakar et al. 2011). Although there is variation in values, studies are in agreement that dried Moringa leaves contain more iron than spinach, more calcium than milk, and more potassium than bananas (Dhakar et al., 2011; Gopalakrishnan et al., 2016). This is the reason behind the application of the Moringa species as natural supplements in Namibia, where Moringa was found to have potential in the treatment of iron deficiency, anaemia, calcium deficiency and potassium depletion (Huber et al, 2017).

Researchers have found that Moringa is a good source antioxidants and polyphenols that reduce tissue damages taking place during physiological processes (Shanmugavel et al., 2018). Studies have shown that the leaves of Moringa have several biological activities such as liver disease, prevention of cardiovascular diseases, anticancer activities (Pari and Kumar, 2002) nervous disorder, skin disorders, antitumor, regulation of thyroid status and inflammation digestive disorders (Shanmugavel et al., 2018).

2.2.3 Medicinal value of *Moringa oleifera*

A study by Singh et al. (2012) reported Moringa to have antibacterial activity, using the disc diffusion method, where 50% ethanolic extracts of Moringa leaves were used. Moringa roots are reported to contain high levels of anti-microbial agents. These agents contain pterygospermin, an antibiotic principle which is responsible for anti-fungal and anti-bacterial activities (Das et al.,

1957). Moringa leaves have anti-tumor potential which might be due to the presence of 3-O-[6′-O-oleoyl- α-D-glucopyranosyl]-β-sitosterol, O-Ethyl-4-[α-L-rhamnosyloxy]benzyl carbamate, niazimicin and 4[α-L-rhamnosyloxy]-benzyl isothiocyanate, which showed significant inhibitory effects on Epstein–Barr virus-early antigen when tested for their potential anti-tumor promoting activity using an in vitro assay (Kumar et al., 2010). Moringa seed extracts have also been reported to have effects on hepatic carcinogen metabolizing enzymes in mice (Bharali et al., 2003).

Juice from Moringa leaves has been reported to have a stabilizing effect on blood pressure. The lowering effect of blood pressure is said to be due to mustard oil glycosides, nitrile and thiocarbamate glycosides isolated from Moringa leaves (Faizi et al., 1995). A study performed on high fat diet fed rats showed that the crude extract of Moringa leaves significantly lowered cholesterol in the serum of the rats (Jain et al., 2010). This was thought to be due to the presence of β-sitosterol, which is a bioactive phytoconstituent (Jain et al., 2010).

Ethanol extract of Moringa leaves was reported to relieve spasms, and this antispasmodic effect might be through calcium channel blockage (Gilani et al., 1992). Ethanolic Moringa extracts possess 4-[α-[L-rhamnosyloxy] benzyl]-o-methyl thiocarbamate [trans], which is responsible for the antispasmodic effect (Gilani et al., 1992). Aqueous and methanolic Moringa extracts showed an antiulcer effect (Pal et al., 1995), which demonstrates that the antiulcerogenic component is abundant in this plant. Extracts (aqueous and alcohol) from Moringa flowers were also reported to have hepatoprotective effects, which may be attributed to the presence of quercetin, a flavonoid known to have hepatoprotective activity (Gilani et al., 1997).

2.2.4 Food applications of *Moringa oleifera*

Moringa leaf powder enriched bread where Moringa leaf powder, seed or flower was incorporated in bread dough prepared from wheat flour, and the nutritional value of bread significantly improved (Chinma et al., 2014). The crude fiber and protein of the bread was reported to have increased by 56% and 54%, respectively, after fortification with 5% Moringa leaf powder (Sengev et al., 2013). Moringa seed flour was used to produce cookies that we similar in pattern and colour as the control. Protein content of the cookies with 10% and 20% Moringa seed flour increased by 45% and 90% as reported by Ogunsina et al. (2010) and were higher than that of the control.

Cheese prepared from buffalo milk was fortified with various concentrations of Moringa leaf powder. Protein content, ash, carbohydrate and fat content increased with an increase in the Moringa concentration (Salem et al., 2013). Cheese fortified with 1%, 2% and 3% Moringa leaf powder increased the protein by 3%, 5% and 8% respectively (Salem et al., 2013). Moringa leaf powder at concentrations of up to 20% were used in the preparation of wheat cake (Kolawole et al., 2013). An increase in nutritional value with an increase in Moringa leaf powder concentration was reported. However, Moringa leaf powder concentrations of above 8% showed a decrease in the acceptability of the product, which was believed to be due to the high chlorophyll content in Moringa (Kolawole et al., 2013).

2.3 *ALOE VERA*

2.3.1 History of *Aloe vera*

Aloe vera plant (Figure 2.2) originates in Tropical Africa and now is cultivated in warm climatic areas of Europe, Asia and America (Nazir and Ahsan, 2017). For decades it has been used by herbalists for the treatment of various human diseases. *Aloe vera* is a spiky cactus like xerophytes. It is a clump forming perennial plant with thick fibrous root which produces large basal leaves, which are filled with a clear, viscous gel. the leaves are usually 12-16 per plant and when mature, the plant weighs about 1.5Kg (Nazir and Ahsan, 2017).

According to Nazir and Ahsan (2017) the aloe plant matures when it is about 4 years old and has a life span of about 12 years. They continue to state that the plant can be harvested every 4 to 6 weeks by removing 3 to 4 leaves. The *Aloe vera* leaves contain amino acids, enzymes, polysaccharides, salicylic acid, minerals, anthraquinones, plant steroids and vitamins, which are all important in maintaining human health (Surjushe et al. 2008; Hossain et al. 2017).

The South African population has been utilizing *Aloe vera* plants for decades for its wound-healing properties and various other diseases (Steenkamp, 2015). *Aloe vera* is easily grown and requires less maintenance and is popular for its medicinal and nutritional properties, as well as its variety of potential uses (Steenkamp, 2015). In South Africa, this plant is cultivated in the Limpopo province and operates with a few farmers who still need assistance in expanding the industry. These farmers produce products such as aloe concentrates, aloe infusions, aloe powders as well as aloe gels (Steenkamp, 2015). They supply products to various industries including food and beverage, nutraceutical, personal care, nutritional supplements, pharmaceutical and animal health.

Figure 2.2. *Aloe vera* plant (Rajeswari et al., 2012)

2.3.2 Nutritional value of *Aloe vera*

A total of 75 potentially active constituents in aloe have been reported. These include vitamins, amino acids, minerals, anthraquinones, sugars, lignins, folic acid, sterols, salicylic acids and saponins (Pankaj et al., 2013). Aloe has been reported to provide 20 of the 22 required amino acids as well as 7 of the 8 essential amino acids. It is a good source of vitamins E, C, A, B12, B6, B3, B2, B1, choline and folic acid (Amin et al., 2018). According to Sevindik et al. (2017) vitamins act as antioxidants and scavenge free radicals. Aloe also contains a wide range of minerals such as magnesium, calcium, manganese, potassium, copper, sodium, chromium, selenium and zinc. These minerals are crucial in ensuring that enzymes function appropriately in different metabolic pathways. Aloe gel contains 8 enzymes which are bradykinase carboxypeptidase, cellulose, lipase, amylase, catalase, aliiase, alkaline phosphate and peroxidase. Bradykinase carboxypeptidase has anti-inflammatory effects, while other enzymes assist in the breaking down of fats and sugars (Pankaj et al., 2013).

2.3.3 Medicinal value of *Aloe vera*

Due to the presence of phytochemicals and nutrients, aloe is known for its health benefits and the treatment of a wide range of diseases. In this section, the effects of aloe on diseases are reviewed. Anti-cancer effects due to glycoprotein (lectins) and polysaccharides which are the two fractions from aloe that are suggested to possess anti-cancer effects (Reynolds and Dweck, 1999).

Acemannan is a major polysaccharide isolated in aloe that has been found to have anti-tumor activity. Its activity has been investigated in different animal species as well as in several in vitro models. Several studies showed aloe's anti-tumor effect through tumour shrinkage, decreased tumour burden and increased survival rates (Boudreau and Beland, 2006).

Anti-diabetic effects because aloe gel preparations in different forms (e.g. juice or in bread) showed a glucose lowering effect when numerous trials were ran in animals and humans (Reynolds and Dweck, 1999). A study was carried out where streptozotocin-induced diabetic rats orally administered aloe gel, a significant decrease in the fasting glucose, plasma and tissue cholesterol, free fatty acids, triglycerides, phospholipids and hepatic transaminases were observed (Rajasekaran et al., 2006). Findings of another study on streptozotocin-induced diabetic rats suggested that the mechanism of aloe gel glucose lowering effect is through enhancing glucose metabolism (Boudreau and Beland, 2006).

Anti-inflammatory effects due to the anti-inflammatory activity of mannose-6-phosphate, which is considered to resemble the effects seen for acetylated mannan in aloe gel. Aloe gel reduces inflammation that is induced by agents through increased infiltration of leucocytes, as well as the promotion of prostaglandin synthesis (Reynolds and Dweck, 1999). A study was carried out to investigate the efficacy of *Aloe vera* in the treatment of Helicobacter pylori-infected rats. The study showed a significant decrease in leucocyte adhesion and tumour necrosis factor α (TNF-α) levels due to the treatment with aloe. Therefore, the results suggest that aloe shows potential in the treatment of inflammatory response (Prabjone et al., 2006).

Several authors have reported that different parts of aloe as well as whole gel have anti-oxidant activities (Amin et al., 2018; Hossain et al., 2017; Pankaj et al., 2013). The anti-oxidant activity in aloe may be due to superoxidase dismutase enzymes, glutathione peroxidase activity and a phenolic anti-oxidant that were found to be present in aloe gel (Langmead et al., 2004). Aloe gel has a dose-dependent anti-oxidant effect, this was observed in two cell-free in vitro systems and by incubation with inflamed colorectal mucosal biopsies (Langmead et al., 2004).

Anti-microbial activity has been reported where several different methods have demonstrated the activity of aloe gel in both Gram-positive and Gram-negative bacteria (Habeeb et al., 2007). Isolated anthraquinones from aloe exudate have shown extensive anti-microbial activity. The anti-

microbial activity of emodin against *Escherichia coli* was believed to be facilitated by inhibition of solute transport in membranes (Alves et al., 2004).

Heart disease activity as Aloe decreases the risk of cardiovascular diseases by stimulating the fibroblasts for making new tissues. Researchers have found that when the fibroblast is stimulated, collagens and proteoglycans are formed thus reducing these risks (Nazir and Ahsan, 2017).

Aloe juice contains various enzymes that are able to speed up cell growth and repair tissue damaged by arthritis (Nazir and Ahsan, 2017). When Aloe is directly applied on to the skin, it penetrates and soothes pain therefore reducing joint and muscle pain caused by arthritis. Studies have found that daily ingestion of aloe prevents and causes a regression of adjutant arthritis (Nazir and Ahsan, 2017).

Numerous mechanisms have been suggested for the wound healing effects of aloe gel. They include promotion of epithelial cell migration, keeping the wound moist, a decrease in inflammation and rapid maturation of collagen (Reynolds and Dweck, 1999). A 5.5 kDa glycoprotein isolated from aloe showed enhanced wound healing effect and cell proliferation on hairless mice (Choi et al., 2001).

2.3.4 Uses of *Aloe vera*

Qualitative improvement of low meat beef burger using Aloe where beef burgers were produced with different concentrations of aloe (0%, 1%, 3% and 5%) and changes in their texture, lipid oxidation, cooking parameters and appeal to consumers were evaluated over 7 days of refrigerated storage (Nafiseh and Hossein, 2015). Findings of the study showed that an increase in Aloe concentration improved the texture, water absorption of the burgers and lipid stability of the burgers. The 3% aloe concentration was found to be more appealing to consumers (Nafiseh and Hossein, 2015).

Another study evaluated the potential use of Aloe gel as edible coating at different concentrations (0%, 5%, 10%, 15% and 20%) to coat tomato fruit that was then stored at 11 °C (Chrysargyris et al., 2016). Quality characteristics were evaluated up to 14 days and quality maintenance was evaluated. Results showed that the 10% concentration showed a decrease in ripening index and thus maintaining the overall quality of the tomato. The 10% concentration also showed an increase

in ascorbic acid content in the tomato, therefore can be considered as a potential treatment for maintaining tomato quality during post-harvest storage (Chrysargyris et al., 2016).

Aloe vera juice can be used in the preparation of beverages such as ready to serve drinks, soft drinks, *Aloe vera* lemon juice, health drinks, aloe sports drink with electrolytes, hangover drink with amino acids, B vitamin and acetaminophen, as well as other products such as tropical juice with *Aloe vera*, diet drink with soluble fiber, cucumber juice with *Aloe vera* and healthy vegetable juice mix have been developed (Eshun and He 2004; Grindlay and Reynolds, 1986; Hamman, 2008). Aloe concentrate prepared to a desired consistency is applied in the production of food products such as jellies, squash and jam. Aloe powder may be used in the production of ice cream, curd and yoghurt (Ahlawat and Khatkar, 2011).

2.3.5 Food applications of *Aloe vera*

Aloe vera gel is prepared from removing the outer rind of the aloe leaf and collecting the gel fillet, which is then washed with distilled water and can be incorporated in the preparation of smoothies, chewing gum, tea granules and candies (Ramachandra and Srinivasa, 2008).

Aloe vera juice is prepared by washing the aloe gel fillet with distilled water and transferring it to the pulper to extract the juice, which is then stored in the refrigerator. This juice is used for the preparation of cucumber juice with *Aloe vera*, sports drink, yogurts, alcohol, sherbet, hangover drink with B-vitamins, health drink, healthy vegetable juice mix, white bread with *Aloe vera* (Eshun and He 2004; Grindlay and Reynolds, 1986; Hamman, 2008)

Aloe vera concentrate is obtained from aloe juice which is concentrated under vacuum at temperatures below 50 °C and under 125 mm Hg vacuum (Ramachandra and Srinivasa, 2008). Aloe concentrate is used to make jellies, squash, jam and can also be incorporated in juice, tea or water (Ahlawat and khatkar, 2011).

Aloe vera powder is prepared by the dehydration method which begins by washing the aloe gel fillet with distilled water and then placed in a humidity chamber where hot air is passed over the fillet to dehydrate them, and then the dry fillet is later on ground into powder and packaged (Ramachandra and Srinivasa, 2008). Aloe powder can be used in the preparation of ice-cream, curd and yogurt.

2.3.6 Safety and toxicological aspects of *Aloe vera* products

Safety and toxicological aspects of aloe products are more focused on aloin, a constituent found in aloe plants that has a laxative effect, can also damage DNA and is a carcinogenic agent (Lachenmeier, 2005). Aloin can be prevented from entering the beverage processing by removing the outer aloe rind, which contains the highest amount of aloin, before extracting the aloe juice (Lachenmeier, 2005). According to Ahlawat and Khatkar (2011) Aloe should not be ingested during pregnancy, lactation or childhood, and by persons suffering from appendicitis, intestinal obstruction or abdominal pain. Because of possible contamination by anthraquinones, oral Aloe gel may cause symptoms of abdominal cramps and diarrhoea.

2.4 PHYTOCHEMICALS

2.4.1 Groups of phytochemicals

Phytochemicals are non-nutritive chemicals which possess disease preventative or protective properties (Abbas et al., 2014). There are three main groups of phytochemicals; Polyphenols, Terpenoids and Thiols.

2.4.1.1 Polyphenols

Phenolic compounds are a diverse, bioactive, and pervasive category of plant secondary metabolites that cover contain a vital part of the human diet and are of interest due to their biological properties (Farzaei et al., 2015). During the past decades, research has determined the health benefits of phenolic compounds. Studies have found that consuming foods rich in polyphenols may reduce the occurrence of cardiosvascular disease (McSweeney and Seetharaman, 2015), such as, colon cancer (Yang et al., 2000), liver disorders (Bose et al., 2008), obesity (Lu et al., 2012) and diabetes (Scarlbert et al., 2005). In plants, these compounds act as defense agents against physiological and environment conditions (Khurana et al., 2013).

Polyphenols are subcategorized as the flavonoids, phenolic acids and other non-flavonoid polyphenols (CANCERNETUK, 2016). Flavonoids make up the largest class of phytochemicals. They play a big role in decreasing disease risk through various physiological mechanisms. Some of these include antiviral, cytotoxic, anti-inflammatory, antioxidant and antimicrobial effects (Thiede and Zidenberg-Cherr, 2016).

Quercetin, catechins, kaempferol, rutin, resveratrol and their derivatives are flavonoids recognized for their excellent health benefits, including the prevention of certain human diseases, especially cancer, diabetes, liver disorder, obesity, infectious diseases and cardiovascular diseases (Farzaei et al., 2015). Fruits such as apples, apricots, pears, grape, berries, and cherries, and vegetables such as tomato, carrot, garlic, celery, cabbage and onion can be used as dietary supplements of phenolic compounds (Pandey and Rizvi, 2009). This is because they contain up to 200-300 mg of phenolic compounds per 100 g of their fresh weight (Pandey and Rizvi, 2009). The presence of phenolic compounds has improved the quality of foods, and these compounds have a low toxicity in the human body, which makes them a safe dietary component. Due to their antioxidant properties, phenolic compounds play an important role in the oxidative stability of foods (Rasouli et al., 2017).

2.4.1.1.1 Quercetin

Quercetin (Figure 2.3) is a flavonoid that has been promoted as an excellent antioxidant in many studies (Chen et al., 2016) It can prevent cardiovascular diseases (Guillermo et al., 2015), has antiulcer, anti-proliferative and antiallergy effects (Al-Jabban et al., 2015), fights influenza A virus (Vaidya et al., 2016)], regulates gene expression (Snyder et al., 2016)] and also possesses anti-inflammatory properties (Hisanaga et al., 2016).

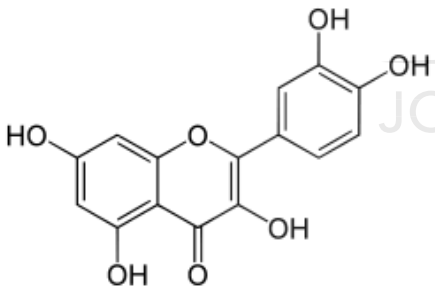

Figure 2.3. Chemical structure of quercetin (Weston and Mathesius, 2013)

2.4.1.1.2 Catechins

Catechins (Figure 2.4) are a group of phytochemicals mostly found in leaves of *Camellia sinensis* (Clouth and Schofer, 2015). Some of the main sources of catechins include green tea, pears, apples, cherries and grapes (Kondo et al., 2002).

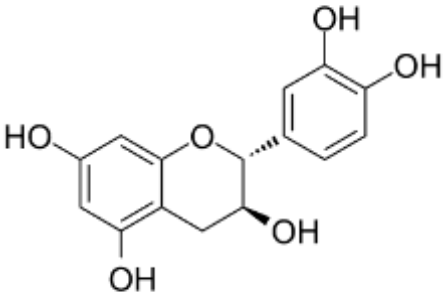

Figure 2.4. Chemical structure of catechin (Bernatoniene and Kopustinskiene, 2018)

According to Rasouli et al. (2016) cardiovascular diseases, cancer and aging are the top three illnesses that are treated with catechins.

2.4.1.1.3 Kaempferol

Kaempferol (Figure 2.5) is a type of phenolic compound found in fruits and vegetables (Calderon et al., 2011). Kaempferol is among the most common flavonol, including quercetin found in foods. The health benefit of kaempferol includes decreasing the incidence of chronic diseases, especially cancer (Huang et al., 2010).

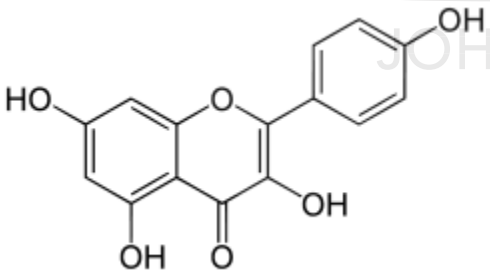

Figure 2.5. Chemical structure of kaempferol (Weston and Mathesius, 2013)

According to studies, there is a potential link between the consumption of foods rich in kaempferol and the reduction of the incidence of developing cardiovascular diseases (Tang et al., 2015), inflammation (Garcia-Mediavilla et al., 2007), obesity and type 2 diabetes (Zang et al., 2015).

2.4.1.1.4 Rutin

Rutin (Figure 2.6), also known as vitamin P is a flavonoid that is part of people's daily diet (Erlund et al., 2000).

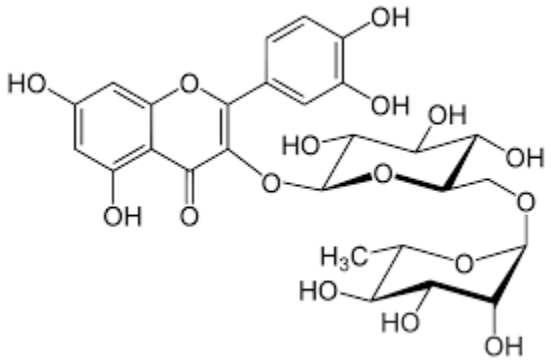

Figure 2.6. Chemical structure of rutin (Georgeta et al., 2016)

Cancer, hypercholesterolemia and hypertension are the three top diseases that can be treated with rutin (Rasouli et al., 2017). Rutin also possesses antioxidant activity (Rasouli et al., 2017).

2.4.1.2 Terpenoids

Terpenoids are the largest group of plant secondary metabolites and are highly diverse in chemical structure (Yazaki et al., 2017). They can be sub-categorized as carotenoids and non-carotenoid terpenoids.

Terpenoids are of great interest due of their ability to prevent cancer, antimicrobial, antiviral, antiparasitic, antifungal and anti-inflammatory effects (Singh and Sharma, 2015). Studies in the past couple of decades have established that terpenes exert anti-inflammatory effects by preventing pro-inflammatory pathways in skin inflammation, obstructive pulmonary disease, ear edema, osteoarthritis and bronchitis (Rufino et al., 2014). Terpenes have also been reported to exert anti-tumorigenic effects, thus proving their potential use for treating tumors by acting as chemotherapeutic agents (Cho et al., 2017)

2.4.1.2.1 Carotenoids

Carotenoids are a class of terpenoids that have various health benefits (Table 2.7). According to Toti et al. (2018) they strengthen the immune system, reduce the risk of degenerative diseases, possess anti-obesity activities and also has antioxidant properties. Types of carotenoids include alpha, beta and gamma carotene, Lutein, Zeaxanthin, Lycopene and Astaxanthin. Carotenoids that

are precursors of vitamin A and non-precursors, such as lycopene, zeaxanthin and lutein, have protective action against cancer (Toti et al., 2018)

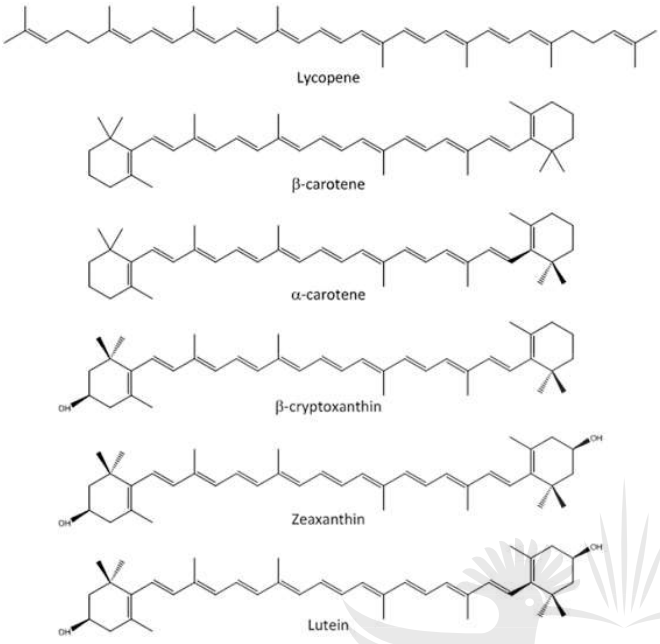

Figure 2.7. Chemical structures of carotenoids (Eldahshan and Singab, 2013)

Clinical and epidemiological studies have established and confirmed that consumption of lycopene-rich diets is associated with a decrease in the risk of developing ovary, lung and prostate cancers, as well as a reduced incidence of cardiovascular diseases and chronic degenerative diseases (Toti et al., 2018).

2.4.1.3 Thiols

This group of phytochemicals includes glucosinolates and indoles.

2.4.1.3.1 Glucosinolates

Glucosinolates are secondary plant metabolites that are popular due to their potential chemopreventative activity (Figure 2.8) (Possenti et al., 2016). More than 120 different glucosinolates have been recognized in plants and a few of these compounds have been considered for the potential use of their metabolic breakdown products as anticarcinogenic agents (Possenti et al., 2016).

Glucosinolates are generally found in vegetables belonging to the Brassicaceae family but can also be found in other plant species such as Moringa, capers and papaya (Possenti et al., 2016).

Glucosinolates have been found to possess benefits that improve health. This includes prevention of cancer incidence and progression and cardiovascular diseases (Possenti et al., 2016).

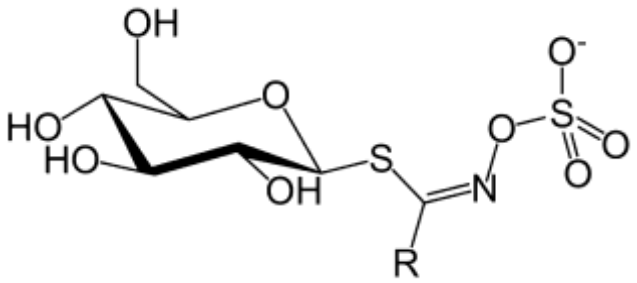

Figure 2.8. General chemical structure of glucosinolate (Fahey et al., 2001)

According to recent studies, indoles and glucosinolates may induce the removal of carcinogens, limit the production of hormones related to cancer, prevent growth of tumors and block carcinogens (Possenti et al., 2016). Isothiocyanates are a type of glucosinolate, and they induce the removal of carcinogens, inhibits tumor growth and works as an antioxidant (Possenti et al., 2016).

2.4.2 Phytochemicals in *Moringa oleifera* and *aloe vera* leaves

Moringa oleifera contains phytochemicals such as flavonoids, phenolic acids, glucosinolates (Amaglo et al., 2010; Coppin et al., 2013) carotenoids and tocopherols (Saini et al., 2014). A number of studies have reported that flavonoids are the main phenolic compounds in *M. oleifera* leaves. The dominant flavonoids appear to be quercetin, kaempferol, apigenin, leteolin and myricetin glycosides (Rodriguez-Perez et al 2015; Castro-Lopez et al., 2017). In another study that investigated phenolic compounds in seven *M. oleifera* cultivars, the major flavonoids identified included quercetin (47.0%), kaempferol (30.0%) and apigenin (20.0%). Elsewhere, quercetin (46.0%), kaempferol (34.0%) and apigenin derivatives (7.7%) were reported as the dominant flavonoids in *M. oleifera* leaves (Rodriguez-Perez et al 2015).

Apart from flavonoids, phenolic acids have been reported in *M. oleifera* leaves at a concentration of approximately 77 – 187 µg per gram dry matter depending on cultivar (Nouman et al., 2016). Caffeoylquinic (45.5% of total phenolic acids) and coumaroylquinic acids (36.4% of total phenolic acids) were reported to be the main phenolic acids in *M. oleifera* leaves in another study (Amaglo

et al 2010). Other authors reported hydroxybenzoic acids (gallic acid and p-hydroxybenzoic acid) as the main phenolic acids in *M. oleifera* leaves (Juhaimi et al., 2017). The differences in the reported phytochemical levels can be attributed to differences in cultivar, extraction solvents, age of the plant and varying climatic and soil conditions (Radha et al., 2014).

Similar to *M. oleifera*, flavonoids have also been identified in *A. vera* extracts (Arunkumar and Muthuselvam, 2009). Flavonoids are thought to be largely responsible for the overall radical scavenging activity in *A. vera*. *Aloe barbadensis* and *Aloe arborescenes* contain about 52 and 93 mg RE/ 100 g flavonoids, respectively (Lucini et al., 2015). Manimegalai and Nithya (2015) reported the presence on alkaloids, flavonoids, steroids, terpenoids, anthraquinone, quinone, tannin and saponin in *A. vera* leaf powder extracted with different solvents. In another study on phytochemical constituents and in vitro radical scavenging activity of different Aloe species, Lucini et al. (2015) reported the presence of hydrocynnamic acids, anthrones and chromones. In addition, these workers identified the phenolic dimer feralolide and flavonoids such as flavones and isoflavones in *A. vera* leaf powder.

It is worth mentioning that the health benefits of *A. vera* cannot be solely ascribed to the presence of phytochemical compounds. Several researchers have highlighted the importance of other biological compounds such as anthraquinones, saccharides, vitamins, enzymes and low molecular weight substances (Christaki and Florou-Paneri, 2010).

2.4.3 Importance of phytochemical and antioxidant activity in foods

Plants consist of non-nutritive chemicals known as phytochemicals which possess disease preventative or protective properties (Abbas et al., 2014). Plants use these phytochemicals for their own protection from deterioration and microbial infection (Abbas et al., 2014). Phytochemicals are also known to possess antioxidant properties. Antioxidants, as well as natural or synthetic food preservatives prevent food from oxidative deterioration on processing and storage (Wilson et al., 2017). Due to their high stability, antioxidants help to maintain the colour, level of nutrients, texture, taste, freshness, aroma, functionality and appeal of the food products to customers (Wilson et al., 2017). These antioxidants prolong shelf-life of products by neutralizing free radicals in foods.

2.5 EFFECTS OF THERMAL TREATMENT ON THE QUALITY OF BEVERAGES

Pasteurization is a thermal processing technique used for shelf-life stability of food and beverages. The thermal processing techniques commonly used are Low temperature long temperature and high temperature short time (Rupasinghe and Juan Yu, 2012). Antioxidant activity in beverages is reduced by high temperature long time treatment (Bansal et al., 2015; Chen et al., 2015). Other studies have shown that ascorbic acid in blended beverages is reduced by high temperature short time treatment (Mena et al., 2013). Carotenoids in beverages are said to be reduced when subjected to high temperature short time treatment (Jimenez-Aguilar et al., 2015) together with total phenolic content (Ucan et al., 2016). Mild temperature long time treatment reportedly reduces flavonoid content in beverages (Saikia et al., 2015). The protein content and total soluble solids of beverage is reduced by high temperature long time treatment (Deboni et al., 2014; Khandpur and Gogate, 2015). Overall, most studies have indicated that the quality of beverages is reduced by high temperature long time treatment (Santhirasegaram et al., 2015).

2.6 CONSUMER ACCEPTABILITY OF PLANT-BASED FUNCTIONAL BEVERAGES

Otu et al. (2013a) carried out a consumer acceptance test for Moringa beverage where they recruited 60 untrained consumers who tested for colour, taste, flavour and overall acceptability using the 9-point hedonic rating scale with 9 and 1 representing the highest and lowest scores, respectively. To optimize the formulation of the Moringa beverage and improve acceptability, pineapple juice and carrot extract were added. Results showed that an increase in Moringa extract levels yielded a formulation that was less preferred by consumers, while the formulation with high level of Pineapple juice was more preferred. The researchers were then able to obtain a highly acceptable formulation ratio of 50:38:12 for Moringa: Pineapple: Carrot, respectively.

Aderinola (2018) developed smoothies made from a blend of banana, apple and pineapple fortified with Moringa leaves at 1.5%, 3% and 3.5% with 0% Moringa product as control. Sensory evaluation was conducted using 20 trained panelists using the 9-point hedonic scale. Panelists tested for flavour, taste, mouthfeel, appearance (colour) and overall acceptability. The control sample was more preferred in all attributes than samples fortified with Moringa leaves. The bitter taste of the Moringa leaves negatively impacted scoring, as well as the greenish colour of the fortified smoothies which was due to the presence of Moringa. As a result, the panelists preferred the white creamy appearance of the control sample. Overall, despite the additional nutrients

obtained from the addition of Moringa leaves in the smoothies, panelists preferred the control sample.

Ramachandran and Nagarajan (2014) developed an aloe gel-papaya functional beverage where the blends were 0% aloe gel, 10% aloe gel, 20% aloe gel and 30% aloe gel incorporated in the papaya beverage. Sensory acceptability was carried out where 20 untrained panelists evaluated the samples for colour, flavour, taste, consistency and overall acceptability on a 5-point hedonic rating scale (Scale: 1 - dislike extremely; 2 - dislike slightly; 3 - neither like nor dislike; 4 - like slightly; 5 - like extremely). The blend with 30% Aloe gel was found to be more acceptable for colour with a value of 4.78 compared to the control (0% aloe gel) with a value of 4.57. The highly sweet taste rendered the control less preferable and the 30% aloe sample was the highest acceptable for taste and flavour. Lower scores for consistency were obtained for the aloe-gel enriched samples which was said to be due to the samples' thin consistency. The overall acceptability identified the 20% and 30% blends to be highly acceptable with values of 4.64 and 4.71, respectively.

Talib et al. (2016) developed an aloe-based soft drink which blended aloe and pear at different levels. Four formulations were developed; 100% pear (control), 10% aloe, 20% aloe and 30% aloe. Sensory acceptability of the formulations was carried out by 10 semi-trained panelists using the 9-point hedonic scale where they rated for colour, taste, flavour, appearance and overall acceptability. An increase in aloe levels turned the drink to a faint colour, therefore the control drink was scored significantly higher (7.08) for colour compared to other formulations. Scores for flavour (7.22), taste (7.12) and appearance of the 20% aloe formulation was similar to that of the control. Similarly, the highest score (7.1) for overall acceptability was seen with the 20% aloe formulation compared to all the other samples.

2.7 STORAGE STABILITY OF PLANT-BASED FUNCTIONAL BEVERAGES

It is essential to develop a beverage that will be stable and safe for consumption over long periods of time. However, various chemical, colour and microbial changes occur within beverages upon storage (Touati et al., 2016). Vitamin C or ascorbic acid is a highly sensitive vitamin that degrades over time. Several researchers have reported a loss in significant amounts of vitamin C upon storage of their plant-based beverages. Singh et al. (2017) and Talib et al. (2016) reported vitamin C losses of 61 % and 70 % in aloe-based beverages, respectively. The loss in vitamin C was

attributed to the high levels of aloe in the beverage which caused the beverage to be more perishable (Talib et al., 2016).

An increase in pH upon storage is common in beverages and such increases lead to a loss of acidity in the beverage (Bhardwaj and Pandey, 2011). The loss in acidity is reported to be due to acid hydrolysis of sugars and polysaccharides due to enzyme invertase that convert non-reducing sugars to reducing sugars (Bhardwaj and Pandey, 2011) as well as converting acids into salts (Bhardwaj and Mukherjee, 2012). Total soluble solids (TSS) refer to the sugar content of a solution and are measured using a refractometer and reported as °Brix (Magwaza and Opara, 2015). Researchers reported a slight increase in the TSS of their beverages upon storage (Hossain et al., 2017; Talib et al., 2016). The increase in TSS was thought to be due to acid hydrolysis of polysaccharides in the beverages (Hossain et al., 2017; Talib et al., 2016). It is believed that a slight increase in TSS over storage is essential in maintaining the quality of the beverage (Bhardwaj and Pandey, 2011).

Plants and plant-based beverages contain compounds such as phytochemicals that degrade upon storage and cause colour changes (Pourcel et al., 2006). Phytochemicals such as flavonoids are known to oxidise, which causes a brown pigment to plants and plant-based beverages and the oxidation reaction is catalyzed by peroxidases and oxidases (Pourcel et al., 2006). Plants have high levels of chlorophyll. In plant-based beverages, chlorophyll deteriorates upon storage which leads to phenophytinisation which is the degradation of the green pigment that leads to the formation of an olive/brown pigment (Dauthy, 1995). Heat and acid are responsible for accelerating the deterioration of chlorophyll (Dauthy, 1995). Anthocyanins are water-soluble reddish pigments that are very common in plants and plant-based beverages (Dauthy, 1995). Anthocyanins are pH-dependent and deterioration is greater in high pH environments (Landi, 2015). Anthocyanins have the ability to form metals such as Cu, Fe, Sn and Al when beverages are stored in metal packaging like cans (Landi, 2015). Therefore, it is important to coat cans with organic linings to prevent the reaction between anthocyanins and metal. Carotenoids include many lipid-soluble compounds and are responsible for red and yellow pigments in plant-based products (Dauthy, 1995). The pigments degrade due to oxidation and can sometimes auto-oxidise when they react with oxygen (Dauthy, 1995). The rate of this reaction is dependent on the presence of antioxidants and prooxidants, heat and light (Dauthy, 1995).

Bacteria and Fungi (consisting of yeast and moulds) are the two major groups found in food and beverages. Generally, bacteria are the quickest developing and even in favourable conditions to both, bacteria will outgrow and exceed fungi (Dauthy, 1995). For microorganisms to cause spoilage, they are influenced by intrinsic and extrinsic factors. Intrinsic factors include water activity, pH, nutrient content, biological structures and antimicrobial constituents of the product (Moral et al., 2017). Extrinsic factors include relative humidity, temperature, gas compositions and relative humidity of the storage environment (Moral et al., 2017). Spoilage of plant-based beverages can be expected to be caused by aciduric bacteria and fermentative yeasts and moulds due to the low pH of these beverages. Otu et. al. (2013b) reported no microbial growth throughout the storage time of Moringa beverage. Similar results were obtained by Talib et al. (2013) and Singh et al. (2013) for their aloe-based beverages. This was due to the preservation techniques they had implemented that included the addition of acids in order to lower the pH of the beverages, addition of preservatives and pasteurization.

2.8 COMBINING MORINGA OLEIFERA AND ALOE VERA IN A BEVERAGE

Moringa and aloe plants have been used to develop functional beverages, however, the idea where the plants are used in combination has not yet been studied. It is important to address whether combining the two medicinal plants in a beverage will increase the products' medicinal and nutritional value. Also, answering questions as to whether the products will be acceptable to consumers and will the shelf-life be affected negatively or positively.

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CHAPTER 3

NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION OF MORINGA-ALOE BEVERAGE BLENDS

ABSTRACT

The aim of the current research was to develop a functional beverage with acceptable nutritional and health promoting attributes by blending *Moringa oleifera* and *A*loe *vera*. Moringa-aloe beverage blends were prepared at 30:70, 50:50, 70:30, 100:0, 0:100, % Moringa: Aloe, respectively. Then, pH, titratable acidity (TA), total soluble solids (TSS), vitamin C, protein, minerals, colour, total phenolic content and total flavonoid content were determined. Results showed that pH ranged from 3.66 - 4.15 with the least and highest pH obtained with 100 % Aloe (100A) and 100% Moringa (100M) beverages, respectively. The TA of the beverages ranged from 0.14 - 0.12 %, while total soluble solids ranged from 0.5-0.8 °Brix. Beverage blends with high Aloe concentration contained high levels of vitamins C due to the high level of the vitamin in Aloe. The highest (343.60 µg/ml) protein content was observed with 100M beverage and the least (74.60 µg/ml) with the 100A beverage. In addition, the concentration of Ca, K, Mg in the beverage blends increased with increase in the concentration of Moringa. The TPC and TFC of the beverage blends improved with increase in Moringa concentration and so did the antioxidant activity when determined with DPPH assay. However, the beverage blends have similar antioxidant activity when with ABTS. Overall, the current study confirms that beverage blends with Moringa have high nutritional quality and possess health promoting properties.

Keywords: *Moringa oleifera*, *Aloe vera*, nutritional properties, phytochemicals, beverages blends

3.1 INTRODUCTION

Moringa oleifera (*M. oleifera*) is a medicinal plant belonging to Moringaceae family and is utilized as a high nutritional supplement for good health, immune booster, food security, livestock feeding and water purification (Mabapa, et al., 2017). This species of Moringa originates in India but has now spread to the tropics and subtropics all over the world (Enwa et al., 2013). Almost all the parts of Moringa plant (flowers, leaves, roots, seeds and bark) can be utilised for therapeutic and medicinal purposes as well as food (Enwa et al., 2013). The *M. oleifera* tree is high in nutrients and phytochemicals (Ashfaq et al., 2012). Dried leaves of *Moringa oleifera* provide four times the amount of carotene as carrots, while fresh leaves contain seven times the amount of vitamin C as oranges (Huber et al 2017; Dhakar et al. 2011). Although there is variation in values, studies are in agreement that dried M. oleifera leaves contain more iron than spinach, more calcium than milk, and more potassium than bananas (Dhakar et al., 2011; Gopalakrishnan et al., 2016).

M. oleifera contains phytochemicals such as flavonoids, phenolic acids, glucosinolates (Amaglo et al., 2010; Coppin et al., 2013) carotenoids and tocopherols (Saini et al., 2014). A number of studies have reported that flavonoids are the main phenolic compounds in *M. oleifera* leaves. The dominant flavonoids appear to be quercetin, kaempferol, apigenin, leteolin and myricetin glycosides (Rodriguez-Perez et al 2015; Castro-Lopez et al., 2017). The antioxidants and polyphenols in *M. oleifera* leaves have been reported to reduce tissue damages taking place during physiological processes (Shanmugavel et al., 2018). They are also involved in several biological activities such as prevention of liver disease, prevention of cardiovascular diseases, anticancer activities (Shanmugavel et al., 2018; Pari and Kumar, 2002) nervous disorder, skin disorders, antitumor, regulation of thyroid status and inflammation digestive disorders (Shanmugavel et al., 2018).

Aloe vera (*A.vera*) is a spiky cactus like xerophytes perennial plant with thick fibrous root and large basal leaves that are filled with a clear, viscous gel (Nazir and Ahsan, 2017). It originates in tropical Africa but now cultivated in warm climatic areas of Europe, Asia and America (Nazir and Ahsan, 2017). For decades, Aloe has been used by herbalists for the treatment of various human diseases. The *A. vera* leaves contain amino acids, enzymes, polysaccharides, salicylic acid, minerals, anthraquinones, plant steroids and vitamins, which are all important in maintaining human health (Surjushe et al. 2008). *A. vera* gel contains many vitamins including important

antioxidant vitamins, A, C and E. Vitamin B1 (thiamine), vitamin B2 (riboflavin), niacin, choline and folic acid are also present (Nazir and Ahsan 2017). *A. vera* juice also contains several enzymes, minerals, sugars, anthraquinones, fatty acids, hormones, 20-22 amino acids and 7 of the 8 essential amino acids (Kar and Bera, 2018).

The juice derived from the *A. vera* plant enables the body to heal itself from cancer (Nazir and Ahsan, 2017). It acts as radiation protectors by inhibiting and healing the damage caused by radio and chemotheraphy on healthy immune cells. This juice also contains various enzymes that are able to speed up cell growth and repair tissue damaged by arthritis, as well as enhancing digestive functioning which then improves the absorption of nutrients and maintains a balance in the blood sugar level (Nazir and Ahsan, 2017). *A. vera* has been used as a resource of functional foods such as yogurt and for the preparation of health drinks including tea (Christaki and Florou-Paneri, 2010). In addition, *A. vera* gel can also be used as an edible coating to prolong the safety and quality of fresh foods (Christaki and Florou-Paneri, 2010).

Similar to *M. oleifera*, flavonoids have also been identified in *A. vera* extracts (Arunkumar and Muthuselvam, 2009). Flavonoids are thought to be largely responsible for the overall radical scavenging activity in *A. vera*. *Aloe barbadensis* and *Aloe arborescenes* contain about 52 and 93 mg RE/ 100 g flavonoids, respectively (Lucini et al., 2015). Manimegalai and Nithya (2015) reported the presence on alkaloids, flavonoids, steroids, terpenoids, anthraquinone, quinone, tannin and saponin in *A. vera* leaf powder extracted with different solvents.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Moringa oleifera fresh leaves were sourced from a farmer in Limpopo province, South Africa. The leaves were washed in cold water and freeze-dried in less than 24 h after collection. The dried plant material was stored in zip-lock bags at room temperature until further use. *Aloe vera* concentrate (food grade) was purchased from a local supplier in Limpopo province. Preservatives (sodium benzoate) and artificial sweeter (stevia), citric acid and xanthan gum were purchased from Sigma Aldrich, Johannesburg, South Africa.

3.2.2 Preparation of Moringa-aloe beverage blends

Dried *M. oleifera* leaves were pulverised using pestle and mortar followed by extraction in distilled water at 62 °C for 30 min. The extract was then filtered through a cheese cloth to obtain a clear extract. Thereafter, it was centrifuged at 3000 rpm for 10 min. Aloe juice was prepared by mixing water and aloe concentrate at a ratio of 9:1. The Moringa extract was then mixed with Aloe juice per the formulations (Table 3.1). Citric acid (Merck, Modderfontein, South Africa), xanthan gum (Sigma, South Africa), sodium benzoate (Merck, South Africa) and stevia were added to the mixture. The beverages were processed following the procedure outlined in Figure 3.1 below which included product pasteurization at 90 °C for 10 min and rapid cooling of the product to approx. 20°C and refrigeration at 4 °C.

Table 3.1. Blend ratios of Moringa-aloe beverage blends

Treatment name	Blend ratio
30M: 70A	30% Moringa : 70% Aloe
50M: 50A	50% Moringa : 50% Aloe
70M: 30M	70% Moringa : 30% Aloe
100M	100% Moringa
100A	100% Aloe

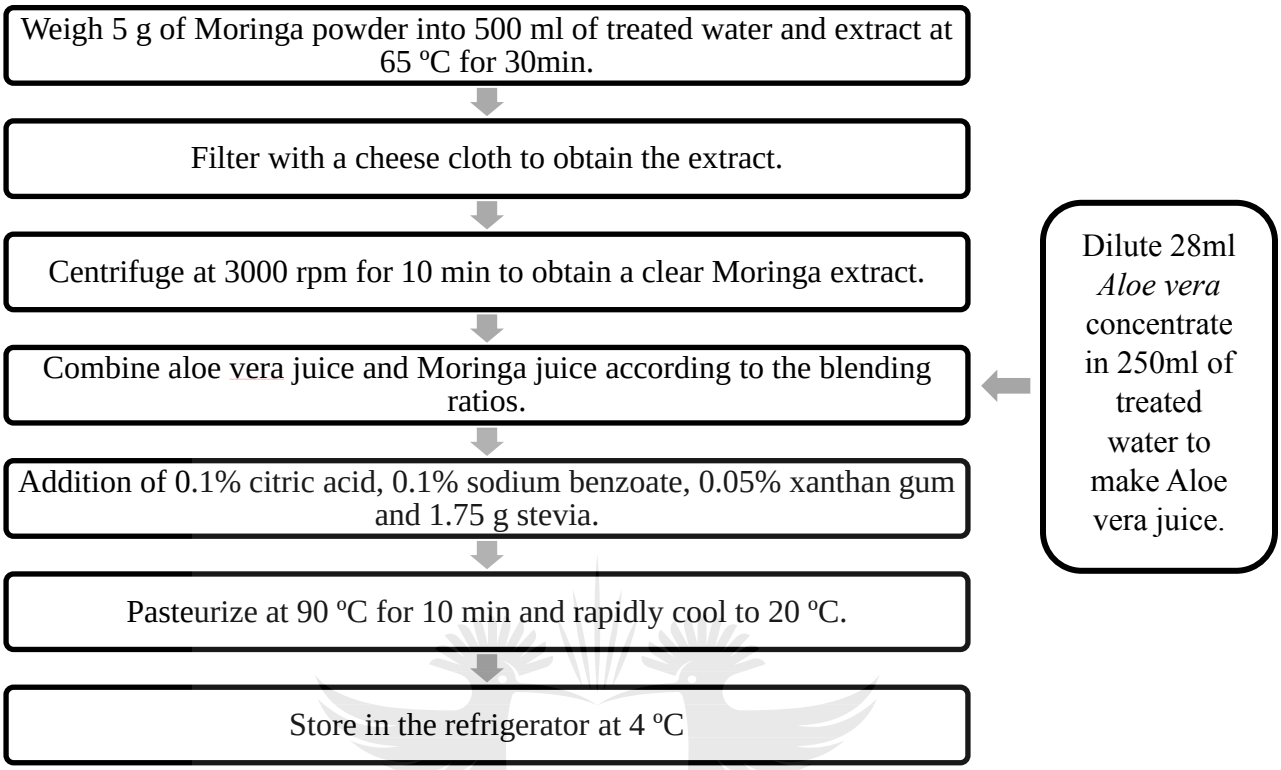

Figure 3.1 Manufacturing process of Moringa-aloe beverage

3.2.3 Quality characteristics of the developed beverage

The proximate composition of the developed beverage was determined in triplicate according to the AOAC (2010) methods. pH was determined with a pH meter, titratable acidity was measured by a titration method where 25ml distilled water was added to a 10ml sample, followed by the addition of phenolphthalein indicator (Sigma-aldrich, United Kingdom). The mixture was titrated against 0.1M Sodium hydroxide (NaOH; Sigma-Aldrich, Sweden) to a faint pink end point. Results were expressed as % citric acid. The total soluble solids content was determined by measuring the °Brix (Handheld Refractometer Atago ATC-IE, Tokyo, Japan) at 20 °C (Wang et al., 2005) based on the official AOAC Official Method 932.12 (AOAC International, 1932). The results were expressed as °Brix. The colour of the samples were measured using a Chroma colour meter (Konica Minolta, Toyko, Japan) and the results expressed as *L*, *a*, *b* values. The mineral content was determined using ICP-AES as outlined by Fernandez-Caceres et al. (2001).

3.2.3.1 Vitamin C Content

The vitamin C content was carried out using a redox titration method (using iodate solution) reported by the University of Cantebury (2014). Briefly, 20 ml sample was added to a conical flask containing 150 ml distilled water, followed by the addition of 5 ml 0.6M potassium iodide (Sigma-Aldrich, Chile), 5 ml 1M hydrochloric acid (Sigma-Aldrich, Austria) and 1 ml 0.5% starch indicator solution (Merck, South Africa). The mixture was titrated against 0.002 M potassium iodate (Merck, South Africa) to a permanent dark blue-black endpoint. The vitamin C content was expressed as mg/100ml ascorbic acid per 100ml aqueous extract.

3.2.3.2 Protein Content

The protein analysis was carried out using a Genesys 20 spectrophotometer (Thermo Fisher Scientific) following the procedure by Lowry et al. (1951). Briefly, 0.2 ml sample was added to a tube containing 0.8 ml distilled water, followed by addition of 5 ml of reagent C (alkaline copper solution) (Associated chemical enterprises, South Africa). The mixture was incubated at room temperature (approx. 22°C) for 10 min. After incubation, 0.5 ml of reagent D (Folin-Ciocalteau solution) (Sigma-Aldrich, Switzerland) was added into the tube, followed by incubation at room temperature for 30 min. Bovine serum albumin (BSA) was used as a standard and absorbance was measured at 660 nm using a spectrophotometer. The protein content was expressed in µg/ml aqueous extract.

3.2.3.3 Total Phenolic Content (TPC)

The total phenolic content was carried out using a spectrophotometer following the procedure reported in previous literature (Fernandes et al., 2015). Briefly, 10 µL sample was added to a test tube containing 500 µl of distilled water followed by addition of 50 µl of folin-ciocalteu reagent (Sigma-Aldrich, Switzerland). After that, 200 µl of 7.5% sodium carbonate (Sigma-Aldrich, India) was added and contents mixed. The reaction mixture was then allowed to stand in the dark for 30 minutes at room temperature (Gong et al., 2019). Gallic acid (Sigma-Aldrich, China) was used as standard and the absorbance was measured at 765 nm using spectrophotometer. The total phenolic content was expressed as mg/L gallic acid equivalent (GAE) of aqueous extract (Al-Owaisi et al., 2014).

3.2.3.4 Total Flavonoid Content (TFC)

Total flavonoid content was determined by the Aluminium Chloride ($AlCl_3$; Sigma-Aldrich, USA) method illustrated by Stankovic (2011). Briefly, 30 µl sample was mixed with 20 µl Sodium Nitrite ($NaNO_2$; Sigma-Aldrich, Germany). The mixture was allowed to stand for 5 min followed by addition of 20 µl of $AlCl_3$ and 100 µl NaOH (Sigma-Aldrich, Sweden) to make a volume of 170 µl. The absorbance was read with the iMark microplate absorbance reader (Bio-Rad laboratories 168-1130) at 450 nm and quercetin (Sigma-Aldrich, India) was used as standard (0-1.0 mg/L). The concentration of flavonoids was expressed as quercetin equivalent (mg quercetin/ L of extract).

3.2.3.5 Determination of antioxidants activity using 2, 2 -diphenyl-1-picrylhydrazyl (DPPH) assay

The ability of the extract to scavenge DPPH was determined as described in previous studies (Stankovic, 2011). In short, DPPH solution (0.1Mm) was prepared by dissolving 1.9 mg of DPPH (Sisco Research Laboratories, India) in 100 ml of methanol (Sigma-Aldrich, Germany) and the mixture was kept in the dark for 30 min. Ten milliliter (10ml) of the prepared DPPH solution was transferred into measuring cylinder and filled up to the 50 ml mark with methanol. The absorbance was measured at 490 nm and this solution was treated as blank. Trolox standard (Sigma-Aldrich, Russia) was prepared by weighing 25 mg of trolox into a 100 ml volumetric flask and filled up to mark with methanol. Trolox standard curve was prepared between 0 and 1000 concentration (mg/ml). The plant extract 40 µL was mixed with 160 µl of the DPPH solution and the resulting solution was shaken in dark for 10 min. The absorbance of the colored products was read at 490 nm and the results expressed in TE µM /L aqueous extract. The lower the absorbance of the reaction mix the higher free scavenging activity.

3.2.3.6 Determination of antioxidants activity using 2, 2-azionobiz 3-ethylbenzothiazoline–6-sulfonic acid (ABTS) assay

With the ABTS assay, a solution of 7 mM ABTS (Sigma-Aldrich, China) was prepared by adding 8 mg of ABTS in 1 ml distilled water and this solution was labelled A. Solution B (2.45 mM Potassium sulfate) was prepared by adding 13.2 mg potassium persulfate (Sisco Research Laboratories, India) in 10 ml distilled water. Equal amounts of solution A and B (1mL) were mixed and incubated for 12 h in the dark at room temperature. The prepared solution A+B mixture was used within 16 h. For analysis, the solution was diluted with methanol to an absorbance of 0.7

± 0.01 at 750 nm. Standard/plant extract (20 µl) was mixed with 180 µl of ABTS free radical cation solution. The mixture was then incubated in the dark for 5 min and the coloured product was read at 750 nm. Trolox (Sigma-Aldrich, Russia) was used as a reference compound to prepare a standard curve between 0 and 1000 concentration (mg/ml). Results were expressed in µM TE/L aqueous extract.

3.2.4 Statistical analysis

Analysis of variance (ANOVA) was used calculate differences between the means using SPSS statistical software version 25 (SPSS/IBM, Chicago, Illinois). The Duncan's Multiple range test was used to determine significant differences between means ($p<0.05$). The results were expressed as means ± standard deviation. All experiments were carried out in triplicate and duplicated at least twice.

3.3 RESULTS AND DISCUSSION

3.3.1 pH, Titratable acidity (TA) and total soluble solids (TSS) of Moringa-aloe beverage blends.

Table 3.2 shows the pH and TA values of the Moringa-aloe beverages and blends. While both methods measure acid, TA measures total acid concentration contained within a food, whereas pH quantifies the concentration of free hydronium ions (H_3O^+) within an aqueous solution (Nielsen, 2010). The highest pH value (4.15) was observed with 100% Moringa (100M) beverage and the lowest (3.66) with the 100% Aloe (100A) beverage. However, there was no statistical difference ($p>0.05$) between the pH values of the 100M beverage and blends with high Moringa (50M: 50A and 70M: 30A). A trend was observed where an increase in Moringa concentration led to an increase in pH. This might be due to Moringa being rich in minerals, thus causing the beverages to be highly alkaline (Arsenault, 2017). The pH values of this study are in accordance to those found by Biswas et al. (2016) who reported pH values between 3.85 and 3.96 for Aloe-pineapple juice. According to Dissanayake (2017), the pH of a beverage should be less than 4.2 in order to prevent microbial growth and increase shelf-life. Since all the beverages in the current study had pH less than 4.2, it can be expected that microbial growth will be limited and subsequently, the beverages will have an extended shelf-life.

There was no significant difference between the TA values of the beverages and blends (Table 3.2). This is in contrast to other reports that indicated that a decrease in pH correlates with an increase in TA (Aftab et al., 2016). The possible reason for this could be that the change in pH of beverage blends studied here was too low to cause changes in TA. Despite this, the TA results of all the beverages and blends were in close agreement with the results reported by Aftab et al. (2016).

Table 3.2. pH, Titratable acidity and Total Soluble solids of Moringa-aloe beverage blends

Beverage	**pH**	**TA**	**TSS (°Brix)**
30M: 70A	3.82 ± 0.03^{ab}	0.13 ± 0.01^{a}	0.6 ± 0.00
50M: 50A	3.94 ± 0.01^{bc}	0.13 ± 0.01^{a}	0.7 ± 0.00
70M: 30A	4.01 ± 0.14^{c}	0.12 ± 0.02^{a}	0.8 ± 0.00
100M	4.15 ± 0.01^{c}	0.12 ± 0.01^{a}	0.8 ± 0.00
100A	3.66 ± 0.01^{a}	0.14 ± 0.01^{a}	0.5 ± 0.00

TA (g/100 mL). Data presented as mean±SD (n=3). Within each column, values not sharing the same superscript letter are significantly different from one another ($P<0.05$). 30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe.

The TSS of the beverages and blends ranged from 0.5 to 0.8. The highest value (0.8 °Brix) was observed for 100M beverage and 70M blend, and the least (0.5 °Brix) in the 100A beverage (Table 3.2). The blends TSS increased with an increase in Moringa concentration. This finding may be as a result of dissolved solids from the Moringa extract, which increased the amount of solids in the blends (Fahey, 2005). The TSS in this study were lower than those found by Aftab et al. (2016) who developed a similar product. This could be due to the 70% °Brix sugar syrup used by the researchers in their product, which thus increased the products TSS. Talib et al. (2016) reported TSS of 1 °Brix for Aloe juice, which is a bit higher than those reported in the present study. High TSS of a food product means better taste of the product, resistance to frost, resistance to disease, high nutrient density and overall high quality (Rane et al., 2016). In the current study, stevia was used as a sugar substitute, which then resulted in low TSS levels in beverages. Stevia is a natural sweetener derived from the leaves of the plant species *Stevia rebaudiana* (Gandhi et al., 2018).

These leaves have been reported to possess properties superior to those of many other highly effective sweeteners (Gandhi et al., 2018). Some of the health benefits of these leaves include anti-hyperglycemic effects where they lower blood glucose levels, anti-hypertensive effects where the leaves prevent complications caused by high blood pressure, as well as preventing dental caries which may lead to tooth decay and other dental infections (Gandhi et al., 2018). Studies have shown that even though sugar is required by the human body, excessive intake of sugar is associated with cancer, obesity, tooth decay and cardiovascular diseases (Rane et al., 2016).

3.3.2 Colour parameters of Moringa-aloe beverage blends

Table 3.3 shows the colour analysis of the Moringa-aloe beverages. The 100M beverage was darker (-0.98 L value) in colour compared with the 100A beverage which had the highest lightness (L value of 2.21). The colour of beverage blends increased in darkness with an increase in Moringa concentration. The Δa* values showed that 100M beverage was greener (-1.88), while the 100A was redder (+0.30). Consequently, the beverage blends with Moringa were greener and an increase in Moringa concentration increased the intensity of the green colour.

The green colour in Moringa may be due to the high amount of chlorophyll present in Moringa leaves (Dubey and Kapoor, 2017). The Δb* values indicated that 100A beverage and all the blends with high concentration of Aloe were bluer. A trend was observed where an increase in Aloe concentration significantly increased the blueness of the beverage blends, while the 100M was yellower with Δb* value of +0.22.

Table 3.3. Colour analysis of Moringa-aloe beverage blends.

Beverage	**ΔL***	**Δa***	**Δb***
30M: 70A	$+1.38 \pm 0.01^{d}$	-0.49 ± 0.01^{d}	-7.71 ± 0.00^{b}
50M: 50A	$+0.50 \pm 0.01^{c}$	-0.97 ± 0.01^{c}	-4.95 ± 0.01^{c}
70 M: 30A	-0.35 ± 0.01^{b}	-1.61 ± 0.00^{b}	-1.21 ± 0.01^{d}
100M	-0.98 ± 0.01^{a}	-1.88 ± 0.01^{a}	$+0.22 \pm 0.00^{e}$
100A	$+2.21 \pm 0.01^{e}$	$+0.30 \pm 0.00^{e}$	-11.47 ± 0.00^{a}

Data presented as mean±SD (n=3). Within each column, values not sharing the same superscript letter are significantly different from one another (P<0.05). 30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe. ΔL* (L* sample minus L* standard) = difference in lightness and darkness (+ = lighter, - = darker): Δa* (a* sample minus a* standard) = difference in red and green (+ = redder, - = greener): Δb* (b* sample minus b* standard) = difference in yellow and blue (+ = yellower, - = bluer).

Multiple sensory properties such as flavor or odour identification and intensity, basic taste perception, quality and consumer acceptance may potentially be influenced by manipulating the colour of a beverage (Mahony, 2001). A study by Capule and Barcelon (2014) reported colour to be an important factor in the acceptability of a ready-to-drink soymilk by consumers. In their study, soymilk with a brown colour was more preferred by consumers when compared to soymilk that was off-white in colour. De-heer et al. (2013) developed a herb infused drink from a blend of Moringa leaves, Roselle calyses and lemon grass leaves. Their sensory evaluation results indicated that the colour of beverages with higher amounts of Roselle (red) were more appealing to consumers (De-heer et al., 2013). This suggests that in the current study, the colour of the beverage blends may influence their acceptance by consumers.

3.3.3 Nutritional value of Moringa-aloe beverage blends.

3.3.3.1 Protein and Vitamin C content of Moringa-aloe beverage blends.

Proteins are an essential nutrient needed by the human body for growth and maintenance (Liyanage et al., 2014). Table 3.4 shows the protein and vitamin C content of Moringa-aloe beverages blends. The 100M beverage contained significantly ($p<0.05$) high protein content (1012.1 µg/ml), while the least protein content occurred with the 100A beverage (74.6 µg/ml). In general, the beverage blends with more Moringa had a corresponding high protein content. A similar trend was observed by Quarcoo (2008) where an increase in Moringa concentration, increased the protein content of Moringa-pineapple-carrot beverage. This trend can be attributed to the high protein content of Moringa leaf powder (27.1g/ 100g) (Gopalakrishnan et al., 2016).

Other researchers have termed Moringa leaves as a good indigenous source of digestible protein (Okiki et al., 2015). In addition, Moringa proteins provide a good proportion of all essential amino acids (Okiki et al., 2015) such as histidine, leucine, tryptophan, threonine, lysine, valine, methionine and phenylalanine (Moyo et al., 2011) which is rarely seen in other plants. Biswas et al. (2016) reported a protein content of 900 – 960 µg/ml for the Aloe-pineapple blended beverages which was lower than that of the 100M beverage in the present study. A trend was observed in their study where an increase in Aloe concentration, increased the protein content of the beverage. However, a protein content of 840 µg/ml was reported for the beverage containing 100% Pineapple: 0% Aloe, which indicated that Aloe was not a major contributor of protein in the Pineapple-Aloe blended beverage. Similarly, in the present study, Aloe was not the major protein contributor in the blends (Table 3.4).

Table 3.4. Protein and Vitamin C content of Moringa-aloe beverage blends.

Beverage	**Protein (µg/ml)**	**Vitamin C (mg/100ml)**
30M: 70A	343.60 ± 0.02^{b}	1.63 ± 0.04^{b}
50M: 50A	529.60 ± 0.04^{c}	1.56 ± 0.02^{b}
70M: 30A	724.10 ± 0.03^{d}	1.41 ± 0.01^{a}
100M	1012.10 ± 0.01^{e}	1.30 ± 0.00^{a}
100A	74.60 ± 0.04^{a}	2.03 ± 0.11^{c}

Data presented as mean±SD (n=3). Within each column, values not sharing the same superscript letter are significantly different from one another ($p<0.05$). 30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe.

Low intake of protein may cause many health complications such as Kwashiorkor, weakness of immune system, impaired mental health, wasting and shrinkage of muscle tissues, and organ failure (Khan et al., 2017). The recommended daily intake of protein is 0.8g per Kg of body weight (Phillips et al., 2007). Therefore, daily consumption of a 100ml of the 100 M beverage and 70M blend may contribute to the daily protein requirement.

Vitamin C, or ascorbic acid is an essential dietary nutrient for the biosynthesis of collagen and a co-factor in the biosynthesis of amino acids, L-carnitine, cholesterol, catecholamines and some peptide hormones (Grosso et al., 2013). The lack of vitamin C leads to Scurvy, a pathological condition leading to connective tissue damage, blood vessel fragility, fatigue, and finally, death (Omeldo et al., 2006).

The 100A beverage contained a significantly ($p<0.05$) high amount of vitamin C (2.03mg/100ml) and the least vitamin C was observed with the 100M beverage with a value of 1.30mg/100ml (Table 3.4). As a result, an increase in Aloe concentration significantly ($p<0.05$) increased the vitamin C content of the blends. The level of vitamin C in 100A beverage was higher than that found by Talib et.al (2016) who reported a vitamin C content of 1.54mg/100ml for *Aloe vera* juice. The difference in results might be due to the use of aloe concentrate to make aloe juice in the present study, compared with the use of aloe gel to make aloe juice by Talib et al. (2016). Aftab et al., (2016) reported a vitamin C content ranging from 135.06-138.34 mg/100ml for Moringa-aloe beverage blends, which was higher than that of the beverages in the current study. This high vitamin C content might be due to the addition of ascorbic acid as an antioxidant in the beverage blends. Madukwe et al. (2013) reported vitamin C levels of 6.26mg/100ml for a Moringa beverage, which was also higher than the vitamin C in the present study.

3.3.3.2 Mineral composition of Moringa-aloe beverage blends.

Minerals form part of the essential nutrients required for proper growth and maintenance of the body (WHO, 2005). Deficiencies in minerals cause numerous health complications. Iron deficiency and prolonged calcium deficiency cause anaemia (Cozzolino, 2007) and osteoporosis (Pravina et al., 2013) respectively. Table 3.5 shows the mineral composition (Na, Ca, K, Mg and Fe) of the beverages and beverage blends. In general, the 100M beverage contained the highest amount of minerals compared to 100A beverage. With all samples, Na, Ca and K were the most abundant minerals while Fe was the least available mineral. The highest Na was observed in the 100A beverage (180.19 mg/L) and the least in the 100M beverage (111.42 mg/L). An increase in aloe concentration, increased Na in the blends. The high amount of sodium is due to the 0.1% of sodium benzoate added as a preservative in the beverages, as well as the sodium benzoate that was already in the Aloe concentrate (as preservative) used to make the Aloe juice. Another trend was

observed where blends with high amount of Moringa (50% and 70%) were high in Ca, K and Mg. The highest Fe was recorded in the 50% Aloe blend and the least in the 30% and 70% Moringa blends.

According to Maizuwo et al. (2017) K, Ca, and Mg are the predominant minerals in the Moringa plant. The beverages and blends in the present study were found to contain higher amounts of Ca, K and Mg compared to the amount reported by Aderinola (2018) in smoothies supplemented with Moringa leaves. The smoothies had Ca, Mg and K values that ranged from 11.21 – 15.53 mg/L, 1.51 – 3.05 mg/L and 17.22 – 25.38 mg/L, respectively. In the present study, Ca, Mg and K levels ranged from 28.04 – 117.92mg/L, 11.60 – 61.29mg/L and 30.84 – 101.94mg/L respectively. In humans, Na and K are needed for proper fluid balance, muscle transmission and nerve transmission (Soetan et al., 2010). Ca is mainly important for healthy bones and teeth, while Mg is needed for protein synthesis as well as nerve transmission and immune system health (Soetan et al., 2010). Fe is a trace element that is required in small concentrations in the body, and it forms part of the haemoglobin that carries oxygen in the body (Soetan et al., 2010).

Table 3.5. Mineral composition (mg/L) of 5 Moringa : aloe beverage blends

Minerals	**30M: 70A**	**50M: 50A**	**70M: 30A**	**100M**	**100A**
Ca	58.72 ± 0.02^{b}	79.28 ± 0.72^{c}	103.93 ± 0.93^{d}	117.92 ± 0.04^{e}	28.04 ± 0.10^{a}
Fe	0.12 ± 0.00^{a}	0.26 ± 0.00^{d}	0.12 ± 0.001^{a}	0.15 ± 0.00^{b}	0.18 ± 0.00^{c}
K	47.43 ± 0.81^{b}	58.78 ± 0.56^{c}	67.42 ± 1.42^{d}	101.94 ± 0.63^{e}	30.84 ± 1.89^{a}
Mg	26.17 ± 0.44^{b}	35.89 ± 0.34^{c}	46.32 ± 1.30^{d}	61.29 ± 1.95^{e}	11.60 ± 0.23^{a}
Na	148.49 ± 0.42^{d}	143.39 ± 3.25^{c}	138.18 ± 2.60^{b}	111.42 ± 0.12^{a}	180.19 ± 1.96^{e}

Data presented as mean±SD (n=3). Within each row, values not sharing the same superscript letter are significantly different from one another ($P<0.05$). 30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe.

Therefore, daily consumption of the Moringa-aloe beverage as part of a balanced diet can help consumers achieve the daily recommended mineral intake levels of Ca (1000mg), Mg (350mg), K (3500mg) and Na (2400mg) (FAO/WHO, 2004), and thus improve the health of the general population.

3.3.4 Total phenolic content, total flavonoid content and antioxidant capacity of Moringa-aloe beverage blends.

Phytochemicals are non-nutritive chemicals that possess disease preventative or protective properties (Abbas et al., 2014). Table 3.6 shows the TPC, TFC and antioxidant activity of beverage blends. The TPC was significantly ($p<0.05$) higher in 100M beverage (18.25 mg GAE/L), while 100A had the least TPC (5.04 mg GAE/L). Moringa plants are rich in phytochemicals such as phenols, flavonoids and alkaloids (Ashfaq et al., 2012; Nazir and Ahsan, 2017). Due to this, the TPC for the blends increased with an increase in Moringa concentration. This trend was also seen in a previous study by Aderinola (2018), following supplementation of smoothies with Moringa leaf extract. However, Aderinola (2018) reported higher TPC in their samples than that of the current study. Studies have found that consuming foods rich in phenolic compounds may reduce the occurrence of cardiosvascular diseases (Yang et al., 2000). The high TPC of Moringa beverage is attributed to the relatively high TPC in Moringa leaves which range from 25 – 144.77 mg/g GAE (Vyas et al., 2015).

Similar to what occurred with TPC, the highest TFC (53.20 mg QE/L) was observed with the 100M beverage and the least (9.69 mg QE/L) with the 100A beverage (Table 3.6). Blends with more Moringa showed high levels of TFC, which indicates that Moringa was the major contributor of flavonoids in the blends. Previous research has reported that Moringa is rich in flavonoids, such as quercetin and kaempferol which make up 47% and 30% respectively, of the total flavonoids found in the plant (Maizuwo et al., 2017). The TFC of the beverage blends of this study was substantially higher than values (10 – 20 mg/L) reported by Aderinola in smoothies with Moringa. The difference could possibly be due to the high Moringa concentration used in this study as compared to the 1.5 – 4.5% Moringa extract used by Aderinola (2018).

Antioxidants are known to prevent food from oxidative deterioration on processing and storage (Wilson et al., 2017). Here, the antioxidant activity of the beverage blends was evaluated using DPPH and ABTS assays (Table 3.6). The DPPH (16.12 µmol/L) of 100M beverage was significantly higher than that of 100A beverage (3.88 µmol/L). The DPPH of the blends increased with an increase in Moringa concentration and this was attributed to the high antioxidant capacity of Moringa. Due to their high stability, antioxidants help to maintain the colour, level of nutrients, texture, taste, freshness, aroma, functionality and appeal of food products (Wilson et al., 2017). Because of this, antioxidants prolong shelf-life of products by neutralising free radicals in foods.

Table 3.6. Phytochemical composition and antioxidant capacity of Moringa-aloe beverage blends

Beverage	TPC (mg GAE/L)	TFC (mg QE/L)	DPPH (µmol TE/L)	ABTS (µmol TE/L)
30M: 70A	10.39± 0.48^{b}	25.51± 2.08^{b}	3.88± 0.38^{a}	43.66± 0.14^{b}
50M: 50A	12.27± 1.42^{bc}	40.53± 1.52^{c}	7.68± 0.18^{c}	43.68± 1.29^{b}
70M: 30A	14.68± 0.48^{c}	51.26± 1.20^{d}	8.09± 0.34^{c}	46.71± 1.40^{b}
100M	18.25± 1.67^{d}	53.20± 0.72^{d}	16.12± 1.10^{d}	43.65± 1.56^{b}
100A	5.04± 1.21^{a}	9.69± 0.43^{a}	5.64± 0.34^{b}	33.78± 2.18^{a}

Data presented as mean±SD (n=3). Within each column, values not sharing the same superscript letter are significantly different from one another ($P<0.05$). 30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe. TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).

In humans, antioxidants are able to scavenge free radicals that attack healthy body cells and thus prevent or reduce the risk of diseases such as cancer and cardiovascular diseases that are majorly promoted by oxidation (Ashadevi and Gotmare, 2015). Therefore, the beverage blends with high antioxidant activity possess high free radical scavenging ability and thus better prevention of diseases.

The ABTS value (43.65 µmol TE/L) of the 100M beverage was significantly higher ($p<0.05$) than that of the 100A beverage (33.78 µmol TE/L). Unlike what occurred with DPPH, there was no significant difference between the antioxidant activities of the beverage blends and 100M beverage when measured with ABTS. This means that the 100M beverage possess the same level of free radical scavenging activity to that of the blends.

Based on the DPPH and ABTS results, it can be suggested that Moringa is the major contributor of antioxidants in the blends. The high antioxidant levels of Moringa are attributed to the plant's high content in phenolic compounds and flavonoids. Rasouli et al. (2017) reported that phenolic compounds possess antioxidant properties, which also play an important role in the oxidative stability of foods. This is in agreement with findings by other researchers that reported Moringa as a good source of antioxidants and polyphenols (Shanmugavel et al., 2018).

3.4 CONCLUSIONS

Moringa addition increases the pH of beverage blends but does not cause significant change on titratable acidity. The protein content and concentration of most minerals improves with increase in Moringa concentration in the beverage blends. However, beverage blends with high Aloe concentration contain high levels of vitamins C due to the high levels of the vitamin in Aloe. The total phenolic content and total flavonoid content of the beverage blends improves with Moringa addition and so does the antioxidant activity when determined with DPPH assay. With ABTS, the beverage blends have similar antioxidant activity. Overall, the current study confirms that beverage blends with Moringa have high nutritional quality and possess health promoting properties.

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CHAPTER 4

SENSORY QUALITY AND STORAGE STABILITY OF MORINGA-ALOE BEVERAGE BLENDS

ABSTRACT

Beverages with improved nutritional and functional properties but were still acceptable to consumers are required. In this study, the consumer acceptability of Moringa-aloe beverage blends was investigated using the 9-point hedonic scale method. In addition, the storage stability of the beverage blends was investigated at 4 °C for 12 weeks. The sensory evaluation results showed that 100 % Aloe (100A) beverage was the most preferred beverage by the taste panel as it was scored highest for taste (7.08), flavour (6.76) and overall acceptability (7.04). The least preferred beverage was 100 % Moringa (100M) followed by the beverage blend with high Moringa concentration (70M: 30A). The highest score for colour (6.48) was observed with 70M: 30A beverage blend and all the other beverage blends were scored high for colour compared to control samples. With storage stability, a slight increase in pH accompanied by a decrease in TA occurred on all beverages after 12 weeks. However, most beverage blends still maintained a pH less than 4.2 at the end of storage. There was no microbial growth observed for total aerobic bacteria and yeast and mold after 12 weeks of storage, possibly due added preservatives and low pH of the products. Therefore, the 30M: 70A beverage blend appears to be the best with respect to consumer acceptability and storage stability.

Keywords: Moringa-aloe beverage blends, sensory quality, storage stability, microbial growth

4.1 INTRODUCTION

Scientifically, sensory science constitutes a set of techniques dealing with human sensory perceptions and reactions to numerous types of beverages, foods and their components that changed from the need for scientifically comprehensive and systematic sensory evaluation (Tuorila and Monteleone, 2009). In the 1940s the US army Corps of Engineers developed consumer or hedonic food acceptance methodologies that attributed to the concept of sensory science (Peryam and Pilgrim, 1957). In recent times, sensory evaluation has been defined as a scientific method used to evoke, measure, analyze and interpret responses to food products as perceived through senses of taste, smell, sight and hearing (Lawless and Heymann, 2010).

There are various methods that may be used for sensory evaluation, consumer acceptability testing and sensory descriptive analysis are the most frequently used. According to Sharif et al. (2017) sensory descriptive analysis comprises of the description and discrimination of product quantitative and qualitative sensory characteristics by trained panelists. Sensory descriptive analysis methods include texture profile, flavour profile, quantitative descriptive analysis and SprectrumTM method (Sharif et al., 2017). Consumer acceptability is one of the most important sensory analysis tests and is usually used to measure the degree of liking or disliking of a product using consumers (Lawless and Heymann, 2010). Other aspects that can be determined during consumer acceptability tests include instrumental information, consumer perception, consumer emotion, relationship between descriptive analysis characteristics and consumers' feelings regarding the product (Venturi et al., 2014).

The role of sensory evaluation has changed significantly over the years. In research and development, along with marketing departments it helps in the formulation and profitable strategy (Sharif et al., 2017). In the early stages of developing a product, it helps to identify important sensory characteristics that drive acceptability. Sensory evaluation can also be useful in establishing product competitors, target consumers and evaluate new concepts for the product. Recently, physical and chemical characteristics of a product influencing sensory attributes can be established by combining data attained from sensory and instrumental testing (Sharif et al., 2017). Sensory evaluation results can determine the effect and risks of scaling up pilot food products to large-scale manufacture. It also gives assurance that superior products are released in the market.

Frequently, sensory evaluation is used to determine the shelf-life of products as product sensory characteristics degrade over time.

The demand for high quality foods by consumers is increasing. As well as consumer expectations that the quality of the food is sustained at a high level throughout the duration between the product purchase and consumption. Shelf-life is an important aspect of all foods. This includes raw materials, ingredients and the final manufactured product. Earle and Earle (2008) define shelf-life as the period whereby the food product will (1) retain physical, chemical, microbiological and sensory characteristics (2) remain safe (3) conform with nutritional data on label declarations, and (4) be acceptable to consumers.

There are countless factors that can influence the shelf-life of products, and these can be categorized into compositional and environmental factors. Compositional factors refer to properties of the final product, such as food composition; pH, type of acid, total acidity, water activity, redox potential (Eh), nutrients, available oxygen, surviving microorganisms, natural microflora; product formulation biochemistry including preservatives. Environmental factors include processing time and temperature; pressure in the headspace; storage and distribution temperature; relative humidity throughout processing, storage, and distribution; Exposure to ultraviolet and Infrared light throughout processing, storage, and distribution; microorganisms in the environment throughout processing, storage, and distribution; and handing by distributer, retailer and consumer (Phimolsiripol and Suppakul, 2016).

4.2 MATERIALS AND METHODS

4.2.1 Sensory evaluation

Sensory evaluation of the Moringa-aloe beverage was carried out by 50 panelists who rated the beverage blends for colour, flavour, taste and overall acceptability using 9 point hedonic rating test method (1=dislike very much, 9=like very much) as recommended by (Ranganna, 1995).

4.2.2 Storage stability studies

Moringa-aloe beverages were subjected to storage studies at refrigerated temperature (4°C) for a period of 12 weeks by drawing samples at 2-week intervals to evaluate changes in pH, TA, TSS, colour and microbial load.

4.2.2.1 Quality characteristics of the developed beverage

The proximate composition of the developed beverage was determined in triplicate according to the AOAC (2010) methods. pH was determined with a pH meter, titratable acidity was measured by a titration method where 25ml distilled water was added to a 10ml sample, followed by the addition of phenolphthalein indicator (Sigma-aldrich, United Kingdom). The mixture was titrated against 0.1M Sodium hydroxide (NaOH; Sigma-Aldrich, Sweden) to a faint pink end point and results were expressed as % citric acid. The total soluble solids content was determined by measuring the °Brix (Handheld Refractometer Atago ATC-IE, Tokyo, Japan) at 20 °C (Wang et al., 2005) based on the official AOAC Official Method 932.12 (AOAC International, 1932). The results were expressed as °Brix. The colour of the samples were measured using a Chroma colour meter (Konica Minolta, Toyko, Japan) and the results expressed as *L*, *a*, *b* values.

4.2.2.2 Microbiological analysis

Microbiological analysis were carried out following the method outlined by Nyambane et al. (2014) with some slight modifications. Briefly, samples were thoroughly mixed by inversion followed by carrying out ten-fold serial dilutions (10^{-1} - 10^{-5}) with sterile 0.1% buffered peptone water (Merck, South Africa). Thereafter, 1 mL sample was pour plated on plate count agar (Merck, South Africa) and incubated at 30 °C for 48 h. Yeast and moulds were enumerated on potato dextrose agar (Merck, South Africa) supplemented with sterile 0.1 g/L chloramphenicol (Merck, South Africa). The inoculated plates were incubated at 25°C for 5 days. Duplicate plates with 25 – 250 colonies were counted.

4.2.3 Statistical analysis

All experiments were carried out in triplicate and each experiment was done in duplicate. One way analysis of variance (ANOVA) was used to performed using SPSS statistical sotfware version 25 (SPSS/IBM, Chicago, Illinois). Results were expressed as means and standard deviations and Duncan's Multiple range test was used to determine significant differences between means ($p<0.05$).

4.3 RESULTS AND DISCUSSION

4.3.1 Sensory evaluation

Table 4.1 shows the consumer scores for colour, flavour, taste and overall acceptability of beverages and beverage blends. The 30M: 70A, 50M: 50A and 70M: 30A beverage blends were compared with two market controls which consisted of store-bought 100% Moringa ($100M_{mc}$) beverage and 100% Aloe ($100A_{mc}$) beverage. The market controls replaced the two controls in the current study (100M and 100A) because the sensory evaluation tests' purpose was to observe how consumers would react to beverage blends in the current study compared to beverages already on the market ($100M_{mc}$ and $100A_{mc}$).

Colour

The colour of a food generates certain product expectations and can influence the way we perceive the product (DLG expert report, 2017). The two market controls ($100M_{mc}$ and $100A_{mc}$) had a water-like colour while the beverage blends in the current study were yellowish. The highest score for colour was seen with the 30M: 70A beverage blend (6.48) and the least with the $100M_{mc}$ beverage (4.76). All the beverage blends (30M: 70A, 50M: 50A, 70M: 30A) were scored significantly higher ($p<0.05$) for colour than both of the controls. This indicated that the colour of the beverage blends in the current study were more preferred by consumers.

Table 4.1. Sensory analysis results of the Moringa-aloe beverages and blends

Beverage	Colour	Flavour	Taste	Overall acceptability
30M: 70A	6.18^{c}	6.04^{d}	6.12^{d}	6.26^{d}
50M: 50A	6.26^{d}	5.82^{c}	5.84^{c}	6.20^{c}
70M: 30A	6.48^{e}	5.54^{b}	5.52^{b}	5.74^{b}
$100M_{mc}$	4.76^{a}	4.72^{a}	4.84^{a}	4.9^{a}
$100A_{mc}$	5.84^{b}	6.76^{e}	7.08^{e}	7.04^{e}

Data presented as means. Within each column, values not sharing the same superscript letter are significantly different from one another ($p<0.05$). 30M: 70A; 30% Moringa: 70% Aloe, 50M: 50A;

50% Moringa: 50% Aloe, 70M: 30A; 70% Moringa: 30% Aloe, $100M_{mc}$; 100% Moringa market control, $100A_{mc}$; 100% Aloe market control.

Flavour

Flavour refers to the taste and aroma perceived when tasting (Jellinek, 1985). Consumers often mention the taste and flavour of a product as being a major factor in their preference decision (Clark, 1998). The highest score for flavour was observed with the $100A_{mc}$ beverage (6.76) and the least with the $100M_{mc}$ beverage (4.72). Increasing the amount of Moringa in the beverage blends gave a flavour that was least liked by consumers. The decrease in flavour score with an increase in Moringa levels might have been due to the strong herbal flavour of Moringa. A similar trend was reported by Otu et al. (2013a).

Taste

The $100A_{mc}$ beverage was rated the highest (7.08) for taste and the least score was observed with the $100M_{mc}$ beverage (4.84), which was expected as the $100M_{mc}$ was also less preferred for flavour. With the beverage blends, the 30M: 70A was the most preferred for taste with a score of 6.12. Similar to what occurred with flavour, increasing Moringa levels led to a taste that was less preferred by consumers.

Overall acceptability

Overall, the most acceptable beverage blend was the $100A_{mc}$ beverage (7.04), followed by the 30M: 70A (6.26), 50M: 50A (6.20), 70M: 30M (5.74) and the least acceptable beverage was the 100M control (4.90). The 100A score for overall acceptability was significantly ($p < 0.05$) higher than the other beverage blends, which was anticipated as it was scored the highest for flavour and taste.

4.3.2 Storage stability of Moringa-Aloe beverage blends

4.3.2.1 pH and TA

The pH and TA of the Moringa-Aloe beverage blends was monitored over 12 weeks at 4 °C. In general, an increase in pH and a decrease in TA was observed with all the samples after 12 weeks storage (Figure 4.1). The highest pH increase occurred with 100A beverage which increased from pH 3.66 – 3.89 after 12 weeks (Figure 4.1A). The least pH increase was observed with 100M

beverage with pH values ranging from 4.15 – 4.33 after the same period of storage. The observed increase in pH was thought to be due to the decrease in acidity of the juices. Similar findings have been reported by Otu et al. (2013b) who observed a significant increase in pH over 16 weeks storage of aloe blended with guava and roselle juice at ambient temperature, as well as Mgaya-Kilima et al. (2014) on roselle fruit juice blends stored for 24 weeks at 4 °C. These researchers attributed the increase in pH to decrease in acidity of the juices. According to Dissanayake (2017), the pH of beverages should be less than 4.2 in order to prevent microbial growth and increase shelf-life. Here, the 100A beverage and all the beverage blends had a final pH of less than 4.2 and thus can be expected to have an extended shelf-life due to limited microbial growth.

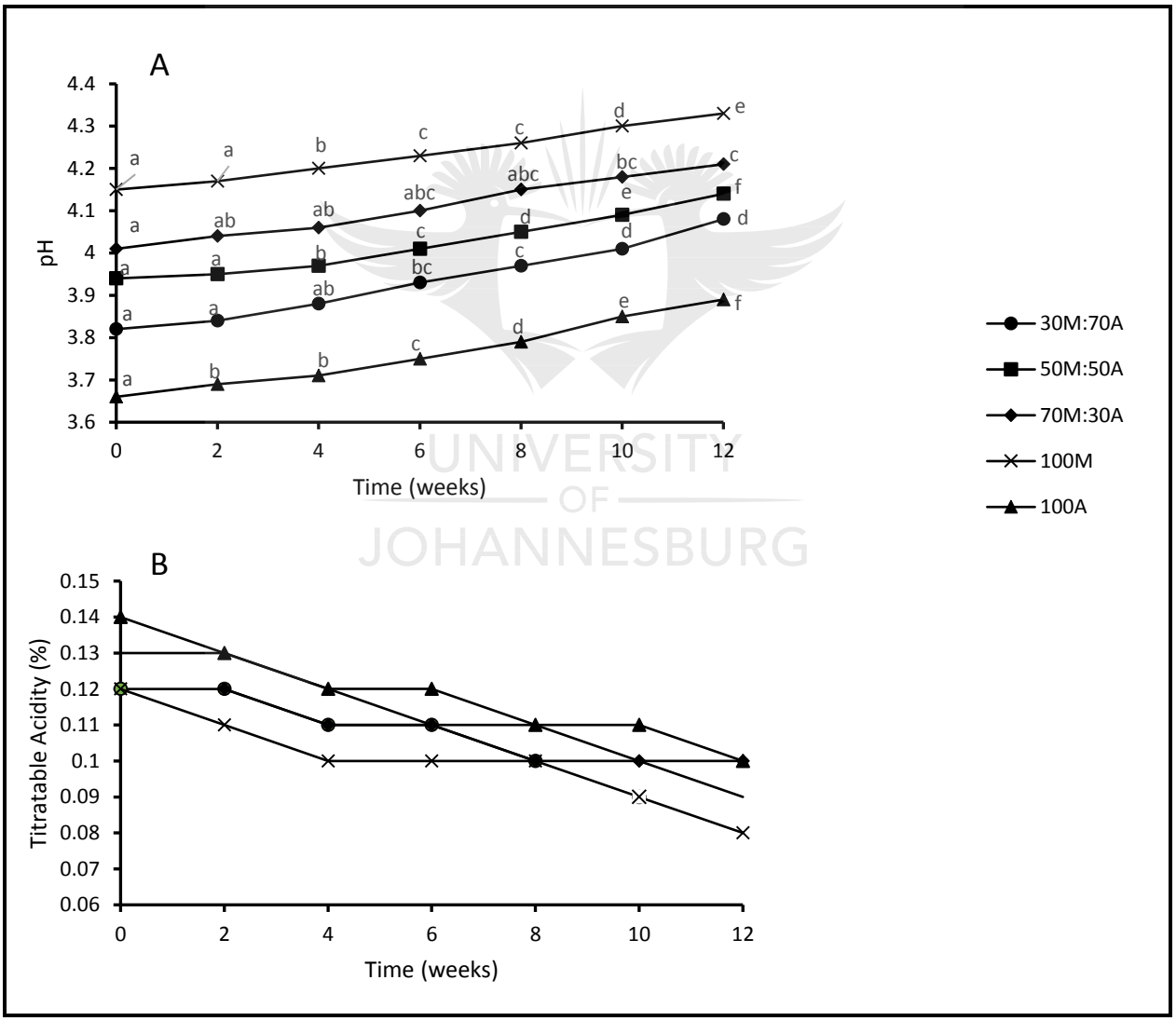

Figure 4.1. The pH (Fig 1A) and TA (Fig 1B) results of the Moringa-aloe beverage blends after 12 weeks at 4 °C. 30M: 70A; 30% Moringa: 70% Aloe, 50M: 50A; 50% Moringa: 50% Aloe, 70M: 30A; 70% Moringa: 30% Aloe, 100M; 100% Moringa, 100A = 100% Aloe.

As indicated, the TA values of all the beverages and beverage blends decreased significantly ($p<0.05$) during the 12 weeks storage (Figure 4.1B). The 70M: 30A beverage blend lost the least acidity (0.03%), while all the beverages and other beverage blends lost a slightly higher acidity (0.04%). The decreased acidity might be due to acidic hydrolysis of sugar and polysaccharides by enzymes particularly invertase, where acid is utilized for converting non-reducing sugar into reducing sugar (Bhardwaj and Pandey, 2011), as well as converting acids into salts (Bhardwaj and Mukherjee, 2012). It can be predicted that the continuous acidic hydrolysis reaction occurring in the beverages will further reduce the acidity of the beverages, thus increasing their pH above pH 4.2 which will reduce the beverages shelf-life by making the beverages a favourable environment for microbial growth.

4.3.2.2 Total soluble Solids (TSS) of Moringa-aloe beverages.

The TSS for the Moringa-aloe beverage blends during the 12 weeks storage at 4 °C is presented in Figure 4.2. An increase in TSS was observed with all the beverages and blends. The highest TSS increase of 0.3 °Brix was observed with the 100A beverage, the 30M: 70A and 50M: 50A beverage blends, followed by the 70M: 30A beverage blend and 100M beverage with a TSS increase of 0.2 °Brix. The increase in TSS might be due to acid hydrolysis of sugar and polysaccharides found in the Moringa and aloe extracts. Bhardwaj and Pandey (2011) suggest that a slight increase in the TSS of a beverage during storage is essential for preservation of the beverage. This is because a high °Brix value gives a product that is resistant to frost, resistant to disease, has a high nutrient density as well as an overall high-quality (Rane et al., 2016). Talib et al. (2016) also observed significant increases in TSS of Aloe based ready to serve (RTS) soft drinks stored for 12 weeks at chilled temperature. They reported that the TSS increase was due to acidic hydrolysis of sugars and polysaccharides in the RTS soft drink. Their TSS increased by 2-6 °Brix and a jackfruit-aloe beverage developed by Hossain et al. (2017) had a TSS increase of 0.59 °Brix after 12 weeks of storage at refrigerated temperature, while in the current study the TSS increased by 0.2-0.3 °Brix. The slight increase in TSS shows that the quality of the beverage blends in the current study was preserved during the 12weeks storage as suggested by Bhardwaj and Pandey (2011).

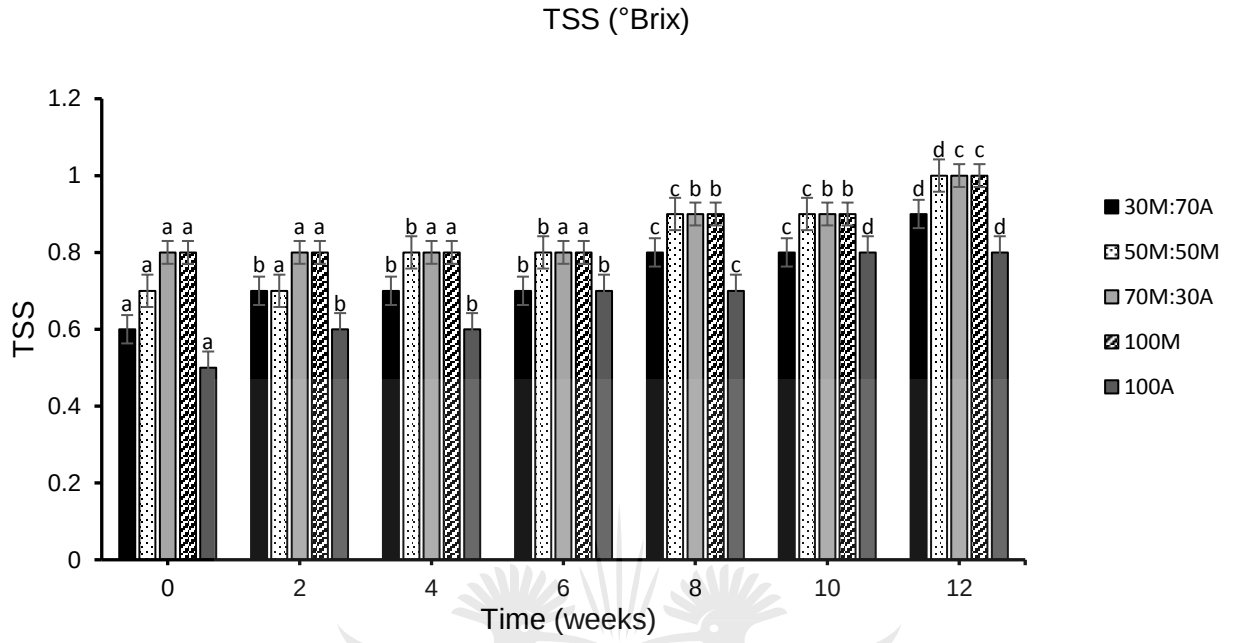

Figure 4.2. Total soluble solids of the Moringa-aloe beverage blends after 12weeks at 4°C. 30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe.

4.3.2.3 Changes in colour of Moringa-aloe blends after 12 weeks storage

Colour is one of the most important attributes of foods and beverages. The first impression of the quality acceptability of certain food is judged upon its appearance (Nielsen, 1998). Table 4.2 shows the colour results of Moringa-aloe beverage blends after 12 weeks storage at 4 °C. The Lightness values (ΔL*) of the 100A beverage increased from 2.21 – 5.38, while that for 100M beverage decreased from -0.98 to -4.31 after 12 weeks storage period. All the blends with Moringa moved towards a darker colour after the same time period. This could have been due to chlorophyll degradation caused by non-enzymatic browning in aqueous environments that contain reducing sugars and proteins (Otu et al., 2013b).

The Δa* (difference in red and green) values for the 100A beverage increased from 0.30 - 1.40 and from -1.88 to -0.38 for the 100M beverage. The green colouration was higher in blends containing Moringa after the 12week storage. This might be an indication that the green pigment (Chlorophyll) of the Moringa was preserved throughout the storage period. Similar results of

beverages containing Moringa remaining greener upon storage were observed by Quarcoo (2008) who stored Moringa-pineapple-carrot blended drinks for 8 weeks at 5°C. The Δb* (difference in yellow and blue) values of the beverages and blends ranged from 0.22 to -12.95, with all the beverages becoming bluer. This shows that storage time and temperature led to a loss in the yellowness of the beverage blends, which might be due to the deterioration of several components such as vitamin C which are vulnerable to deterioration upon storage.

Table 4.2. Colour parameters of Moringa-aloe beverages and blends after 12 weeks at 4°C

Parameters	**ΔL***		**Δa***		**Δb***	
Storage time	0 weeks	12 weeks	0 weeks	12 weeks	0 weeks	12 weeks
30M: 70A	1.38^{d}	-1.48^{d}	-0.49^{d}	-1.21^{b}	-7.71^{b}	-10.88^{b}
50M: 50A	0.50^{c}	-2.81^{c}	-0.97^{c}	-0.35^{d}	-4.95^{c}	-3.82^{c}
70M: 30A	-0.35^{b}	-6.29^{a}	-1.61^{b}	-1.80^{a}	-1.21^{d}	-2.11^{d}
100M	-0.98^{a}	-4.31^{b}	-1.88^{a}	-0.38^{c}	0.22^{e}	-2.01^{e}
100A	2.21^{e}	5.38^{e}	0.30^{e}	1.40^{e}	-11.47^{a}	-12.95^{a}

30M: 70A = 30% Moringa: 70% Aloe, 50M: 50A = 50% Moringa: 50% Aloe, 70M: 30A = 70% Moringa: 30% Aloe, 100M = 100% Moringa, 100A = 100% Aloe. ΔL* (L* sample minus L* standard) = difference in lightness and darkness (+ = lighter, - = darker): Δa* (a* sample minus a* standard) = difference in red and green (+ = redder, - = greener): Δb* (b* sample minus b* standard) = difference in yellow and blue (+ = yellower, - = bluer).

4.3.2.4 Microbiological analysis of Moringa-aloe beverage blends after 12 weeks

The total aerobic count (TAC) and yeast and mould count of Moringa-aloe beverage blends was monitored over 12 weeks period at 4°C. The TAC is generally used to determine the freshness of a product by detecting spoilage and non-spoilage microorganism, as well as to validate sanitation procedures during processing (Sperber and Doyle, 2009). Yeasts and moulds are known to cause various degrees of deterioration and decomposition in foods. They have an advantage of dominating other microorganisms in low pH foods due to their wide pH requirement range for

growth (Aneja et al., 2014). The low pH and high acidity of the Moringa-aloe beverage blends makes them susceptible to spoilage by fermentative yeasts, moulds and some aciduric bacteria.

As shown in Table 4.3, there was no microbial growth was detected with both TAC and yeast and mould after 12 weeks storage at 4 °C. This indicates that the processing and preservation methods (low pH, addition of sodium benzoate, pasteurization and storage at 4°C) applied were successful in preserving the quality of the beverage blends. Generally, pathogenic microorganisms grow very slowly or do not grow at all at pH levels below 4.6 (USDA, 2012) and the pH of the beverage blends in the current study was below 4.6 for the entire storage period. Sodium benzoate is a commonly used preservative in beverages that effectively inhibits the growth of fungi and bacteria during storage (Tsay et al., 2007). Pasteurization eliminates vegetative cells, and the benefit of pasteurizing in containers reduces the risk of product contamination after packaging (Petruzzi et al., 2017). Further, the storage of the product at low temperature (4 °C) is thought to have inhibited the proliferation of spoilage microorganisms (USDA, 2012). All these factors are believed to have contributed collectively to elimination and prevention of microbial growth in the beverage blends.

4.3.3 CONCLUSIONS

Sensory evaluation of Moringa-aloe beverage blends indicate that high concentration of aloe in the beverage blends impacts positively on their sensory acceptability by consumers in terms of flavour, taste and overall acceptability. In contrast, increasing the concentration of Moringa makes the beverage blends less desirable to consumers. Despite this, all the beverage blends were scored high for colour compared to the control samples. With storage stability, a slight increase in pH accompanied by a decrease in TA occurs on the beverages over time. However, most beverage blends still maintain a pH of less than 4.2 after 12 weeks. There was no microbial growth for total aerobic bacteria and yeast and mold after 12 weeks of storage, possibly due added preservatives and low pH of the products. Therefore, the 30M: 70A beverage blend appears to be the best with respect to consumer acceptability and storage stability.

Table 4.3. Microbial analysis of Moringa-aloe beverages and blends after 12 weeks storage at 4°C

		Storage Period (weeks)						
Item	**Beverage**	**0**	**2**	**4**	**6**	**8**	**10**	**12**
TAC	30M: 70A	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
	50M: 50M	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
	70M: 30 A	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
	100M	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
	100 A	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
Yeast & mould	30M: 70A	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml
	50M: 50M	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml
	70M: 30 A	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml
	100 M	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml
	100 A	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml	>10 cfu/ml

TAC: Total aerobic count; 30M: 70A -30% Moringa: 70% Aloe; 50M: 50A - 50% Moringa: 50% Aloe; 70M: 30A - 70% Moringa: 30% Aloe; 100M - 100% Moringa; 100A - 100% Aloe.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1 Nutritional and phytochemical constituents of Moringa-aloe beverage blends

There is an increase in the demand of functional foods due to deteriorating health of the general population. The aim of the study was to develop a functional beverage by blending *Moringa oleifera* extract and *Aloe vera* juice at different proportions. Results showed that the beverage blends had pH values ranging from 3.66 - 4.15, which was excellent since Dissanayake (2017) reported that the pH of a beverage should be less than 4.2 in order to prevent microbial growth and thus increase shelf-life. The titratable acidity (TA) of the beverages ranged from 0.12 - 0.14 g/ 100g and there was no significant difference ($p>0.05$) in TA between the beverage control samples and beverage blends. A recent study investigated the effect of pH and TA of commercial beverages on *in vivo* salivary pH after beverage consumption (Tenuta et al., 2014). Their results concluded that salivary pH is influenced more by TA than pH after consumption of acidic beverages. In the context of the current study, this suggest that the TA of the beverage blends will not influence salivary pH differently since all beverages had TA that was statistically similar.

The total soluble solids (TSS) is a crude but important measure of the amount of sugar in a beverage since it partly includes vitamins, fructans, proteins, pigments, phenolics, and minerals (reviewed by Magwaza and Opara, 2015). In this study, the TSS of the beverages was lower than that of other studies due to the fact that stevia was used as a sugar substitute (Aftab, et al., 2016; Talib et al. 2016). This will be good for commercial purposes since the South African government enforced sugar tax on sugar-sweetened beverages in April 2018. The colour of the beverage blends increased in darkness and greenness with increase in *M. oleifera* concentration. The latter was thought to be due to high level of chlorophyll in Moringa leaves. In general, colour is known to influence acceptance of beverages by consumers. The protein content and the concentration of most minerals increased with Moringa addition. However, beverages with high Aloe concentration contained high levels of vitamin C.

The beverage blends with more Moringa contained high total phenolic content and total flavonoid content. Phenolics and flavonoids are reported by several researchers to exhibit anti-cancer, anti-

tumor, anti-inflammatory, antidiabetic and antioxidant activities (Shanmugavel et al., 2018; Boudreau and Beland, 2006). This suggest that beverage blends with Moringa may confer these benefits to consumers. The beverage blends of this study were also found to exhibit antioxidant activity with highest antioxidant activity observed with high Moringa concentration when determined with DPPH assay. With ABTS, the beverage blends exhibited similar ($p<0.05$) antioxidant activity. Antioxidants scavenge free radicals that may damage cells and cause degenerative diseases in humans. In food, antioxidants neutralize free radicals which then prolongs the shelf-life of the product (Wilson et al., 2017). It can therefore, be suggested that the Moringa-aloe beverage blends may confer the afore-mentioned health benefits associated with antioxidants.

5.2 Sensory quality and storage stability of Moringa-aloe beverage blends

Consumer acceptability was carried out here to determine how consumers would react to the beverage blends (30M: 70A, 50M: 50A and 70M: 30A) of the current study compared to beverages already in the market, referred to as market controls ($100M_{mc}$ and $100A_{mc}$) throughout the document. Consumer acceptability results showed that the $100A_{mc}$ beverage was the most preferred as it was scored highest for taste (7.08), flavour (6.76) and overall acceptability (7.04), followed by the 30M: 70A beverage blend with scores of 6.04, 6.12 and 6.26 for flavour, taste and overall acceptability, respectively. The least preferred beverage was the $100M_{mc}$. Overall, results showed that an increase in Moringa concentration negatively affected the acceptability of the beverage blends, which may be due to the strong herbal flavour of Moringa. However, it worth mentioning that increase in Moringa concentration was accompanied by increase in nutritional quality and phytochemical content before. Since consumer acceptability highly influences purchasing of the beverages, the best beverage blend would be 30M: 70A beverage blend. It is important to note that despite having low protein content, TFC and mineral content compared to the other beverage blends, this beverage blend contained the highest vitamin C content among the blends. Also, it contained more than 4-fold protein content than 100A beverage.

The storage stability of the beverage blends was determined at 4°C for a period of 12 weeks. This temperature was chosen based on literature to represent the best temperature that will ensure extended shelf life of the product. Temperature abuse may however, occur at retail level or during transportation of food products to the market. In this study, storage stability was monitored through

observing changes in pH, TA, TSS, colour and microbial quality. An increase in pH occurred with all beverages with the highest final pH (4.33) observed in 100M beverage. The fact that pH of all beverages was below pH 4.6 after 12 weeks was good because growth of most microorganism is inhibited below pH 4.6 (USDA, 2012). Microbiological analysis of the beverage blends agreed with this as no counts were observed for total aerobic bacteria and yeast and molds. As expected, the increase in pH was accompanied by a decrease in TA possibly due to acid hydrolysis of sugar and polysaccharides by enzymes (Bhardwaj and Pandey, 2011). Similarly, an increase in TSS was observed with all beverages at the end of storage. Increases in TSS during storage have been associated with improved storage stability of beverage products. With regards to microorganisms, this increases the osmotic potential and thus negatively affect microbial growth.

The colour of the beverage blends was throughout the storage period, which might have been due to degradation of chlorophyll or non-enzymatic browning in a solution that contains sugars and proteins. There was no microbial growth throughout the 12-week storage period, which indicates that preservation methods were successful in inhibiting microbial growth and preserving the beverage blends. Overall, the Moringa-aloe beverage has the potential for commercial exploitation as a refreshing health drink.

5.3 Conclusions and recommendations

To our knowledge, this is the first study that investigated the potential of blending *Moringa oleifera* leaf extract and *Aloe vera* juice at different proportions for development of a functional beverage. Beverage blends with Moringa have high protein content, mineral content, TPC, TFC and antioxidant activity when determined with DPPH assay. However, beverage blends with more Aloe have high vitamin C content compared to beverages with high Moringa concentration. Sensory evaluation indicates that beverage blends with high concentration of Aloe are more preferred by consumers, while increase in Moringa extract makes the beverages less desirable to consumers. The beverages blends appeared stable after 12 weeks at 4°C as shown by the lack of microbial growth, slight changes in pH and TA. Overall, the current study shows that beverage bends with high concentration of Moringa have improved nutritional quality and possess health promoting properties. However, they are least preferred by consumers. Therefore, the 30M: 70A

beverage seems to be the best with respect consumer acceptability, storage stability and in part, nutritional quality and health promoting properties.

Future research must investigate the shelf life of the product at various temperatures to ascertain what happens in cases where there is temperature abuse. Such investigations must be accompanied by monitoring the microbiological quality, pH, TA, colour, loss of vitamins and phytochemical profile at various temperatures. The phytochemical profile of the beverage blends must also be determined using liquid chromatography mass spectrometry. This will help to identify the major phytochemicals that are improved with blending of the two plants. Lastly, the storage stability tests and sensory evaluation study must be conducted for more than 12 weeks to determine the exact shelf life of the product.

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APPENDIX A: STANDARD CURVE FIGURES FOR PHYTOCHEMICALS

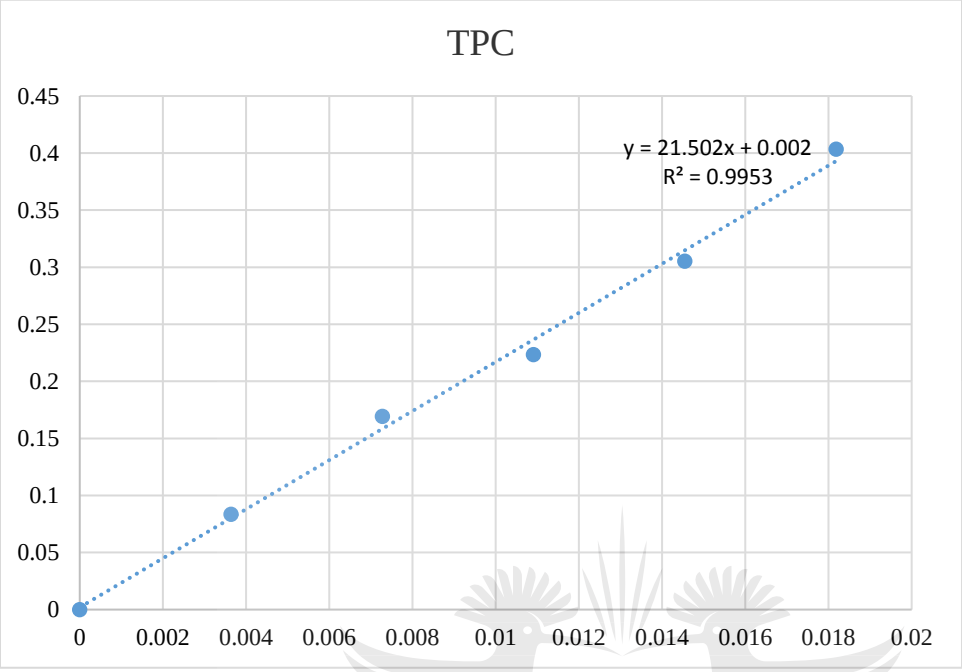

Figure A1: TPC standard curve

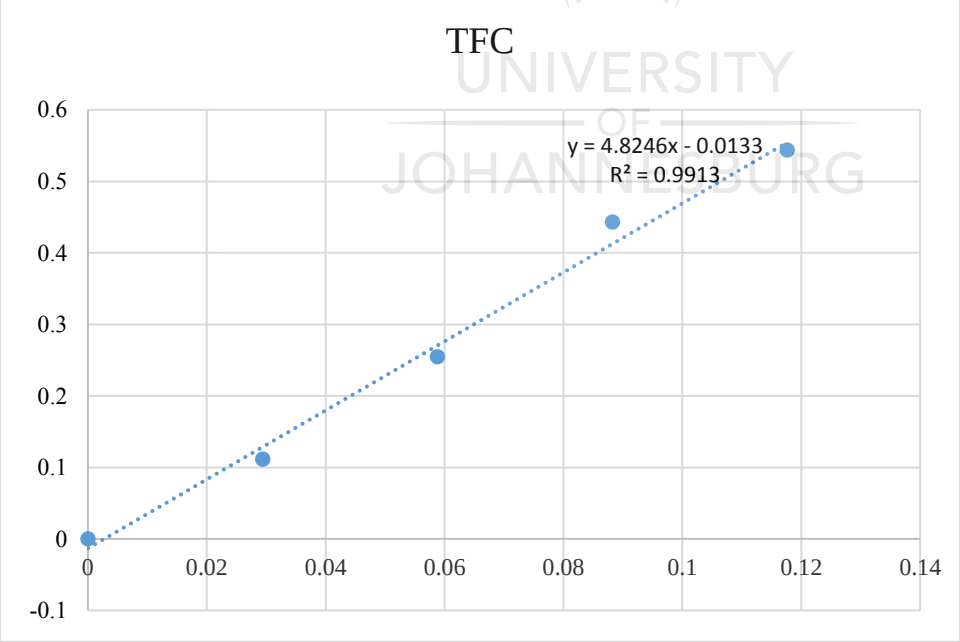

Figure A2: TFC standard curve

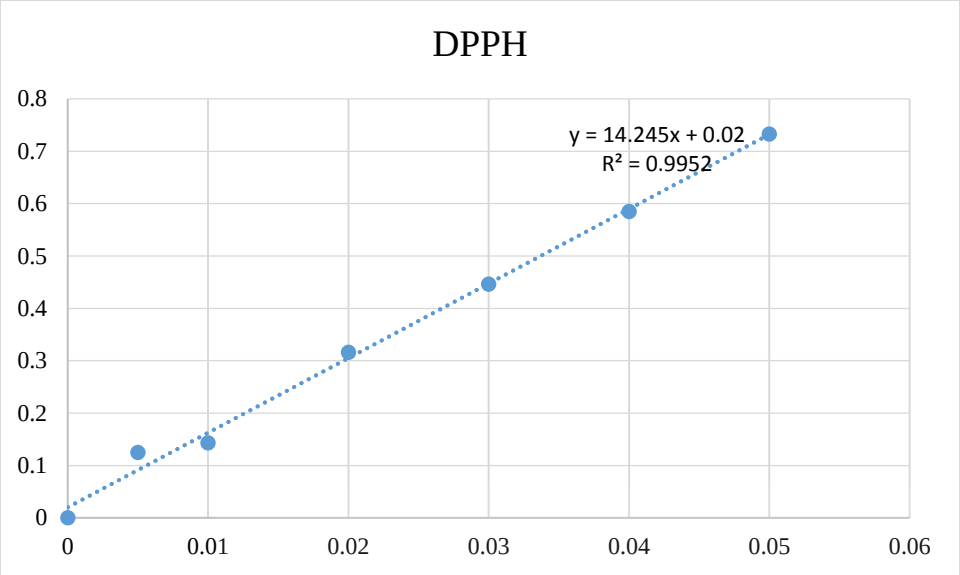

Figure A3: DPPH standard curve

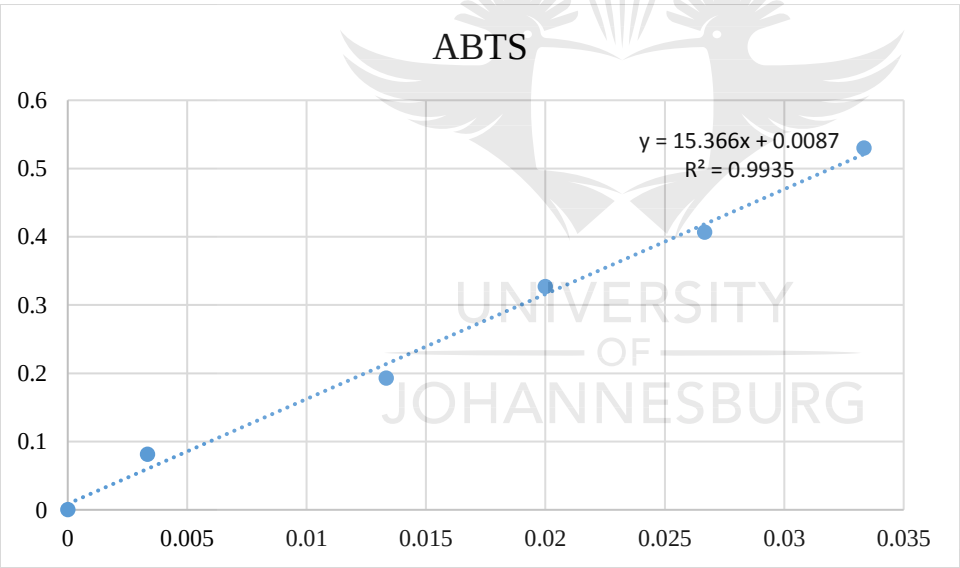

Figure A4: ABTS standard curve

APPENDIX B: ETHICS APPROVAL

FACULTY OF SCIENCE

FACULTY ETHICS COMMITTEE

Ethics Reference Number: 2019-10-10/ Dlamini_Thobakgale

Researcher:	**Thobakgale MR** (201326909)
Supervisor:	Dr BC Dlamini
Department:	Biotechnology and Food Technology
Project Title:	**Quality characteristics of a functional beverage developed from *Moringa oleifera* and *Aloe vera***
Programme:	**MSc Food Technology**

18 October 2019

Dear Dr Dlamini

Re: Feedback on your application for ethical clearance

Status – Approval

With reference to your application for ethical clearance to use humans for testing/ research purposes that served on 10 October 2019, the Ethics Committee of the Faculty of Science, University of Johannesburg, reviewed and approved the application on condition that all permits and relevant documents are in place. Please also send these documents to the Ethics Committee for record purposes.

Sincerely yours

Bettine van Vuuren
Chair: Faculty of Science Ethics Committee
University of Johannesburg

APPENDIX C: SENSORY SCORE SHEET

Score sheet

Hedonic rating scale

Panelist name…..…….…… Date……..

Sample characteristics: Colour, Flavour, Taste and Overall acceptability.

Score value assigned:

1 – Dislike extremely 4 – Dislike slightly 7 – Like moderately

2 – Dislike very much 5 – Neither like nor dislike 8 – Like very much

3 – Dislike moderately 6 – Like slightly 9 – Like extremely

Sample Characteristics	Sample codes				
	010	020	030	040	050
Colour					
Flavour					
Taste					
Overall acceptability					
Comments:					

APPENDIX D: CONSENT FORM FOR SENSORY ANALYSIS

FACULTY OF SCIENCE

Department of Biotechnology & Food Technology

2019

LETTER OF CONSENT TO PARTICIPATE CONSENT

I……..(Full names of participant) hereby confirm that I understand the contents of this Sensory Evaluation and the nature of the research project and I consent to participating in the research project. I understand that I am at liberty to withdraw from the research study project at any time, should I so desire.

……. .....

SIGNATURE OF PARTICIPANT DATE