CHEMISTRY AND BIOTRANSFORMATION OF *Uapaca kirkiana* PULP IN DEVELOPMENT OF A FUNCTIONAL FOOD USING A PROBIOTIC

By

CHAWAFAMBIRA ARMISTICE

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Promotor : Prof MM Sedibe
Co-promotor : Dr A Mpofu
Co-promotor : Dr MC Achilonu

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DECLARATION

I, **Chawafambira Armistice**, student number , do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree DOCTOR TECHNOLOGIAE: AGRICULTURE, is my own independent work, and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

Signature

19 February 2020
Date

DEDICATION

To God Almighty, my sanctuary, my rock on which I stand, my protector and redeemer, full of love and joy, the ultimate source of inspiration, wisdom, knowledge, and understanding. *Ebenezer* this far you have taken me, glory be unto you, Lord. To my adoring wife Carol, thanks for being a pillar of strength when it seemed tough and impossible. I could not have done this without you by my side. Michael and Michealla you always brought a smile when I was stressed. Thank you guys and I salute you.

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ABSTRACT

Modern advances in understanding the relationship between nutrition and health have resulted in the development of the concept of functional foods, which is a new practical approach earmarked to promote optimal health status. Underutilised wild fruits have great potential to improve overall human nutrition and help to mitigate malnutrition related problems faced by most communities in Sub-Saharan Africa. This research focused on the chemistry and biotransformation of *Uapaca kirkiana* pulp in the development of a functional food using a probiotic, *Lactobacillus rhamnosus* yoba. *Lactobacillus rhamnosus* yoba is a Gram negative, lactic acid bacterium, and generic probiotic of *L. rhamnosus* GG. The specific objectives of the study were the following: to determine the bioactive compounds, physiochemical properties, and functional potential of a highly nutritious, but underutilized *U. kirkiana* Muell. Arg (wild loquat) fruit; to produce a probiotic jam; to determine the functional properties of the jam and the bioaccessibility of iron and zinc, and its sensorial qualities. Ripe fruits were obtained from the Bikita, Gokwe, and Kazangarare areas in Zimbabwe and the bioactive phytochemical constituents, physicochemical properties and functional characteristics of the fruit pulp were analysed. The total soluble sugars, individual sugars and mineral contents in the fruit pulp were determined. Ascorbic acid was determined using the 2,6-Dichlorophenolindophenol (DCPIP) titration test. The total phenolic, tannin, and flavonoid contents were analysed using the Folin-Ciocalteu test, tannin binding test, and vanillin test, respectively. A composite pulp sample was obtained and its physicochemical properties (vitamin C, total titratable acid (TTA), pH, total soluble solids (TSS), antioxidant activity (AOA), moisture, and % pectin) were analysed before jam making. A probiotic jam was developed using the formulation- 55 % (wt/vol) pulp, 46 % (wt/vol) sugar, 1.5 % (wt/vol) pectin, and 0.5 % (wt/vol) citric acid. After preparation of the jam, the probiotic, *L. rhamnosus* yoba was inoculated into the jam, and the control jam sample was inoculated with distilled water. The viability of *L. rhamnosus* yoba in the jam was determined before consumption. Functional properties (vitamin C, total titratable acid (TTA), pH, total soluble solids (TSS), antioxidant activity (AOA), and moisture) of the jam inoculated with *L. rhamnosus yoba* were determined. Iron and zinc bioaccessibility in the probiotic jam were analysed using the *in vitro* simulated

digestion protocol. The sensory evaluation of the jam was conducted by trained (n = 20) and untrained (n = 130) panellists. Sensory attributes, including taste, appearance, aroma, spreadability, mouthfeel, and texture were scored using a 9 point hedonic scale. A triangle test and preference test for overall acceptance were conducted. Pulp yield ranged from 12.15 ± 0.16 g/100 g to 15.09 ± 0.27 g/100 g and was significantly different (F = 158.71, $p < 0.0001$) in all fruits from the three study areas, and accounted for 96 % of the variation in the fruit. The TTA (0.3–0.48 g/kg) and pH (4.3–4.6) values of the pulp were significantly different (F = 12.58; $P<0.0001$ and F = 15.66, $P< 0.0001$, respectively) in fruits obtained from the three sampling areas. Fruit properties varied amongst the three study site and this was contributed by pH (74 %) level and TTA (69 %) content. The TSS (sugar content) was significantly different (F = 4.66, $P < 0.0071$) and accounted for 45 % of the fruit variation. There was a strong relationship between TTA and pH ($r^2 = 0.79$); TTA and antioxidant ($r^2 = 0.72$); and pH and phosphorus ($r^2 =0.81$). The iron content ranged between 11.25 ± 0.52 mg/100 g to 12.16 ± 0.54 mg/100 g. Phosphorus, sodium and iron accounted for approximately 73 %, 50 %, and 43 % of the variation, respectively. The vitamin C content accounted for 27 % of the variation. Fructose was the dominant sugar. Tannins, flavonoids, and gallotannins were present. The fruit pulp had a total phenolic content of 67.0–82.5 µg GAE/g. Principal components 1 and 2 which represented physiochemical and functional properties of the pulp had eigenvalues of 5.59 and 2.13, and a variability of 37.31 % and 14.17 %, respectively. The jam inoculated with *L. rhamnosus* yoba had a vitamin, TTA, brix, and moisture content of 0.34 ±0.02 mg/100 g, 2.2 ± 0.11, 68.5 ± 0.2, and 34.8 ± 1.2, respectively. The fruit pulp had an antioxidant activity of 35 ± 1.02 %. Immediately after production, the jam inoculated with *L. rhamnosus yoba* had an iron and zinc content of 4.13 ± 0.52 mg/100 g and 0.36 ± 0.02 mg/100 g, respectively. The jam inoculated with *L. rhamnosus* yoba exhibited high fructose and sucrose content of 12.84 ± 0.21 g/100 g and 24.61 ± 0.12 g/100 g, respectively. Further, the jam inoculated with *L. rhamnosus* yoba had a TTA content of 2.2 at d 0 (after production), 2.37 ± 0.01 at d 4, and 2.48 ± 0.02 at d 7 of storage (25 °C). The jam inoculated with *L. rhamnosus* yoba had an iron bioaccessibility of 6.55 ± 0.36 % and a zinc bioaccessibility of 16.1 ± 0.50 %. The use of *L. rhamnosus* yoba in the jam showed a 4 % and 2 % increase in the iron and zinc bioaccessibility, respectively. *L. rhamnosus* yoba jam had mean scores of 7.5, 7.0, 6.0, and 6.5 for spreadability, taste, appearance, and mouthfeel, respectively. The jam inoculated with *L. rhamnosus* yoba had an overall acceptance score of 7.5 (*n = 120).* The good chemical and functional properties of the

fruit pulp resulted in the utilisation of the fruit pulp in producing a probiotic jam through the biotransformation of nutrients. The fruit jam was able to deliver 6.2 ± 0.2 log CFU/mL live *L. rhamnosus* yoba cells, which make it a good probiotic food with possible functional benefits.

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CHAPTER ONE
BACKGROUND AND INTRODUCTION

1.1 Background of the study

Sub-Saharan Africa is home to some of the most nutritionally insecure people in the world; notably, women and children suffer from insufficient intake of protein and energy, and lack of micronutrients (Stevens *et al.*, 2012; WHO, 2017). In Zimbabwe, the prevalence of stunting was 26 % in 2018 and it remains above the acceptable global threshold of 20 % (NNS, 2018). In addition, 35 districts had prevalence greater than the national average (26 %), while 14 districts are in the high prevalence category (30–39 %), according to the World Health Organization (WHO) standards. The prevalence of wasting and malnourishment are 2.5 % and 8.8 %, respectively (NNS, 2018). Mineral undernutrition, particularly iron and zinc deficiencies have been reported to be the prevalent nutritional problems worldwide (Platel and Srinivasan, 2016). A WHO report (2015) states that iron deficiency is the most common micronutrient deficiency that affects over 30 % of the global population , with women and children representing a greater percentage of effected individuals in developing nations.

The Zimbabwe Demographic Health Surveys (2016) report showed that children aged 6–59 months and women, 15–49 years old, living in drought-prone areas exhibited iron deficiency and anaemia rates of 37.5 % and 31.2 %, respectively. According to the UNICEF (2018) one out of every three children under the age of five years is iron deficient, 72 % of the children are living with iron deficiency, and 26 % of the women in the reproductive age group are anaemic. The malnourished children are susceptible to disease, may suffer from cognitive impairments, have poorer educational outcomes, and are likely to experience reduced productivity in their endeavours (Mpofu *et al.*, 2014).

The use of indigenous fruits has great potential to improve nutrition. In sub-Saharan Africa, indigenous fruits play an important role in providing food, well-being, and financial stability (Ngadze *et al.*, 2017). Traditional foods (wild fruits) are safe and nutritious and communities can afford to buy it (Mpofu *et al.*, 2014). The poor and vulnerable women and children, especially in drier, rural areas have been known to intensively use wild fruits in Zimbabwe (Campbell, Luckert

and Scoones, 1997). *Uapaca kirkiana* also known as wild loquat is an underutilised indigenous fruit that is well-adapted to the miombo ecological zone in sub-Saharan Africa and is consumed as part of the diet (Akinnifesi *et al.*, 2004; Saka *et al.*, 2004; Nhukarume *et al.*, 2010; Bille, Shikongo-Nambab and Cheikhyoussef, 2013; Mpofu *et al.*, 2014) during times of droughts (Mithöfer and Waibel, 2003; Legwaila *et al.* 2011), especially in semi-arid areas.

Due to diverse species, soil differences, and environmental patterns, indigenous fruits have varied functional properties and are rich in phytochemicals. Phytochemicals are a group of non-nutritive, active biological compounds, such as phenolic acids, carotenoids, and flavonoids (Fernandes *et al.*, 2011; Alasalvar and Shahidi, 2012) that have been found to have health protective properties, for example, providing protection against aging, inflammation, and certain cancers (Shofian *et al.*, 2011). Their protective effects can be attributed to their ability to act as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and or metal chelators (Ikram *et al.*, 2009). However, there are no reports regarding the health benefits of other phytoconstituents that are present in the fruit. Therefore, I determined the functional and phytochemical properties of the *U. kirkiana* fruit pulp. Such data was vital as a guide for processing the fruit into a functional food. While filling the gaps in research regarding the indigenous knowledge systems on the fruit, the research also documented the functional, phytochemical, and nutritional properties, and the mineral bioaccessibility of the *U. kirkiana* fruit pulp and of the *U. kirkiana* probiotic jam containing *L. rhamnosus* yoba.

Structurally, *Uapaca kirkiana* fruit has a fleshy brown skin with a juicy pulp (Moombe *et al.*, 2014). *U. kirkiana* fruit is a source of protein (Akinnifesi *et al.*, 2008, Ndabikunze, Masambu and Tiisekwa, 2010, and Vinceti *et al.*, 2013), carbohydrate (Stadlmayr *et al.*, 2013), and sugar (Akinnifesi *et al.*, 2008, Ndabikunze, Masambu, and Tiisekwa, 2010, and Vinceti *et al.*, 2013). The fruit contains essential sugars (Stadlmayr *et al.*, 2013) and is a good source of minerals, including iron, zinc, calcium, and potassium (Ndabikunze, Masambu and Tiisekwa, 2010). Therefore, wild loquat fruit could be used as an inexpensive source of iron in the diets of pregnant women and children. The fruit ripens in November (Mithöfer and Waibel, 2003). Ripe fruits are normally picked from the ground and/or plucked from the tree for consumption and marketing (Mithöfer and Waibel, 2003; Akinnifesi *et al.* 2004). Unripe fruits are kept in the soil to induce

ripening (Maroyi, 2013). The ripe fruits are often sold at the roadside and in most rural markets in sub-Saharan Africa. The fruit is of socio-economic importance amongst the rural and urban poor. *U. kirkiana* was found to be the most preferred indigenous fruit among farmers and consumers in Zambia (Akinnifesi *et al.*, 2004; Franzel, Akinnifesi and Ham, 2008; Kalaba, Chirwa and Prozesky, 2009; Moombe *et al.*, 2014) because of its sweetness and nutritional value (Saka *et al.*, 2007; Ramadhani and Schmidt, 2008), and thus has better market growth prospects and characteristic uses (Ramadhani and Schmidt, 2008).

Developing a functional food from *U. kirkiana* fruit pulp using probiotic technology would aid in the provision of a healthy nutrient-dense food to the poor communities of sub-Saharan Africa. A report of the Functional Food Centre by Martirosyan and Singh (2015) defines a functional food as a natural or processed food that contains known or unknown biologically active compounds, which are defined as, “effective and non-toxic compounds that provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease.” The Food and Agricultural Organisation and the World Health Organisation (FAO/WHO, 2001) define probiotics as, “live microorganisms, which when consumed as part of food in adequate amounts, confer a health benefit on the host beyond inherent general nutrition, including the improvement of the intestinal microbial balance."

Lactobacillus rhamnosus GG is a lactic acid bacterium and is the most studied probiotic (von Ossowski *et al.*, 2010; Kort and Sybesma, 2012). A probiotic is generally regarded as safe (GRAS) and meets the requirements of clinical trials (FAO/WHO, 2001). Szajewska *et al*., (2007) have shown that *L. rhamnosus* GG can result in reduced treatment days for acute diarrhoea in children in ten European countries. There is no report regarding the use of the probiotic, *L. rhamnosus* yoba in *U. kirkiana* fruit pulp processing in sub-Saharan Africa. Incorporation of *L. rhamnosus* yoba into *U. kirkiana* fruit pulp-based food would help to produce a functional food that will improve nutrient absorption in the intestinal mucosa.

Of late, the idea of providing access to the use of probiotics for populations, especially in the developing world has led to the introduction of generic probiotics (Kort and Sybesma, 2012). *L. rhamnosus* yoba is one example of a generic probiotic of *L. rhamnosus* GG. An isolate was

obtained from a commercial product containing *L. rhamnosus* GG and was identified, and confirmed using 16S rRNA sequencing (Kort and Sybesma, 2012). This isolate had been deposited at the Belgian Co-ordinated Collections of Microorganisms/Laboratorium voor Microbiologie Gent (BCCM/LMG) culture collection under the name *L. rhamnosus* yoba (Kort and Sybesma, 2012). In this thesis, *L. rhamnosus* yoba was used in the production of a functional food (probiotic jam).

1.2 Problem Statement

U. kirkiana fruit has the potential to improve the nutritional status, food security, and the livelihood of rural populations in sub-Saharan Africa, especially in the drier, rural regions of Zimbabwe. However, the contribution of indigenous fruits to nutritional requirements and poverty reduction efforts is often unrecognised. The *U. kirkiana* fruit is underutilised and the traditional claims with respect to its nutritional and health benefits need to be verified and validated by scientific data. It is possible to improve the nutritional and economic benefits of the fruit at a household level by processing the fruit into different products and commercialising them as health food products. Indigenous knowledge systems regarding the processing of *U. kirkiana* fruit at a rural household level need to be verified, upgraded, and optimised as they are diverse as well as unreliable. Thus, it is imperative to study the compounds with nutritive and health beneficial in the fruit and determine their value and accessibility.

The impact of processing of the fruit pulp on the physicochemical, nutritional, functional, and sensorial properties, and digestibility of the food products is not well documented. *U. kirkiana* is a popular indigenous fruit that has adapted to the drier areas of Zimbabwe such as the Gokwe and Bikita districts, which are known to have a high prevalence of micronutrient deficiencies. Traditionally, the fruit is eaten when ripe, without processing. Application of new food processing technologies is therefore of paramount importance in order to provide the communities with healthy foods that can help reverse these micronutrient deficiencies. There is need to explore the use of probiotics (*L. rhamnosus* yoba) in processing the underutilised *U. kirkiana* fruit into a functional food. After establishing the presence of compounds with significant health and nutritive benefits in the food, it is necessary to determine the bioaccessibility of the essential minerals (iron and zinc). This becomes very important as the presence of compounds with health or nutrients

benefits in food material does not automatically render them bioaccessible for absorption after digestion in the intestines.

1.3 Justification

This study focus on *U. kirkiana* fruit is important as the species is deemed essential and opens the possibility for utilising its nutritional and functional properties. The research will achieve a scientific understanding of the potential nutritional and health benefits of *U. kirkiana* fruit by identification and characterisation of its key health promoting components. Determining the bioactive compounds, functional properties, nutritional composition, and bioaccessibility of minerals will help to substantiate the indigenous knowledge claims on the value of the fruit and guide future value addition efforts accordingly. Modern day value addition and product development should be guided by strong nutritional knowledge and how the nutrients behave during processing and subsequent digestion, hence the need to determine the bioaccessibility of essential micronutrients (iron and zinc). As the *U. kirkiana* fruit trees are widely distributed in Zimbabwe, its presence in drought-prone areas and semi-arid regions will hlep the people known to be affected by poor nutritional status in these areas.

The fruit can also providing essential micronutrients (iron and zinc), alleviating food insecurity, and improve livelihoods and health benefits. There is great potential in processing the *U. kirkiana* fruit pulp into a functional food with nutritional and possible health benefits. A probiotic jam from *U. kirkiana* fruit pulp that contains *L. rhamnosus* yoba, will act as a vehicle that provides beneficial, live microbes to the body, and its action will potentially improve micronutrient bioaccessibility once consumed. This study will ensure improved sensorial qualities of the *U. kirkiana* fruit-based functional food.

1.4 Objectives

1.4.1 Main Objective

The main objective of the study was to determine the chemistry and biotransformation of *U. kirkiana* pulp in the development of a functional food using a probiotic.

1.4.2 Specific Objective

The specific objectives of the study were:

- To determine the physicochemical and nutritional composition of ripe *U. kirkiana* fruit pulp from selected areas,
- To determine the functional and bioactive properties of the composite fruit pulp.
- To develop a fruit jam incorporating a probiotic, *L. rhamnosus* yoba,
- To determine the effect of processing on the biotransformation of bioactive compounds, nutritional, and functional properties of the functional food (probiotic jam),
- To determine the effects of incorporating *L. rhamnosus* yoba on the bioaccessibility of iron and zinc and
- To determine the sensory qualities and customer preferences with respect to the *L. rhamnosus* yoba fruit jam.

1.5 Hypotheses

H_0: *L. rhamnosus* yoba use has no effect on biotransformation of bioactive compounds, and the nutritional and functional properties of the functional food (probiotic *U. kirkiana* jam)**.**

H_0: *L. rhamnosus* yoba use has no effect on the bioaccessibility of iron and zinc.

H_0: *L. rhamnosus* yoba use has no effect on the sensory qualities and customer preferences with respect to the probiotic *U. kirkiana* jam.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Indigenous fruit: *Uapaca kirkiana*

In sub-Saharan Africa, indigenous fruits play a vital role as a food source (Ngadze *et al.*, 2017). Indigenous fruits are mainly used to supplement the diet by many rural folks (Mithofer and Waibel, 2003; Akinnifesi *et al.*, 2004; Saka *et al.*, 2004; Nhukarume *et al*., 2010; Legwaila *et al.*, 2011; Bille, Shikongo-Nambab and Cheikhyoussef, 2013; Mpofu *et al.*, 2014;), and have the potential to mitigate nutritional deficiencies (Campbell *et al.*, 2002). Consumption of the fruits is important amongst the poor and vulnerable groups in the community (Maghembe *et al.*, 1998; Cunningham, 2002; Tiisekwa *et al.*, 2004) because they cannot afford to buy food to feed themselves. Traditional foods (wild fruits) have a history of being safe and nutritious and affordable (Mpofu *et al.*, 2014). The *U. kirkiana* fruit tree is closely related to other species such as *U. bengelensis, U. nitida, U. pilosa, and U. robynsii*, which are not commonly found in Zimbabwe (Ngulube, 1996). The fruit tree is mainly distributed in semi-dry and dry areas although it can grow in some relatively wet areas of Zimbabwe. The species, *U. kirkiana* (Figure 2.1A) is one of the most dominant and abundant wild fruit tree in Zimbabwe (Ngulube and Hall, 1995; Kadzere *et al.*, 2001). The tree produces fruits which ripen during the period October to February in Zimbabwe (Figure 2.1B).

Figure 2.1: *Uapaca kirkiana* tree (A), *U. kirkiana* fruits (B), and *U. kirkiana* pulp and seed (C).

The fruit is known by different names from different locations and countries; wild loquat (English), *msuku/nkusu* in Malawi, Tanzania, and Zambia, and *mahobohobo* or *mazhanje* in Zimbabwe (Akinnifesi *et al.*, 2004). The fruit is oval shaped (Figure 2.1C), and contains seeds and a yellowish pulp (Moombe *et al.*, 2013). Unripe fruits usually have a relatively poor taste (Kadzere *et al.*, 2001). The ripe fruit has a sweet taste (Mithofer and Waibel, 2003; Maroyi, 2014). Wild loquat fruit can produce a pulp yield of 28.6 % of total fruit weight (Ndabikunze *et al.*, 2010). In Zimbabwe, the poor and vulnerable woman and children, especially from drier rural areas have been cited as intensive users of these wild fruits (Campbell, Luckert and Scoones, 1997) due to their good sensorial attributes, availability, and perceived health benefits (Chifamba, 2011). Domestication of the fruit tree can therefore be suggested (Mwamba, 1988; Maghembe *et al.*, 1993; Katsvaga *et al.*, 2007).

2.2 Nutritional composition of *U. kirkiana* fruit pulp

Nutritionally, *U. kirkiana* fruit contains protein (Akinnifesi *et al.*, 2008; Ndabikunze *et al.*, 2010; Vincete *et al.*, 2013), carbohydrates (Sufi and Kaputo, 1977; Saka, Msonthi and Sambo, 1992), and essential sugars (Akinnifesi *et al.*, 2008; Ndabikunze *et al.*, 2010; Vincete *et al.*, 2013). Macronutrient composition of *U. kirkiana* fruit compares well with other wild fruits from sub-Saharan Africa (Table 2.1). The fruit has a total carbohydrate content in the range of 28.7–92 g/100 g EP (Ngulube, 1996), which is greater than that found in *Ziziphus mauritiana* and *Irvingia edulis* fruits, but less than that found in *Adansonia digitata* and *Tamarindus indica* (Table 2.1). In a study by Saka and Msonthi (1994), the whole fruit was found to contain 86.5 % total carbohydrate, 8.4 % fibre, 1.1 % fat, 1.8 % crude protein, and 27.4 % DM. A review on specific sugars noted that *U. kirkiana* fruit contains 41 g/100 g glucose, 27 g/100 g fructose, 15 g/100 g sucrose, 2 g/100 g xylose, and traces of galactose, raffinose, and ribose (Sufi and Kaputo, 1977). These sugar levels are higher than those of exotic fruits such as apples, which were found to contain 5.7 g/100 g fructose, 0.6 g/100 g glucose, and 0.57 g/100 g sucrose (Ngadze *et al.*, 2017). Studies by Ngulube (1996), and Malaisse and Parent (1984) reported that related species of *U. kirkiana* had a carbohydrate content of 890 g kg^{-1}, 829 g kg^{-1}, 883 g kg^{-1}, and 912 g kg^{-1} (*U. bengelensis, U. nitida, U. pilosa*, and *U. robynsii* species, respectively). These observations explain the fact that other related species of *U. kirkiana* have a carbohydrate content that is almost identical to that of

Adansonia digitata and *Tamarindus indica*. A comparative study of the nutritional content of selected exotic fruits and *U. kirkiana* has noted that the fruit has a higher carbohydrate content (28.7 g/100 g EP) as compared to that of most exotic fruits (Table 2.2). Furthermore, *U. kirkiana* fruit compares well in energy provision and has a high caloric value of 523 Kcal/KJ. This suggests that the fruit is an excellent source of energy as compared to other exotic fruits, such as apples, oranges, and mangoes.

A review on analysis of the protein content data amongst the major indigenous fruits of sub-Saharan Africa indicated that *U. kirkiana* had a protein content range of 0.3 g/100 g EP (Table 2.1) to 0.9 g/100 g EP (Ngulube, 1996; Jones, Lineback and Levine, 2006). This reviewed data showed that *U. kirkiana* is a poor source of protein as compared to other fruits such as *Adansonia digitata*. Furthermore studies by Malaisse and Parent (1984) on the *Uapaca* species reported a protein content of 0.8 g / 100 g for *U. nitida* and 0. 4g / 100 g for *U. kirkiana*. . Reviewed sources indicated that *U. kirkiana* has a fat content of 4 g kg^{-1} (Table 1), which was not significantly different from that of most indigenous fruits. The fat content was noted to be as high as 1.1 g/100 g EP (Saka, Msonthi and Sambo, 1992) in *U. kirkiana* fruit the, making it a relatively high fat content fruit as compared to other exotic fruits (Table 2.3) and indigenous fruits (Table 2.1). However, the *U. pilosa* species has the highest fat content of 34 g kg^{-1} (Malaisse and Parent, 1984; Ngulube, 1996). *U. kirkiana* has a fibre content of 2.0 g/100 g EP (Table 2.1). Dietary fibre intake reduces the risk of stroke (Steffen *et al.*, 2003; Whelton *et al.*, 2005), hypertension (Keenan *et al.*, 2002; Montonen *et al.*, 2003; Anderson, 2004), diabetes (Lairon, Arnault and Bertrais, 2005; Birketvedt *et al.*, 2005), and obesity (Brown *et al.*, 1999; Watzl, Girrbach and Roller, 2005), and potentially improves the immune system. The relatively high fibre content in the edible portion of the fruit could be correlated with the reported higher micronutrient contents (Table 2.2). The ash content ranged from 0.8 g/100 g EP (Table 1) to 3.2 g/100 g EP (Stadlmayr *et al.*, 2013). The fruit had an ash content that was comparable to that of *Ziziphus mauritiana* and *Irvingia edulis*. Ngulube *et al.* (1996) reported energy values of 13,780 KJ kg^{-1}, 14,200 KJ kg^{-1}, 14,620 KJ kg^{-1}, 14,820 KJ kg^{-1}, and 14,620 KJ kg^{-1} in *U. bengelensis, U. kirkiana, U. nitida, U. pilosa, and U. robynsii,* respectively. In a comparative study by Stadlmyar *et al.* (2013), the *U. kirkiana* fruit had high energy values of 523 Kcal kJ-1, which was higher than that of *Ziziphus mauritiana* (184 Kcal kJ^{-1}), *Vitex doniana*, (474 Kcal kJ^{-1}), and *Irvingiaga bonensis*, (364 Kcal kJ^{-1}). Comparative studies

on the water content showed that *U. robynsis*, a related species of *U. kirkiana* has the highest water content of 83.0 g/100 g (Ngulube *et al.*, 1996). Findings from literature indicate that *U. kirkiana* fruit has a fresh fruit water content of more than 50 %, suggesting a high water activity in the fruit, which can affect its microbiological activity, shelf life, and storage quality. The fresh fruit pulp had a total plate count (aerobic bacteria) of 2.2 x 10^3 as compared to *S. berrea* pulp and *A. digitata* pulp that had a total plate count of 2.4 x 10^3 (Ndabikunze *et al.*, 2010).

Table 2.1: Nutritional composition of some indigenous fruits of sub-Saharan Africa.

Nutritional composition of selected indigenous fruit of Southern Africa per 100 g EP								
Fruit Name	[a]Energy (kcal/KJ)	Water (g)	[b]Proteins (g)	[c]Fats (g)	[d]CHOs (g)	[e]Fibre (g)	Ash (g)	Reference
Adansonia digitata	1380	10	2.3	0.7	74.9	7.8	4.8	(Osman, 2004; Abdel-Rahm, Mohammed and Mohammed, 2011)
Sclerocarya berrea	2703	87	0.9	0.5	3.7	2.9	3.8	(Jaenicke and Thiong'o, 2000; Thiongo's, Kingori and Jaenicke, 2002)
Uapaca kirkiana	523	72	0.3	0.4	28.7	2.0	0.8	(Malaisse and Parent, 1984; Saka, Msonthi and Sambo, 1992)
Ziziphus mauritiana	184	92	0.35	0.7	8.3	1.2	0.8	(Saka, Msonthi and Sambo, 1992; Lockett, Calvert and Grivetti, 2000)
Tamarindus indica	1160	28	3.8	1.1	60.4	6.0	2.4	(Soloviev *et al.*, 2004; Fentahun and Hager, 2009)
Strychnos cocculoides	1315.4	78.8	3.2	0.3	16.8	25.2	0.5	(Ngadze *et al.*, 2017)

[a]Metabolisable energy calculated based on protein 4 (kcal/17 KJ), fat 9(kcal/37 KJ), CHOs 4(kcal/ 17 KJ), Fibre 2(kcal/8 KJ)
[b]Protein values obtained using Kjeldahl method
[c]Fat values by Soxhlet method
[d]CHOs calculated by difference method
[e]Fibre represents crude fibre

Table 2.2: Comparison of the nutritional content of selected exotic fruits and Uapaca kirkiana per **100 g edible portion (EP).**

	Water (g)	CHOs (g)	Protein (g)	Fibre (g)	Fat (g)	Calories (kcal/KJ)	Vitamin (mg)	References
Wild loquat	72	28.7	0.3	2.0	0.4	523	16.8	(Malaisse and Parent, 1984; Saka, Msonthi and Sambo, 1992)
Apples	85.3	15.25	0.19	2.7	0.36	207	5.7	(Jensen *et al.*, 2015)
Mangoes	84	15	1.0	1.6	0.4	255	53	(Kazii, Yadaw and Agele, 2015)
Oranges	87	10.6	1.0	1.8	-	198	49	(Kazii, Yadaw and Agele, 2015)
Avocados	81	7.0	2.0	0.2	15.4	523	8.80	(USDA, 2011)
Peaches	89	7.9	1.0	1.4	-	151	8	(Kazii, Yadaw and Agele, 2015)

The review of literature indicated that *U. kirkiana* fruit pulp has a mean iron content of (11.8 mg/100 g EP), zinc (1.3 mg/100 g EP), sodium (10 mg/100 g EP), calcium (17 mg/100 g EP), potassium (375 mg/100 g EP), and magnesium (39 mg/100 g EP) (Table 2.3). From the reviewed data in Table 2.3, it is evident that *U. kirkiana* fruit can be used as an important source of iron and zinc because its iron content (11.8 mg/100 g EP) is higher than that in most indigenous fruits and its zinc content shows a no significant difference with respect to wild fruits. A comparative analysis of mineral content of *U. kirkiana* and exotic fruits revealed that *U. kirkiana* is an excellent source of iron, zinc, magnesium, and sodium, as shown in Table 2.4. These comparative results in Table 2.4 make *U. kirkiana* the best source of essential minerals and can be used to improve iron and zinc nutrition upon consumption of the fruit. Therefore, there is a need to carry out more mineral assays and to ascertain their bioaccessibility and/or bioavailability in the human body up on

consumption. The studies on vitamin C content revealed that the fruit has an ascorbic acid content of 14.5 mg g^{-1} (Stadlmayr *et al.*, 2013), while Saka and Msonthi (1994) reported a vitamin C content of 168 mg kg^{-1} in the fruit fresh weight. Ndabikunze *et al.*, (2010) noted that the fruit pulp has a vitamin C content of 208 mg kg^{-1}, which is comparative to that of *V. mombassae* (410.3 mg kg^{-1}). Vitamin C acts as a cofactor, and protecting the oxidation of low-density lipoproteins (LDLs) and promotes the absorption of iron in the ileum (Padayatty and Levine, 2001). The WHO (1999) reported that the daily body requirement of vitamin C is between 45 mg to 80 mg. Consuming 247 mg of vitamin C daily improves iron absorption by 35 % (Cook and Reddy, 2001). Therefore, the *U. kirkiana* fruit can aid in iron absorption because of its relatively high vitamin C content.

Table 2.3: Comparative mineral content of selected indigenous fruits of sub-Saharan Africa.

Mineral composition of selected indigenous fruits of sub-Saharan Africa per 100 g EP									
Fruit Name	[a]Ca (mg)	[a]Fe (mg)	[a]Mg (mg)	[a]P (mg)	[a]K (mg)	[a]Na (mg)	[a]Zn (mg)	Vit. C (mg)	References
Adansonia digitata	401	8.1	285	62	2120	20.4	1.87	273	(Osman, 2004; Abdel-Rahm, Mohammed and Mohammed, 2011)
Uapaca kirkiana	17	11.8	39	15	375	10	1.3	16.8	(Malaisse and Parent, 1984; Saka, Msonthi and Sambo, 1992)
Sclerocarya berrea	67	8.0	33	-	601	2.7	5.19	167	(Jaenicke and Thiong'o, 2000; Thiongo's, Kingori and Jaenicke, 2002)
Ziziphus mauritiana	23	0.82	5	19	256	6	0.03	13.6	(Saka, Msonthi, and Sambo, 1992; Lockett, Calvert and Grivetti, 2000)
Tamarindis indica	192	4.4	66	87	933	30.2	3.1	15.5	(Soloviev *et al.*, 2004; Fentahun and Hager, 2009)
Strychnos cocculoides	46.5	70.5	137.2	116.5	959.2	4.5	0.4	34.2	(Ngadze *et al.*, 2017)

[a]Mineral values were obtained using atomic absorption spectroscopy and some calorimetric methods.

Table 2.4**:** Comparison of mineral content of selected exotic fruits and Uapaca kirkiana per 100 **g EP.**

Mineral content of selected exotic fruits and *U. kirkiana* per 100 g EP									
	Fe (mg)	Zn (mg)	Mg (mg)	Ca (mg)	K (mg)	P (mg)	Cu (mg)	Na (mg)	References
Wild liqout	11.8	1.3	39	17	375	15	0.1	10	(Malaisse and Parent, 1984; Saka, Msonthi and Sambo, 1992)
Apples	0.18	0.04	6.0	7.0	115	12.0	0.041	-	(Jensen *et al.*, 2015)
Mangoes	0.50	0.30	7.0	7	250	13	0.11	2	(Soloviev *et al.*, 2004)
Oranges	0.40	0.20	11	29	145	24	0.05	-	(Soloviev *et al.*, 2004)
Avocados	0.61	0.68	29.0	13.0	507	54	-	8.0	(Fentahun and Hager, 2009)
Peaches	0.20	0.1	6.0	6	186	19	0.07	-	(Soloviev *et al.*, 2004)

Table 2.5: *U. kirkiana* composition with recommended dietary allowance (RDA) and adequate intake (AI) for children (1-9yrs), children (9–13 yrs), adolescents (14–18 yrs), pregnant females (all ages).

		CHOs	Protein	Fibre	Zn	Fe	Mg	Ca	Vit C
		g/day	g/day	g/day	mg/ day	mg/ day	mg/ day	mg/ day	mg/ day
***U. kirkiana* composition**		**28.7**	**0.3**	**2.0**	**1.3**	**11.8**	**39**	**17**	**16.8**
RDA children (1-9yrs)	Man and Women	80[b]	25[b]	10[a]	5[b]	7[b]	130[b]	1000[b]	52[b]
% contribution of the fruit in children		**35**	**1.2**	**0.2**	**26**	**168**	**30**	**1.7**	**32**
RDA children (9–13 yrs)	Man	130 [b]	34 [b]	12 [a]	8 [b]	8 [b]	240 [b]	1300 [b]	45 [b]
	Women	130 [b]	34 [b]	12 [a]	8 [b]	8 [b]	240 [b]	1300 [b]	45 [b]
% contribution of the fruit in children		**22.0**	**0.8**	**16.6**	**16.2**	**147.5**	**16.2**	**1.3**	**37.3**
RDA adolescents (14–18 yrs)	Man	130 [b]	55 [b]	16 [a]	11 [b]	11 [b]	410 [b]	1300 [b]	75 [b]
	Women	130 [b]	52 [b]	17 [a]	9 [b]	15 [b]	360 [b]	1300 [b]	65 [b]
% contribution of the fruit in adolescents		**22.0**	**0.5**	**16.6**	**13**	**90.7**	**10.1**	**1.3**	**24**
RDA pregnant	All ages	175 [b]	41 [b]	28 [a]	12 [b]	27 [b]	350 [b]	1000 [b]	85 [b]
% contribution of the fruit in pregnant females		**16.4**	**0.7**	**7.1**	**10.8**	**43.7**	**11.1**	**1.7**	**16.7**

[a] : Adequate intake (AI) values [b] : Recommended dietary allowances (RDA) values

2.3 Functional properties of the fruit

U. kirkiana fruit has a higher mean pulp yield of 283.4 ± 3.91 g kg^{-1}, which was comparable to *S. berrea* (161.9 ± 1.97 g kg^{-1}), *A. digitata* (202.4 ± 4.4 g kg^{-1}), and *V. mombassea* (186.0 ± 4.59 g kg^{-1}) (Ndabikunze *et al.*, 2010). Ndabikunze *et al.* (2010) also noted that *U. kirkiana* fruit pulp has a high total soluble solids (TSS) content of 169 ± 0.14 g kg^{-1} as compared to *S. berrea* (133.0 ± 0.19 g kg^{-1}), *A. digitata* (116.3 ± 0.16 g kg^{-1}), and *V. mombassea* (123.3 ± 0.16 g kg^{-1}). The TSS values obtained by Ndabikunze *et al.* (2010) of the *U. kirkiana* fruit compare positively with those of other tropical fruits and mangoes (140 g / 100g) when used in the processing of commercial juices. This suggests the potential use of the fruit as an ingredient in juice production. Ndabikunze *et al.* (2010) noted that *U. kirkiana* pulp possessed a mean pH value of 4.67 ± 0.04. The mean pH value of the fruit pulp was 3.0–3.5 (Ndabikunze *et al.*, 2010), and is a critical attribute in its processing into products such as juices and jam. The mean total titratable acidity (TTA) of the fruit pulp was 0.5 ± 0.02 g kg^{-1} (Ndabikunze *et al.*, 2010). The acid content of ripe fruit pulp affects the biotransformation of nutrients during processing and product stability in juices. Therefore, there is need to improve the acid levels when processing the *U. kirkiana* in juice processing (FAO, 1999).

2.4 Bioactive compounds

Phytochemicals are a group of non-nutritive, active biological compounds, for example phenolic acids, flavonoids, and carotenoids (Fernandes *et al.*, 2011; Alasalvar and Shahidi, 2012), that have been found to confer health protective properties, such as preventive actions against aging, inflammation and certain cancers (Shofian *et al.*, 2011). Their protective property is attributed to their ability to act as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and metal chelators (Ikram *et al.*, 2009). *U. kirkiana* fruit contains tannins, which gives it an astringent taste (Muchuweti, Ndhlala and Kasiamhuru, 2006). Tannins reduce blood pressure, speed up blood clotting, reduce serum lipid levels, and adjust immune responses (Bele, Jadhav and Kadam, 2010). In wine, tannins exhibit potent antioxidant effects against low-density lipoprotein (LDL) (Bele, Jadhav and Kadam, 2010). Muchuweti, Ndhlala and Kasiamhuru (2006) reported that ripe and unripe sun-dried *U. kirkiana* pulp had tannin concentrations of 0.020 mg g^{-1} and 0.025 mg g^{-1}, respectively.

Golding *et al.* (1998) reported that the biochemical events that occur during the ripening of banana involve the conversion of starch into sugars, flesh softening, and aroma development (Gross *et al*, 1976), and in some cases, tannin biotransformation by UV light produces bioactive metabolites. The embryo part of the *U.kirkiana* fruit had the highest tannin concentration of 0.045 mg g^{-1}. Flavonol concentrations were found to be 0.4 mg g^{-1} and 0.3 mg g^{-1} in sun and oven-dried pulp samples, respectively (Muchuweti, Ndhlala and Kasiamhuru, 2006). Flavonoids are non-nutrient, bioactive compounds (Harnly *et al.*, 2006) that are absent in the fruit flesh (Aherne and Obrien, 2002), and help to inhibit the oxidation of low-density lipoproteins (LDL) cholesterol (Huxley and Neil, 2003; Rapizzi *et al.*, 2004; El-Sayed *et al.*, 2006; Chen *et al.*, 2007; Hollman, Geelen and Kromhout, 2010). In the reviewed experimental designs, a methanolic solution was used as the extraction medium; quantification of polyphenols was done using the Folin-Ciocalteu reagent, and that of flavonoids was measured by the vanillin assay.

2.5 Mineral bioaccessibility

Few studies have focused on the mineral composition in *U. kirkiana* fruit (Stadlmayr *et al.*, 2013). However, in terms of nutrition, it is not just enough to determine the total mineral content; it is therefore important and necessary to determine the bioaccessibility of these minerals, in other words, the amount of minerals that is released from the food matrix during gastrointestinal digestion that becomes accessible and available for absorption in the ileum. Minerals are important for the normal functioning of an organism. Barros, Ferreira and Genovese (2012) noted that minerals such as iron, calcium, zinc, copper, sodium, potassium, magnesium, boron, manganese, and sulphur were present in the fruit pulp and peel of citrus fruits. *In vitro* digestion simulation methods have long been used to determine nutrient bioaccessibility as an alternative to *in vivo* digestion simulation methods (Guerra *et al.*, 2012). Dialysis and solubility methods are used for determining nutrient bioaccessibility *in vivo* (Xia *et al.*, 2017b). The availability and accessibility of some minerals is decreased due to the presence of antinutritional factors such as phytates; oxalates, tannins, and fibres possibly interfere with the accessibility and availability of minerals (Amalraj and Pius, 2015). Phytic acid tends to complex itself with minerals, forming insoluble compounds that decrease the bioavailability and absorption of these minerals by the organism (Pereira, 2010). Processing of

food by heating has been used as a method to reduce the activity and content of phytates in food (Embaby, 2011). Research by Silva de Lima *et al.* (2014) on the mineral bioaccessibility in apple juice showed that the bioaccessibility of copper, iron, and zinc were 15 %, 11.5 %, and 3.7 %, respectively. No research has been conducted on the mineral bioaccessibility of *U. kirkiana* fruit**..**

2.6 Fruit processing

Over the years, the *U. kirkiana* fruit has been mostly eaten fresh, but is sometimes processed into juices, squashes, wines, sweet beer, porridge, jam (Figure 2.3), and cakes (Ngulube and Hall, 1995). The fruit is used for the production of Masuku, a local brew in Zambia (Muchuweti, Ndhlala and Kasiamhuru, 2006). Furthermorethere is no evidence of commercial processing of the fruit in Zimbabwe. Processing of fresh fruit is necessary because of the high rate of fruit perishability. Production of *U. kirkiana* juice involves cutting the fruit skin, pulping the crude mixture in a mortar and pestle, sieving it through a 800 µm sieve, diluting the pulp with cool boiled water, adding sucrose and preservatives, pasteurising at 90 °C for 15 min (TBS, 1985), and cooling and storage (25–32 °C) (Ndabikunze *et al.*, 2010).

Pasteurisation reduced the vitamin C content in *U. kirkiana* fruit juice by 55 % (from 45.57 ± 3.24 to 21.10 ± 3.30 mg kg^{-1}) (Ndabikunze *et al.*, 2010). The observed loss in vitamin C levels was attributed to the high pasteurization temperatures (Cradall, Upandhyaya and Davis, 1990). During juice storage, the vitamin C content decreased significantly (by 40 %) with time. The same trend was observed in citrus fruits (Kadzere *et al.*, 2006). However, despite the reported decrease in the vitamin C content over time, the vitamin C content of *U. kirkiana* juice was within the allowed amounts recommended for an adult (60 mg per day, Lutham, 1997). It is important to note that most households are unaware of the processing technologies that may be suitable to meet their needs, regardless of the technologies are being implemented in the rest of the World. Most people rely on their indigenous knowledge systems of processing the fruit at a household level. In addition, there is a need to upgrade and improve the processing techniques. Tiisekwa *et al.*, (2004) recommended that if households are trained on good processing methods in their locality, the fruit could be better processed for home consumption, even at a commercial level.

2.7 Sensory qualities of the fruit

Moombe *et al.* (2014) reported that *U. kirkiana* was the most preferred indigenous fruit and had an overall mean ranking score of 3 out of 5 in Zambia. In Malawi, the fruit was most preferred because of its sweetness and nutritional value (Ngulube, Hall and Maghembe, 1995; Campell, Luckert and Scooner, 1997; Saka *et al.*, 2008; Haq, Bowe and Dunsiger, 2008; Ntupanyama *et al.*, 2008). Ntupanyama *et al.* (2008) reported the fruit was liked for the following parameters: preference level of sweetness (38 %), vitamins (23 %), snack value (18 %), hunger satisfaction (16 %), leisure/habit (3 %), and thirst quenching (2 %). Sweetness suggests the presence individual sugars in the fruit. Consumers regarded taste, cleanliness and flavour as major indicators on which to base their liking of the fruit (Ramadhani and Schmidt, 2008). Studies indicated that 55 % of the consumers selected the fruit based on taste, while 32 % selected the fruit based on fruit colour (Ntupanyama *et al.*, 2008).

Jam made from the fruit had a higher mean acceptance of 4.0, as compared to that of *Strychnos cocculoides* fruit jam (Saka *et al.*, 2007). Juices made from *Uapaca* composite had the highest mean preference scores with respect to appearance (3.77) and mouth feel (4.33) among *Strychnos cocculoides* composite (3.33 and 3.39), mango composite (2.9 and 3.5), and baobab composite (3.45 and 3.39) juices (Iranbakhsh, Ebadi and Zare, 2009). Juices made from *U. kirkiana* had a higher colour score of 5.36 and 6.19 as compared to those of *V. mombassea* (4.56 and 4.02), *S. berrea* (4.63 and 4.37), and *A. digitata* (4.87 and 5.49) fruits (Ndabikunze, Masambu and Tiisekwa, 2010).

2.8 Fruit Marketing

U. kirkiana fruit collectors, retailers and vendors usually collect the fruits for selling from naturally grown forests near their homes, and very few people collect the fruits from trees at their homesteads. The marketing system of the fruits is not characterized by a clear separation of marketing activities. This is because anyone can become a fruit collector, a vendor and a consumer at the same time thereby making it difficult to dentify the fruit collectors who sale to vendors, identify vendors who then sale to consumers. Fruit collectors tend to use scotch carts, bicycles, buses, and sometimes by hired pick-up trucks to transport the fruit to markets

In most situations, vendors and retailers buy the *U. kirkiana* fruits from fruit collectors and from the people with the fruit tree at their home and transport them to urban markets where they sell them to other vendors, and to customers (Figure 2.2). In this review, retailers are defined as formal traders with permanent selling places in urban markets, and who pay the tax. Literature shows that as compared to retailers, vendors sold most of the indigenous fruits. Vendors are the informal traders who sell fruits along the highways, on roadsides, in streets, and at the peripheries of the market places, where they do not have to pay taxes (Ramadhani and Schmidt, 2008). Unlike most exotic fruits (*Mangifera indica, Prunus persica, Psidium guajava*), which have a marketing system in place, these indigenous fruits (*U. kirkiana* and *S. cocculoides*) lack product differentiation at the production level (Figure 2.2) as there is no grading, packing or washing of the collected fruits prior to sale at the market (Ramadhani and Schmidt, 2008). Furthermore, the marketing process involves the sale of fruits of mixed sizes (small, medium, large), different colours (brown, yellow), and different levels of freshness. In addition, the marketing of indigenous fruits in Zimbabwe is conducted without an established formal pricing system. This resulted in *U. kirkiana* and other fruits such as *S. cocculoides* having varied prices based on the regional locations of the markets. Fruit prices in urban markets were higher than those in semi-urban areas/growth points and rural markets (Ramadhani and Schmidt, 2008).

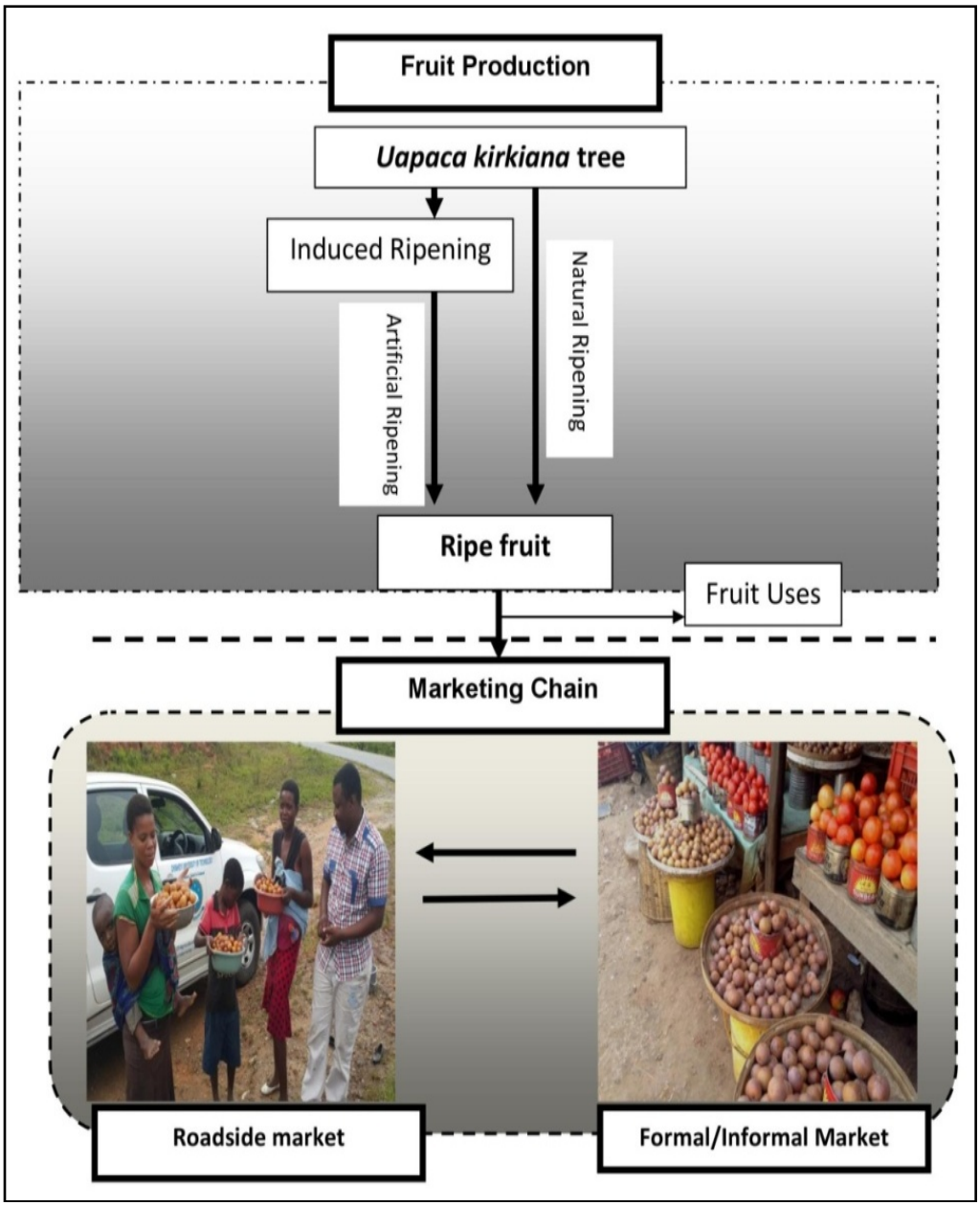


Figure 2.2: *U. kirkiana* fruit marketing.

2.9 Post-harvest handling

Post-harvest darkening of the fruit is due to the impact damage during harvesting, and is an undesirable quality characteristic of the fruit and affects the degree of liking by consumers. Subtropical fruits such as *Litchi chinensis* darken due to heat stress and the same is also true for the *U. kirkiana* fruits that undergo post-harvest darkening in October due to higher

temperatures, sunlight intensities, and low relative humidity (Kadzere *et al.*, 2006). The skin of the fruit is sift when fully ripened and the heat caused the fruit to darken (Hughes and Haq, 2003). The fruits are collected and carried in *museke* baskets to reduce post-harvest losses in Zambia. It is evident that the harvesting methods used reduce fruit quality. Saka *et al.*, (2004) supported these findings and reported that nutrient and quality loss of the fruit occurs at all stages, right from harvesting to marketing. Hughes and Haq (2003) reported post-harvest losses of 40–60 %. Furthermore, post-harvest losses could be attributed to a lack of knowledge regarding fruit handling. The handling and uses of *U.kirkiana* fruit are summarised in Figure 2.3.

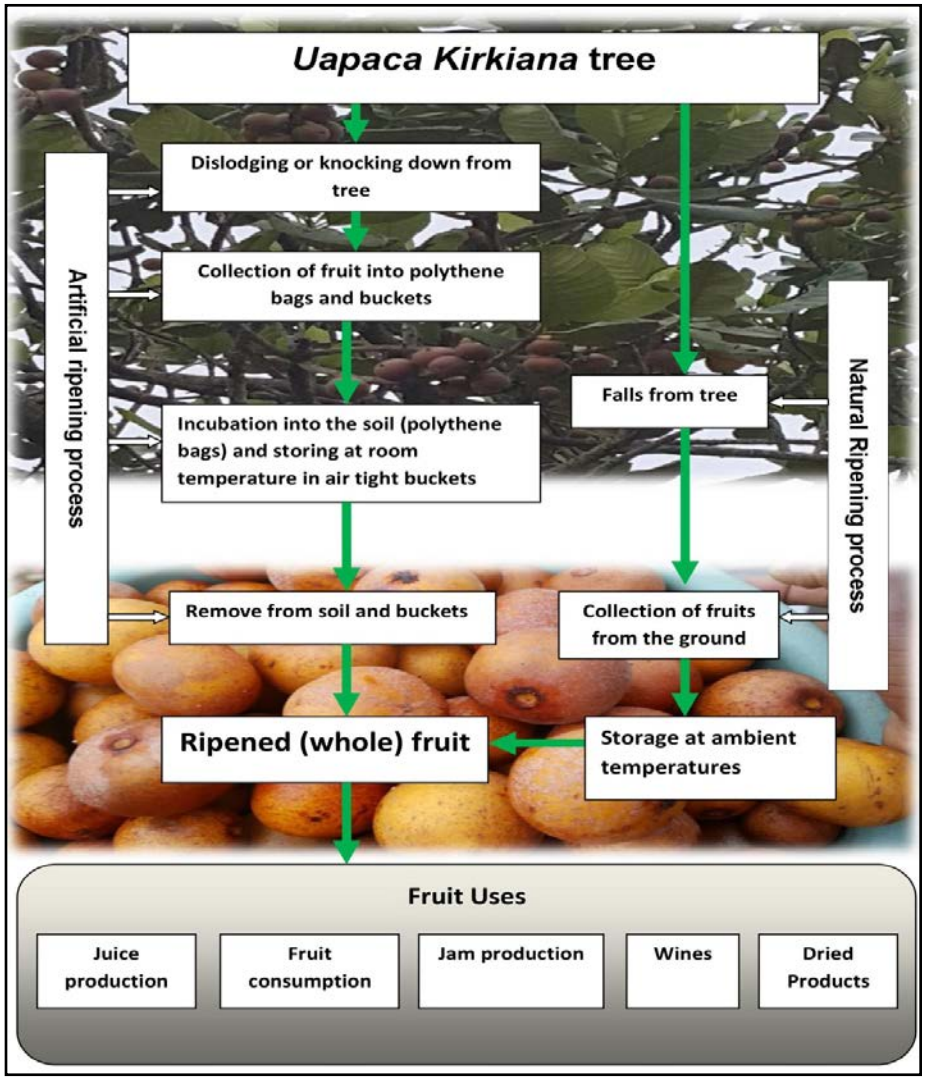


Figure 2.3: *U. kirkiana* fruit handling and use

2.10 Micronutrients

All over the world, plant-based foods that include fruits and vegetables serve as important ingredients in the human diet (Global Dietary Database, 2010). Plant-based food systems not only supply macronutrients, but also are the main sources of micronutrients such as minerals to human and populations. Micronutrients are defined as chemical elements that are necessary to perform vital functions by organisms (Berdanier, Dwyer and Heber 2013; IFPRI, 2016). Minerals are classified as essential nutrients because they cannot be synthesized by the human body, and therefore must be supplied through the diet. Over two billion people in the world have been found to suffer from 'hidden hunger', a form of micronutrient malnutrition that results in poor health, low worker productivity, high rates of mortality and morbidity, increased rates of coronary heart disease, cancer, stroke, and diabetes, and permanent impairment of cognitive abilities in infants born to micronutrient-deficient mothers (Welch and Grahamn, 1999). Micronutrient deficiency is estimated to affect over two billion people globally and ranks among the primary risk factors for causing death in humans (IFPRI, 2016). In Zimbabwe, iron and zinc deficiencies are widespread in people living in rural areas, along with other deficiencies such as calcium, vitamin A, iodine, and selenium (Gagada *et al.*, 2009; ZDHS, 2016).

2.10.1 Iron

Iron is an important element involved in the metabolic processes of living organisms. It is a key component of hundreds of proteins and enzymes in humans (Beard and Dawson, 1997; Wood and Ronnenberg, 2006). Iron is required for growth, psychomotor development, and the upkeep of the immune system (Wood and Ronnenberg, 2005). Iron enables the transportation of oxygen from the lungs to different body tissues by haemoglobin—contained within red blood cells—and carries electrons within cells. In humans, approximately 70 % of the iron is present in red blood cells as haemoglobin. Haemoglobin is a conjugated protein composed of four units, each containing one haem group and one protein chain (Brody, 1999). Haem is an iron-containing compound; the major sources of haem iron are haemoglobin and myoglobin, which are obtained by the consumption of meat and meat products.

Haem, which is present in animal-based foods, contains iron that is complexed with the porphyrin ring of either haemoglobin or myoglobin (Pizarro *et al.,* 2016). Specific receptors on the microvilli of enterocytes mediate the absorption of iron complexed with haem. Once absorbed, the iron is removed from the complex by haem oxygenase (Fuqua, Vulpe and Anderson, 2012). Non-haem iron is present in plant-based foods and is transported across the mucosal membrane by a divalent metal transporter 1 (DMT1) in its ferrous state (Fe^{2+}) together with protons. Non-haem iron in its ferric state (Fe^{3+}) must be reduced to its ferrous state before being absorbed (Vulpe and Anderson, 2012; Sitrin, 2014).

Iron deficiency is a condition in which iron stores are depleted and results in anaemia that can cause impaired cognitive development, impaired immune mechanisms, reduced work capacity, reduced learning ability, and increased morbidity rates (Abbaspour *et al.,* 2014). This condition is more dangerous during pregnancy because it is associated with increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight (Abbaspour *et al.,* 2014). The Zimbabwe Demographic Health Survey (2016) reported that anaemia caused by iron deficiency continues to be a problem in both rural and urban areas of Zimbabwe.

The Recommended Dietary Allowances (RDA) for iron varies from 0.2 mg in infants to 27 mg in pregnant women (Table 2.6).

Table 2.6: The Recommended Dietary Allowances for iron.

Life stage	Age	Men (mg/day)	Women (mg/day)
Infants	0–6 months	0.27	0.27
Infants	7–12 months	11	11
Children	1–3 years	7	7
Children	4–8 years	10	10
Children	9–13 years	8	8
Adolescents	14–18 years	11	15
Adults	19–50 years	8	18
Adults	≥ 51 years	8	8
Pregnancy	All ages		27
Breast-feeding	≤ 18 years		10
Breast-feeding	≥ 19 years		9

2.10.2 Zinc

Zinc plays an important role in the human body. Millions of people globally, have limited access to zinc-rich foods and this has resulted in insufficient levels of zinc in their diets. The presence of zinc inhibitors such as phytates that are common in plant-based diets is reported as a causal factor of the lack of zinc in the human body (Bhowmik *et al.*, 2010). Zinc absorption is mainly inhibited by inositol hexa- (and penta-) phosphate or phytate (Hambidge *et al.*, 2010). Zinc is required for the action of many enzymes that are involved in the metabolism of proteins, carbohydrates and fats in the body (Bhowmik *et al.*, 2010). It is important for cell division and during the synthesis of DNA and protein. Zinc also plays an important role in wound healing, taste acuity, connective tissue growth and maintenance, immune system function, prostaglandin production, bone mineralization, aids in thyroid function, blood clotting, cognitive functions, foetal growth, sperm production, and maintenance of normal serum testosterone (Deshpande, Joshi and Giri 2013). Further, zinc is also vital for cell signalling and influences hormone release and transmission of nerve impulses (Chatterjea and Schinde, 2005; Deshpande, Joshi and Giri 2013). The main signs of zinc deficiency are growth retardation, pale skin, fatigue,

low blood pressure, diarrhoea, hair loss, retarded bones, severe weight loss, and white spots under the finger nails (Deshpande, Joshi and Giri 2013). The RDA for zinc varies from 2 mg in infants to 12 mg in adults **(**Table 2.7).

Table 2.7: The Recommended Dietary Allowances for zinc varies from 2 mg in infants to 12 mg in adults.

Life stage	Age	Men (mg/day)	Women (mg/day)
Infants	0–6 months	2	2
Infants	7–12 months	3	3
Children	1–3 years	3	3
Children	4–8 years	5	5
Children	9–13 years	8	8
Adolescents	14–18 years	11	9
Adults	≥19 years	11	8
Pregnancy	≤18 years	-	12
Pregnancy	≥19 years	-	11
Breast-feeding	≤18 years	-	13
Breast-feeding	≥19 years	-	12

2.11 Phenolic compounds

Phenolic compounds are naturally occurring organic compounds that contain at least one aromatic ring with one or more hydroxyl groups bonded to the ring. Naturally, these phenolic compounds occur as conjugates with monosaccharides and polysaccharides bonded to one or more phenolic groups (Harborne, 1998).

2.11.1 Classification of phenolic compounds

Phenolic compounds exist in many groups and the main dietary phenolic compounds are phenolic acids, flavonoids, and tannins. Phenolic acids comprise hydroxybenzoic and hydroxycinnamic acids.

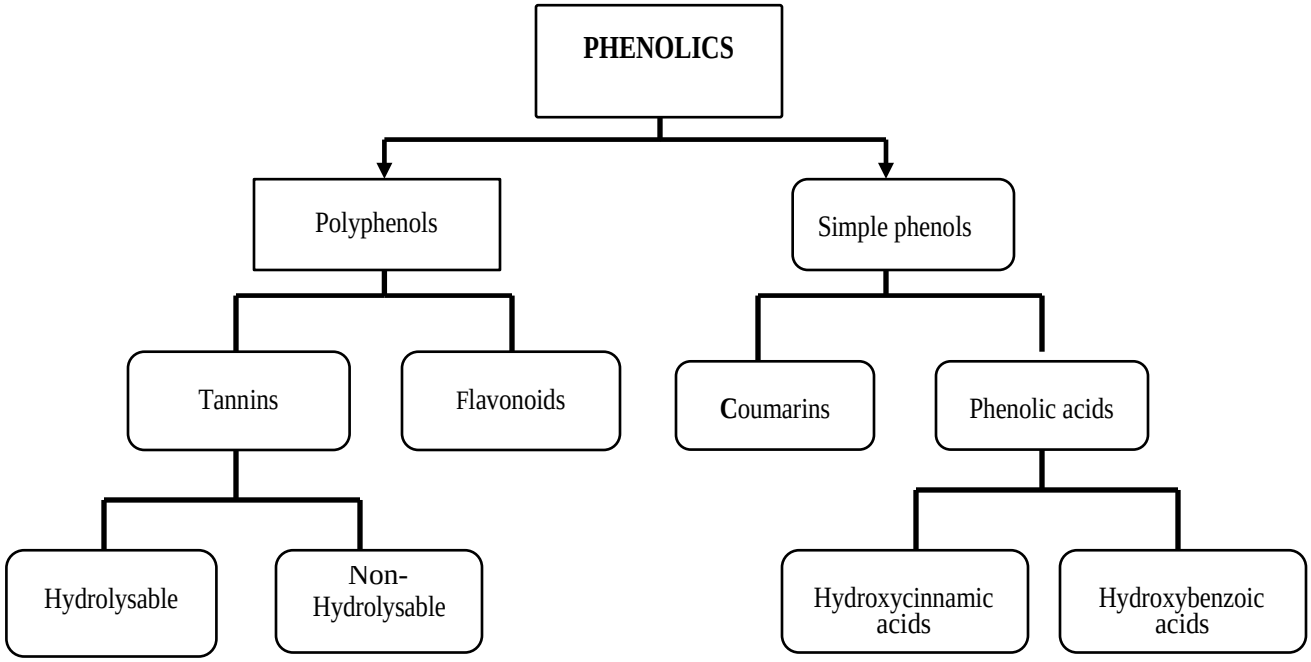


Figure 2.4: Groups of phenolic compounds **(**adopted from Anantharaju *et al.*, 2016).

2.11.2 Hydroxybenzoic acids

Structurally, hydroxybenzoic acid comprises *p*-hydroxybenzoic acid (Figure 2.5).

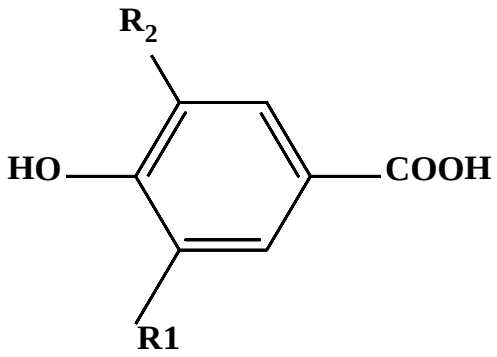
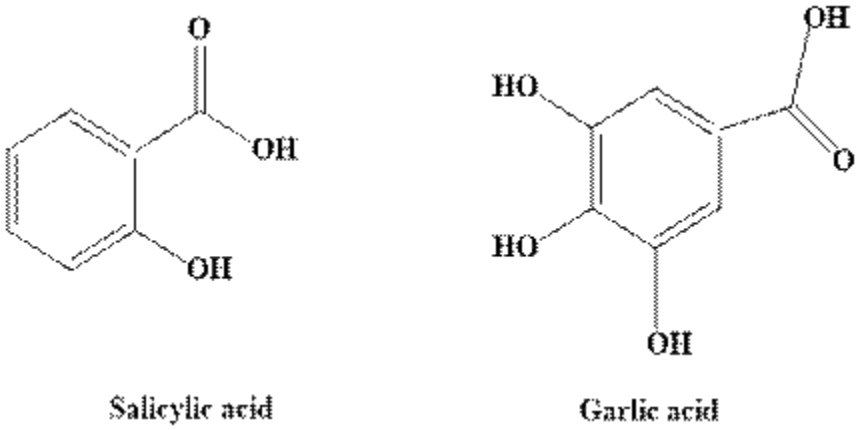


Figure 2.5: p-Hydroxybenzoic; R1= R2=H, vanillic; R1= methyl group, R2=H, protocatechuic acid; R1= OH, R2=H and syringic acid; R1= R2 =methyl group (adopted from Aljadi and Yusoff, 2002)

Hydroxybenzoic acids exist in a soluble and insoluble form when bound to sugars and lignin, respectively. Salicylic acid (2-hydroxybenzoate) is another hydroxybenzoic acid. Gallic acid occurs as a trihydroxyl derivative that plays a part in the production of hydrolysable gallotannins.



Salicylic acid Garlic acid

Figure 2.6: Structure of Salicylic acid and Garlic acid (adopted from Aljadi and Yusoff, 2002)

2.11.3 Hydroxycinnamic acids

Hydroxycinnamic acids exist in many conjugated forms that are esters of esters of hydroxyacids such as quinic, shikimic, and tartaric acids, and sugar derivatives. These conjugated forms arise from enzymatic and/or chemical hydrolysis of phenolic compounds during their tissue extraction process. The naturally occurring hydroxycinnamic acids in fruits include *p*-coumaric, caffeic, ferulic, and sinapic acids as shown in Figure 2.7 (Galvez, Reid and Gonner, 1997; Aljadi and Yusoff, 2002).

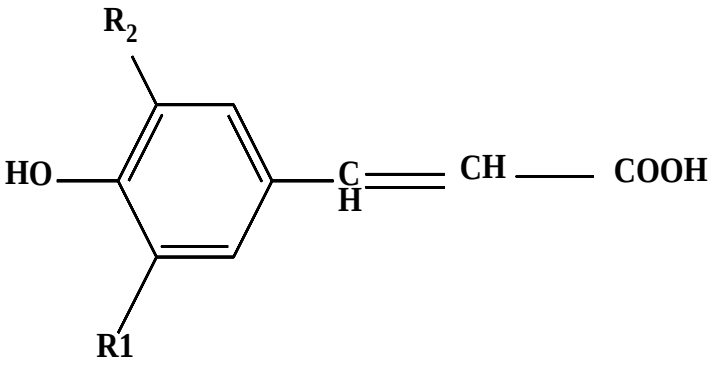


Figure 2.7: p-Coumaric acid; R1= R2 = H, caffeic acid; R1= OH, R2 = H, ferulic acid; R1= Ome, R2 = H and sinapic acid; R1= R2 = Ome (adopted from Galvez, Reid and Gonner, 1997; Aljadi and Yusoff, 2002).

2.11.4 Flavonoids

Flavonoids are secondary plant metabolites that comprise a C15-(C6-C3-C6) flavone nucleus. The flavone nucleus is contained within a heterocyclic ring system that originates from the linkage between the phenylalanine (ring B) and polyketide biosynthesis (ring A) via an oxygen containing a pyran or pyrone ring (ring C) (Harborne, 1998; Maraisi *et al*., 2006).

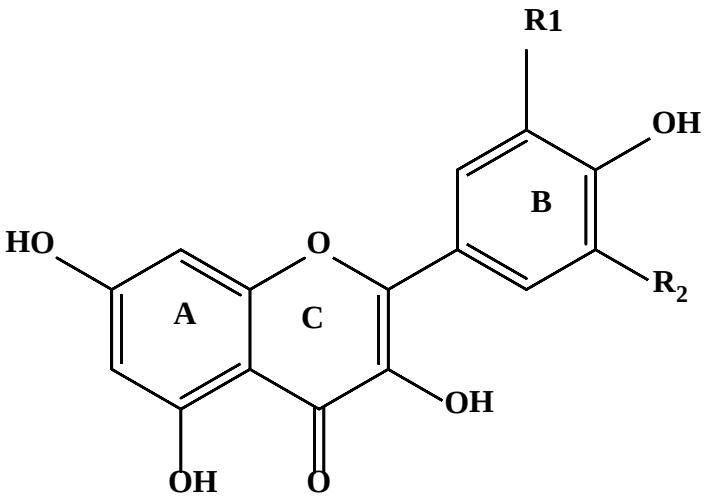


Figure 2.8: Flavonoid skeleton with rings A, B and C.

Flavonoids are pale yellow compounds and have poor solubility in water (Maraisi *et al*., 2006). They exist in foods as O-glycosides, and D- glucose is the most common sugar residue that binds to carbon number 3 (Macheix *et al.*, 1990). Other sugars such as D-galactose, L-rhamnose, L- arabinose, D-xylose, and D-glucuronic acid are also present. Major structures of flavonoids are represented in Figure 2.9.

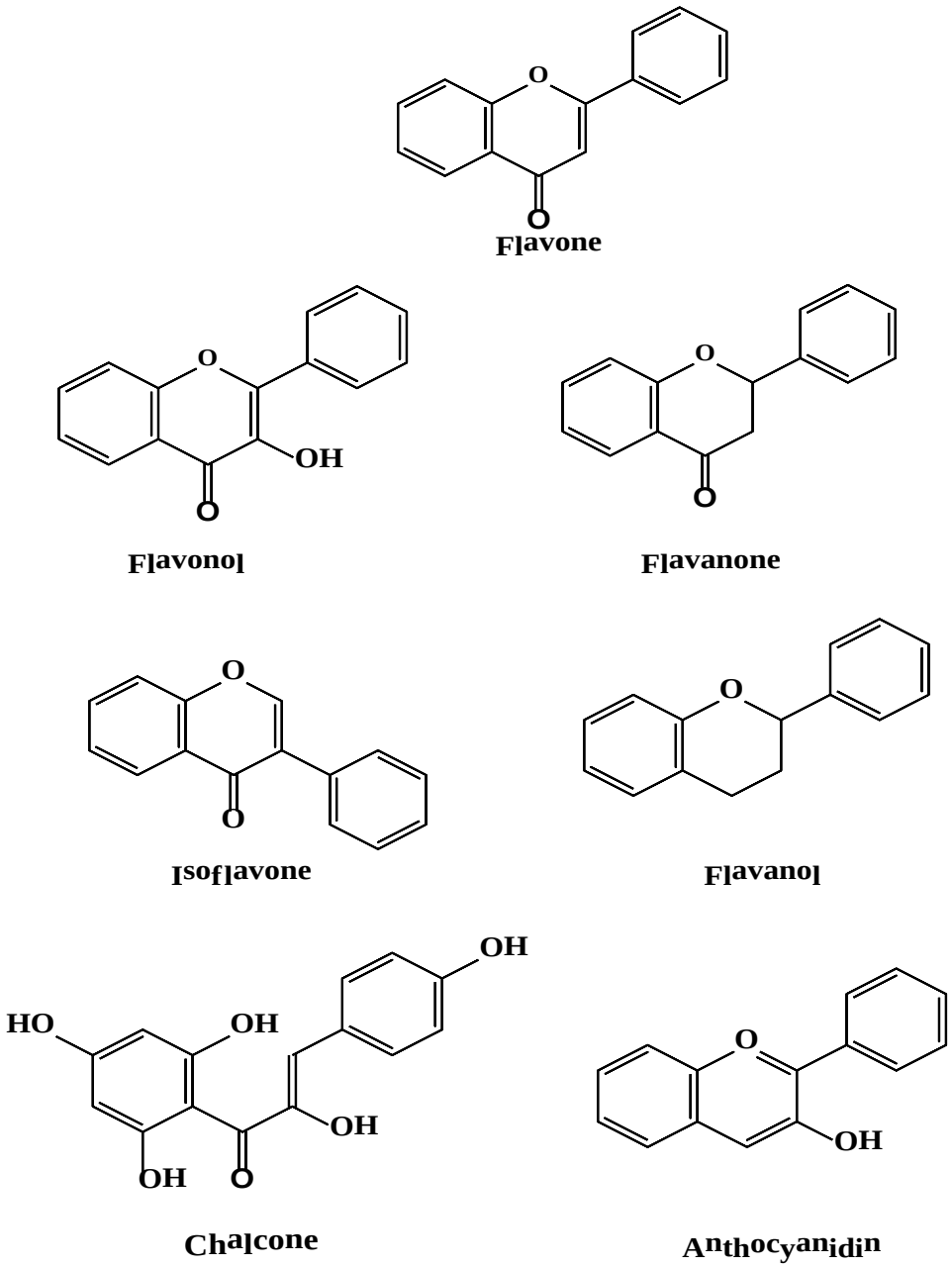


Figure 2.9: Structures of major flavonoids (adopted from Maraisi *et al*., 2006)

2.11.5 Flavanols

Flavanols occur in plants and form an essential part of the diet. The widely distributed species includes quercetin, kaempferol, myricetin, and isorhamnetin (De Man, 1999) as shown in Figure 2.10. Quercetin plays an important role as a strong reducing agent and together with vitamin C, vitamin E, and carotenoids, helps to protect the body against oxidative stress. It acts as a strong antioxidant and prevents the occurrence of diseases linked to oxidative stress, such as cancer, cardiovascular diseases, inflammation, and other degenerative diseases (Ames, Shigenaga and Hagen, 1993). Dietary quercetin in high concentrations has been associated with the inhibition of the growth of malignant cells by arresting the cell cycle in the late-G1-phase or by causing apoptosis of malignant cells (Yao *et al.*, 2004; Lakhanpal and Rai, 2007).

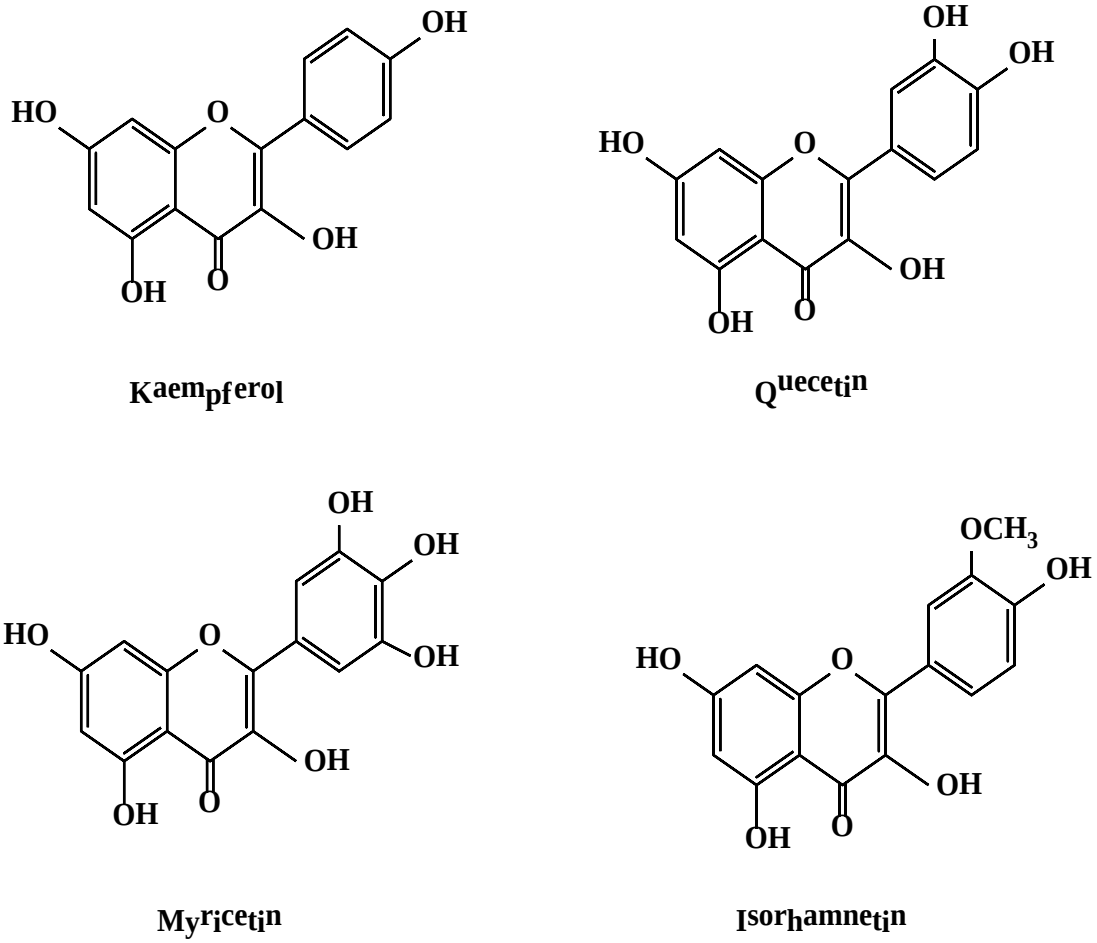


Figure 2.10: The structures of kaempferol, myricetin, quercetin, and isorhamnetin (adopted from De Man, 1999).

2.11.6 Tannins

Tannins are naturally occurring uncrystallisable colloids with a distinct astringent quality; their main property is to bind and precipitate gelatine (Yoshida and Hatano, 2000). They significantly affect the nutritive value of food (Mueller-Harvey, 2001). Tannins are responsible for the astringent taste in wines or unripe fruits. They are divided into three classes and these are the hydrolysable tannins (HT), proanthocyanidins (PAs) or condensed tannins (CT), and mixed tannins (Jose, Isaza and Yoshida, 2001). Structurally, hydrolysable tannins (HTs) contain a polyol, mainly D-glucose that is esterified with galloyl groups. In gallotannins, the hydroxyl groups (-OH) of the D-glucose can be partially or totally esterified with gallic acid and ellagitannin (Yoshida *et al.*, 1999; Hargerman, 2002).

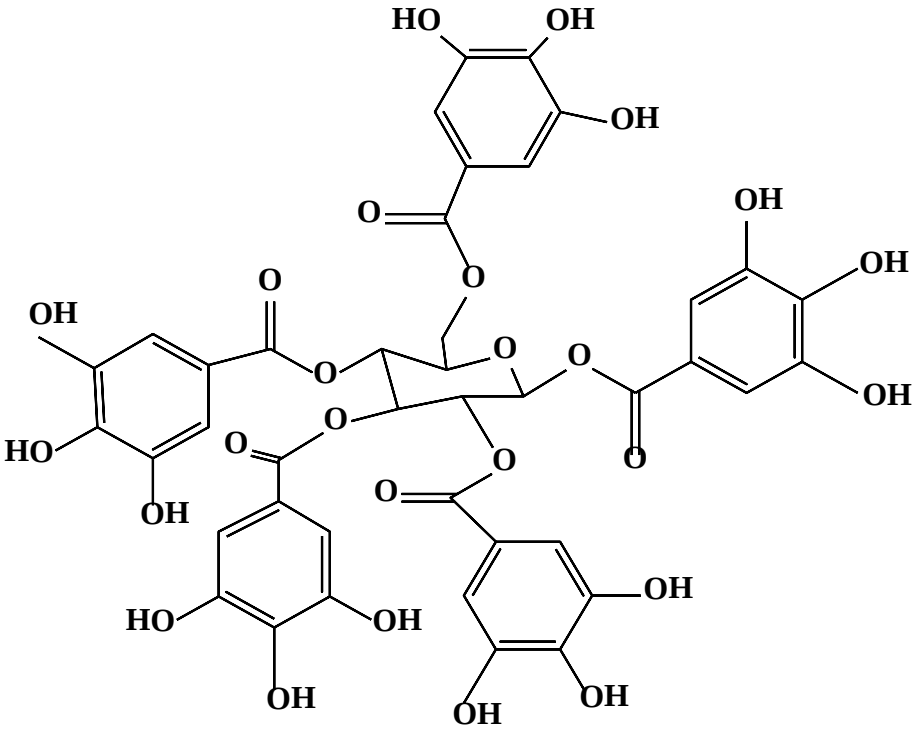


Figure 2.11: Penta galloyl-D-glucose (adopted from Hargerman, 2002).

2.11.7 Proanthocyanidins (PAs)

Proanthocyanidins (PAs) are also referred to as condensed tannins (CT); carbon-carbon bonds link the oligomers of flavonoid units (flavan-3-ol) as represented in Figure 2.12. They cannot be broken down by hydrolysis.

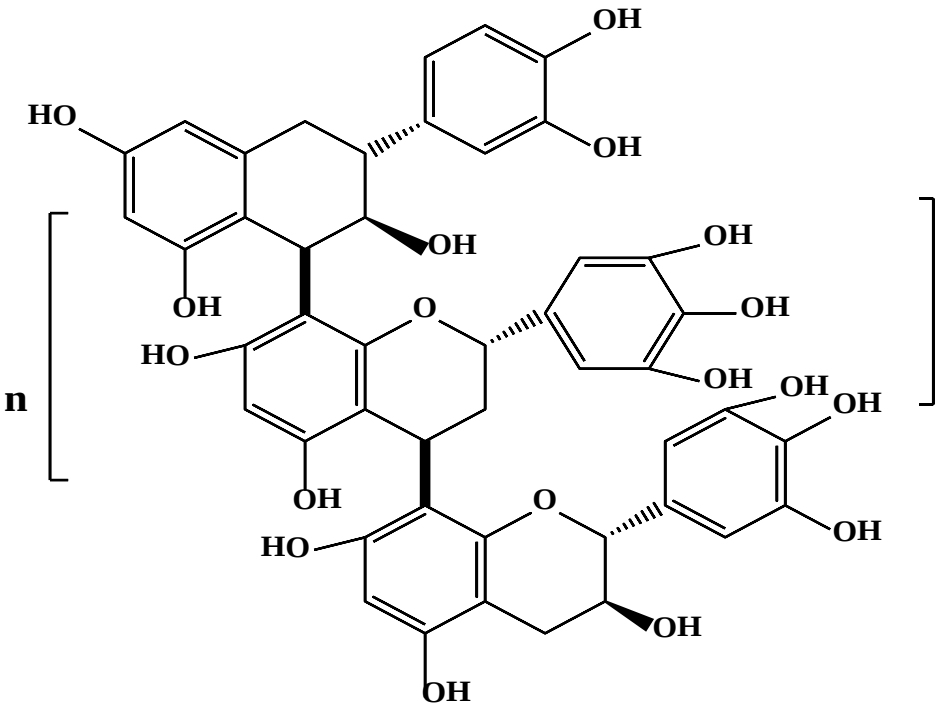


Figure 2.12: Proanthocyanidins (PAs).

2.12 Probiotics

Recently, the utilisation of specific bacterial species that offer health benefits to humans is increasing. Beneficial bacterial species that were identified in the gastrointestinal (GI) tract are termed probiotics, which refer to live microorganisms that confer a health benefit to the host when consumed in adequate amounts as part of food (Hill *et al.,* 2014). Oral intake of live bacteria or as part of dried supplements has been the primary route of administering probiotics in humans. Probiotics must be consumed as a part of the food matrix that allows them to survive in intestinal conditions, exposure to bile, and must remain viable (live probiotic cells) in the food to confer their health benefit. A probiotic food must have a minimum of 6 log CFU/g viable cells (Shah, 2000; Adikhari, Mustapha and Grun, 2003). The number of viable cells of the probiotic bacteria is important in determining the quality and functionality of a probiotic food product (Gionchetti *et al.,* 2007). The most common probiotic bacterial species include

the lactobacilli such as *L. rhamnosus GG, L. acidophilus*, and bifidobacteria (Daly and Davis, 1998).

Studies on the nutritional and therapeutic potential of probiotics have been increasing, with renewed interest from many researchers. Many studies (Lee *et al.,* 1999; Hartley *et al.,* 2001) have suggested the use of lactobacillus species and have reported that their fermented products provide many nutritional and therapeutic benefits to the consumers (Lee *et al.,* 1999; Reid *et al.,* 2001; and MacFarlane and Cummings, 2002). The potential benefit of consuming fermented dairy products containing viable probiotic bacteria has been well documented (Gill and Guarner, 2004), and was primarily mediated through alterations of the microecology of the gastrointestinal tract. The benefits of using probiotics in the fermentation of milk and its products include improvements in the nutritive values of food (the synthesis of vitamins or release of free amino acids), development of desirable organoleptic properties, improvements in food preservation, control of serum cholesterol levels (Lin *et al.,* 1989), and improvement of prophylactic properties (O'Sullivan *et al.,* 1992, Morelli, 2000). Probiotics provide physiological functions and benefit the human body through their growth and activity (Figure 2.13).

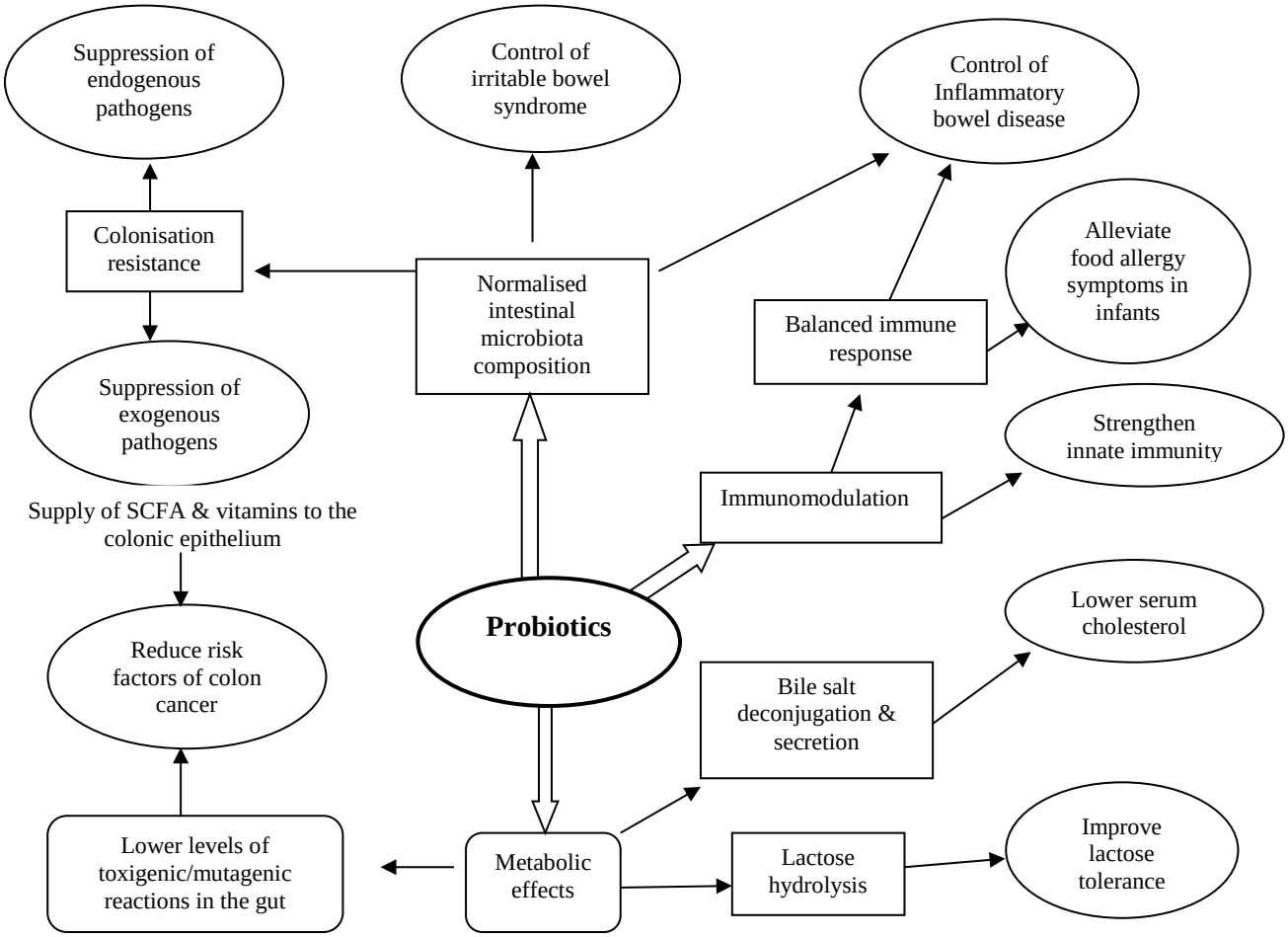


Figure 2.13: Various health benefits of consumption of probiotics **(**adopted from Shah, 2015**).**

2.12.1 *Lactobacillus rhamnosus* GG

L. rhamnosus belongs to a group of gram-positive bacteria, and is non-spore forming, non-motile, catalase negative, and microaerophilic (Valík *et al.*, 2008)**.** Its growth is promoted by the presence of folic acid, niacin, pantothenic acid, riboflavin, calcium, and an optimum pH range of 6.4–4.5. It exists either as single rods or multiple rods arranged as short chains; the cell sizes are, 0.8–1.0 μm (width) and 2.0–4.0 μm (length) (Wood and Holzapfel, 1995). *L. rhamnosus* GG is known to counteract pathogenic bacteria (Petrova *et al.*, 2018), and fungi in the urogenital tract (Reid, 2017). The history of *L. rhamnosus* use in dairy products has made it a mode of delivery of probiotics in the human body (Lazzi *et al.*, 2014). In Africa, probiotics

have made the production of yoghurt, and that of other fermented foods easy (Franz *et al.*, 2014). Guandalini *et al.* (2000) confirmed the beneficial effect of *L. rhamnosus* GG on children suffering from acute, watery diarrhoea.

Furthermore, evidence exists regarding the beneficial effects of *L. rhamnosus* GG in preventing and treating antibiotic-associated diarrhoea (Ruszczynski, Radzikowski and Szajewska, 2008), gastrointestinal and upper respiratory tract infections in children (Hojsak *et al.*, 2010), and rotavirus diarrhoea (Grandy *et al.*, 2010), and with respect to inhibiting growth and adhesion of enteropathogens (Mack *et al.*, 1999; Gopal *et al.*, 2001). A study by Corcoran *et al.* (2005) revealed that *L. rhamnosus* GG had a high tolerance to the acidic conditions prevalent in the stomach. The bacterium was also reported to survive the conditions prevalent in the intestinal passage (Sandholm-Mattila *et al.*, 1999). *L. rhamnosus* GG could efficiently adhere to the human colonic mucosa (Rinkinen *et al.*, 2003); it briefly inhabits the gastrointestinal tract after being delivered into the body (Sandholm-Mattila *et al.*, 1999; Tuomola, Ouwehand and Salminen, 2000).

2.13 Bioaccessibility/bioavailability of iron and zinc

Upon consumption, nutrients present in food are released from the matrix, converted into absorbable units by the digestion process, absorbed into the bloodstream, and are then transported to particular target tissues (Boland, Golding and Singh, 2014). When a nutrient fraction is released from the food matrix and is available for absorption, it is termed as being bioaccessible, whereas a nutrient fraction, when absorbed and available to be used for physiological functions and storage is termed as being bioavailable. Bioaccessible nutrients are determined using *in vitro* methods while bioavailable nutrients are determined *in vivo* or in cell cultures (Fernandez-Garcıa, Carvajal-Lerida and Perez-Galvez, 2009). Haem iron has a relatively high bioavailability and non-haem iron has a lower bioavailability of 15–35 % and 2–20 %, respectively, due to dietary factors and the presence of other food compounds (Hurrell and Egli, 2010). This becomes a concern, especially for people in rural Zimbabwe who rely on plant foods as their source of nutrition. It is therefore imperative to conduct research and to find solutions for improving iron bioavailability from plant-based foods (Hunt, 2003).

Ascorbic acid enhances iron absorption and when present in the food, compounds, such as tannins, phytic acid, polyphenols, calcium, and peptides from partially digested proteins act as the main inhibitors of its absorption (Hurrell and Egli, 2010). A study by Hemalatha, Platel and Srinivasan (2007a) on zinc bioaccessibility from a group of cereals and pulses used the equilibrium dialysis protocol with simulated gastrointestinal digestion, and reported that zinc bioaccessibility was 5.5 % from sorghum (*Sorghum vulgare*) and 21.4 % from rice, whereas in pulses it was 27 % from whole green gram (*Phaseolus aureus*) and 56.5 % from decorticated chickpea. The bioaccessibility of zinc from pulses was higher than that from cereals; this was attributed to the inhibitory effects of natural factors present in cereals, such as phytate, calcium, and dietary fibre, which are present in higher amounts in cereals than in pulses (Hemalatha, Platel and Srinivasan, 2007a). It was also observed that iron bioaccessibility from cereals was 4 % in case of sorghum and 8 % in case of rice; while from pulses it ranged between 1.8 % (cowpea; *Vigna catjung*) and 10.2 % (beans; *Phaseolus vulgaris*). This suggests that the bioaccessibility of zinc may be less affected by the presence of dietary factors compared to that of iron. The bioaccessibility of zinc and iron from composite staple grain meals regularly consumed in India was determined using a simulated gastrointestinal digestion protocol and equilibrium dialysis (Bhavyashree *et al.*, 2009). Wheat-based (*Triticum aestivum*) meal had the highest iron bioaccessibility of 4.7 % and finger millet (*Eleusine coracana*) meal had the lowest iron bioaccessibility of 1.5 % (Bhavyashree *et al.*, 2009). Zinc bioaccessibility was highest in rice meals and lowest in sorghum-based meals (0.31 %) (Bhavyashree *et al.*, 2009). The bioaccessibility of iron and zinc was lower in finger millet-based meals, which was attributed to the antinutrient effect of tannins. *In vitro* studies on iron and zinc bioaccessibility from many foods have been carried out in recent years. However, studies on the iron and zinc bioaccessibility from fruit-based jam, particularly those prepared from *U. kirkiana* fruits are scarce, and to our knowledge, *in vitro* methods for investigating mineral bioaccessibility were only developed for fruit juices (Hazell and Johnson, 1987), fortified citric fruit juices (Haro-Vicente *et al.*, 2006), and fruit juices with milk and cereals (Perales *et al.*, 2006; 2007).

2.14 Antinutrients affecting mineral bioaccessibility and bioavailability

Antinutrients refer to a group of natural or synthetic substances that affect nutrient absorption (Cammack *et al.*, 2006). An antinutrient hinders the bioaccessibility and bioavailability of the

nutrient from food (Figure 2.14). Antinutrients occur in large quantities in plant-based foods and they play a significant physiological role as a chemical defence against many insects and pests (Svarc-Gajic, 2013). Andrews *et al.* (2014) and Sulibursk a and Krejpcio (2014) have reported the effects of dietary fibres, phytic acid, and polyphenols on iron and zinc bioavailability/bioaccessibility using *in vitro* studies.

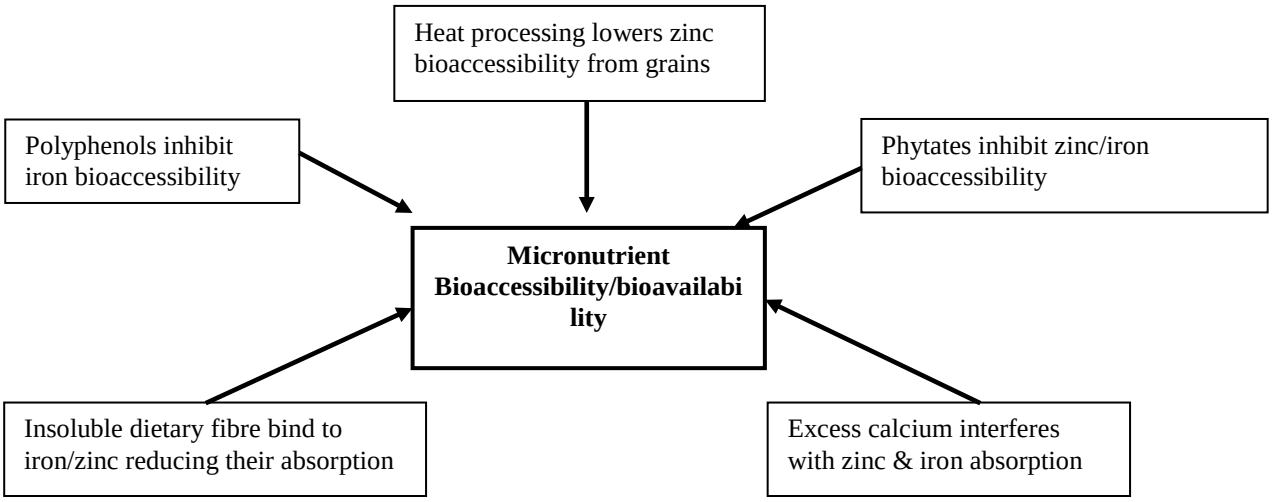


Figure 2.14: Inhibitors of iron and zinc micronutrient bioavailability (adopted from Sandberg, 2002; Gupta, Jyothilakshmi and Prakash, 2006).

2.14.1 Dietary fibre

Dietary fibre refers to the edible carbohydrate monomers that naturally occur in foods; more than ten monomeric units cannot be hydrolysed by endogenous enzymes in the ileum of humans (Joint FAO/WHO Food Standards Programme Commission, 2016). Dietary fibres are classified into water-soluble and water-insoluble fibres based on the solubility in water with a specific pH. Soluble dietary fibre is present in fruits and vegetables at higher concentrations whereas insoluble dietary fibres are present in high amounts in cereals and legumes (de Almeida Costa *et al.*, 2006). Some *in vitro* studies have reported that the binding of minerals could be influenced by the source of fibre, the physicochemical properties of dietary fibre and pH (Platt and Clydesdale 1987; Persson *et al.*, 1987). The solubility of the fibre affects mineral bioaccessibility and bioavailability.

Pectin is a soluble dietary fibre that naturally occurs in the cell wall of plant-based foods, such as fruits, vegetables, and legumes (Chan *et al.*, 2017). It is a heteropolysaccharide that mainly contains galacturonic acid (GalA) residues, which contain methoxyl esters groups and neutral sugars as side chains (Voragen *et al.*, 2009). Pectin can exist as a polynomic compound and has the ability to bind to cations when the non-methylesterified GalA residues are ionized. This interaction between divalent cations and pectin is summarised by the egg-box model (Caffall and Mohnen 2009) shown in Figure 2.15.

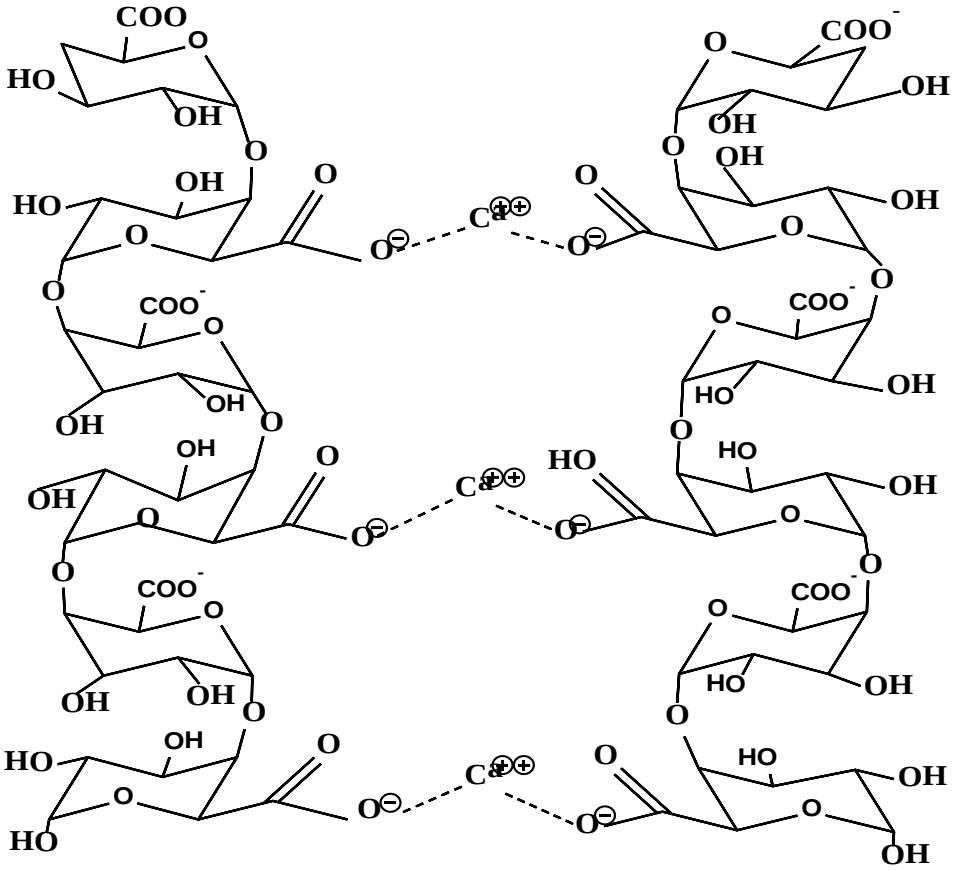


Figure 2.15: The egg-box model of pectin and calcium crosslinking (adopted from Caffall and Mohnen, 2009).

Bosscher *et al.* (2003) evaluated the iron, calcium, and zinc availability in infant formula that was supplemented with soluble dietary fibre fractions using an *in vitro* dialysis model. They found that pectin decreases iron bioaccessibility. In addition, Kyomugasho *et al.* (2015) and Celus *et al.* (2018) noted a decrease in iron, calcium, and zinc bioaccessibility with increasing electrostatic pectin-interactions, using *in vitro* studies.

2.14.2 Phytic acid

Phytic acid (myo-inositol hexaphosphate) (IP6) or phytate functions as the main form of phosphorus storage in many plant tissues (Figure 2.16).

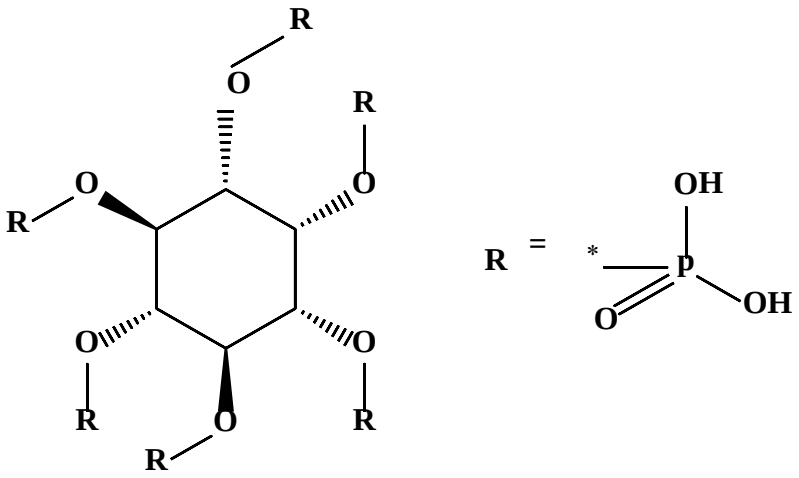


Figure 2.16: Basic chemical structure of phytic acid (adopted from Kumar *et al.*, 2010).

Phytic acid is produced as the plant seed matures and its total phosphorus content, which comprises many cations such as iron, zinc, magnesium, calcium and potassium is 60–90 % in dormant seeds (Frossard *et al.*, 2000; Kumar *et al.*, 2010). In presence of phytase, phytic acid is hydrolysed into inositol penta-(IP5), tetra-(IP4) or triphosphate (IP3). Phytic acid gets converted into a strongly negatively charged ion at a pH range greater than 2.0, and forms stable complexes with other mineral ions such as iron, calcium, zinc, and magnesium via electrostatic interactions (Kumar *et al.*, 2010). Stronger complexes are formed when phytic acid binds to copper and zinc as compared to those formed with iron and calcium (Oberleas and Chan, 1997). Monogastric animals and humans fail to digest these complexes and phytates because they lack the enzyme, phytase (Hurrell and Egli, 2010). During digestion,

gastrointestinal secretions might contain ions which may bind to phytic acid in the gastrointestinal tract (Boland, Golding and Singh, 2014). An *in vitro* study by Lestienne *et al.* (2005) revealed a significant improvement in both iron and zinc bioaccessibility from whole pearl millet flour after the breakdown and removal of phytic acid.

2.14.3 Polyphenols

Polyphenols are an essential component of the human diet, are naturally present in plant tissues; they serve as protective agents against ultraviolet radiation and the harmful actions of pathogens (Pandey and Rizvi, 2009). Polyphenolics present in plants occur as flavonoids, which are low molecular weight phenolic compounds that include anthocyanins and tannins. Tannic acid is the main tannin compound that comprises a hepta- to octa-galloyl-β-d-glucose; addition of other galloyl groups causes esterification to a pre-existing β -1, 2, 3, 4, 6-pentagalloyl-d-glucose core (Liu *et al.*, 2005) as shown in Figure 2.17.

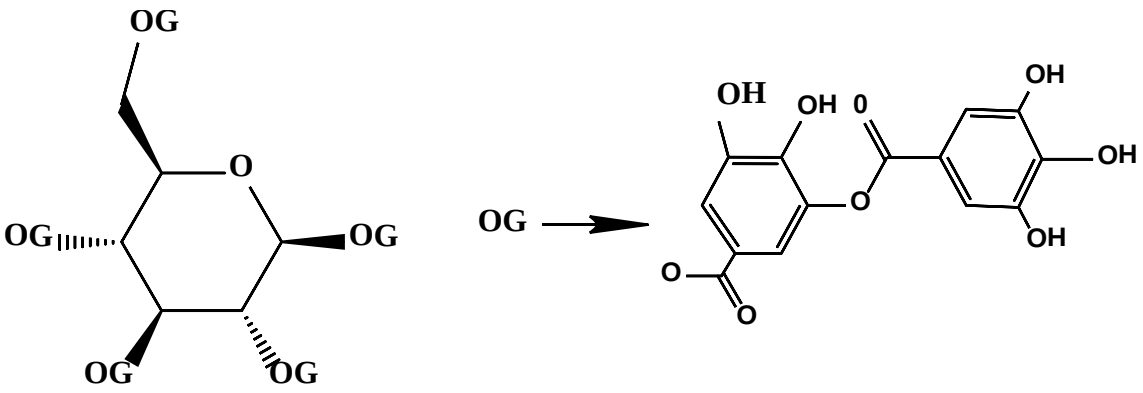


Figure 2.17: Chemical structure of tannic acid (adopted from Xu, Han and Wang, 2019).

Polyphenols can have metal chelating properties depending on the flavonoid structure. If a food matrix or digestive tract contains polyphenols and metal cations, interactions with carboxyl (COOH) groups and hydroxyphenyl will occur, resulting in an iron-polyphenol complex as illustrated in Figure 2.18.

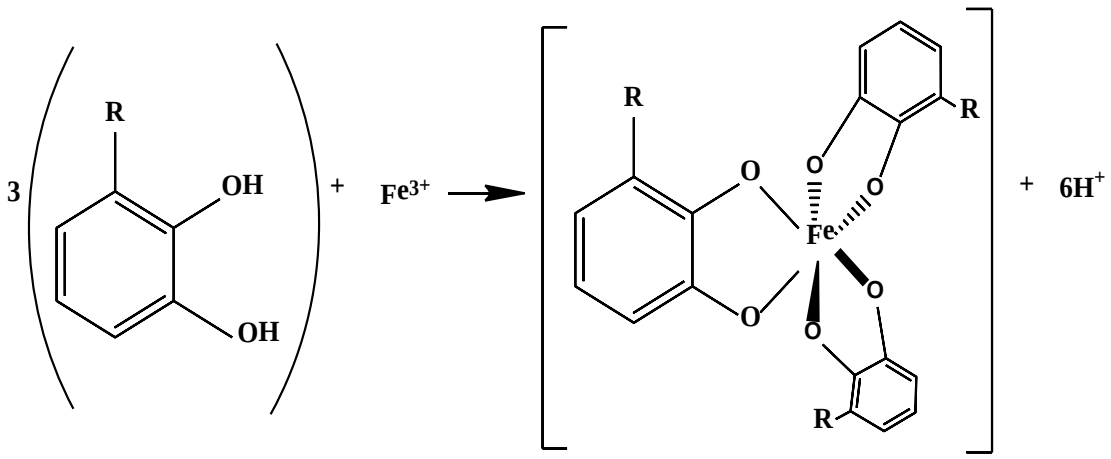


Figure 2.18: Octahedral coordination geometry of iron-polyphenol complexes. Gallols, R = OH; catechols, R = H (adopted from Perron and Brumaghim, 2009).

The high iron chelating ability of flavonoids (Ryan and Hynes 2007; Mladenka *et al.*, 2011) as well as tannins (Iffat, Maqsood and Fatima, 2005; Karamac, 2009) has been reported in some *in vitro* studies. The chelation of iron that primarily occurs in the lumen of the gastrointestinal tract (GIT) during the digestion of tannins and flavonoids has been reported to reduce iron absorption from the human diet (Layrisse *et al.*, 2000; Petry *et al.*, 2010).

2.15 Impact of food processing on bioaccessibility of iron and zinc from plant foods

Food processing is important because of the following: 1) it helps to improve the stability and safety of food, 2) it produces a preferred product, 3) it induces flavours, and 4) it enhances palatability. Although processing has many benefits, it can be disadvantageous as it may cause detrimental changes in colour, flavour, texture, and smell (Considine *et al.*, 2008). Food processing effects the nutritional quality of foods, and thus possibly affects nutrient bioavailability/bioaccessibility (Table 2.8). As for minerals, their nutritional quality in food hinges on their quantity, bioaccessibility, and bioavailability (Reddy and Love, 1999). Food processing can have a positive or negative effect on the mineral content. During processing of foods, minerals are either removed through leaching and physical separation, or enriched by the introduction of ingredients through the processing equipment and packing material (Alegria

et al., 2015). Furthermore, food processing has a huge effect on the release of minerals from the food matrix.

2.15.1 Heat processing

The heating of food can cause the softening and loosening of the food matrix and improves the digestibility of macronutrients, thereby making them more accessible to digestive enzymes. The process of loosening and softening the food matrix is thought to result in the release of minerals bound to proteins such as iron, thus assisting in their absorption (Lombardi-Boccia *et al.*, 1995). In addition, the heating of food can reduce the effects of natural inhibitory factors such as phytates and soluble dietary fibre that inhibit mineral absorption, thereby improving their bioavailability.

Table 2.8: Influence of domestic food processing methods on the bioaccessibility of iron and zinc from food grains.

Process	Iron bioaccessibility	Zinc bioaccessibility
Food processing methods		
Pressure-cooking	Enhanced	Decreased
Microwave cooking	Enhanced	Decreased
Germination	Enhanced	Decreased
Fermentation	Enhanced	Enhanced
Malting	Enhanced	Enhanced
Exogenous factors		
Amchur	Enhanced	Enhanced
Citric acid	Enhanced	Enhanced
Soy protein isolate (SPI)	Decreased	Enhanced
Sodium chloride	Effect of SPI countered	Effect of SPI potentiated
Therapeutic levels of iron and calcium		Decreased

(Adopted from Hemalatha *et al.*, 2007b, 2007c, 2009; Platel *et al.*, 2010).

During processing, heat treatment (cooking, pasteurisation, sterilisation, microwave heating, and roasting) techniques have different temperature-time combinations and modes (dry or wet heating systems) (Wang and Sun, 2016). Furthermore, wet heat treatments can cause the heat stable antinutrients (phytic acid and polyphenols) to leach out of the outer layers of seeds (Wang *et al.,* 2010). Rehman and Shah (2005) reported a reduction in tannin and phytic acid content (21–27 % and 24–35 %, respectively) in pulses upon cooking. Thermal processing of food was reported to have an effect on dietary fibre content (Dhingra *et al.,* 2012).

Generally, heat treatment has an effect on the ratio of soluble and insoluble fibres to the total dietary fibre content (Elleuch *et al.,* 2011). Heating of plant-based foods causes the fibres to depolymerise so the soluble fibres with lower molecular weight would leach out (Sila *et al.,* 2009). The mineral binding capacity of pectin is reduced when it is depolymerised (Kyomugasho *et al.,* 2015). Furthermore, the heat process can cause inactivation of beneficial enzymes such as phytase and consequently, mineral bioaccessibility and bioavailability of the food is greatly reduced (Raes *et al.,* 2014). Hemalatha, Platel and Srinivasan (2007b) noted that the heating of food grains had different effects on zinc and iron bioaccessibility. Microwave heating and pressure cooking enhanced iron and zinc bioaccessibility from cereals and pulses, whereas the bioaccessibility of zinc was significantly reduced. The bioaccessibility of iron was 7 % and 12 % from pressure-cooked wheat and rice, respectively. Pressure-cooking decreased zinc bioaccessibility from finger millet and rice by 63 % and 57 %, respectively (Hemalatha, Platel and Srinivasan, 2007b).

2.15.2 Fermentation

The fermentation process depends on the activity of microorganisms as they produce a range of metabolites that inhibit the growth and survival of unwanted microbes in food (Ross, Morgan and Hill, 2002). However, fermented foods are preferred because of their sensory qualities, for example, flavour, aroma, and texture rather than due to their preservative benefits and safety (Holzapfel, 2002). The fermentation process can improve mineral bioavailability by reducing the effect of phytic acid, which inhibits mineral bioavailability (Duhan, Khetarpaul and Bishnoi, 2004). In addition, fermentation can produce organic acids, which form soluble and absorbable complexes with minerals, thus preventing the formation of insoluble complexes with phytic acid

(Sokrab, Ahmed and Babiker, 2014). Furthermore, the production of organic acids like lactic acid lowers the pH, which promotes the endogenous phytase and polyphenol oxidase activity (Towo, Matuschek and Svanberg, 2006).

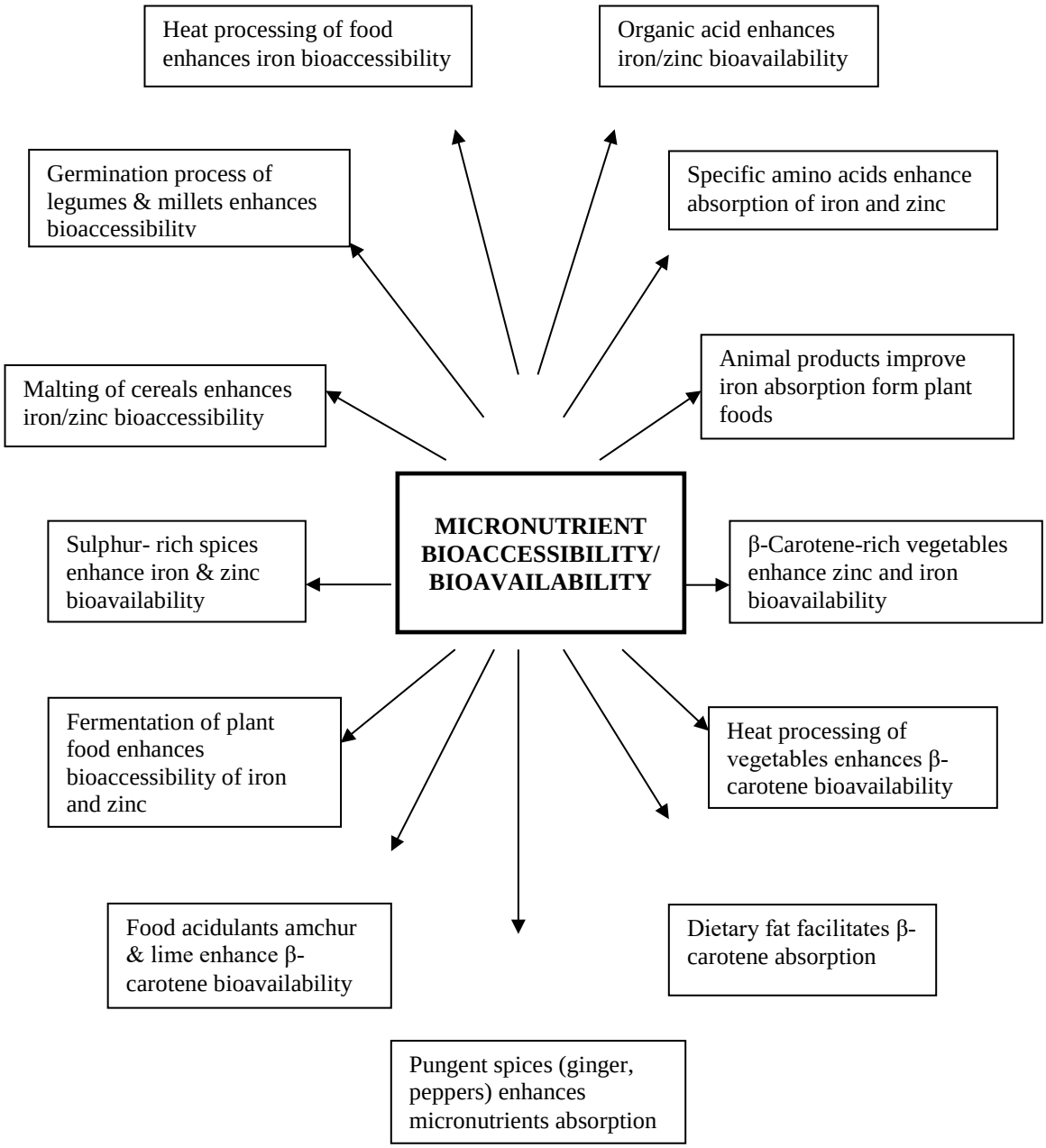


Figure 2.19: Factors that promote micronutrient bioavailability (adopted from Gupta, Jyothilakshmi and Prakash, 2006).

2.16 *In vitro* bioaccessibility protocols

In recent years, there has been extensive use of *in vitro* screening studies for determining nutrient bioaccessibility and bioavailability of different foods (Etcheverry, Grusak and Fleige, 2012; Minekus *et al.*, 2014). Bioaccessibility is defined as the quantity of an ingested nutrient that is accessible for absorption after digestion and release from the food matrix, whereas bioavailability refers to the quantity of an ingested nutrient that is released from the food matrix after digestion and absorption, and is available for functional use in tissues of the body (Etcheverry, Grusak and Fleige, 2012). Currently, the use of *in vitro* protocols in scientific disciplines such as food science and nutrition has gained much recognition. In addition, *in vitro* assays are quicker, less expensive, need little labour, and are not subject to ethical constraints whereas *in vivo* assays, that use human subjects and live animals, are costly, resource-intensive, and ethically controversial (Minekus *et al.*, 2014). *In vitro* protocols favour mechanistic studies and hypothesis building, and allow for replication, easy sampling, and mimics the gastrointestinal digestion process (Vardakou *et al.*, 2011). However, *in vitro* assays do not involve consideration of host-related parameters, such as nutrient status, genotype, physiological state, and secretion of hydrochloric and gastric acid that can potentially affect nutrient absorption. The protocol comprises an *in vitro* digestion process that mimics the three main phases of the human digestive system, that is, the oral phase, gastric and intestinal digestion.

In the gastric phase of digestion, the pH must be adjusted to 2 (to mimic the gastric pH of an adult) and 4 (to simulate the gastric pH of an infant), upon the addition of the pepsin enzyme obtained from porcine stomach. This is because pepsin gets denatured and consequently loses its activity at pH $\geq$ 5. Neutralisation of food samples to pH 5.5–6 occurs at the beginning of intestinal digestion prior to the addition of pancreatic enzymes such as pancreatic amylase, ribonuclease, lipase, proteases (trypsin), and bile salts (emulsifiers), and is then re-adjusted to pH 6.5–7. The oral phase precedes the gastric phase and is sometimes used to mimic the action of alpha-amylase, which breaks down the glycosidic bonds of starch. The oral phase involves the use of force and centrifugation to simulate the chewing process (Minekus *et al.*, 2014).

2.17 Jam making process

Jam is made from the fruit base and addition of sugar, pectin, and acid. This involves boiling of fresh or pre-cooked fruits in a solution of sugar until water evaporates to about 35 % and the mixture forms a gel upon cooling (Abdel, 2012). The presence of pectin at a pH of 3.2–3.4 and a high sugar concentration will result in the formation of a viscous, semi-solid (gel) (Abdel, 2012). Generally, jam making involves weighing of the fruit pulp and heating whilst stirring constantly. Then, the heat is turned off and pectin is added to the fruit pulp while stirring to avoid lump formation or clotting. After the pectin has fully dissolved, sugar is added and is stirred until it dissolves. The jam mixture is heated and stirred. Citric acid is then added near the finishing point. The finishing point is determined by removing samples at specific intervals and checking for TSS. When the jam reaches the desired TSS content of 68 % BRIX, the heat is turned off, and the foam is removed. The jam is transferred into sterile jars. Filling is done rapidly to prevent the temperature of the jam from falling. After filling, the jars are sealed with sterile lids (Sun, 2011).

2.17.1 Pectin

Pectin is a heteropolysaccharide that occurs naturally in the cell walls of fruits and vegetables, thus giving them structure. The polysaccharides of pectin are complex and are present in primary cell walls, particularly the non-woody parts of native plants (Braidwood, Breuer and Sugimoto, 2014). The main sources of commercial pectin are the peels of citrus fruits and to a lesser extent, the peels of apples (May, 2010). When cooked at a high temperature in the presence of sugar and acid, pectin forms a gel, giving jam and jellies a semi-solid texture when they cool (Srivastava and Malyiya, 2011). Pectin located in the middle lamella helps cement the walls of adjacent cells (Albersheim *et al*., 2010). During fruit ripening, protopectin is converted into pectin that is water soluble (Wills and Golding, 2016). In addition, the pectin helps the ripening fruits to remain firm and maintain their shape. As a fruit becomes overripe, the pectin is broken down into water-soluble simple sugars by the enzymes, pectinase and pectin esterase (Braidwood, Breuer and Sugimoto, 2014). As a result the overripe fruit becomes soft and begins to lose its shape (Barclay, Sandall and Shwide-Slavin, 2014).

2.17.1.1 Chemical structure of pectin

Structurally, pectin is a linear polysaccharide that is both polydisperse and polymolecular in nature. The composition of pectin is varied due to the differences in sources and method of isolation. The chemical structure of pectin consists of D-galacturonic acid (Figure 2.20**)** moieties joined by α-(1-4) glycosidic bonds (Srivastava and Malviya, 2011). These uronic acids have carboxyl groups, some of which are naturally present as methyl esters, and others are commercially treated with ammonia to produce carboxyamide groups (Srivastava and Malviya, 2011).

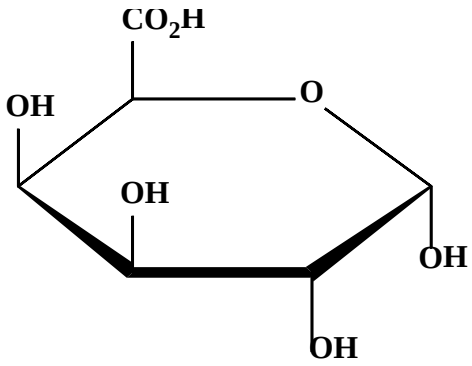


Figure 2.20: Galacturonic acid (adopted from Cosgrove, 2005).

The polygalacturonic acid chain is partly esterified with methyl groups, and the free acid groups may be partly or fully neutralized with sodium, potassium, or ammonium ions. The degree of esterification (DE) is dependent on species, tissue, and maturity. The classes based on the DE comprise high methoxyl (HM) pectins and low methoxyl (LM) pectins, which are either conventionally demethylated or amidated (Wicker *et al.*, 2014). The DE for the commercial HM pectins typically ranges from 60 % to 75 % and those for LM pectins range from 20 % to 40 % (Thibault and Ralet, 2003).

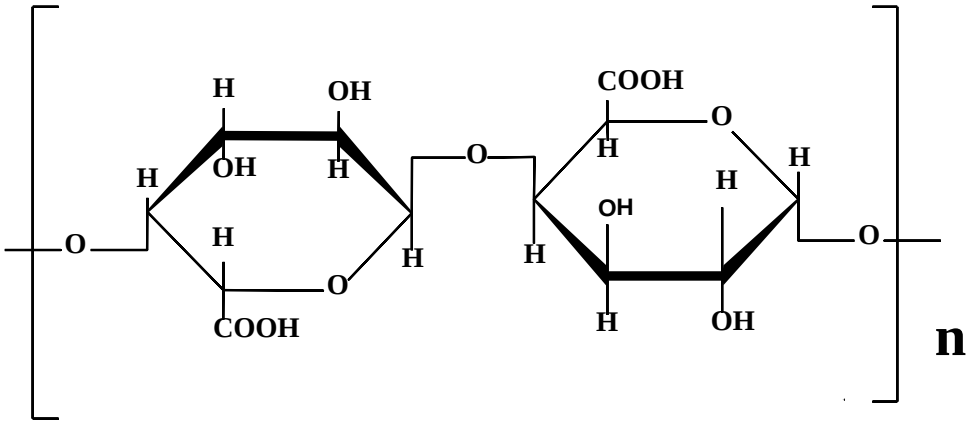


Figure 2.21: Polygalacturonic acid (adopted from Kashyap *et al.*, 2001).

2.17.2 Gelling properties of pectin

Gel formation is caused by hydrogen bonding between free carboxyl groups present on the pectin molecules and between hydroxyl groups of the neighbouring molecules. In a neutral or slightly acid dispersion of pectin molecules, most of the unesterified carboxyl groups are present as partially ionized salts. Upon the addition of an acid, the carboxylic ions are converted to mostly unionized carboxylic acid groups (Sriamornsak, 2003). A decrease in the number of negative charges lowers the attraction between pectin and water molecules and the repulsive forces between pectin molecules. Sugar further decreases the hydration of the pectins by competing for water (Sriamornsak, 2003). These conditions decrease the ability of pectins to stay in a dispersed state. The unstable dispersal of less hydrated pectin forms a gel upon cooling, which is a continuous network of pectin that holds the aqueous solution. The DE affects the rate of gel formation. Other factors that influence gelation of pectin are pH, the presence of other solutes, molecular size, degree of methoxylation, the number and arrangement of side chains, and the charge density on the molecule (Thakur *et al*., 1997). HM pectin sets more rapidly, requires a minimum amount of soluble solids, and a pH of approximately 3.0 in order to form gels (Sriamornsak, 2003). HM pectin gels are thermally reversible, hot water-soluble, and often contain a dispersion agent such as dextrose to prevent lumping. LM pectins produce gels independent of sugar content (Thomas *et al.*, 2013). They are not as sensitive to pH as the HM pectins. LM pectins require the presence of a controlled amount of calcium or other divalent cations for gelation. Although sugar is not essential for gel formation by LM pectins,

small amounts of 10–20 % sugar tends to decrease syneresis and adds desirable firmness to the gel (Thomas *et al.*, 2013). The amount of LM pectin required to form a gel is reduced when sugar is present. A high concentration of sugar that is 60 % or higher interferes with gel formation because the dehydration of sugar favours hydrogen bonding and decreases cross-linking by due to the chemical bonding with anions (Sundar *et al*., 2012).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental design

Ripe *U. kirkiana* fruits were collected from domesticated trees in Gokwe, Bikita, and Kazangarare communal areas (Figure 3.1). Gokwe is a semi-dry region located 18.22°S 28.93°E in Agro farming region 4 and receives a total rainfall of 450–650 mm. Its soils are regosol and basaltic vertisols. Bikita is a dry region located 20. 5 S° 31.37° E in Agro farming region 4 and receives 450 - 650 mm of rainfall. Kazangarare is located 16.30°S 29.56°E in Agro farming region 3 and receives 650–800 mm of rainfall (Metrological Services Department of Zimbabwe, 2018). Permission was sought from the local leaders (councillors) in each area to carry out the research. Consent was obtained from all households and families that participated in the research. In each area, 10 domesticated trees were selected from a total of 5 wards in each area. In each ward two domesticated fruit trees were randomly selected from trees belonging to families that were willing to participate in this study. Fruit were collected between the months of December 2016 - February 2017 and again in December 2017 – February 2018.

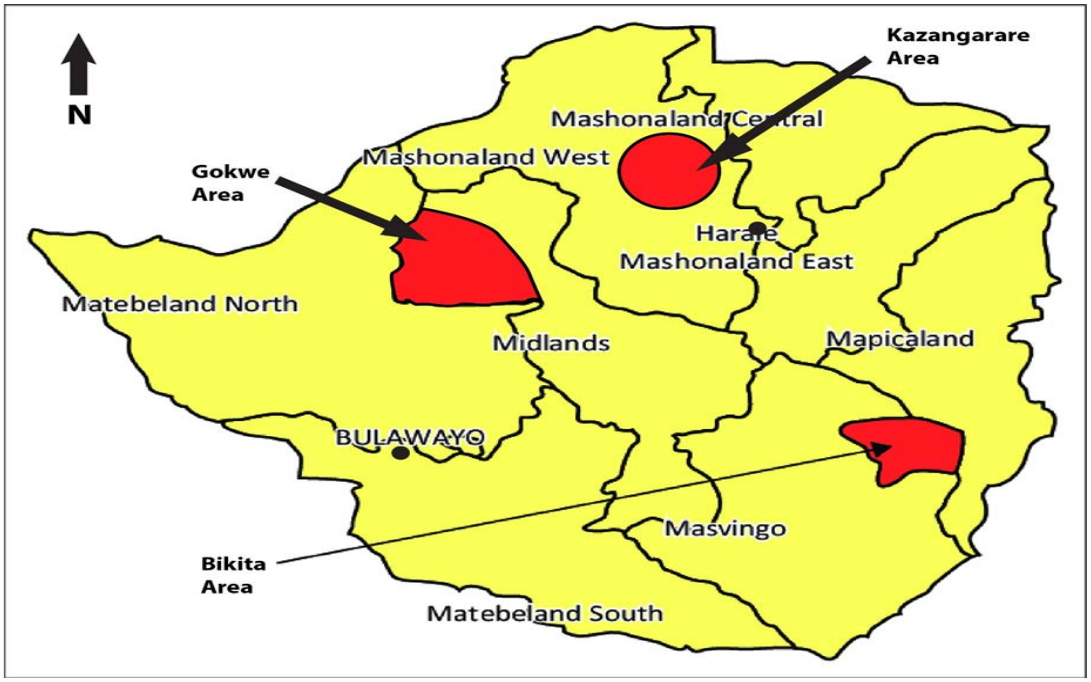

Figure 3.1: Map showing sampling areas (Bikita, Gokwe, and Kazangarare) of *U. kirkiana* fruits in Zimbabwe.

3.2 Sample preparation

Samples of 100 ripe fruits that had fallen from different parts of the tree were randomly collected from -from the ground. A total of 1000 fruits with a net mass of 8kg were collected in each area. The fruits were transported in clean polythene bags and were stored at room temperature (25 ^{o}C) in a Laboratory. From each area, trees highly preferred by households were chosen using randomised design and were used to obtain the fruits. The fruits were cut open and the seeds and skins were removed. The fruit was then pulped using a mortar and pestle, and the crude pulp mixture was sieved through an 800 μM sieve in the laboratory to obtain a composite pulp sample from each area. The fruits were weighed before and after pulping to determine the pulp yield (g / kg). The pulp sample was used to analyse its functional properties (pH, TSS, dry matter, sugars, acidity, mineral content, and vitamin C), fruit attributes (weight, diameter, length, and pulp yield), and bioactive compounds. A composite sample was prepared and used in the production of probiotic jam.

3.3 Physicochemical and nutritional analysis of the fruit and pulp

3.3.1 Determination of fruit diameter and length

Fruit diameter and length were determined using a method adopted from Katsvanga *et al.*, (2007). A hand caliper (Model: Accurate - 2ss) was used to measure fruit diameter and length of nine randomly selected fruits.

3.3.2 Determination of pH

The pH was determined according to AOAC standard method using a digital pH meter (BOECO, Germany: Model BT-675). The glass electrode of the pH meter was calibrated using standard buffer solutions (pH 4 and pH 7) before use (AOAC, 2000).

3.3.3 Determination of Total Soluble Solids

Total soluble solids (TSS) content of the *U. kirkiana* pulp were determined according to AOAC standard method (AOAC, 2000) using a bench brix refractometer (Nieuwkoop BV: Model MA871) and distilled water was used to calibrate and rinse-off residual sample after each reading.

3.3.4 Determination of the dry matter

Dry matter was determined using a method adopted from Magaia *et al.* (2013) with a modification in temperature. A 2 g sample was dried in an automatic oven in a crucible and incubating at 100 ^{o}C overnight until constant moisture loss.

3.3.5 Determination of the moisture content

Moisture content was determined using a method adopted from AOAC (2000). A dry crucible was weighed and 5 g of the sample was transferred into the crucible. The sample was placed in a hot oven at 102 °C for 5 hours and then cooled in a desiccator and re-weighed. The moisture content was determined using the following formula:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where W_1= weight of crucible (g); W_2 = weight of crucible (g) + fresh sample (g); W_3 = weight of crucible (g) + dried sample (g).

3.3.6 Determination of individual sugars (glucose, fructose, and sucrose)

The glucose, fructose, and sucrose content was determined according to a method described by Minekus, (2014) using a Sucrose/D-Fructose/D-Glucose assay kit (Megazyme: K-SUFRG 06/14) protocol as shown in Table 3.1. Samples were placed in plastic cuvettes (10 mm light path) and a colorimetric measurement was used to analyse the sample at absorbance at 340 nm using a UV-vis spectrometer.

Table 3.1: **Protocol for individual sugar assays (adopted from Megazyme, 2014).**

Pipette into cuvettes	Blank sucrose sample	Sucrose sample	Blank D-glucose/D-fructose sample	D-glucose/ D-fructose sample
Solution of 6* (β-fructosidase) sample solution	0.2 mL -	0.20 mL 0.10 mL	- -	- 0.10 mL
Mix **. Incubate for 5 mins (NOTE: before pipetting solution 6. First warm to (25–30 °C). **Then Add:**				
Distilled water (25 °C) Solution 1 (buffer) Solution 2 ($NADP^+$/ ATP)	2.00 mL 0.10 mL 0.10 mL	1.90 mL 0.10 mL 0.10 mL	2.20 mL 0.10 mL 0.10 mL	2.10 mL 0.10 mL 0.10 mL
Mix **. Read absorbance of solution (A_1) after approximately 3 minutes and start the reaction by the addition of:				
Suspension 3 (HK/G6P-DH)	0.02 mL	0.02 mL	0.02 mL	0.02 mL
Mix **. Read the absorbance of solution (A_2) at the end of the reaction (approx. 5 min). If the reaction is not stopped after 5 min, continue to read the absorbance at 2 min intervals until the absorbance remains the same over 2 min***. **Then add:**				
Suspension 4 (PGI)	-	-	0.02 mL	0.02 mL
Mix **. Read the absorbance of solution (A_3) after approximately 10 min.				

3.3.7 Determination of the Total Titratible Aacid

Pulp and/or jam acidity (expressed as total titratable acidity) was determined according to AOAC standard method by titrating 10 g of sample dissolved in 100 mL distilled water against 0.1 M NaOH solution. Development of a pink colour was recorded as the end point using phenolphthalein as an indicator (AOAC, 2000).

3.3.8 Pectin extraction

Extraction of pectin was carried out according to a method described by Tang *et al*. (2011). Ripe fruit was cut with a knife, the seeds were removed and the pulp was collected and dried. Forty grams of dried fruit was weighed using an electrical balance and placed into a beaker. Acid water was prepared by mixing 40 g citric acid with 200 mL of water in a beaker until the pH reached 3. The acid water was added to the beaker containing dried sample and mixed. Extraction was done using water at 90 °C for 3 hours. After 3 h, the samples were cooled to approximately 55 °C and filtered into a beaker using a muslin cloth. Isolation of pectin was carried out using 95 % ethanol as the precipitating agent. One volume of extract was added to ethanol in a ratio of 1:1. Pectin was filtered through a Whatman filter paper and washed with excess 96 % ethanol and cold water to further remove any remaining impurities. Finally, the precipitate was dried at 50 °C in a hot oven (UL 40, Memmert) for 10 h. After drying the precipitate was placed in a desiccator for cooling. The pectin was then ground into powder using a pestle and motor, then sieved and stored in a cool dry place. The experiments were performed in triplicate to ensure consistency. The percentage yield of extracted pectin was calculated as follows:

$$\text{Pectin yield (\%)} = \frac{P}{Q} \times 100$$

where: P = the amount of extracted pectin in grams (g), Q = the initial amount of fruit sample (40 g).

3.3.9 Determination of minerals (zinc and iron)

The mineral analysis was determined according to a method adopted from Altundag and Tuzen, (2011) using an Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES) (Agilent 5100) which allows for simultaneous detection of minerals. Samples were prepared by digestion in concentrated solutions of HNO_3 and H_2SO_4, followed by addition of ultrapure H_2O_2 to complete digestion. Residual pulp was filtered off where necessary. The digested samples were then fed into the automated ICP-OES by vacuum operated pipes and the results were recorded from the print out. The data were standardised per 100 g fresh pulp weight (Altundag and Tuzen, 2011)

3.3.10 Determination of vitamin C (ascorbic acid)

The ascorbic acid concentration was determined by the DCPIP (Dichlorophenolindophenol) titration test according to a method adopted from Nyanga *et al.* (2013). DCPIP solution was prepared by dissolving 0.25 g of the 2,6-Dichlorophenolindophenol in 500 mL of distilled water. Exactly 0.21 g of sodium bicarbonate was then added to the solution and allowed to dissolve. The resulting solution was finally diluted to a litre with distilled water. About 10 mL of the sample juice was pipetted into a 100 mL volumetric flask and mixed with 40 mL of 5 % acetic acid. After 20 minutes, water was added up to the 100 mL mark. The resulting solution (with sample) was then titrated against the prepared standard DCPIP.

3.3.11 DPPH radical scavenging activity of pulp

The radical scavenging activity was determined using a method adopted from Kuda *et al.* (2005) with modifications. Methanolic solution of DPPH (1.5 mL, 1 mM) was mixed with 0.1 mL sample and incubated at 25 °C for 25 min. The sample was mixed at five equal time intervals during the incubation period. The absorbance was determined at 517 nm on a Spectronic Genesys Spectrophotometer after calibration with methanol. Ascorbic acid (0.1 M) was used as a reference control. The scavenging activity was calculated as the percentage decrease in absorbance with time using the following equation:

$$\text{DPPH radical scavenging activity} = \frac{\text{Ab}_{\text{control}} - \text{Ab}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100$$

where $\text{Ab}_{\text{control}}$ = Absorbance of control and $\text{Ab}_{\text{sample}}$ = Absorbance of sample

3.4 Bioactive compounds in fruit pulp

3.4.1 Extraction of polyphenols

The extraction method was adopted from Makkar (1999) with a few modifications. Sun dried fruit pulp (2 g) was ground and placed in 50 mL Eppendof plastic tubes on ice. Extract prepared in the solvent (50 % methanol in distilled water (1:1 v/v) (10 mL)) was subjected to ultrasonication for 15 min. Using a bench centrifuge, the tubes were centrifuged (MLC-3000) for 10 min at 1610 x g. After separation, the supernatant was collected, filtered, and analysed.

3.5 Folin-Ciocalteu assay for total phenolics in pulp

Total phenolic compounds were determined using a method adopted from Makkar (1999) and modified. A sample (50 µL) was diluted to a total volume of 1 L using distilled water. Then, 1 N Folin-C reagent (500 µL) and sodium carbonate (2.5 mL) were added. The mixture was incubated for 40 min at 25 ^{o}C room temperature and the absorbance was measured at 725 nm using a Spectronic- Genesys spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) against a methanol blank. A standard curve of gallic acid plotted between the concentrations of 2.5–50 µg was used to determine the total phenolic content (Figure 2). The total phenolic content of the fruit pulp was expressed in µg of gallic acid equivalence (GAE)/g dry weight (DW).

3.6 Tannin binding assay for tannins present in the pulp

The amount of tannins was determined by using a method adopted form Makkar and Goodchild (1996) with modifications. Polyvinylpolypyrrolidone (PVPL) (1 g) was dissolved in 1 mL distilled water and 1 mL sample was added to this mixture. The mixture was then vortexed and incubated for 15 min at 4 ºC, following which it was centrifuged (Microyn Digital Bench-top Centrifuge) at 1107 x *g* and the total content of phenolic compounds in the supernatant was determined by measuring the absorbance at 725 nm using a Spectronic- Genesys spectrophotometer. The tannin content was calculated as follows:

Tannin content (mg/g) = Total content of phenolic compounds before binding with PVPL - Total content of phenolic compounds after binding with PVPL.

3.7 Vanillin assay for flavonoids in pulp

The sample (5 µL) was diluted in distilled water to a final volume of 1 mL in a test tube. To the diluted sample, 2.5 mL of methanol-HCl (1:1 v/v) and 2.5 mL of vanillin reagent (0.5 g/25 mL) were added. The mixture was vortexed for 15 min and allowed to stand. The absorbance of the sample was measured at 500 nm using a Spectronic Genesys spectrophotometer against a blank of 50 % methanol. The total flavonoid (proanthocyanidin) content was calculated from a calibration curve, and the result was expressed as catechin equivalent per g dry weight as recommended by Porter *et al.* (1986).

3.8 Data analysis

Analysis of Variances (ANOVA) was conducted to determine whether there are any statistically significant differences between the means of independent groups (physicochemical properties) of the pulp from three study sites. The least significant differences (LSD) test was used to compare between the means of the physicochemical properties. Multivariate analysis was performed to identify the most significant fruit pulp attribute responsible for variations in sampling area using the XLSTAT statistical computer package (Version 2015.04.36025). Principal component analysis (PCA) was used to discriminate individual pulp attributes. Pearson's correlation coefficients were used to show the correlation between the pulp attribute in each area. The chi-square distribution was used to analyse sensory evaluation results from the triangle test and the student's *t*-test was used for the preference testing.

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CHAPTER FOUR

BIOACTIVE COMPOUNDS AND FUNCTIONAL POTENTIAL OF *Uapaca kirkiana* (MUELL. ARG.) FRUITS

Abstract

Bioactive compounds and functional properties of a highly nutritious but underutilized *Uapaca kirkiana* fruit collected from semi-dry areas of Zimbabwe were carried out. Some important bioactive and functional compounds in the fruit pulp such as tannins, flavonoids, sugars, ascorbic acid, total titratable acids, dry matter, antioxidant activity and essential minerals were analysed. The presence of total phenolic content, tannin, and flavonoid contents were evaluated using Folin Ciocalteau test, tannin binding test and vanillin test respectively. The pulp yield of the fruit ranged from 12.15 to 15.09 g/100 g. The biochemical and functional parameters obtained include total titratable acid (TTA) of 0.3 - 0.48 g/kg and pH of 4.3 - 4.6. Iron content ranged from 11.25 to 12.16 mg/100 g. Fructose was the dominant sugar (10.12 - 11.0 g/100g). The fruit pulp had a total phenolic content of 67.0–82.5 µg GAE/g. Our studies have revealed that *U. kirkiana* fruit is an excellent source of Fe, phenolics, vitamin C, sugars and therefore is recommended for a functional food source.

4.1 INTRODUCTION

Wild loquat is an underutilised indigenous wild fruit found in the miombo ecological zone in sub-Saharan Africa. The fruit is oval shaped, yellow-brown, and possessing a fleshy skin with a juicy pulp (Moombe *et al.,* 2014). The fruits ripen in November (Mithofer and Waibel 2003) and are normally picked from the ground or removed from the tree for consumption (Mithofer and Waibel 2003; Akinnifesi *et al.,* 2004). The unripe fruits are buried into the soil to induce ripening (Maroyi, 2013). The ripe fruits are often sold at roadsides and at most at the rural markets in Sub-Saharan Africa. The fruit is of important socio-economic value amongst the rural and urban poor. Wild loquat was found to be the most preferred indigenous fruit tree among farmers and consumers in Zambia (Akinnifesi *et al.,* 2004; Franzel *et al.,* 2008; Kalaba *et al.,* 2009; Moombe *et al.,* 2014). The fruit is most preferred because of its sweet taste and nutritional value (Saka *et al.,* 2007; Ramadhani and Schmidt, 2008) and has a better market growth prospects and characteristic uses (Ramadhani and Schmidt, 2008). The fruit is a food

resource to many rural households (Akinnifesi *et al.,* 2004; Saka *et al.,* 2004; Nhukarume *et al.,* 2010; Bille *et al.,* 2013; Mpofu *et al.,* 2014) especially during times of droughts (Mithofer and Waibel 2003; Legwaila *et al.,* 2011). The underprivileged and vulnerable groups of the society in drier areas have been cited as intensive consumers of wild fruits (Campbell *et al.,* 1997).

The fruit is noted to be a good source of protein (Akinnifesi *et al.,* 2008; Ndabikunze *et al.,* 2010; Vinceti *et al.,* 2013), energy (Stadlmayr *et al.,* 2013) and sugar (Akinnifesi *et al.,* 2008; Ndabikunze *et al.,* 2010; Vinceti *et al.,* 2013) for the local consumers. Stadlmayr *et al.,* 2013 reported the proximate composition of the fruit as follows; water (72.6 g / 100 g), carbohydrates (28.7 g / 100 g), proteins (0.5 g / 100 g), fat (0.4 g / 100 g), calories (523 kcal / kJ), Ash (1.1 g / 100 g), fiber (2.3 g / 100 g) ,vitamin C (16.8 mg / 100 g) and is a good source of iron, zinc, calcium and potasium (Ndabikunze *et al.,* 2010).

Owing to diverse species of *U. kirkiana*, the indigenous plant has wide-ranging functional properties as well as rich phytochemical constituents. Phytochemicals are a group of non-nutritive, active biological compounds such as phenolic acids, carotenoids, flavonoids (Fernandes *et al.,* 2011; Alasalvar and Shahidi, 2012) and tannins. These compounds have been found to confer health-beneficial attributes to their consumers such as prevention against inflammation and some cancers (Shofian *et al.,* 2011). Their protective characteristic is due to their ability to act as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and /or metal chelators (Ikram *et al.,* 2009). Unfortunately, indigenous knowledge on wild edible fruits is not yet adequately documented, particularly within Africa, though some attempts have been made in the past to document the uses of certain wild fruits as reported by Shava (2005). Therefore, to fill this research gap in indigenous knowledge system, the present research aims at determining the bioactive compounds and functional properties of the *Uapaca kirkiana* fruit.

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

Sample collection was carried out as described in Chapter 3, Section 3.1.

4.2.2 Pulp extraction

Pulp extraction was carried out using as described in Chapter 3 in Section 3.2.

4.2.3 Biochemical and functional characteristics of *U. kirkiana* fruit pulp

4.2.3.1 Determination of fruit diameter and length

Fruit diameter and length were determined using a method described in Section 3.3.1.

4.2.3.2 Determination of pH

The pH was determined according to AOAC standard method described in Section 3.3.2.

4.2.3.3 Determination of sugar TSS

Total soluble solids (TSS) content of the *U. kirkiana* pulp was determined using a method decribes in Section 3.3.3.

4.2.3.4 Determination of dry matter

Dry matter was determined using a method described in Section 3.3.4.

4.2.3.5 Determination of sugars (glucose, fructose and sucrose) present in the pulp

Glucose, fructose and sucrose content were determined using a sucrose/D-fructose/D-glucose assay kit (Megazyme: K-SUFRG 06/14) according to a method decribed in Section 3.3.6.

4.2.3.6 Determination of the pulp acidity

Pulp acidity (expressed as total titratable acidity) was determined according to a method described in Section 3.3.7.

4.2.3.7 Determination of minerals

The mineral analysis was determined according to a method described in Section 3.3.9.

4.2.3.8 Determination of Vitamin C (ascorbic acid)

The ascorbic acid concentration was determined by a method described in Section 3.3.10.

4.2.4 Bioassay of bioactive phytochemicals of *U. kirkiana* fruit pulp

4.2.4.1 Extraction of polyphenols

Polyphenols were extracted using a method described in Section 3.4.1.

4.2.4.2 Total phenolic compounds measurement

Analyses of phytochemical content are usually used to identify the active phenolic constituents, including flavonoids of the plant parts. Total phenolic compounds were determined using a method described in Section 3.5.

4.2.4.3 Tannin binding assay

Tannins were determined following the method described in Section 3.6.

4.2.4.4 Antioxidant activity

The radical scavenging activity was determined using a method described in Section 3.3.11.

4.2.4.5 Flavonoid content

Flavonoid content determined using Vanillin assay as described in Section 3.7.

4.2.5 Statistical Analysis

ANOVA was carried out to determine if there is any statistically significant difference between the means of fruit properties. The least significant differences (LSD) test was used to compare the means of the physicochemical properties. Multivariate analysis was performed to identify the most significant fruit pulp attribute responsible for variations in sampling area using XLSTAT statistical computer package (Version 2015.04.36025). The principal component analysis (PCA) was used to differentiate individual pulp attributes. Pearson's correlation coefficients were used to show the relationship between the pulp attribute in each area.

4.3 RESULTS

4.3.1 Fruit characteristics

Fruits from Kazangarare had the highest fruit length of 50.17 ± 1.16 mm and diameter of 47.12 ± 2.03 mm as indicated in Table 4.1. Pulp yield was highest in fruits from Kazangarare (15.09 ± 0.27 g/100 g) and lowest in fruits from Bikita (12.15 ± 0.16). Fruit pulp yield was significantly different at ($P < 0.05$) in all areas and fruit length was not significantly different in Bikita and Gokwe.

Table 4.1: Fruit characteristics (n = 9).

Study site	Fresh mass (g)	Fruit length (mm)	Fruit diameter (mm)	Pulp Yield (g/100 g)
Bikita	23.56 ± 1.13^{a}	31.45 ± 0.46^{a}	30.73 ± 0.46^{a}	12.15 ± 0.16^{c}
Gokwe	26.43 ± 0.67^{b}	31.23 ± 0.31^{a}	34.16 ± 0.32^{b}	14.27 ± 0.36^{b}
Kazangarare	34.20 ± 2.11^{c}	50.17 ± 1.16^{b}	47.12 ± 2.03^{c}	15.09 ± 0.27^{a}

Mean ± standard deviations are reported. Means with identical superscripts in a row are not significantly different at $p < 0.05$.

4.3.2 Functional properties of fruit pulp

Fruit pulp had a pH 4.35 ± 0.11, 4.42 ± 0.09, and 4.42 ± 0.09 for Kazangarare, Gokwe, and Bikita, respectively as indicated in Figure 4.1. Total Titratible Acid was highest in fruit pulp from Bikita (0.48 ± 0.04 g/kg) (Figure 4.2). Dry Matter was highest in fruits obtained from Kazangarare (29.38±0.94 %) as shown in Figure 4.3. Fruits from Gokwe had the highest vitamin C content of 16.03 ± 0.69 mg / 100g (Figure 4.4). Fruits from Kazangarare had the highest pulp total soluble solid content of 21.87 ± 1.03 g/100 g (Figure 4.5). The fruit pulp with the highest AOA of 36.68 ± 0.46 was found in fruits from Bikita; fruit pulp with the lowest AOA was found in fruits from Kazangarare (34.96 ± 0.86) (Figure 4.6). The pulp had a pectin content of 0. 21 ± 0.05 %. Dry matter, pH, vitamin C, and TTA were not significantly different ($P < 0.05$) in all fruit pulps from Kazangarare and Gokwe.

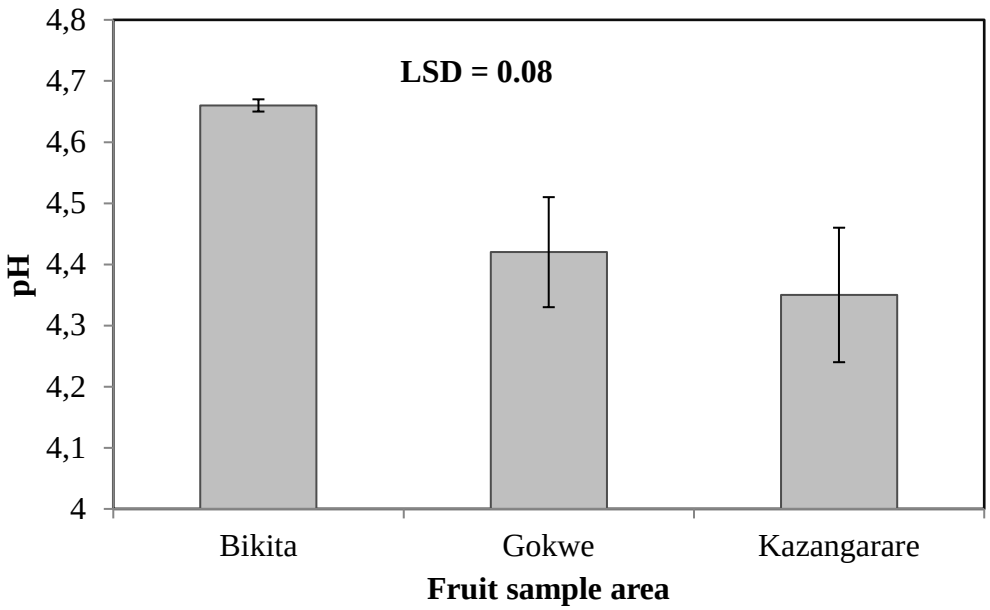

Figure 4.1: pH content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

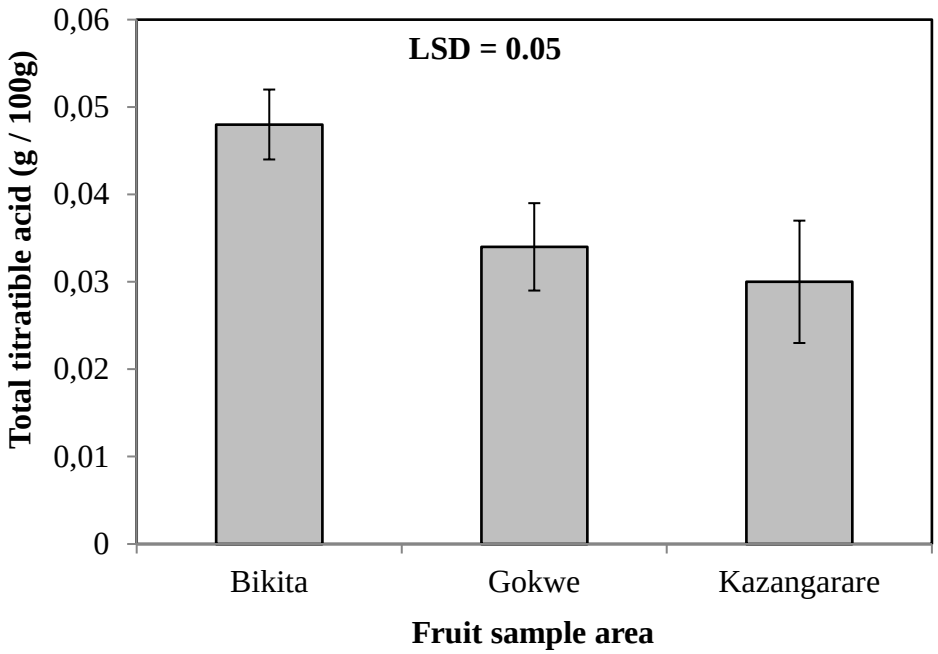

Figure 4.2: Total titratable acid content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

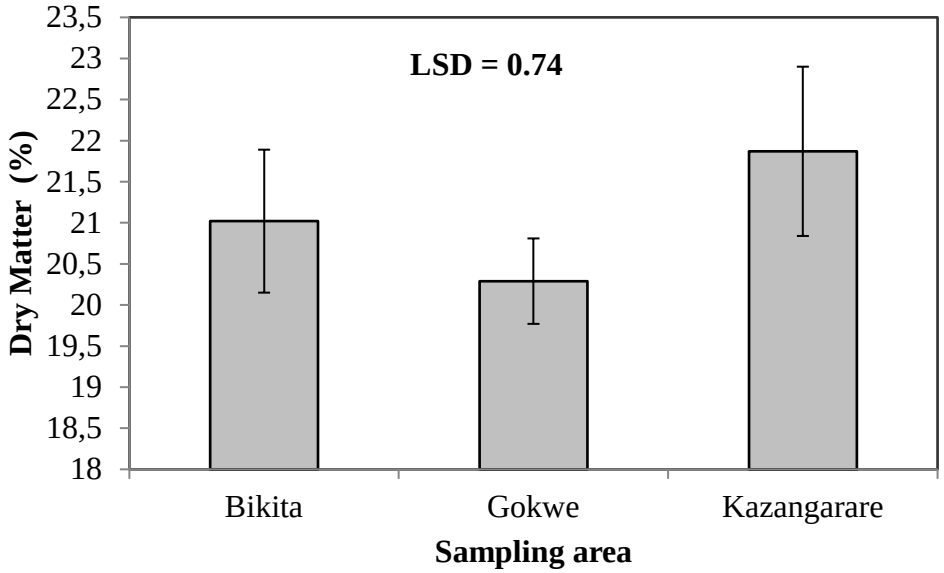

Figure 4.3: Dry Matter content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

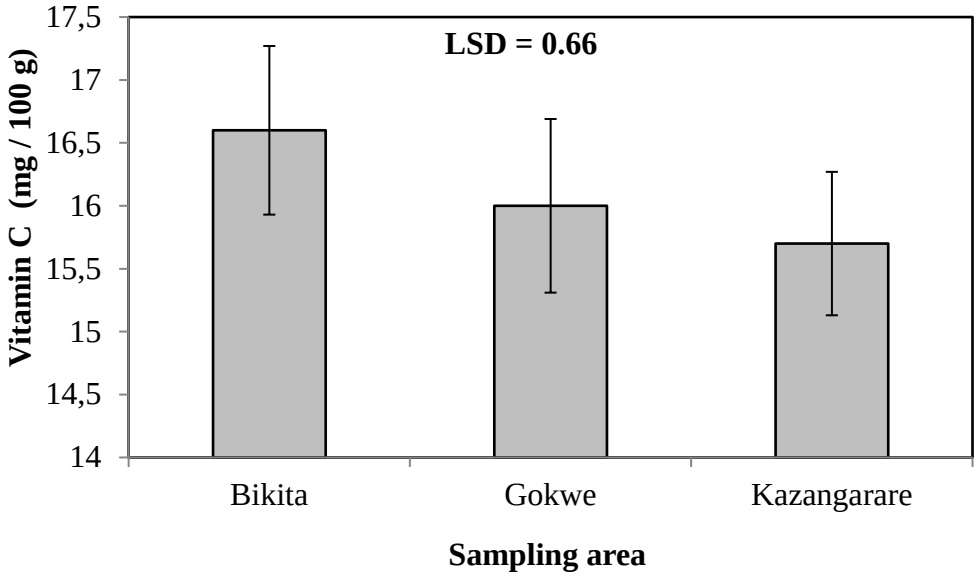

Figure 4.4: Vitamin C content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

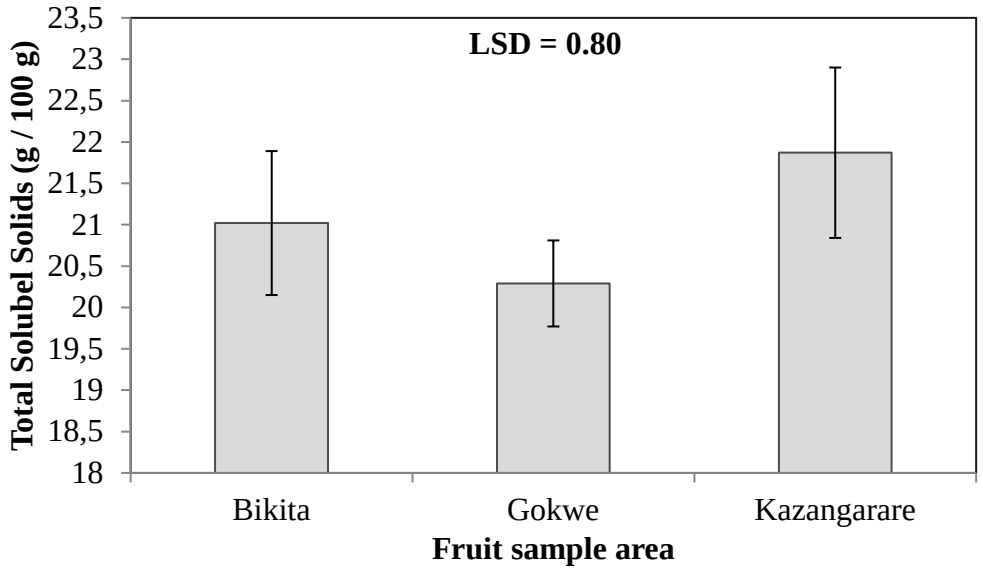

Figure 4.5: Total Soluble Solids content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

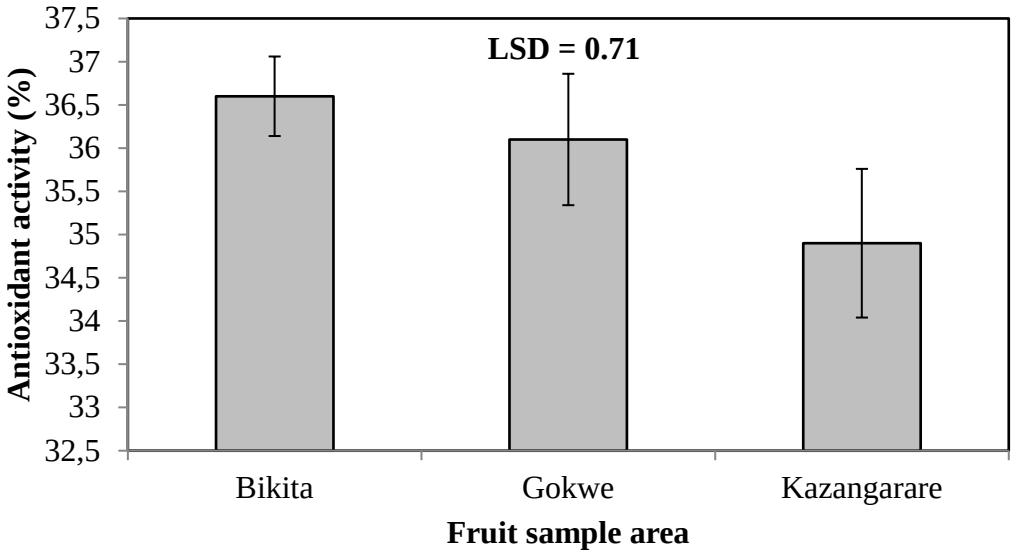

Figure 4.6: Antioxidant Activity (AOA) in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

4.3.3 Gallic acid standard curve for total phenolic content assay in the pulp

The absorbance values for gallic acid that was used to correlate total phenolics in the fruit pulp were 0.08, 0.3, 0.7, and 1.7 for garlic acid concentrations 2.5 µg, 10 µg, 20 µg, and 50 µg respectively as shown in Figure 4.7.

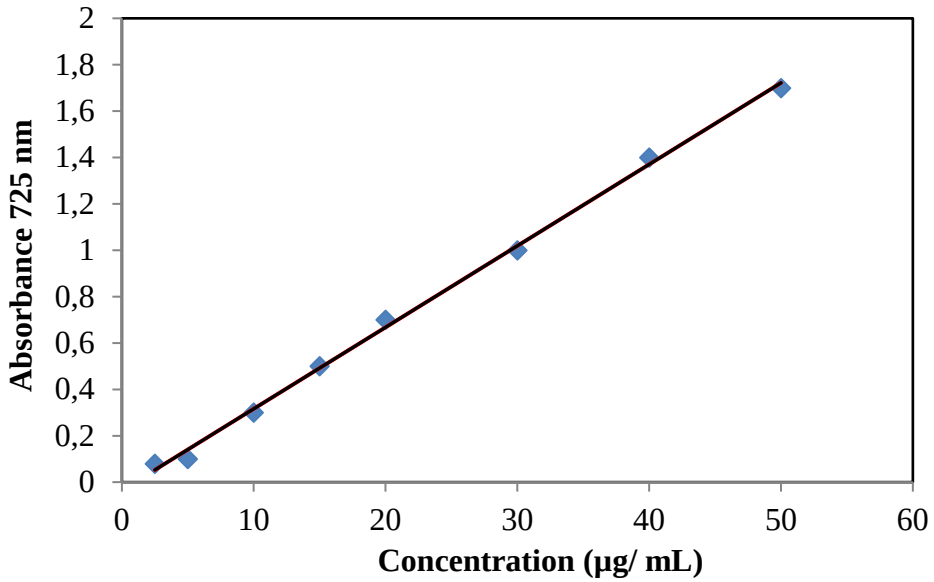

Figure 4.7: Gallic acid standard curve for total phenolic content assay.

4.3.4 Total phenolic content of *U. kirkiana* fruit pulp

Fruits obtained from the Bikita area had the highest total phenolic content of 82.5 µg GAE/g in the pulp, whereas fruits from Gokwe had the lowest total phenolic content of 67 µg GAE/g as indicated in Table 4.2.

Table 4.2: Total phenolic content of *U. kirkiana* fruit pulp.

Location	Absorbance	Concentration (µg/mL)	TPC (µg GAE/g)
Kazangarare	0.53	14.90	74.5
Gokwe	0.48	13.40	67.0
Bikita	0.60	16.80	82.5

4.3.5 Mineral Composition of Fruit pulp

The iron content was 12.06 ± 0.41 mg/100 g, 11.25 ± 0.52 mg/100 g, and 12.16 ± 0.54 mg/100 g for Bikita, Gokwe, and Kazangarare fruit pulp samples, respectively; for zinc content, the mean values were 0.87 ± 0.17 mg/100 g, 0.88 ± 0.11 mg/100 g, and 0.94 ± 0.13 mg/100 g for Bikita, Gokwe, and Kazangarare fruit pulp samples, respectively as shown in Table 4.3. There was no significant difference ($P < 0.05$) in the zinc content from the three areas.

Table 4.3: Mineral composition of *U. kirkiana* fruit pulp (mg/100 g)

Minerals (mg)	Sampling area		
	Bikita	Gokwe	Kazangarare
Ca	16.91 ± 0.38^{ab}	16.43 ± 0.95^{b}	17.26 ± 0.36^{a}
Fe	12.06 ± 0.41^{a}	11.25 ± 0.52^{b}	12.16 ± 0.54^{a}
Zn	0.87 ± 0.17^{a}	0.88 ± 0.11^{a}	0.94 ± 0.13^{a}
Mg	35.01 ± 1.56^{a}	35.13 ± 0.87^{a}	28.72 ± 9.70^{b}
Na	9.6 ± 0.33^{a}	9.78 ± 0.26^{a}	9.08 ± 0.33^{b}
P	15.06 ± 0.18^{a}	14.20 ± 0.54^{b}	13.42 ± 0.49^{c}
K	383.07 ± 4.22^{a}	390.5 ± 4.35^{a}	439.8 ± 162.32^{a}
Cu	0.94 ± 0.11^{a}	0.88 ± 0.07^{ab}	0.8 ± 0.8^{b}

Mean ± standard deviations are reported. Means with identical superscripts in a row are not significantly different at $p < 0.05$.

4.3.6 Sugar content in *Uapaca kirkiana* fruit pulp

The individual sugar content analysis result reveals: mean glucose content ranged from 4.3 to 4.64 g/100 g; sucrose content ranged from 7.08 to 7.62 g/100 g and fructose content range from 10.12 to 11.0g/100g (Table 4.4).

Table 4.4: Sugar content of *Uapaca kirkiana* fruit pulp.

Location	Glucose (g/100 g)	Sucrose (g/100 g)	Fructose (g/100 g)
Bikita	4.64 ± 0.23[a]	7.08 ± 0.16[a]	10.12 ± 0.24[b]
Gokwe	4.35 ± 0.47[a]	7.62 ± 0.45[b]	11.00 ± 0.35[a]
Kazangarare	4.60 ± 0.20[a]	7.10 ± 0.26[a]	10.93 ± 0.21[a]

Mean± standard deviations are reported. Means with identical superscripts in a row are not significantly different at $p < 0.05$.

4.4 DISCUSSION

4.4.1 Fruit characteristics

The results for fruits characteristics are presented in Table 4.1. Fruit weight ranged from 23.56 g to 34.20 g and showed a statistically significant difference in fruits from the three areas. The variation in the fruit weight could be attributed to the presence of organic compounds. The variability in fruit characteristics can be attributed to climatic, edaphic, genetic, and cultural factors (Kelly and Senou, 2017). The higher fruit length observed in fruit from the Kazangarare area could be explained by genetic attributes of the fruit in that area when compared to other sites. A fruit length of (31.23 ± 0.31 mm) was observed in Gokwe and showed a no statistically significant difference compared to observations by Katsvanga *et al.* (2007). Katsvanga *et al.* (2007) noted a fruit length of 26.50 ± 0.77 mm in Gokwe. Pulp yield ranged from 12.15 ± 0.16 g/100 g to 15.09 ± 0.27 g/100 g (Table 4.1) and was lower compared to a pulp yield of 28.34 ± 0.39 g/100 g of *U. kirkiana* fruits growing in Tanzania (Ndabikunze *et al.*, 2010). Pulp yield was significantly different ($F = 158.71$, $p < 0.05$) between the three areas and accounted for 96 % of the variation ($R^2 = 0.96$).

4.4.2 Functional properties

Functional properties of the fruit pulp are presented in Figures 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6. The TTA (0.3–0.48 g/kg) and pH (4.3–4.6) values were significantly different between the three areas studied ($F = 12.58$; $P < 0.0001$ and $F = 15.66$, $P < 0.005$). Approximately 74 % and 69 % of the variation in pulp attributes was attributed to pH ($R^2 = 0.74$) and TTA ($R^2 = 0.69$), respectively. The TTA and pH values were in agreement with the TTA (4.67) and pH (0.5 g/kg) values reported by Ndabikunze Masambu, and Tiisekwa (2010), and are indicators of the

organoleptic quality of the fruit (Harker *et al.*, 2002; Bugaud *et al.*, 2011). Ndabikunze *et al.* (2011) reported a pH of 4.67 ± 0.04 in *U. kirkiana* pulp (n = 4). The TTA can be attributed to the presence of organic acids, such as, malic (Chen, Liu and Chen, 2009) and citric acids present in most ripe fruits (Seymour, Taylor and Tucker, 1993). A positive relationship between moisture content and TTA content in ripe fruits has been reported by many authors (Des Gachons *et al.*, 2004; De La Hera-Orts *et al.*, 2005; Thakur and Singh, 2012).

Total soluble solids content ranged from 20.29 ± 0.52 g/100 g to 21.87 ± 1.03 g/100 g (Figure 4.5) and was significantly different ($F = 4.66$, $P < 0.005$). Approximately 45 % of the variation in pulp attributes was attributed to TSS ($R^2 = 0.45$). Total soluble solids values obtained in the study as shown in Figure 4.5 were higher compared to TSS values of 16.9 ± 0.014 g/100 g reported by Ndabikunze, Masambu and Tiisekwa (2010); it was also higher than that of mangoes (*Mangifera indica*) (14 g/100 g, Belitz and Grosch, 1999), which indicates the potential of *U. kirkiana* fruit in juice and jam making. Katsvanga *et al.* (2007) noted that fruits from areas that experience humid conditions with warm nights often have higher TSS levels and lower fruit acidity, which might explain the trend observed although temperature assays of the areas were not measured to ascertain the possibility its effect. The TSS content in the fruit pulp could also be attributed to the effect of sunlight received in the area during the ripening stage (Leakey and Newton, 1994), which is when the conversion of starch to sucrose and reducing sugars occurs (Alston, 1992); this is correlated to the Brix. The vitamin C content ranged from 15.74 ± 0.57 mg/100 g to 16.63 ± 0.67 mg/100 g (Figure 4.4). The data were not significantly different ($F = 2.04$, $P < 0.05$), and the vitamin C content only accounted for 27 % of the variations in pulp attributes ($r^2 = 0.27$). The observed vitamin C content shown in Figure 4.4 was lower than that reported by Stadlmayr *et al.* (2013) and Ndabikunze, Masambu and Tiisekwa (2010) in the *U. kirkiana* fruit and in other indigenous fruits such as marula (*Sclerocarya birrea*, 128.3 mg/100 g) and baobab (*Adansonia digitate*, 141.3 mg/100 g) (Amarteifio and Mosase, 2006). Fruit pulps had pectin content in the range of 0.18 ± 0.1 to 0.24 ± 0.02 %. Pectin plays an important role in gel formation during the processing of pulp into products such as jam and jellies (Barclay, Sandall and Shwide-Slavin, 2014).

The mean DPPH radical-scavenging activity ranged from 34.96 ± 0.86 % and 36.68 ± 0.46 % (Figure 4.6). There was a strong relationship (r = 0.72) between TTA and AOA of the pulp

(Table 5). The low antioxidant content could be attributed to the presence of phenolic compounds in the pulp. Ndlala *et al.* (2008) reported a high AOA of 43.05 ± 1.34 percent in *U. kirkiana* fruit peel samples (80 µL).

4.4.3 Mineral composition

Mineral content results of the fruit pulp are indicated in Table 4.3. The iron (Fe) content range was 11.25 ± 0.52 mg/100 g to 12.16 ± 0.54 mg/100 g (Table 4.3). There was a significant difference in iron content ($F = 4.20$, $P < 0.05$), magnesium ($F= 3.17$, $P < 0.05$), sodium ($F= 5.59$, $P < 0.05$), and phosphorus ($F = 15.11$, $P < 0.05$). Phosphorus, sodium and iron accounted for approximately 73 %, 50 %, and 43 % of the variation, respectively.

There was a strong correlation ($r^2 = 0.81$) between pH and phosphorus content. The iron and zinc values were higher compared to mean values reported by Ndabikunze, Masambu and Tiisekwa (2010) but are in agreement with values noted in the *U.kirkiana* fruit (Stadlmayr *et al.,* 2013). The fruit pulp has comparable iron content to the given RDA values of 12–19 mg/100 g and is a good iron and zinc source compared to other indigenous fruits such as (*A. digitata*; 0.10 mg/100 g iron and 0.14 mg/100 g zinc; *V. infausta*; 0.09 mg/100 g iron and 0.02 mg/100 g zinc) (Amarteifio and Mosase, 2006). Iron and zinc deficiencies are a major problem in sub-Saharan Africa, especially in rural Zimbabwe (Gadaga, Madzima and Nembaware, 2009; ZIMSTAT, 2016)

4.4.4 Individual sugars

The mean glucose content ranged from 4.3 ± 0.46 g/100 g to 4.64 ± 0.23 g/100 g; mean sucrose content ranged from 7.08 ± 0.16 g/100 g to 7.62 ± 0.45 g/100 g and mean fructose content ranged from 10.12 ± 0.24 g/100 g to 11.0 ± 0.34 g/100 g (Table 4.4). Fructose was the dominant sugar. The variability in the mean sugar content could be attributed to differences in the maturity index of the fruit as the sugar content often varies based on the ripening stage (Bahramian *et al.,* 2011; Lee *et al.,* 2013). Furthermore, during ripening, sugars (glucose) accumulate rapidly (Sweetman *et al.,* 2009) through the process of gluconeogenesis and fruits from water stressed areas tend to accumulate sugars and organic acids (Hummel, Pantin and Sulpice, 2010). This was supported by Gautier *et al.* (2008), who noted that temperature and solar radiation has a huge influence on the accumulation of sugars in the fruit; temperature

ranges of 26 to 30 °C result in increased TSS during ripening due to changes in carbohydrate biosynthesis and increased transpiration rates.

4.4.5 Phytochemicals

The total phenolic content (TPC) of the fruit samples ranged from 67–82.5 µg GAE/g as indicated in Table 4.2**.** The TPC could be attributed to the presence of other non-phenolic compounds in the pulp, such as sugars, amines and organic acids, which can reduce the Folin-Ciocalteu reagent (Prior, Wu and Schaich, 2005). There was no significant difference in the tannin concentrations ($P < 0.05$; LSD =0.0014) in samples from the three areas. The samples from Bikita and Gokwe showed a significant difference (mean difference > LSD) in tannin levels among the two areas. Muchuweti, Ndhlala and Kasiyamhuri (2006) noted a tannin concentration of 0.018 mg/g in ripe sun-dried *U. kirkiana* fruit pulp; sun drying resulted in loss of tannins. Muchuweti, Ndhlala and Kasiyamhuri (2006) reported a difference of 0.125 mg/g DM when the fruit is ripe. This is evidence that tannins are lost during fruit ripening. There was no significant difference in the flavonoid concentrations in all samples ($P < 0.05$). Muchuweti, Ndhlala and Kasiyamhuri (2006) noted a flavonoid content of 0.004 mg/g DM in fruit pulp prepared from ripe *U. kirkiana*. Ndlala *et al.* (2007a) reported a flavonoid content of 202 µg catechin / g and 41µg catechin / g in *Sclerocarya birrea* and *Flacourtia indica* fruit pulp respectively. There was no significant difference in gallotannin concentrations in samples ($F = 49.46$; $P < 0.001$) obtained from all areas. Mean gallotannin concentrations were not significantly different in all areas (LSD = 0.057). Muchuweti, Ndhlala and Kasiyamhuri (2006) reported a 0.0067 mg/g DM gallotannin in fruit pulp from ripe fruits, which has been reported to have many biological activities, such as anticancer, antioxidant, anti-inflammatory, anti-hyperglycaemic, lipid lowering, and antimicrobial activities (Patel and Goyal, 2011).

4.5 CONCLUSION

The study has shown that *Uapaca kirkiana* fruit has good physicochemical and biochemical functional properties. The pulp yield and the TSS values of the fruit are high. The investigation also established the fruit as a good source of micronutrients especially iron. The fruit has acceptable amounts of phenolic compounds and good vitamin C content: powerful antioxidants that are purported to be healthy food components. Therefore, it is recommended that the *U.*

kirkiana fruit should be used to produce nutritive functional foods. However, comprehensive bioassay and toxicity analyses should be conducted to establish the fruit's full biochemical value.

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CHAPTER FIVE

FUNCTIONAL AND NUTRITIONAL PROPERTIES OF *U.KIRKIANA* FRUIT JAM CONTAINING A PROBIOTIC, *Lactobacillus rhamnosus* YOBA.

Abstract

A probiotic fruit jam was developed using an underutilised fruit, *U. kirkiana* fruit to benefit the resource-poor populations in southern Africa from a functional food. *U. kirkiana* fruit pulp is mainly consumed in rural southern Africa, making it an ideal food matrix to carry probiotics. A process to develop the probiotic fruit jam was designed. Ripe *U. kirkiana* fruits were obtained from domesticated trees that were mostly preferred by households residing in semi-dry rural areas. The fruits were pulped and the crude pulp mixture was sieved through an 800 µm sieve. Pectin content of the pulp was determined. A probiotic jam was developed using the formulation- 55 % (wt/vol) pulp, 46 % (wt/vol) sugar, 1.5 % (wt/vol) pectin, and 0.5 % (wt/vol) citric acid. The fruit pulp was mixed with sugar in a stainless steel pot and cooked at 110 °C. Citric acid was added and stirred gently whilst cooking until it reached 55 Brix. Pectin was then added and the mixture was continuously stirred until it reached 68 Brix. The jam was inoculated with probiotic *Lactobacillus rhamnosus* yoba and left to ferment for 24 h, while the growth of the bacterial culture was monitored. The physicochemical and functional properties (pH, TSS, sugars, acidity, iron content, zinc content, and vitamin C), and probiotic viability of the jam inoculated with *L. rhamnosus* yoba were analysed. The jam inoculated with *L. rhamnosus* yoba had a vitamin C, TTA, Brix, and moisture content of 0.34 ±0.02 mg/100 g, 2.2 ± 0.11, 68.5 ± 0.2, and 34.8 ± 1.2, respectively. Immediately after production, the jam inoculated with *L. rhamnosus* yoba had an iron and zinc content of 4.13 ± 0.52 mg/100 g and 0.36 ± 0.02 mg/100 g, respectively. The jam inoculated with *L. rhamnosus* yoba exhibited high fructose and sucrose content of 12.84 ± 0.21 g/100 g and 24.61 ± 0.12 g/100 g, respectively. Further, the jam inoculated with *L. rhamnosus* yoba had a TTA content of 2.2 at d 0 (after production), 2.37 ± 0.01 at d 4, and 2.48 ± 0.02 at d 7 of storage (25 °C). The fruit jam was able to deliver 6.2 ± 0.2 log CFU/mL live *L. rhamnosus* yoba cells, which make it a good probiotic food.

5.1 INTRODUCTION

A probiotic bacterium is defined as a live microorganism that is able to move through the gastrointestinal tract passage, reach the intestinal tract in its active form, and in adequate viable numbers that will positively affect the health of the host (Franz *et al.,* 2011a). The probiotic must be consumed as part of food in order to confer their health benefits (WHO, 2002; Mpofu *et al.,* 2014). Probiotic uses have an estimated world market share of 15 billion USD (Bhadoria and Mahapatra, 2011) and take a great fraction of the world market for 'functional foods' (Figueroa-González *et al.,* 2011). 'Functional foods' refer to foods that contain ingredients which improve physiological and health conditions such as probiotics, prebiotics, minerals, and vitamins (Franz *et al.,* 2014). The mode of action of probiotics especially on health improving properties still lack complete understanding although suggested actions relate to immunemodulation, anticarcinogenic, and antimutagenic processes, fighting pathogens, improvement of lactose intolerance symptoms, decreasing blood cholesterol levels, preservation of intestinal mucosa conditions, and improved periodontal health (Reid *et al.,* 1995; Burns and Rowland, 2000; Holzapfel and Schillinger, 2002; Isolauri, 2004; Hummelen *et al.,* 2010; Gupta, 2011; Kumari *et al.,* 2011). Many meta-analysis studies have shown positive effects of specific probiotic strains on treatment of diarrheal disease among children (van Niel *et al.,* 2002; Allen *et al.,* 2004, 2010; Szajewska *et al.,* 2007a,b; Guandalini, 2008, 2011; Salari *et al.,* 2012).

The use of probiotic strains such as *Lactobacillus rhamnosus* GG in food products in sub-Saharan African is not well documented and these products are yet to be sold (Kort *et al.,* 2015). Sub-Saharan Africa has the highest percentage of chronically malnourished people in the world (OECD-FAO, 2011) and highest under -five year's old mortality rate of 98 deaths per 1000 live births (UN Interagency Group for Child Mortality estimation, 2013). Diarrhoea is one of the leading causes of poor health and childhood mortality in sub-Saharan Africa and accounts for 37 % of childhood deaths (Kalipeni, 2000). More studies on the lactic acid bacterium *L. rhamnosus* GG have reported recognizable health benefits of the probiotic bacterium once consumed, including the treatment of diarrhoea in children (Kort *et al.,* 2015). Meta-analyses of clinical research with *L. rhamnosus* GG bacterium have indicated the ability of the probiotic

bacterium in shortening the diarrheal phase of rotavirus infection by one day upon after oral intake (de Roos and Katan, 2000; Allen *et al.*, 2010).

The utilization of local food materials such as wild fruits and incorporating probiotics may act as a possible way by which the health of the children can be improved in Africa (Franz *et al.*, 2014). Food processing may also improve food quality, minimise postharvest losses, detoxifying the food, and increase the dietary intakes of macro and micronutrients, hence alleviating malnutrition (Holzapfel, 2002). This was supported by a study by Mpofu *et al.*, (2015) who reported the effective suppression of five food pathogens in fermented dairy products with *L. rhamnosus* yoba 2012 in Zimbabwe.

Lately, generic probiotics are being studied as a practical solution to gain access to probiotics for use in food processing by many rural folks in most rural parts in Africa (Kort and Sybesma, 2012). This study was aimed at determining the functional and nutritional properties of *U. kirkiana* fruit jam containing a probiotic, *L. rhamnosus* yoba. *L. rhamnosus* yoba 2012 is a generic probiotic obtained after isolation of *L. rhamnosus* GG using a commercial food product (Kort and Sybesma, 2012). *L. rhamnosus* yoba was used in this study. In order to enable growth of the *L. rhamnosus* yoba bacterium, this experiment used *U. kirkiana* fruit pulp as a source of degradable sugars.

5.3 MATERIALS AND METHODS

5.3.1 Sample collection and Pulp extraction

Sample collection and pulp extraction were carried out according to a method described in Section 3.1 and 3.2.

5.3.2 Pectin extraction

Extraction of pectin was carried out according to a method described in Section 3.3.8.

5.3.3 Determination of the moisture content

Moisture content was determined using a method described in Chapter 3 on section 3.3.5.

5.3.4 Production of jam

The preparation of the *U. kirkiana* fruit jam is represented in the flow chart depicted in Figure 23**.** The production process was adopted from Randazzo *et al.* (2013) with a few modifications. In the formulation process, the jam comprised 55 % composite fruit pulp, 43 % sugar, 1.5 % pectin, and 0.5 % citric acid. In producing the jam, a composite fruit pulp was mixed with sugar in a stainless steel pot and cooked at 110 °C until all the sugar had dissolved. The mixture was checked for the right consistency using the spoon test. Citric acid was added and stirred gently whilst cooking until it reached 55 Brix. Commercial pectin was then added to avoid clotting or formation of lumps. The mixture was continuously stirred whilst being cooked. Brix measurements were conducted at regular intervals during cooking using a hand refractometer until it reached 68 %. The hot jam mixture was filled into sterilised bottles (340 g) and capped. The bottles were then dipped in boiling water, cooled and stored at room temperature. After cooling, the jam bottles were inoculated with a fresh probiotic culture of *L. rhamnosus* yoba and stored at room temperature (25 °C). In a control experiment, distilled water was boiled, cooled and inoculated into the jam.

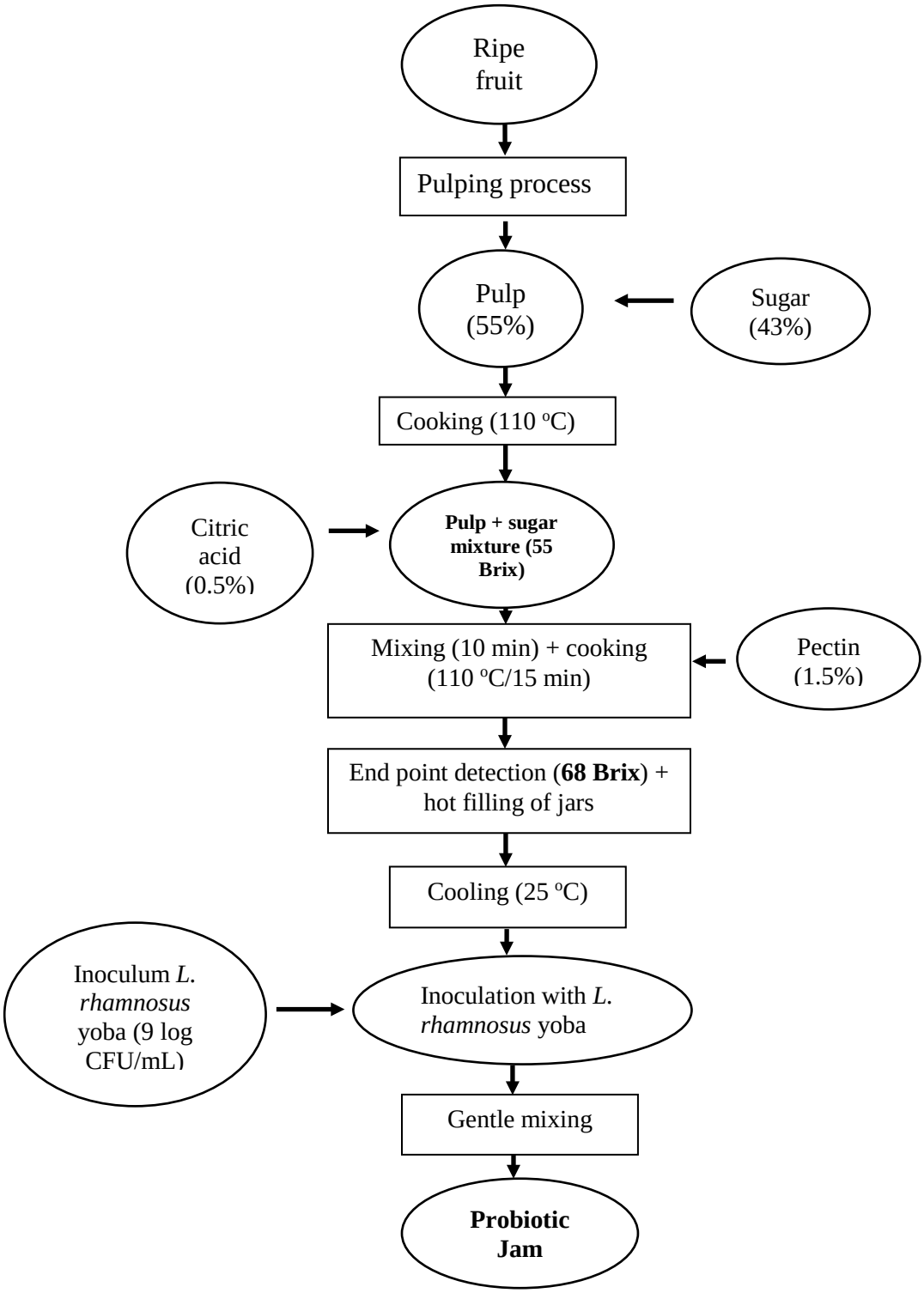

Figure 5.1: Flow chart depicting the production of probiotic jam (functional food).

The physicochemical and functional properties (pH, TSS, sugars, acidity, iron and zinc content, and vitamin C), and probiotic viability of the jam inoculated with *L. rhamnosus* yoba and control jam samples were analysed.

5.3.5 Medium and inoculum for probiotic jam

An isolate of *L. rhamnosus* yoba (Kort and Sybesma, 2012) was used in the experiment. The *L. rhamnosus* yoba strain was obtained in a sachet from the Yoba for Life Foundation, Amsterdam, Netherlands and stored at −80 °C. The *L. rhamnosus* yoba strain was reactivated by sub-culturing anaerobically in MRS broth at 37 °C for 18 h. *L. rhamnosus* yoba utilises sugar and it supports its growth. *U. kirkiana* fruit pulp was mixed with sugar, boiled and subsequently cooled to room temperature (25 °C), and was then used to cultivate *L. rhamnosus* yoba. Fermentable sugar is normally added to stimulate and promote growth (Jyoti, Suresh and Venkatesh, 2003; Gaudreau, Champagne and Jelen, 2005). *L. rhamnosus* yoba was then precultured in the medium and incubated at 37 °C for 36 h until the number of live cells reached above 9 log CFU/mL. This probiotic culture was used for preparing the probiotic jam.

5.3.6 Inoculation of probiotic cultures into the jam

Sterilised tubes (100 g) containing the *U. kirkiana* fruit jam were opened under aseptic conditions, and the jam was inoculated with a (0.25 mL) fresh probiotic culture. The cell suspensions were gently mixed with the jam. The jam was stored at 25 °C.

5.3.7 Enumeration of *L. rhamnosus* yoba into the probiotic jam

Enumeration of viable L. *rhamnosus* yoba in the probiotic jam was carried out using a protocol adopted from Mpofu *et al.* (2014). *L. rhamnosus* yoba was enumerated in the produced probiotic jam before it was consumed and evaluated by the sensory panellists. One millilitre of sample was aseptically taken from the jam to be served to a consumer. Serial decimal dilutions were carried out in a peptone physiological salt solution (pH 7.0, 8.5 g/L NaCl, and 1 g/L neutralized bacteriological peptone from Oxoid). Diluents of 100 µL were plated in triplicate onto de Man, Rogosa and MRS agar (1.2 % agar, bacteriological peptone from Oxoid, added to de Man, Rogosa and Sharpe broth, Merck). MRS agar plates were incubated at 37 °C under anaerobic conditions in Gas Pack anaerobic jars (Becton Dickinson Microbiology Systems, Baltimore, Maryland, USA). All colonies on the MRS agar were counted and results were expressed as colony forming units per millilitre (CFU/mL) of *L. rhamnosus* yoba, taking into account the dilution factors.

5.3.8 Physicochemical analyses of the jam inoculated with *L. rhamnosus* yoba

5.3.8.1 Determination of pH

The pH of the fruit and the jam samples were determined using a method described in Section 3.3.2. Measurements were taken from day of jam production up to d 7.

5.3.8.2 Determination of individual sugars (glucose, fructose, and sucrose)

The glucose, fructose, and sucrose content were determined using a Sucrose/D-Fructose/D-Glucose assay kit (Megazyme: K-SUFRG 06/14) according to a procedure described in Section 3.3.6.

5.3.8.3 Determination of minerals (zinc and iron) in the jam

Iron and zinc content in the jam was determined according to a method described in Section 3.3.9.

5.3.8.4 Determination of vitamin C (ascorbic acid)

Ascorbic acid concentration in the jam was determined by a method described in Section 3.3.10.

5.3.8.5 DPPH radical scavenging activity of pulp

The radical scavenging activity of the jam was determined using a method described in Section 3.3.11.

5.4 RESULTS

5.4.1 Functional Properties of *L. rhamnosus* yoba jam and composite pulp

The jam inoculated with *L. rhamnosus* yoba had a vitamin C, TTA, Brix, and moisture content of 0.34 ±0.02 mg/100 g, 2.2 ± 0.11, 68.5 ± 0.2, and 34.8 ± 1.2, respectively as shown in Table 5.2. The composite pulp sample had an antioxidant activity of 35 ± 1.02 %. The control sample had a low pH of 3.3 ± 0.10. There was no significant difference ($p< 0.05$) between the jam inoculated with *L. rhamnosus* yoba and control jam sample with respect to the TTA and Brix contents just after production.

Table 5.1: Bio-chemical and Functional properties of functional food (probiotic jam) and composite pulp

Functional Properties	Jam with *L. rhamnosus* yoba	Control jam sample	Composite pulp
Vitamin C (mg/100 g)	0.34 ±0.02^{a}	0.28 ± 0.03^{b}	17.4 ± 0.13^{c}
TTA	2.2 ± 0.11^{a}	2.1 ± 0.10^{a}	0.4 ± 0.02^{b}
pH	3.5 ± 0.12^{a}	3.3 ± 0.10^{b}	4.3 ±0.02^{c}
Brix	68.5 ± 0.2^{a}	68.0 ± 0.1^{a}	20.6 ±0.12^{c}
Antioxidant activity (%)	3.7 ± 1.12^{a}	3.3 ± 1.0^{b}	35 ±1.02^{c}
Moisture content	32.8 ± 1.1^{a}	32.5 ± 1.2^{a}	72.2±0.3^{b}
% Pectin	-	-	0.25± 0.05^{a}

Mean ± standard deviations are reported. Means with identical superscripts in a row are not significantly different at $p < 0.05$.

5.4.2 Mean iron content in jam and composite pulp

The jam inoculated with *L. rhamnosus* yoba had an iron content of 4.13 ± 0.52 mg/100 g immediately after production (Figure 5.2). The composite pulp used to produce the jam had an iron content of 11.7 ± 0.03 mg/100 g. The control jam (inoculated with distilled water) sample had an iron content of 4.03 ± 0.14 mg/100 g. There was no significant difference ($p < 0.05$) in the iron content between the inoculated and control jam.

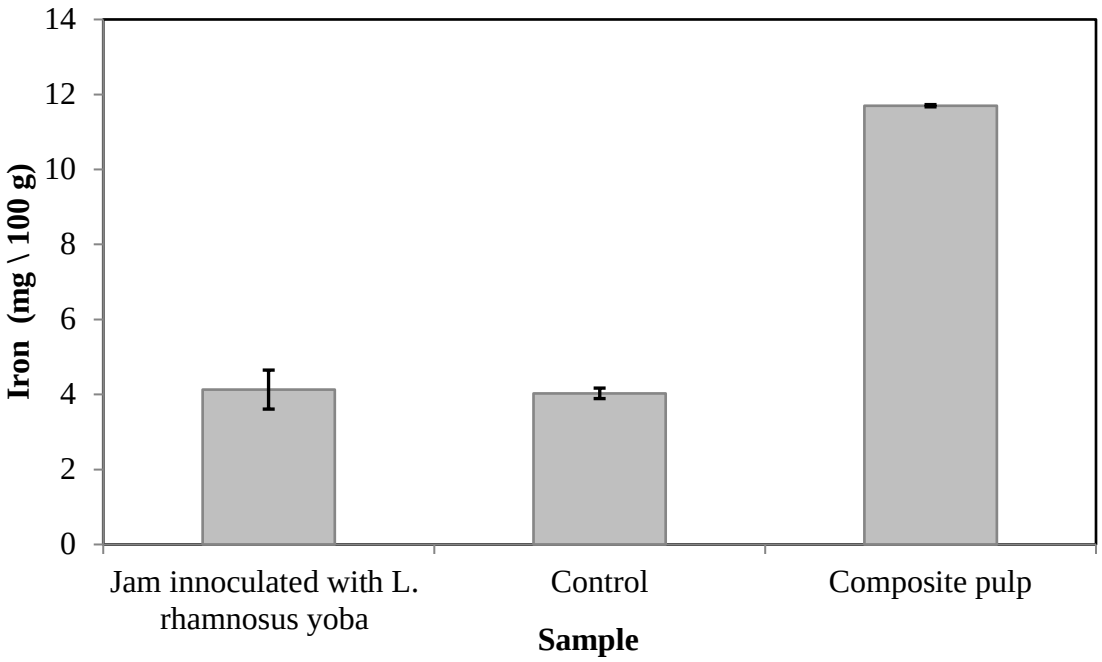

Figure 5.2: Iron content in the *L. rhamnosus* yoba jam.

5.4.3 Zinc content in jam and composite pulp

The jam inoculated with *L. rhamnosus* yoba had a zinc content of 0.36 ± 0.02 mg/100 g as indicated in Figure 5.3. The composite pulp used to produce the jam had a zinc content of 0.83 ± 0.02 mg/100 g. The control jam (inoculated with distilled water) had a zinc content of 0.34 ± 0.01 mg/100 g. There was no significant difference ($p < 0.05$) in zinc content between the inoculated and control jam.

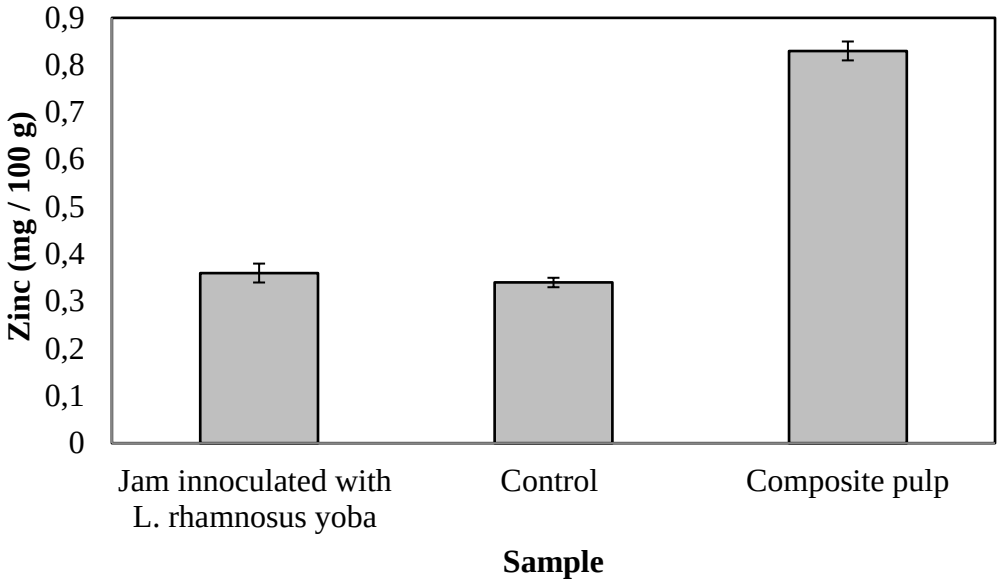

Figure 5.3: Zinc content in the jam inoculated with *L. rhamnosus* yoba.

5.3.4 Mean sugars in jam and composite pulp

The jam inoculated with *L. rhamnosus* yoba had the highest fructose and sucrose contents of 12.84 ± 0.21 g/100 g and 24.61 ± 0.12 g/100 g, respectively (Figure 5.4). The control jam had a sucrose content and fructose content of 23.4 ± 0.1 g/100 g and 12.62 ± 0.06 g/100 g, respectively. Fructose and sucrose contents were statistically different at ($P < 0.05$) in all the samples.

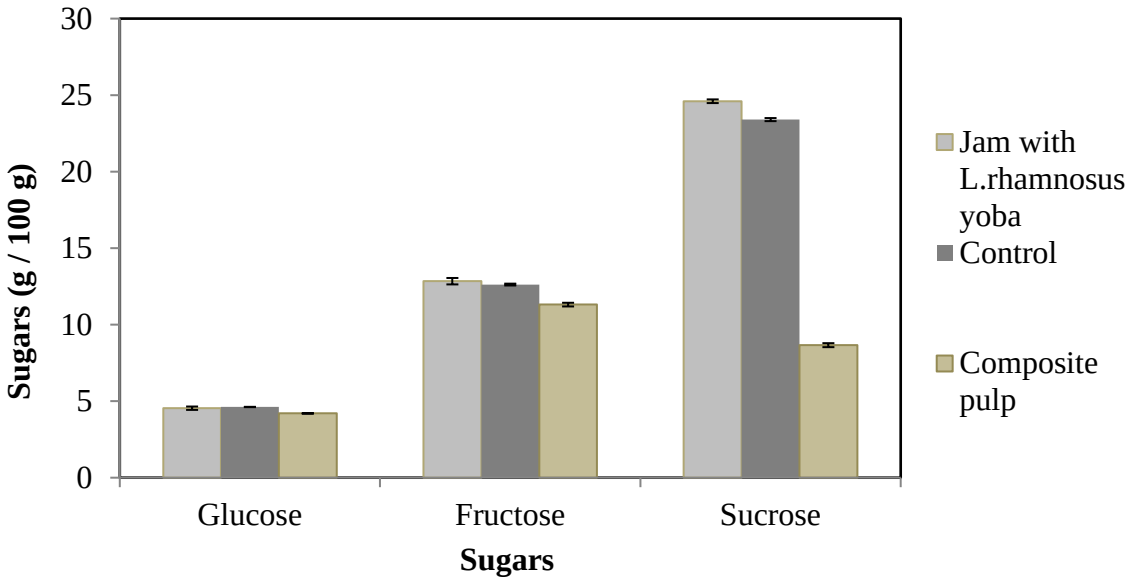

Figure 5.4: Sugar contents in the jam inoculated with *L. rhamnosus* yoba.

5.4.4 TTA content in *L. rhamnosus* yoba jam over 7 d in storage (25 ºC)

Both the jam inoculated with *L. rhamnosus* yoba and control jam had a TTA content of 2.2±0.01 at d 0 (after production) as shown in Figure 5.5. *L. rhamnosus* yoba jam had a TTA content of 2.37 ± 0.01 and 2.48 ± 0.02 at d 4 and 7, respectively in storage (25 °C) as indicted in Figure 5.5. TTA contents were significantly different in jam inoculated with *L. rhamnosus* yoba and control jam at ($p < 0.05$).

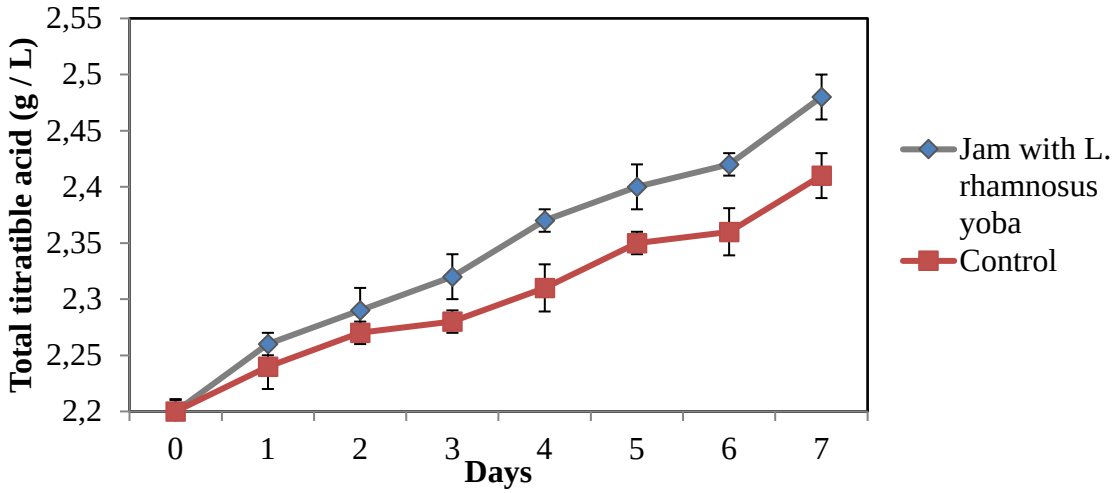

Figure 5.5: Total Titatable Acids content in the jam inoculated with *L. rhamnosus* yoba over 7 d in storage (25 °C).

5.5 DISCUSSION

5.5.1 Production of jam

5.5.1.1 Gel formation and pH

Titratable acidity and pH influence gel formation during the production of jam. During the production of the jam that was then inoculated with *L. rhamnosus* yoba, gel formation was desirable. This suggests that the presence of high methoxyl pectins in the pulp was complimented by the addition of commercial pectin that was added during the production process. The addition of citric acid helped to regulate the pH of the jam. The use of citric acid in setting the pH at approximately 2.8–3.2 affected the viscosity of the final product (Nwosu et al., 2014). Further, at this pH range (2.8–3.2) there is improvement in gel formation, flavour enhancement, and shelf life of the product (Featherstone, 2016). The *L. rhamnosus* yoba jam had a higher pH, which resulted in a firm gel that was found to be desirable by the panellists when they evaluated the jam. There was a significant difference between the jam formulations ($P < 0.05$). The *L. rhamnosus* yoba and control jam had a pH of 3.5 ±0.12 and 3.3 ±0.1, respectively. They were noted to be acidic having a pH range of 3.4–3.6, which is in accordance with the required pH limit (3.6) of the Food and Agriculture Organisation of the United Nations. The pH of jam is usually in the range of 2.8–3.4 and is mainly dependent on the fruit (Nwosu et al., 2014).

During the production of jam, the setting or gelling process requires three main ingredients, pectin, acid, and water (Nwosu et al., 2014). This supports the use of these ingredients in the production of the *L. rhamnosus* yoba jam. In the jam system, pectin acts as a gelling agent that causes a physical transformation through aggregate bonding changes that result in a syrup solidifying into a solid gel. Sugar and acid act as the major agents that bring about this physical transformation, whereas water acts a solvent into which sugar, pectin and acid are dissolved (Featherstone, 2016). Attempts to explain the gelling mechanism of the sugar, pectin, and acid system have been made (Nwosu et al., 2014). The solubility of pectin during gel formation is one of the theories to explain the gelling mechanism in jam production. Pectin is readily water soluble at a concentration of 25 %. The addition of sugar during the production of the L. rhamnosus yoba jam allowed the precipitation of natural pectin molecules in the pulp and the commercial pectin that was added, due to the dehydrating effect of the sugar. Because of their

negative charge, the pectin molecules in solution repel each other. The concentration of hydrogen ions is higher at low pH, which tends to supress the ionisation of the galacturonate carboxyl group, and the tendency of the negatively charged carboxyl group to repel each other. With the addition of citric acid, that is, lowering the pH through the addition of more hydrogen ions, the negative charge of the pectin molecules is reduced and subsequently enables the hydrogen bonding of adjacent pectin molecules (Featherstone, 2016). This results in the precipitation of pectin molecules to form a web that traps water and solutes in the network (Featherstone, 2016).

Furthermore, the production of a satisfactory gel was dependent mainly on the pH of the jam during production and on the use of appropriate quantities and concentrations of pectin and sugar. Therefore, in the formulation, 0.5 % citric acid and 1.5 % pectin were used to produce a good gel. The addition of 1.5 % pectin was necessitated by the fact that the composite pulp used in jam making had a relatively low pectin content of 0.25 ± 0.05 %. In addition, a low pH is important in jam for inhibiting the proliferation of spoilage bacteria, fungi, and moulds. The effect of pH is explained in Figure 5.6, where a pH < 2.4 and > 3.6 will produce weak and no gels, respectively. The optimum pH for the growth of L. rhamnosus is 6.4–6.9 (Liew et al., 2005). At a pH range of 3.4-4.4, its growth the lowest (Helland, Wicklund and Narvhus, 2004). The pH of the jam after production was not favourable for the active growth of bacteria and suggests a slowed growth rate. However, there was still 9 log CFU/mL of live cells before consumption. Such a low pH ensures the microbiological safety of the jam as a low pH inhibits the growth and survival of many food pathogens and microbes (International Commission on Microbiological Specifications for Foods, 2002).

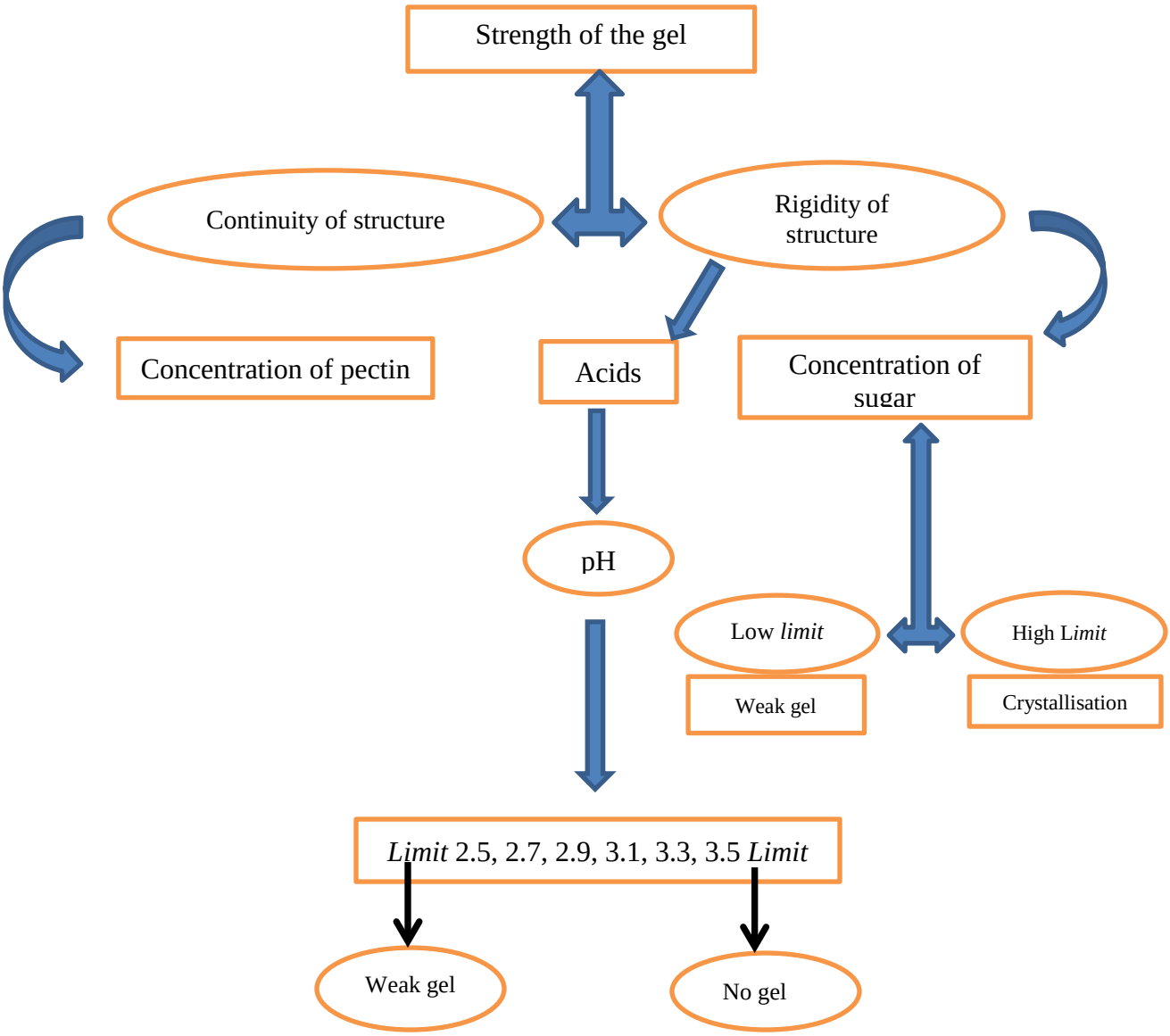

Figure 5.6: Effect of pH on gel formation.

5.5.2 Total titratable acid (TTA)

There was a significant difference in the TTA of the jam, and it was noted to range from 2.1–2.5 over a 7 d storage period, which is in accordance with the standard value associated with good quality jam. Ndabikunze *et al.* (2011) reported a percentage TTA content of 0.05 ± 0.02 in *U. kirkiana* pulp (n = 4). Acidity of the pulp is an important aspect in jam making as it has an influence on gel formation. Acid is one of the essential ingredients that are required for the gelling of jam. Fruits naturally contain acids, mainly citric acid, but other acids such as malic acid and tartaric acid can also be found in a number of fruits. The source of the TTA noted in the jam could be attributed to the presence of natural acids in the fruit, but these are present in very low quantities to support jam making. As such, to achieve a desirable pH, citric acid is mainly added at a low concentration to balance and improve the pH (Featherstone, 2016). Acids play an important role in the setting of pectin and gel formation, as explained in Section 5.6.1. A more acidic pH ensures that the carboxyl groups present in the jam mixture are not be ionised, thereby lowering the repulsive forces (Featherstone, 2016).

5.5.3 Moisture content of jam formulations

There was no significant difference in the moisture content of the jam formulations ($P < 0.05$). *L. rhamnosus* yoba jam had a moisture content of 32.8 ± 1.1 % and the control jam had a moisture content of 32.5 ± 1.2 %. These observed moisture content of the jam was within expected limits of 30.9–34.4 %, which are useful in maintaining the storage quality of jam and the shelf life (Ashaye and Adeleke, 2009). Greater the moisture content, higher the water activity, which tends to promote the growth of many spoilage bacteria, fungi, and moulds (Ashaye and Adeleke, 2009). In addition, the use of sugar as an ingredient in producing the jam resulted in sugar binding to water molecules, which reduces the amount of available water in the jam. This will ensure that the jam is tightly packed after production.

5.5.4 Total soluble solids

The Brix level was 68.5 ± 0.2 and 68.0 ± 0.1 in the *L. rhamnosus* yoba and control jam, respectively. These values are in agreement with a study by Ndabikunze *et al.* (2011) who reported a TSS of 68.53 in *U. kirkiana* jam (n = 3) made with commercial pectin. The brix measurement is a ratio (wt/wt) of water to sugar (TSS) in the food material. Brix is mainly

determined in fruit pulps and their products such as jam, juices, and jellies. The Brix content normally changes due to physiological conditions present in the fruit. In jam making, the TSS is critical for good gel formation and for preservation of the jam. A good jam must have a final TSS in the range 65–68 %. The Brix values of the *L. rhamnosus* yoba jam were in agreement with this specified range. Lower Brix contents of < 65 % have been found to affect the shelf life. Furthermore, the jam will have a runny consistency, making it ideal for the growth of bacteria and moulds. Higher TSS contents of > 68 % will cause the sugar to form crystals and the formation of a very stiff gel. In order to overcome this effect, the Brix of the jam was monitored regularly during production and the endpoint of boiling/cooking of the jam was reached when the Brix level reached 68 %. High Brix can also be attributed to the presence of natural enzymes (pectinase) and the heat treatment used in processing, which enables the breakdown of the insoluble pectin from the complex polysaccharides into simpler sugars (Kumar, 2015). The TSS levels in *U. kirkiana* pulp might be due to the ripeness of the fruits before processing, which was also reported by Fweja (2002), and Kansci, Koubala and Mbome Lape (2003) for mango fruits at different stages of ripening.

5.5.5 Individual sugars (glucose, sucrose, fructose)

The jam inoculated with *L. rhamnosus* yoba had the highest fructose and sucrose contents of 12.84 ± 0.21 g/100 g and 24.61 ± 0.12 g/100 g, respectively. This is explained by the addition of sugar during the production of the jam. In the formulation, 43 % was sugar (sucrose). Fructose was the dominant simple sugar. The higher sugar contents could be attributed to the breaking down of the pulp matrix, which releases soluble fractions (Kumar, 2015). The difference in the sugar content between the jam and the composite pulp might be the result of differences in the maturity index of sampled fruits during the sampling process. This is because the sugar content often differs in fruits due to differences in the ripening stage, when sucrose is hydrolysed into glucose and fructose (Bahramian, *et al.*, 2011; Lee, *et al.*, 2013).

5.5.6 Vitamin C

The vitamin C content was recorded as 0.34 ± 0.02 mg/100 g, 0.28 ± 0.03 mg/100 g, and 17.4 ± 0.13 mg/100 g for the jam inoculated with *L. rhamnosus* yoba, control jam sample, and composite pulp, respectively. The drastic decrease in vitamin C levels in the *L. rhamnosus* yoba jam could be attributed to the processing temperatures used during the production of the jam.

Vitamin C is highly sensitive to heat, especially when heated to above 70 °C, where it tends to leach out into the surrounding solution due to its solubility (Igwemmar, Kolawole and Imran, 2013). Vitamin C is an essential nutrient for the human body and its importance cannot be undermined. Some of its physiological functions include lowering the risk of cancer, healing of wounds, reduction in susceptibility to infections, formation of bones and teeth, and iron absorption (Yang *et al.,* 2009; Kagawa *et al.,* 2009).

5.5.7 Extraction of pectin in composite pulp

The percentage yield of pectin from the composite pulp was 0.24 ± 0.05 %. Ndabikunze *et al.* (2011) reported a pectin content of 0.28 ± 0.05 % in *U. kirkiana* fruits collected from the Iringa forest areas in Tanzania. This pectin content was higher compared to that of *V. mombassae* and *S. birrea* fruits, which had pectin contents of 0.12 ± 0.05 % and 0.17 ± 0.08 %, respectively (Ndabikunze *et al.,* 2011). The pectin yield was extracted at 90 °C for 3 h and depends on temperature and time. The use of heat treatment, which weakened the structure of the fruits, could have resulted in an increase in the interaction between the acidic solution and the pulp matrix during the extraction process, hence resulting in an effective pectin yield. An extraction temperature of 90 °C was appropriate because it encouraged the loss of energy through vaporization, but a very high temperature of 100 °C and above can cause degradation of pectin—as pectin is composed of α-1,4-linked units of galacturonic acid—yielding pectin of lower molecular weight, which is unstable (Ania *et al.,* 2012). Extraction at lower temperatures (< 80 °C) can result in production of pectin with a low viscosity and poor diffusion between phases, hence resulting in a slow rate of extraction and lower yields of pectin. Studies by Drusch (2007) and Udonne, Ajani and Akinyemi (2016) revealed that a low pH of 3 produces a high pectin yield irrespective of the plant material.

5.5.8 Enumeration of probiotic bacteria

Viable counts of *L. rhamnosus* yoba in jam were determined before the consumption and sensory evaluation experiment. The viable plate count of *L. rhamnosus* yoba was found to be 6.2 ± 0.2 log CFU/mL. The jam was able to deliver a live *L. rhamnosus* yoba bacterial cell count that was over 6 log CFU/mL, making it a probiotic food (Kajander *et al.,* 2008), although the bacterial counts were lower than that noted by Mpofu *et al* (2014) in the probiotic, mutandabota (8.8 ± 0.5 log CFU/mL). This suggests that the fruit pulp allowed the survival of

L. rhamnosus yoba. Stadlymayr *et al.* (2013) reported the proximate composition of *U. kirkiana* fruit pulp as, crude protein 0.3 g/100 g, fibre 2.1 g/100 g, fat 0.4 g/100 g, ash 0.8 g/100 g, and carbohydrates 28.7 g/100 g. The growth of the *L. rhamnosus* yoba was promoted by the presence of sugars (CHO) which supplied carbon. Morphological characterisation by microscopy showed that the *L. rhamnosus* yoba cells present in the jam just before consumption were round, pale yellow, and curved. These findings are supported by Mpofu *et al.* (2014) who noted similar bacterial morphology in *L. rhamnosus* yoba *mutandabota*.

5.6 CONCLUSION

A fruit jam inoculated with *Lactocillus rhamnosus* yoba was produced. The formulation had 55 % (wt/vol) pulp, 46 % (wt/vol) sugar, 1.5 % (wt/vol) pectin, and 0.5 % (wt/vol) citric acid. Additionally, the probiotic jam had 6.2 ± 0.2 log CFU/mL viable *L. rhamnosus* yoba cells at time of consumption. The result makes the probiotic jam a probitoc food since it contained a viable cell conectration in excess of 6 log cfu/mL. This study has revealed that an underutilised fruit was successfully used to culture and grow a probiotic bacterium thus allowing for further use of other indigenous fruits as in producing local probiotic foods. There is also a potential of using probiotics in traditional foods and enhance access to probiotics for rural folks who need them. This study has managed to produce a functional food that contained *Lactobacillus rhamnosus* yoba, an isolate of *L.rhamnosus* GG, with a viability in access of the recommended intake level upon consumption.

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CHAPTER SIX

IRON AND ZINC BIOACCESSIBILITY, SENSORY PROPERTIES, AND CUSTOMER PREFERENCE OF *U. Kirkiana* FRUIT JAM CONTAINING *Lactobacillus rhamnosus* YOBA

Abstract

Lactobacillus rhamnosus yoba, a generic probiotic of *L. rhamnosus* GG was cultured in *U.kirkiana* fruit jam to allow rural communities in semi-dry region to benefit from consuming a probiotic food. A probiotic jam was developed using the formulation - 55 % (wt/vol) *U. kirkiana* fruit pulp, 46 % (wt/vol) sugar, 1.5 % (wt/vol) pectin, 0.5 % (wt/vol) citric acid, and *L. rhamnosus* yoba was inoculated at 0.25 % (wt/vol). Iron and zinc bioaccessibility was determined using the Infogest *in vitro* digestion protocol that simulated the oral phase, gastric phase and intestinal phase. Sensory evaluation was conducted by a panel (n=140) to determine product preference and acceptance. The probiotic jam had 6.2 ± 0.2 log CFU/mL viable *L. rhamnosus* yoba cells. The jam inoculated with *L. rhamnosus* yoba had an iron bioaccessibility of 6.55 ± 0.36 % and a zinc bioaccessibility of 16.1 ± 0.50 %. The use of *L. rhamnosus* yoba in the jam showed a 4 % and 2 % increase in the iron and zinc bioaccessibility, respectively. *L. rhamnosus* yoba jam had mean scores of 7.5, 7.0, 6.0, and 6.5 for spreadability, taste, appearance, and mouthfeel, respectively. There was a significant difference ($p = 0.02$) in customer preferences for probiotic jam and jam with no probiotic. The jam inoculated with *L. rhamnosus* yoba had an overall acceptance score of 7.5 (*n = 120)*. The probiotic jam was accepted by most rural consumers of the fruit as a sustainable functional food that can deliver viable *L. rhamnosus* yoba cells with potential health benefits.

6.1 INTRODUCTION

Deficiencies of micronutrients, especially iron and zinc are the most occurring nutritional problems all over the world (Platel and Srinivasan, 2016), and widely prevalent in most developing countries (Rousseau *et al.,* 2019). Micronutrient deficiencies are often referred to as ‘hidden hunger’ because they are less visible than macronutrients deficiencies (Platel and Srinivasan, 2016). Iron is essential in the synthesis of haemoglobin and myoglobin (Cilla *et al.*, 2009). Zinc plays an important role in gene regulation and apoptosis (Truong-Tran *et al.*, 2000).

Iron and zinc absorption occurs in the small intestine (Sitrin, 2014). Heme specific receptors sites on microvilli of enterocytes and a carrier (ZnT-1) mediates the absorption of iron and zinc respectively (Roohani *et al.,* 2013; Rousseau *et al.,* 2019). Zinc deficiencies have been reported to cause neural tube defects in infants (Dey *et al.,* 2010), higher rates of respiratory tract pneumonia in infants (Barnett *et al.,* 2010), diarrhoea (Luabeya *et al.,* 2007), and child stunting (Umeta *et al.,* 2003). Currently, in vitro assays are mainly used to evaluate mineral bioaccessibility in foods (Rousseau *et al.,* 2019) through simulations of the digestion process (Minekus *et al.,* 2014). Bioaccessibility refers to a nutrient fraction that is released from the food matrix and available for absorption (Fernandez-Garcıa, Carvajal-Lerida, and Perez-Galvez, 2009; Rousseau *et al.,* 2019). There is evidence of enhanced bioaccessibility of iron from plant foods due to household food processing techniques such as heat treatment and fermentation (Platel and Srinivasan, 2016). Of late, in vitro bioaccessibility of iron and zinc in many foods samples have been conducted by solubility assays in fruit juices (Cilla *et al.,* 2009), but studies on probiotic fruit jam especially foods containing *L.rhamnosus* yoba are scarce.

Uapaca kirkiana fruit is a good source of sugar, energy, and essential minerals (Akinnifesi *et al.,* 2008; Ndabikunze *et al.,* 2010; Vinceti *et al.,* 2013). Stadlmayr *et al. (*2013) reported the proximate composition of the fruit as follows; water (72.6 g / 100 g), carbohydrates (28.7 g / 100 g), proteins (0.5 g / 100 g), fat (0.4 g / 100 g), calories (523 kcal / kJ), ash (1.1 g / 100 g), fiber (2.3 g / 100 g) and vitamin C (16.8 mg / 100 g). Many studies have reported the benefits of consuming *L. rhamnosus* GG. Clinical trials of LGG have shown benefits in prevention and treatment of upper respiratory tract infections, gastrointestinal infections, and diarrhoea in children (Guandalini *et al.,* 2000; Grandy *et al.,* 2010; Hojsak *et al.,* 2010). The use of *L. rhamnosus* yoba could significantly change the organoleptic properties, preferences and acceptability of the jam by consumers. Sensorial qualities of the food product have a major influence on product preferences amongst other factors. Mattila–Sandholm *et al.* (2002) noted that sensory aspects of probiotic foods and technological factors are of utmost importance in promoting the consumption of functional foods. Studies on determining the sensorial characteristics and preferences of jam containing *L. rhamnosus* GG are scarce, even though the bacterium is the most studied probiotic (Gorbach and Goldin 1989; Kort and Sybesma 2012). The aim of this investigation was to determine the effects of incorporating *L. rhamnosus* yoba

in jam on bioaccessibility of iron and zinc and to evaluate the sensory qualities and customer preferences of the *L. rhamnosus* yoba fruit jam.

6.2 MATERIALS AND METHODS

6.2.1 Ingredients for *U. kirkiana* fruit jam preparation

Fruits were collected according to a method described in Section 3.1. The pulping process was carried out using an assay described in Section 3.2. Citric acid and commercial pectin were obtained from the Department of Food Science and Technology Laboratory at Chinhoyi University of Technology, Zimbabwe.

6.2.2 Preparation of *U.kirkiana* fruit jam

The formulation process of the jam comprised 55 % (wt/vol) composite fruit pulp, 43 % (wt/vol) sugar, 1.5 % (wt/vol) pectin, and 0.5 % (wt/vol) citric acid. In producing the jam, a composite fruit pulp was mixed with sugar in a stainless steel pot and cooked at 110 °C until all the sugar had dissolved. Citric acid was added and stirred gently whilst cooking until it reached 55 Brix. Commercial pectin was then and the mixture was continuously stirred whilst being cooked until it reached 68 Brix.

6.2.3 Inoculum and production of probiotic jam

An isolate of *L. rhamnosus* yoba (Kort and Sybesma, 2012) was used in the experiment. The *L. rhamnosus* yoba strain was obtained in a sachet from the Yoba for Life Foundation, Amsterdam, Netherlands and stored at −80 °C. The *L. rhamnosus* yoba strain was reactivated by sub-culturing anaerobically in MRS broth at 37 °C for 18 h. *L. rhamnosus* yoba utilises sugar and it supports its growth. *U. kirkiana* fruit pulp was mixed with sugar, boiled and subsequently cooled to room temperature (25 °C), and was then used to cultivate *L. rhamnosus* yoba. *L. rhamnosus* yoba was then precultured in the medium and incubated at 37 °C for 36 h until the number of live cells reached above 9 log CFU/mL. Sterilised tubes (100 g) containing freshly produced *U. kirkiana* fruit jam were opened under aseptic conditions, and the jam was inoculated with a (0.25 mL) fresh probiotic culture. The cell suspensions were gently mixed with the jam. The jam was stored at 25 °C.

6.2.4 Iron and zinc *in vitro* bioaccessibility assay

The bioaccessibility of iron and zinc was determined using the Infogest *in vitro* digestion protocol (Minekus *et al.*, 2014). The initial iron and zinc content in the probiotic jam were measured before and after simulated gastrointestinal digestion, using an assay described in section 3.3.9. The oral, gastric, and intestinal phases of simulated gastrointestinal digestion were used (Figure 6.2).

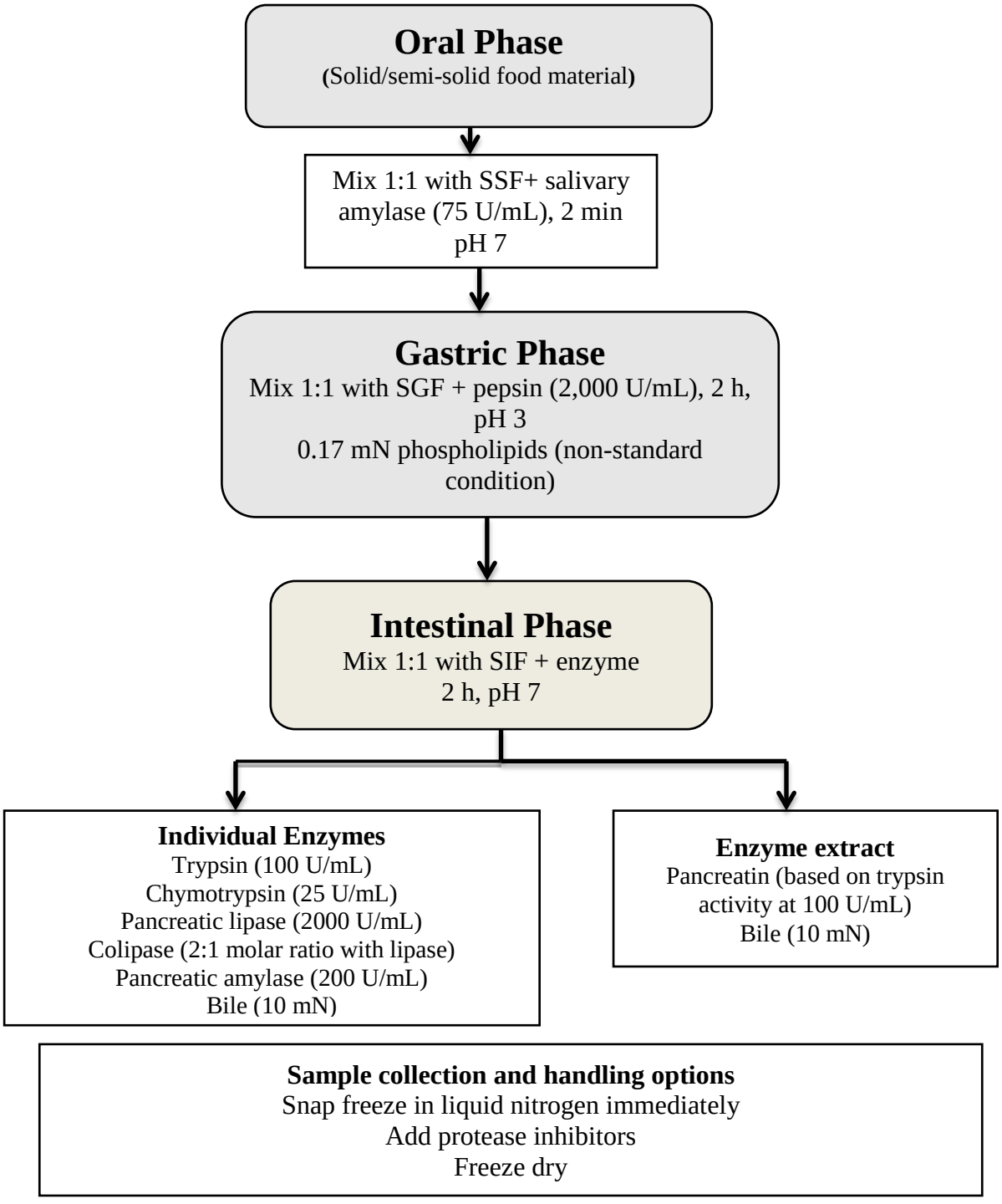

Figure 6.2: A summary flow diagram illustrating the Infogest in vitro digestion protocol (adapted from Minekus *et al.*, 2014).

6.2.4.1 Oral phase

A jam sample of 5 g was mixed with 4 mL of simulated salivary fluid (SSF). To this sample, 0.95 mL of Milli-Q water was added, followed by addition of 25 µL of $CaCl_2$ solution and 25 µL of α-amylase (75 units / mL). The resultant mixture was incubated for 2 min at 37 ˚C in a shaking water bath.

6.2.4.2 Gastric phase

In this simulated digestion phase, 7.5 mL of simulated gastric fluid (SGF), 1.6 mL pepsin solution (2000 units/mL), and 5 µL of $CaCl_2$ solution were added to the mixture from the oral phase. The pH of the mixture was adjusted to pH 3 by adding approximately 0.8 mL of 6 M hydrochloric acid. The resultant mixture was incubated for 2 min at 37 ˚C in a shaking water bath.

6.2.4.3 Intestinal phase

In the intestinal phase of simulated digestion, solutions were added in the following sequence to the mixture from the gastric phase; 11 mL of simulated intestinal fluid (SIF), 5 mL of pancreatin solution (100 units/mL), 2.5 mL of bile solution (10 mM), and 40 µL of $CaCl_2$. The pH of the mixture was adjusted to pH 7 by adding 1M NaOH and the mixture was incubated for 2 hrs at 37 ˚C in a shaking water bath. A sample of 1 mL was collected after simulated intestinal digestion and pipetted into capped micro-centrifuge tubes. The sample was then analysed for the mineral content using ICP. Bioaccessibility (%) of the element was calculated as follows:

$$\text{Bioaccessibility (\%)} = 100 \times Y/Z$$

Where: Y is the element content of the bioaccessible fraction (mg mineral/100 g), and

Z is the total mineral (zinc or iron) content (mg/100 g) (Hemalatha, Platel and Srinivasan, 2007a)

Table 6.1: Preparation of simulated digestion fluids (SDF).

Stock Constituents conc.			SSF pH 7 Vol. of Stock	SSF pH 7 Conc In SSF	SGF pH 3 Vol. of Stock	SGF pH 3 Conc. in SIF	SIF pH 7 Vol. of Stock	SIF pH 7 Conc. In SIF
	g L^{-1}	mol L^{-1}	mL	mmol L^{-1}	mL	mmol L^{-1}	mL	mmol L^{-1}
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH_2PO_4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
$NaHCO_3$	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	_	_	11.8	47.2	9.6	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.5	0.15	0.4	0.1	1.1	0.33
For pH adjustment								
	mol L^{-1}	mL		mmol L^{-1}	mL	mmol L^{-1}	mL	mmol L^{-1}
NaOH	1	_		_	_	_	_	_
HCl	6	0.09		1.1	1.3	15.6	0.7	8.4
$CaCl_2(H_2O)_2$ is added to the final mixture of SDF and sample								
	g L^{-1}	mol L^{-1}	mmol L^{-1}		mmol L^{-1}		mmol L^{-1}	
			1.5		0.15		0.6	
$CaCl_2(H_2O)_2$	44.1	0.3	(0.75*)		(0.075*)		(0.3*)	

6.2.5 Sensory evaluation

A trial was was conducted without the actual panelists to determine the way the taste panel was supposed to carry out the evaluation and how the samples were supposed to be presented. In the actual sensory evaluation process, 140 taste panels were selected using a systematic random sampling. Demographic information about the taste panelists was collected. Consent forms were signed by the panellists. A score card and instructions to the panelists were translated in the local Shona language for easy understanding of the sensory process by the panelists. Panelists were not allowed to discuss their results during the sensory evaluation process. Panelists were presented with a sample weighing 25 g each. Samples were served in small paper plates covered with aluminium foil.

6.2.5.1 Triangle test

The ability of trained and untrained panellists to discriminate between probiotic jam samples and ordinary jam samples was calculated using a triangle test. The respondents/panellists were

drawn from three areas, Gokwe (n = 40), Bikita (n = 40), and Kazangarare (n = 40) (Total untrained panellists: n = 120) and trained panellists from Staff lecturers and students from the Department of Food Science and Technology, Chinhoyi University of Technology (CUT), Chinhoyi, Zimbabwe (Total trained panellists: n = 20). Panellists/respondents had to sign non-disclosure and agreement forms before carrying out the tests. Temporary booths made of a cardboard box were used by untrained panellists in the Gokwe, Bikita, and Kazangarare areas and open-ended booths were used at the Chinhoyi University of Technology, Post-Harvest Department Laboratory. The samples given to respondents were coded using six combinations (A1B, 1AB, 1BA, B1A, BA1, and AB1). The coded samples were given randomly to panellists together with a glass of water to rinse out the residual taste of the previous sample. The panellists were provided with a score card and were asked to identify the odd sample among the three samples given to each panelist.

6.2.5.2 Preference test

Trained panellists comprising staff and students (*n = 20*) from the Department of Food Science and Technology, Chinhoyi University of Technology (CUT) were used to choose between the jam samples in terms of sweetness, colour, aroma, texture, and overall acceptance. The panellists were given two samples to analyse using a 9 point hedonic scale score card. The panellists were asked to score for a sensory attribute and indicate their overall product preference. A glass of water was provided to rinse out residual food sample in the mouth.

6.2.6 Statistical Analysis

Statistical analysis was done using XLSTAT and Genstat 14.0 for Windows statistical package. ANOVA was used to determine significant differences in means of unrelated data sets. Descriptive statistics for the data which included means, percentages, and variances were calculated. Probabilities for triangle taste tests were computed to analyse the triangular taste test data.

6.3 RESULTS

6.3.1 Iron bioaccessibility in *L. rhamnosus* yoba jam

Jam that was inoculated with *L. rhamnosus* yoba had an iron bioaccessibility of 6.55 ± 0.36 % (Table 6.2). Iron bioaccessibility was significantly different from that of the control jam ($p < 0.05$).

Table 6.2: *In vitro* digestion on iron content of the jam inoculated with *L. rhamnosus* yoba.

	Iron content (mg/100 g)			
Sample	Undigested (Total content)	After digestion	Bioaccessible Portion	Bioaccessibility %
L. rhamnosus yoba Jam	4.13 ± 0.22[a]	3.86 ± 0.14[b]	0.27 ± 0.08[a]	6.55 ± 0.36[a]
Control	4.03 ± 0.41[a]	3.92 ± 0.03[b]	0.11± 0.38[b]	2.7± 0.92[b]

Mean ± standard deviations are reported. Means with identical superscripts in a column are not significantly different at $p < 0.05$.

6.3.2 Zinc bioaccessibility in *L. rhamnosus* yoba jam

Zinc bioaccessibility of the *L. rhamnosus* yoba jam was significantly different from that of the control jam ($P < 0.05$). The jam inoculated with *L. rhamnosus* yoba had a zinc bioaccessibility of 16.1 ± 0.50 % (Table 6.3).

Table 6.3: *In vitro* digestion on zinc content of the jam inoculated with *L. rhamnosus* yoba.

	Zinc content (mg/100 g)			
Sample	Undigested (Total content)	After digestion	Bioaccessible Portion	Bioaccessibility %
L. rhamnosus yoba Jam	0.68 ±0.02[a]	0.57±0.01[a]	0.11±0.01[a]	16.1±0.50[a]
Control	0.64±0.03[b]	0.55±0.02[a]	0.09±0.01[b]	14.0±0.33[b]

Mean ± standard deviations are reported. Means with identical superscripts in a column are not significantly different at $p < 0.05$.

6.3.3 Demographic information of sensory panelists

The probiotic jam inoculated with *L. rhamnosus* yoba can be consumed by all ages in rural communities as indicated in Figure 6.3. Age groups 11 – 15 years, 16 – 20 years, and 26 – 30 years had 25, 20, and 15 panelist respectively. Age group 66+ years had 1 participant.

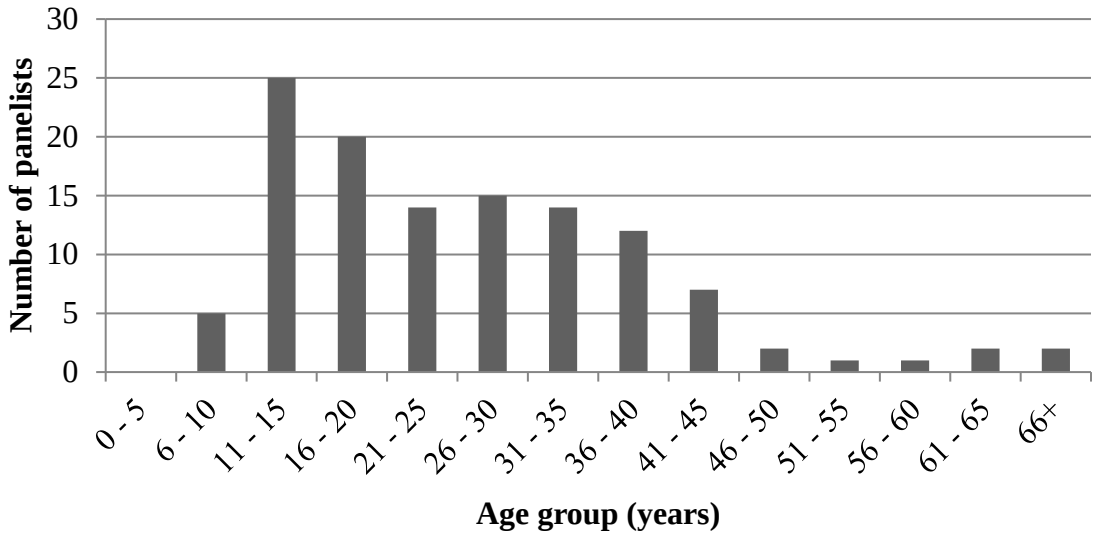

Figure 6.3: Age distribution of panelists

6.3.4 Triangle test (discrimination between *L. rhamnosus* yoba and control jam)

There was a significant difference ($P < 0.05$) between the trained and untrained panellists with respect to their ability to discriminate between the *L. rhamnosus* yoba and the control jam. Using a triangle test, 60% and 36% of the trained and untrained panelist were able to discriminate the jam inoculated with *L. rhamnosus* yoba respectively. The triangle test showed that 58% and 12% of the trained and untrained panelist were able to discriminate the control jam respectively. Forty percent (40%) and 74% of the trained and untrained panelist were unable to discriminate the jam inoculated with *L. rhamnosus* yoba respectively. Forty two (42%) percent and 88% of the trained and untrained panelist were unable to discriminate the control jam respectively.

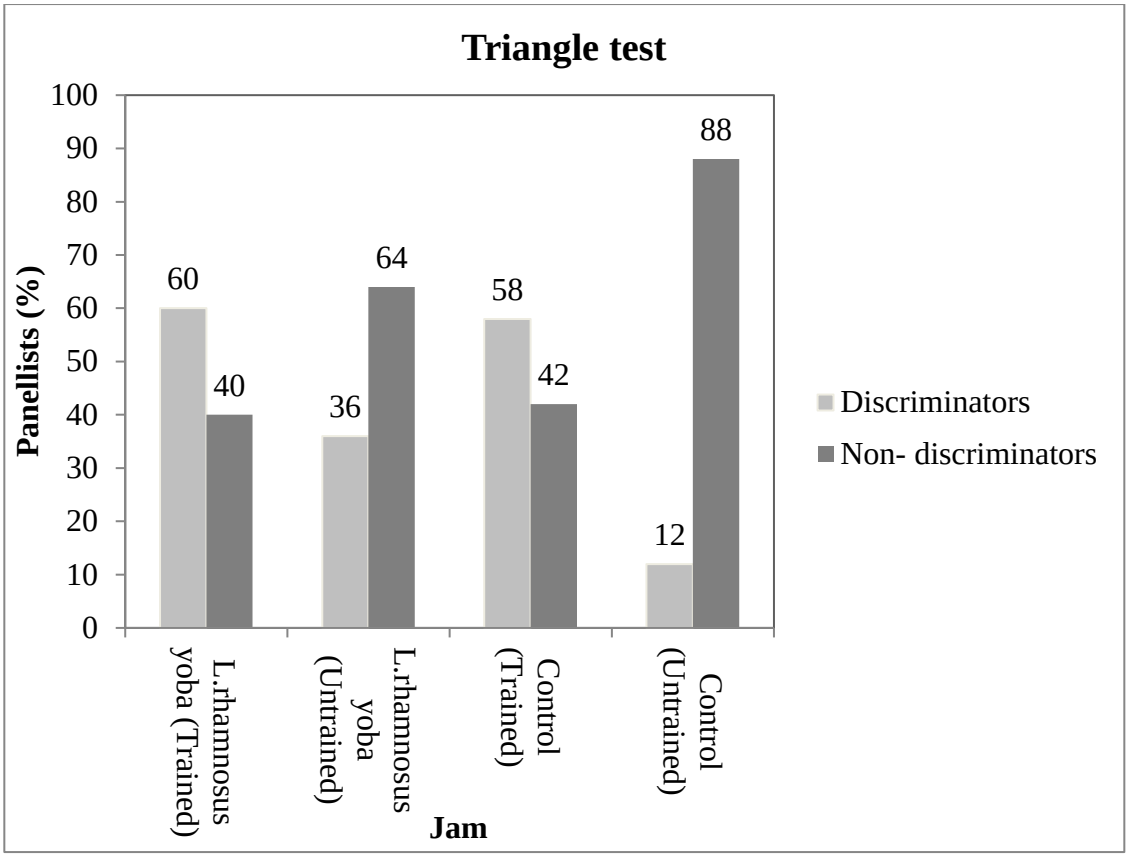

Figure 6.4: Triangle test (discrimination between *L. rhamnosus* yoba and control jam)

6.3.5 Preference test (discrimination between *L. rhamnosus* yoba and control jam)

The jam inoculated with *L. rhamnosus* yoba had a mean preference score of 7.5; 6.5; and 7 on spreadability, texture, and mouthfeel respectively. Control jam had a mean preference score of 7; 6; and 6.5 on spreadability, texture, and mouthfeel respectively. Jam inoculated with L.rhamnosus yoba had an overall acceptance score of 7.5.

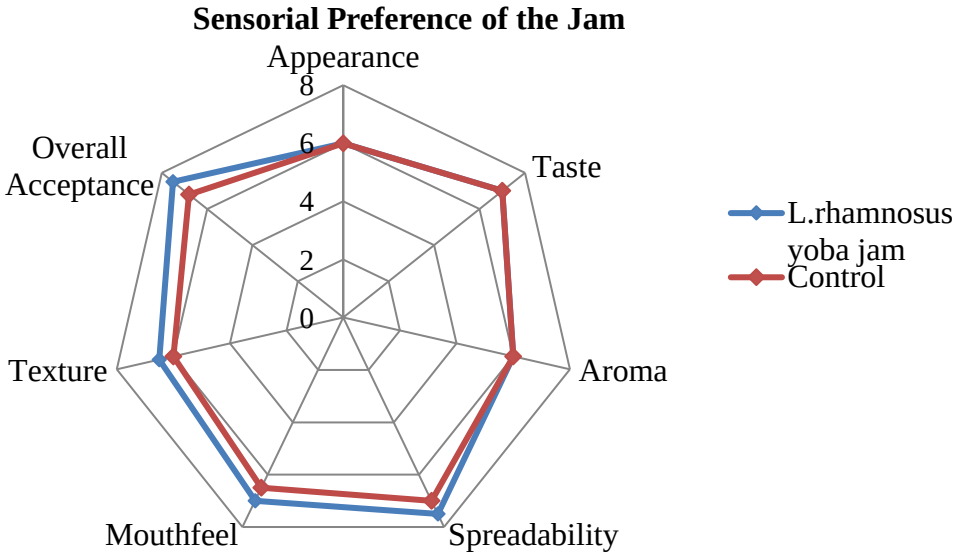

Figure 6.5: Preference test on inoculated jam with *L.rhamnosus* and control jam.

6.4 DISCUSSION

6.4.1 *In vitro* bioaccessibility of iron and zinc

Iron plays an important role in the human body, particularly in the formation of red blood cells. The iron bioaccessibility from the *L. rhamnosus* yoba jam was recorded as 6.55 ± 0.36 % and that from the control as 2.7 ± 0.92 %, revealing a 4 % increase in the bioaccessibility of iron when the jam was inoculated with *L. rhamnosus* yoba. Zinc bioaccessibility in *L. rhamnosus* yoba and the control jam was 16.1 ± 0.5 % and 14 ± 0.33 %, respectively. This translated to an increase of 2 % in zinc bioaccessibility when *L. rhamnosus* yoba was inoculated into the jam. This could be attributed to the action of bacteria as it produced degradation enzymes that acted on the food matrix to release the bound zinc. Furthermore, the effect of processing during jam-making cause breakdown of complex polysaccharides from the food matrix due to action of pectinase will release the bound minerals. Khouzam, Pohl and Lobinski (2011) reported a bioaccessibility of 6.7–12.7 % for essential minerals in different fruits and vegetables. The bioaccessibility of iron was low (6.55 ± 0.36 %), which suggests the presence of inhibiting compounds such as phytates and carbonate salts during fruits maturation, which may chelate and form insoluble complexes with iron resulting in impaired iron bioaccessibility

(Khouzam, Pohl and Lobinski, 2011). This fruits contains organic acids such as malic and oxalic acids and these might have complexed the iron and zinc during fruit maturation. Phenolic compounds also have an effect on mineral bioaccessibility. The fruit pulp had a total phenolic content of 67–82.5 µg GAE/g. This could explain the low bioaccessibility of iron.

Zinc is an essential micronutrient in the human body and is involved in many metabolic processes catalysed by different enzymes. Its deficiency may lead to retarded growth and dermatitis (Deshpande, Joshi and Giri, 2013). The ZDHS (2016) report states that the RDA for zinc and iron ranges from 3–11 mg/100 g and from 13–19 mg/100 g, respectively among sexes and age groups. During sample preparation, the action of pectinase might explain the release of zinc from the pectin matrix. *U. kirkiana* contains relatively high levels of calcium and its presence has been found to inhibit the bioaccessibility of other minerals such as zinc. Phytates that build up in the fruit pulp during the maturation process can also affect zinc bioaccessibility (Hambidge *et al.*, 2010).

6.4.2 Demographic information

Age group 0-5 years had no participants because they could not comprehend the sensory evaluation process. The sensory evaluation was performed by panelists with minimum years of 10. The population distribution in the sample areas indicated that age groups 11-15 and 16-20 years were the most common as compared to older ages (ZimStats, 2010; WHO, 2013). The gender distribution of the panelists was 71 women and 49 man. Rural areas in Zimbabwe have more women than men because most man has left the rural areas to go to urban areas in search of employment (Olivieri *et al.*, 2008).

6.4.3 Triangle test (discrimination between *L. rhamnosus* yoba and control jam)

The triangle test is a sensory evaluation technique that uses a discriminative technique to determine differences between samples or to select qualified panellists for a specific test. There were significant differences in the results of the triangle test ($P < 0.05$) between the ability of the trained ($n = 20$) and untrained panellists ($n = 130$) to identify the odd sample from the inoculated and control jam. The trained panellists exhibited a significantly higher success rate of 60 % compared to 40 % for the untrained panellists with respect to the correct identification of the *L. rhamnosus* yoba jam. This could be attributed to the fact that the training and experience of the panellists played a role in the proper discrimination of the samples during evaluation.

6.5 CONCLUSION

Lactobacillus rhamnosus yoba was successfully cultured in *U.kirkiana* fruit jam to produce a functional food. The probiotic jam was able to deliver 6.2 ± 0.2 log CFU/mL viable *L. rhamnosus* yoba cells on consumption to the consumers. The fruit jam inoculated with *L. rhamnosus* yoba had an iron bioaccessibility of 6.55 ± 0.36 % and a zinc bioaccessibility of 16.1 ± 0.50 %. The use of *L. rhamnosus* yoba in the jam showed a 4 % and 2 % increase in the iron and zinc bioaccessibility, respectively thereby making the probiotic jam an excellent source of iron and zinc needed for physiological body functions and in mitigating iron and zinc deficiencies in most rural communities. The findings of sensory evaluation indicated that the probiotic jam had mean scores of 7.5, 7.0, 6.0, and 6.5 for spreadability, taste, appearance, and mouthfeel, respectively. The main sensory descriptors used on the probiotic jam were ‘a sweet taste’ and ‘excellent spreadability’. A sweet taste is important because people prefer sweet taste and the probiotic would benefit children nutritionally and improve their gastrointestinal health. A paired difference test showed a significant difference ($p = 0.02$) in customer preferences for probiotic jam and jam with no probiotic. The probiotic jam had an overall acceptance score of 7.5 ($n = 120$). Utilisation of *U. kirkiana* fruit pulp in development of a probiotic food will make most rural people access essential minerals (iron and zinc) and improve their health especially the vulnerable children and women.

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CHAPTER SEVEN

MULTIVARIATE ANALYSIS ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF *U. Kirkiana* FRUIT PULP FROM SEMI-DRY AREAS

Abstract

The study was aimed at evaluating the effect of different locations on physiochemical and functional properties of *Uapaca kirkiana* fruit that grows in a relatively hot and a semi-arid area of Zimbabwe. The fruits were obtained from domesticated trees in Kazangarare, Gokwe and Bikita communal areas. Physiochemical and functional properties of the fruit pulp were analysed. Multivariate analysis was performed to determine the eigenvalue of the correlation matrix. Principal component 1 and Principal component 2 accounted for the most variability. Principal component 1 and 2 which represented physiochemical and functional properties of the pulp had eigenvalues of 5.59 and 2.13, and a variability of 37.31 % and 14.17 %, respectively. The attributes that contributed positively to the most variability among the sampling areas in PC 1 were pH, Vitamin C, antioxidant activity (AOA), TTA, magnesium, sodium, potassium, and copper, with pulp yield having a negative effect. In PC 2, calcium and iron content in the pulp contributed most to the variability, while the sodium content had a negative effect. Variability in fruit pulp properties were attributed to pH (74 %) and TTA (69 %). There was a strong relationship between TTA and pH (r^2 = 0.79); TTA and antioxidant (r^2 = 0.72); and pH and phosphorus (r^2 =0.81). Phosphorus, sodium and iron accounted for approximately 73 %, 50 %, and 43 % of the variation, respectively. TTA and phosphorus content had a positive effect on pulp yield. Physicochemical and functional properties of the fruit pulp make it a good ingredient for producing a functional food, despite the differences in the characteristics of the area were the trees grow. The fruit is an excellent source of mineral (phosphorus, sodium and iron), vitamin C, antioxidants, and sugars.

7.1 INTRODUCTION

In Sub-Saharan Africa, most indigenous fruits play vital roles in food provision, health, and financial stability of most rural households especially during drought periods (Akinnifesi, 2001; Jamnadass *et al.,* 2011; Ngadze *et al.,* 2017). *Uapaca kirkiana* fruit locally referred to as Mazhanje in Shona and Umhobohobo in Ndebele, has traditionally been used to supplement diets and nutritive benefits and its pulp being used as a starting ingredient for many products (Akinnifesi *et al.,* 2004). The fruit pulp of *U. kirkiana* can be consumed fresh (Ngulube and Hall, 1995) and can be fermented to produce the local brew, masuku wine in Zambia (Muchuweti *et al.,* 2006) Of the five naturally occurring *Uapaca spp* in Sub-Saharan Africa, *U. kirkiana spp* is the commonly consumed fruit tree and is a good source of sugar, energy, and essential minerals (Akinnifesi *et al.,* 2008; Ndabikunze *et al.,* 2010; Vinceti *et al.,* 2013).

Multivariate analysis was used to minimise redundancy in the data on physiochemical and functional properties of *Uapaca kirkiana* fruit pulp and to determine the eigenvalues of the correlation matrix (Maji and Shaibu, 2012). Principal components analysis (PCA) is a data analysis method that uses a factor method (El-Bakry and Hazem, 2007). In PCA, large data sets are replaced with smaller data sets. In the process, associations or correlations between variables are highlighted and variables with less variability are determined from those with high variability (Yu, 2005; Kara, 2009). Hidden variables are termed factors or components (Helmy and Taweel, 2009). Components become useful when there is high variability among variables, thereby making it insufficient to discriminate between the samples for evaluation. Multivariable tools are then applied to set patterns, to remove data redundancy, and to identify significant associations between variables (Adam, 2000; Maji and Shaibu, 2012). Original variables are clustered together from the data sets (Abbe *et al*., 2017). PCA was used in this research to show the correlation between fruit pulp attributes (physiochemical and functional) and sample area. The eigenvalues and loading factors were used to understand the correlation matrix, covariance, and performance of the variables.

7.2 MATERIALS AND METHODS

7.2.1 Sample preparation

Sample collection and preparation was carried using a procedure described in Section 3.1 and 3.2

7.2.2 Multivariate analysis

Multivariate analysis was performed to identify the most significant fruit pulp attribute responsible for variations in sampling area using the XLSTAT statistical computer package (Version 2015.04.36025). Principal component analysis (PCA) was used to discriminate individual pulp attributes. Pearson's correlation coefficients were used to show the correlation between the pulp attribute in each area.

7.3 RESULTS AND DISCUSSION

7.3.1 Multivariate analysis

Principal component analysis grouped physicochemical attributes of the fruit pulp into 15 principal components (Appendix 1). The attributes that contributed positively to the most variability among the sampling areas in PC 1 were pH, Vitamin C, antioxidant activity (AOA), TTA, magnesium, sodium, potassium, and copper, with pulp yield having a negative effect. Principal component 2 (PC2) had an eigenvalue of 2.13 and accounted for 14.17 % of the variability (Appendix 1). In PC 2, calcium and iron content in the pulp contributed most to the variability in the sampling areas, while the sodium content had a negative effect. The biplot clearly indicates that there was a grouping of samples from specific areas. Kazangarare samples were grouped together based on the Brix (TSS), potassium, pulp yield, and iron and calcium contents. Bikita samples were clustered together with respect to the pulp pH, TTA, copper, AOA, Vitamin C, and phosphorus content, whereas Gokwe samples were grouped together based on the magnesium content (Figure 7.1).

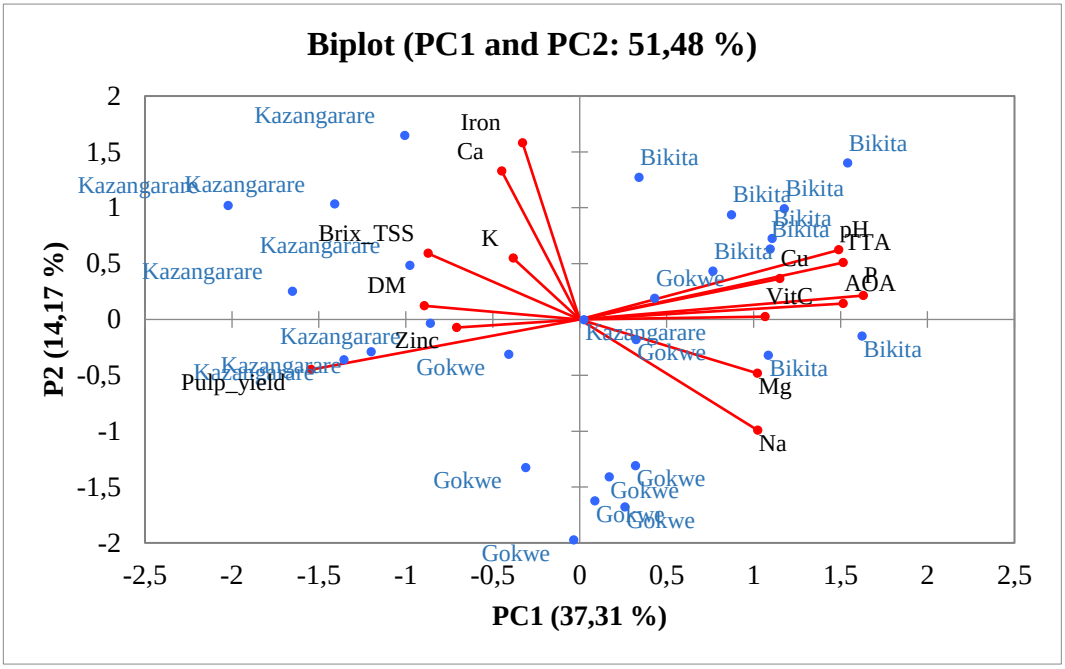


Figure 7.1: Principal component biplot showing variation of fruit pulp characteristics in the sampling areas.

7.3.2 Correlation analysis of fruit pulp attributes

As shown in Figure 7.2 and Table 7.1, the pH content of the fruit pulp showed a significant positive correlation with phosphorus (r^2 = 0.813) content, TTA (r^2 = 0.796) content and AOA (r^2 = 0.626) content. TTA had a positive correlation with phosphorus (r^2 = 0.668) content.

Table 7.1: Correlation analysis for 15 quantitative fruit pulp attributes.

Variable	pH	Brix (TSS)	DM	Vit C	AOA	Pulp yield	Ca	Fe	Zn	TTA	Mg	Na	P	K	Cu
pH	**1**														
TSS	-0.286^{NS}	**1**													
DM	-0.182^{NS}	0.086 ^{NS}	**1**												
Vit C	**0.534*****	-0.289 ^{NS}	-0.204 ^{NS}	**1**											
AOA	**0.626******	-0.433 ^{NS}	-0.372 ^{NS}	0.265^{NS}	**1**										
Pulp yield	-0.803^{NS}	0.153 ^{NS}	0.329 ^{NS}	-0.496^{NS}	-0.603 ^{NS}	**1**									
Ca	0.011 ^{NS}	0.150 ^{NS}	0.072 ^{NS}	-0.114^{NS}	-0.086 ^{NS}	0.162 ^{NS}	**1**								
Fe	0.116 ^{NS}	0.321 ^{NS}	0.156 ^{NS}	-0.190^{NS}	-0.107 ^{NS}	-0.077^{NS}	**0.506*****	**1**							
Zn	-0.186^{NS}	0.235 ^{NS}	0.212 ^{NS}	-0.327^{NS}	-0.329 ^{NS}	0.128 ^{NS}	-0.176 ^{NS}	0.146^{NS}	**1**						
TTA	**0.792******	-0.178 ^{NS}	-0.278 ^{NS}	**0.474****	**0.726******	-0.774^{NS}	- 0.108 ^{NS}	0.045 ^{NS}	-0.352	**1**					
Mg	0.232 ^{NS}	-0.188 ^{NS}	**-0.489** ^{NS}	0.372 ^{NS}	**0.490****	-0.356^{NS}	- 0.266 ^{NS}	-0.327 ^{NS}	-0.042	0.291	**1**				
Na	0.258 ^{NS}	-0.392 ^{NS}	-0.082 ^{NS}	0.068 ^{NS}	**0.492****	-0.373^{NS}	**-0.559** ^{NS}	-0.477 ^{NS}	-0.296	**0.382**	0.217	**1**			
P	**0.813******	-0.440 ^{NS}	**-0.464** ^{NS}	**0.530*****	**0.611******	-0.814^{NS}	-0.141 ^{NS}	-0.017 ^{NS}	-0.281	**0.668******	**0.383**	**0.406**	**1**		
K	-0.255^{NS}	0.163 ^{NS}	0.230 ^{NS}	-0.131^{NS}	0.086 ^{s}	0.166 ^{NS}	0.147 ^{NS}	0.214 ^{NS}	-0.024	0.023	-0.103	-0.168	-0.268	**1**	
Cu	**0.402****	-0.278 ^{NS}	-0.339 ^{NS}	0.065 ^{NS}	**0.636******	-0.508^{NS}	-0.037 ^{NS}	0.153 ^{NS}	-0.208	**0.462****	**0.416**	0.317	**0.477****	0.030	**1**

*NS= Not significant at a P value of 0.05, * significant at a P value ≤ 0.05, ** significant at a P value ≤ 0.01, *** significant at a P value ≤ 0.001, **** Significant at a P value ≤ 0.0001*

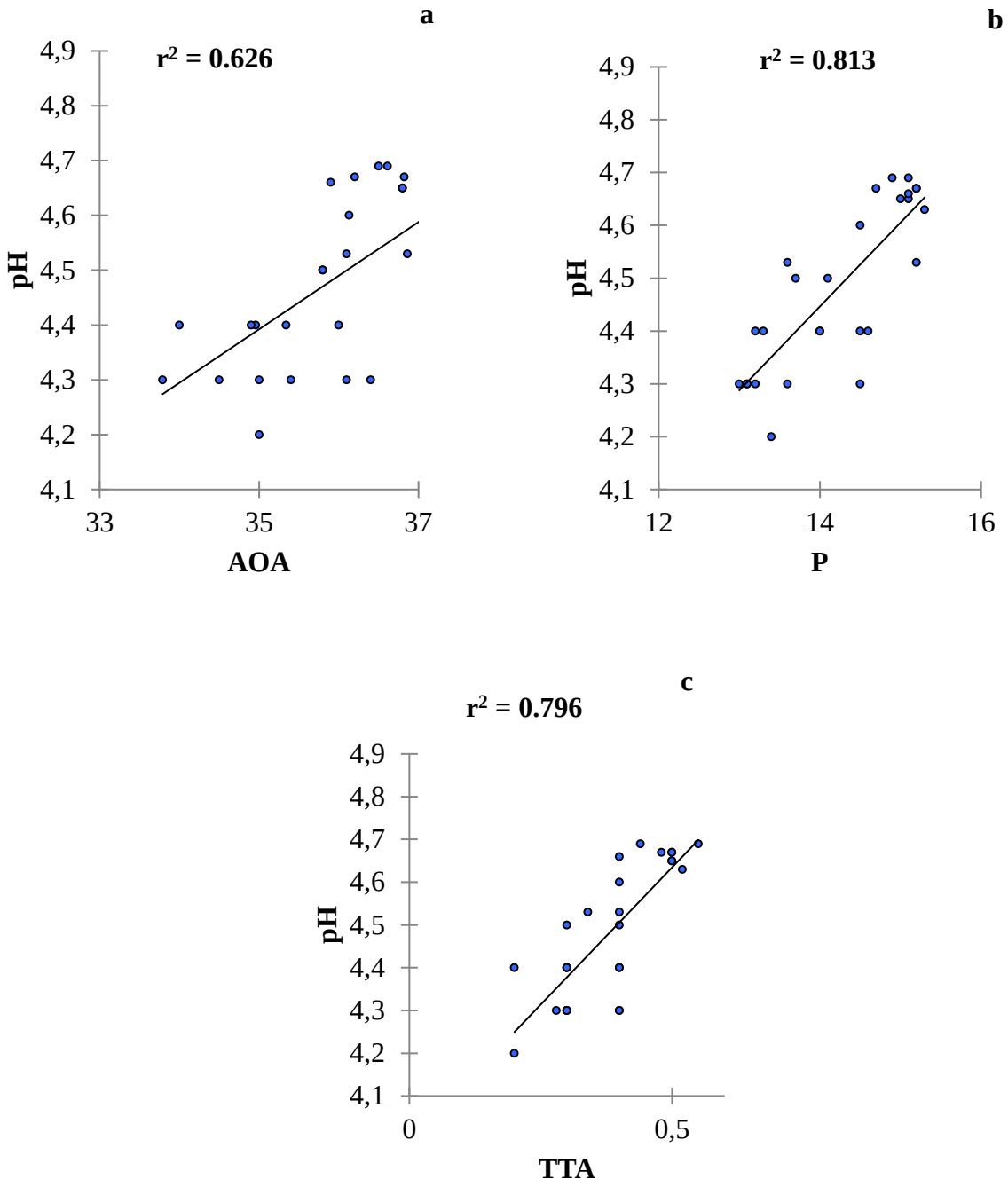


Figure 7.2: The correlation between pH and AOA (a), the correlation between pH and phosphorus (b), and the correlation between pH and TTA (c).

As shown in Figure 7.3 and Table 7.1, AOA had a significant positive correlation with TTA (r^2 = 0.726) content, copper (r^2 = 0.636) content, and phosphorus (r^2 = 0.611) content in the fruit pulp. TTA had a positive correlation with phosphorus (r^2 = 0.668) as shown in Figure 39 and Table 17.

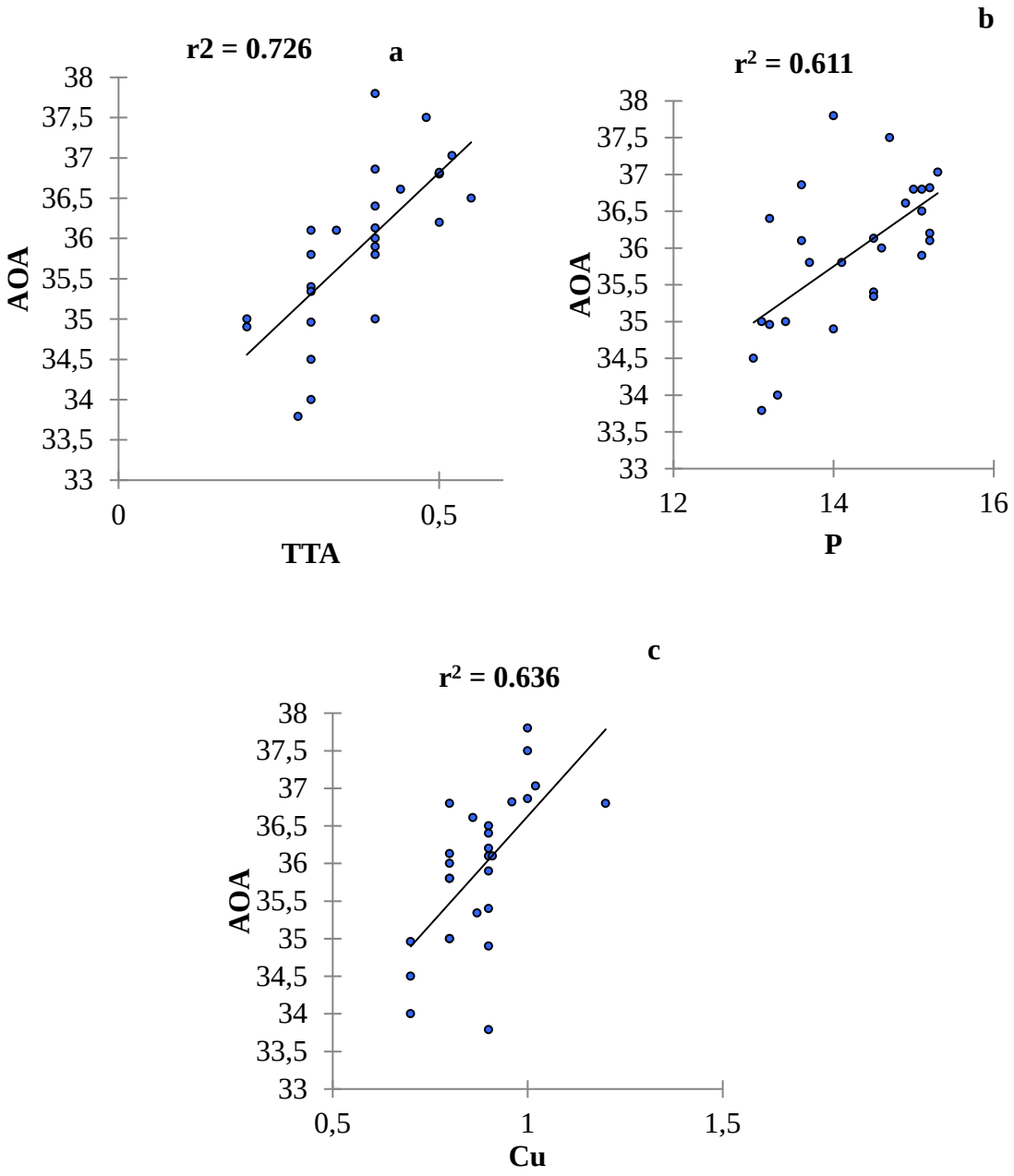


Figure 7.3: The correlation between AOA and TTA (a), the correlation between AOA and phosphorus (b), and the correlation between AOA and copper (c).

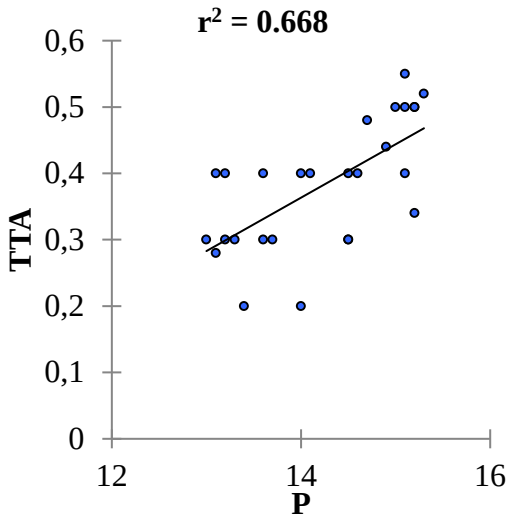
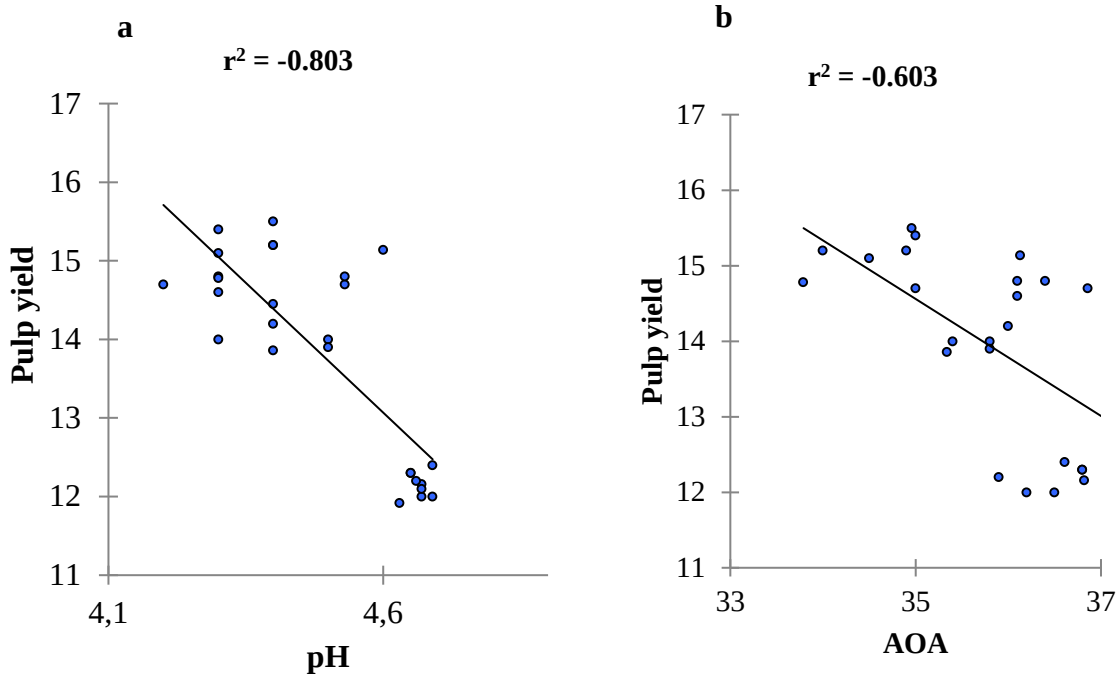


Figure 7.4: The correlation between TTA and phosphorus content in fruit pulps.

As shown in Figure 7.5 and Table 7.1, pulp yield showed a negative correlation with pH (r^2 = -0.803) content and AOA (r^2 = -0.603). Pulp yield also exhibited negative correlations with TTA (r^2 = -0.774) and phosphorus (r^2 = -0.814).



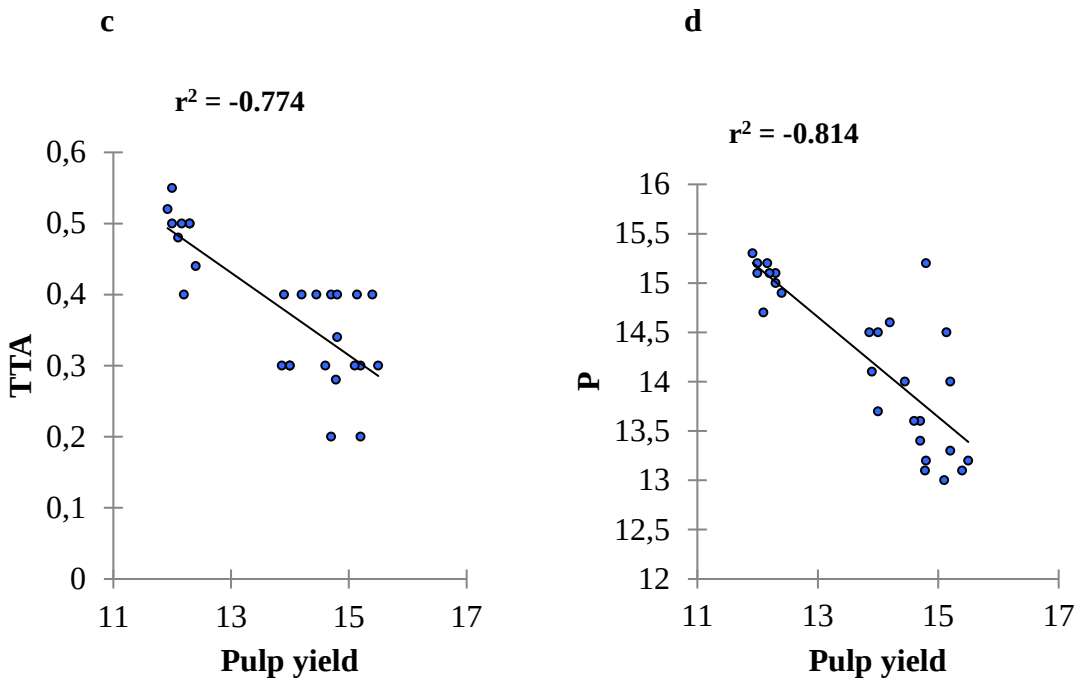
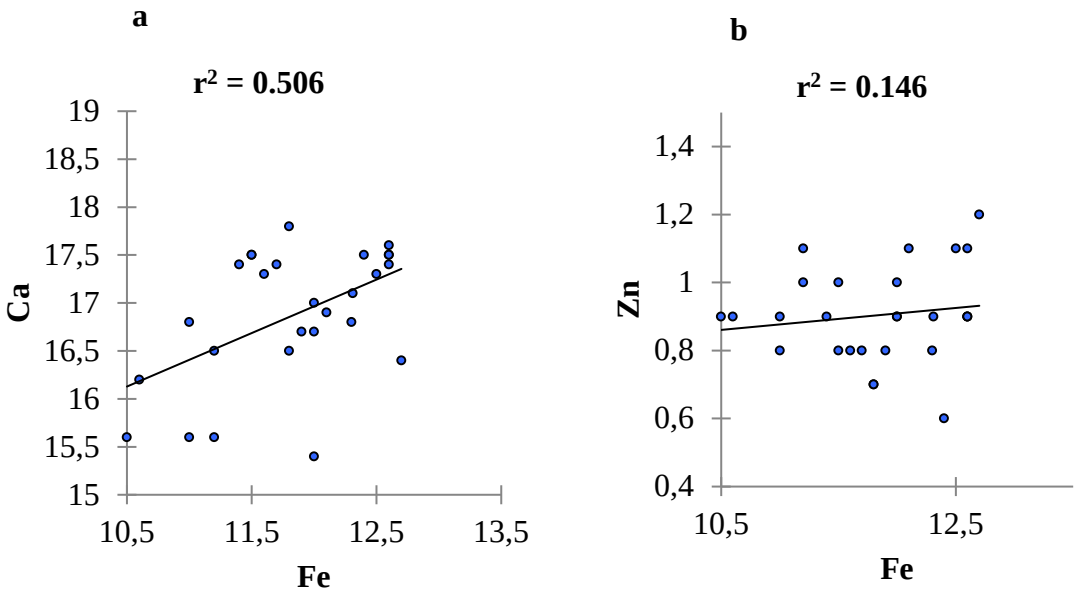


Figure 7.5: The correlation between pulp yield and pH (a), the correlation between pulp yield and AOA (b), the correlation between TTA and pulp yield (c), and the correlation between pulp yield and phosphorus (d).

As shown in Figure 7.6 and Table 7.1, iron content had a slight positive correlation with calcium (r^2 = 0.506) content, a weak negative correlation with sodium (r^2 = -0.477) content, and a very weak positive correlation with the zinc (r^2 = 0.146) content in the fruit pulp.



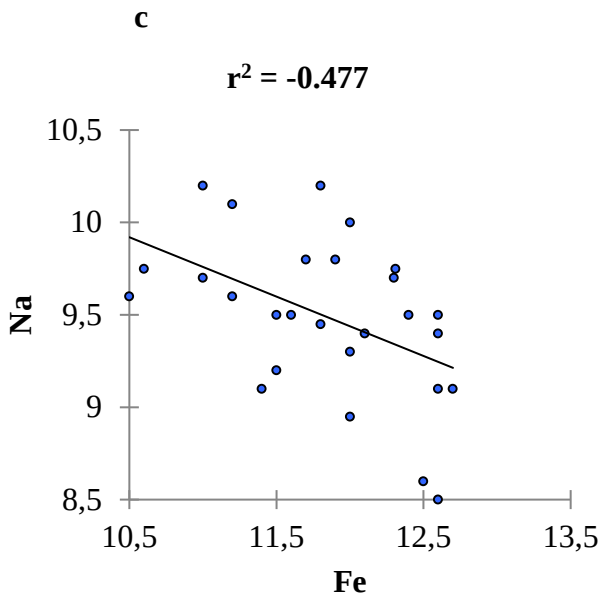


Figure 7.6: The correlation between iron and calcium (a), the correlation between iron and zinc (b), and the correlation between iron and sodium (c).

7.3.3 Analysis of variance (pulp yield)

Total titratable acidity and phosphorus content had correlation values of $R^2 = 0.663$ and $R^2 = 0.758$ respectively. ANOVA model showed that only parameters TTA and phosphorus content in the fruit were significant ($P < 0.0001$) in the model (Table 7.2).

Table 7.2: Analysis of variance (Pulp yield)

	DF	Sum of squares	Mean squares	F	P value
Model	2	32.846	16.423	37.631	**< 0,0001**
Error	24	10.474	0.436		
Corrected Total	26	43.320			

7.3.4 Relationship between TTA and Phosphorus on pulp yield in fruits

The computed effect of all pulp attributes on pulp yield showed that only TTA and phosphorus contents had an effect on the pulp yield for the fruit as represented by the correlation equation. The equation represented the relationship between TTA and phoshorus contents in the pulp. TTA content has the greatest effect on pulp yield meaning the higher the TTA content in the fruit the lower the pulp yield.

Pulp yield = 28, 2233912721278 - 5, 53115217852001*TTA - 0, 862299285234017*P

7.3.5 Model parameters (pulp yield)

TTA content had a significant difference on pulp yield content (P < 0.005). Phosphorus content had a significant difference on pulp yield content (P< 0.001). All other pulp attributes had no significant effect on pulp yield content in the fruit (Table 7.3).

Table 7.3: Model parameters (Pulp yield)

Source	Value	Standard error	t	P value
Intercept	28.223	2.675	10.550	**< 0,0001**
pH	0.000	0.000		
Brix_TSS	0.000	0.000		
DM	0.000	0.000		
VitC	0.000	0.000		
AOA	0.000	0.000		
Ca	0.000	0.000		
Iron	0.000	0.000		
Zinc	0.000	0.000		
TTA	-5.531	1.796	-3.079	**0.005**
Mg	0.000	0.000		
Na	0.000	0.000		
P	-0.862	0.217	-3.983	**0.001**
K	0.000	0.000		
Cu	0.000	0.000		

7.4 CONCLUSION

Multivariate analysis indicated that Principal component 1 (PC1) and PC 2 which represented physicochemical and functional properties of the pulp had eigenvalues of 5.59 and 2.13, and a variability of 37.31 % and 14.17 %, respectively. Variability in fruit pulp properties were attributed to pH (74 %) and TTA (69 %). Pulp attributes, pH, Vitamin C, antioxidant activity (AOA), TTA, magnesium, sodium, potassium, and copper contributed positively to the most variability in PC 1 among the sampling areas. In PC 2, calcium and iron content in the pulp contributed positively to the variability. Phosphorus, sodium and iron accounted for approximately 73 %, 50 %, and 43 % of the variation, respectively. TTA and phosphorus content had a positive effect on pulp yield. Physicochemical and functional properties of the fruit pulp play a vital role in processing of the fruit into a functional food.

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CHAPTER EIGHT

CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

This study has shown that the *U. kirkiana* fruit has good physicochemical and functionality properties. The fruit has a high pulp yield (15.5 g/100 g), TSS (23.2 g/100 g), pH (4.4 ± 0.09), AOA (30.6 ± 0.46), TTA (0.48 ± 0.04) and pectin (0.24 ± 0.02 %). The study also revealed that the fruit is a good source of micronutrients especially iron (12.3 mg/100 g) and can potentially help to curb the problem of iron related malnutrition. The fruit acts as a source of vitamin C (16.9 mg/100 g) and Antioxidant activity. This will allow the fruit to have possibility of helping in contrillong the effect of free radicals once consumed as part of a diet. Fructose was the dominant sugar in the pulp and it aided in improving sensorial attributes of the jam that contain the fruit. The fruit had a low content of polyphenols such as tannins, flavonoids and gallotannins. Principal component 1 and 2 had eigenvalues and variability of 5.59, 2.13 and 37.31 %, 14.17 %, respectively. The study also revealed that factors that contribute to variability of physicochemical properties in fruit pulps include pulp pH, vitamin C, antioxidant activity, titratable acid, phosphorus, copper, and sodium contents. Jam inoculated with *L. rhamnosus* yoba had a vitamin C, TTA, total soluble solids , and moisture content of 0.34 ± 0.02 mg/100 g, 2.2 ± 0.11, 68.5 ± 0.2, and 34.8 ± 1.2 which improves the functionality of the jam The study noted that the fruit was a good and ideal environment that allowed the growth of probiotic bacteria. The probiotic, *L. rhamnosus* yoba was able to degrade the pulp matrix and release bound minerals which can be supported by the mineral results in the jam (iron- 4.13 ± 0.52 mg/100 g and zinc -0.36 ± 0.02 mg/100 g). *L. rhamnosus* yoba can degrade complex sugars into simple sugars hence the high fructose and sucrose contents of 12.84 ± 0.21 g/100 g and 24.61 ± 0.12 g/100 g observed in the jam. *L. rhamnosus* yoba can also ferment pulp sugars into organic acids as indicated in the jam which had a TTA content of 2.2 at d 0 (after production), 2.37 ± 0.01 at d 4 and 2.48 ± 0.02 at day 7 in storage (25 ^{o}C). The study also notes that action of the probiotic, *L. rhamnosus* yoba can aid in iron bioaccessibility **(**6.55 ± 0.36 %) and zinc bioaccessibility (16.1 ± 0.50 %). The use of *L. rhamnosus* yoba in the jam showed a 4 % and 2 % increase in the iron and zinc bioaccessibility,

respectively. The use of the probiotic, *L. rhamnosus* yoba in fruit jam has a potential in improving sensorial attributes of the product such as spreadability, taste, appearance, and mouthfeel.
Jam inoculated with *L. rhamnosus* yoba had an overall acceptance score of 7.5 (n = 150*)*. The good functional properties of the fruit pulp can result in the utilisation of the fruit to produce a probiotic jam with possible functional benefits. The fruit jam was able to deliver 6.2 ± 0.2 log CFU/mL live *L. rhamnosus* yoba cells, which make it a good probiotic food.

8.2 Recommendations

Fruit jam inoculated with *L. rhamnosus* yoba is a good source of nutrition and especially that of some micronutrients, however further tests on improvement on the jam in terms of its shelflife, product stability during storage must be conducted with a future objective of commercialising the jam so that it can be consumption as part of a diet and potentially help to mitigate malnutrition problems for rural communities. Rural communities living in dry and semi-dry regions must be educated on current methods of food processing, especially the use of *L. rhamnosus* yoba, creating the probiotic bank, and food safety. This study further recommends research on:

1. Identification of the specific fruit tree species locally and their functionality at a molecular level.
2. The use of the *L. rhamnosus* yoba bacteria in processing the fruit into other fermented foods such as wines and other beverages.

APPENDICES

Appendix 1. Principal component analysis of *U. kirkiana* fruit pulp properties

Variable	F1	F2	F3	F4
Eigenvalue	5.597	2.126	1.326	1.250
Variability (%)	37.312	14.171	8.841	8.331
Cumulative %	37.312	51.483	60.324	68.655
Factor loadings*				
pH	**0.810**	0.338	-0.254	-0.200
TSS	-0.474	0.321	-0.163	0.350
DM	-0.486	0.067	0.141	**-0.540**
Vit. C	**0.580**	0.014	-0.406	-0.279
AOA	**0.824**	0.077	0.389	0.086
Pulp_yield	**-0.840**	-0.244	0.188	-0.053
Ca	-0.244	**0.721**	0.038	-0.124
Fe	-0.179	**0.859**	0.003	0.094
Zn	-0.385	-0.039	-0.312	0.448
TTA	**0.824**	0.277	0.070	-0.111
Mg	**0.556**	-0.262	-0.026	0.554
Na	**0.556**	**-0.539**	0.323	-0.195
P	**0.887**	0.116	-0.202	-0.053
K	-0.208	0.298	**0.669**	0.033
Cu	**0.626**	0.198	0.407	0.348

*TSS: Total soluble solids, DM: dry matter, Vit. C: vitamin C, AOA: antioxidant activity, TTA: total titratable acidity..