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.

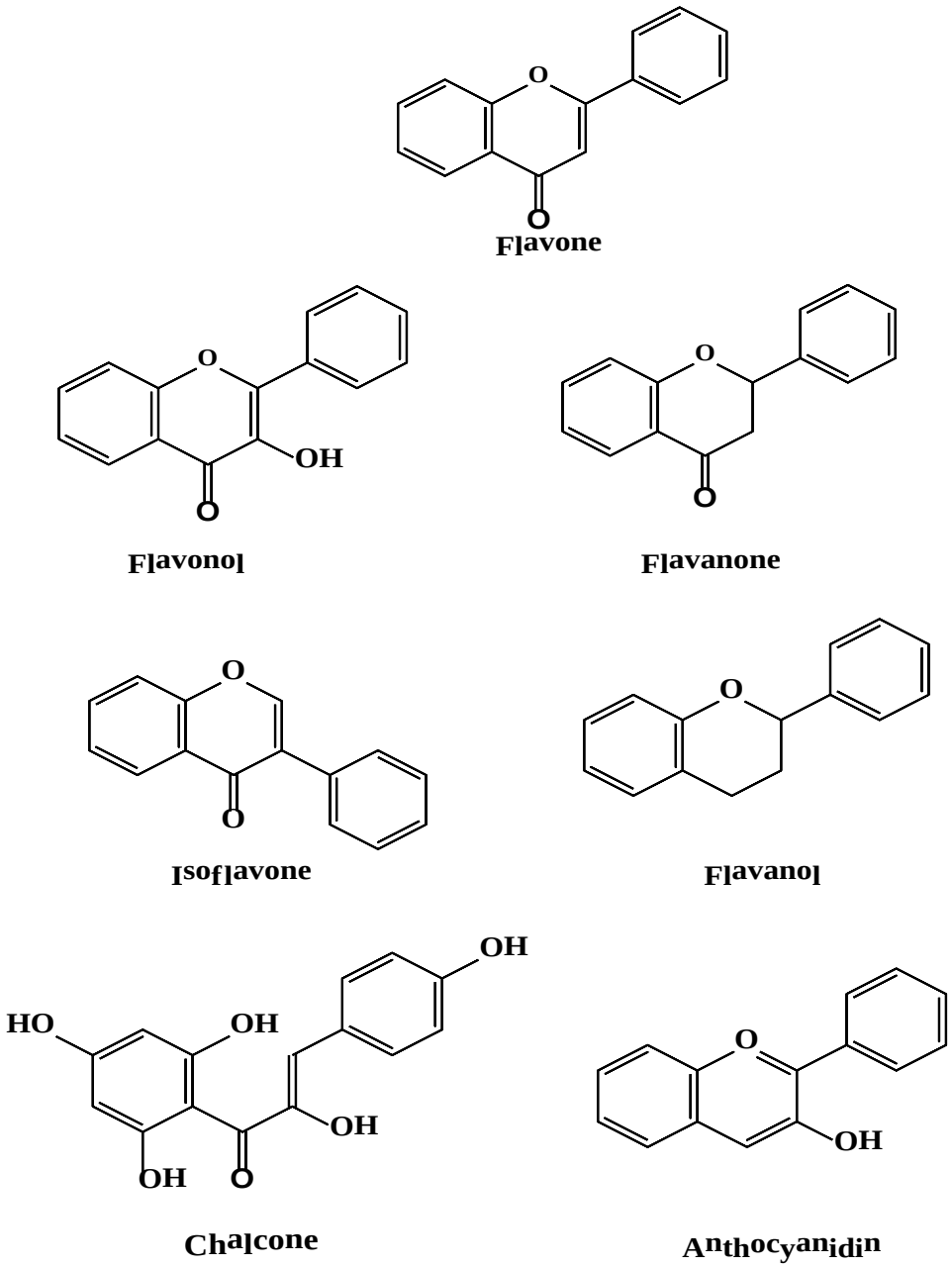


Figure 2.9: Structures of major flavonoids (adopted from Maraisi *et al*., 2006)

© Central University of Technology, Free State

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).

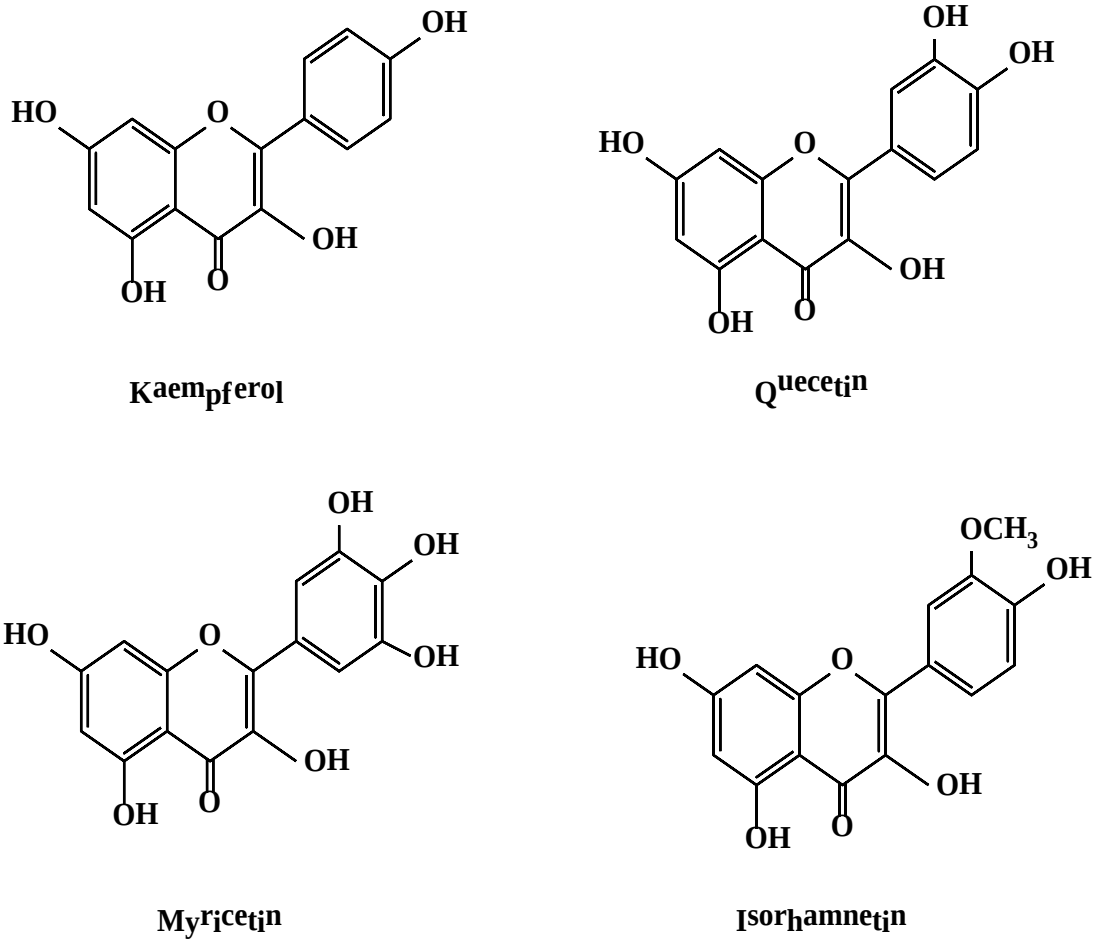


Figure 2.10: The structures of kaempferol, myricetin, quercetin, and isorhamnetin (adopted from De Man, 1999).

© Central University of Technology, Free State

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).

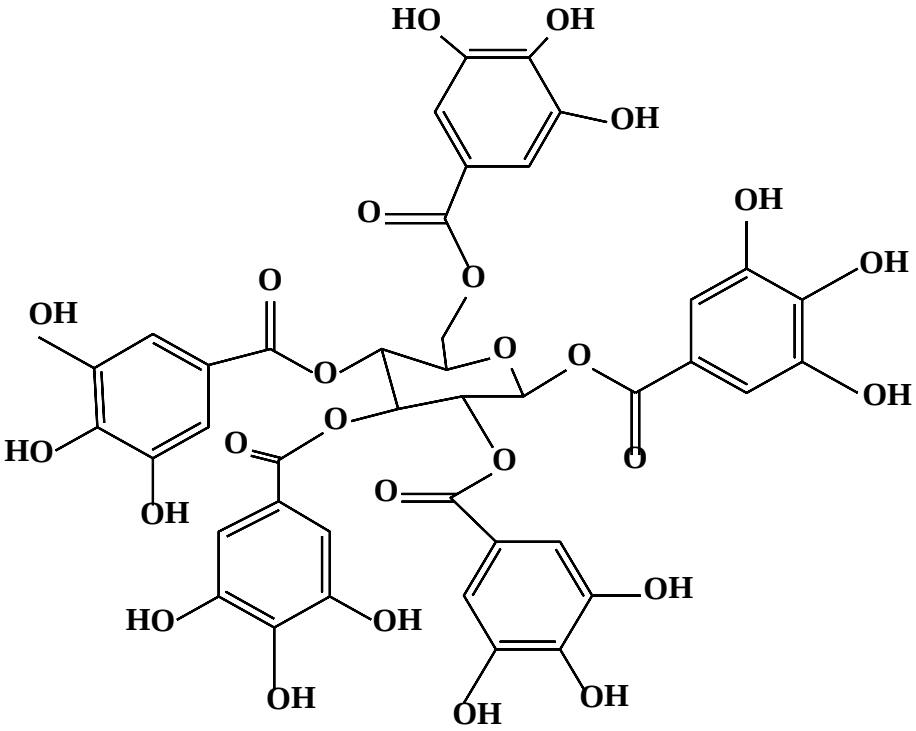


Figure 2.11: Penta galloyl-D-glucose (adopted from Hargerman, 2002).

© Central University of Technology, Free State

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.

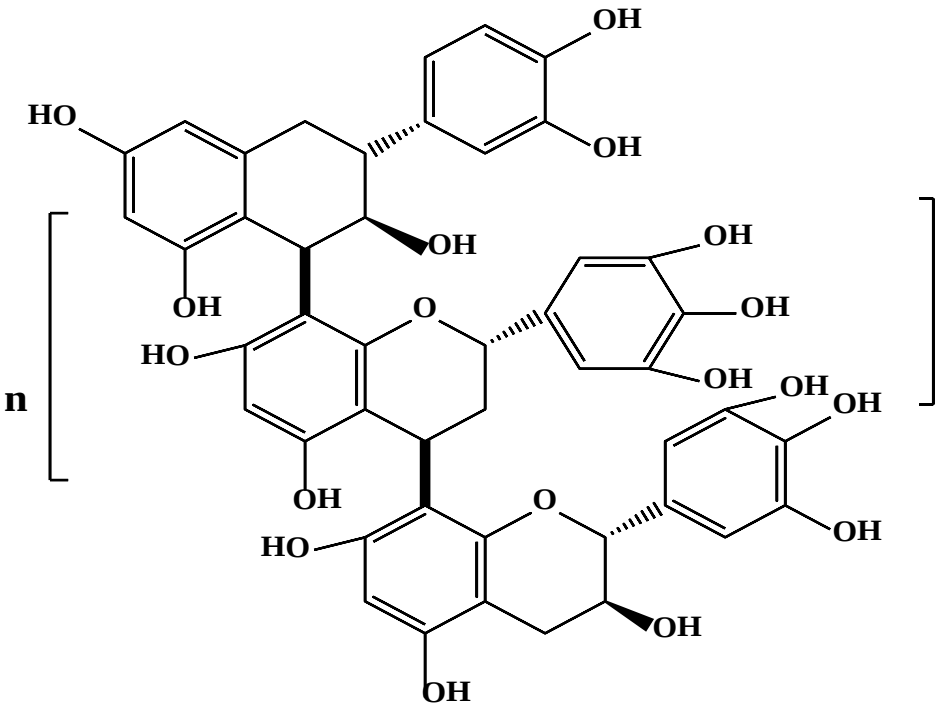


Figure 2.12: Proanthocyanidins (PAs).

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

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

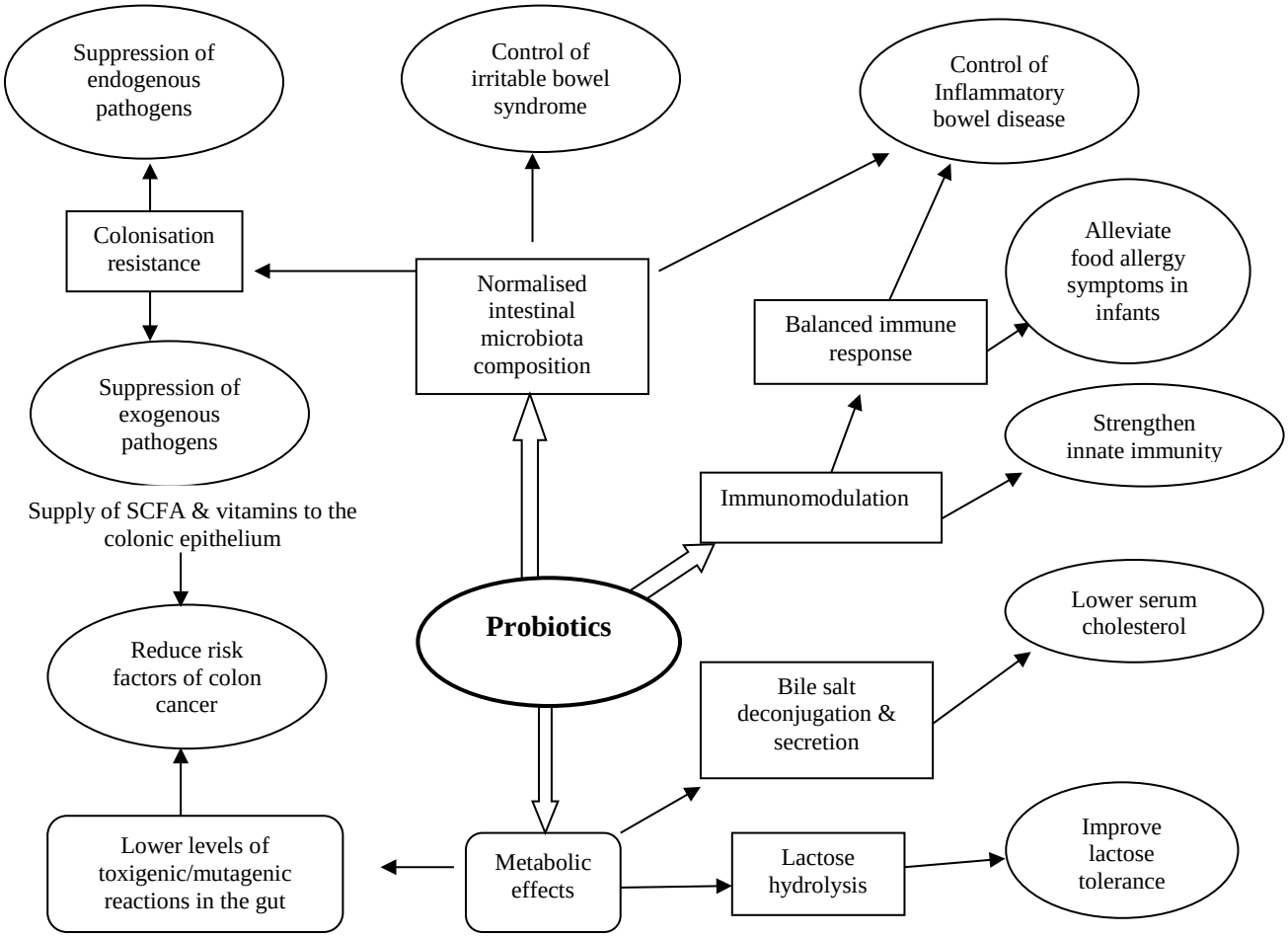


Figure 2.13: Various health benefits of consumption of probiotics **(**adopted from Shah, 2015**).**

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

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

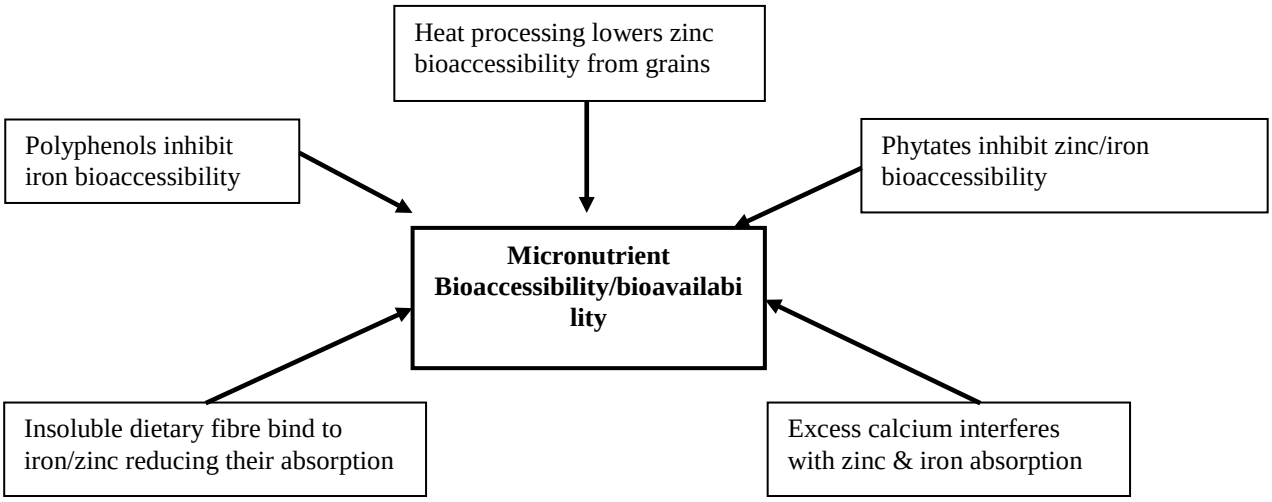


Figure 2.14: Inhibitors of iron and zinc micronutrient bioavailability (adopted from Sandberg, 2002; Gupta, Jyothilakshmi and Prakash, 2006).

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.

© Central University of Technology, Free State

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.

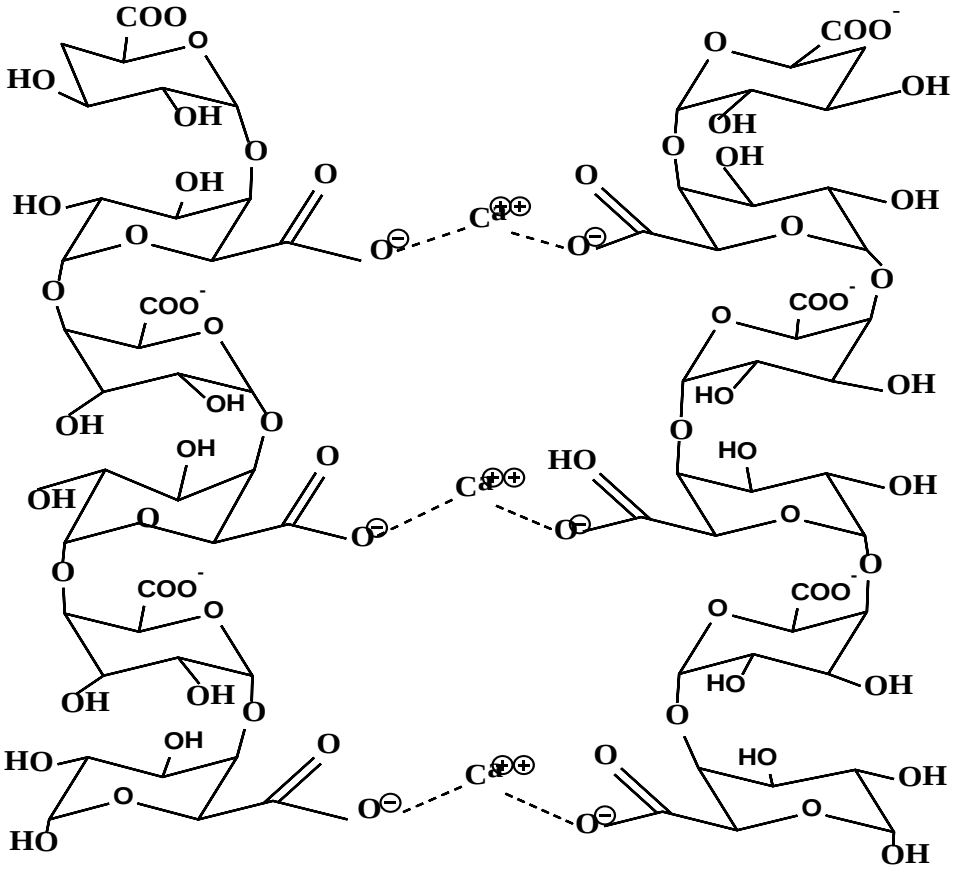


Figure 2.15: The egg-box model of pectin and calcium crosslinking (adopted from Caffall and Mohnen, 2009).

© Central University of Technology, Free State

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).

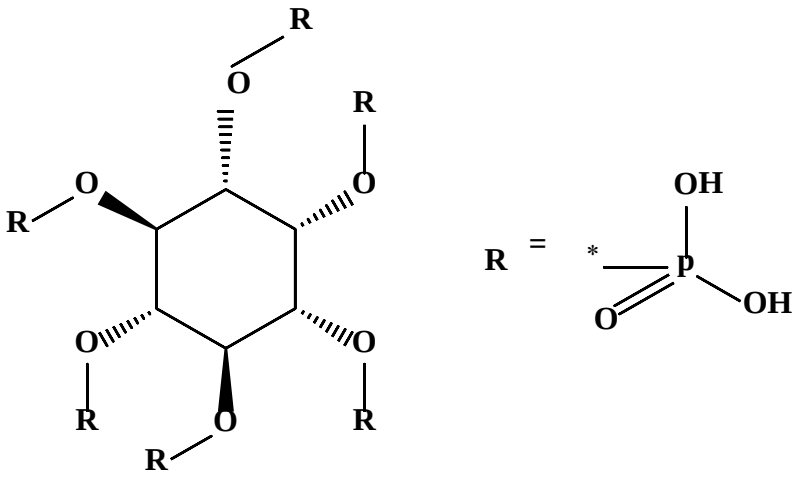


Figure 2.16: Basic chemical structure of phytic acid (adopted from Kumar *et al.*, 2010).

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,

© Central University of Technology, Free State

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.

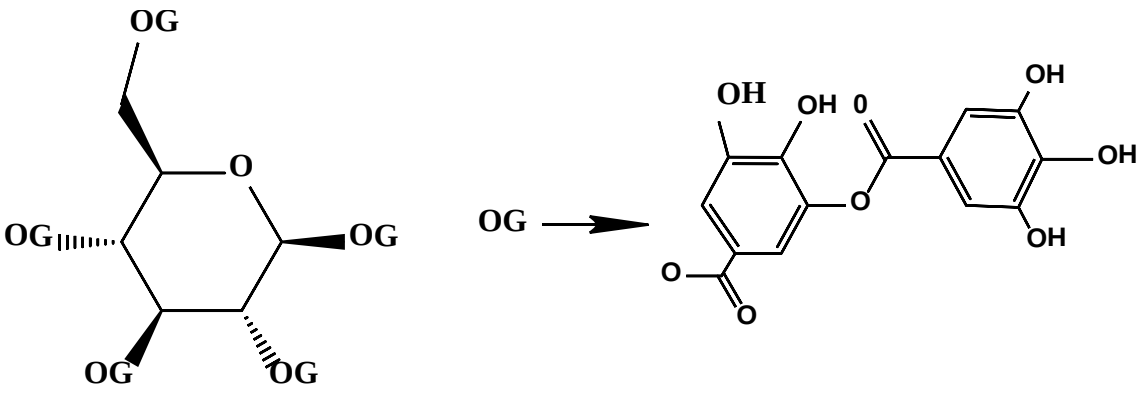


Figure 2.17: Chemical structure of tannic acid (adopted from Xu, Han and Wang, 2019).

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.

© Central University of Technology, Free State

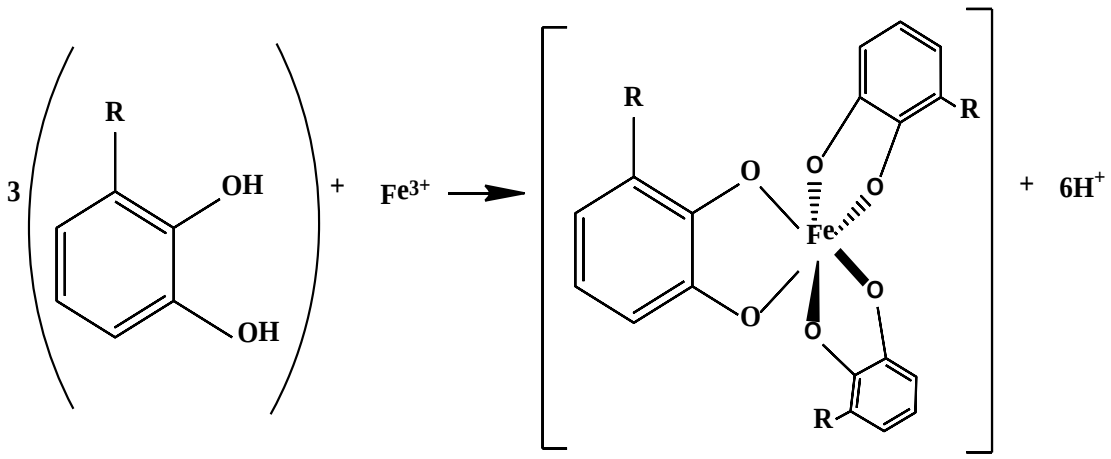


Figure 2.18: Octahedral coordination geometry of iron-polyphenol complexes. Gallols, R = OH; catechols, R = H (adopted from Perron and Brumaghim, 2009).

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

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

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

© Central University of Technology, Free State

(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).

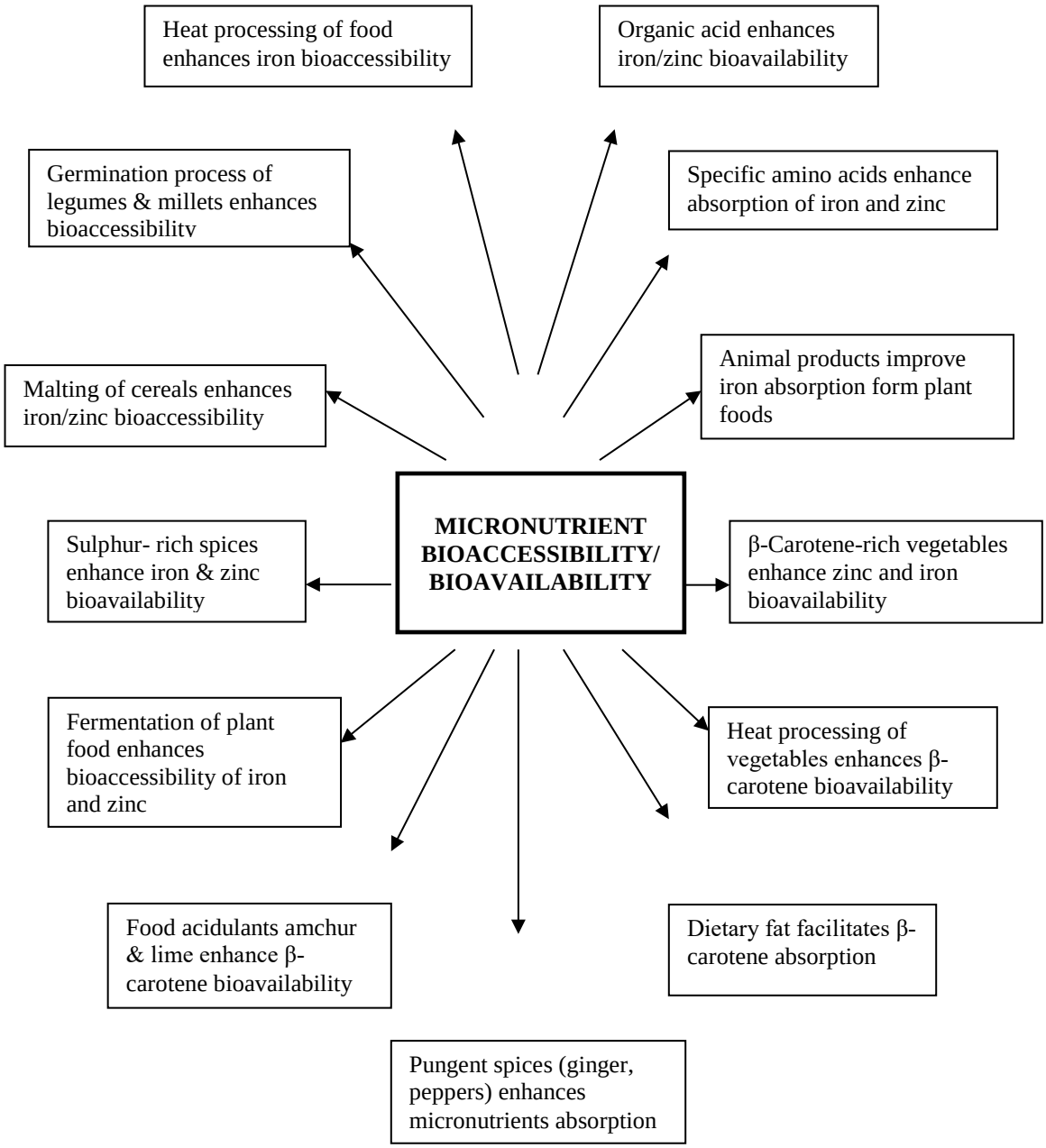


Figure 2.19: Factors that promote micronutrient bioavailability (adopted from Gupta, Jyothilakshmi and Prakash, 2006).

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

Alegria, A., Cilla, A., Farre, R. and Lagarda. M. J. 2015. Inorganic nutrients. In *Handbook of food analysis*, eds. Nollet, L. M. L. and Toldra, F., 3rd ed. Boca Raton, FL: CRC Press, Taylor and Francis. pp. 733–753.

Aljadi, A. M. and Yusof, K. M. 2002. Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medicine*. 3, 229–236.

Amalraj, A. and Pius, A. 2015. Bioavailability of calcium and its absorption inhibitors in raw and cooked green leafy vegetables commonly consumed in India--an *in vitro* study. *Food Chemistry*. 170, 430 –436.

Ames, B. N., Shigenaga, M. K. and Hagen, T. M. 1993. Oxidants, antioxidants and degenerative diseases of aging. *Proceedings of National Academy of Science USA.* 90, 7915–7922.

Anantharaju, P., Gowda, P. C., Vimalambike, M. G. and Madhunapantula, S. V. 2016. An overview on the role of dietary phenolics for the treatment of cancers. *Nutrition Journal.* 15, 99.

Anderson, J. W. 2004. Whole grains and coronary heart disease: the whole kernel of truth. *American Journal of Clinical Nutrition.* 80, 1459–1460.

Andrews, M., Briones, L., Jaramillo, A., Pizarro, F. and Arredondo. M. 2014. Effect of calcium, tannic acid, phytic acid and pectin over iron uptake in an *in vitro* caco-2 cell model. *Biological Trace Element Research.* 158, 122–127.

Barclay, A., Sandall, P. and Shwide-Slavin, C. 2014. *The ultimate guide to sugars and sweeteners: discover the taste, use, nutrition, science, and lore of everything from agave nectar to xylitol*. New York, NY: The Experiment.

Barros, H. R., Ferreira, T. A. and Genovese, M. I. 2012. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chemistry.* 134, 1892–1898.

Beard, J. L. and Dawson, H. D. 1997. Iron. In: *Handbook of nutritionally essential minerals*. O'Dell, B. L. and Sunde, R. A., eds. New York: Marcel Dekker. pp. 275–334.

Bele, A. A., Jadhav, V. M. and Kadam, V. J. 2010. Potential of tannins: a review. *Asian Journal of Plant Science.* 9, 209–214.

Berdanier, C. D., Dwyer, J. T. and Heber. D. 2013. *Handbook of nutrition and food*, 3rd ed. Boca Raton, FL: CRC Press, Taylor and Francis.

© Central University of Technology, Free State

Bhavyashree, S. H., Prakash, J., Platel, K. and Srinivasan, K. 2009. Bioaccessibility of minerals from cereal-based composite meals and ready-to-eat foods. *Journal of Food Science and Technology*. 46, 431–435.

Bhowmik, D., Chiranjib, K. P. and Kumar, S. 2010. A potential medicinal importance of zinc in human health and chronic disease. *International Journal of Pharmacy and Biomedical Science*. 1, 5–11.

Bille, P., Shikongo-Nambab, M. and Cheikhyoussef, A. 2013. Value addition and processed products of three indigenous fruits in Namibia. *African Journal of Food, Agriculture, Nutrition and Development.* 13, 7192–7212.

Birketvedt, G. S., Shimshi, M., Erling, T. and Florholmen, J. 2005. Experiences with three different fiber supplements in weight reduction. *Medical Science Monitor*. 11, 15–18.

Boland, M. M., Golding, M. and Singh. H. 2014. *Food structures, digestion and health*. Cambridge, MA: Academic Press, Elsevier.

Bosscher, D., Van Caillie-Bertrand, M., Van Cauwenbergh, R. and Deelstra, H. 2003. Availabilities of calcium, iron and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions. *Nutrition.* 19, 641–645.

Braidwood, L., Breuer, C. and Sugimoto, K. 2014. My body is a cage: mechanisms and modulation of plant cell growth. *New Phytologist.* 201, 388 –402.

Brody, T. 1999. *Nutritional Biochemistry*, 2nd ed. San Diego, CA: Academic Press, Elsevier.

Brown, L., Rosner, B., Willett, W. W. and Sacks, F. M. 1999. Cholesterol lowering effects of dietary fiber: a meta-analysis. *American Journal of Clinical Nutrition.* 69, 30–42.

Caffall, K. H. and Mohnen. D. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research.* 344, 1879–1900.

Cammack, R., Atwood, R. Campell, P. Parish, H. Smith, A.Vella, F. and Stirling. J. 2006. Oxford Dictionary of Biochemistry and Molecular Biology (2 ed.). Oxford University Press.

Campbell, B. M., Jeffrey, S., Kozanayi, W., Luckert, M., Mutamba, M. and Zindi, C. 2002. *Household livelihoods in semi-arid regions: options and constraints*. Bogor, Indonesia: CIFOR.

© Central University of Technology, Free State

Campbell, B. M., Luckert, M. and Scoones, I. 1997. Local level valuation of savannah resources: a case study from Zimbabwe. *Economic Botany*. 51, 59–77.

Celus, M., Kyomugasho, C., Salvia-Trujillo, L., Van Audenhove, J., Van Loey, A. M., Grauwet, T. and Hendrickx, M. E. 2018. Interactions between citrus pectin and Zn^{2+} or Ca^{2+} and associated *in vitro* Zn^{2+} bioaccessibility as affected by degree of methylesterification and blockiness. *Food Hydrocolloids.* 79, 319–330.

Chan, S. Y., Choo, W. S., Young, D. J. and Loh, X. J. 2017. Pectin as a rheology modifier: origin, structure, commercial production and rheology. *Carbohydrate Research.* 161, 118–139.

Chatterjea, M. N. and Shinde, R. 2005. *Text book of Medical Biochemistry*, 6th edition. New Delhi, India: Jaypee Brothers Medical Publishers.

Chen, C. H., Liu, T. Z., Chen, C. H., Wong, C. H., Chen, C. H., Lu, F. J. and Chen, S. C. 2007. The efficacy of protective effects of tannic acid, gallic acid, ellagic acid, and propyl gallate against hydrogen peroxide-induced oxidative stress and DNA damages in IMR-90 cells. *Molecular Nutrition and Food Research.* 51, 962–968.

Chifamba, E. 2011. Cultivation and commercialization of indigenous fruit trees to improve household food security in dry regions of Buhera Zimbabwe, *Journal of Sustainable Development in Africa.* 13, 95–108.

Considine, K. M., Kelly, A. L., Fitzgerald, G. F., Hill, C. and Sleator, R. D. 2008. High-pressure processing-effects on microbial food safety and food quality. *FEMS Microbiology Letters.* 281, 1–9.

Cook, J. D. and Reddy, M. B. 2001. Effect of ascorbic acid intake on non heme-iron absorption from a complete diet. *American Journal of Clinical Nutrition.* 73, 93–98.

Corcoran, B. M., Stanton, C., Fitzgerald, G. F. and Ross, R. P. 2005. Survival of probiotic *Lactobacilli* in acidic environments is enhanced in the presence of metabolizable sugars. *Applied and Environmental Microbiology.* 71, 3060–3067.

Cosgrove, D.J. 2005. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* 6:850-861

Cradall, P. G., Upandhyaya, J. K. and Davis, K. C. 1990. Portable, low cost equipment for small fruit juice processing. *International Journal of Food Science and Technology.* 25, 583 – 589.

© Central University of Technology, Free State

Cunningham, A.B. 2002. *Applied Ethnobotany: People, Wild Plant Use and Conservation Manuals*. London and Sterling, VA: WWF and Earthscan Publications.

Daly, C. and Davis, R. 1998. The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health. *Agricultural and Food Science.* 7, 219–250.

de Almeida Costa, G., Queiroz-Monici, E. K. S., Machado Reis, S. M. P. and de Oliveira, A. C. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry.* 94, 327–330.

de Lima, A. C., Soares, D. J., da Silva, L. M., de Figueiredo, R. W., de Sousa, P. H. and de Abreu Menezes, E. 2014. *In vitro* bioaccessibility of copper, iron, zinc and antioxidant compounds of whole cashew apple juice and cashew apple fibre (*Anacardium occidentale* L.) following simulated gastro-intestinal digestion. *Food Chemistry.* 161, 142–147.

De Man, M. 1999. *Principles of food chemistry*, 3rd ed. New York, USA: Springer.

Deshpande, J. D., Joshi, M. M. and Giri, P. A. 2013. Zinc: the trace element of major importance in human nutrition and health. *International Journal of Medical Science and Public Health.* 2, 1–6.

Dhingra, D., Michael, M., Rajput, H. and Patil, R. T. 2012. Dietary fibre in foods: A review. *Journal of Food Science and Technology.* 49, 255–266.

Duhan, A., Khetarpaul, N., and Bishnoi, S. 2004. HCl-extractability of zinc and copper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *Journal of Food Composition and Analysis.* 17, 597–604.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. and Attia, H. 2011. Dietary fibre and fibre-rich by-products of food processing: characterisation, technological functionality and commercial applications: a review. *Food Chemistry.* 124, 411–421.

El-Sayed, I. H., Lotfy, M., El-Khawaga, O. Y., Nasif, W. A. and El-Shahat, M. 2006. Prominent free radicals scavenging activity of tannic acid in lead-induced oxidative stress in experimental mice. *Toxicology and Industrial Health*. 22, 157–163.

Embaby, H. E. 2011. Effect of heat treatments on certain antinutrients and *in vitro* protein digestibility of peanut and sesame seeds. *Food Science and Technology Research.* 17, 31–38.

© Central University of Technology, Free State

Etcheverry, P., Grusak, M. A. and Fleige, L. E. 2012. Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology.* 3, 317.

FAO, 1997. *Guidelines for small-scale fruit and vegetable processors*. FAO Agricultural Services Bulletin 127. Cifford Hay on Wye, Hereford, UK: Peter Midway Technology Ltd.

Fentahun, M. T. and Hager, H. 2009. Exploiting locally available resources for food and nutritional security enhancement: wild fruits diversity, potential and state of exploitation in the Amhara region of Ethiopia. *Food Security.* 1, 207–219.

Fernandes, F. A., Rodrigues, S., Law, C. L. and Mujumdar, A. S. 2011. Drying of exotic tropical fruits: a comprehensive review. *Food and Bioprocess Technology.* 4, 163–185.

Fernandez-Garcıa, E., Carvajal-Lerida, I. and Perez-Galvez. A. 2009. *In vitro* bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research.* 29, 751–760.

Franz C. M., Huch M., Mathara J. M., Abriouel H. and Benomar, N. 2014. African fermented foods and probiotics. *International Journal of Food Microbiology.* 190, 84–96.

Frossard, E., Bucher, M., Machler, F., Mozafar, A. and Hurrell, R. 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture.* 80, 861–879.

Fuqua, B. K., Vulpe, C. D. and Anderson, G. J. 2012. Intestinal iron absorption.

Gadaga, T., Madzima, R. and Nembaware, N. 2009. Status of micronutrient nutrition in Zimbabwe: a review. *African Journal of Food, Agriculture, Nutrition and Development.* 9, 502–522.

Galvez, J. M., Reid, B., and Gonner, A. H. 1997. Analytical studies on Tara tannins. *Holzforschung*. 51, 235–243.

Gill, H. S. and Guarner, F. 2004. Probiotics and human health: a clinical perspective. *Postgraduate Medical Journal.* 80, 516–526.

Gionchetti, P., Rizzello, F., Morselli, C., Poggioli, G., Tambasco, R., Calabrese, C., Brigidi, P., Vitali, B., Straforini, G. and Campieri, M. 2007. High-dose probiotics for the treatment of active pouchitis. *Diseases of the Colon and Rectum.* 50, 2075–2082.

© Central University of Technology, Free State

Global Dietary Database. 2010. *Dietary Data by Region*. [online] Available at: https://www.globaldietarydatabase.org/dietarydata- by-region.html [Accessed 23 May 2019].

Golding, J. B., Shearer, D., Wyllie, S. G. and McGlasson, W. B. 1998. Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. *Postharvest Biology and Technology*. 14, 87–98.

Gopal, P. K., Prasad, J., Smart, J. and Gill, H. S. 2001. *In vitro* adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. *International Journal of Food Microbiology*. 67, 207–216.

Grandy, G., Medina, M., Soria, R., Teran, C. G. and Araya, M. 2010. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double blind, controlled trial using two different probiotic preparations in Bolivian children. BMC *Infectious Diseases*. 10, 253–266.

Gross, J., Carmon, M., Lifshitz, A. and Costes, C. 1976. Carotenoids of banana pulp, peel and leaves. *Food Science Technology*. 9, 211–214.

Guandalini, S., Pensabene, L., Zikri, M. A., Dias, J. A., Casali, L. G., Hoekstra, H., Kolacek, S., Massar, K., Micetic-Turk, D., Papadopoulou, A., de Sousa, J. S., Sandhu, B., Szajewska, H. and Weizman, Z. 2000. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *Journal of Pediatric Gastroenterology and Nutrition*. 30, 54–60.

Guerra, A., Etienne-Mesmin, L., Livrelli, V., Denis, S., Blanquet-Diot, S. and Alric, M. 2012. Relevance and challenges in modeling human gastric and small intestinal digestion *Trends in Biotechnology*. 30, 591–600.

Gupta, S., Jyothilakshmi, A. and Prakash, J. 2006. Comparative analysis of influence of promoters and inhibitors on *in vitro* available iron using two methods. *International Journal of Food Science and Nutrition*. 57, 559–569.

Hambidge, M. K., Miller, L. V., Westcott, J. E., Sheng, X. and Krebs, N. F. 2010. Zinc bioavailability and homeostasis. *American Journal of Clinical Nutrition*. 91, 1478S–1483S.

© Central University of Technology, Free State

Haq, N., Bowe, C. and Dunsiger, Z. 2008. Challenges to stimulating the adoption and impact of indigenous fruit trees in tropical agriculture. In: Akinnifesi, F. K., Leakey, R.B., Ajayi, O. C., Sileshi, G., Tchoundjeu, Z., Matakala, P. and Kwesiga, F. R., (eds). *Indigenous fruit trees in the tropics domestication, utilization and commercialization*. Wallingford, UK: CABI Publishing. pp. 50–69.

Harborne, J. B. 1998. *Phytochemical methods: A guide to modern techniques of plant analysis*, 3rd ed. London, UK: Chapman and Hall.

Hargerman, A. E. 2002. *Tannin chemistry*. [online] Available at: https://www.users.miamioh.edu/hagermae/ Miami University, USA.

Harnly, J. M., Doherty, R. F., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Bhagwat, S. and Gebhardt, S. 2006. Flavonoid content of U.S. fruits, vegetables, and nuts. *Journal of Agricultural and Food Chemistry*. 54, 9966–9977.

Haro-Vicente, J.F., Martinez-Gracia, C. and Ros, G. 2006. Optimization of in vitro measurement of available iron from different fortificants in citric fruit juices. *Food Chemistry* 98: 639–648.

Hartley, D., Marcos, A., Rosado, J., Rubeglio, E. and Tannock, G. 2001. *Fermented Foods and Healthy Digestive Functions*. Montrouge, France: John Libbey Eurotext.

Hazell, T. and Johnson, I.T. 1987. Effects of food processing and fruit juices on in vitro estimated iron availability from cereals, vegetables and fruits. *Journal of the Science of Food and Agriculture* 38: 73–82.

Hemalatha, S., Gautam, S., Platel, K. and Srinivasan, K. 2009. Influence of exogenous iron, calcium, protein and common salt on the bioaccessibility of zinc from cereals and legumes. *Journal of Trace Elements and Medical Biology*. 23, 75–83.

Hemalatha, S., Platel, K. and Srinivasan, K. 2007. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *Journal of Trace Elements and Medical Biology*. 21, 1–7.

Hemalatha, S., Platel, K. and Srinivasan, K. 2007a. Zinc and iron content and their bioaccessibility in cereals and pulses consumed in India. *Food Chem*istry. 102, 1328–1336.

© Central University of Technology, Free State

Hemalatha, S., Platel, K. and Srinivasan, K. 2007c. Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. *European Journal of Clinical Nutrition.* 61, 342–348.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J. and Pot, B. 2014. Expert consensus document. The International Scientific Association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology.* 11, 506–514.

Hojsak, I., Snovak, N., Abdovic, S., Szajewska, H., Misak, Z. and Kolacek. S. 2010. *Lactobacillus rhamnosus* GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double blind, placebo controlled trial. *Clinical Nutrition.* 29, 312–316.

Hollman, P. C., Geelen, A. and Kromhout, D. 2010. Dietary flavonol intake may lower stroke risk in men and women. *Journal of Nutrition.* 140, 600–604.

Holzapfel, W. H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*. 75, 197–212.

Hughes, A. and Haq, N. 2003. Promotion of indigenous fruit trees through improved processing and marketing in Asia. *International Forestry Review.* 5, 176–181.

Hunt, J.R. 2003. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets, *The American Journal of Clinical Nutrition*, 78:633S–639S

Hurrell, R. and Egli, I. 2010. Iron bioavailability and dietary reference values. *American Journal of Clinical Nutrition*. 91, 1461S–1467S.

Huxley, R. R. and Neil, H. A. 2003. The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. *European Journal of Clinical Nutrition.* 57, 904–908.

Iffat, A. T. K., Maqsood, Z. T. and Fatima, N. 2005. Study of complex formation of Fe (III) with tannic acid. *Journal of the Chemical Society of Pakistan.* 27, 174–177.

Ikram, E. H. K., Eng, K. H., Jalil, A. M.M., Ismail, A., Idris, S., Azlan, A., Nazri, H. S. M., Diton, N. A. M. and Mokhtar, R. A. M. 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of food Composition and Analysis*. 22, 388–393.

© Central University of Technology, Free State

International Food Policy Research Institute (IFPRI). 2016. *Global Nutrition Report 2016: From Promise to Impact: Ending Malnutrition by 2030*. Washington, DC., USA: International Food Policy Research Institute.

Iranbakhsh, A., Ebadi, M. and Zare, Z. 2009. The contribution of indigenous fruit trees in sustaining rural livelihoods and conservation of natural resources. *Journal of Horticulture and Forestry.* 1, 001–006.

Jaenicke, H. and Thiong'o, M. K. 2000. Preliminary nutritional analysis of marula (*Sclerocarya birrea*) fruits from two Kenyan provenances. *Acta Horticulturae.* 531, 245–249.

Jensen, E. N., Buch, A., Ravn-Haren, G. and Dragsted, L. 2015. Mini-review: the effects of apples on plasma cholesterol levels and cardiovascular risk – a review of the evidence, *The Journal of Horticultural Science and Biotechnology*. 84, 34–41.

Joint FAO/WHO Food Standards Programme Commission. 2016. *CODEX Alimentarius (CODEX) Guidelines on Nutrition Labelling CAC/GL 2-1985*, as Last Amended 2016, Rome.

Jones, J. R., Lineback, D. M. and Levine, M. J. 2006. Dietary reference intakes: implications for fiber labeling and consumption: a summary of the International Life Sciences Institute North American Fiber Workshop. *Nutrition Reviews.* 64, 31–38.

Jose, H., Isaza, M. and Yoshida, T. 2001. Tetrameric ellagitannins from *Monochaetum multiflorum*. *Heterocycles.* 55, 29–32.

Journal of Trace Elements and Electrolytes in Health and Disease. 26, 115–119.

Kadzere, I., Hove, L., Gatsi, T., Masarirambi, M. T., Tapfumaneyi, L., Maforimbo, E., Magumise, I., Sadi, J. and Makaya, P. R. 2001. *Current practices on post-harvest handling and traditional processing of indigenous fruits in Zimbabwe*. A Final Technical Report. Department of Research & Specialist Services, Ministry of Agriculture and Rural Resettlement, Harare, Zimbabwe. p. 66.

Kadzere, I., Watkins, C. B., Merwin, I. A., Akinnifesi, F. K., Saka, J. D. K. and Mhango, J. 2006. Harvesting and post-harvest handling practices and characteristics of *Uapaca kirkiana* (Muell. Arg.) fruits: a survey of roadside markets in Malawi. *Agroforestry Systems.* 68, 133–142.

© Central University of Technology, Free State

Karamac, M. 2009. Chelation of Cu (II), Zn (II), and Fe (II) by tannin constituents of selected edible nuts. *International Journal of Molecular Sciences.* 10, 5485–5497.

Kashyap, D. R., Vohra, P. K., Chopra, S. and Tewari, R. 2001. Applications of pectinases in the commercial sector: a review. *Bioresource Technology*. 77, 215–227.

Katsvanga, C. A. T., Jim, L., Gwenzi, D., Muhoni, L., Masuka, P. and Moyo, M. 2007. Characterisation of community identified *Uapaka kirkiana* phenotypes for domestication. *Journal of Sustainable Development in Africa.* 9, 356–366.

Kazii, N. A., Yadav, J. P. and Agale, M. G. 2015. Nutritional value of fruits, *Scholary Research Journal for Interdisciplinary Studies.* 3, 2937–2943.

Keenan, J. M., Pins, J. J., Frazel, C., Moran, A. and Turnquist, L. 2002. Oat ingestion reduces systolic and diastolic blood pressure in patients with mild or borderline hypertension: a pilot trial. *Journal of Family Practice*. 51, 369–375.

Kumar, V., Sinha, A. K., Makkar, H. P. S. and Becker, K. 2010. Dietary roles of phytate and phytase in human nutrition: a review. *Food Chemistry.* 120, 945–959.

Kwesiga, F. and Mwanza, S. 1994. Under-exploited wild genetic resources: the case of indigenous fruit trees in eastern Zambia. In: *Improvement of indigenous fruit trees of the miombo woodlands of southern Africa*. Maghembe, J. A., Ntupanyana, Y. and Chirwa, P. (eds.). Nairobi, Kenya: ICRAF.Kwesiga, F. R. 2008. *Indigenous Fruit Trees in the Tropics: Domestication, Utilization and Commercialization*. Wallingford, UK: CABI Publishing.

Kyomugasho, C., Willemsen, K., Christiaens, S., Van Loey, A. and Hendrickx. M. 2015. Pectin-interactions and *in vitro* bioaccessibility of calcium and iron in particulated tomato-based suspensions. *Food Hydrocolloids.* 49, 164–175.

Lairon, D., Arnault, N. and Bertrais, S. 2005. Dietary fiber intake and risk factors for cardiovascular disease in French adults. *American Journal of Clinical Nutrition.* 82, 1185–1194.

Lakhanpal, P. and Rai, D. K. 2007. Quercetin: a versatile flavonoid. *Proceedings of National Academy of Science USA.* 90, 8032–8055.

Layrisse, M., Garcıa-Casal, M. N., Solano, L., Baron, M. A., Arguello, F., Llovera, D., Ramırez, J., Leets, I. and Tropper, E. 2000. Iron bioavailability in humans from breakfasts enriched

© Central University of Technology, Free State

with iron bis-glycine chelate, phytates and polyphenols. *The Journal of Nutrition*, 130, 2195–2199.

Lazzi C., Turroni S., Mancini A., Sgarbi E., Neviani E. and Brigidi, P. 2014. Transcriptomic clues to understand the growth of *Lactobacillus rhamnosus* in cheese. *BMC Microbiology*, 7, 28.

Lee, Y. K., Nomoto, K., Salminen, S. and Gorbach, S. L. 1999. *Handbook of Probiotics*. New York, NY: John Wiley & Sons.

Legwaila, G., Mojeremane, W., Madisa, M., Mmolotsi, R. and Rampart, M. 2011. Potential of traditional food plants in rural household food security in Botswana. *Journal of Horticulture and Forestry*. 3, 171–177.

Lestienne, I., Besancon, P., Caporiccio, B., Lullien-Pellerin, V. and Treche. S. 2005. Iron and zinc *in vitro* availability in pearl millet flours (*Pennisetum glaucum*) with varying phytate, tannin, and fiber contents. *Journal of Agricultural and Food Chemistry.* 53, 3240–3247.

Lin, S. Y., Ayres, J. W., Winkler, W. and Sandine, W. E. 1989. Lactobacillus effects on cholesterol: *in vitro* and *in vivo* results. *Journal of Dairy Research.* 72, 2885–2889.

Liu, X., Kim, J., Li, Y., Li, J., Liu, F. and Chen. X. 2005. Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. *The Journal of Nutrition*, 135, 165–171.

Lockett, C. T., Calvert, C. C. and Grivetti, L. E. 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. *International Journal of Food Sciences and Nutrition.* 51, 195–208.

Lombardi-Boccia, G., De Santis, N., Di Lullo, G. and Carnovale, E. 1995. Impact of processing on Fe dialysability from bean (*Phaseolus vulgaris* L.). *Food Chemistry*. 53, 191–195.

Lutham, M. C. 1997. *Human Nutrition in the Developing World.* FAO Food and Nutrition Series No. 29. Rome, Italy: FAO.

MacFarlane, G. T. and Cummings, J. H. 2002. Probiotics, infection and immunity. *Current Opinion in Infectious Disease.* 15, 501–506.

Macheix, J. J., Fleuriet, A. and Billot, J. 1990. *Fruit phenolics*. Boca Raton, FL, USA: CRC press.

© Central University of Technology, Free State

Mack, D. R., Michail, S., Wei, S., McDougall, L. and Hollingsworth. M. A. 1999. Probiotics inhibit enteropathogenic *Escherichia coli* adherence *in vitro* by inducing intestinal mucin gene expression. *American Journal of Physiology*. 276, 941–950.

Maghembe, J. A., Kwesiga, F., Ngulube, M. and Prins, H. 1993. "Domestication potential of indigenous fruit trees in Makoka, Malawi". Paper prepared at the *International Conference on Tropical Trees Potential for Domestication,* held at Heroit- Watt University, Edinburgh.

Maghembe, J. A., Simons, A. J., Kwesiga. F. and Rarieya, M. 1998. *Selecting indigenous fruit trees for domestication in southern Africa: priority setting with farmers in Malawi., Tanzania, Zambia and Zimbabwe*. Nairobi: International Centre for Research in Agroforestry. p. 94.

Malaisse, F. and Parent, G. 1985. Edible wild vegetable products in the Zambezian woodland area: a nutritional and ecological approach. *Ecology of Food and Nutrition.* 18, 43–82.

Maraisi, J. P. J., Deavours, B., Dixon, R. A. and Ferreira, D. 2006. The stereochemistry of flavonoids. In *The science of flavonoids*. Groteworld, E. (ed.). New York, USA: Springer.

Maroyi, A. 2013. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *Journal of Ethnobiology and Ethnomedicine.* 9, 31.

May, C. D. 2010. Industrial pectins: sources, production and applications. *Carbohydrate Polymers.* 12, 79–99.

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W., and Brodkorb, A. 2014. A standardised static *in vitro* digestion method suitable for food - an international consensus. *Food and Function.* 5(6), 1113–24.

Mithöfer, D. and Waibel, H. 2003. Income and labour productivity of collection and use of indigenous fruit tree products in Zimbabwe. *Agroforestry Systems*. 59: 295–305.

Mladenka, P., Macakova, K., Filipsky, T., Zatloukalova, L., Jahodar, L., Bovicelli, P., Silvestri, I.P., Hrdina, R. and Saso. L. 2011. *In vitro* analysis of iron chelating activity of flavonoids. *Journal of Inorganic Biochemistry.* 105: 693–701.

© Central University of Technology, Free State

Montonen, J., Knekt, P., Jarvinen, R., Aromaa, A. and Reunanen, A. 2003. Whole-grain and fiber intake and the incidence of type 2 diabetes. *American Journal of Clinical Nutrition* 77: 622 – 629.

Moombe, K. B., Cori, H., Clarke, J. S., Franzel, S. and Ackerman, P. 2014. Consumer preferences for *Uapaca kirkiana* fruits in Zambia. *Forests, Trees and Livelihoods*. 23: 238–260.

Morelli, L. (2000). *In vitro* selection of probiotic lactobacilli: a critical appraisal. *Current Issues in Intestinal Microbiology.* 1:59–67.

Mpofu, A., Linnemann. A. R., Nout M. J. R., Zwietering, M. H. and Smid, E. J. 2014. Mutandabota, a food product from Zimbabwe: processing, composition, and socioeconomic aspects. *Ecology of Food and Nutrition*. 53: 24–41.

Muchuweti, M., Ndhlala, A. R. and Kasiamhuru, A. 2006. Analysis of phenolic compounds including tannins, gallotannins and flavanols of *Uapaca kirkiana* fruit. *Food Chemistry.* 94: 415–419.

Mueller-Harvey, I. 2001. Analysis of hydrolysable tannins. *Animal Feed Science and Technology*. 91: 3–20.

Mwamba, C. K. 1988. Studies in *Uapaca kirkiana Muell. Arg. (*Euphorbiaceae*). TIRC/NCRSR Tech Report*. Kitwe Zambia.

Ndabikunze, B. K., Masambu, B. N. and Tiisekwa, B.M. 2010. Vitamin C and mineral contents, acceptability and shelf life of juice prepared from four indigenous fruits of the Miombo woodlands of Tanzania. Journal of Food, Agriculture and Environment. 8: 91–96.

Ngadze, R. T., Linnemann, A. R., Nyanga, L. K., Fogliano, V. and Verkerk, R. 2017. Local processing and nutritional composition of indigenous fruits: the case of monkey orange (Strychnos spp.) from Southern Africa. *Food Reviews International*, 33: 123–142.

Ngulube, M. R. 1996. Ecology and Management of *Uapaca kirkiana* in Southern Africa. PhD Thesis, University of Wales, Bangor.

Ngulube, M. R., Hall, J. B. and Maghembe, J. A. 1996. *Uapaca kirkiana* (Euphorbiaceae): a review of silviculture and resource potential. *Journal of Tropical Forest Science.* 8: 395 – 411.

© Central University of Technology, Free State

Ngulube, M. R., Hall, J. B. and Maghembe, J.A. 1995. Ecology of a miombo fruit tree: *Uapaca kirkiana* (Euphorbiaceae). *Forest Ecology and Management.* 77: 107–117.

Nhukarume, L., Chikwambi, Z., Muchuweti, M. and Chipurura, B. 2010. Phenolic content and antioxidant capacities of *Parinari curatelifolia, Strychnos spinosa* and *Adansonia. Journal of Food Biochemistry.* 34: 207–221.

Ntupanyama, Y. M., Mwase, F. W., Stedje, B., Kwapata, M. B., Bokosi, J. M. and Hvoslef-Eide, A. K. 2008. Indigenous knowledge of rural communities in Malawi on socio-economic use, propagation, biology, biodiversity and ecology of *Uapaca kirkiana* Muell. Arg. *African Journal of Biotechnology*. 7: 2386–2396.

O'Sullivan, M. G., Thornton, G., O'Sullivan, G. C. and Collins, J. K. 1992. Probiotic bacteria: myth or reality. *Trends in Food Science and Technology.* 3: 309–314.

Oberleas, D. and Chan. H. C. 1997. Cation complexation by phytate. *Trace Elements and Electrolytes.* 14: 173 – 176.

Osman, M. A. 2004. Chemical and nutrient analysis of baobab (*Adansonia digitata*) fruit and seed protein solubility. *Plant Foods for Human Nutrition.* 59: 29 – 33.

Padayatty, S. J. and Levine, M. 2001. New insights into the physiology and pharmacology of vitamin C. *Canadian Medical Association Journal.* 164: 353–355.

Pandey, K. B. and Rizvi, S. I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity.* 2: 270–278.

Perales, S., Barbera´, R. Lagarda, M. J. and Farre´, R. 2006. Bioavailability of zinc from infant foods by in vitro methods (solubility, dialyzability and uptake and transport by Caco-2 cells). *Journal of the Science of Food and Agriculture* 86: 971–978.

Perales, S., Barbera´, R. Lagarda, M. J. and Farre´, R. 2007. Availability of iron from milk-based formulas and fruit juices containing milk and cereals estimated by in vitro methods (solubility, dialysability) and uptake and transport by Caco-2 cells. *Food Chemistry* 102: 1296–1303

Pereira, R. 2010. Eficiência de uma fitase bacteriana na liberação de fósforo fítico em dietas de frangos de corte.Mestrado Dissertação, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba

© Central University of Technology, Free State

Perron, N. R. and Brumaghim, J. L. 2009. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics.* 53: 75 – 100.

Persson, H., Nair, B. M., Frolich, W., Nyman, M. and Asp. N. G. 1987. Binding of mineral elements by some dietary fibre components—*In vitro* (II). *Food Chemistry.* 26:139 –148.

Petrova, M., Macklaim, J., Wuyts, S., Verhoeven, T., Vanderleyden, J., Gloor, G., Lebeer, S. and Reid, G. 2018. Comparative genomic and phenotypic analysis of the vaginal probiotic *Lactobacillus rhamnosus* GR-1. *Frontiers in Microbiology.* 9: 1278.

Petry, N., Egli, I., Zeder, C., Walczyk, T. and Hurrell. R. 2010. Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. *The Journal of Nutrition.* 140: 1977–1982.

Pizarro, F., Olivares, M., Valenzuela, C., Brito, A., Weinborn, V., Flores, S. and Arredondo, M. 2016. The effect of proteins from animal source foods on heme iron bioavailability in humans. *Food Chemistry.* 196: 733–738.

Platel, K., Eipeson, S. W. and Srinivasan, K. 2010. Bioaccessible Mineral Content of Malted Finger Millet (*Eleusine coracana*), Wheat (*Triticum aestivum*), and Barley (*Hordeum vulgare*). *Journal of Agricultural Food Chemistry*. 58: 8100–8103.

Platt, S. R. and Clydesdale. F.M. 1987. Mineral binding characteristics of lignin, guar gum, cellulose, pectin, and neutral detergent fiber under simulated duodenal pH conditions. *Journal of Food Science.* 52: 1414 –1419.

Raes, K., Knockaert, D., Struijs, K. and Van Camp, J. 2014. Role of processing on bioaccessibility of minerals: influence of localization of minerals and antinutritional factors in the plant. *Food Science and Technology.* 37: 32–41.

Ramadhani, T. 2002. Marketing of indigenous fruits in Zimbabwe. PhD Thesis. University of Hannover, Germany.

Ramadhani, T. and Schmidt, E. 2002. "Marketing analysis of *Uapaca kirkiana* indigenous fruits in Zambia: which is the way forward". Paper presented at the *Regional Agroforestry Conference on Agroforestry*, May 20-24, Pretoria, South Africa.

Ramadhani, T. and Schmidt, E. 2008. Marketing of Indigenous Fruits in Southern Africa. In: Akinnifesi, F. K., Leaky, R. R. B., Ajayi, O. C., Silesh, G., Tchoundjeu, Z., Matakala, P.

© Central University of Technology, Free State

and Kwesiga, F. R. (eds.) *Indigenous Fruit Trees in the Tropics Domestication, Utilization and Commercialization*. Wallingford, UK: CAB International. pp. 224–236.

Rapizzi, E., Fossati, S., Moroni, F. and Chiarugi, A. 2004. Inhibition of poly (ADP-Ribose) glycohydrolase by gallotannin selectively up-regulates expression of proinflammatory genes. *Molecular Pharmacology.* 66: 890 – 898.

Reddy, M. B. and Love, M. 1999. Nutritional quality of vitamins and minerals. In *Impact of processing on food safety*. Jackson, L. S., Knize, M. G., and Morgan, J. N. (eds). New York: Kluwer Academic/Plenum Publishers. pp. 99–106.

Rehman, Z. and Shah, W. H 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry*. 91: 327 – 331.

Reid, G. 2017. The development of probiotics for women's health. *Canadian Journal of Microbiology*. 63: 1 – 9.

Reid, G., Beuerman, D., Heinemann, C. and Bruce, A. W. 2001. Probiotic Lactobacillus dose required to restore and maintain a normal vaginal flora. *FEMS Immunology and Medical Microbiology*. 32: 37 – 41.

Rinkinen, M., Jalava, K., Westermarck, E., Salminen, S. and Ouwehand. A. C. 2003. Interaction between probiotic lactic acid bacteria and canine pathogens: a risk factor for intestinal *Enterococcus faecium* colonization. *Veterinary Microbiology*. 92:111–119.

Ross, R. P., Morgan, S. and Hill. C. 2002. Preservation and fermentation: past, present and future. *International Journal of Food Microbiology*. 79:3–16.

Ruszczynski, M., Radzikowski, A. and Szajewska, H. 2008. Clinical trial: effectiveness of *Lactobacillus rhamnosus* (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Alimentary Pharmacology and Therapeutics*. 28: 154–161.

Ryan, P. and Hynes, M. J. 2007. The kinetics and mechanisms of the complex formation and antioxidant behaviour of the polyphenols EGCg and ECG with iron (III). *Journal of Inorganic Biochemistry*. 101:585–593.

Saka, J. D. K. and Msonthi, J. D. 1994. Nutritional value of edible fruits of indigenous wild trees in Malawi. *Forest Ecology and Management.* 64:245–248.

Saka, J. D. K., Isabel, R., Akinnifesi, F., Victoria, N. V. and Mhango, J. 2007. Physicochemical and organoleptic characteristics of *Uapaca kirkiana, Strychnos cocculoides, Adansonia*

© Central University of Technology, Free State

digitata and *Mangiferia indica* fruit products. *International Journal of Food Science and Technology.* 42: 836–841.

Saka, J. D. K., Kadzere, I., Ndabikunze, B. K., Akinnifesi, F. K. and Tiisekwa, B. P. M. 2008. Product development, nutritional value, processing and utilization of indigenous fruits from Miombo Ecosystem. In Akinnifesi, F. K., Leakey, R. R. B., Ajayi, O. C., Sileshi, G., Tchoundjeu, Z., Matakala, P. and Kwesiga, F. R. (eds) *Indigenous Fruit Trees in Southern Africa: Domestication, Use, and Commercialisation*, Wallingford, UK: CAB International, pp. 288–309.

Saka, J. D. K., Msonthi, J. D. and Sambo, E. Y. 1992. Dry matter, acidity and ascorbic acid contents of edible wild fruits growing in Malawi. *Tropical Science,* 32:217–221.

Saka, J. D. K., Swai, R., Mkonda, A., Schomburg A., Kwesiga F. and Akinnifesi, F. K. 2004. "Processing and utilisation of indigenous fruits of the miombo in southern Arica". In *Proceedings of the Regional Agroforesty Conference on Agroforestry Impacts on Livelihoods in Southern Africa: Putting Research into Practise.* South Africa, World Agroforesty Centre.

Sandberg, A. 2002. Bioavailability of minerals in legumes. *British Journal of Nutrition.* 88: 281–285.

Sandholm-Mattila, T., Blum, S., Collins, J. K., Crittenden, R., de Wos, W., Dunne, C., Fonden, R., Grenov, G., Isolauri, E., Kiely, B., Marteau, P., Morelli, L., Ouwehand, A., Reniero, R., Saarela, M., Salminen, S., Saxelin, M., Schiffrin, E., Shanahan, F., Vaughan, E. and von Wright, A. (1999). Probiotics: towards demonstrating efficacy. *Trends in Food Science and Technology.* 10: 393–399.

Shah, A. 2015. Probiotic bacteria: functional properties and awareness towards Indian customers in consuming probiotic food supplements. *Asian Academic Research Journal of Multidisciplinary.* 2:392–403.

Shah, N. P. 2000. Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science.* 83: 894–907.

Shava, S. 2005. Research on indigenous knowledge and its application: a case of wild plants in Zimbabwe. *Southern African Journal of Environmental Education.* 22: 73–86.

Shofian, N. M., Hamid, A. A., Osman, A., Saari, N., Anwar, F., Pak Dek, M. S. and Hairuddin, M. R. 2011. Effect of freeze-drying on the antioxidant compounds and antioxidant

© Central University of Technology, Free State

activity of selected tropical fruits. *International Journal of Molecular Sciences.* 12: 4678–4692.

Sila, D. N., Van Buggenhout, S., Duvetter, T., Fraeye, I., De Roeck, A., Van Loey, A. and Hendrickx. M. 2009. Pectins in processed fruits and vegetables: part II-structure-function relationships. *Comprehensive Reviews in Food Science and Food Safety.* 8: 86–104.

Sitrin, M.D. (2014). Absorption of water-soluble vitamins and minerals. In *The gastrointestinal system*. Leung, P.S. (ed). Netherlands: Springer. pp. 211–234.

Sokrab, A. M., Ahmed, I. A. and Babiker, E. E. 2014. Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. *Journal of Food Science and Technology.* 51: 2608–2615.

Soloviev, P., DaoudaNiang, T., Gaye, A. and Totte, A. 2004. Variabilit ´e des caract`eres physico chimiques des fruits de trois esp`eces ligneuses de cueillette r ´ecolt´es au S´en´ egal: Adansonia digitata, Balanites aegyptiaca et Tamarindus indica. *Fruits*. 59: 109–119.

Sriamornsak, P. 2003. Chemistry of pectin and its pharmaceutical uses: a review. *Silpakorn University International Journal*, 3: 206–228.

Srivastava, P. and Malviya, R. 2011. Sources of pectin, extraction and its applications in pharmaceutical industry− an overview. *Indian Journal of Natural Products and Resources*. 2: 10–18.

Stadlmayr, B., Charrondiere, U. R., Eisenwagen, S., Jamnadass, R., and Kehlenbeck, K. 2013. Nutrient composition of selected indigenous fruits from sub-Saharan Africa. *Journal of the Science of Food and Agriculture*. 93: 2627–2636.

Steffen, L. M., Jacobs, D. R., Stevens, J., Shahar, E., Carithers, T. and Folsom, A. R. 2003. Associations of whole-grain, refined grain, and fruit and vegetable consumption with risks of all cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *American Journal of Clinical Nutrition.* 78: 383–390.

Sufi, N. A. and Kaputo, M. T. 1977. Identification and determination of free sugars in *Uapaca kirkiana* fruits. *Zambia Journal of Science and Technology*. 2: 23–25.

Sulibur ska, J. and Krejpcio. Z. 2014. Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts. *Journal of Food Science and Technology.* 51: 589–594.

© Central University of Technology, Free State

Sundar Raj, A., Rubila, S., Jayabalan, R. and Ranganathan, T. 2012. A review on pectin: chemistry due to general properties of pectin and its pharmaceutical uses. *Scientific Reports*, 1, 550. doi:10.4172/scientificreports.550.

Sun-Waterhouse, D. 2011. The development of fruit-based functional foods targeting the health and wellness market: a review. *International Journal of Food Science and Technology*. 46: 899–920.

Svarc-Gajic, J. 2013. Antinutrients in food. In *Nutritional insights and food safety*, Mandic, A., Sakac, M. and Misan, A. (eds). Hauppauge, NY: Nova Science Publishers. pp. 1–37.

TBS 1985. *Tanzania Standard*. Orange Juice Specification, Tanzania Bureau of Standards, Dar-es-Salaam, Tanzania.

Thakur, B. R., Singh, R. K., Handa, A. K. and Rao, M. A. 1997. Chemistry and uses of pectin: a review. *Critical Reviews in Food Science and Nutrition.* 37: 47–73.

Thibault, J. F. and Ralet, M. C. 2003. Physico-chemical properties of pectins in the cell walls and after extraction. In *Advances in pectin and pectinase research*. Voragen, F., Schols, H., and Visser, R. (eds) Dordrecht: Springer.

Thiong'o, M., Kingori, S. and Jaenicke, H. 2002. The taste of the wild: variation in the nutritional quality of marula fruits and opportunities for domestication. *Acta Horticulturae.* 575: 237–244.

Thomas, S., Durand, D., Chassenieux, C. and Jyotishkumar, P. 2013. *Handbook of biopolymer-based materials: from blends and composites to gels and complex networks*. Thomas, S., Durand, D., Chasssenieux, C. and Jyotishkumar, P. (eds) Weinheim, Germany: John Wiley & Sons.

Tiisekwa, B. P. M., Ndabikunze, B. K., Samson, G. and Juma, M. 2004. "Suitability of some indigenous tree fruits for manufacturing juices and jams in Tanzania". *Agroforestry impacts on livelihoods in southern Africa: Putting research into practice. Proceedings of the Regional Agroforestry Conference* held in Warmbaths, South Africa 20−24 May, 2002. World Agroforestry Centre (ICRAF), Nairobi, Kenya, pp. 331−335.

Tontisirin, K., Nantel, G. and Bhattacharjee. L. 2002. Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proceedings of the Nutrition Society.* 61: 243–250.

© Central University of Technology, Free State

Towo, E., Matuschek, E., and Svanberg. U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and *in vitro* accessible iron. *Food Chemistry.* 94: 369–376.

Tuomola, E. M., Ouwehand, A. C. and Salminen, S. J. 2000. Chemical, physical and enzymatic pre-treatments of probiotic lactobacilli alter their adhesion to human intestinal mucus glycoproteins. *International Journal of Food Microbiology.* 60: 75–81.

USDA, 2011. Avocado, almond, pistachio and walnut composition. Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 24. U.S. Department of Agriculture. Washington, DC.

Valík, L., Medved'ová, A. and Liptáková, D. 2008. Characterization of the growth of *Lactobacillus rhamnosus* GG in milk at suboptimal temperature. *Journal of Food Nutrition Research.* 47: 60–67.

Vardakou, M., Mercuri, A., Naylor, T. A., Rizzo, D., Butler, J. M., Connolly, P. C., Wickham, M. S. J. and Faulks, R. M. 2011. Predicting the human *in vivo* performance of different oral capsule shell types using a novel *in vitro* dynamic gastric model. *International Journal of Pharmaceutics.* 419: 192–199.

Vinceti, B., Ickowitz, A., Powell, B., Kehlenbeck, K., Termote, C., Cogill, B. and Hunter, D., 2013. The contributions of forest foods to sustainable diets. *Unasylva*. 64: 54–64.

Voragen, A. G. J., Coenen, G. J., Verhoef, R. P. and Schols. H. A. 2009. Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry.* 20: 263–275.

Wang, L. and Sun, D. W. 2016. Heat and mass transfer in thermal food processing. In *Thermal food processing: New technologies and quality issues*, ed. Sun, D. W. 2nd ed. Boca Raton, FL: CRC Press.

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R. and Gawalko. E. J. 2010. Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Research International.* 43: 589–594.

Watzl, B., Girrbach, S. and Roller, M. 2005. Inulin, oligofructose and immunomodulation. *British Journal of Nutrition.* 93: 49 – 55.

Welch, R. M. and Graham, R. D. 1999. A new paradigm for world agriculture: human needs productive, sustainable, nutritious, *Field Crops Research.* 60: 1–10.

© Central University of Technology, Free State

Whelton, S. P., Hyre, A. D., Pedersen, B., Yi, Y., Whelton, P. K. and He, J. 2005. Effect of dietary fiber intake on blood pressure: a metal analysis of randomized, controlled clinical trials. *Journal of Hypertension.* 23: 475–481.

WHO 1999. Management of Severe Malnutrition. A Manual for Physicians and Other Senior Health Workers. World Health Organization, Geneva.

Wicker, L., Kim, Y., Kim, M. J., Thirkield, B., Lin, Z. and Jung, J. 2014. Pectin as a bioactive polysaccharide–extracting tailored function from less. *Food Hydrocolloids.* 42: 251–259.

Wills, R. and Golding, J. 2016. *Postharvest: an introduction to the physiology and handling of fruit and vegetables*. 6th ed. Sydney, NSW: UNSW Press / Oxfordshire, UK CABI Wallingford [ISBN: 9781742247854].

Wood, B. J. B. and Holzapfel, W. H. 1995. *Genera of lactic acid bacteria*. London, UK: Blackie Academic and Professional [ISBN 075140215X].

Wood, R. J. and Ronnenberg, A. G. 2005. Iron. In: *Modern nutrition in health and disease*. 10th ed. Shils, M. E., Shike, M., Ross, A. C., Caballero, B. and Cousins, R. J., (eds). Philadelphia: Lippincott Williams and Wilkins. pp. 248–270.

Xia, Q., Wang, L., Xu, C., Mei, J. and Li, Y. 2017b. Effects of germination and high hydrostatic pressure processing on mineral elements, amino acids and antioxidants *in vitro* bioaccessibility, as well as starch digestibility in brown rice (*Oryza sativa* L.). *Food Chemistry*. 214: 533–542.

Xu, W., Han, E. and Wang. Z. 2019. Effect of tannic acid on corrosion behavior of carbon steel in NaCl solution. *Journal of Materials Science and Technology.* 35: 64–75.

Yao, L. H., Jiang, Y. M., Shi, J., Barberan, T., Thomas-Barberan, F. A., Datta, N., Singanusong, R. and Chen, S. S. 2004. Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition.* 59: 113–122.

Yoshida, T. and Hatano, T. 2000. What is "Polyphenol"? *Food Style.* 4: 35–38.

Yoshida, T., Amakura, Y., Yokura, N., Ito, H., Hipo, J., Isaza J.H., Ramirez, S., Pelaez, D. P. and Renner, S. S. 1999. Oligomeric hydrolysable tannins from *Tibouchina multifora*. *Phytochemistry*, 52: 1661–1666.

© Central University of Technology, Free State

Zimbabwe Demographic and Health Survey (ZDHS), 2016. *Key Indicators*. Rockville, Maryland, USA: Zimbabwe National Statistics Agency (ZIMSTAT) and ICF International.

© Central University of Technology, Free State

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.

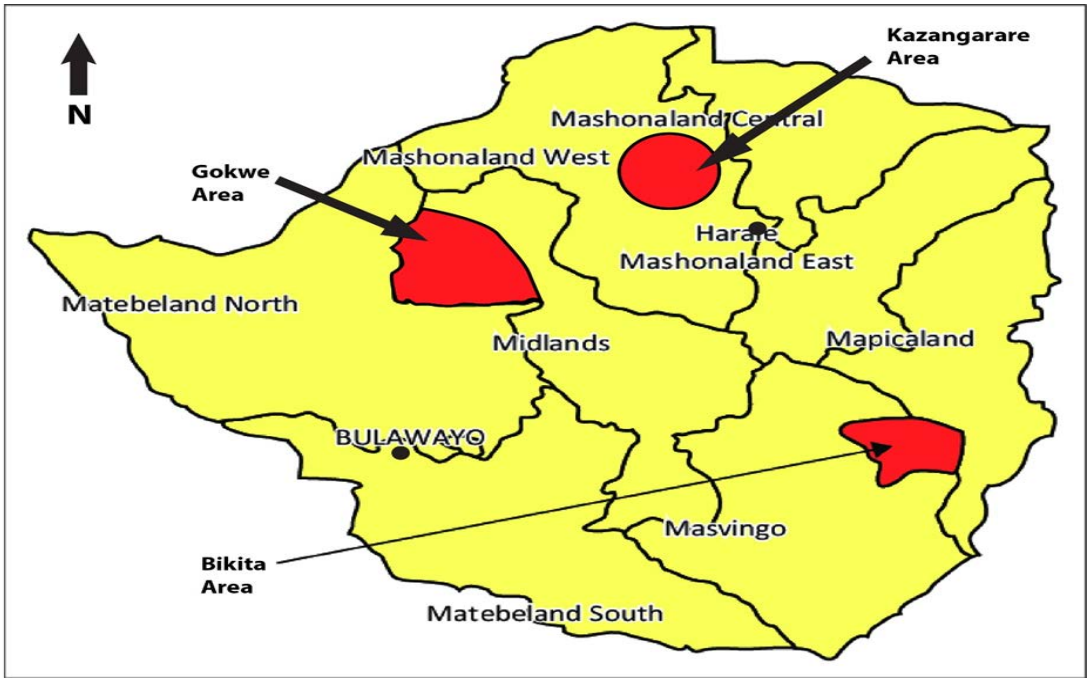


Figure 3.1: Map showing sampling areas (Bikita, Gokwe, and Kazangarare) of *U. kirkiana* fruits in Zimbabwe.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

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)

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

3.9 REFERENCES

Altundag, H and Tuzen, M. 2011. Comparison of dry wet and microwave digestion methods for the multi element determination in some dried fruit samples by ICP-OES. *Food and Chemical Toxicology Food and Chemical Toxicology* 49: 2800-2807

Association of Official Analytical Chemistry. 2000. Official method of analysis (AOAC) International. 17th edition. AOAC International. Maryland. USA.

Katsvanga, C. A. T., Jim, L., Gwenzi, D. Muhoni, L., Masuka, P., & Moy, M. (2007). Characterisation of community identified *Uapaka kirkiana* phenotypes for domestication. *Journal of Sustainable Development in Africa*, 9:356-366.

Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y. (2005). Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *Journal of Food Composition and Analysis.* 18: 625–633.

Magaia, T., Uamusse, A., Sjöholm, I. and Skog, K. 2013. Proximate Analysis of Five Wild Fruits of Mozambique. *The Scientific World Journal*. 2013:1-6.

Makkar, H. P. S. (1999). Quantification of tannins in tree foliage. A laboratory manual for the FAO/IAEA coordinated research project on 'Use of Nuclear and Related Techniques to Develop Simple Tannin Assay for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on the Tanniniferous Tree Foliage'. Vienna: Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. pp. 1–29.

Makkar, H. P. S. and Goodchild, A. V. (1996). Quantification of tannins: a laboratory manual. International center for agricultural research in dry areas, Aleppo, Syria. 25 p.

Megazyme, 2014. *Sucrose, D-Fructose and D-Glucose assay procedure (K-SUFRG 06/14),* Megazyme International Ireland.

Metrological Services Department of Zimbabwe, 2018. www.msd.org.zw. [Accessed 20 April 2018].

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W. and Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food - an international consensus. *Food and Function.* 5:

© Central University of Technology, Free State

1113–1124.

Nyanga, L.K., Gadaga, T.H., Nout, M.J.R., Smid, E.J., Boekhout, T. and Zwietering, M. H. 2013. Nutritive value of masau (Ziziphus mauritiana) fruits from Zambezi Valley in Zimbabwe. *Food Chemistry* 138: 168-172

Porter, L. J., Hrstich, L. N. and Chan, B. G. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 25: 223–230

Tang, P. Y., Wong, C. J., & Woo, K. K. (2011). Optimization of pectin extraction from peel of dragon fruit (Hylocereus polyrhizus). *Asian Journal of Biological Sciences*, 4: 189-195.

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

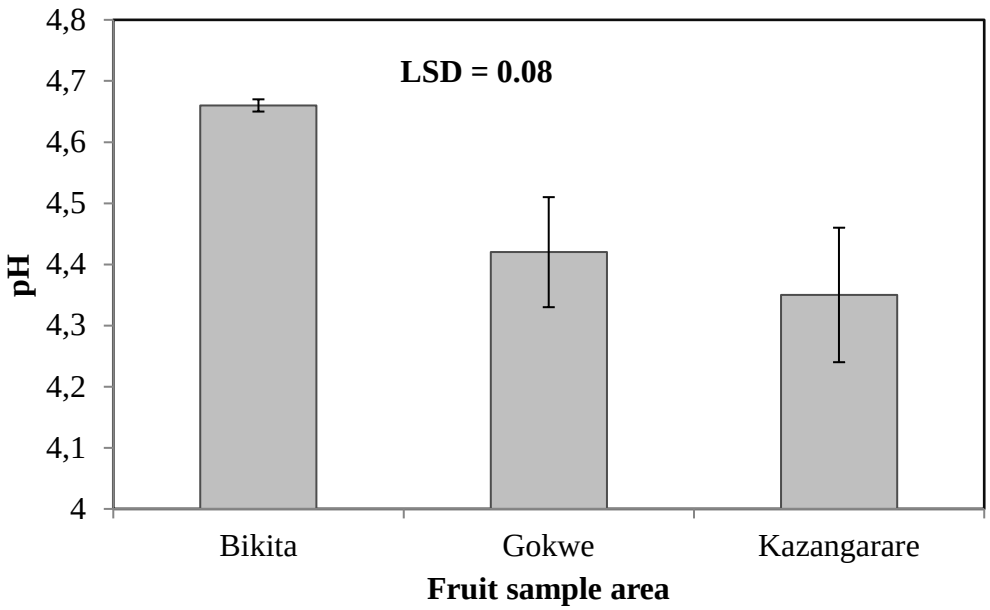


Figure 4.1: pH content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

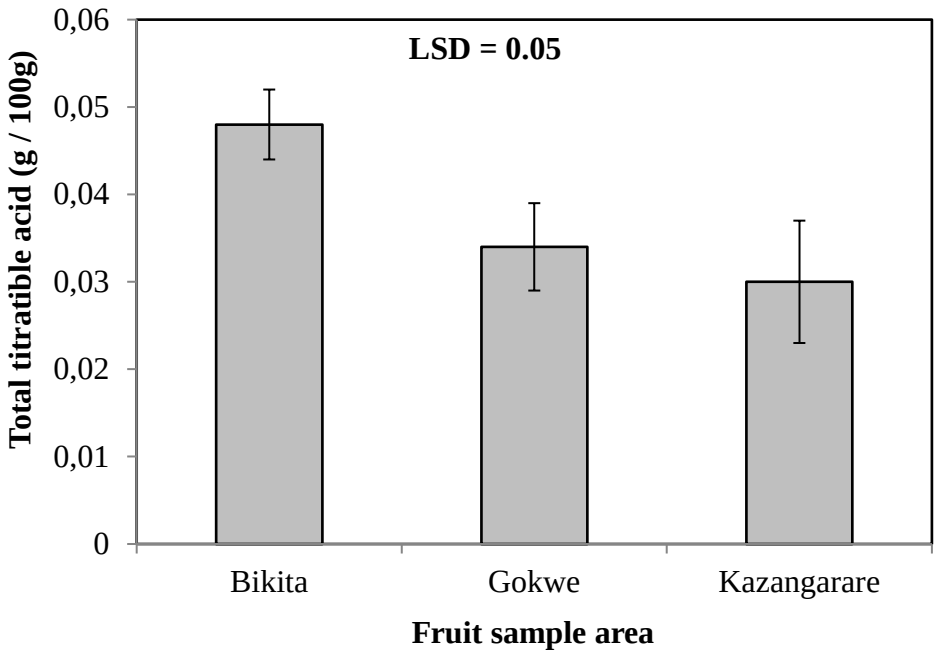


Figure 4.2: Total titratable acid content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

© Central University of Technology, Free State

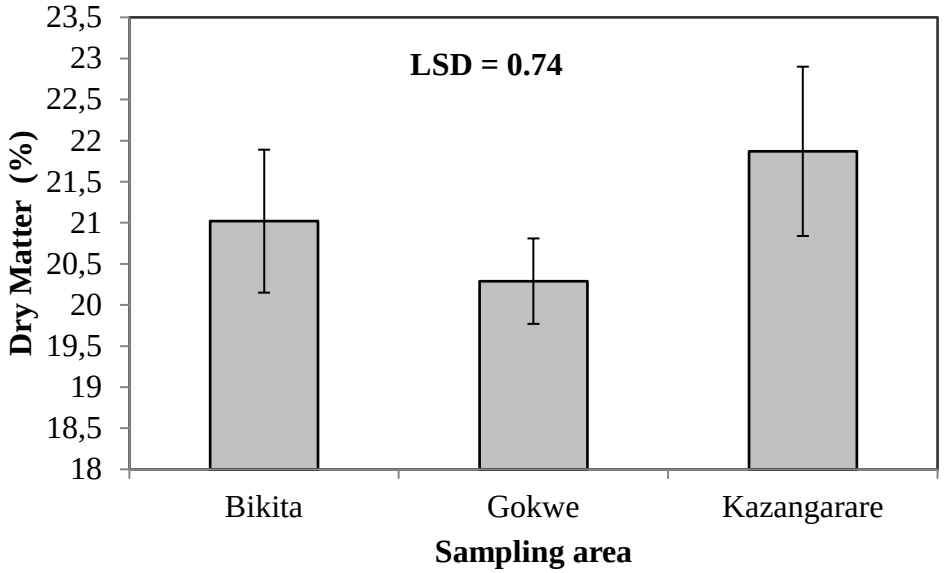


Figure 4.3: Dry Matter content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

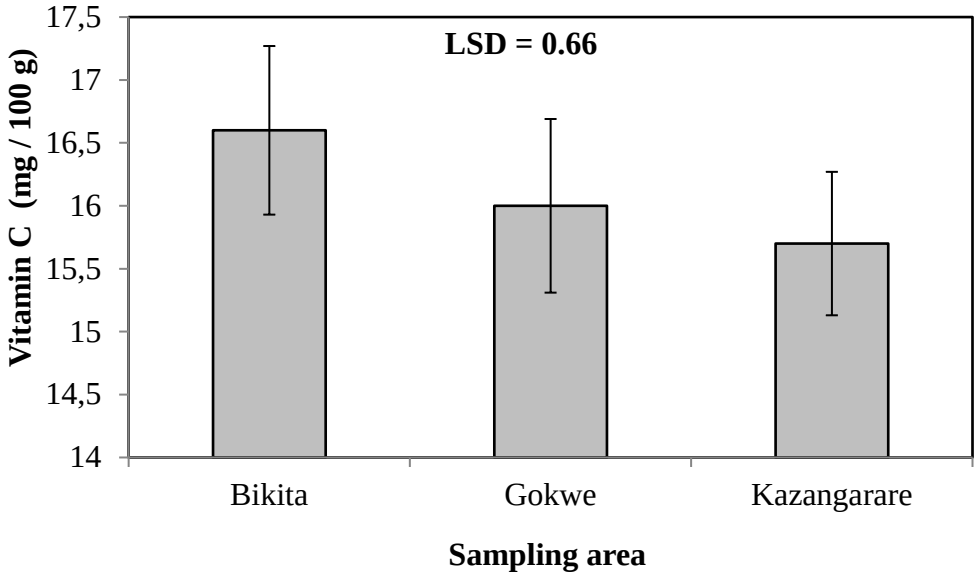


Figure 4.4: Vitamin C content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

© Central University of Technology, Free State

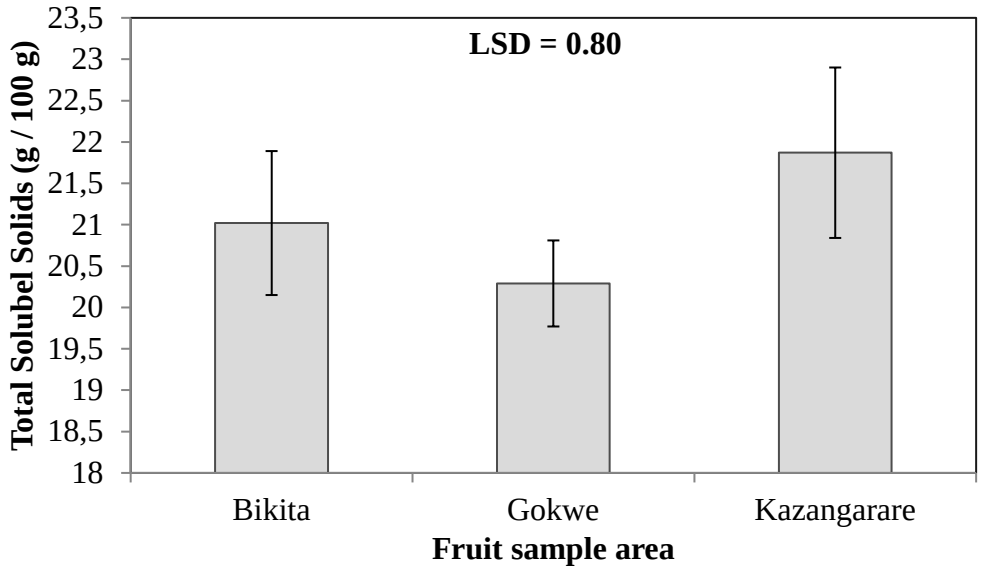


Figure 4.5: Total Soluble Solids content in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

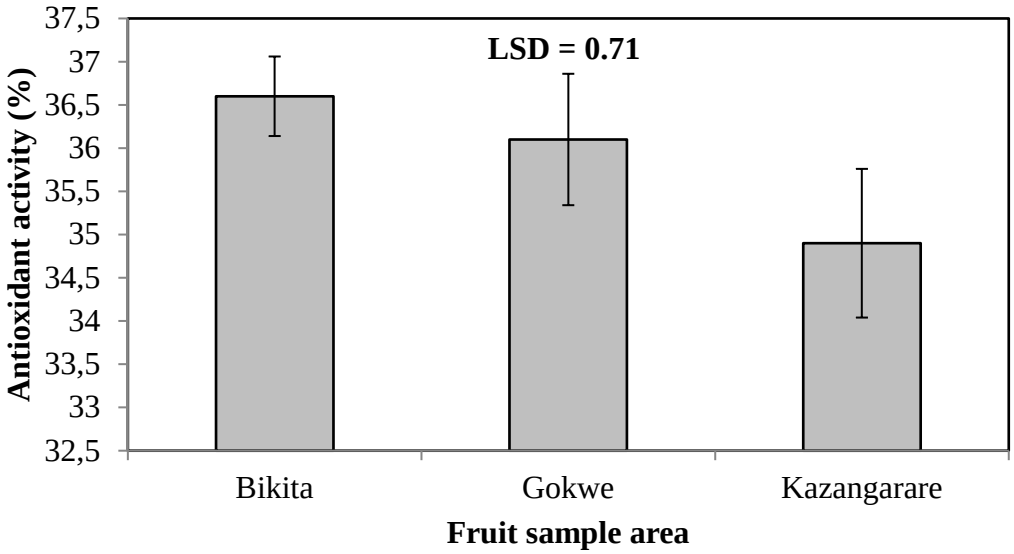


Figure 4.6: Antioxidant Activity (AOA) in *U. kirkiana* fruit pulp samples from Bikita, Gokwe, and Kazangarare.

© Central University of Technology, Free State

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.

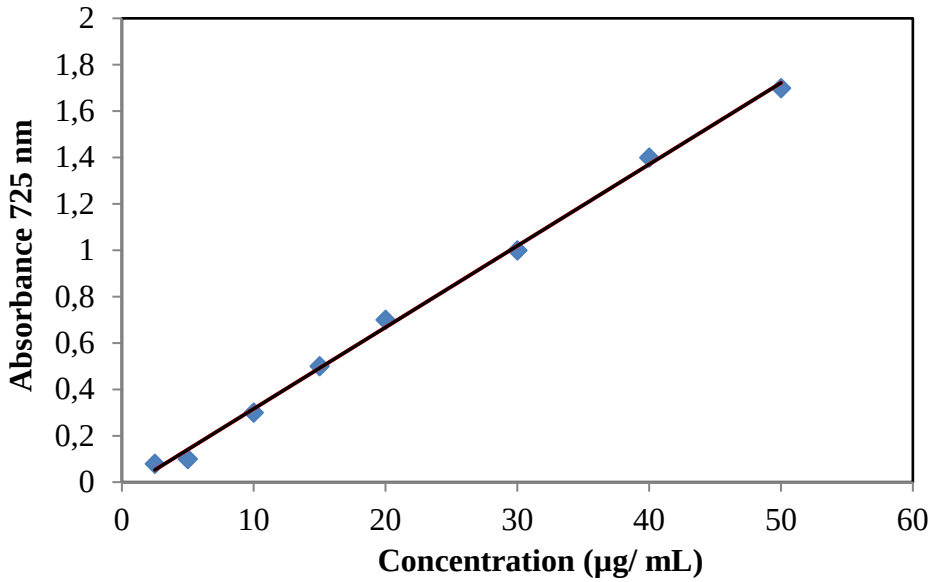


Figure 4.7: Gallic acid standard curve for total phenolic content assay.

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

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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

© Central University of Technology, Free State

(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

© Central University of Technology, Free State

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.*

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

4.6 REFERENCES

Akinnifesi, F. K., Kwesiga, F., Mhango, J., Mkonda, A., Chilanga, T, and Swai, R. 2004. Domesticating priority miombo indigenous fruit trees as a promising livelihood option for smallholder farmers in southern Africa. *Acta Horticulturae*. 632: 15–30.

Akinnifesi, F. K., R. R. B. Leakey, O. Ajayi, G, Sileshi, Z. Tchoundjeu, P. Matakala and F.R. Kwesiga. 2008. In: Indigenous fruit trees in the tropics: Domestication, utilization and commercialization. CABI, Wallingford: UK. http://doi: 10.1079/9781845931100.0000.

Alasalvar, C. and F. Shahidi. 2012. Composition, phytochemicals, and beneficial health effects of dried fruits: an overview. Dried Fruits: Phytochem. Health. Eff., 1-19.

Alston, F. H. 1992. Flavour improvement in apples and pears through plant breeding. Phytoparasitica 20: 33-41.

Amarteifio, J. O. and M. O. Mosase. 2006. The chemical composition of selected indigenous fruits of Botswana. *Journal of Applied Sciences and Environmental Management*. Mgt. 10: 43 –47.

Baba, S. A. and Malik, S.A 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*. 9: 449–454.

Bahramian, S., Azin, M., Chamani, M and Gerami, A. 2011. Optimization of enzymatic extraction of sugars from Kabkab Date fruit. *Middle East Journal of Scientific Research*. 7: 211-216.

Belitz, D. and Grosch. W. 1999. Food Chemistry. 2nd ed. Springer-Verag, Berlin: Germany.

Bille, P, M. Shikongo-Nambab and A. Cheikhyoussef. 2013. Value addition and processed products of three indigenous fruits in Namibia. *African Journal of Food, Agriculture, Nutrition and Development*. 13: 7192-7212.

Bugaud, C., Deverge, E., Daribo, M.O., Ribeyre, F., Fils-lycaon, B., Mbeguine, A. and Mbeguine, D. 2011. Sensory characterisation enabled the first classification of dessert bananas. J. Sc. Food Agric. 91: 992–1000.

Campbell, B., Luckert, M. and Scoones. I.1997. Local level valuation of savannah resources: a case study from Zimbabwe. *Economic Botany*. 51: 57–77.

© Central University of Technology, Free State

Chen, F. X., Liu X.H, and Chen L.S. 2009. Developmental changes in pulp organic acid concentration and activities of acid-metabolising enzymes during the fruit development of two loquat (*Eriobotrya japonica Lindl.*) cultivars differing in fruit acidity. Food Chem. 114, 657–664.

Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.P., Bordenave,L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen, C., Vivin, P. and S. Delrot. 2011. Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: A review. *American Journal of Enology and Viticulture*. 62: 413-425.

De La Hera-Orts, M., Martinez-Cutillas, A., Lopez-Roca, L. and Gomez-Plaza. E. 2005. Effect of moderate irrigation on grape composition during ripening. Spanish. *Journal of Agricultural Research*. 3: 352–361.

Des Gachons, C. P., Leeuwen, C.V., Tominaga, T., Soyer, J. P.Gaudillere, J.P. and Dubourdieu. D.2004. Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis viniferaL cv Sauvignon blanc* in field conditions. J. Sci. Food Agric. 85: 73–85. Doi.org/10.1002/jsfa.1919.

Fernandes, F. A., Rodrigues, S., Law, C.L. and Mujumdar.A.S. 2011. Drying of exotic tropical fruits: A comprehensive review. *Food Biotechnology*. 4: 163-185.

Franzel, S., Akinnifesi, F. K. and Ham. C.2008. Setting priorities among indigenous fruit species: Examples from three regions in Africa. In: Indigenous fruit trees in the tropics: Domestication, utilization and commercialization. Edited by: Akinnifesi, F. K., Leakey, R. R. B., Ajayi, O. C., Sileshi, G., Tchoundjeu, Z., Matakala, P. and Kwesiga, F. R. World Agroforestry Centre: Nairobi. CAB International Publishing. Wallingford: UK. 1–27.

Gadaga, T. H., Madzima, R. and Nembaware. N. 2009. Status of micronutrient nutrition in Zimbabwe: A review. Afr. J. Food Agric. Nutr. Dev. 9: 502-522.

Gautier, H., V. Diakou-Verdin, C. Benard, M. Reich, M. Buret, F. Bourgaud, J. L. Poessel, C. Caris-Veyrat and M. Genard. 2008. How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? J. Agric. Food Chem. 56: 1241–1250.

Harker, F., K. Marsh, H. Young, S. Murray, F. Gunson and S. Walker. 2002. Sensory interpretation of instrumental measurements 2: Sweet and acid taste of apple fruit. Postharvest Bio. Technol. 24: 241–250.

© Central University of Technology, Free State

Hummel, I., F. Pantin, R. Sulpice, M. Piques, G. Rolland, M. Dauzat, A.´l. Christophe, M. Pervent, M. Bouteille´, M. Stitt, Y. Gibon and B. Muller. 2010. Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: An integrated perspective using growth, metabolite, enzyme, and gene expression analysis. Plant Physiol. 154: 357–372.

Ikram, E. H. K., K. H. Eng, A. M. M. Jalil, A. Ismail, S. Idris, A. Azlan, H. S. M. Nazri, N. A. M. Diton and M. RAM. 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis*. 22: 388-393.

Kalaba, F. K., P. W. Chirwa and H. Prozesky. 2009. The contribution of indigenous fruit trees in sustaining rural livelihoods and conservation of natural resources. *Journal of Horticulture and Forestry*. 1: 1 - 6.

Katsvanga, C. A. T., Jim, L., Gwenzi, D. Muhoni, L., Masuka, P., and Moy, M. (2007). Characterisation of community identified *Uapaka kirkiana* phenotypes for domestication. *Journal of Sustainable Development in Africa*, 9:356 - 366.

Kelly, B. A. and O. Senou 2017. Variation of leaf and fruit characteristics of *Vitellaria paradoxa* (shea tree) according to agronomical performance along south-north climatic gradient in Mali. Afr. J. Plant Sci. 11: 142 - 150.

Kennedy, J. A., M. A. Matthews and A. L. Waterhouse. 2000. Changes in grape seed polyphenols during fruit ripening. Phytochemistry 55: 77–85.

Leakey, R. R. B. and A. C. Newton. 1994. Domestication of *Cinderella* Species as the start of a woody-plant revolution. In: Tropical Trees: The Potential for domestication and rebuilding of forest resources. Edited by Leakey, R. R. B and A. C. Newton. HMSO: London.

Lee, P. R., R. M. Tan, B. Yu, P. Curran and S. Q. Liu. 2013. Sugars, organic acids, and phenolic acids of exotic seasonable tropical fruits. J. Nutr. Food Sci., 43: 267-276.

Legwaila, G., W. Mojeremane, M. Madisa, R. Mmolotsi and M. Rampart. 2011. Potential of traditional food plants in rural household food security in Botswana. J. Hortic. For. 3: 171-177.

Makkar, H. P. S. 2000. Roles of tannins and saponins. In: Effects of antinutrients on the nutritional value of legume diets. Edited by Krogdahl, A., S. D. Mathiesen and I. F. Pryme. European Union. Brussels: Belgium. 8:103 –114.

© Central University of Technology, Free State

Maroyi, A. 2013. Traditional use of medicinal plants in south-central Zimbabwe: Review and perspectives. J. Ethnobiol. Ethnomed. 9-31: 1-18. Doi: 10.1186/1746-4269-9-31.

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W. and Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food - an international consensus. *Food and Function.* 5: 1113 – 1124.

Mithofer, D. and H. Waibel. 2003. Income and labour productivity of collection and use of indigenous fruit tree products in Zimbabwe. 295 - 305.

Moombe, K. B., H. Cori, J. S. Clarke, S. Franzel and P. Ackerman. 2014. Consumer preferences for *Uapaca kirkiana* fruits in Zambia. Forests, Trees and Livelihoods. 23: 248–260.

Mpofu, A., A. R. Linnemann, M. J. R. Nout, M. H. Zwietering and E. J. Smid. 2014. Mutandabota, a food product from Zimbabwe: Processing, composition, and socioeconomic aspects. Ecol. Food Nutr. 53: 24-41.

Muchuweti, M., A. R. Ndhlala and A. Kasiyamhuri. 2006. Analysis of phenolic acids including tannins, gallotanins and flavonols in *Uapaca kirkiana* fruits. Food Chem. 94: 415–419.

Ndabikunze, B. K., B. N. Masambu and B. M. Tiisekwa. 2010. Vitamin C and mineral contents, acceptability and shelf life of juice prepared from four indigenous fruits of the Miombo woodlands of Tanzania, J. Food Agric. Environ. 8: 91 – 96.

Ndlala, A. R., A. Kasiyamhuru, C. H. Mupure, K. Chitindingu, M. A. N. Benhura and M. Muchuweti. 2007. Phenolic composition of *Flacourtia indica*, Opuntiamegacantha and Sclerocarya birrea. Food Chem. 103: 82-87.

Ndlala, A. R., K. Chitindingu, C. H. Mupure, T. Murenje, F. Ndlala, M. A. N. Benhura and M. Muchuweti. 2008. Antioxidant properties of methanolic extracts from *Diospyros mespiliformis* (jackal berry), *Flacourtia indica* (Batoka plum), *Uapaca kirkiana* (wild loquat) and *Ziziphus mauritania* (yellow berry) fruits. Int. J. Food Sci. Technol. 43: 284-288.

© Central University of Technology, Free State

Nhukarume, L., Z. Chikwambi, M. Muchuweti and B. Chipurura. 2010. Phenolic content and antioxidant capacities of *Parinari curatelifolia, Strychnos spinose and Adansonia digitata*. J. Food Biochem. 34: 207-221.

Patel, S. S. and R. K. Goyal. 2011. Cardioprotective effects of gallic acid in diabetes-induced myocardial dysfunction in rats. Pharmacogn. Res. 4: 239-245.

Ramadhani, T. and E. Schmidt. 2008. Marketing of indigenous fruits in Southern Africa. In: Indigenous Fruit Trees in the Tropics Domestication, Utilization and Commercialization. Eds. Akinnifesi, F. K., Leaky R. R. B., Ajayi O. C., Silesh G., Tchoundjeu Z., Matakala P., Kwesiga F. R.. Columns Design Ltd. Reading: UK.

Saka, J. K., R. Swai, A. Mkonda, A. Schomburg, F. Kwesiga and F. K. Akinnifesi. 2004. Processing and utilisation of indigenous fruits of the miombo in Southern Africa. In proceedings of the Agroforestry impacts on Livelihoods in Southern Africa: Putting Research into Practice conference. Aventura Resorts Warmbaths, South Africa: World Agroforestry Centre publication.

Saka, J., I. Rapp, F. Akinnifesi, V. Ndolo and J. Mhang. 2007. Physicochemical and organoleptic characteristics of *Uapaca kirkiana, Strychnos cocculoides, Adansonia digitate* and *Mangifera indica* fruit products. Int. J. Food Sci. Technol. 42: 836–841.

Seymour, G. B., J. Taylor and G. A. Tucker. 1993. Biochemistry of fruit ripening. London: Chapman & Hall.

Shava, S. 2005. Research on indigenous knowledge and its application: A case of wild plants in Zimbabwe. Southern Afr. J. Environ. Educ. 22: 73 – 86.

Shofian, N. M., A. A. Hamid, A. Osman, N. Saari, F. Anwar, M. S. Par Dek and M. R. Hairuddin. 2011. Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. Int. J. Mol. Sci. 12: 4678-4692.

Stadlmayr, B., U. R. Charrondiere, S. Eisenwagen, R. Jamnadassand and K. Kehlenbeck. 2013. Nutrient composition of selected indigenous fruits from sub-Saharan Africa, J. Sci. Food Agric. 93: 2627 – 2636. Doi: 10.1002/jsfa.6196.

Sweetman, C., L. G., Deluc, G. R., Cramer, C. M., Ford and K. L. Soole. 2009. Regulation of malate metabolism in grape berry and other developing fruits. Phytochemistry 70: 1329–1344.

© Central University of Technology, Free State

Thakur, A. and Z. Singh. 2012. Responses of ‘Spring Bright’ and ‘Summer Bright’ nectarines to deficit irrigation: Fruit growth and concentration of sugars and organic acids. Sci. Hortic. 135: 112–119

Vaishnav, P. and A. L. Demain. 2010. Unexpected applications of secondary metabolites. Biotechnol. Adv. 29: 223-229.

Vinceti, B., A. Ickowitz, B. Powell, K. Kehlenbeck, C. Termote, B. Cogill and D. Hunter. 2013. The contributions of forest foods to sustainable diets. *Unasylvia*. 64: 54 – 64.

ZIMSTAT. 2016. Zimbabwe national statistics agency and ICF International. Zimbabwe Demographic and Health Survey 2015: Key Indicators. Rockville, Maryland: USA.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

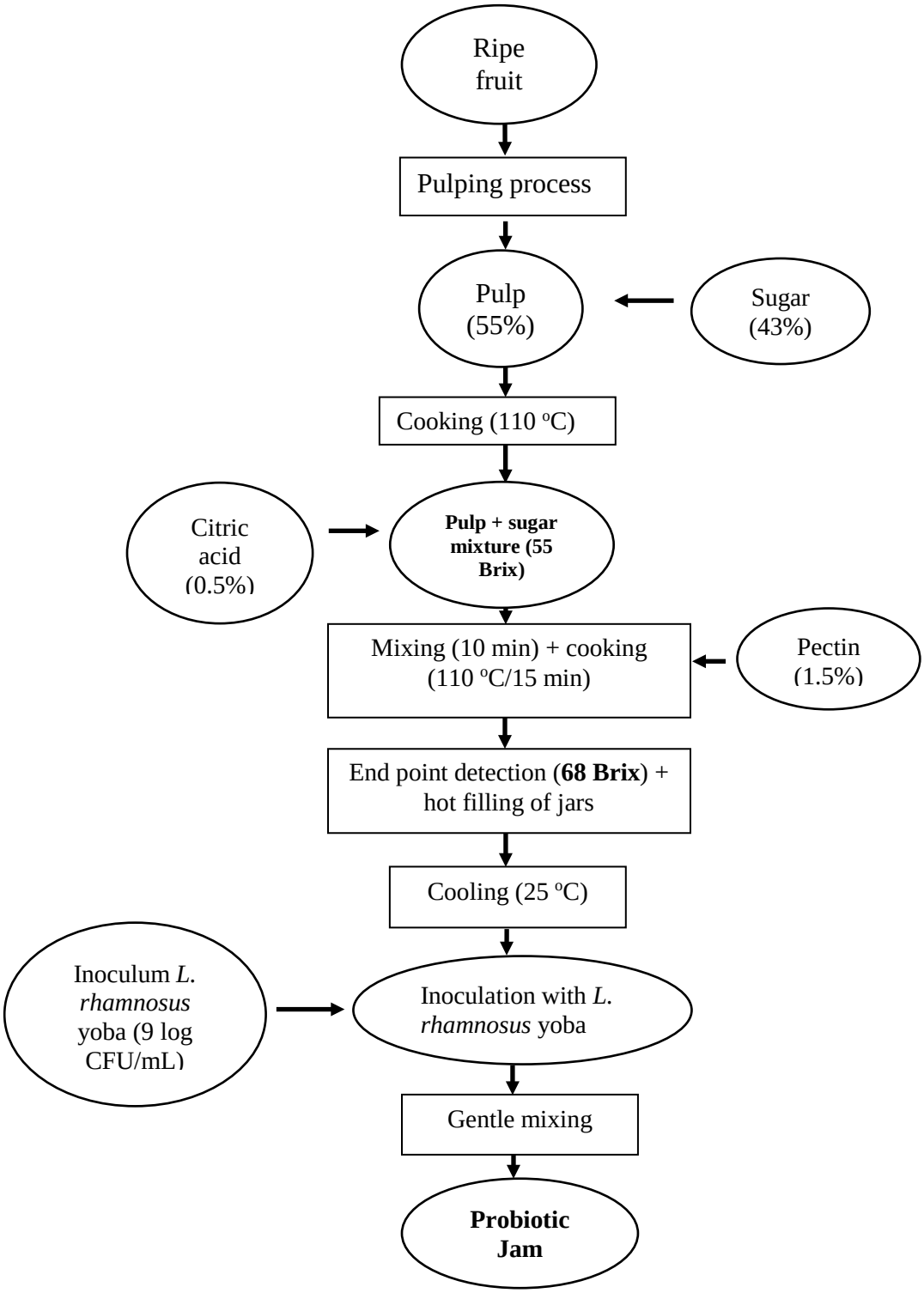


Figure 5.1: Flow chart depicting the production of probiotic jam (functional food).

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

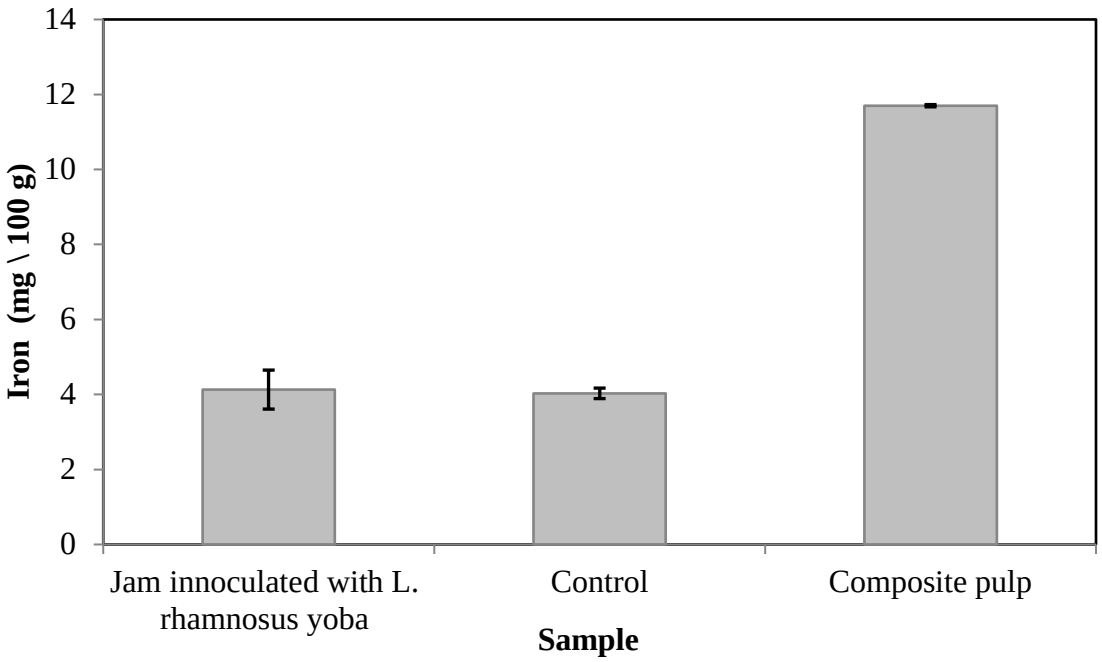


Figure 5.2: Iron content in the *L. rhamnosus* yoba jam.

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.

© Central University of Technology, Free State

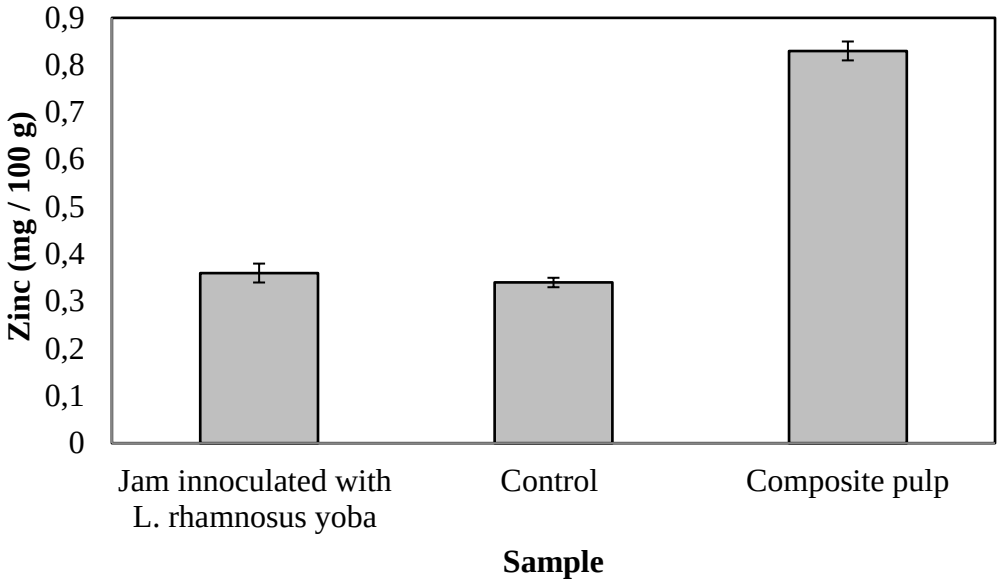


Figure 5.3: Zinc content in the jam inoculated with *L. rhamnosus* yoba.

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.

© Central University of Technology, Free State

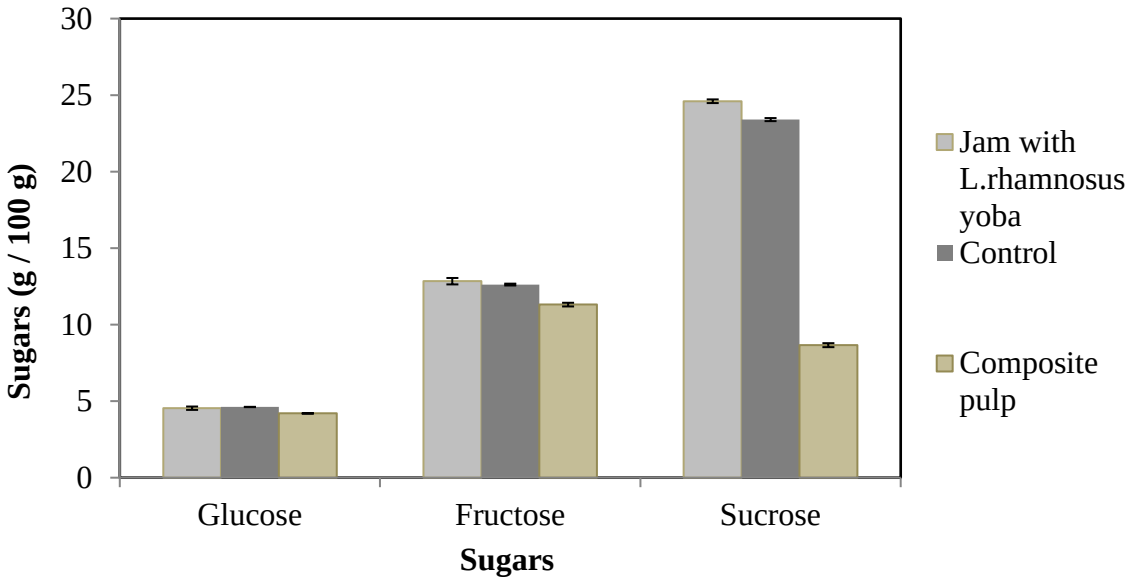


Figure 5.4: Sugar contents in the jam inoculated with *L. rhamnosus* yoba.

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 ($p < 0.05$).

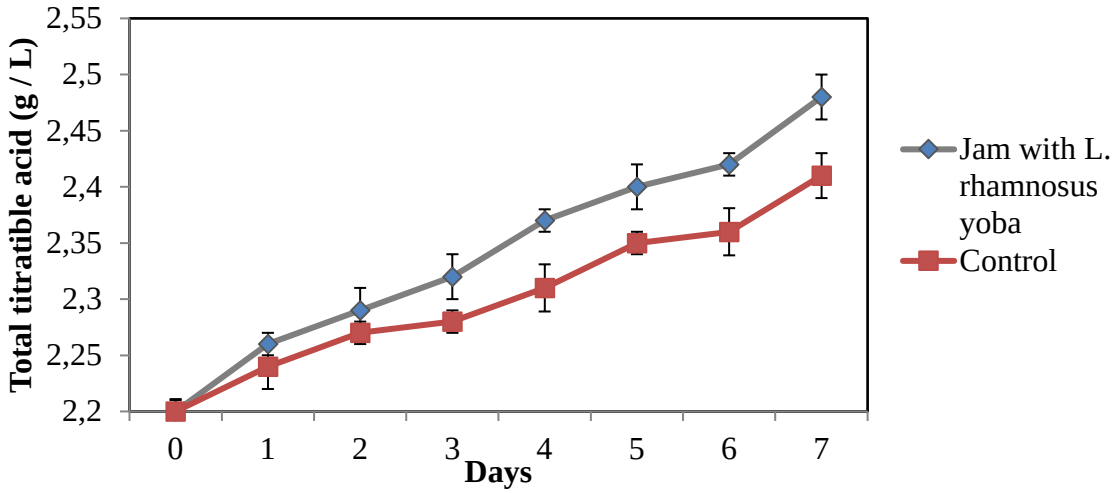


Figure 5.5: Total Titatable Acids content in the jam inoculated with *L. rhamnosus* yoba over 7 d in storage (25 °C).

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

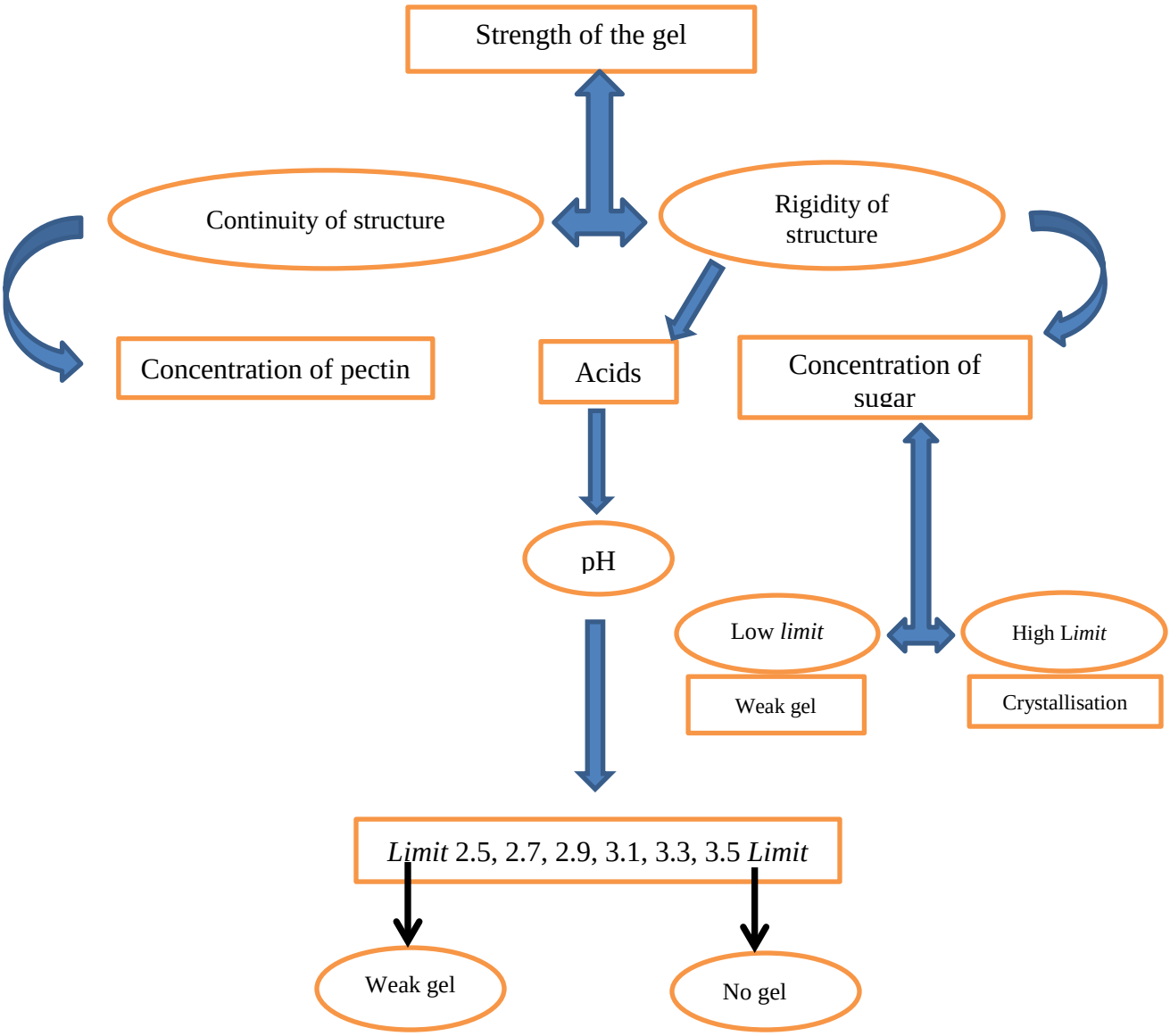


Figure 5.6: Effect of pH on gel formation.

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

5.7 REFERENCES

Allen, S. J., Martinez, E.G., Gregorio, G.V., Dans, L. F. 2010. Probiotics for treating acute infectious diarrhoea. *The Cochrane Database of Systematic Reviews* 11:1 – 98

Allen, S.J., Okoko, B., Martinez, E. G., Gregorio, G.V. Dans, L.F. 2004. Probiotics for treating infectious diarrhoea (Cochrane Review). *The Cochrane Database of Systematic Reviews* 3:1 – 38

Ania A.O., Mustapha M., Barau O.A., Mamman, Amina Z., Hauwa H., Hauwa M.S.U and Yagana, B.A. 2012. Extraction and characterization of pectin from peels of lemon (citrus lemon), grape fruit (citrus paradise) and sweet orange (Citrus sinensis). *British Journal of Pharmacology and Toxicology* 3: 259-262.

Ashaye, O. A. and Adeleke, T. O. 2009 Quality attributes of stored Roselle jam. *International Food Research Journal* 16: 363-371.

Bahramian, S., Azin, M., Chamani, M. Gerami, A. 2011. Optimization of Enzymatic Extraction of Sugars from Kabkab Date Fruit. *Middle East Journal of Scientific Research* 7: 211-216.

Bhadoria, P. B. S. and Mahapatra, S. C. 2011. Prospects, technological aspects and limitations of probiotics — a worldwide review. *European Journal of Food Research and Review*. 1:23 – 42

Burns, A. J. Rowland, I. R. 2000. Anti-carcinogenicity of probiotics and prebiotics. *Current Issues in Intestinal Microbiology.* 1:13 – 24

de Roos, N.M. Katan, M.B. 2000. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *American Journal of Clinical Nutrition* 71: 405 – 411

Drusch, S. 2007. Sugar beet pectin: A novel emulsifying wall component for microencapsulation of lipophilic food ingredients by spray-drying. *Food Hydrocolloids* 21: 1223-1228.

FAO/WHO, 2002. Guidelines for the evaluation of probiotics in food. Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report. (ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf).

© Central University of Technology, Free State

Featherstone, S. 2016. A Complete Course in Canning and Related Processes*: Processing Procedures for Canned Food Products.* 14th Edition. Woodhead Publishing. Series in Food Science, Technology and Nutrition.

Figueroa-González, I., Quijano, G., Ramírez, G. Cruz-Guerrero, A. 2011. Probiotics and prebiotics — perspectives and challenges. *Journal of the Science of Food and Agriculture* 91:1341 – 1348

Franz, C.M., Huch, M., Mathara, J.M., Abriouel, H., Benomar, N., Reid, G., Galvez, A. Holzapfel, W.H. 2014. African fermented foods and probiotics. *International Journal of Food Microbiology* 190: 84 – 96

Franz, C.M.A.P., Huch, M., Abriouel, H., Holzapfel, W.H. Gálvez, A. 2011a. Enterococci as probiotics and their implications in food safety. *Int. J. Food Microbiol* 151:125 –140

Fweja, L. W. 2002. Composition, Sensory characteristics and aroma profile of off vine ripened mango (*Mangifera indica* L.). MSc Thesis, Sokoine Unversity of Agriculture, Morogoro, Tanzania

Gaudreau, H., Champagne, C. P. and Jelen, P. (2005). The use of crude cellular extracts of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 to stimulate growth of a probiotic *Lactobacillus rhamnosus* culture in milk. *Enzyme and Microbial Technology.* 36:83–90.

Guandalini, S. 2008. Probiotics for children with diarrhea: an update. *Journal of Clinical Gastroenterology* 42: 53 –57

Guandalini, S. 2011. Probiotics for prevention and treatment of diarrhea. *Journal of Clinical Gastroenterology* 45: 149–153.

Gupta, G. 2011. Probiotics and periodontal health. *Journal of medicine and life* 4:387 – 394

Helland, M. H., Wicklund, T. and Narvhus, J. A. (2004). Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. *International Dairy Journal.* 14:957– 965

Hemalatha, S., Platel, K. and Srinivasan, K. (2007). Zinc and iron content and their bioaccessibility in cereals and pulses consumed in India. *Food Chemistry*. 102: 1328–1336.

Holzapfel, W.H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology* 75: 197 – 212

© Central University of Technology, Free State

Holzapfel, W.H. & Schillinger, U. 2002. Introduction to pre- and probiotics. *Food Research International* 35: 109 –116

Hummelen, R., Changalucha, J., Butamanya, N. L., Cook, A., Habbema, J.D.F., Reid, G. 2010. Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 to prevent or cure bacterial vaginosis among women with HIV. *International Journal of Gynecology & Obstetrics* 111: 245 – 248

International Commission on Microbiological Specifications for Foods. 2002. Microbiological Testing in Food safety Management: Microorganisms in Food 7. New York, USA, Springer Science.

Isolauri, E. 2004. Probiotics - Immunomodulatory potential against allergic disease. *Journal of Food Science* 69: 135 – 143

Jyoti, B. D., Suresh, A. K. and Venkatesh, K. V. (2003). Diacetyl production and growth of *Lactobacillus rhamnosus* on multiple substrates. *World Journal of Microbiology and Biotechnology*. **19**, 509 –514

Kagawa, Y., Higasa, S., Tsujimura, M., Komatsu, F., Yanagisawa Y. & Iwamoto, S. 2009. Human Specific Vitamin C Metabolism and Xenobiotic Polymorphism: The Optimal Nutrition. In: *Handbook of Vitamin C Research*. Eds: Kucharski, H., Zajac, J. Nova Science Publishers, Inc.

Kajander, K., Myllyluoma, E., Rajilicstojanovic, M., Kyronpalo, S., Rasmussen, M., Jarvenpa, S., Zoetendal E.G., de Vos, W.M., Vapaatalo, H. Korpela, R. 2008. Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. *Alimentary Pharmacology & Therapeutics* 27: 48-57

Kalipeni, E. 2000. Health and disease in southern Africa: a comparative and vulnerability perspective. *Social Science & Medicine* 50: 965 – 983

Kansci, G., Koubala, B.B., Israel Mbome Lape, I.M. 2003. Effect of ripening on the composition and the suitability for jam processing of different varieties of mango (*Mangifera indica)*. *African Journal of Biotechnology* 9: 301-306.

Kort, R. Sybesma, W. 2012. Probiotics for everybody. *Trends Biotechnol* 30: 613 – 615

© Central University of Technology, Free State

Kort, R. Sybesma, W. 2015. A novel consortium of *Lactobacillus rhamnosus* and *Streptococcus thermophilus* for increased access to functional fermented foods. *Microbial Cell Factories* 14:195

Kumar, S. 2015. Role of enzymes in fruit juice processing and its quality enhancement *Advances in Applied Science Research* 6: 114-124.

Kumari, A. Catanzaro, R. Marotta, F. 2011. Clinical importance of lactic acid bacteria: a short review. *Acta Biomedica* 82:177 – 180

Lee, P. R., Tan, R. M., Yu, B., Curran, P. Liu, S. Q. 2013. Sugars, organic acids, and phenolic acids of exotic seasonable tropical fruits. *Journal of Nutrition and Food Science*, 43: 267-276

Liew, S.L., Ariff, A.B., Raha, A.R., Ho, Y.W. 2005. Optimization of medium composition for the production of a probiotic microorganism, *Lactobacillus rhamnosus*, using response surface methodology. *International Journal of Food Microbiology*. 102:137-142

Mpofu, A., Linnemann, A.R., Nout, M.J., Zwietering, M.H., Smid, E.J., den Besten, H.M. 2015. Inactivation of bacterial pathogens in yoba mutandabota, a dairy product fermented with the probiotic Lactobacillus rhamnosus yoba. *International Journal of Food Microbiology* 217: 42 – 48

Mpofu. A., Linnemann, A.R., Sybesma, W., Kort, R., Nout, M.J., Smid, E. J. 2014. Development of a locally sustainable functional food based on mutandabota, a traditional food in southern Africa. *Journal of Dairy Science* 97:2591–2599.

Ndabikunze, B. K., Masambu B. N., Tiisekwa, B. P. M. and Issa-Zacharia, A. 2011. The production of jam from indigenous fruits using baobab (*Adansonia digitata* L.) powder as a substitute for commercial pectin. *African Journal of Food Science* 5: 168-175

Nwosu, J. N., Udeozor, L. O., Ogueke, C. C., Onuegbu, N., Omeire, G. C. and Egbueri, I. S. 2014. Extraction and Utilization of Pectin from Purple Star-Apple (Chrysophyllum cainito) and African Star-Apple (Chrysophyllum delevoyi) in Jam Production. *Austin Journal of Nutrition and Food Science* 1: 1003-1009.

OECD/FAO, 2011. OECD-FAO Agricultural outlook 2011– 2020. OECD Publishing and FAO, (http://dx.doi.org/10.1787/agr_outlook-2011-en).

© Central University of Technology, Free State

Randazzo, C. C., Pitino, I., Licciardello, F., Muratore, G. and Caggia, C. (2013). Survival of *Lactobacillus rhamnsus* probiotic strains in peach jam during storage at different temperatures. *Foodo Science and Technology* 33:652–659.

Reid, G. Bruce, A.W. Taylor, M. 1995. Instillation of Lactobacillus and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. *Microecology and therapy* 23: 32 – 45

Salari, P. Nikfar, S. Abdollahi, M. 2012. A meta-analysis and systematic review on the effect of probiotics in acute diarrhea. *Inflamm. Allergy Drug Targets* 11: 3 – 14

Szajewska, H. Skorka, A. Dylag, M. 2007a. Meta-analysis: Saccharomyces boulardii for treating acute diarrhoea in children. *Alimentary Pharmacology & Therapeutics* 25: 257 – 264

Szajewska, H., Skorka, A., Ruszczynski, M., Gieruszczak-Bialek, D. 2007b. Meta-analysis: lactobacillus GG for treating acute diarrhoea in children. *Alimentary Pharmacology & Therapeutics* 25: 871 – 881

Udonne, J. D., Ajani, O. O. and Akinyemi, O. P. 2016. A Comparative Study of Extraction of Pectin from Wet and Dried Peels Using Water Based and Microwave Method. *International Journal of Scientific and Engineering Research* 7: 416-432

UN Interagency Group for Child Mortality estimation, 2013. Levels & trends in child mortality Report http://www.childinfo.org/files/Child_Mortality_Report_2013.pdf.

Van Niel, C.W. Feudtner, C. Garrison, M.M. Christakis, D.A. 2002. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 109: 678 – 684

Yang, D., Zhang, J. Z., Fu, S., Xue, Y. and Hu, J. 2009. Evolution process of polymethacrylate hydrogels investigated by rheological and dynamic light scattering techniques. Colloids and Surfaces A: *Physicochemical and Engineering Aspects* 353:197-203

© Central University of Technology, Free State

Central University of Technology, Free State

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).

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

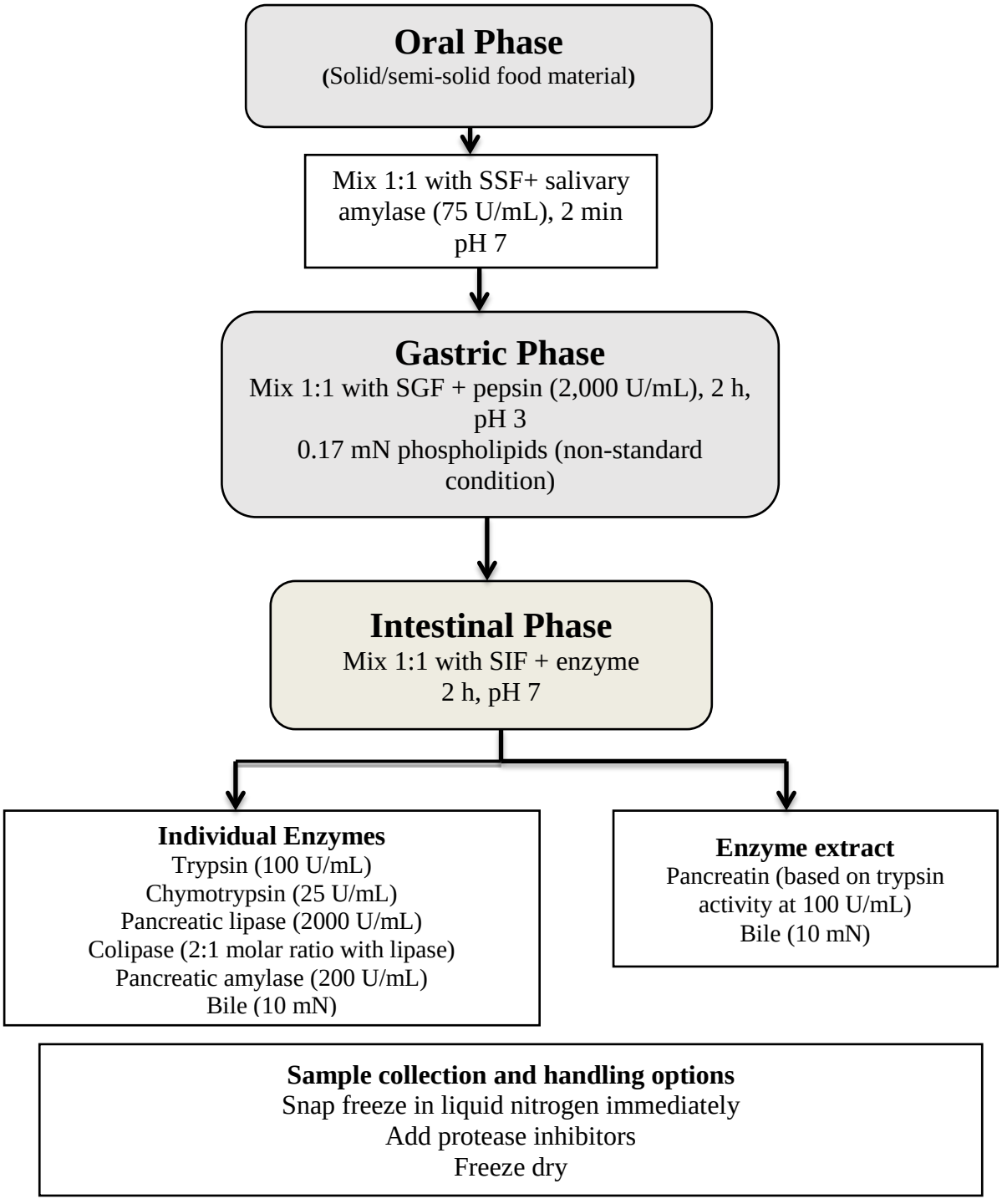


Figure 6.2: A summary flow diagram illustrating the Infogest in vitro digestion protocol (adapted from Minekus *et al.*, 2014).

© Central University of Technology, Free State

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)

© Central University of Technology, Free State

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

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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 $p < 0.05$.

© Central University of Technology, Free State

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.

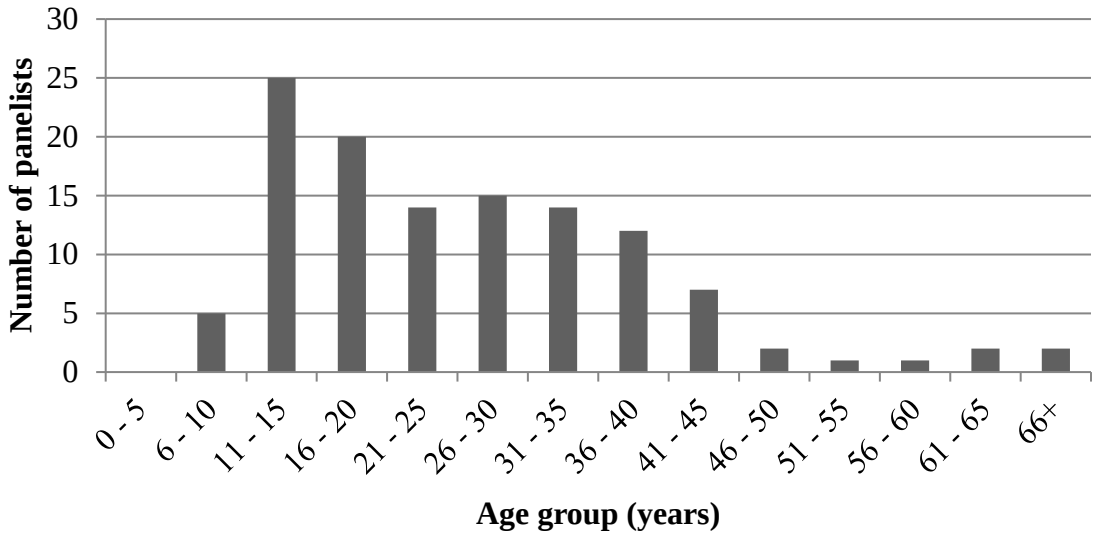


Figure 6.3: Age distribution of panelists

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.

© Central University of Technology, Free State

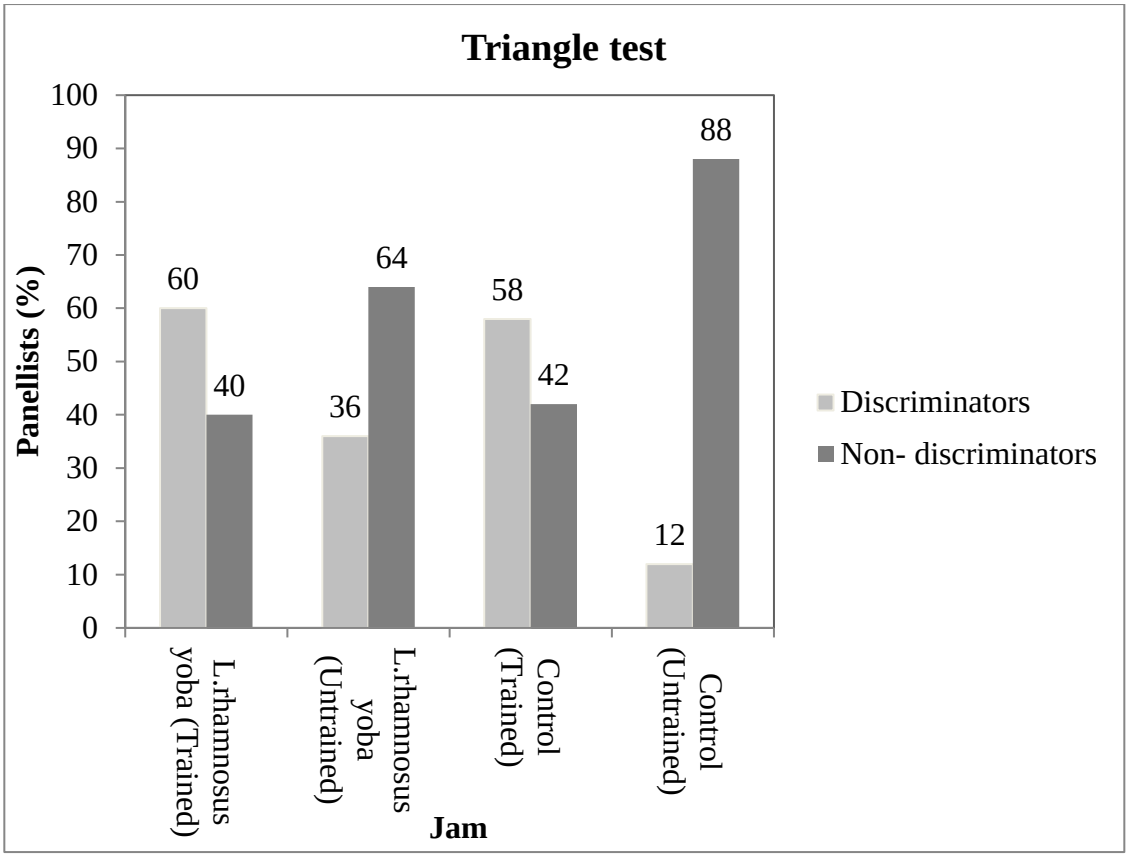


Figure 6.4: Triangle test (discrimination between *L. rhamnosus* yoba and control jam)

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.

© Central University of Technology, Free State

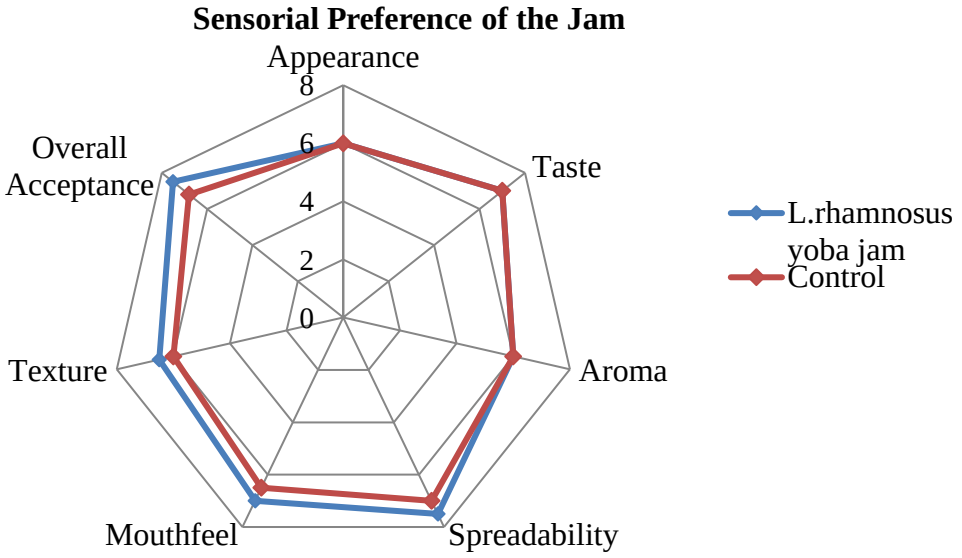


Figure 6.5: Preference test on inoculated jam with *L.rhamnosus* and control jam.

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

© Central University of Technology, Free State

(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).

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

6.6 REFERENCES

Akinnifesi, F. K., Leakey, R. R. B., Ajayi, O., Sileshi, G., Tchoundjeu, Z., Matakala, P. and Kwesiga, F.R. 2008. In: Indigenous fruit trees in the tropics: Domestication, utilization and commercialization. CABI, Wallingford: UK.

Barnett, J.B., Hamer, D.H. and Meydani, S.N. 2010. Low zinc status: a new risk factor for pneumonia in the elderly? *Nutrition Reviews.* 68: 30 – 37.

Cilla, A., Garcıa-Nebot, M.J., Perales, S., Lagarda, M. J., Barbera, R. and Farre, R. 2009. In vitro bioaccessibility of iron and zinc in fortified fruit beverages. *International Journal of Food Science and Technology* 44:1088 – 1092.

Deshpande, J. D., Joshi, M. M. and Giri, P. A. 2013. Zinc: the trace element of major importance in human nutrition and health. *International Journal of Medical Science and Public Health.* 2:1–6.

Dey, A.C., Shahidullah, M., Mannan, M. A., Noor, M. K., Saha, L. and Rahman, S. A. 2010. Maternal and neonatal serum zinc level and its relationship with neural tube defects. *Journal of Health, Population and Nutrition 28*: 343–350.

Fernandez-Garcıa, E., Carvajal-Lerida, I. and Perez-Galvez, A. 2009. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research* 29:751–760.

Gorbach, S.L. and Goldin, B.R. 1989. US Patent No. 4,839,281, Washington, DC. Patent and Trademark Office, USA patent application.

Grandy, G., Medina, M., Soria, R., Teran, C.G. and Araya. M. 2010. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. *BMC Infectious Diseases* 10: 253 – 266.

Guandalini, S. Pensabene, L. Zikri, M.A. Dias, J. Casali, L.G. Hoekstra, H. Kolacek, S. Massar, K. Micetic-Turk, D.. Papadopoulou, A. de Sousa, J.S. Sandhu, B. Szajewska, H. and Weizman. Z. 2000. *Lactobacillus* GG administered in oral rehydration solution to with acute diarrhea: A multicenter European trial. *Journal of Pediatric Gastroenterology and Nutrition* 30: 54 – 60.

© Central University of Technology, Free State

Hambidge, M. K., Miller, L. V., Westcott, J. E., Sheng, X. and Krebs, N. F. 2010. Zinc bioavailability and homeostasis. *American Journal of Clinical Nutrition.* 91:1478S–1483S.

Hemalatha, S., Platel, K. and Srinivasan, K. (2007a). Zinc and iron content and their bioaccessibility in cereals and pulses consumed in India. *Food Chem*istry. 102:1328–1336.

Hojsak, I., Snovak, N., Abdovic, S., Szajewska, H., Misak, Z. and Kolacek. S. 2010. *Lactobacillus rhamnosus* GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: A randomized, double-blind, placebo-controlled trial. *Clinical Nutrition* 29:312 – 316.

Khouzam, R.B., Pohl, P. Lobinski, R. 2011. Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables. *Talanta* 86: 425- 428

Kort, R. and Sybesma, W. 2012. Probiotics for everybody. *Trends Biotechnology* 30:613 – 615

Luabeya, K. K., Mpontshane, N., Mackay, M., Ward, H., Elson, I., Chhagan, M., Tomkins, A., Van den Broeck, J. and Bennish, M. L. 2007. Zinc or multiple micronutrient supplementation to reduce diarrhea and respiratory disease in South African children: a randomized controlled trial. *PLoSONE* 2:e541.

Mattila- Sandholm, T. *et al.*, 2002.Technological challenges for future probiotic foods. *International Dairy Journal* 12:173-182

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S. Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J. Ménard, O. Recio, I, Santos, C. N. Singh, R. P. Vegarud, G. E. Wickham, M. S. Weitschies, W. Brodkorb, A. 2014. A standardised static in vitro digestion method suitable for food - an international consensus. *Food and Function*, 5: 11, 13-24.

Ndabikunze, B. K., Masambu, B. N. and Tiisekwa. B. M. 2010. Vitamin C and mineral contents, acceptability and shelf life of juice prepared from four indigenous fruits of the Miombo woodlands of Tanzania, *Journal of Food, Agriculture and Environment* 8: 91 – 96.

© Central University of Technology, Free State

Platel, K. and Srinivasan, K. 2016. Bioavailability of micronutrients from plant foods: An update. *Critical Reviews in Food Science and Nutrition* 8398:1608–1619

Roohani, N., Hurrell, R., Kelishadi, R. and Schulin. R. 2013. Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences* 18:144 – 157.

Rousseau, S., Kyomugasho, C., Celus, M., Hendrickx, M.E.G. and Grauwet, T. 2019. Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing, *Critical Reviews in Food Science and Nutrition.*

Sitrin, M.D. 2014. Absorption of water-soluble vitamins and minerals. In The gastrointestinal system, ed. P.S. Leung, 211–234. Netherlands: Springer.

Stadlmayr, B., Charrondiere, U. R., Eisenwagen, S., Jamnadassand, R. and Kehlenbeck. K. 2013. Nutrient composition of selected indigenous fruits from sub-Saharan Africa. *Journal of the Science of Food and Agriculture* 93: 2627 – 2636.

Truong-Tran, A. Q., Ho, L. H., Chai, F., Zalewski, P. D. 2000. Cellular Zinc Fluxes and the Regulation of Apoptosis/Gene-Directed Cell Death, *The Journal of Nutrition*, 130:1459S–1466S.

Umeta, M., West, C.E., Verhoef, H., Haidar, J. and Hautvast, J. G. 2003. Factors associated with stunting in infants aged 5-11 months in the DodotaSire District, rural Ethiopia. *Journal of Nutrition* 133: 1064 –1069.

Vinceti, B., Ickowitz, A., Powell, B., Kehlenbeck, K., Termote, C., Cogill, B. and Hunter, D. 2013. The contributions of forest foods to sustainable diets. *Unasylvia* 64: 54 – 64

Zimbabwe Demographic and Health Survey (ZDHS) (2016). *Key Indicators*. Rockville, Maryland, USA: Zimbabwe National Statistics Agency (ZIMSTAT) and ICF International.

Olivieri, F., Semproli, S., Pettener, D. Toselli, S. 2008. Growth and malnutrition of rural Zimbabwean children (6-17 years of age). *American Journal of Physical Anthropology* 136:214-22.

WHO. 2013. World health statistics 2013- Indicator compendium. World Health Organization, Geneva, Switzerland.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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).

© Central University of Technology, Free State

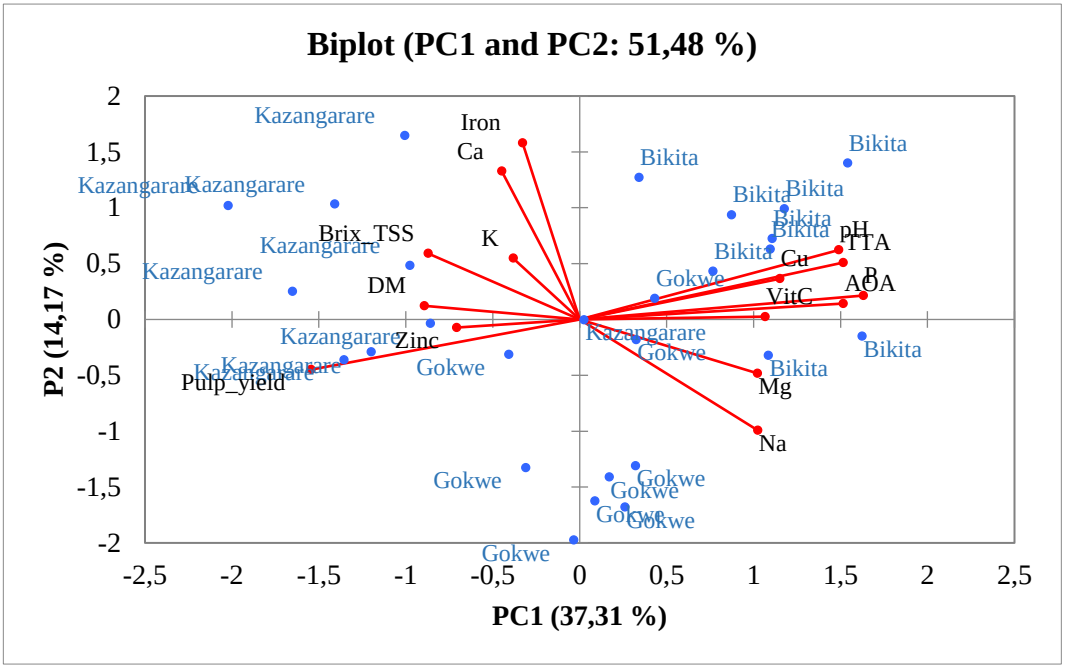


Figure 7.1: Principal component biplot showing variation of fruit pulp characteristics in the sampling areas.

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.

© Central University of Technology, Free State

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*

© Central University of Technology, Free State

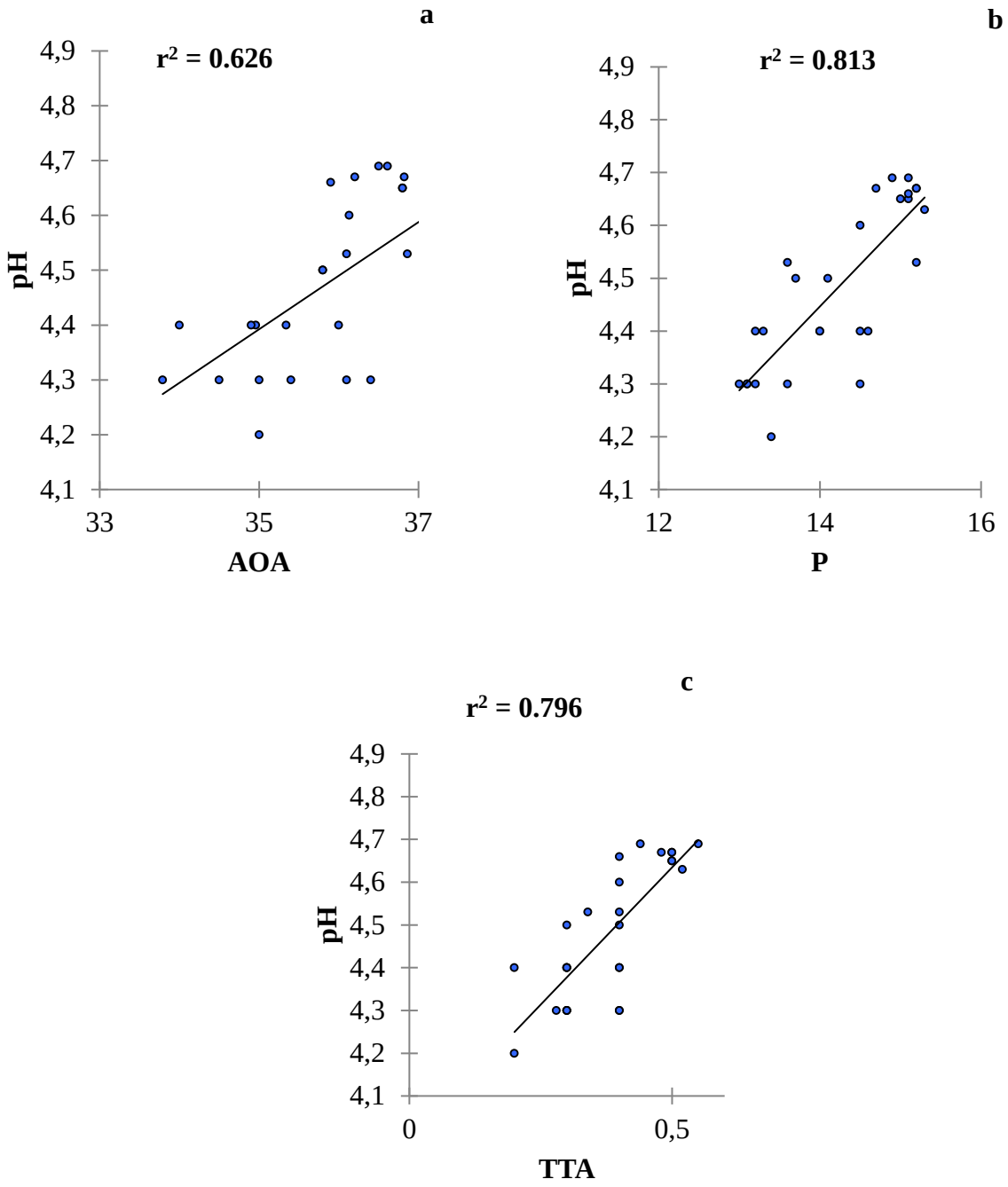


Figure 7.2: The correlation between pH and AOA (a), the correlation between pH and phosphorus (b), and the correlation between pH and TTA (c).

© Central University of Technology, Free State

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.

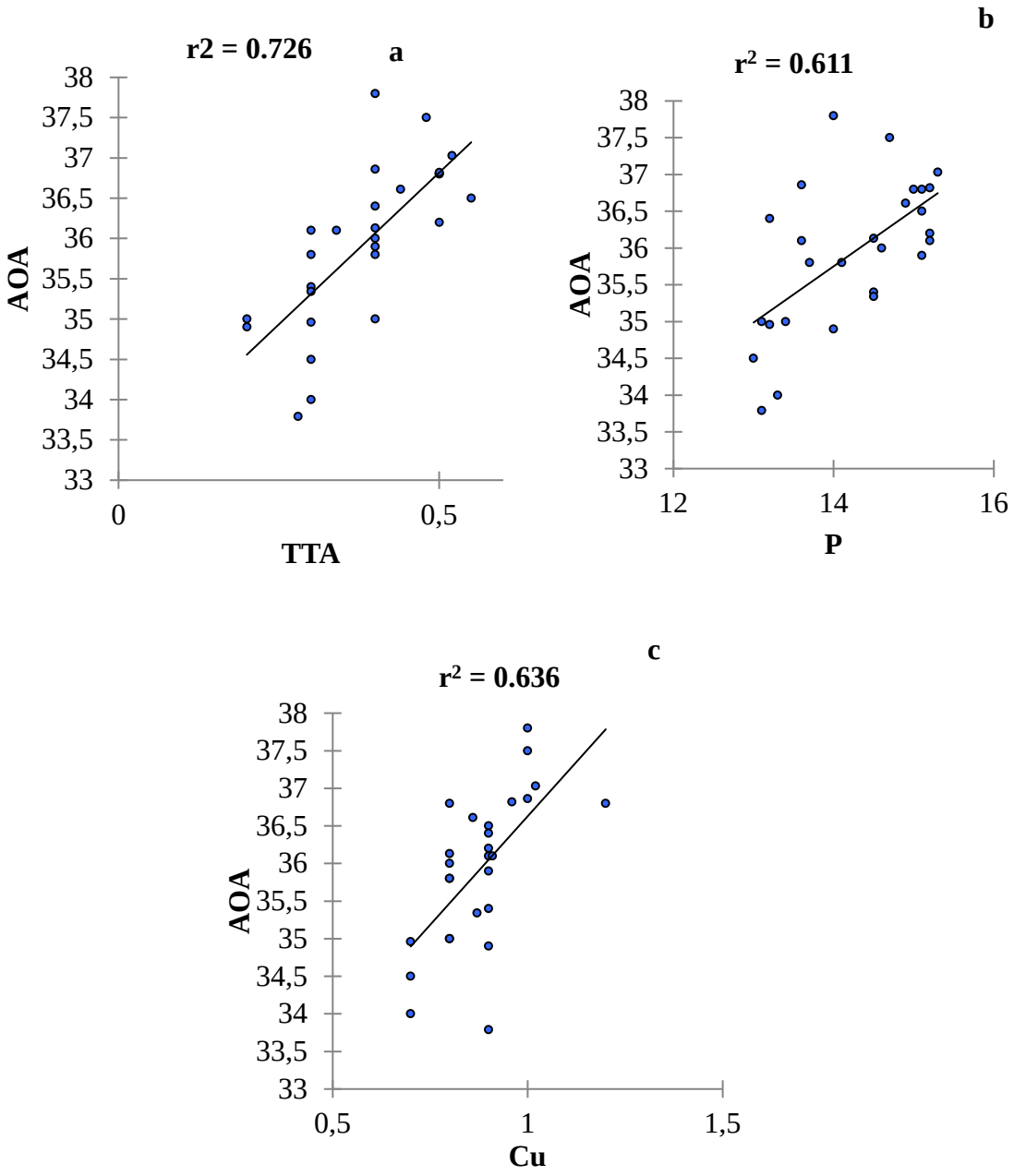


Figure 7.3: The correlation between AOA and TTA (a), the correlation between AOA and phosphorus (b), and the correlation between AOA and copper (c).

© Central University of Technology, Free State

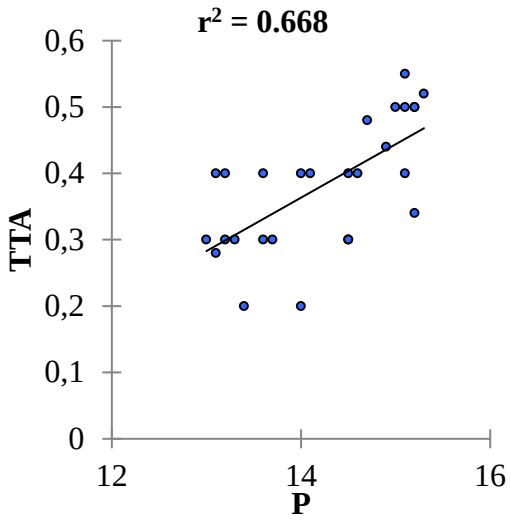
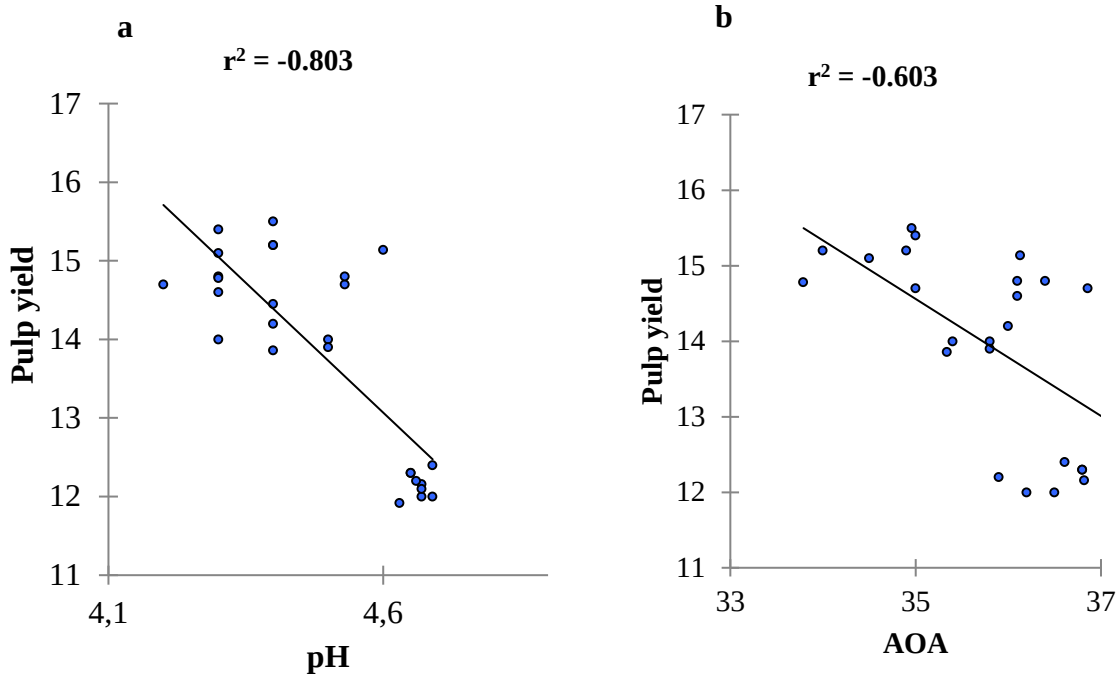


Figure 7.4: The correlation between TTA and phosphorus content in fruit pulps.

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).



© Central University of Technology, Free State

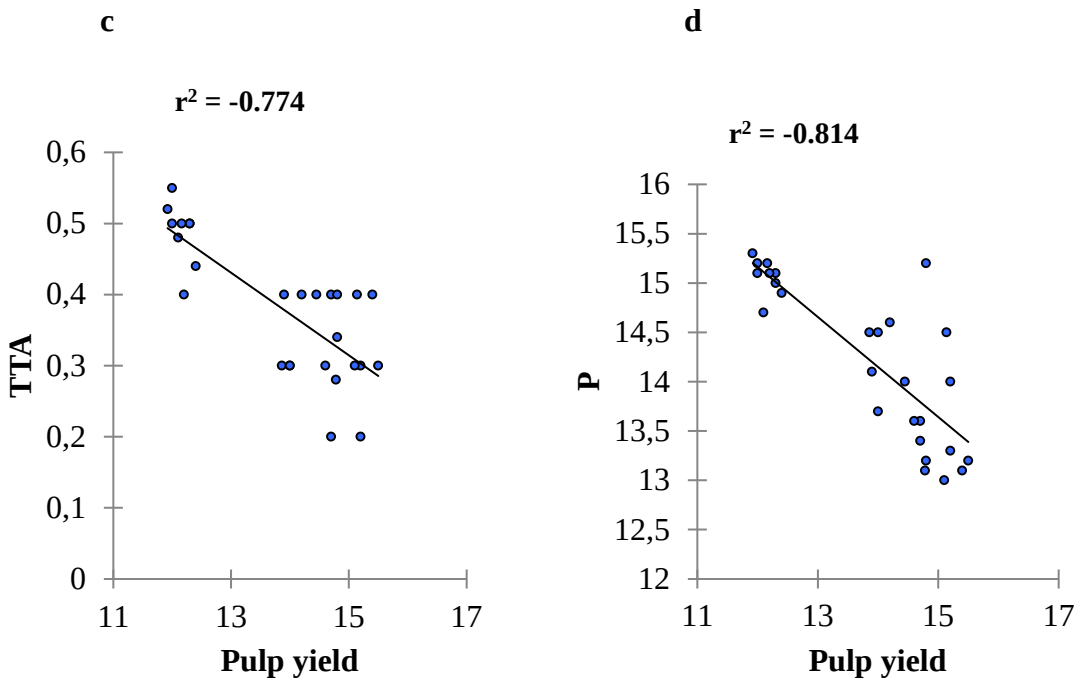
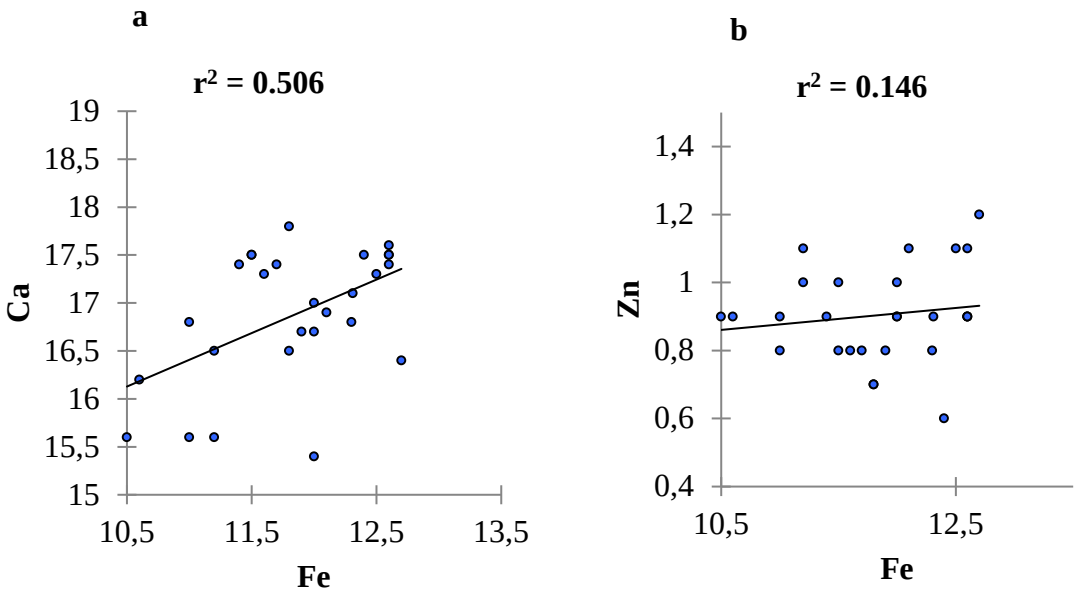


Figure 7.5: The correlation between pulp yield and pH (a), the correlation between pulp yield and AOA (b), the correlation between TTA and pulp yield (c), and the correlation between pulp yield and phosphorus (d).

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.



© Central University of Technology, Free State

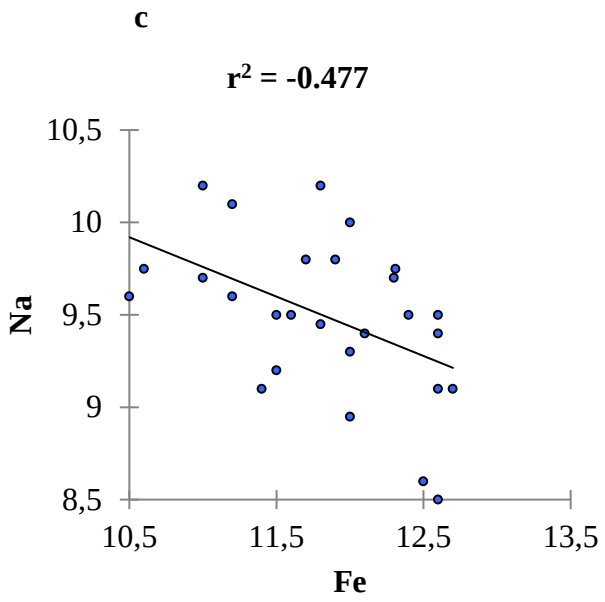


Figure 7.6: The correlation between iron and calcium (a), the correlation between iron and zinc (b), and the correlation between iron and sodium (c).

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			

© Central University of Technology, Free State

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		

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

7.5 REFERENCES

Abbe, E., Fan, J., Wang, K. and Zhong, Y. 2017. Entry wise eigenvector analysis of random matrices with low expected rank. [online] Available at: https://arxiv.org/abs/1709.09565 [Accessed 28 September 2018].

Adam, B. 2000. A principal component analysis of patients, diseases and treatment variables: a new prognostic tool in breast cancer after mastectomy. *Science Direct* 5:83–89.

Akinnifesi, F. K. 2001. Domestication – tapping the unexplored wealth of indigenous fruit trees in Malawi. *Horticulture* 3: 9 - 14.

Akinnifesi, F. K., Kwesiga, F., Mhango, J., Mkonda, A., Chilanga, T. and Swai, R. 2004. Domesticating priority miombo indigenous fruit trees as a promising livelihood option for smallholder farmers in southern Africa. *Acta Horticulture* 632:15 – 30

Akinnifesi, F. K., Leakey, R. R. B., Ajayi, O., Sileshi, G., Tchoundjeu, Z., Matakala, P. and Kwesiga, F.R. 2008. In: Indigenous fruit trees in the tropics: Domestication, utilization and commercialization. CABI, Wallingford: UK.

El–Bakry, and Hazem, M. 2007. New Fast Principal Component Analysis for Face Detection. *J. Adv. Computational Intelligence and Intelligent Informatics* 11:195 –201.

Helmy, A. K. and Taweel, G. H. S. 2009. Authentication scheme based on principal component analysis for satellite images. *International Journal of Signal Processing, Image Processing and Pattern Recognition* 2:1–14

Jamnadass, R.H., Dawson, I.K., Franzel, S., Leakey, R.R.B., Mithöfer, D., Akinnifesi, F.K., and Tchoundjeu, Z. 2011. Improving livelihoods and nutrition in sub-Saharan Africa through the promotion of indigenous and exotic fruit production in smallholders' agroforestry systems. *International Forestry Review* 13:338-354

Kara, D. 2009. Evaluation of trace metal concentration in some herbs and herbal teas by principal component analysis. *Food Chemistry* 114:347–354

Maji, A. T and Shaibu, A. A. 2012. Application of principal component analysis for rice germplasm characterization and evaluation. *Journal of Plant Breeding and Crop Science* 4:87– 97

© Central University of Technology, Free State

Muchuweti, M., Ndhlala, A. R. and Kasiamhuru, A. 2006. Analysis of phenolic compounds including tannins, gallotannins and flavanols of *Uapaca kirkiana* fruit. *Food Chemistry* 94: 415 – 419.

Ndabikunze, B. K., Masambu, B. N. and Tiisekwa. B. M. 2010. Vitamin C and mineral contents, acceptability and shelf life of juice prepared from four indigenous fruits of the Miombo woodlands of Tanzania, *Journal of Food, Agriculture and Environment* 8: 91 – 96.

Ngadze, R. T., Linnemann, A. R., Nyanga, L. K., Fogliano, V. and Verkerk, R. 2017. Local processing and nutritional composition of indigenous fruits: the case of monkey orange (Strychnos spp.) from Southern Africa. *Food Reviews International* 33: 123 – 142.

Ngulube, M. R., Hall, J. B. and Maghembe, J.A. 1995. Ecology of a miombo fruit tree: *Uapaca kirkiana* (Euphorbiaceae). *Forest Ecology and Management.* 77:107–117

Vinceti, B., Ickowitz, A., Powell, B., Kehlenbeck, K., Termote, C., Cogill, B. and Hunter, D. 2013. The contributions of forest foods to sustainable diets. *Unasylvia* 64: 54 – 64

Yu, P. 2005. Application of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feeding structure and feeding molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) micro *spectroscopy. Journal of Agriculture and Food Chemistry* 53:7115 –7127

© Central University of Technology, Free State

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,

© Central University of Technology, Free State

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.

© Central University of Technology, Free State

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..

© Central University of Technology, Free State