EFFECTS OF ENZYME MACERATION ON PHYSICOCHEMICAL PROPERTIES AND MICRONUTRIENT BIOACCESSIBILITY OF *STRYCHNOS COCCULOIDES* PRODUCTS (JUICE/PORRIDGE)

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BY

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DECLARATION

I **QHUBEKANI NYONI** hereby declare that I am the sole author of this thesis; I also authorise Midlands State University to lend this thesis to other institutions or individuals solely for the purposes of scholarly research.

Signature: ………………………… Date: ……………………………

APPROVAL

This dissertation entitled 'Effects of enzyme maceration on physicochemical properties and micronutrient bioaccessibility of *STRYCHNOS COCCULOIDES* products (juice/porridge)' meets the regulation governing the award of the Master of Science degree in Food Science and Nutrition at Midlands State University and is approved for its contribution to knowledge and literal presentation.

Supervisor: …………………………   Date: ……………………………

DEDICATION

To God Almighty my refuge, my strong tower, my anchor in troubled waters, the supreme source of inspiration, wisdom, knowledge and understanding. *Ebenezer* this far you have taken me, glory and praise be to you most high. To my loving wife, 'ANOTHER MILESTONE IS DONE', wouldn't have made it this far without your support. I salute!

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ABSTRACT

Information on the nutritional content and health-promoting properties of African indigenous fruits is very limited. Although the limited available literature does point towards wealth in essential nutrients, micronutrients and antioxidants there is still a need to fill the gap to ascertain health and nutritional claims that have been passed on from previous generations. Studies on the impact of processing of the fruit pulp on physicochemical, nutritional properties, and digestibility thereof are still very scarce and fragmented. Hence, the focus of this study was to explore enzyme (pectinase) maceration as a processing option to enhance physicochemical properties, antioxidant activity, antimicrobial potential and bioaccessibility of selected micronutrients in *Strychnos cocculoides* (*S, cocculoides*) juice/pulp. In this context, enzyme maceration refers to the breakdown of pectin that occurs in fruit pulp as complex structural polysaccharides into simpler, soluble compounds by the action of a commercial mixture of pectinases. Enzyme maceration has been reported to be beneficial in improving juice yield and enhances release of bioactive compounds into resultant juice from fruit pulps. Since the juice is commonly consumed with maize-meal porridge, the *S. cocculoides* enriched porridge was also incorporated into the study. Physicochemical properties were assayed using standard methods, mineral analysis by ICP-OES, phenolic compound assay by the Folin Ciocalteau method, antioxidant activity by DPPH radical scavenging ability, antimicrobial activity by the disc diffusion method and bioaccessibility was assayed using the Infogest digestion protocol. Sensory evaluation was also done to gauge the acceptance of the enzyme macerated samples. The physicochemical properties of the enzyme macerated juice and enzyme porridge were more appreciable compared to their non-enzyme counterparts. The mineral content was highest in the pulp (9.12 mg/100g for iron and 2.04 mg/100g for zinc) followed by enzyme macerated juice (8.89 mg/100g for iron and 2 mg/100g for zinc). The enzyme porridge also recorded higher levels of mineral content. Vitamin C content was affected by the thermal processing used in juice extraction and porridge preparation, although the enzyme juice still contained appreciable vitamin C content (9.45 mg/100g). The total phenol content was also higher in the enzyme juice with recorded value of 3327.75 mg/100g. The same trend was observed for antioxidant and antimicrobial activities with the enzyme macerated juice recording higher results (76.4 % for DPPH radical scavenging AOA). The bioaccessibility of iron, zinc, ascorbic acid and total phenolic compounds was higher in the enzyme treated samples, with values of 28.76 %, 18.14 %, 37.89 % and 36.75 % respectively in enzyme macerated juice. The bioaccessibility of micronutrients was significantly lower ($p < 0.05$) in the porridge samples, clearly highlighting the effect of the food matrix in determining bioaccessibility. The enzyme macerated samples had higher acceptance on sensory evaluation. The observed results in this study are mostly attributable to the breakdown of the complex polysaccharide, pectin, into simpler more volatile compounds such as galacturonic acid. From the observed results it can be concluded that enzyme macerated *S. cocculoides* juice is an excellent source of some bioaccessible micronutrients and phenolic compounds; hence its consumption should be encouraged especially in nutrition-related intervention programmes. However further research is still needed to identify individual specific phenolic compounds in *S. cocculoides* juice and how they are affected by enzyme maceration and in-vitro digestion. The work done in this study can also be employed for other indigenous fruits to promote their utilisation and increase their value.

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LIST OF ACRONYMS

AES	-	Atomic Emission Spectrometer
ANOVA	-	Analysis Of Variance
AOA	-	Antioxidant Activity
TSS	-	Total Soluble Solids
DCPIP	-	Dichloroindophenol
DPPH	-	2, 2-diphenyl-1-picrylhydrazyl
HPLC	-	High Performance Liquid Chromatography
ICP-OES	-	Inductively Coupled Plasma – Optical Emission Spectrometer
PE	-	Pectinesterase
PG	-	Polygalacturonases
PL	-	Polygalacturonate lyase
PMG	-	Polymethylgalacturonase
TPC	-	Total Phenol Content
ZDHS	-	Zimbabwe Demographic Health Survey

CHAPTER 1

1.0 INTRODUCTION

Information on the nutritional content and health-promoting properties of African indigenous fruits is very limited. Although the limited available literature does point towards wealth in essential nutrients, micronutrients and antioxidants (Ndlala, Kasiyamhuru, Mupure, Chitindingu, Benhura, & Muchuweti, 2007; Hassan, Abdulmumin, Umar, Ikeh, & Aliero, 2014; Mpofu, Linnemann, Nout, Zwietering, & Smid, 2014; Oikeh, Omoregie, Oviasogie, & Oriakhi, 2016), there is still a need to fill the gap to ascertain health and nutritional claims that have been passed on from previous generations. In Sub-Saharan Africa some indigenous fruits are vital for food provision, health and financial stability of rural households especially during drought spells (Akinnifesi, 2001; Jamnadass, Dawson, Franzel, Leakey, Mithöfer, Akinnifesi, 2011; Ngadze, Linnemann, Nyanga, Fogliano, & Verkerk, 2017).

Strychnos cocculoides (*S. cocculoides*) locally referred to as Matamba in Shona and Umkhemeswane in Ndebele, is one such fruit that has been used traditionally for nutritive and health benefits with its juice and/or pulp being used as a starting ingredient for various products (Ngadze et al., 2017). The fruits are commonly referred to as 'monkey oranges' and they are indehiscent, oval shaped and have a thick woody shell that is yellow or orange coloured. The fruit pulp is fleshy, bright yellow or brown with hard seeds imbedded within it (Bisset, 1970). *Strychnos spp.* has reportedly been used to treat gastrointestinal ailments (Mwamba, 2006) sore throats, abdominal pains, sore eyes, gonorrhoea and genital warts (Maroyi, 2013). *Strychnos spp.* is a member of the Loganiaceae family; there are 75 *Strychnos* species that have been recognised in Africa, 22 of which are edible (Southampton Centre for Underutilised Crops, 2006). In Southern Africa the most commonly consumed

species include *S. innocua* (Mwamba, 2006), *S. cocculoides*, *S. pungens*, and *S. spinosa* (Bisset, 1970; Southampton Centre for Underutilised Crops, 2006).

The fruit pulp of *S. cocculoides* and *S. spinosa* can be consumed fresh or can be added to maize-meal to create a soft porridge, mixed with water to make sweet beverages, dried to be consumed in future or used in jam and marmalade production (Ngadze et al., 2017), where products made depend upon species. The pulp of *S. cocculoides* fruits is high in colour intensity (rich brown-orange colour) (Ngadze et al., 2017) and this maybe related to high antioxidant activity. There is thus an expectation that the pulp (juice) maybe rich in phytochemicals (Kalt, 2005). The potential health benefits of phytochemicals have been linked to their antioxidant activity. It has been reported that phenolics are potent antioxidants to free radicals and reactive oxygen species which are known as causative agents of some chronic human diseases (Chen and Yen, 2007).

Of the four commonly consumed *Strychnos spp.* in Southern Africa, *S. cocculoides* has the highest mean content of iron with reported values of up to 70.5mg/100g (Ngadze et al., 2017). Thus, *S. cocculoides* juice also has the potential to provide iron when consumed by pregnant or lactating women and children. Iron deficiency has been reported as the most widespread form of malnutrition; as it affects about 2 billion people worldwide (IFPRI, 2016). In Zimbabwe, iron deficiency anaemia continues to be a problem, with 37.6% of male children and 35.9% of female children aged 6 – 59 months; and 25.6% (rural), 28.6% (urban) of pregnant and lactating women being reported to be anaemic iron deficient (ZDHS, 2016). Matebeleland South Province and Midlands Province have the highest prevalence of iron deficiency, reported as 43.1% and 31.2% respectively for women aged between 15 – 49 years (ZDHS, 2016). Micronutrients and antioxidants (phenolic compounds) have been associated with lowered incidences of degenerative diseases (such as cancer, arthritis, immune system

decline, brain dysfunction and cataracts) (Agte and Tarwadi, 2005; Bello, Falade, Adewusi, & Olawore, 2008).

In this context enzyme maceration refers to the breakdown of pectin that occurs in fruit pulp as complex structural polysaccharides into simpler soluble compounds. Pectin is responsible for the turbidity characteristic of fruit pulp. Tapre & Jain (2014) defined pectin substances as, "complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by α (1-4) linkage; side chains include L-rhamnose, arabinose, galactose and xylose; and the carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or completely neutralized by sodium, potassium or ammonium ions". In this study a commercial mixture of pectinases was used consisting of pectinesterases (PE), depolymerases (polymethylgalacturonases (PMG), polygalacturonases (PG)) and cleaving enzymes (polymethylegalacturonate lyases (PGL), polygalacturonate lyases (PL)). The roles of pectolytic enzymes in the fruit processing industry include increase in yields; improved liquefaction, clarification and filterability of juices; maceration and extraction of plant tissues; and releasing flavour, enzymes, proteins, polysaccharides, starch and phenolic compounds (Landbo, Kaack and Meyer, 2007; Sandri et al., 2012). Enzyme maceration has been reported to be beneficial in improving juice yield (Sandri, Fontana, Barfknecht & Da Silveira, 2012) and enhances release of bioactive compounds into resultant juice from fruit pulps (Laroze and Zuniga, 2010). Enzyme treatment of fruit pulp has the potential to improve bioaccessibility of nutrients by breakdown of the pectin-rich matrix (Kumar, 2015). It is thus important to explore enzyme treatment as an option in improving the quality of *S. cocculoides* pulp/juice.

S. cocculoides juice has a relatively low pH of about 3.5 (Saka, Rapp, Akinnifesi, Ndolo, Mhango, 2007); this suggests antimicrobial potential although the juice maybe susceptible to fungal deterioration over long storage periods. In a study conducted by Saka et al., 2007,

processed juice recorded no microbial growth under various storage conditions, suggesting antimicrobial potential. Nutrient bioaccessibility provides data on the fraction of the nutrients released from the food matrices during gastrointestinal digestion that is their potential bioavailability (Minekus, Alminger, Alvito, Balance, Bohn, Bourlieu, Carrière, Boutrou, Corredig, Dupont, Dufour, Egger, Golding, Karakaya, Kirkhus, Le Feunteun, Lesmes, Macierzanka, Mackie, Marze, McClements, Ménard, Recio, Santos, Singh, Vegarud, Wickham, Weitschies, Brodkorb, 2014). Currently, studies on assessment of bioaccessibility of nutrients in indigenous fruits are very scarce and sporadic. Hence the aims of this study are to fortify nutritional value and health claims for *S. cocculoides* consumption; improve the physicochemical and bioaccessibility properties by enzyme treatment and possibly contribute to assisting relevant stakeholders in increasing awareness on potential remedies to reduce micronutrient (iron and zinc) deficiencies especially in rural populations in Zimbabwe.

1.1 Problem statement

S. cocculoides fruit has the potential to be utilised commercially/traditionally as a starting ingredient for various products which could in turn convey health benefits and improve the nutritional status of rural populations (Ngadze et al, 2017). However, some of the traditional claims on the nutritive value and health benefits of *S. cocculoides* fruit are yet to be substantiated by scientific data. It is thus imperative to investigate the nutritive and health beneficial constituents of the fruit to determine their content and accessibility. The impact of processing of the fruit pulp on physicochemical, nutritional properties, and digestibility thereof is not well documented. The current traditional procedure used for juice extraction employs use of high temperatures over long periods may be detrimental to nutrient content and quality traits. There is need to explore processing options (in this case enzyme maceration) that improve or ensure maintenance of quality and health beneficial traits. After

ascertaining the presence of significant health beneficial and nutritive components there is an added necessity to determine the bioaccessibility of the identified components. This is very vital since the presence of nutrients or health beneficial components does not necessarily translate to their bioaccessibility for absorption after gastrointestinal digestion. The bioaccessibility of nutrients from the pulp/juice may be affected when it is added or served with another product. The juice is commonly served with maize-meal porridge (mutandabota) (Ngadze et al, 2017); hence the porridge was used in this study to elucidate the effects of adding the juice to another food product.

1.2 Research questions

1. Does processing (enzyme maceration) have an effect on physicochemical and nutritional properties of *S. cocculoides* juice/porridge?
2. Does pasteurized, enzyme macerated *S. cocculoides* juice possess antimicrobial properties?
3. What are the effects of adding enzyme macerated and unmacerated *S. cocculoides* juice as a starting ingredient to a locally consumed product (maize-meal porridge) on bioaccessibility of phenolic compounds and micronutrients (iron and zinc)?
4. Does enzyme maceration affect the sensorial properties of *S. cocculoides* juice/maize-meal porridge?

1.3 Broad objective

To determine the effect of enzyme maceration on physicochemical properties of *S. cocculoides* juice/porridge and *in-vitro* bioaccessibility of selected micronutrients.

1.4 Specific objectives

1. To conduct physicochemical and nutritional analysis **(pH, brix, dry matter, individual sugars - glucose, fructose and sucrose, minerals - iron and zinc), ascorbic acid and total phenol content)** of enzyme macerated *S. cocculoides* juice and after addition to maize-meal porridge.
2. To determine the antioxidant activity and antimicrobial activity of enzyme macerated and non-macerated *S. cocculoides* juice.
3. To determine the *in vitro* bioaccessibility of phenolic compounds and micronutrients (iron and zinc) in *S. cocculoides* juice and a *S. cocculoides* enriched product (maize-meal porridge).
4. To determine the acceptance of an enzyme macerated *S. cocculoides* enriched product (maize-meal porridge) by sensory evaluation.

1.5 Hypotheses

1. **H_0:** Enzyme maceration has no effect on physicochemical and nutritional properties of *S. cocculoides* juice/porridge.

2. **H_0:** Enzyme maceration has no effect on antioxidant activity and antimicrobial activity of *S. cocculoides* juice.

3. **H_0:** The addition of enzyme macerated *S. cocculoides* juice to maize-meal porridge has no effect on the bioaccessibility of phenolic compounds and micronutrients (ascorbic acid, iron and zinc).

4. **H_0:** Enzyme maceration of *S. cocculoides* juice has no effect on product acceptance when added to maize-meal porridge.

1.6 Significance of the study

To the researcher

Completion of this study has brought a better scientific understanding of the nutritional and health benefit potential of *S. cocculoides* juice/pulp by identification and characterisation of the key health promoting components and bioaccessibility thereof. This has laid the groundwork for further study on other indigenous foods/diets to improve nutritional value and enhance bioaccessibility by exploring different processing methods. The researcher has also gained invaluable experience in analytical methods such as mineral analysis (icp-oes), and in-vitro bioaccessibility assays.

To the University

This research work has added to the body of knowledge on *S. cocculoides* fruit and thus contributes towards research output for the Department of Food Science and Nutrition (MSU). Determining the effects of enzyme maceration on *S. cocculoides* contributes towards substantiating the indigenous knowledge claims on the value of the fruit and guide future value addition efforts that can be employed by the university accordingly. Modern day value addition and product development should be guided by strong nutritional knowledge and how the nutrients behave during processing and subsequent digestion, hence the importance of determining bioaccessibility.

To the community/consumer

Enzyme macerated *S. cocculoides* juice and/or value added products have immense potential in providing essential micronutrients and health benefits to many rural families in Zimbabwe. The fruits can also serve as an alternative source of income, especially in times of cultivated crop shortages. Since the fruit trees are widely distributed in drought prone areas in Zimbabwe this study could inspire the use of *S. cocculoides* in nutrition-related intervention

programmes and policies, especially in the fight against micronutrient (iron) deficiency. Enzyme macerated *S. cocculoides* also has the potential to provide much needed polyphenols (phenolic acids) to the rural community; and help in curbing the rising trends of degenerative and chronic diseases in Zimbabwe.

1.7 Scope of the study

The study was carried out over one harvest season (August – December 2016). To assess health beneficial and nutritive components focus was placed on phenolic compounds, micronutrients (ascorbic acid, iron and zinc), antioxidant activity and antimicrobial activity in *S. cocculoides* fruit. Preliminary experiments (in press) show that phenolic acids are the dominant phenolic compounds in *S. cocculoides* fruit. Bioaccessibility assays were limited to gastric and intestinal phases of simulated gastrointestinal digestion and did not include the oral phase as there is no expected significant digestion of the *S. cocculoides* juice at the oral phase. However for the *S. cocculoides* enriched porridge the oral phase was included.

1.8 Delimitations

Sample collection was limited to rural Lower Gweru (19.23° S, 29.25° E), a communal settlement in the Midlands Province of Zimbabwe. Lower Gwelo has an altitude of 1273M and is characterized by low rainfall (annual rainfall <200mm). It is classified under the agro-ecological region IV and requires extensive irrigation facilities to support crop production.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Nutritional importance of indigenous fruits

Indigenous foods play an important role in the livelihoods of many rural societies in sub-Saharan Africa (Jamnadass et al., 2011; Legwaila, Mojeremane, Madisa, Mmolotsi, & Rampart, 2011; Mpofu et al., 2014; Ngadze et al., 2017). During periods of food shortages many rural households rely on wild resources including edible fruits to meet their nutritional needs (Legwaila et al., 2011; Ngadze et al., 2017). However, the contribution of indigenous fruits to nutritional requirements and poverty reduction efforts is often unrecognised. Most indigenous fruits are underutilised and available nutrient composition data is scarce and lacks sufficient scientific depth (Charrondière, Stadlmayr, Rittenschober, Mouille, Nilsson, & Medhammar, 2013). Knowledge on the nutritional composition of indigenous fruit trees is vital in order to stimulate utilisation of these species in nutrition-related intervention programmes and policies. Information on nutritional composition is also important in assessing the contribution of these fruits to nutrient intake estimations (Jamnadass et al., 2011). Jamnadass *et al* in 2011 compiled a comprehensive review on nutrient composition of indigenous fruits consumed in sub-Saharan Africa and most species reviewed had significant amounts of macronutrients and micronutrients such as (vitamin C, iron, magnesium, zinc, phosphorus and potassium).

The protein content of most of the fruits is relatively low ranging from 1.3g/100g for *Adansonia digitata* to 3.7g/100g for *Sclerocarya birrea* (Amarteifio & Mosase, 2006). For *Vangueria infausta* protein contents of 3.0g/100g and 5.7g/100g were reported by Mateke (2001) and Saka *et al*. (1994) respectively. Fat content also is very low with reported values of 0.4g/100g in *Uapaca kirkiana,* 0.7g/100g in *Ziziphus mauritiana*, 0.5g/100g in *Adansonia*

digitata and 0.8g/100g in *Tamarindus indica* (Jamnadass et al., 2011). Carbohydrate content is mostly high with values ranging from 26.3g/100g in *Vitex doniana* (Saka *et al*., 1994) to 74.9g/100g in *Adansonia digitata* (Amarteifio & Mosase, 2006). Sugar composition in fruit pulp is mainly comprised of the hexose monosaccharides glucose and fructose and the disaccharide sucrose. It should however be noted that fruit pulp from different fruit trees varies in both sugar concentration and composition (Lotz & Schondube, 2006).

Indigenous fruits are excellent sources of micronutrients, hence the focus of this study on iron, zinc and ascorbic acid (vitamin C). Amarteifio and Mosase (2006) reported vitamin C content in fresh sample of 67.7mg/100g *Vangueria infausta* and 141.3mg/100g *Adansonia digitata*. In Jamnadass' *et al* (2011) review it was reported that *Adansonia digitata, Sclerocarya birrea* and *Tamarindicus indica* had mean iron contents of 6.2mg/100g, 3.4 mg/100g and 3.1mg/100g respectively. In a separate study it was reported that *Adansonia digitata, Sclerocarya birrea* and *Vangueria infausta* have zinc contents of 0.14mg/100g, 0.13mg/100g and 0.02mg/100g respectively (Amarteifio & Mosase, 2006). Although it is clear that indigenous fruits are a rich source of nutrients, marked compositional differences are often observed within the same fruit species without plausible discussions. It can be postulated that the differences arise mainly due to different analytical methods (and precision thereof) and possibly geographical location (Jamnadass et al., 2011).

2.2 *Strychnos spp*. (Monkey orange): Consumption and nutritional composition

The 'monkey orange' (*Strychnos* spp.) belongs to the Loganiaceae family and is native to the tropical and subtropical regions of Africa (Bisset, 1970). When water resources are limited the tree remains dormant hence the ability to thrive in drought prone and semi-arid regions. In Africa alone up to seventy five species have been officially acknowledged and twenty of

those species are known to produce edible fruits (Southampton Centre for Underutilised Crops, 2006). Monkey oranges are seasonal and normally harvested from August to December, a time of food crop shortages in Zimbabwe. The most regularly utilised species in Southern Africa include *S. cocculoides*, *S. pungens*, *S. spinosa* (Bisset, 1970) and *S. innocua* (Mwamba, 2006). When ripe, *S. cocculoides* fruits can easily be identified by their physical features which include indehiscent, oval shaped, yellow or orange coloured thick woody shells. The weight of one single whole fruit can vary from around 145g to 383g. Peeling or cracking open the thick woody shell reveals fleshy pulp with numerous hard seeds imbedded in it. The pulp may be bright yellow or brown in colour, juicy in texture and sweet and/or sour taste (Ngadze et al., 2017).

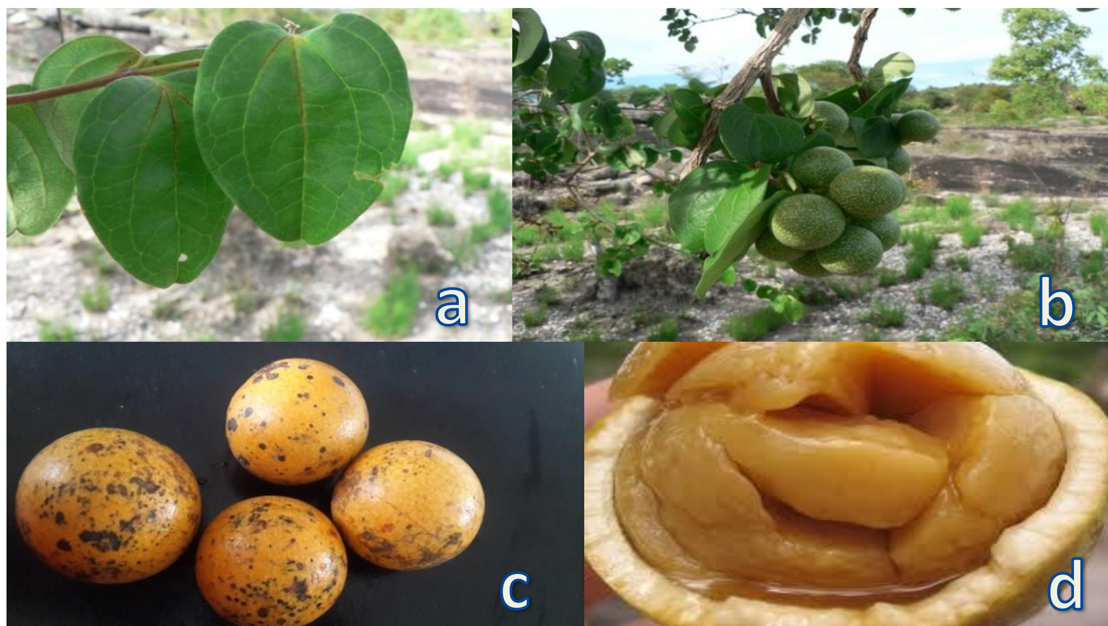


Figure 1: *Strychnos cocculoides* fruit (a: leaves; b: unripe fruits; c: ripe fruits; d: pulp)

2.2.1 Processing and consumption trends

Monkey oranges have a pleasurable sweet/sour taste and flavour and are commonly consumed by rural communities in Southern Africa (Ngadze et al., 2017). The sweetness of the fruit is dependent on sugar composition which in turn is dependent on the stage of ripening (Lee, Tan, Yu Curran, Liu, 2013). The characteristic sour taste can be attributed to

the presence of organic acids and the relatively high acidity. A survey was carried out where *S. cocculoides* was placed first among other indigenous fruits in Zimbabwe according to its role in food security provision, commercialization, taste, and abundance (Akinnifesi, Leakey, Ajaui, Sileshi, Tchoundjeu, Matakala & Kwesiga, 2007). The fruit can be consumed fresh and unprocessed after peeling/cracking the shell or processed into various products. The food products made are species dependent and for *S. cocculoides* the most common is a maize meal sweet porridge (Ngadze et al., 2017), hence its use in this study. The fruit pulp can also be dissolved in water to make sweet drinks, dried to preserve for later consumption or used to produce jams and marmalades (Saka et al., 2007). The pulp is also consumed for medicinal purposes, where it is mixed with honey to cure sore throats, colds and flu; or mixed with other plant extracts to treat some sexually transmitted illnesses (Maroyi, 2013).

Traditionally monkey orange juice extraction is done manually by mashing with the aid a hand held whisk or wooden spoon. Water is then added and the mixture is boiled for several minutes followed by filtration (Saka et al., 2007). The residue left after filtration can be used for jam making. There is no need of adding pectin during jam or marmalade production as the inherent pectin depolymerisation in monkey orange allows the jams to spread well. Drying increases the shelf life by reducing water activity thus lowering bacterial proliferation. Sun drying is normally used and nutrients that are sensible to heat, light and oxygen might be lost (Santos and Silva, 2008). The severe thermal treatments that are used during juice extraction and processing may result in disagreeable changes in nutritional, functional and sensorial value (Ngadze et al., 2017). Moderate thermal processing has the potential of releasing bound phytochemicals but if extreme temperatures are used this could lead to the phytochemicals being lost to the surrounding solution (Nyanga, Nout, Gadaga, Boekhout & Zwietering, 2008).

2.2.2 Nutritional composition of fruit pulp/flesh

The reported values for macronutrient composition of monkey orange species vary markedly within and between species (Ngadze et al., 2017). For carbohydrate content *S. innocua* has values of 15.4g/100g up to 61g/100g dry weight (dw), *S. spinosa* has values of 15.2g/100g up to 42.1g/100g dw and *S. cocculoides* has values of 16.8g/100g up to 19.6g/100g dw. This variance can be attributed to the inefficiency of analytical method used as carbohydrates were calculated by the difference method. For protein and fat in *S. cocculoides,* Tumeo, Mhango, & Munthali (2008) reported very low and nutritionally insignificant values of 0.3g/100g for both nutrients. Ash content has been reported for *S. spinosa* (1.8g/100g dw) and *S. cocculoides* (0.5g/100g dw) (Arnold, Wells, & Wehmeyer, 1985).

S. innocua and *S. cocculoides* are potential significant sources for Zn (28.7mg/100g) and Fe (70.5mg/100g) respectively (Ngadze et al., 2017). There is a need to validate the mineral contents (available data maybe too old) and digestibility of these minerals as they have the potential to improve human nutrition for mineral deficient populations. In their review Ngadze et al (2017) also reported vitamin C content of 34.2mg/100g dw in *S. cocculoides* which is comparable to oranges (50mg/100g dw) and strawberries (59 mg/100 g dw). The baobab fruit (*A. digitata*) has superior vitamin C content of 141.3mg/ 100g (Amarteifio & Mosase, 2006), so does *S. spinosa* with 88mg/100g dw (Ngadze et al., 2017). *S. spinosa* has been reported to have phenolic content, radical quenching ability and flavonoids, expressed as catechin equivalence relatively comparable to baobab nectar (Nhukarume, Chikwambi, Muchuweti, & Chipurura, 2010). High colour intensity is commonly related to a high total antioxidant capacity of a product (Kalt, 2005), hence monkey orange species potentially have high antioxidant capacity as they have bright orange-brown coloured pulp. The anti-nutrient levels of *S. spinosa* were reported to be too low for any nutritional importance and were

below the established toxic level (Bello et al., 2008). Below is a table illustrating the potential nutrient contribution of *S. cocculoides* fruit pulp to different population categories.

Table 1: Monkey orange (*S. cocculoides*) composition with RDI for children 4-8, pregnant females 19-50, adult males 19-50 and adult females 19-50 years (*Adapted from Ngadze et al., 2017*)

	CHO g/day	**Protein g/day**	**Zn mg/day**	**Fe mg/day**	**Vit. C mg/day**
RDI children 4-8 years	***130***	***19***	***5***	***10***	***25***
S. cocculoides pulp composition	18.2	3.5	0.4	70.5	34.2
% contribution	14	18	8	705	137
RDI Pregnant female 19-50 years	***175***	***71***	***11***	***27***	***85***
S. cocculoides pulp composition	18.2	3.5	0.4	70.5	34.2
% contribution	10.4	5	4	261	40
RDI adult male 19-50 years	***130***	***56***	***11***	***8***	***90***
S. cocculoides pulp composition	18.2	3.5	0.4	70.5	34.2
% contribution	14	6	4	881	38
RDI adult female 19-50 years	130	46	8	18	75
S. cocculoides pulp composition	18.2	3.5	0.4	70.5	34.2
% contribution	14	8	5	392	46

2.3 Significance of micronutrients (iron, zinc, vitamin C)

Micronutrients are substances such as vitamins or minerals that are essential in minute amounts to orchestrate a range of physiological functions (IFPRI, 2016). They play vital roles in human nutrition, including but not limited to prevention and treatment of several diseases, as well as optimizing physical and mental function (Gadaga, Madzima, & Nembaware, 2009). Micronutrient deficiency is among the primary risk factors for death in humans across the globe, affecting an estimated over two billion people (IFPRI, 2016). In Zimbabwe iron and zinc deficiencies are especially prevalent in rural populations along with deficiencies in calcium, vitamin A, iodine, and selenium (Gagada et al., 2009; ZDHS, 2016). It is this status quo that roots the interests of this study in the presence and bioaccessibility thereof, of micronutrients in indigenous foods (*S. cocculoides*) that are readily available to the rural population to prevent or reduce 'hidden hunger' (micronutrient deficiency).

2.3.1 Iron

Iron is an important micronutrient that plays a vital role in the metabolism of most living organisms. It is needed for growth (maintenance of physical activity and work capacity), psychomotor development and maintenance of the immune system (Wood & Ronnenberg, 2005). In humans, iron is a crucial constituent of numerous proteins and integrated enzyme systems. Iron facilitates the transport of oxygen from the lungs to various body tissues by red blood cell haemoglobin and also transports some electrons within cells. About 70% of iron in the human body occurs in the red blood cells as haemoglobin (molecule composed of four units, each containing one heme group and one protein chain) (Brody, 1999). The structure of haemoglobin allows it to transport oxygen in the lungs and partially unload it in the tissues. Myoglobin is similar but has only one heme unit. Cytochromes (iron-containing enzymes), also have one heme group and one globin protein chain. These enzymes serve as electron carriers within the cell and their structures do not allow for reversible transport oxygen. Other

important roles for these iron-containing enzymes include the production of steroid hormones and bile acids, detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters (dopamine and serotonin systems in the brain) (Yip & Dallman, 1996). When in excess iron is stored in the liver as ferritin.

Dietary iron comprises of iron bound to the heme prosthetic group of heme iron proteins (animal source) and of iron bound to cysteine residues of non-heme iron proteins (plant source) (Hurrell & Egli, 2010). The major sources of heme iron are hemoglobin and myoglobin from the consumption of meat and meat products, whereas nonheme iron is obtained from plant foods such as cereals, pulses, legumes, fruits, and vegetables. Despite the apparent wealth of dietary iron food sources, iron bioavailability continues to be a quandary for many nutrition scientists especially iron from food plant sources. The bioavailability of heme iron is relatively high (15%-35%) and dietary factors have little effect on its absorption, whereas nonheme iron absorption is much lower (2%-20%) and it is influenced by the presence of other food components (Hurrell & Egli, 2010). This becomes a huge area of concern in populations where plant foods are the main sources of nutrition as is the case in rural Zimbabwe. There is thus a need for research to find ways of improving iron bioavailability in plant based foods (Hunt, 2001). The main inhibitors of iron absorption include tannins, phytic acid, polyphenols, calcium, and peptides from partially digested proteins, whereas ascorbic acid enhances iron absorption (Hurrell & Egli, 2010).

Iron deficiency is a condition in which iron stores are depleted and signs of a compromised supply of iron to tissues are apparent. Although Iron deficiency does exist without anaemia most functional deficits occur with the development of anaemia (Wood & Ronnenberg, 2005). The effects of iron deficiency anaemia can include impaired cognitive development and immunity mechanisms, reduced work capacity, reduced learning ability and increased rates of morbidity (Abbasppour et al., 2014). During pregnancy iron deficiency is associated

with increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight (Abbasppour et al., 2014). The main causes of iron deficiency include low intake of bioavailable iron (vegetarian diets), increased iron requirements during rapid growth, pregnancy, menstruation, and excess blood loss caused by various pathologic infections (Zimmermann & Hurrell, 2007). In Zimbabwe iron deficiency anaemia continues to be a problem in both rural and urban areas. From the Zimbabwe Demographic Health Survey (2016) it was reported that 37.6% of male children and 35.9% of female children aged between 6 – 59 months were anaemic iron deficient. Pregnant and lactating women are the other vulnerable group with prevelances of 25.6%, and 28.6% for rural and urban areas respectively. Matebeleland South Province and Midlands Province have the highest prevalence of iron deficiency, reported as 43.1% and 31.2% respectively for women aged between 15 – 49 years (ZDHS, 2016). The Recommended Dietary Allowance (RDA) for iron varies from 0.2mg in infants to 27mg in pregnant females (Table 2).

Table 2: The Recommended Dietary Allowance (RDA) for Iron

Life stage	Age	Males mg/day	Females mg/day
Infants	0-6 months	0.27	0.27
Infants	7-12 months	11	11
Children	1-3 years	7	7
Children	4-8 years	10	10
Children	9-13 years	8	8
Adolescents	14-18 years	11	15
Adults	19-50 years	8	18
Adults	51 years and older	8	8
Pregnancy	All ages	-	27
Breast-feeding	18 years and younger	-	10
Breast-feeding	19 years and older	-	9

2.3.2 Zinc

Zinc is an essential micronutrient that is required for the metabolic activity of numerous enzymes involved in the metabolism of protein, carbohydrate and fat in the human body (Bhowmik et al., 2010). It is also essential for cell division and plays a vital role in the synthesis of DNA and protein. Other roles of zinc are related to wound healing, taste acuity, connective tissue growth and maintenance, immune system function, prostaglandin production, bone mineralization, proper thyroid function, blood clotting, cognitive functions, foetal growth, sperm production and maintenance of normal serum testosterone (Deshpande et al., 2013). Zinc finger proteins have been reported in regulation of gene expression by acting as transcription factors. Zinc is also involved in cell signalling and influences hormone release and transmission of nerve impulses (Chatterjea et al., 2005; Deshpande et al., 2013).

The major sources of dietary zinc include meat based products such as chicken, lamb, beef, rabbit meat, oysters, scallops, blackfish and animal liver. Although plant foods are regarded as poor sources of zinc significant amounts can be found in mushrooms, day lily flowers, edible fungus, cabbage, black sesame, black rice, dates, hazelnut, ebony and other vegetables, food crops and fruit (Deshpande et al., 2013). Globally, millions of people may have insufficient levels of zinc in their respective diets due to limited access to zinc-rich foods (animal products, oysters and shellfish) and the presence of zinc inhibitors, such as phytates, common in plant-based diets (Bhowmik et al., 2010). Several physiological factors, particularly the quantity of zinc ingested, determine the quantity of zinc absorbed and the efficiency of absorption. However, the main dietary factor known to inhibit zinc absorption is inositol hexa- (and penta-) phosphate or phytate (Hambidge et al., 2010). The main symptoms of zinc deficiency may include growth retardation, low blood pressure, retarded bones, poor appetite, poor sense of smell and taste, severe loss of weight, pale skin, diarrhoea, hair loss, fatigue, and white spots under finger nails (Deshpande et al., 2013).

Zinc deficiency has an effect on various organ systems, including the integumentary, gastrointestinal, central nervous system, immune, skeletal, and reproductive systems. It may lead to dysfunction of both humoral and cell-mediated immunity thus increases susceptibility to infection. Zinc deficiency may also be responsible for delayed sexual maturation and impotence, mental changes in the form of apathy and depression, delayed healing of wounds and sight-related problems such as conjunctivitis, corneal opacities, macular degeneration and night blindness. The Recommended Dietary Allowance (RDA) for zinc varies from 2mg in infants to 12mg in adults (Table 3).

Table 3: The Recommended Dietary Allowance (RDA) for Zinc

Life stage	Age	Males mg/day	Females mg/day
Infants	0-6 months	2	2
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	18 years and younger	-	12
Pregnancy	19 years and older	-	11
Breast-feeding	18 years and younger	-	13
Breast-feeding	19 years and older	-	12

2.3.3 Ascorbic acid (Vitamin C)

Vitamin C is an essential micronutrient with various significant biological functions. It is a cofactor for the biosynthesis of collagen, carnitine, neurotransmitters and peptide hormones (Yang, Liu, & Parry, 2009). Ascorbic acid is a water-soluble potent antioxidant with the ability of eliminating reactive oxygen and nitrogen species (Kagawa, Higasa, Tsujimura, Komatsu, Yanagisawa, & Iwamoto, 2009). Minute quantities of vitamin C can protect

indispensable molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids; from impairment by free radicals and reactive oxygen species that are produced during normal metabolic processes. Vitamin C also protects us by preventing the development of nitrosamines, the cancer causing chemicals that stem from the nitrates contained in many foods (Kagawa et al., 2009). Ascorbic acid has specific roles in two groups of enzymes: the copper-containing hydroxylases and the 2-oxoglutarate-linked iron-containing hydroxylases. Dopamine β-hydroxylase is a copper-containing enzyme involved in the synthesis of the catecholamines norepinephrine (noradrenaline) and epinephrine (adrenaline) from tyrosine in the adrenal medulla and central nervous system. Proline and lysine hydroxylases (iron-containing) are essential for the post-synthetic modification of pro-collagen in the formation of mature, insoluble, collagen (Kagawa et al., 2009). Vitamin C also helps the body to absorb iron and to break down histamine, the inflammatory component of many allergic reactions (Yang et al., 2009)

Table 4: The Recommended Dietary Allowance (RDA) for Vitamin C

Life stage	Age	Males mg/day	Females mg/day
Infants	0-6 months	40	40
Infants	7-12 months	50	50
Children	1-3 years	15	15
Children	4-8 years	25	25
Children	9-13 years	45	45
Adolescents	14-18 years	75	65
Adults	19 years and older	90	75
Smokers	19 years and older	125	110
Pregnancy	18 years and younger	-	80
Pregnancy	19 years and older	-	85
Breast-feeding	18 years and younger	-	115
Breast-feeding	19 years and older	-	120

The main dietary sources of vitamin C include citrus fruits, green peppers, red peppers, strawberries, tomatoes, broccoli, brussels sprouts, turnip and other leafy vegetables. Fish and milk also contain small amounts of vitamin C. There is a gradual decline in the amount of vitamin C as foods age and during prolonged heat treatment processing (Kagawa et al., 2009). Vitamin C deficiency is associated with a specific ailment, scurvy. The symptoms relating to scurvy become apparent when plasma ascorbate levels are under 0.2 mg/100 ml and they include include swollen or bleeding gums and haemorrhages under the skin. The Recommended Dietary Allowance (RDA) for Vitamin C varies from 40mg in infants up to 120mg in breast feeding females (Table 4).

2.4 Antioxidant activity

The term antioxidant defines a substance (present in low concentrations) that delays or prevents oxidation of a given oxidisable substrate (Coinu, Carta, Urgeghe, Mulinacci, Pinelli, Franconi, & Romani, 2007). Antioxidants are categorised into two major classes that is endogenous antioxidants and exogenous antioxidants. Endogenous antioxidants include antioxidant enzymes, iron binding and transport proteins and other compounds affecting signal transduction and gene expression. The common exogenous antioxidants include vitamin E, vitamin C and glutathione. In addition to vitamin E and vitamin C, phenolic compounds can function as antioxidants. The antioxidant properties of certain plant extracts have been attributed partly to their phenolic compound contents (Coinu *et al.,* 2007). There is growing evidence to suggest that the disease prevention properties of plant and plant extracts are essentially attributable to the antioxidant activity of phenolic compounds naturally present in the plant. The antioxidant activity of phenolic compounds is considered to be correlated to scavenging free radicals; chelating transition-metals involved in free-radical production; and inhibiting the enzymes participating in free-radical generation.

The main function of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and reactive oxygen species (ROS) are present in biological systems from a wide variety of sources. Reactive oxygen species can be produced in the human body to carry out important physiological functions including energy production and signal transduction for cellular communication. However a large excess of ROS can damage some biological targets and oxidative stress plays a causative role in a variety of diseases including cancer, heart disease, immuno-modulatory diseases and other chronic degenerative diseases associated with aging (Li, Fang, Choi, Wang, & Yang, 2013). Hence, there is a need for dietary antioxidants to supplement the body's mechanisms to remove ROS. Fruits are a rich source of antioxidants (polyphenols, vitamin C, carotenoids and tocopherols) that can prevent the formation of free radicals (ROS) (Ellong, Billard, Adenet, & Rochefort, 2015). It has been reported that phenolic compounds have superior antioxidant activity when compared to ascorbic acid (Vitamin C) (Yang, Sang, Lambert, & Lee, 2008; Bouayed & Bohn, 2010; Bouayed et al., 2011b, 2011c).

2.5 Phenolic compounds (Phenolics)

2.5.1 Classification

Phenolic compounds (polyphenols) are compounds that possess an aromatic ring bearing one or more hydroxy (-OH) substituents (Wrolstad, 2005). In plants, phenolic compounds mainly exist in their mono-glycosylated form with glucose as the predominant glycosyl moiety, however, arabinose, galactose, rhamnose and xylose are also common (Manach, Scalbert, Morand, Remesy, & Jiménez, 2004). Phenolic compounds can also be conjugated with aliphatic organic acids, amines, lipids, oligosaccharides or other substituents. Thousands of phenolic compounds have been identified in plants, with differences in complexity of structure, conjugation, hydroxylation and methoxylation contributing to the comprehensive

assortment of naturally occurring phenolic compounds (Wrolstad, 2005). Phenolic compounds have been categorised into many different classes but the main dietary phenolics include phenolic acids, flavonoids, and tannins. Flavonoids are a diverse group of secondary plant metabolites that comprise of flavonols, flavanols, flavanones and flavones. For tannins, the major classes include the hydrolysable and condensed tannins. Phenolic acids include hydroxybenzoic and hydroxycinnamic acids (Harbone, 1998; Anantharaju, Gowda, Vimalambike, & Madhunapantula, 2016).

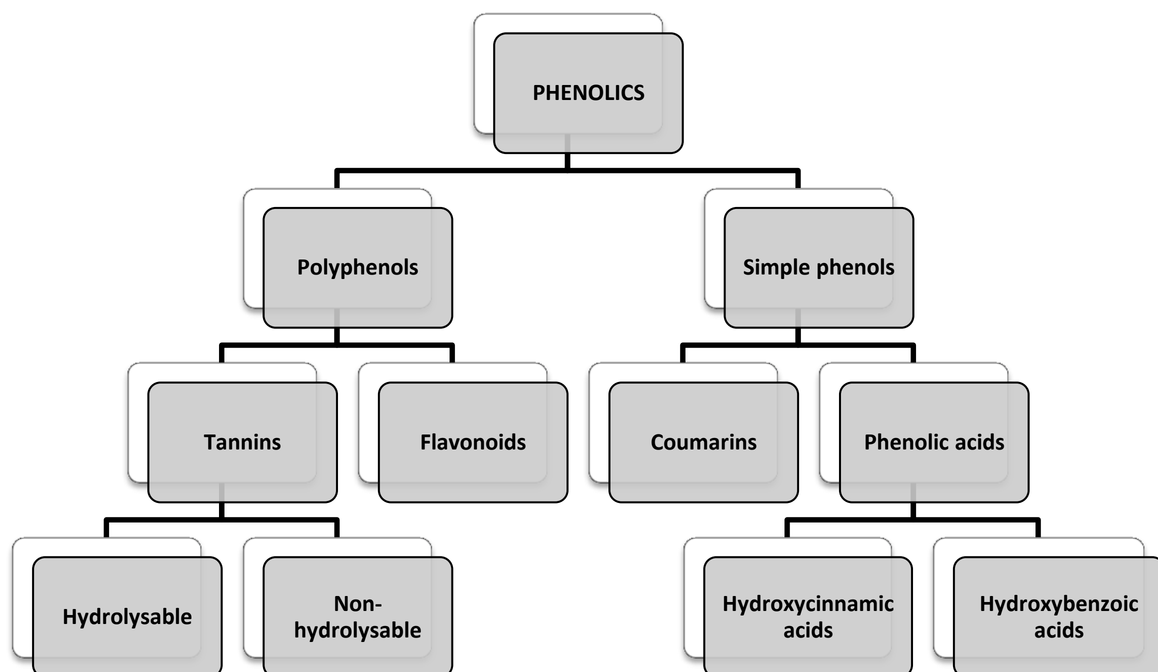


Fig. 2: Simple classification of phenolic compounds (Anantharaju *et al., 2016*)

2.5.2 Importance of phenolic compounds

Phenolic compounds are secondary metabolites that serve important functions in protection and physiology of the plant including but not limited to, filtering harmful ultraviolet (UV) radiation from damaging the plant; defence mechanism after exposure to microorganisms;

toxic to the larvae of fruitworm; lignification therefore influencing structure; involved in colour formation for flowers and fruits; and others attract insects for pollen dispersal in some flowers (Manach et al., 2004; Wrolstad, 2005; Stervbo, Vang & Bonnesen, 2007; Vaishnav & Demain, 2010). Due to their antioxidant activity numerous phenolic compounds have attested vitality as antibacterial/antifungal agents, anticancer drugs, cholesterol-lowering agents, immunosuppressant, antiparasitic agents, herbicides, diagnostics, and tools for research (Vaishnav & Demain, 2010).

Gallic acid has the ability of reducing allergic symptoms by acting as an antihistamine (Maggi-Capeyron, Ceballos, Cristol, Delbosc, Le Doucen, Pons, Leger, & Descomps, 2001). It has been proven that some caffeic acid derivatives (dicaffeoylquinic and dicaffeolytartaric acids) are potent and selective inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase (Robins, 2003). Ferulic acid rarely occurs in its free from in plants but rather it occurs as conjugates of mono- and oligosaccharides, polyamines, lipids and polysaccharides. Physiological functions associated with ferulic acid include antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anticancer activities (Goleniowski, Bonfill, Cusido, & Palazón, 2013). Chlorogenic acid possesses various health properties that can be linked to the treatment of metabolic syndrome. These properties include antilipedic, antidiabetic, antiobesity antihypertensive antioxidant and anti-inflammatory activities (Kaur, 2014).

Wang, Rentian, Bowman, Penhallegon, Ding, & Lu (2005) reported that some fruit phenolic extracts induced cancer cell apoptosis and suppressed cell damage from UV radiation when applied to cell cultures. Correlation between consumption of phenolic compounds and improved health status has been reported in several epidemiological studies (Knekt, Kumpulainen, Jarvinen, Rissanen, Heliovaara, Reunanen, Hakulinen, & Aromaa, 2002; Nichenametla, Taruscio, Barney, & Exon, 2006). Phenolic compounds also contribute to sensorial quality and the organoleptic properties typically ascribed to phenolic compounds

are astringency, and bitterness (Kyle and Duthie, 2006). Besides there contribution to sensory properties, there is substantial interest in phenolic compounds with respect to their antioxidant and free radical scavenging abilities; and subsequent potential to increase the shelf-life of the food. In developed food industries, manufacturers use food-grade phenolic antioxidants to avert the deterioration of product quality and nutritional value due to oxidation (Shahidi and Naczk, 2004). The roles of phenolic compounds in human nutrition and health are wide and far reaching; the future can only be brighter as more discoveries are being achieved.

2.5.3 Phenolic compound composition in fruits and effects of processing

In a study conducted by Sun, Chu, Wu and Liu (2002) it was deduced that apple (296mg/100g) contained the highest phenolic content followed by red grape (201mg/100g), pineapple (94mg/100g), banana (90mg/100g), peach (84mg/100g), lemon (82mg/100g), orange (81mg/100g), pear (71mg/100g) and grapefruit (50mg/100g). Berry fruits are considered better sources of potential health-promoting phenolic compounds (especially flavonoids and phenolic acids) with reported values as follows; Bilberry (*Vaccinium myrtillus*) 3 300mg/100 g dw (Kahkonen, Hoipa & Heinonen, 2001) and 844mg/100g fresh weight(fw) (Maatta-Riihinen et al., 2004); blueberry 376mg/100g fw (Kalt et al., 2001); cranberry 527mg/100g fw (Sun et al., 2002); blackcurrant berry 2230 to 2790mg/100g dw (Kahkonen et al., 2001); blackberry (*Rubus sp.*) 383 to 844mg/100g fw (Siriwoharn & Wrolstad, 2004); and Strawberry fruit (*Fragaria* spp.) 1600 to 2410mg/100g dw (Kahkonen et al., 2001). In Zimbabwe studies by Ndlala et al., (2007 a,b) focused on wild fruits and the following total phenolic compounds were reported; *Ximenia caffra* (2280.73mg/100g), *Artobotrys brachypetalus* (2230.56mg/100g), *Syzygium cordatum* (200.56mg/100g), *Sclerocarya birrea* (22620mg/100g) and *Flacourtia indica* (3340mg/100g).

Processing procedures such as canning, drying, heating, enzymatic clarification and fermentation can affect phenolic compound composition and concentrations in fruit juice (Lu and Foo, 1997; Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007). Other domestic preparation procedures such as chopping, shredding, peeling, boiling and cooking have been reported to decrease the phenolic content in fruits. Aguilar-Rosas et al. (2007) studied the conventional pasteurization of apple juice at 90 °C for 30s and observed a reduction in the total phenolic compounds of about 32%, as compared to the untreated juice. Sentandreu et al. (2007) reported that thermal pasteurisation (90 °C for 30 s) of orange juice had negligible effects on its phenolic substances content. Vegara et al. (2013) reported that the clarification process reduced the content of total monomeric and individual anthocyanins, and increased the antioxidant activity of pomegranate juice. Storage of apple juice (11 months) has been reported to decrease phenolic acid content (Gliszczynska-Swiglo & Tyrakowska, 2003) and a decrease in p-coumaric acid content has also been observed in frozen red raspberries (Mullen et al., 2002). However, apples (Annurca variety) have shown marked increase in chlorogenic acid after four months storage: from 101 to 144 mg/kg fresh weight (Napolitano, Cascone, Graziani, Ferracane, Scalfi, Di Vaio, Ritieni, & Fogliano, 2004). Studies on phenolic compound content and effects of processing thereof in indigenous fruits (of Zimbabwe) are still very scarce and fragmented.

2.6 Nutrient Bioaccessibility

The accurate evaluation of the suitability of recommended dietary intakes of nutrients entails not only information of the nutrient composition of the foods consumed, but also the degree to which the nutrient existing in the diet is (bio)accessible for absorption and utilization by the body (Aggett, 2010). Often the term ‘bioaccessibility’ is used interchangeably with ‘bioavailability’. However for the purposes of this study bioavailability is defines as the

absorbable fraction of a nutrient that can be used for specific physiological functions in organs; whereas bioaccessibility defines the proportion of nutrients that are released from a given food matrix and their consequent availability for absorption in the gastrointestinal (GI) tract (Failla, Thakkar & Kim, 2009). The course of rendering a nutrient bioaccessible includes the processes of chewing (mastication) and initial digestion of the food in the mouth, followed by mixing with acid and more enzymes in the gastric juice after swallowing, and lastly release into the small intestine (key site of nutrient absorption). It should be noted that digestion of the food matrix continues in the small intestine with the aid of further enzymes supplied by the pancreatic juice (Holst & Williamson, 2008).

Nutrient bioaccessibility is vastly variable and is subject to many factors including physiochemical properties; the food matrix in which the nutrient is embedded; the composition of other food constituents that either enhance or inhibit absorption and host-related factors (such as state of health, genetic factors, age and lifestyle) (Aggett, 2010). The bioaccessibility of macronutrients (carbohydrates, proteins, fats) is usually not a problem given normal physiological function; it is mostly very high up to 90% of the amount ingested. However, the bioaccessibility of micronutrients such as vitamins and minerals and can vary extensively (Aggett, 2010).

2.6.1 Iron bioaccessibility

Iron is absorbed into the mucosal cells of the small intestine (duodenum) by an active and saturable process. The effectiveness of iron absorption is amplified with iron deficiency and reduced when erythropoiesis is depressed (Abbaspour, Hurrell, & Kelishadi, 2014). The type of iron (heme or non-heme) has a colossal effect on the bioaccessibility. Approximately 90% of dietary iron is consumed in the non-heme form. However, as a result of its low bioavailability it constitutes a lesser amount of iron essentially absorbed into the human body. The bioaccessibility of heme iron is reasonably high (35%) and dietary factors have little

effect on its absorption, whereas nonheme iron absorption can be as low as 2% and it is largely influenced by the presence of dietary components primarily as a result of luminal interactions. (Hurrell & Egli, 2010). The haem iron molecule is absorbed intact into the mucosal cell as the haem proteins have a protective effect against the digestive system.

The presence of soluble enhancers such as ascorbic acid and inhibitors such as phytates, polyphenols and calcium, consumed during the same meal has a significant influence on the amount of non-heme iron absorbed. In plant-based diets, phytate is the main inhibitor of iron absorption (Hurrel and Egli, 2010), and its effect is dose dependent starting at very low concentrations of 2-10mg/meal (Abbaspour et al., 2014). Food processing methods, such as milling, heating, soaking, germination and fermentation that degrade phytate, will enhance absorption of dietary iron. Calcium and dairy products also have inhibitory effects on non-heme iron absorption, but it also has the ability to inhibit heme iron absorption. Animal proteins such as milk proteins, egg proteins, and albumin, have been shown to possess inhibitory effects on iron absorption and proteins from soybean also decrease iron absorption (Abbaspour et al., 2014).

Ascorbic acid (Vitamin C) increases iron absorption in part by acting as weak chelators and consequently solubilizing the metal in the duodenum. The enhancing effect of Vitamin C is also as a result of its capability to reduce ferric to ferrous iron. Ascorbic acid enhances the absorption of both native and fortification iron and also overcomes the inhibitory effect of phytate, polyphenols, and the calcium and proteins in milk products. It should however be noted that the enhancing effect of ascorbic acid in fruits and vegetables is often varied due to the differing polyphenol composition (Abbaspour et al., 2014). Ascorbic acid is the main enhancer in vegetarian diets, however processing techniques involving cooking, industrial processing, and storage may degrade ascorbic acid and reduce its enhancing effect on iron absorption (Teucher, Olivares & Cori, 2004)

2.6.2 Zinc bioaccessibility

The absorption of zinc responds to the total amount of zinc ingested. When more zinc is consumed, there is a diminishing return in the effectiveness of uptake of zinc, although the net absorption continues to increase as a function of the dose (Tudor, Zalewski, & Ratnaike, 2005). Besides the physiological effects of the amount of zinc ingested, phytate also impairs the bioaccessibility of zinc by chelating to the metal. At low zinc intakes, in the absence of phytate, more than 50% of dietary zinc can be absorbed. However for high phytate diets absorption seldom exceeds 20 % (Prasad, 2009. Fermentation reactions that reduce phytate content may enhance the bioaccessibility of zinc (Tudor et al., 2005). In aqueous solutions iron impairs the absorption of zinc, but this interaction is reduced when iron is added to an animal protein meal. The bioaccessibility of zinc from human milk is high (40% in adults), lower (30%*)* from cows' milk based formula and cows' milk, and even less (14 %) from an infant soya formula (high phytate). The bioaccessibility in zinc in human milk can be attributed to low molecular weight ligands such as citrate or to specific proteins (Chung, Stookey & Dare, 2008).

Excretion of endogenous zinc, primarily intestinal excretion, also merits attention with respect to zinc bioavailability. The quantity of endogenous faecal zinc plays an important role in the maintenance of zinc homeostasis and is determined by both the quantity of recently absorbed zinc and zinc status (Prasad, 2009). Host-related factors such as intestinal diseases (celiac disease, Crohn's disease, protein-energy malnutrition, and intestinal parasitoses) that induce mal-absorption affect the uptake of dietary zinc (Tudor et al., 2005). The lowest absorption of zinc is usually evident in developing countries, where diets are based on cereals and legumes with high phytate content and negligible amounts of animal protein.

2.6.3 Vitamin C bioaccessibility

The bioaccessibility of dietary vitamin C represents the fraction of the micronutrient that is available for absorption by the intestines after gastrointestinal digestion (Michels, Hagen & Frei, 2013). Fruit and vegetables are endowed with abundant micronutrients (vitamins and minerals), dietary fiber, and phytochemicals (flavonoids, phenolic acids) and these may affect the bioaccessibility of vitamin C. Vitamin C interacts with vitamin E by reducing the tocopheroxyl radical and regenerating native tocopherol and some studies have reported that vitamin E is able to preserve vitamin C *in vivo* (Tanaka, Hashimoto, Tokumaru, Iguchi, & Kojo, 1997). Dietary vitamin C improves the bioaccessibility of non-heme iron largely due to its ability to reduce iron from its ferric to ferrous state; nonetheless it is still unclear whether iron can affect vitamin C bioaccessibility. Iron has been shown to increase the uptake of vitamin C in-vitro (cultured intestinal cells), however in-vivo human studies have shown no effect of iron intake on vitamin C bioavailability (Scheers & Sandberg, 2011). Numerous *in vitro* studies have shown that different flavonoids can inhibit vitamin C uptake by their respective transporters. For example quercetin and myricetin can inhibit the uptake of vitamin C into cultured monocytic and lymphocytic (Carr and Vissers, 2013). Obviously any processing procedure including but not limited to heat treatment that reduces the amount of vitamin C will affect the subsequent bioaccessibility by reducing the amount available for digestion (Yang et al., 2009)

2.6.4 Bioaccessibility of phenolic compounds

Numerous studies on the existence of various types of bioactive compounds with antioxidant properties in fruits have been published; however, researchers have only recently begun to assess the potential contribution of these bioactive compounds after their consumption (Haminiuk, Maciel, Plata-Oviedo, & Peralta, 2012). The bioaccessibility of polyphenols from fruits can be defined as the fraction of the antioxidant compound that is released from the

food matrix after digestion consequently becoming available for intestinal absorption (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). The procedures involved in fruit processing may affect phenolic compound content and alter fruit microstructure hereby influencing access and availability of the phenolic componds (Haminiuk et al., 2012).

The chemical structure of phenolic compounds and their interactions with various macromolecules such as proteins and dietary fibres affect their bioaccessibility (Yang et al., 2008; Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). A vast number of phenolic compounds must undergo enzymatic hydrolysation before absorption. Acylation, conjugation, molecular size and solubility also determine bioaccessibility. Phenolic compounds with high molecular weights are often predicted to be poorly absorbed (Yang et al., 2008). High fibre fruits can decrease the bioaccessibility of phenolic compounds; nonetheless these antioxidants do continue to contribute to a healthy antioxidant environment in the large intestine (Palafox-Carlos et al., 2011). The interactions between phenolic compounds and constituents of the gastrointestinal system and colonic microflora play a vital role in their bioaccessibility. Many phenolic compounds only become bioaccessible after digestion by hepatic enzymes and interaction with microflora. Since there are no esterases produced in human tissues, esterified hydroxycinnamic acids require the action of esterases of the colonic microflora for the cleavage of ester bonds which consequently proffers bioaccessibility (van Duynhoven et al., 2011). Saura-Calixto et al. (2007) reported that approximately 48% of dietary polyphenols are bioaccessible in the small intestine, whilst 42% become bioaccessible in the large intestine.

2.7 Enzyme treatment of fruit juice

The most significant class of enzymes used in fruit and vegetable processing is the pectinases. Since the early 1930's pectinases have been used in the production of wines and fruit juices, and they are currently an essential part of fruit juice industries, as well as having several biotechnological uses. (Sandri et al., 2012; Tapre & Jain, 2014). These enzymes are capable of breaking down pectin that occurs in fruit pulp as complex structural polysaccharides that are responsible for the turbidity characteristic of fruit pulp. Tapre & Jain (2014) defined pectic substances as, "complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by α (1-4) linkage; side chains include L-rhamnose, arabinose, galactose and xylose; and the carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or completely neutralized by sodium, potassium or ammonium ions". Commercial pectinases are usually a mixture of three classes of enzymes which include pectinesterases (PE), depolymerases (polymethylgalacturonases (PMG), polygalacturonases (PG)) and cleaving enzymes (polymethylegalacturonate lyases (PGL), polygalacturonate lyases (PL)) (Tapre & Jain, 2014).

The roles of pectolytic enzymes in the fruit processing industry include increase in yields; improved liquefaction, clarification and filterability of juices; maceration and extraction of plant tissues; and releasing flavour, enzymes, proteins, polysaccharides, starch and phenolic compounds (Landbo, Kaack and Meyer, 2007; Sandri et al., 2012). Kumar (2015) reported that the application of enzyme treatment may be of great importance with regard to increase in phenolic compounds content in juices. Pectinases are also used in the production of high quality fruit purees by softening the skins and tissues. The enzymes can be used in deskinning of oranges as opposed to sodium hydroxide treatment which may result in substantial loss of soluble solids. Pectinases have also been reported in the extraction of sugar from date fruits (Bahramian, Azin, Chamani & Gerami, 2011).

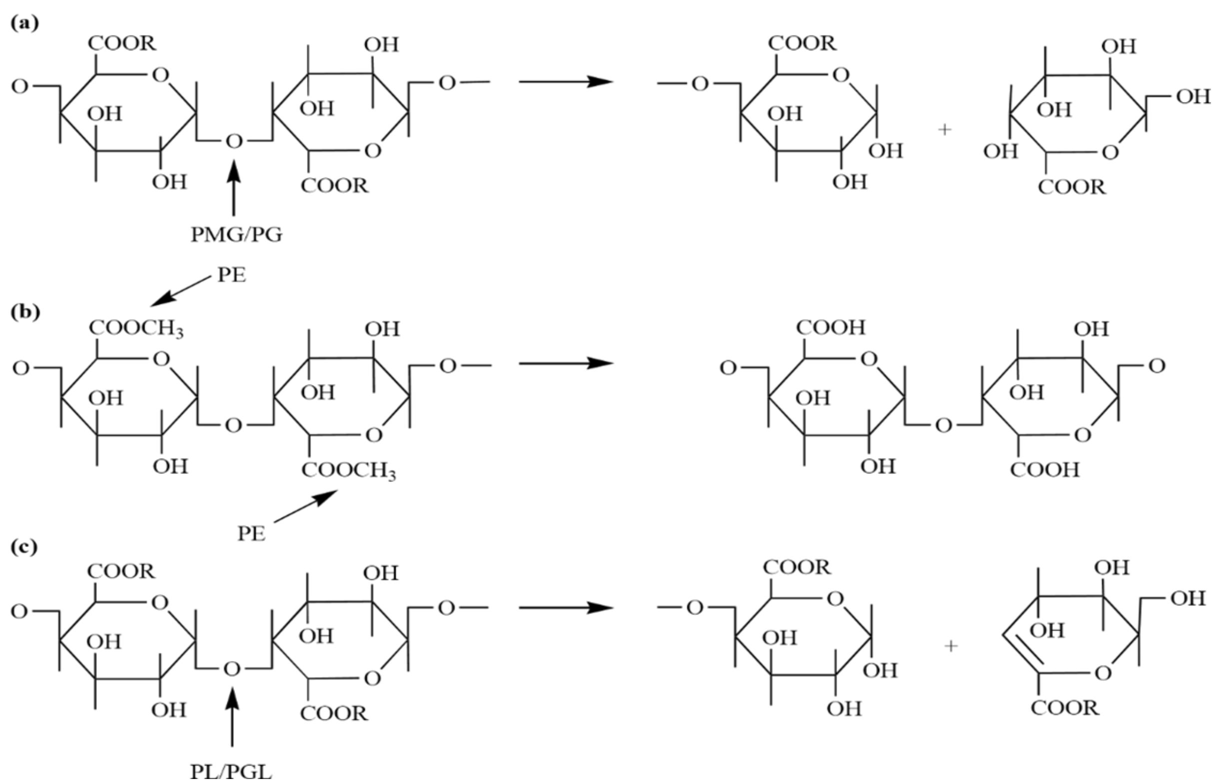


Fig. 3: Mechanism of action for different of pectinases: (a) PMG: hydrolytic cleavage of α(1,4)-glycosidic bonds; PG: hydrolysis of α(1,4)-glycosidic linkages (b) PE: deesterification of the methoxyl group (c) PL/PGL: cleavage of α-1,4-glycosidic linkage

2.8 Principles of some assays used

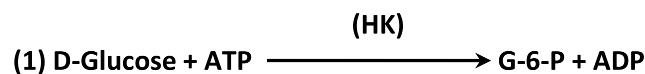
2.8.1 Sugar kit (glucose, fructose and sucrose)

D-glucose, D-fructose and sucrose are found naturally occurring in various plant and food products. In plants, D-glucose and D-fructose occur as free sugars in sucrose, and in an assortment of oligosaccharides (galactosyl-sucrose oligosaccharides and fructo-oligosaccharides) and polysaccharides such as fructans (inulins), starch, 1,3 and 1,4-β-D-glucans and cellulose. They are present in significant quantities in fruits. The principle of the assay (Megazyme D-glucose, D-fructose and sucrose assay kit) used in this study is outlined below. The content of D-glucose is assayed prior to and after hydrolysis of sucrose by β-

fructosidase (invertase), whereas the D-fructose content of the given sample is determined subsequent to the determination of D-glucose, after isomerisation by phosphoglucose isomerase (PGI) (Megazyme, 2014).

2.8.1.1 D-Glucose determination

Hexokinase (HK) catalyses the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) to glucose-6-phosphate (G-6-P), at the same time there is concurrent formation of adenosine-5'-diphosphate (ADP) (1).

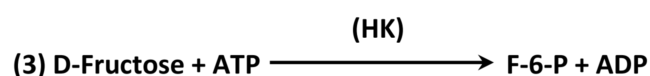


In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidised by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (2) (Megazyme, 2014).

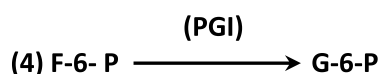
The amount of NADPH produced in this reaction is stoichiometric with the amount of D-glucose; hence the NADPH is measured by the increase in absorbance at 340 nm (Megazyme, 2014).

2.8.1.2 D-Fructose determination

Hexokinase also possesses the capability of catalysing the phosphorylation of D-fructose to fructose-6-phosphate (F-6-P) by adenosine-5'-triphosphate (ATP) (3).



The F-6-P is subsequently converted to G-6-P by PGI (4).



G-6-P reacts with NADP+ forming gluconate-6-phosphate and NADPH, leading to a further rise in absorbance that is stoichiometric with the amount of D-fructose (Megazyme, 2014).

2.8.1.3 Hydrolysis of Sucrose

At pH 4.6, sucrose is hydrolysed by β-fructosidase to D-glucose and D-fructose.

The D-glucose in the sample following hydrolysis of sucrose (total D-glucose) is determined as described above. The sucrose content is calculated from the difference in D-glucose concentrations before and after hydrolysis by β-fructosidase (Megazyme, 2014).

2.8.2 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

In recent years, instrument manufacturers have started naming ‘atomic emission spectrometers’ (AES) instruments as ‘optical emission spectrometers’ (OES) due to the fact that they measure light that is emitted when excited atoms return to the ground state (Miller, 2008). The ICP-OES is a powerful instrument that is used for the determination of metals in a various sample types. The basic principle is centred on the spontaneous emission of photons from atoms and ions that have been excited in a radiofrequency discharge.

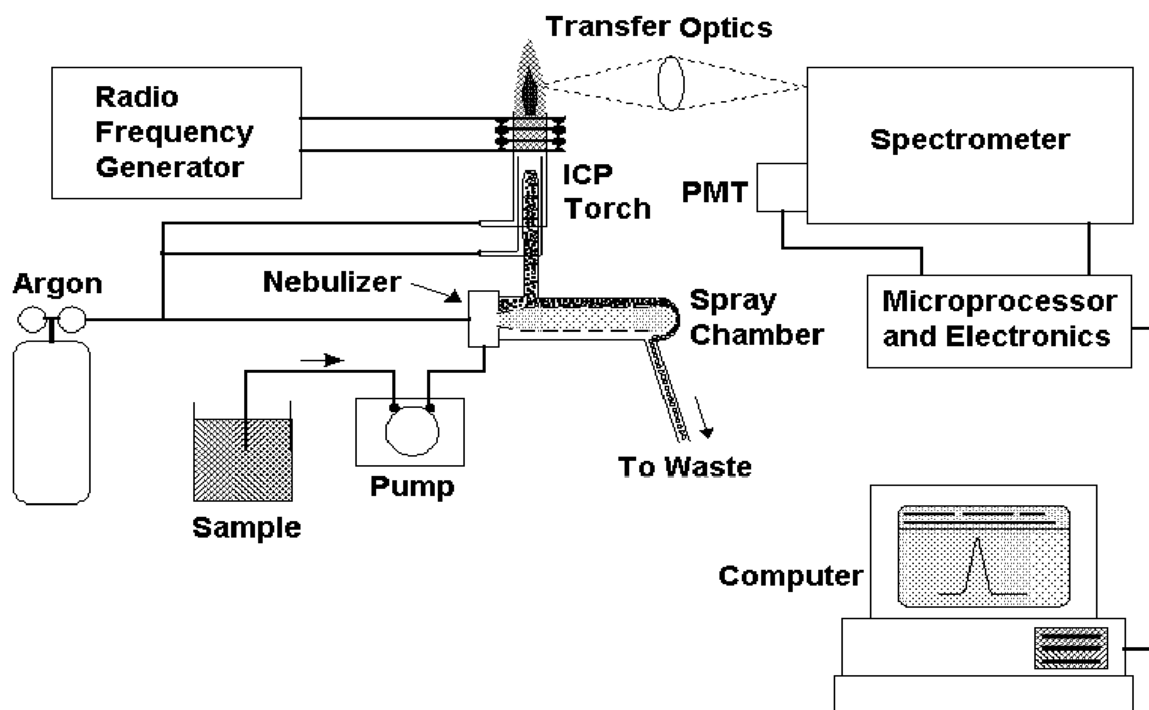


Fig. 4: Schematic representation of the ICP operating principle (*Adapted from Hou & Jones, 2000*)

It is ideal for liquid and gas samples as they can be injected directly into the instrument, whereas for solid samples extraction and/or acid digestion is necessary before sample insertion. On insertion, the sample solution is transformed to an aerosol that is subsequently directed to the central channel of the plasma. The inductively coupled plasma (ICP) can withstand temperatures of up to 10 000°C, hence the aerosol is rapidly vaporised and elements in the analyte are liberated as free atoms in the gaseous state. Further energy is imparted to the atoms by the collisional excitation within the argon-based plasma, promoting them to excited states. Adequate energy is repeatedly available to transform the atoms into ions followed by the promotion of the ions to their respective excited states. When the atomic and ionic excited state species relax and revert back to their respective ground states through the emission of a photon. The resultant photons possess distinctive energies, determined by the quantized energy level structure for the specific atoms or ions. Hence the individual elements can be identified by the wavelength of the photons. Quantitative analysis is

facilitated since the total number of photons is directly proportional to the concentration of the originating element in the sample (Hou & Jones, 2000).

The advantages of ICP over other excitation sources are attributable to its proficiency in efficient and reproducible vaporization, atomization, excitation, and ionization for a wide variety of elements in different samples. This is principally due to the high operating temperature in the observation zones of the ICP. Some of the most beneficial characteristics of ICP include; high operating temperature, high electron density (10^{14}–10^{16} cm^{-3}), significant degree of ionization for several elements, concurrent multi-element assays, low background emission and low chemical interference, high stability (leading to accuracy and precision), excellent detection limits for most elements (0.1–100 ng mL^{-1}), applicability to refractory elements and lastly cost-effective analysis (Hou & Jones, 2000; Miller, 2008).

2.8.3 Folin-Ciocalteu method (total phenol content)

The Folin-Ciocalteau assay is one of the simplest procedures available for the quantification of total phenolic content in products. The constituents of the Folin-Ciocalteau phenol reagent include heteropoly acids, phosphomolybdic and phosphotungstic acids in which the molybdenum and the tungsten are in the 6+ oxidation state. The assay is a colorimetric method based on electron transfer reactions between the reagent and phenolic compounds. When the reagent reacts with a reducing agent, the molybdenum blue and the tungsten blue are formed and the mean oxidation state of the metals is between 5 and 6 (Agbor, Vinson & Donnelly, 2014).

2.8.4 High performance liquid chromatography (HPLC)

Adequate sample preparation and efficient phenolic compound extraction is essential to the success of phenolic acid assays using HPLC. These pre-steps are largely dependent on the nature of the sample matrix and the chemical properties of the phenolics, including molecular structure, polarity, concentration, number of aromatic rings and hydroxyl groups. Some

samples may need to be dried (freeze-drying, air-drying, oven drying) to enhance phenolic compound extraction. It should also be noted that complexes with proteins, carbohydrates and/or other elements may hinder complete extraction (Khoddami, Wilkes and Roberts, 2013). Factors that may affect yield include extraction time, temperature, solvent-to-sample ratio, the number of repeat extractions and solvent type. The most common extraction solvents are water, acetone, ethyl acetate, alcohols (methanol, ethanol and propanol) and their mixtures (Garcia-Salas, Morales-Soto, Segura-Carretero, & Fernández-Gutiérrez, 2010).

HPLC is the most favoured technique separation and quantification of phenolic compounds. Sample purification, mobile phase, column types and detectors all have a heavy bearing on HPLC analysis (Garcia-Salas et al., 2010). The main mobile phases used for HPLC quantification of phenolic compounds are acetonitrile and methanol, or their aqueous forms. It is important to ensure that the pH of the mobile phase is kept around 2 – 4 to avoid the ionization of phenolics (Lee, 2008). Various classes of phenolic compounds can be detected using a normal phase C18 or reversed phase (RP-C18) column 10–30 cm in length, 3.9–4.6 mm internal diameter and 3–10 µm particle size (Robbins, 2003). The detectors of choice for phenolic compound identification are UV-VIS and photodiode array (PDA) detectors at wavelengths 190–380 nm, but other detectors can be used (de Villiers Kalili, Malan, & Roodman, 2010).

2.8.5 DPPH radical-scavenging (antioxidant activity)

DPPH (α, α-diphenyl-β-picrylhydrazyl) (violet in solution) free radical scavenging method offers one of the most common and simplest methodologies for evaluating the antioxidant potential of a compound, an extract or other biological sources. The simplicity is evident in the procedure, wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period. However, with the advancement of technology various modifications to suit given requirements have been developed, although

the basic principle remains the same (Kedare & Singh, 2011). The principle is based on the extent of the scavenging capacity of antioxidants towards DPPH ($C_{18}H_{12}N_5O_6$). The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. When the DPPH solution is mixed with a substance that can donate a hydrogen atom a loss of the violet colour is observed as the DPPH turns to its reduced form. The primary reaction can be represented as;

Z• + AH = ZH + A•, *where Z• is the DPPH radical and AH is the antioxidant (donor molecule).*

2.8.6 *In-vitro* bioaccessibility assays

Over the years, *in-vitro* screening approaches have been developed for determining the bioaccessibility and bioavailability of various nutrients from foods (Vardakou, Mercuri, Naylor, Rizzo, Butler, Connolly, Wickham, & Faulks, 2011; Etcheverry, Grusak, & Fleige, 2012; Minekus et al., 2014). Bioaccessibility refers to the amount of an ingested nutrient that is potentially accessible for absorption and is reliant mainly on digestion and release of the nutrient from the food matrix; whereas bioavailability defines the amount of an ingested nutrient that is absorbed and available for physiological functions at the target cells/tissues (Etcheverry et al., 2012). For the purposes of this study bioaccessibility assays will be used. *In-vitro* assays are fast gaining scientific recognition and are broadly used in various areas of food and nutritional sciences. The use of *in-vivo* methods, that is human trials and animal models, is often costly, resource intensive, and ethically disputable. On the other hand, *in-vitro* methods are considerably quicker, less expensive, require less labour, and are mostly void of ethical constraints (Minekus et al., 2014). *In-vitro* models are suited for mechanistic studies and hypothesis building as they allow for reproducibility, choice of controlled conditions and relatively easy sampling at the required site of gastrointestinal digestion (Vardakou et al., 2011). However it has to be noted that with *in-vitro* models it is mostly

impossible to factor in host-related variables that have the potential to influence nutrient absorption. These variables may include nutrient status, genotype, physiological state (such as pregnancy, lactation, and obesity), chronic and acute infectious disease states, secretion of hydrochloric acid, gastric acid, and/or other intrinsic factor, are impossible to factor in *in vitro* assays.

The basic procedural principle involves an *in-vitro* digestion that is performed to simulate the human digestive system that is the oral phase, gastric and intestinal digestion. For the gastric phase of digestion it is necessary to adjust the pH to 2 (to simulate the gastric pH of an adult) and 4 (to simulate the gastric pH of an infant), on addition of pepsin (derined from porcine stomach). This is necessary because the pepsin begins to denature and subsequently loses activity pH $\geq$ 5. At the beginning of intestinal digestion, the samples are neutralized to pH 5.5–6 prior to the addition of pancreatin (pancreatic enzymes such as pancreatic amylase, lipase, ribonuclease, and proteases such as trypsin) and bile salts (emulsifiers), and lastly re-adjusted to pH 6.5–7. The other digestion step that is sometimes used to simulate the oral phase is the digestion by alpha-amylase (breakdown of glycosidic bonds of starch molecules). This precedes the gastric phase and usually involves physical interventions such as centrifugation to mimic mastication (Minekus et al., 2014).

CHAPTER 3

3.0 METHODOLOGY

3.1 Experimental design

Ripe *S. cocculoides* fruits were obtained from Lower Gweru (19.23° S, 29.25° E) a communal settlement in the Midlands Province of Zimbabwe. Lower Gweru is located about 40 km to the North-West of Gweru city and stretches for a further 50 km to the west. This area is characterised by low and erratic rainfall patterns with annual rainfall of less than 200mm, synonymous with a generally dry climate. It is classified under the agro-ecological region IV (Zimbabwe) and requires extensive irrigation facilities to support crop production.

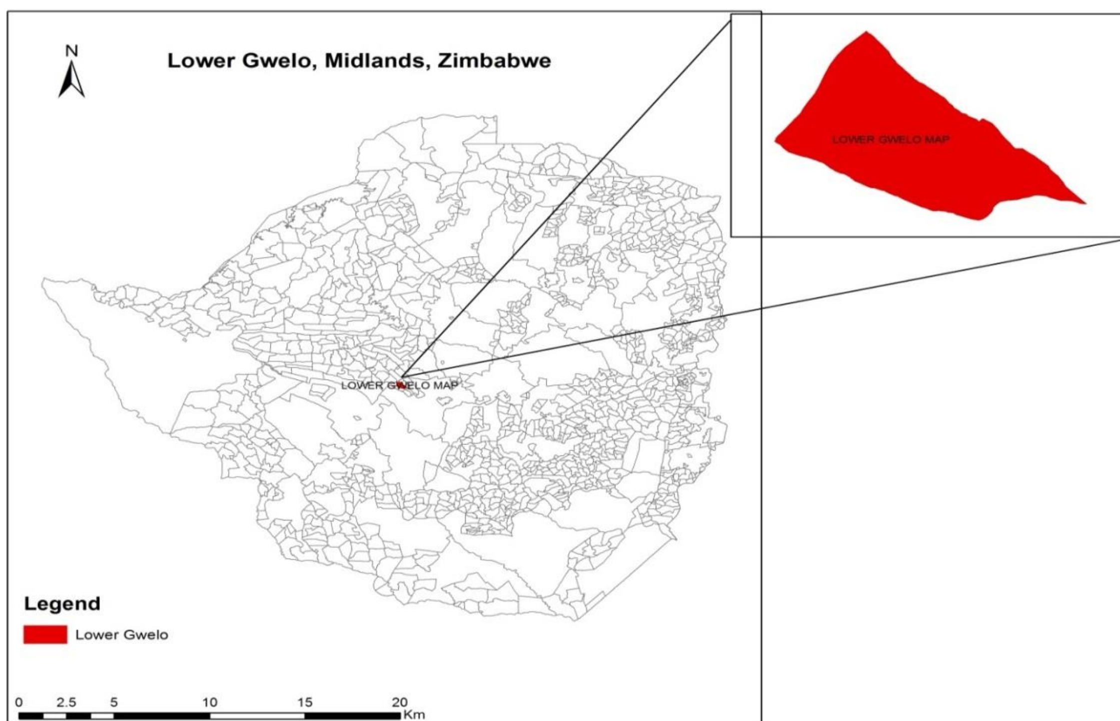


Fig. 5: Map indicating the sampling area

Sample fruits were randomly obtained from trees in the study area assuming random and normal distribution with each unit having an equal chance of being included. Before obtaining the pulp the fruits were rinsed thoroughly in distilled water and all defective fruits were excluded. A total of 100 individual ripe fruits were used in this study.

3.2 Sample preparation

The original pulp (with seeds embedded in it) was retrieved from the ripe fruits by cracking open the woody shells separating the pulp from the shells manually and stored in sealable plastic sample bags at -20°C. Two different methods were used for sample preparation (juice extraction) aimed at laboratory analysis. For the first method (non-enzyme macerated juice) the frozen fruit pulp was thawed and individual fruits blended in a blender to separate the seeds from the pulp. The resultant pulp was then mixed with distilled water at a ratio of 1:1 and incubated in a water bath at 100°C for 5 hours. For the second method (enzyme macerated juice), subsequent to blending and mixing with water, the pulp was incubated at pre-optimized conditions for enzyme (pectinase) maceration; that is temperature at 45°C, incubation time of 5 hours and enzyme concentration at 0.5 %. The enzyme was then inactivated by placing in a 90°C water bath for 5 minutes. The enzyme macerated and non-enzyme juices were used to prepare porridge samples by boiling the juice and adding mealie-meal at the ratio of 2:1 respectively, and then stirring until gelatinisation. All treatments and analysis were done in triplicate to reduce bias and ensure consistency.

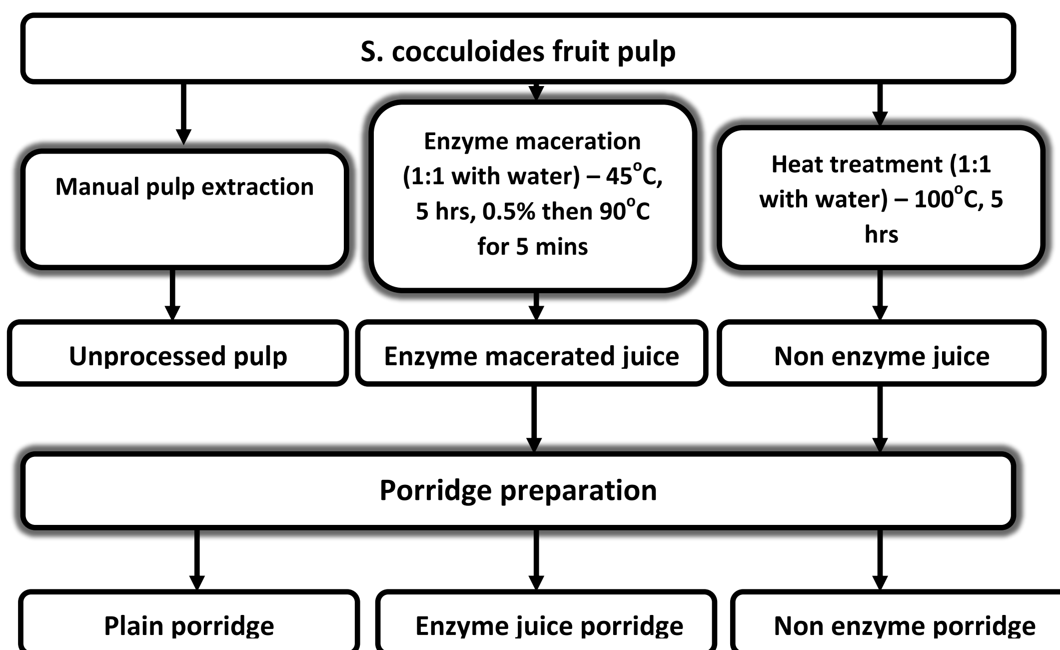


Fig. 6: Overview of sample preparation

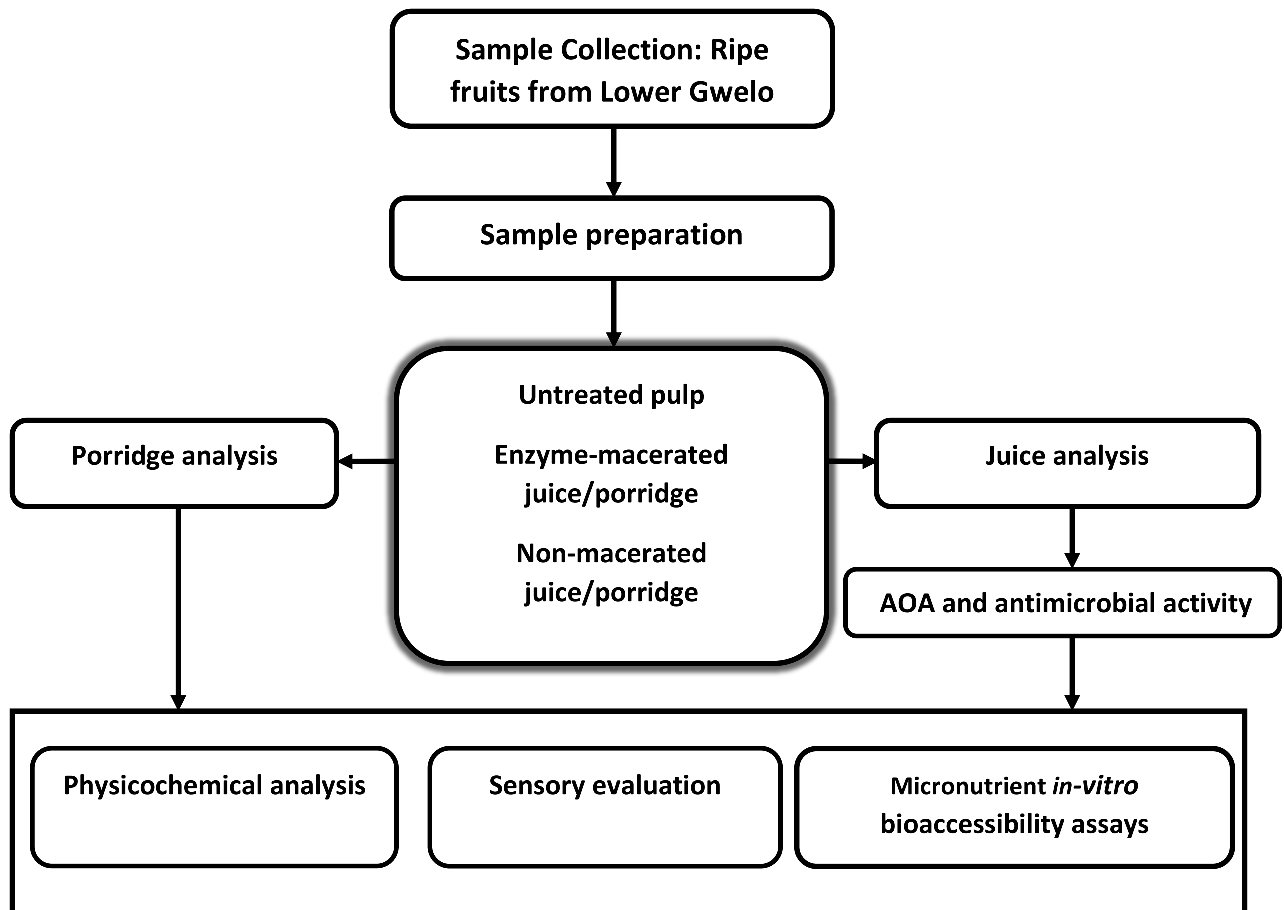


Fig. 7: Illustration of the methodology overview

3.2 Physicochemical and nutritional analysis

3.2.1 pH

The pH of the samples was determined with the aid of a digital pH meter (BOECO, Germany: Model BT-675). The electrode of the pH meter was standardized using standard buffer solutions (pH 4 and pH 7) before use. The electrode was continuously rinsed with distilled water and wiped with absorbent tissue after each reading.

3.2.2 Brix (TSS)

The Brix was determined using a bench refractometer (Nieuwkoop BV: Model MA871). Distilled water was used to rinse off residual sample after each reading.

3.2.3 Dry matter

Dry matter was determined using the automatic oven drying method by placing the sample in a crucible and incubating at 100°C overnight until constant moisture loss.

3.2.4 Individual sugars (glucose, fructose and sucrose)

Glucose, fructose and sucrose content in samples was determined using a Sucrose/D-Fructose/D-Glucose assay kit (Megazyme: K-SUFRG 06/14) that allows for colorimetric measurement with absorbance (uv/vis spectrometer) read at 340nm. The kit consists of 6 bottles with different reagents as shown below.

Fig. 8: Sucrose/D-Fructose/D-Glucose assay kit

Bottle 1: Buffer 1 (25 mL, pH 7.6) plus sodium azide (0.02% w/v) as a preservative.

Bottle 2: NADP+ plus ATP.

Bottle 3: Hexokinase plus glucose-6-phosphate dehydrogenase suspension, (4.1 mL).

Bottle 4: Phosphoglucose isomerase suspension (2.25 mL).

Bottle 5: D-Glucose plus D-fructose standard solution (5 mL, 0.2 mg/mL of each sugar).

Bottle 6: β-Fructosidase (pH 4.6), lyophilised powder.

All bottles were used as supplied except for bottle 2 and bottle 6 which were first dissolved in 22 ml and 20 ml of distilled water respectively. The procedure used is illustrated in the table below. Plastic cuvettes (1 cm light path) were used and absorbance read at 340 nm.

Pipette into cuvettes	Blank sucrose sample	Sucrose sample	Blank D-glucose/ D-fructose sample	D-Glucose/ D-fructose sample
solution 6* (β-fructosidase)	0.20 mL	0.20 mL	-	-
sample solution	-	0.10 mL	-	0.10 mL
Mix**, incubate for 5 min (NOTE: before pipetting solution 6, first warm it to 25-30°C). **Then add:**				
distilled water (at ~ 25°C)	2.00 mL	1.90 mL	2.20 mL	2.10 mL
solution 1 (buffer)	0.10 mL	0.10 mL	0.10 mL	0.10 mL
solution 2 ($NADP^+$/ATP)	0.10 mL	0.10 mL	0.10 mL	0.10 mL
Mix**, read absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:				
suspension 3 (HK/G6P-DH)	0.02 mL	0.02 mL	0.02 mL	0.02 mL
Mix**, read the absorbances of the solutions (A_2) at the end of the reaction (approx. 5 min). If the reaction has not stopped after 5 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min***. **Then add:**				
suspension 4 (PGI)	-	-	0.02 mL	0.02 mL
Mix**, read the absorbances of the solutions (A_3) after approx. 10 min.				

Fig. 9: Procedure for individual sugar assays (*Adapted from Megazyme, 2014*)

The concentration of glucose, sucrose and fructose was calculated as follows:

$$c = \frac{V \text{ x } MW}{\varepsilon \text{ x } d \text{ x } v} \text{ x } \Delta A \qquad [g/L]$$

Where: V = final volume [mL]; MW = molecular weight of the substance assayed [g/mol]; ε = extinction coefficient of NADPH at 340 nm (6300 [l x mol-1 x cm-1]); d = light path [cm]; v = sample volume [mL].

3.2.5 Protein analysis

For crude protein analysis the automated Kjeldahl method was used. The sample (5 g) was placed into the Kjeldahl flask (tubes) with 10 g potassium sulphate, copper sulphate catalyst

tablets and 25 mL concentrated sulphuric acid. The mixture was left to digest at 420^oC for 60 minutes. This was followed by cooling the tubes to around 50^oC – 60^oC by addition distilled water. About 80 mL of 40% sodium hydroxide were then added to the digested sample with heat applied to release ammonia, which was in turn collected into boric acid solution (40 mg/L) with 0.5 g bromosocresol green and 0.1 mL methyl red in 100 mL of 95% ethanol. The boric acid mixture was titrated against 0.1 M HCl. The crude protein content was read from the display screen.

3.2.6 Mineral analysis (iron and zinc)

Mineral analysis was done with the aid of 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 were necessary. The digested samples were then fed into the automated ICP-OES by vacuum operated pipes and results recorded from the print out.

3.2.7 Vitamin C (ascorbic acid)

Ascorbic acid concentration was determined by the DCPIP titration test. DCPIP solution was prepared by dissolving 0.25g of 2, 6 dichloindophenol in 500ml of water. Exactly 0.21g of sodium bicarbonate was then added to the solution and allowed to melt. The resulting solution was finally diluted to a litre with distilled water. About 10ml of the sample juice was pipetted into a 100ml volumetric flask and mixed with 40ml of 5% acetic acid. After 20 minutes, water was added up to the mark. The resulting solution (with sample) was then titrated against the prepared standard DCPIP.

3.2.8 Total phenol content

Phenols extraction was done by stirring 1 mL sample with 9 mL of 80% methanol for a period of 30 minutes at room temperature. The resultant mixture was then centrifuged at 10 000 x *g* for 10 minutes. Total phenol content was determined by the Folin-Ciocalteu method with gallic acid as a standard. About 5 ml of demi water was pipetted into a 25 ml volumetric flask, followed by addition of 1 ml sample/calibration sample/blank. This was followed by addition of 1ml Folin-Ciocalteu reagent and then addition of 1 ml saturated Na_2CO_3. The volume was then adjusted to 25 ml and the flask swirled for uniform distribution. Absorbance was measured at 750 nm after 15 minutes. A standard (gallic acid) calibration curve of absorbance against concentration was plotted and used to calculate phenol content.

3.3 Antioxidant activity and antimicrobial assays

3.3.1 DPPH radical-scavenging activity (Antioxidant activity)

DPPH radical-scavenging activity was performed by the method described by Akter et al. (2010). DPPH solution (3.9 ml of the 6 x 10^{-5}) was pipetted into a test tube. This was followed by addition of 0.1 ml of the sample/blanks. The resulting mixture was covered and incubated in a water bath at temperature of 25°C for 30 minutes. The sample was mixed at 5 equal time intervals during the incubation. The absorbance was determined at 515 nm after calibrating the spectrophotometer with methanol. Trolox (6 hydroxy 2,5,7,8 tetramethylchroman 2-carboxylic acid) was used as the reference compound.

DPPH radical scavenging activity (%) = [(Abscontrol- Abssample) /Abscontrol] x 100

3.3.2 Antimicrobial activity

The disc diffusion method was used to test for antimicrobial potential in *Strychnos cocculoides* juice samples against the following bacterial species: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella spp.* and fungal species *Candida albicans and Aspergillus niger*. Filter paper discs were prepared and soaked to saturation in each of the sample juices. The broth cultures of the test organism were streaked on nutrient agar plates using sterile cotton swabs. The discs were then aseptically placed on the agar plates and incubated at 37°C for 24 hrs. The diameters of zones of inhibition obtained were measured and recorded according to CLSI, 2006.

3.4 In-vitro bioaccessibility assay

The bioaccessibility of the phenolic compounds and micronutrients (ascorbic, iron and zinc) was determined using the Infogest in-vitro digestion protocol (Minekus et al, 2014). Antioxidant activity, phenolic compounds, ascorbic acid, iron and zinc content were measured before and after simulated gastrointestinal digestion, using the methods described in section 3.2. For the liquid samples only supernatant digest at gastric and intestinal phases of simulated gastrointestinal digestion were used. The oral phase was included only for the *S. cocculoides* enriched porridge. Porridge samples were freeze dried prior to analysis.

3.4.1 Oral phase

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

3.4.2 Gastric phase

For the gastric phase of simulated digestion, 7.5 mL of simulated gastric fluid (SGF), 1.6 mL pepsin solution (2000 units/mL) and 5 µL of $CaCl_2$ solution were used either by adding to the mixture from oral phase or direct to the liquid samples (*S. cocculoides* juice). The pH was then adjusted to around 3 by addition of about 0.8 mL of 6M hydrochloric acid. The resultant mixture was also incubated in a shaking water bath for 2 hours at 37˚C.

3.4.3 Intestinal phase

For the intestinal phase of simulated digestion, the following solutions were added in sequence to the product from the gastric phase; 11 mL of simulated intestinal fluid (SIF), 5 mL of pancreatin solution (100units/mL), 2.5 mL of bile solution (10mM) and 40 µL of $CaCl_2$. Where necessary the pH was adjusted to around 7 by addition of NaOH, followed by incubation for 2 hours at 37˚C in a shaking water bath. After simulated intestinal digestion samples (1 mL) were collected and pipetted into capped micro-centrifugal tubes. The samples were immediately snap frozen in liquid nitrogen and preserved for further analysis.

Table 5: Preparation of simulated digestion fluids (SDF)

			SSF		SGF		SIF	
			pH 7		pH 3		pH 7	
Constituent	**Stock conc.**		**Vol. of stock**	**Conc. In SSF**	**Vol. of stock**	**Conc. In SIF**	**Vol. of stock**	**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}**
$CaCl_2(H_2O)_2$	44.1	0.3		1.5 (0.75*)		0.15 (0.075*)		0.6 (0.3*)

*** corresponding Ca^{2+} concentration in the final digestion mixture.**

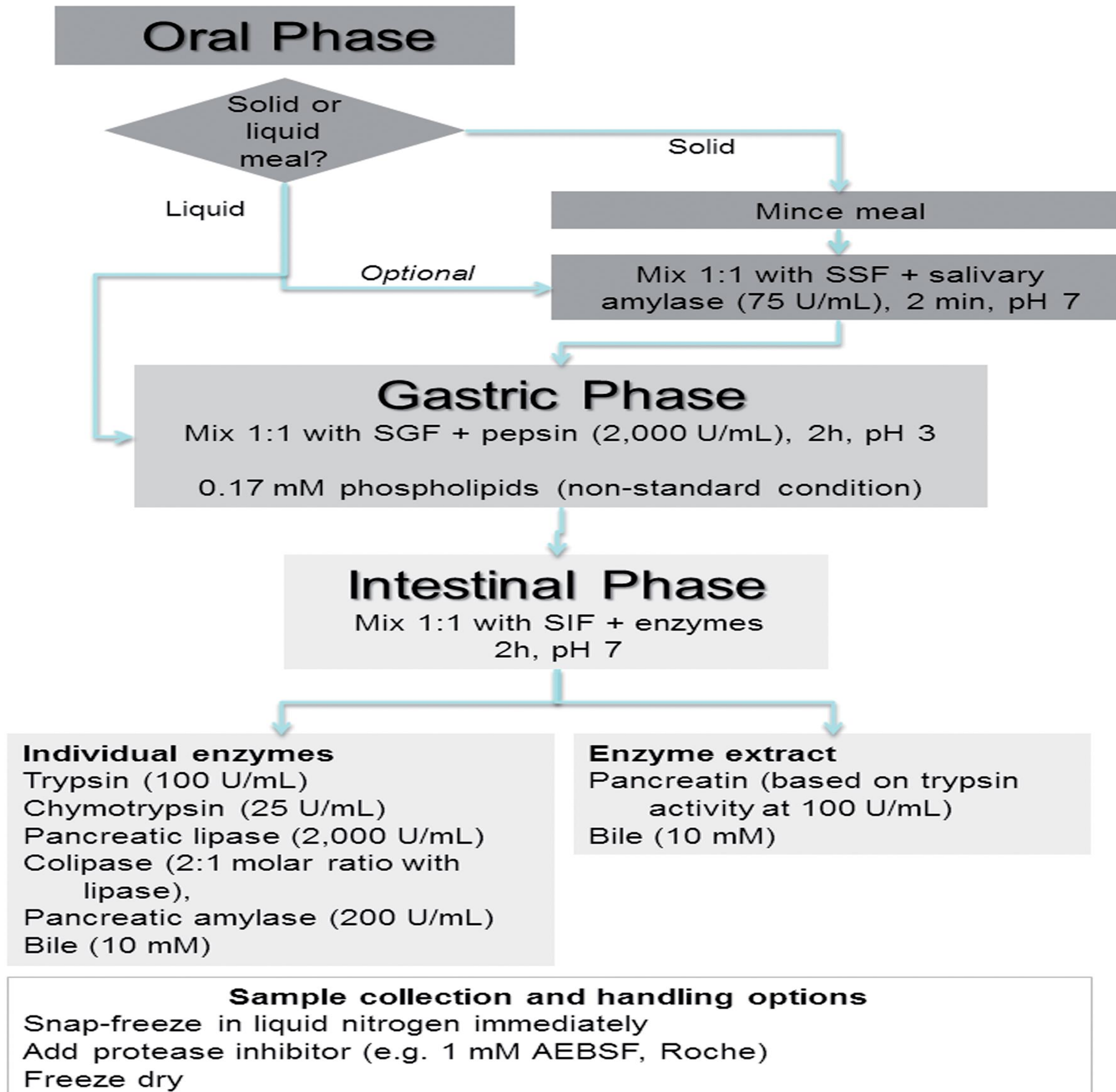


Fig. 10: A summary flow diagram illustrating the Infogest in-vitro digestion protocol. (*Adapted from Minekus et al., 2014*)

3.5 Sensory evaluation

3.5.1 Triangle test

Trained and untrained panelists were used to test for the ability by consumers to discriminate between enzyme macerated and non-enzyme juice/porridge by use of the triangle test.

Panelist /judges were drawn from six villages in Lower Gweru (Untrained: n = *132*) and from Chinhoyi University of Technology (CUT) students and lecturers (Trained: n = *12*). Non-disclosure and consent forms were signed before commencement of the tests. Makeshift booths made of cardboard box were used in Lower Gweru and open-ended booths were used at CUT (Fig. 13). The samples that were used for evaluation were coded using six combinations (ABB, BAA, AAB, BBA, ABA, and BAB). The respondents were randomly given three coded samples and a glass of water to mask the taste of the previous sample. After going through all three samples the respondents were given a score sheet and asked to select the odd sample.

Fig. 11: Makeshift (cardboard box) sensory evaluation booths used in Lower Gweru (a, b) and open-ended booths used at CUT (c, d)

3.5.2 Preference test

Trained panelists from CUT students and lecturers (Trained: n = *12*) were used to determine preference between the different samples (juices/porridge) in terms of sweetness, astringency, colour, aroma and overall acceptance. The panelists were provided with two samples at a

given time and a score sheet. They were requested to indicate their preference for the given samples according to designated sensory attributes including overall product acceptance. A 9 point hedonic scale from 'dislike extremely' (score 1) to 'like extremely' (score 9) was used. Water was provided and used as a palate cleanser after each sample.

3.6 Data analysis

All analysis was done in triplicate and results are expressed as means and standard deviations. ANOVA (SPSS 16th Edition) followed by LSD test was used to establish significant difference between means at 5% for different physicochemical properties and antimicrobial activity. For sensory evaluation; the chi-square distribution was used to evaluate results from the triangle test and the student's *t*-test was used for the preference test.

CHAPTER 4

4.0 RESULTS

4.1 Effects of enzyme maceration on physicochemical properties

4.1.1 pH

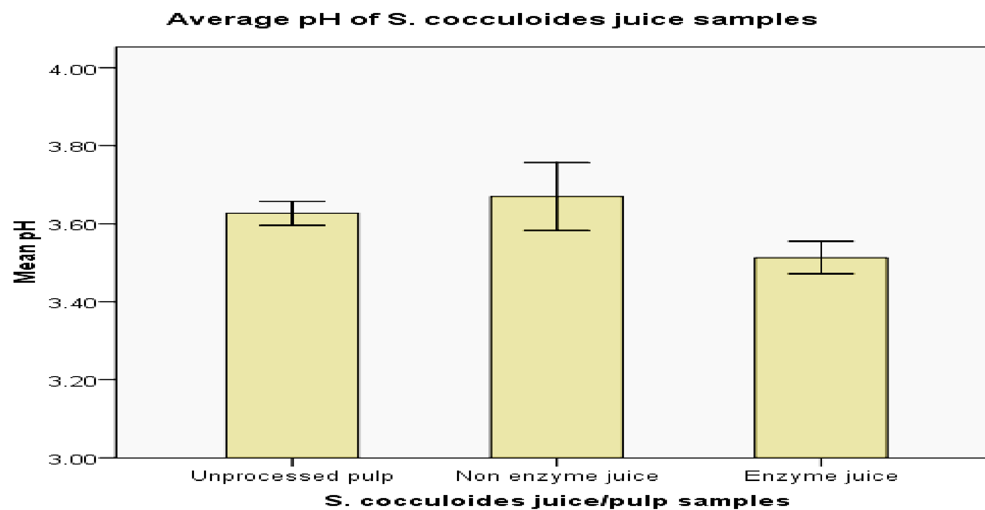


Fig. 12: Effect of enzyme treatment on pH of *S. cocculoides* juice/pulp samples

The average pH of *S. cocculoides* juice samples was 3.63 ± 0.02, 3.67 ± 0.04 and 3.51 ± 0.02 for the unprocessed pulp, non-enzyme macerated juice and enzyme macerated juice respectively. The pH of the enzyme juice was significantly lower ($p < 0.05$) than both the pulp and the non-enzyme juice. There was significant difference among all the sample means.

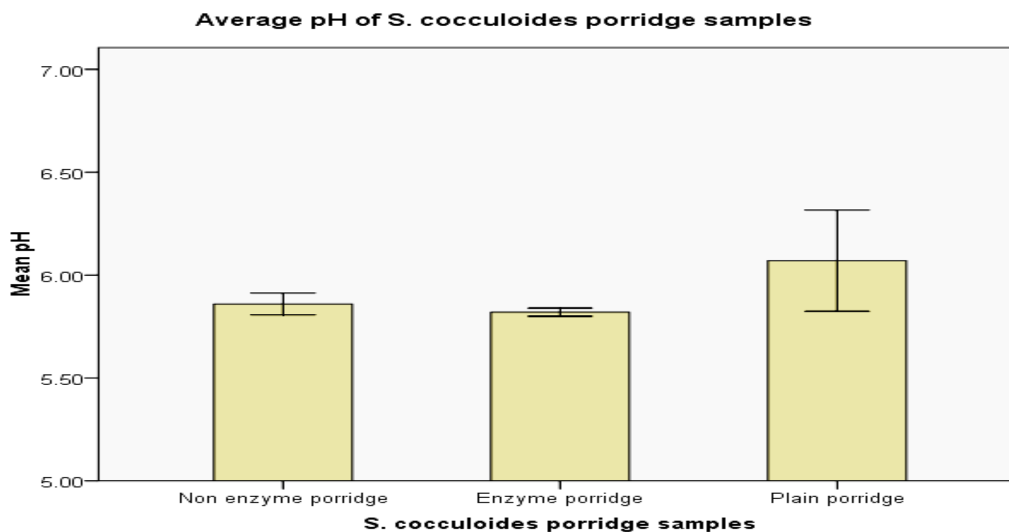


Fig. 13: Effect of enzyme treatment on pH of *S. cocculoide*s enriched porridge

The average pH of *S. cocculoides* porridge samples was 5.86 ± 0.03, 5.82 ± 0.01 and 6.07 ± 0.12 for the non-enzyme porridge, enzyme porridge and plain porridge respectively. The enzyme porridge recorded the lowest pH although there was no significant difference ($p >$ 0.05) in mean pH between the non-enzyme porridge and enzyme porridge.

4.1.2 Brix

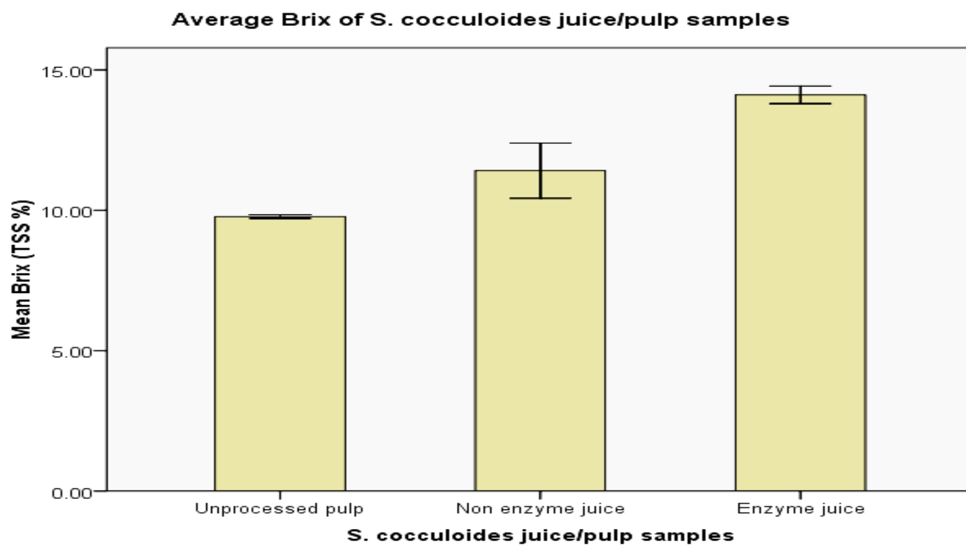


Fig. 14: Effect of enzyme treatment on Brix of *S. cocculoides* juice/pulp samples

There was an increase in brix° from unprocessed pulp to non-enzyme juice up to the enzyme juice; that is from 9.78 ± 0.03, 11.41 ± 0.49, 14.11 ± 0.16 respectively. There was significant difference ($p < 0.05$) in all sample means.

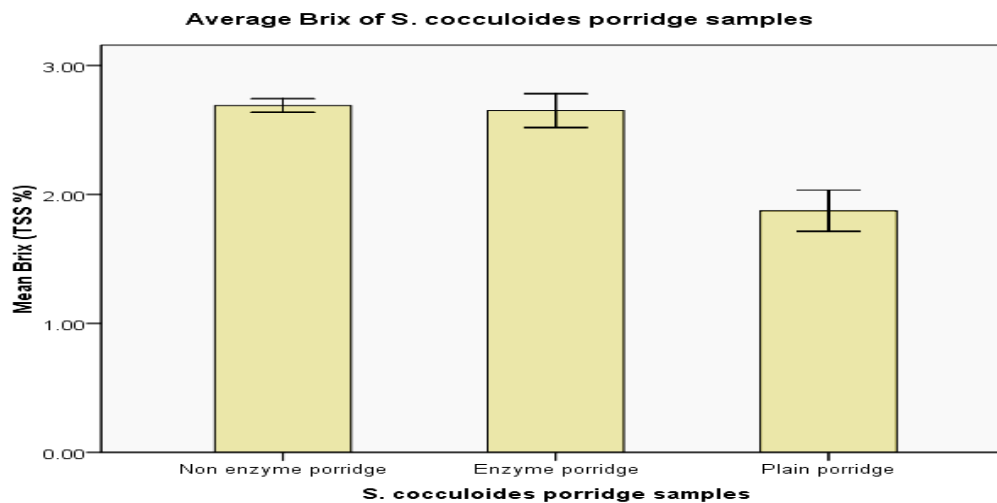


Fig. 15: Effect of enzyme treatment on Brix of *S. cocculoides* enriched porridge

The average brix° for the porridge samples was 2.69 ± 0.03, 2.65 ±0.07, and 1.87 ± 0.08 for the non-enzyme porridge, enzyme porridge and plain porridge respectively. There was no significant difference ($p > 0.05$) between the non-enzyme porridge and the enzyme porridge. However brix° for plain porridge was significantly lower than the other two samples.

4.1.3 Dry matter

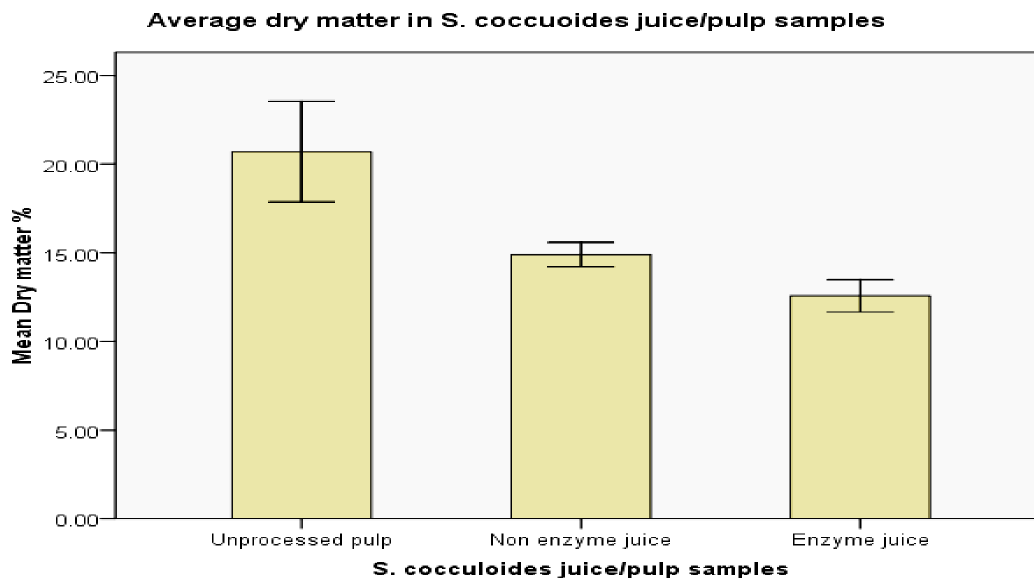


Fig. 16: Effect of enzyme treatment on dry matter of *S. cocculoides* juice/pulp samples

The dry matter was recorded as 20.70 ± 1.42 %, 14.90 ± 0.34 % and 12.58 ± 0.46 % for the pulp, non-enzyme juice and enzyme juice respectively. There was significant difference ($p < 0.05$) in all sample means.

For the porridge samples the dry matter was recorded as 62.43 ± 0.64 %, 61.04 ± 1.07 % and 59.46 ± 1.13 % for the non-enzyme porridge, enzyme porridge and plain porridge respectively (**Fig. 19**). There was no significant difference ($p > 0.05$) between sample means of dry matter between all three porridge samples.

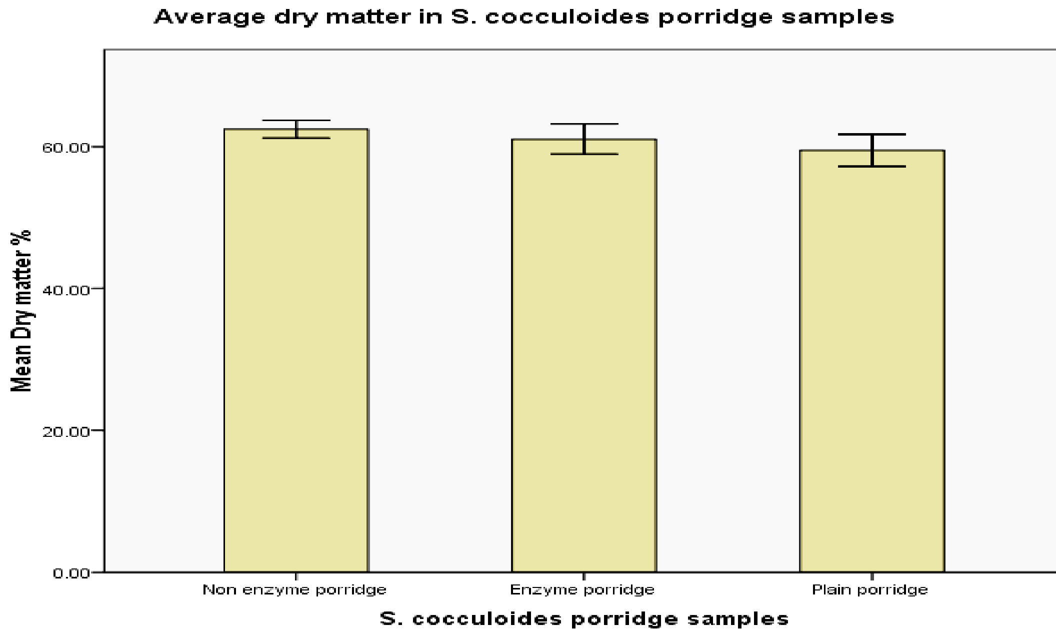


Fig. 17: Effect of enzyme treatment on dry matter of *S. cocculoides* enriched porridge

4.1.4 Individual sugars (glucose, sucrose fructose)

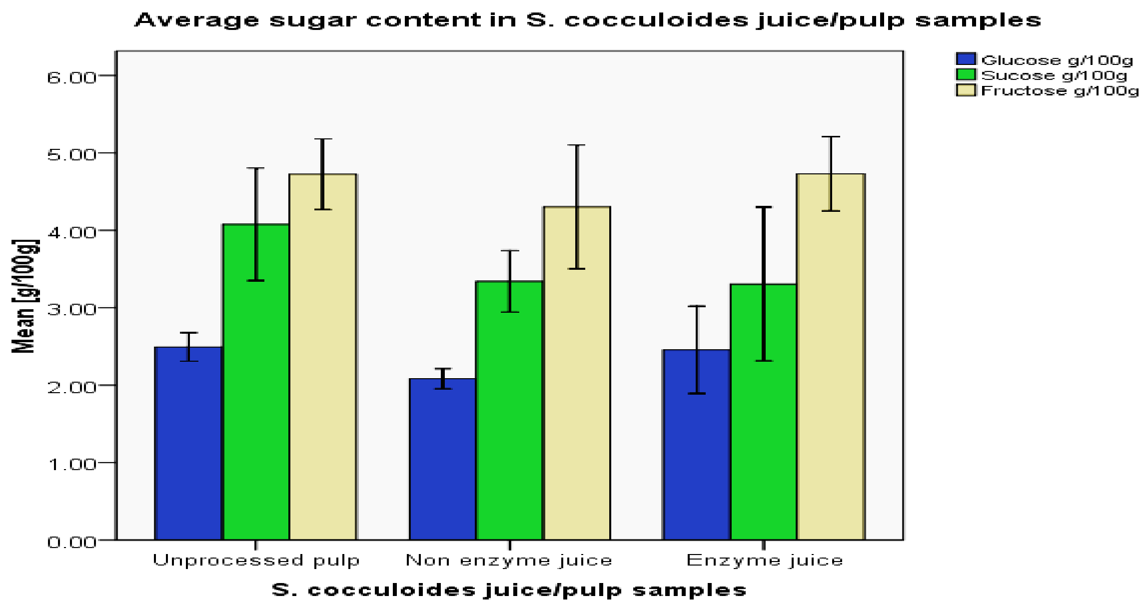


Fig. 18: Effect of enzyme treatment on sugar composition of *S. cocculoides* juice/pulp samples

Fructose was the dominant sugar in all juice samples, however there was high variability characterised by large standard deviations. The recorded values of individual sugar content in unprocessed pulp were 2.49 ± 0.16 mg/100g, 4.04 ± 1.07 mg/100g and 4.76 ± 0.29 mg/100g

for glucose, sucrose and fructose respectively; in non-enzyme juice the content was 2.08 ± 0.11 mg/100g, 3.34 ± 0.35 mg/100g and 4.24 ± 0.4 mg/100g for glucose, sucrose and fructose respectively; and in enzyme juice the content was 2.23 ± 0.38 mg/100g, 3.31 ± 0.86 mg/100g and 4.73 ± 0.59 mg/100g for glucose, sucrose and fructose respectively. There was significant difference ($p < 0.05$) between all sample means.

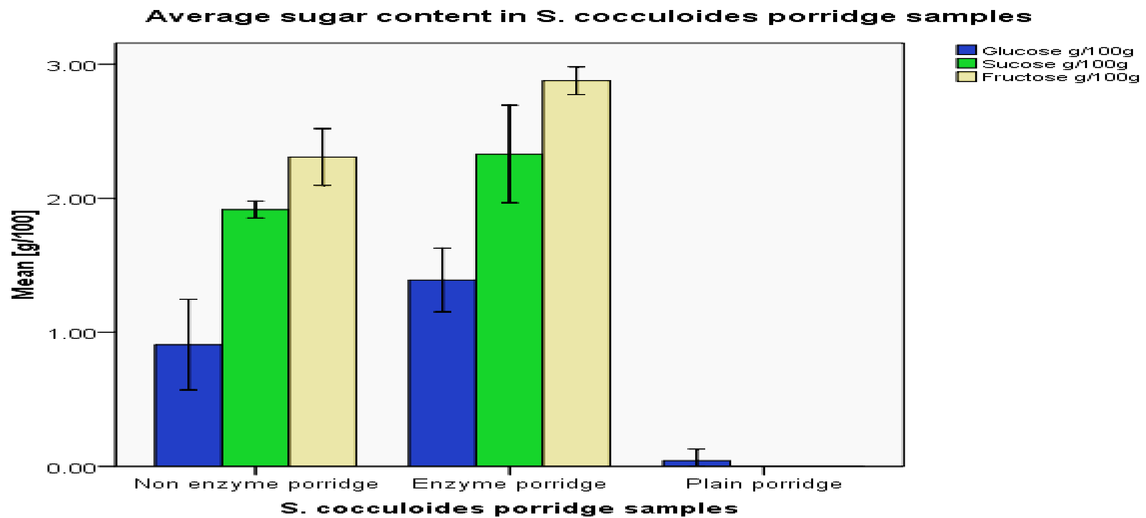


Fig. 19: Effect of enzyme treatment on sugar composition of *S. cocculoides* enriched porridge

For the porridge samples plain porridge had the lowest sugar content recorded only for glucose at 0.04 ± 0.08 mg/100g. The recorded values of individual sugar content in non-enzyme porridge were 0.91 ± 0.29 mg/100g, 1.92 ± 0.06 mg/100g and 2.31 ± 0.18 mg/100g for glucose, sucrose and fructose respectively; and for enzyme porridge the content was 1.39 ± 0.21 mg/100g, 2.33 ± 0.31 mg/100g and 2.88 ± 0.09 mg/100g for glucose, sucrose and fructose respectively. There was significant difference ($p < 0.05$) between all sample means.

4.1.5 Protein content

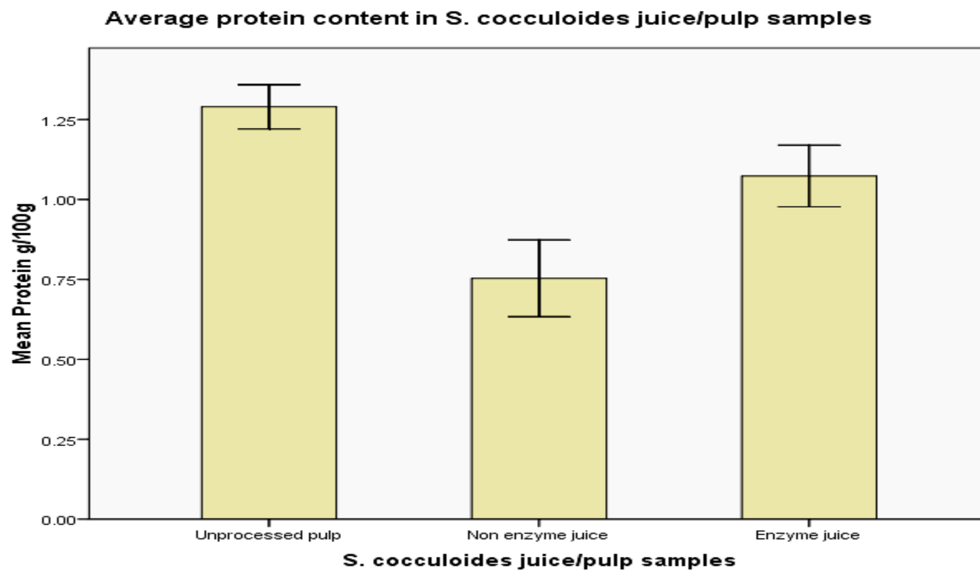


Fig. 20: Effect of enzyme treatment on protein content in *S. cocculoides* juice/pulp samples

The protein content in the juice samples ranged from 1.29 ± 0.04 g/100g, 0.75 ± 0.10 g/100g to 1.07 ± 0.08 g/100g for unprocessed pulp, non-enzyme macerated juice and enzyme macerated juice respectively. There was significant difference ($p < 0.05$) between protein content means in all the samples.

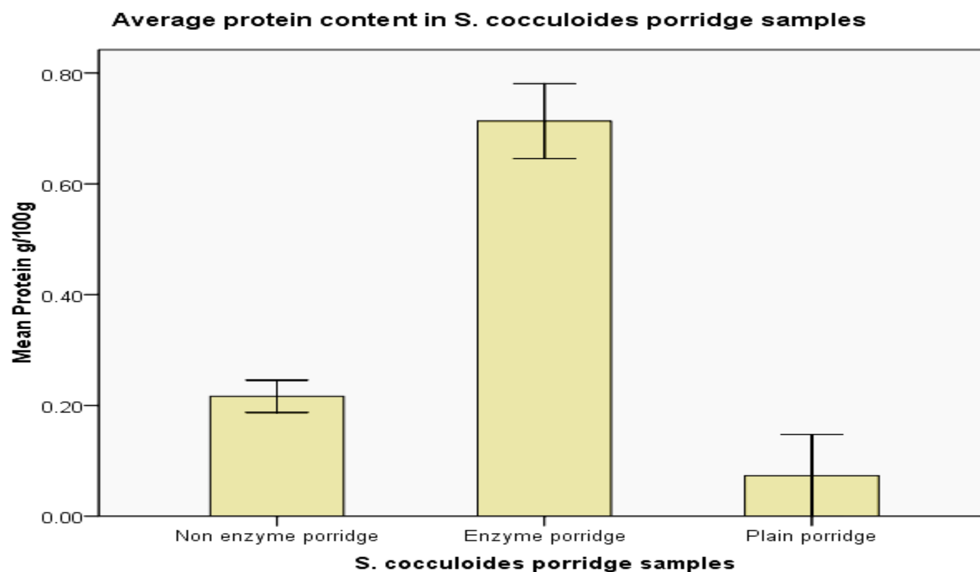


Fig. 21: Effect of enzyme treatment on protein content in *S. cocculoides* enriched porridge

The protein content in the porridge samples ranged from 0.22 ± 0.03 g/100g, 0.71 ± 0.06 g/100g to 0.07 ± 0.08 g/100g for non-enzyme porridge, enzyme porridge and plain porridge

respectively. There was significant difference ($p < 0.05$) between protein content means in all the samples.

4.1.6 Mineral analysis (iron and zinc)

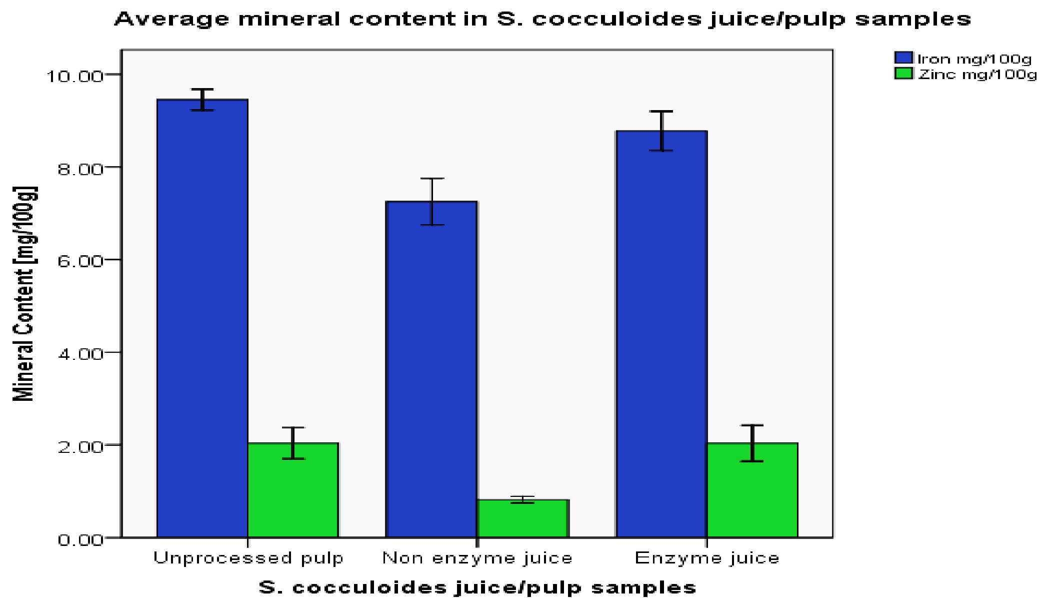


Fig. 22: Effect of enzyme treatment on iron and zinc content in *S. cocculoides* juice/pulp samples

The iron content was 9.12 ± 0.50 mg/100g, 7.25 ± 0.25 mg/100g and 8.89 ± 0.42 mg/100g for pulp, non-enzyme juice and enzyme juice respectively; whereas for zinc content the mean values were 2.04 ± 0.17 mg/100g, 0.82 ± 0.04 mg/100g and 2.0 ± 0.19 mg/100g for pulp, non-enzyme juice and enzyme juice respectively. There was no significant difference ($p > 0.05$) in zinc content between the pulp and enzyme juice samples. The means of all other samples were significantly different.

For the porridge samples the iron content was 5.76 ± 0.57 mg/100g, 4.70 ± 0.44 mg/100g and 1.76 ± 0.08 mg/100g for the enzyme porridge, non-enzyme porridge and plain porridge respectively; whereas for zinc content the mean values were 1.11 ± 0.03 mg/100g, 0.60 ± 0.04 mg/100g and 0.30 ± 0.03 mg/100g for enzyme porridge, non-enzyme porridge and plain porridge respectively (**Fig. 25**). There was significant difference ($p < 0.05$) between all samples.

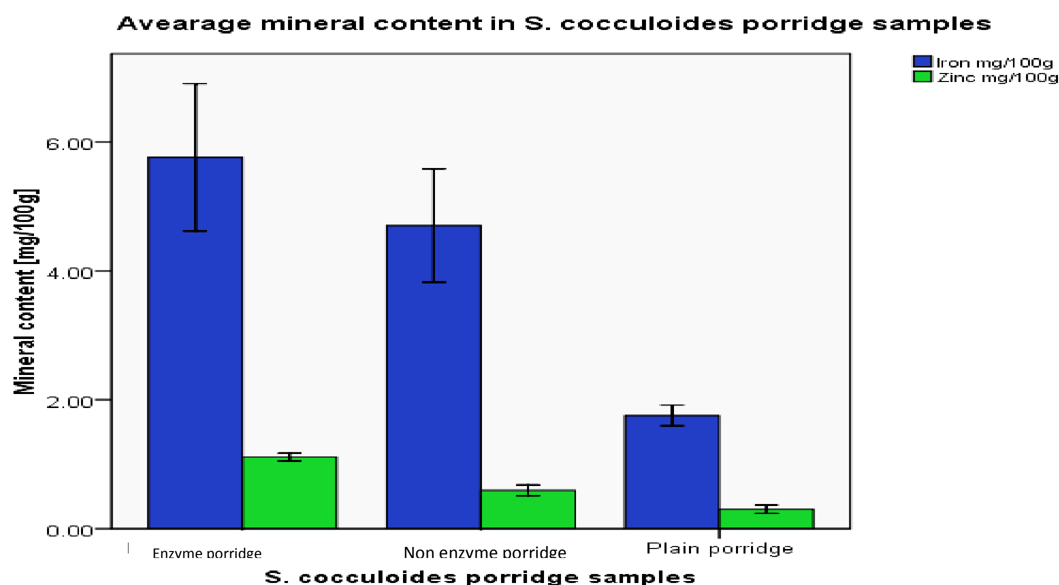


Fig. 23: Effect of enzyme treatment on iron and zinc content in *S. cocculoides* enriched porridge

4.1.7 Vitamin C (Ascorbic acid)

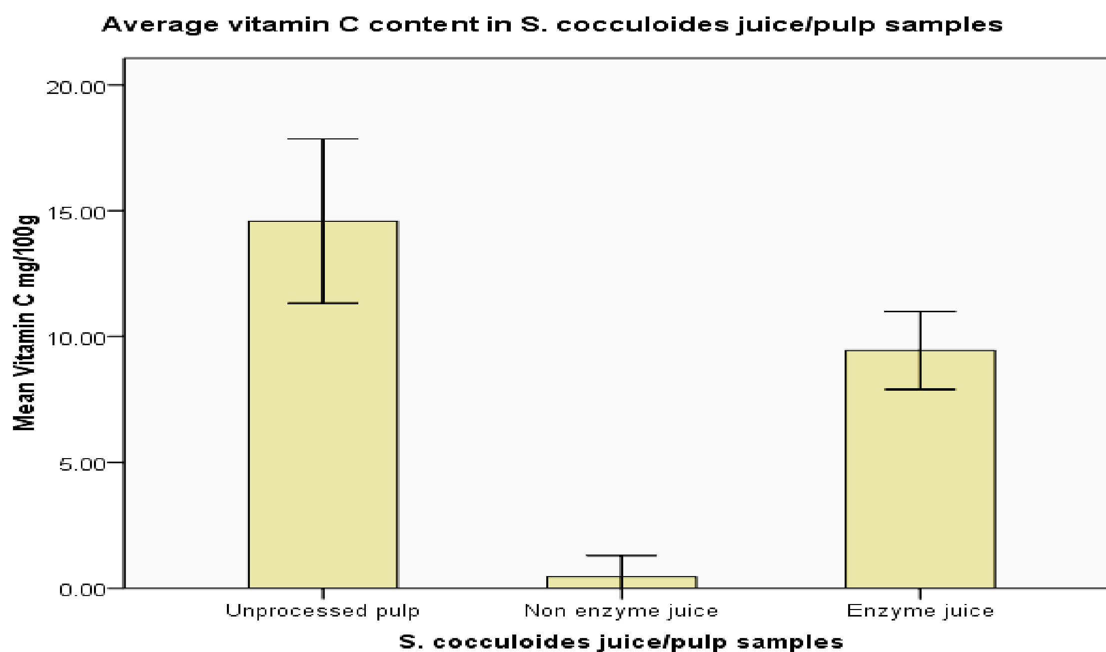


Fig. 24: Effect of enzyme treatment on vitamin C content of *S. cocculoides* juice/pulp samples

The vitamin C content of the juice samples was recorded as 14.58 ± 1.64 mg/100g, 0.46 ± 0.42 mg/100g and 9.45 ± 0.77 mg/100g for pulp, non-enzyme juice and enzyme juice respectively. There was significant difference ($p < 0.05$) in vitamin C content of all samples.

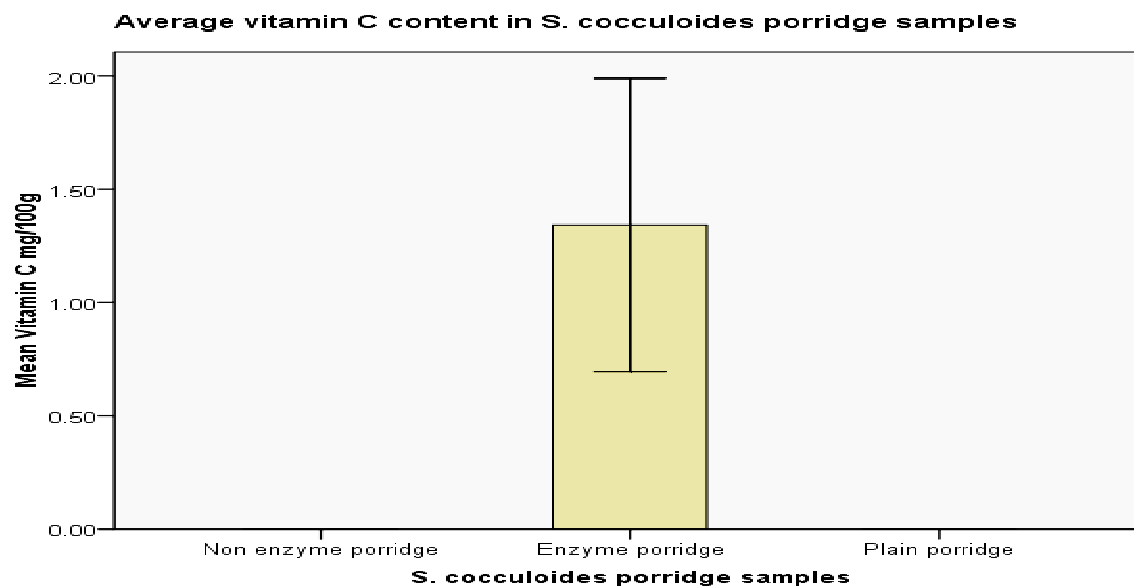


Fig. 25: Effect of enzyme treatment on vitamin C content of *S. cocculoides* enriched porridge

For the porridge samples vitamin C was only detected in the enzyme porridge at 1.34 ± 0.32 mg/100g.

4.1.8 Total phenol content

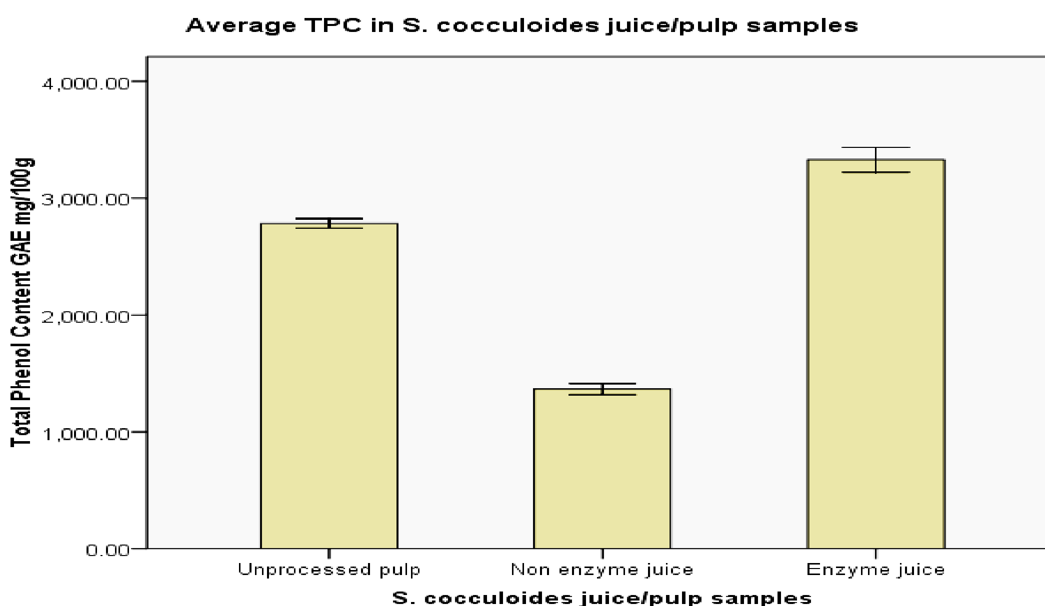


Fig. 26: Effect of enzyme treatment on total phenol content of *S. cocculoides* juice/pulp samples

The total phenol content of was recorded as 2783.45 ± 20.65 mg/100g, 1365.57 ± 23.64 mg/100g and 3327.75 ± 52.85 mg/100g for pulp, non-enzyme juice and enzyme juice

respectively. There was significant difference ($p < 0.05$) in total phenol content of all juice samples.

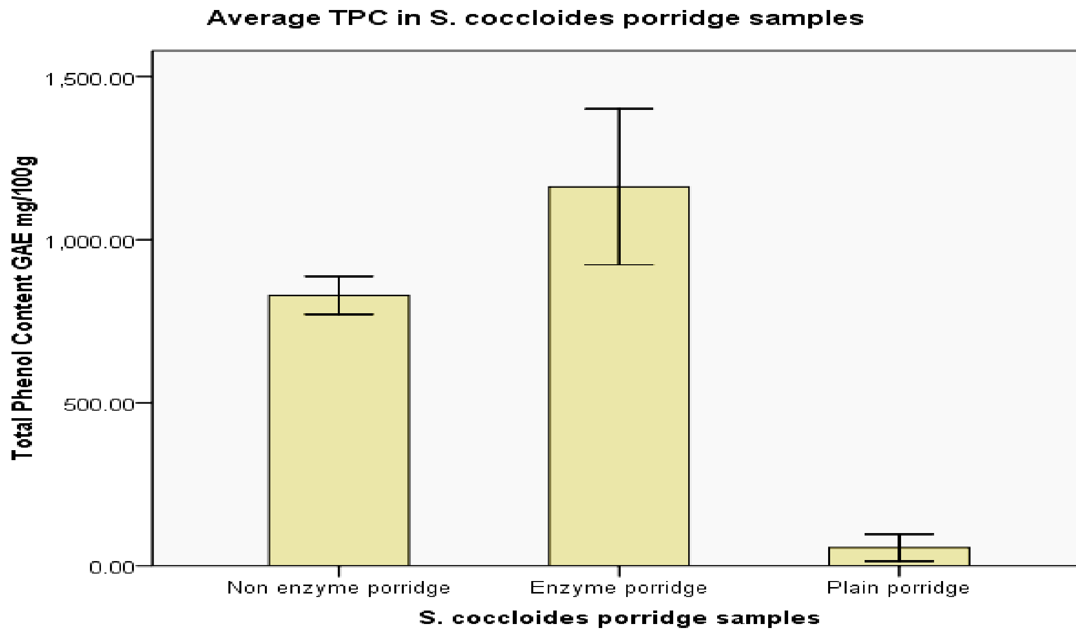


Fig. 27: Effect of enzyme treatment on total phenol content of *S. cocculoides* enriched porridge

For the porridge samples the total phenol content was recorded as 829.15 ± 29.36 mg/100g, 1162.57 ± 119.41 mg/100g and 56.34 ± 20.73 mg/100g for non-enzyme porridge, enzyme porridge and plain porridge respectively. There was significant difference ($p < 0.05$) in total phenol content of all porridge samples.

4.2 Effects of enzyme maceration on antioxidant and antimicrobial activities

4.2.1 DPPH radical-scavenging activity (antioxidant activity)

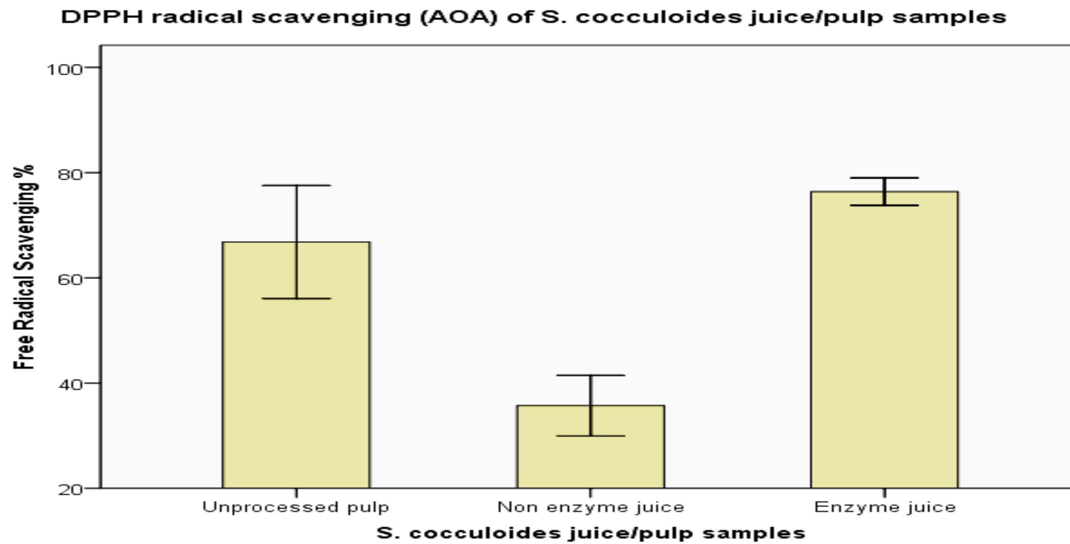


Fig. 28: Effect of enzyme treatment on AOA of *S. cocculoides* juice/pulp samples

The DPPH radical-scavenging activity was recorded as 66.82 ± 5.36 %, 35.72 ± 2.88 % and 76.40 ± 1.30 % for pulp, non-enzyme juice and enzyme juice respectively. There was significant difference ($p < 0.05$) in antioxidant activity between all juice samples.

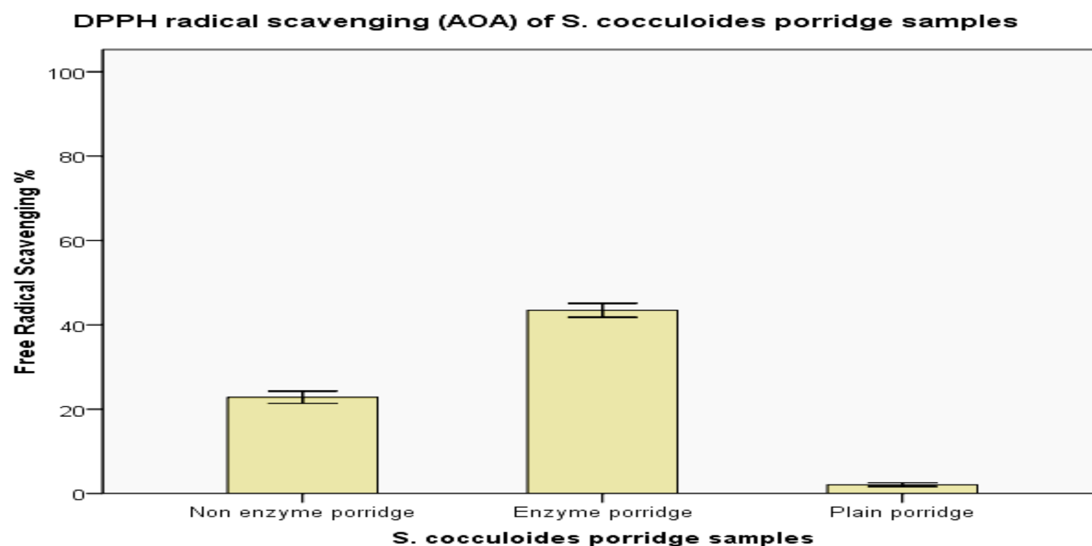


Fig. 29: Effect of enzyme treatment on AOA of *S. cocculoides* enriched porridge

For the porridge samples the DPPH radical-scavenging activity was recorded as 22.82 ± 0.73 %, 43.44 ± 0.83 % and 2.10 ± 0.23 % for non-enzyme porridge, enzyme porridge and plain porridge respectively. There was significant difference ($p < 0.05$) in antioxidant activity between all juice samples, with plain porridge recording the lowest.

4.2.2 Antimicrobial activity

Table 6: Antimicrobial activity of S. cocculoides juice/pulp samples

	Zone of inhibition in mm					
	Bacteria				Fungi	
	S. aureus	*P. aerugonisa*	*E. coli*	*Salmonella spp.*	*C. albicans*	*A. niger*
Unprocessed Pulp	13.33 ± 0.58^{b}	15.33 ± 1.53^{b}	12 ± 1.00^{b}	-	2.33 ± 0.58^{b}	2.33 ± 0.58^{a}
Non enzyme juice	4.33 ± 0.58^{a}	3.67 ± 0.58^{a}	3 ± 1.00^{a}	-	1.33 ± 0.58^{a}	-
Enzyme juice	17 ± 0.58^{c}	16.33 ± 1.53^{b}	18.67 ± 0.58^{c}	1.33 ± 0.58^{a}	3.33 ± 0.58^{c}	5.33 ± 0.58^{b}
Tetracycline (ctrl)	18 ± 1.00^{c}	24.5 ± 0.58^{c}	18.5 ± 1.53^{c}	-	-	16 ± 0.58^{c}

Similar letters in the same column indicate that means are not significantly different ($p < 0.05$)

The enzyme macerated juice recorded the largest diameters (zone of inhibition) for all microbes followed by unprocessed pulp and lastly non-enzyme juice as shown in Table 6. There was no significant difference ($p > 0.05$) in zones of inhibition for the control (tetracycline) and enzyme juice against *E. coli* and for unprocessed pulp and enzyme juice against *P. aerugonisa*.

4.3 Effects of enzyme maceration on in-vitro bioaccessibility of selected micronutrients

4.3.1 Vitamin C

Table 7: Effects of in vitro digestion on vitamin C content of *S. cocculoides* juice/porridge samples

	Vitamin C (mg/100g)			
	[1]In-vitro digestion phase			
Sample	Undigested	Gastric	Intestinal	Bioaccessibility %
Non Enzyme Juice	0.46 ± 0.42[c]	0.35 ± 0.35[b]	0.08 ± 0.08[a]	17.39 ± 0.56**
Enzyme juice	9.45 ± 0.77[c]	7.37 ± 0.92[b]	3.58 ± 0.55[a]	37.89 ± 1.73***
Non Enzyme Juice Porridge	N.D.	N.D.	N.D.	-
Enzyme Juice Porridge	1.01 ± 0.58[c]	0.60 ± 0.31[b]	0.11 ± 0.27[a]	10.89 ± 0.47*

Similar letters in the same row indicate that means are not significantly different ($p < 0.05$)

The enzyme juice recorded the highest in-vitro bioaccessibility of vitamin C (37.89 %), followed by non-enzyme juice (17.39 %) and lastly enzyme juice porridge (10.89). Vitamin C was not detected in non-enzyme porridge. There was significant difference ($p < 0.05$) in bioaccessibility of vitamin C between samples.

4.3.2 Total phenol content (TPC)

Table 8: Effects of in vitro digestion on TPC of *S. cocculoides* juice/porridge samples

	TPC (mg/100g)			
	[1]In-vitro digestion phase			
Sample	Undigested	Gastric	Intestinal	Bioaccessibility%
Non Enzyme Juice	1365.57 ± 23.64[b]	1498.22 ± 8.89[c]	259.58 ± 54.09[a]	19.01 ± 0.03*
Enzyme Juice	3327.75 ± 52.85[b]	3410.72 ± 55.57[c]	1222.90 ± 29.72[a]	36.75 ± 0.18***
Non Enzyme Juice Porridge	829 ± 29.36[a]	786.36 ± 81.90[c]	203.51 ± 22.21[b]	24.55 ± 5.09**
Enzyme Juice Porridge	1162.12 ± 119.41[c]	982.02 ± 31.98[b]	245.18 ± 44.29[a]	21.10 ± 4.12**

Similar letters in the same row indicate that means are not significantly different ($p < 0.05$)

There was an overall increase in total phenol content between the undigested and gastric phase for the non-enzyme juice and enzyme juice. However there was a decrease of total

phenol content after simulated intestinal digestion giving overall bioaccessibility of 19.01 % and 36.75 % for non-enzyme juice and enzyme juice respectively. Bioaccessibility of 24.55 % and 21.10 % was recorded for non-enzyme juice porridge and enzyme juice porridge respectively. There was no significant difference ($p > 0.05$) in bioaccessibility of total phenol count in the porridge samples.

4.3.3 Antioxidant activity (AOA)

Table 9: Effects of in vitro digestion on AOA of *S. cocculoides* juice/porridge samples

	AOA (DPPH radical scavenging %)			
	[1]In-vitro digestion phase			
Sample	Undigested	Gastric	Intestinal	Bioaccessibility %
Non Enzyme Juice	35.72 ± 2.88[b]	39.14 ± 1.40[c]	12.33 ± 2.18[a]	34.52 ± 0.05**
Enzyme juice	76.40 ± 1.30[b]	78.70 ± 0.96[c]	35.08 ± 0.94[a]	45.92 ± 0.32****
Non Enzyme Juice Porridge	22.82 ± 0.73[b]	21.82 ± 0.97[b]	6.19 ± 0.41[a]	27.13 ± 0.11*
Enzyme Juice Porridge	43.44 ± 0.83[c]	39.66 ± 0.77[b]	15.70 ± 1.31[a]	36.14 ± 0.1***

Similar letters in the same row indicate that means are not significantly different ($p < 0.05$)

There was an increase in AOA from the undigested sample to the gastric digests of 35.72 ± 2.88 % to 39.14 ± 1.40 % and 76.40 ± 1.30 % to 78.70 ± 0.96 % for non-enzyme juice and enzyme juice respectively. After intestinal digestion the residual AOA was 12.33 ± 2.18 % and 35.08 ± 0.94 % for non-enzyme juice and enzyme juice respectively. However, for the porridge samples there was decrease in AOA along the digestion phases. The bioaccessibility of the four samples was recorded as 34.52 ± 0.05 %, 45.92 ± 0.32 %, 27.13 ± 0.11 % and 36.14 ± 0.1 % for non-enzyme juice, enzyme juice, non-enzyme juice porridge and enzyme juice porridge respectively.

4.3.4 Minerals (Iron and zinc)

Table 10: Effects of in vitro digestion on iron content of *S. cocculoides* juice/porridge samples

	Iron content (mg/100g)		
	[1]In-vitro digestion		
Sample	Undigested	After digestion	Bioaccessibility %
Non Enzyme Juice	7.25 ± 2.25[b]	1.65 ± 0.58[a]	16.09 ± 0.12***
Enzyme juice	8.89 ± 5.12[b]	3.29 ± 0.27[a]	28.76 ± 0.21****
Non Enzyme Juice Porridge	4.70 ± 0.44[b]	0.23 ± 0.13[a]	3.99 ± 0.35*
Enzyme Juice Porridge	5.76 ± 0.57[b]	0.45 ± 0.57[a]	9.57 ± 0.24**

Similar letters in the same row indicate that means are not significantly different ($p < 0.05$)

Iron bioaccessibility was least for non-enzyme juice porridge and highest for enzyme juice. All means were significantly different ($p < 0.05$) across all digestion phases.

Table 11: Effects of in vitro digestion on zinc content of *S. cocculoides* juice/porridge samples

	Zinc content (mg/100g)		
	[1]In-vitro digestion		
Sample	Undigested	After digestion	Bioaccessibility %
Non Enzyme Juice	0.82 ± 0.04[b]	0.07 ± 0.05[a]	8.53 ± 1.45**
Enzyme juice	2.00 ± 0.19[b]	0.37 ± 0.07[a]	18.14 ± 0.67****
Non Enzyme Juice Porridge	0.60 ± 0.04[b]	0.05 ± 0.06[a]	0.90 ± 0.33*
Enzyme Juice Porridge	1.11 ± 0.03[b]	0.17 ± 0.04[a]	16.67 ± 0.14***

Similar letters in the same row indicate that means are not significantly different ($p < 0.05$)

Zinc bioaccessibility was least for non-enzyme juice porridge and highest for enzyme juice. All means were significantly different ($p < 0.05$) across all digestion phases.

4.4 Effects of enzyme maceration on sensory attributes of *S. cocculoides* juice/porridge samples

4.4.1 Triangle test (Discrimination between enzyme and non-enzyme macerated)

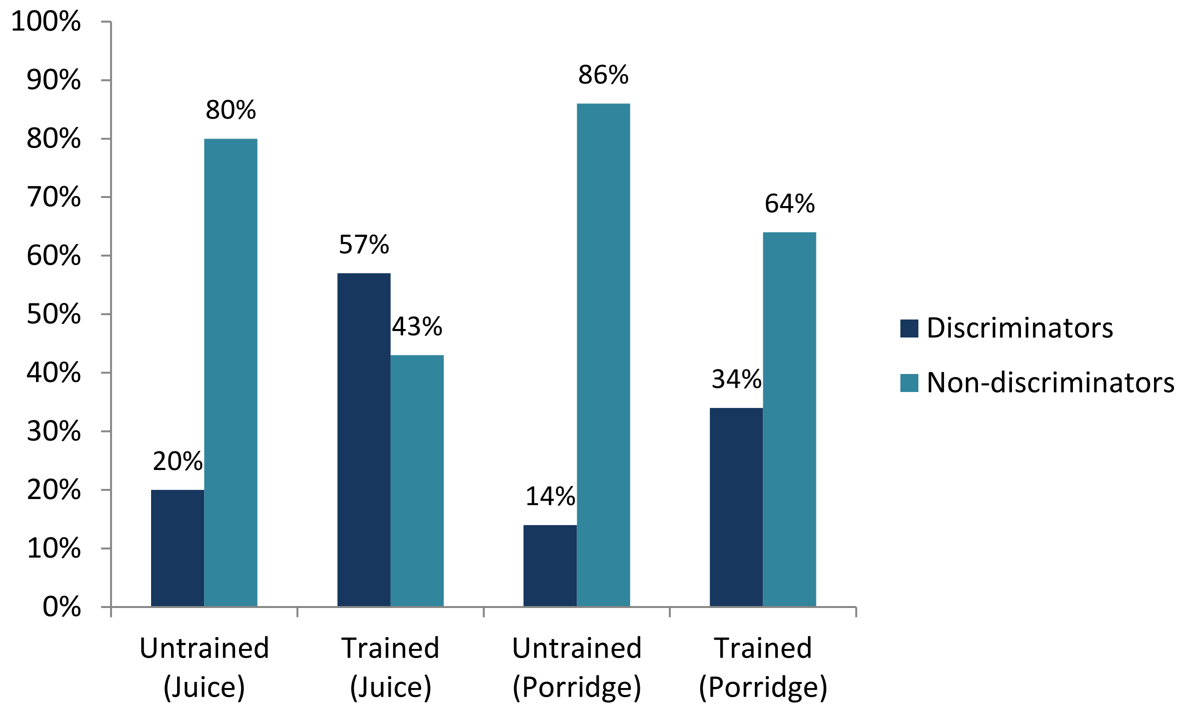


Fig. 30: Sensorial discrimination between enzyme and non-enzyme macerated *S. cocculoides* juice/porridge by trained (*n = 12*) and untrained panelists (*n = 132*)

There was significance difference ($p < 0.05$) between the trained and untrained panelists in the ability to pick out the odd sample for both the juice and the porridge with trained panelists exhibiting a significantly higher success rate.

4.4.2 Preference test (between enzyme and non-enzyme macerated *S. cocculoides* juice/porridge

There was significant difference ($p < 0.05$) in preference between enzyme juice porridge and non-enzyme juice porridge with the enzyme porridge being the most preferred (Fig. 33); and between enzyme juice and non-enzyme juice with the enzyme juice being the most preferred (Fig. 34).

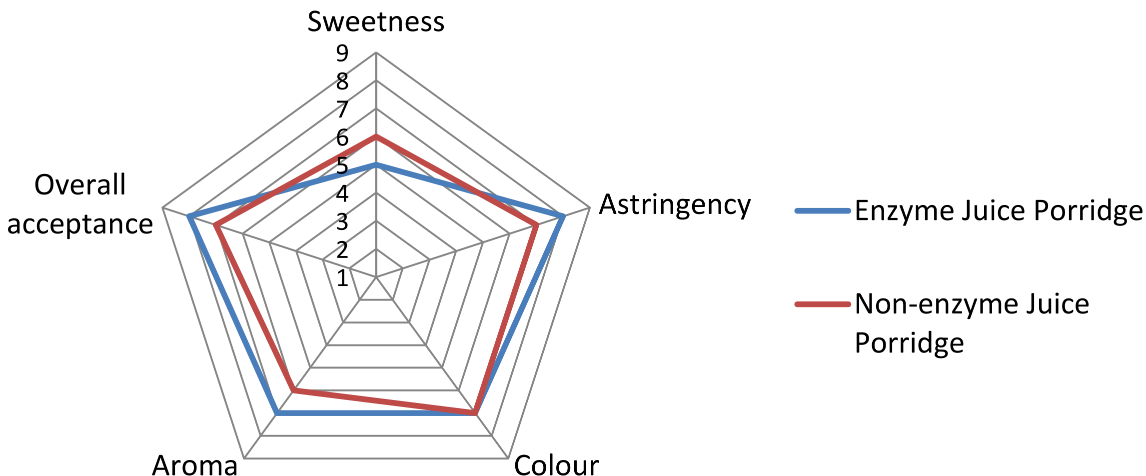


Fig. 31: Preference of different sensorial attributes of *S. cocculoides* enriched porridge samples

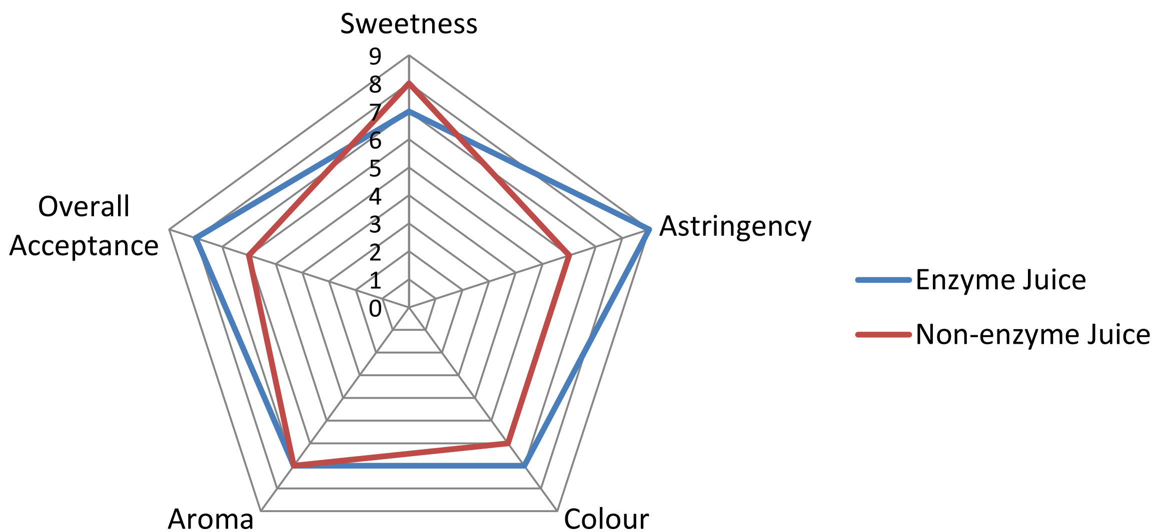


Fig. 32: Preference of different sensorial attributes of *S. cocculoides* juice samples

CHAPTER 5

5.0 DISCUSSION

5.1 Effect of enzyme maceration on different physicochemical properties

Different physicochemical properties were assayed and results are discussed below. To the best of the researcher's knowledge, data on physicochemical properties of *S. cocculoides* is still limited and scarce.

5.1.1 pH

The enzyme macerated juice recorded the lowest average pH value (3.51 ± 0.02) compared to unprocessed pulp (3.63 ± 0.02) and non-enzyme macerated juice (3.67 ± 0.04) (Fig. 12). The lower pH in enzyme macerated juice can be attributed to the breakdown of complex polysaccharides (pectin) of the fruit pulp into simpler compounds chief of which is galacturonic acid (Kumar, 2015). The results are in agreement with Saka et. al (2007), who reported mean pH of 3.53 ± 0.05 for *S. cocculoides* juice (albeit non-enzyme macerated). The difference in pH between non-enzyme porridge (5.86 ± 0.03) and enzyme porridge (5.82 ± 0.01) was not significant ($p > 0.05$) (Fig. 13), depicting that the maize-meal could have had a masking effect on the pH levels of juice samples. However the pH of the plain porridge (6.07 ± 0.12) was significantly higher clearly showing the effect of *S. cocculoides* juice in reducing the pH of the porridge samples.

5.1.2 Brix (TSS)

The brix levels increased from unprocessed pulp (9.78 ± 0.03) to non-enzyme juice (11.41 ± 0.49) up to the enzyme juice (14.11 ± 0.16) (Fig. 14). This increase in brix can be attributed to the enzyme and heat treatment which facilitate the breakdown of the insoluble pectin into simpler soluble compounds such as simpler sugars from the complex polysaccharides (Kumar, 2015). The obtained values are different from Saka et al. (2007) who recorded 32.3

± 1.9. The enzyme juice has the highest brix levels since the pectinase used is more potent in pectin breakdown than heat alone. There was no significant difference ($p > 0.05$) in brix between the non-enzyme porridge and the enzyme porridge, although brix for plain porridge was significantly lower than the other two samples (Fig. 15) Overall brix for porridge samples was significantly lower than for juice samples due to the addition of insoluble gelatinised starch from the maize-meal.

5.1.3 Dry matter

The enzyme juice recorded the least dry matter followed by non-enzyme juice and then unprocessed pulp (Fig. 16). This can also be attributed to the breakdown of pectin in to soluble by-products (Tapre and Jain, 2014; Kumar, 2015). However there was no significant difference ($p < 0.05$) between sample means of dry matter between all three porridge samples (Fig. 17). This could also be attributed to the masking effect of the gelatinised starch in the maize-meal (Onyango, 2014).

5.1.4 Individual sugars (glucose, sucrose fructose)

There was high variability in sugar content between samples characterised by large standard deviations (Fig. 18, 19). Nonetheless fructose was the dominant sugar in most of the samples assayed. The enzyme treated samples recorded higher sugar contents, which can be attributed to the breakdown of the pulp matrix to release soluble fractions (Kumar, 2015). The variability of the sugar content can be attributed to the maturity index of sample fruits as sugar content often varies according to ripening stage, where sucrose is broken down to fructose and glucose (Bahramian, 2011; Lee, 2013). Acquiring sample fruits with the same maturity index is often problematic.

5.1.5 Protein content

As expected protein content was very low in all samples with the unprocessed pulp recording the highest content of 1.29 ± 0.04 g/100g, non-enzyme juice (0.75 ± 0.10 g/100g) and enzyme juice (1.07 ± 0.08 g/100g) (Fig. 20). The protein content in the porridge samples was even lower. These values are lower than the values highlighted in a review by Ngadze et al. (2017), where mean protein content 3.5 g/100g (Fig. 21)). The unprocessed pulp recorded the highest protein content and was lower in other samples as prolonged heating at high temperatures caused denaturation.

5.1.6 Iron and zinc content

The iron content was 9.12 ± 0.50 mg/100g, 7.25 ± 0.25 mg/100g and 8.89 ± 0.42 mg/100g for pulp, non-enzyme juice and enzyme juice respectively; whereas for zinc content the mean values were 2.04 ± 0.17 mg/100g, 0.82 ± 0.04 mg/100g and 2.0 ± 0.19 mg/100g for pulp, non-enzyme juice and enzyme juice respectively (Fig. 22). For the porridge samples the iron content was highest in the enzyme porridge (5.76 ± 0.57 mg/100g) and this porridge has the potential to be utilised in the fight against iron deficiency in the rural areas where consumption of maize-meal porridge is rife. For zinc content the mean values were 1.11 ± 0.03 mg/100g, 0.60 ± 0.04 mg/100g and 0.30 ± 0.03 mg/100g for enzyme porridge, non-enzyme porridge and plain porridge respectively (Fig. 23).

Iron and zinc deficiencies continue to be a problem in Zimbabwe especially in rural populations (Gagada et al., 2009; ZDHS, 2016). From the results observed it can be deduced that enzyme treatment enhances mineral content. Although these values indicate appreciable levels of mineral content (given RDA: Iron = 13 - 19 mg/100g; Zinc = 3 - 11 mg/100g (ZDHS, 2016)), they fall short of the averages reported by Ngadze et al (2017), that is 70.5 mg/100g for iron and 0.4 mg/100g for zinc. On the other hand the obtained values are in agreement with Saka et al. (2007) who recorded 2.13 ± 0.33 mg/100g for zinc in *S.*

cocculoides juice. Amarteifio & Mosase (2006) reported significantly lower contents of iron and zinc in other indigenous fruits (*A. digitata*; 0.10 mg/100g for iron and 0.14 mg/100g for zinc; *V. infausta*; 0.09 mg/100g for iron and 0.02 mg/100g for zinc), hence *S. cocculoides* is a better source of iron and zinc.

5.1.7 Vitamin C

The importance of vitamin C as an essential nutrient can never be over stated. Besides being involved in the synthesis of collagen and some hormones, it has also been associated with lower cancer risk, wound healing, reduction in susceptibility to infections, formation of bones and teeth, iron absorption and prevention of scurvy and cardiovascular diseases (Yang et al., 2009; Kagawa et al., 2009).The vitamin C content of the juice samples was recorded as 14.58 ± 1.64 mg/100g, 0.46 ± 0.42 mg/100g and 9.45 ± 0.77 mg/100g for pulp, non-enzyme juice and enzyme juice respectively (Fig. 24). For the porridge samples vitamin C was only detected in the enzyme porridge at 1.34 ± 0.32 mg/100g (Fig. 25). The trend shows drastic reduction of vitamin C content with increase in processing temperature (45°C followed by 90°C - enzyme juice, 100°C non-enzyme juice). Further heating in porridge preparation resulted in further reduction. Vitamin C is highly sensitive to heat especially more than 70°C, where it tends to leach out into surrounding solution due to its solubility (Igwemmar, Kolawole & Imran, 2013). Ngadze et al. (2017) reported a range 34.2 mg/100g to 88 mg/100g of vitamin C content in monkey orange fruits. This is significantly lower than the values obtained in this study. When compared to other indigenous fruits in the region, that is, marula (*Sclerocarya birrea*) (128.3 mg/100g) and baobab (*Adansonia digitata*) (141.3 mg/ 100g) (Amarteifio & Mosase, 2006) vitamin C content of *S. cocculoides* juice is also significantly lower. The recommended RDA for vitamin C is 46mg/d (mean) (ZDHS, 2016)

5.1.8 Total phenol content

The total phenolic compound content was determined by the Folin-Ciocalteu method for all the samples. The total phenol content increased after enzyme maceration (3327.75 ± 52.85 mg/100g) from the unprocessed pulp (2783.45 ± 20.65 mg/100g), and was lowest in the non-enzyme juice (1365.57 ± 23.64 mg/100g) (Fig. 26). Enzyme maceration releases the bioactive compounds from the pectic matrix hereby increasing the total phenol yield (Sharma, Patel & Sharma, 2014; Kumar, 2015). The same trend was observed for the porridge samples. Prior, Wu, & Schaich (2005) reported the ability of non-phenolic compounds (such as sugar, amines and organic acids) to reduce the Folin-Ciocalteu reagent. Hence, these could have also contributed to the high TPC values. The interest in phenolic compounds is fast gaining pace largely to their ubiquitous nature in fruits and vegetables, and their antioxidant properties. Consumption of foods with sufficient phenolic compounds has been linked to various health-beneficial properties such as (but not limited to antibacterial/antifungal, anticancer, cholesterol-lowering and immunosuppressant activities (Vaishnav & Demain, 2010). Studies by Ndlala et al., (2007 a,b) also showed high contents of phenolic compounds where they concentrated on the following indigenous fruits of Zimbabbwe; *Ximenia caffra* (2280.73mg/100g), *Artobotrys brachypetalus* (2230.56mg/100g), *Syzygium cordatum* (200.56mg/100g), *Sclerocarya birrea* (22620mg/100g) and *Flacourtia indica* (3340mg/100g). When compared to tropical fruits indigenous fruits are better sources of phenolic compounds as Su et al. (2002) the following for total phenol content of some tropical fruits; apple (296mg/100g), red grape (201mg/100g), pineapple (94mg/100g), banana (90mg/100g), peach (84mg/100g), pear (71mg/100g) and grapefruit (50mg/100g).

5.2 Effects of enzyme maceration on antioxidant and antimicrobial activities

The DPPH radical scavenging assay was chosen to access the antioxidant activity of samples due to the relatively cheap cost and ease of completion. The antioxidant activity, measured as

DPPH scavenging activity was highest in the enzyme macerated juice (76.40 ± 1.30 %) and lowest in the non-enzyme juice (35.72 ± 2.88 %) (Fig. 28). This can be attributed to the total phenol content that also followed the same trends as a result of release from the pectin matrix. There was a strong linear correlation (r = 0.87; $p < 0.05$) between antioxidant activity and total phenol content. Due to the observed results we can infer that the antioxidant activity of *S. cocculoides* is largely due to the presence of phenolic compounds. The antioxidant activity of phenolic compounds has been reported by various authors (Wang et al., 2005; Vaishnav & Demain, 2010; Sharma et al., 2014; Kumar, 2015).

The microbial strains used showed higher susceptibility to the enzyme juice compared to the other samples. Overall, the gram + strains such as *S. aureus* exhibited the highest susceptibility with zones of inhibition ranging from 4.33 mm to 17 mm (Control: 18 mm) (Table. 6). *Salmonella* spp. recorded the lowest diameter of zones of inhibition for all the juice samples, with only enzyme juice displaying antimicrobial activity at 1.33 mm (zone of inhibition). Studies on antimicrobial properties of indigenous fruits are still very scarce and fragmented. However when compared to other tropical fruits of the same pH such as citrus fruits (Oikeh et al., 2016) *S. cocculoides* juice possesses significantly higher antimicrobial activity. The lower antibacterial activity for the gram-negative test organisms can be attributed to the periplasmic space (rich in peptidoglycans) and the lipopolysaccharide layer of the outer membrane. These present a barrier against foreign substances (Cheruiyot, Olila & Kateregga, 2009). For the fungal strains (*C. albicans* and *A. niger*) the zones of inhibition were significantly lower ($p < 0.05$) when compared to the bacterial strains. *A. niger* showed higher susceptibility compared to *C. albicans*.

The antioxidant and antimicrobial properties can also be linked to the medicinal properties of *S. cocculoides* reported by Maroyi (2013), in his review on the use of traditional medicinal plants (including roots and bark) in Zimbabwe. These properties of *S. cocculoides* have the potential to be utilised in nutrition-related intervention programmes and help in curbing

degenerative and chronic diseases in Zimbabwe, especially in rural areas where the fruit trees are found in abundance.

5.3 Effects of enzyme maceration on in-vitro bioaccessibility of selected micronutrients

In-vitro bioaccessibility/bioavailability assays are broadly being used in various areas of food and nutritional sciences as they are mostly faster, less expensive, require less labour, and are usually void of ethical constraints (Minekus et al., 2014). On the other hand, in-vivo methods (human trials and animal models) can be very expensive, resource intensive, and ethically questionable. In this study the Infogest in-vitro digestion protocol as outlined in section **3.4** was used. To the best of the researcher's knowledge, bioaccessibility assays of micronutrients in Zimbabwean indigenous foods are still extremely scarce.

5.3.1 In-vitro bioaccessibility of Vitamin C

Together with phenolic compounds, vitamin C significantly contributes to the antioxidant activity of numerous foods (Barba et al., 2012). In this study the bioaccessibility of vitamin C was 37.89 %, 17.39 % and 10.89 % for enzyme juice, non-enzyme juice and enzyme juice porridge respectively (Table. 7). Vitamin C was not detected in the plain porridge and non-enzyme porridge. Noteworthy losses in the bioaccessibility of vitamin C were recorded for the enzyme juice porridge compared to the juices. This can be attributed to the denser food matrix of the porridge when compared to juice samples. The enzyme macerated juice exhibited the highest bioaccessibility, as enzyme treatment with mild thermal treatment promotes the release of nutrients from the food matrix by cell rupture/separation and most importantly by the breakdown of the pectin (Kumar, 2015). The instability of vitamin C at high temperatures has been widely reported (Igwemmar et al., 2013) thus accounting for the significant difference ($p < 0.05$) in bioaccessibility between the enzyme juice (extracted at 45°C) and non-enzyme juice (extracted at 90°C)

5.3.2 In-vitro bioaccessibility of total phenol content

To fully understand the dynamics involved in release of phenolic compounds during digestion, it is vital to consider their location in the sample being assayed. In plant cells they are usually found in vacuoles and the apoplast, conjugated form with either mono-/polysaccharides or proteins (Bohn et al., 2015). The bioaccessibility of total phenolic compounds ranged from 19.1 % in the non-enzyme juice to 36.75 % in the enzyme juice (Table 8). Bouayed et al. (2011) reported 55 % as the bioaccessibility of phenolic compounds in apple. The enzyme juice exhibited significantly higher ($p < 0.05$) bioaccessibility of TPC compared to all the other samples. This can be attributed to the release of phenolic compounds from the pectin rich matrix by the action of the enzyme (pectinase) (Kumar, 2015). However there was no significant difference ($p > 0.05$) between the porridge samples, attributable to the effect of adding maize-meal to the matrix.

There was a notable increase in TPC after gastric digestion for the juice samples. This can be attributed to the low pH in the gastric environment which results in acidic hydrolysis of the phenolic glycosides to their aglycon forms during gastric digestion. The aglycon forms are more potent reducers of the Folin-Ciocalteu reagent (Bouayed et al., 2011a,b). The observed drastic decrease of total phenol content at the intestinal phase could be due to degradation of the phenolic compounds in the weak alkaline environment (pH 7.4) of this digestion phase. Some phenolic compounds have been reported to be highly sensitive to alkaline conditions (Bouayed et al., 2011a,b).

5.3.3 In-vitro bioaccessibility of antioxidant activity (AOA)

Prior to in-vitro digestion, antioxidant activity was recorded as 35.72 ± 2.88 %, 76.40 ± 1.30 %, 22.82 ± 0.73 % and 43.44 ± 0.83 % for non-enzyme juice, enzyme juice, non-enzyme juice porridge and enzyme juice porridge respectively (Table 9). The trend observed for AOA is the same with that of total phenol content where enzyme treated samples observed higher

bioaccessibility compared to their counterparts. As expected (from TPC results) there was an increase in AOA after gastric digestion as the acidic conditions enhance the AOA of phenolic compounds by conversion of phenolic glycosides to their aglycon forms. This also is in line with the assertions made by Bouayed et al. (2011) that aglycones phenolics are more potent antioxidants than their glycoside forms.

5.3.4 In-vitro bioaccessibility of iron and zinc

Iron is required by the human body particularly for the formation of red blood cells. The RDA for iron ranges from 13 - 19 mg/100g across different sexes and age groups (ZDHS, 2016). In this study iron bioaccessibility was recorded as 16.09 ± 0.12 %, 28.76 ± 0.21 %, 3.99 ± 0.35 % and 9.57 ± 0.24 % for non-enzyme juice, enzyme juice, non-enzyme juice porridge and enzyme juice porridge respectively (Table 10). The enzyme treated samples recorded higher bioaccessibility when compared to non-enzyme samples. This can also be attributed to the breakdown of complex polysaccharides by the pectinase to release the minerals. Khouzam, Pohl, and Lobinskib (2011) reported bioaccessibility of 6.7% to 12.7% for essential minerals in different fruits and vegetables (albeit non-enzyme macerated). The values obtained in this study for enzyme macerated juice are significantly higher. However, largely the bioaccessibility of iron was very low across all samples. This could be due to the presence of phytates (especially porridge samples), organic acids, carbonate salts and some phenolic acids which may chelate and form insoluble complexes with the metal hence impaired iron bioaccessibility (Khouzam et al., 2011).

Zinc is an essential micronutrient that plays a vital role in numerous metabolic processes and is postulated to be a key element in the function of over 300 body enzymes. Zinc deficiency may result in retarded growth patterns, irregular bone formation and dermatitis (Deshpande et al., 2013). The RDA for zinc ranges from 3 - 11 mg/100g across different sexes and age groups (ZDHS, 2016). The observed zinc bioaccessibility in this study was 8.53 ± 1.45 %,

18.14 ± 0.67 %, 0.90 ± 0.33 % and 16.67 ± 0.14 % for non-enzyme juice, enzyme juice, non-enzyme juice porridge and enzyme juice porridge respectively (Table 11). As observed with iron bioaccessibility, the enzyme treated samples recorded higher bioaccessibility and this can also be attributed to the release of the zinc from the pectin matrix by the action of the pectinase during sample preparation. The low zinc bioaccessibility across all samples can be attributed to the occurrence of other minerals such as iron and calcium which may retard bioaccessibility. As with iron, phytates can also affect zinc bioaccessibility (Hambidge et al., 2010).

5.4 Effects of enzyme maceration on sensory properties

5.4.1 Triangle test (Discrimination between enzyme and non-enzyme macerated)

The triangle test is an important sensory evaluation technique that applies discriminative methods to gauge differences between samples or to select qualified panelists for a specific test. As expected from the observed results there was significance difference ($p < 0.05$) between the trained ($n = 12$) and untrained panelists ($n = 132$) in the ability to pick out the odd sample for both the juice and the porridge (Fig. 30). The trained panelists exhibited a significantly higher success rate of 57 % compared to 20 % for the trained panelists, for the juice samples. This can be attributed to the training and experience from the trained panel as some of the trained panelists had to be assisted during evaluation. However, discrimination was lower for both groups (34 % trained, 14 % untrained) with the porridge samples although the trained panelist score was higher. This can be attributed to the masking effect caused by addition of maize-meal and subsequent starch gelatinisation.

5.4.2 Preference test (between enzyme and non-enzyme macerated *S. cocculoides* juice/porridge

Only the trained panel was used for the preference test. There was significant difference ($p < 0.05$) in preference between enzyme juice porridge and non-enzyme juice porridge; and between enzyme juice and non-enzyme juice with the enzyme treated samples being the most preferred based on overall acceptance results. In terms of sweetness both the non-enzyme juice and non-enzyme porridge scored higher than their enzyme treated counterparts. This could be attributed to the masking of the sweetness by the reduced pH in enzyme treated samples. As previously discussed the enzyme treatment produces galacturonic acid from the breakdown of pectin and releases other organic acids (Kumar, 2015). This can also be attributed to the higher score of astringency in enzyme treated samples. The panelists described the porridge as being similar in sensorial quality to 'sour' maize-meal porridge where the maize-meal is fermented for a few days before cooking. The colour of the enzyme macerated juice was more appealing as the pectinase reduces some haziness and cloudiness by clarification (breakdown of pectin) (Tapre & Jain, 2014). However, for the porridge similar colour and aroma scores were recorded for both samples, attributable to the masking effect of the gelatinised maize-meal. For the juice samples, aroma score was higher for the enzyme juice and this could be due to the breakdown of pectin into simpler more volatile compounds such as esters and organic acids that contribute to the intense aroma (Tapre & Jain, 2014; Kumar, 2015).

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Enzyme maceration has a significant ($p < 0.05$) effect on physicochemical properties of *S. cocculoides* juice/porridge. Enzyme macerated juice is a good source of iron (8.89 ± 0.42 mg/100g) and contains appreciable levels of zinc (2.0 ± 0.19 mg/100g) and vitamin C (9.45 ± 0.77 mg/100g). Although iron was present in lower amounts in the enzyme porridge, significant content was recorded (5.76 ± 0.57 mg/100g). The enzyme macerated juice and the enzyme porridge have the potential to contribute to the fight against iron deficiency in the rural areas of Zimbabwe. Enzyme maceration also enhances the phenolic composition, antioxidant and antimicrobial activities of *S. cocculoides* juice, hence conferring health promoting properties. The bioaccessibility of the assayed micronutrients improved significantly with enzyme maceration of *S. cocculoides* juice, attributable to the breakdown of the complex polysaccharide pectin. Addition of *S. cocculoides* juice lowered the bioaccessibility of the assayed micronutrients, although bioaccessibility was significantly higher with enzyme porridge. In terms of sensorial properties, both the enzyme macerated juice and enzyme porridge where more appreciable than their non-macerated counterparts.

6.2 Recommendations

Enzyme macerated *S. cocculoides* juice is an excellent source of some micronutrients and phenolic compounds; hence its consumption should be encouraged especially by juice manufacturers and in nutrition-related intervention programmes and policies by researchers, non-governmental organizations across all relevant levels of society. Further research to identify individual specific phenolic compounds should be done as these can be isolated and used to enrich other foods. Similar studies (enzyme maceration) should be explored for other

indigenous fruits to improve nutritional value and enhance bioaccessibility with the aim of promoting their utilisation and increasing their value. Value addition and product development of *S. cocculoides* pulp/juice can be employed and be guided by strong nutritional knowledge of how the nutrients behave during processing and subsequent digestion.

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APPENDICES

Appendix 1: Sensory evaluation questionnaire

Number of panelist………………………… Date…………………………

Product……………………….

Please assess the given sample and indicate the chosen score for each attribute and indicate it by putting an **X**

Nine point hedonic scale

	ATTRIBUTES				
SCORE	**COLOUR**	**ASTRINGENCY**	**SWEETNESS**	**AROMA**	**OVERALL ACCEPTANCE**
9.Like					
8.Like very					
7.Like little					
6.Like					
5.Neither					
4.Dislike					
3.Dislike little					
2.Dislike very much					
1.Dislike extremely					

Suggestions/comments………………………………………………………………………………
…..……………………………………………………………………………………………………
………

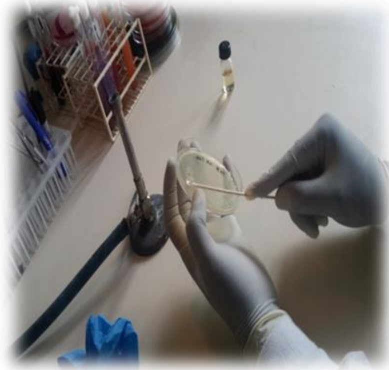
You are provided with two samples of fruit juice please tick in the box of the sample you like most

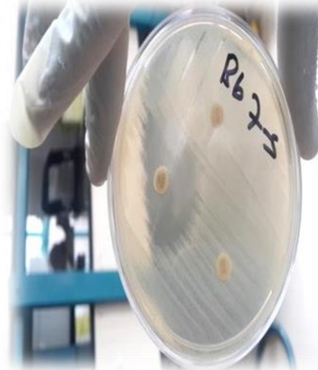
SAMPLE X2X ☐ **SAMPLE Y2Y** ☐

Suggestions/comments………………………………………………………………………………
…..……………………………………………………………………………………………………
………

Appendix 2: Images of some analytical equipment and observed results

pH meter **Freeze dryer** **Drying column**





Antimicrobial assays

Kjedahl – protein analysis **Preparation of simulated digestion fluids**

Traditional preparation of *S. cocculoides porridge*

Sensory evaluation

UV/VIS Spectrophotometer

Appendix 3: Raw results

	Sample	Treatment	pH	Brix (TSS%)	Dry matter %	Vit. C mg/100g	Protein g/100g	Glucose g/100g	Sucrose g/100g	Fructose g/100g	TPC GAE mg/100g	AOA %	Iron mg/100g	Zinc mg/100g
Unprocessed pulp	1	1	3.63	9.80	22.32	23.25	0.81	2.67	4.67	7.32	1760.51	70.52	13.33	2.23
Unprocessed pulp	2	1	3.61	9.74	19.67	19.98	0.65	2.45	5.76	6.74	1800.56	69.28	12.55	1.96
Unprocessed pulp	3	1	3.64	9.79	20.10	21.00	0.69	2.36	6.80	7.11	1789.29	60.67	13.48	1.92
Non enzyme juice	4	2	3.64	11.98	14.73	0.00	0.56	2.05	3.99	5.65	240.89	37.89	10.45	0.81
Non enzyme juice	5	2	3.65	11.14	14.67	0.56	0.48	1.99	4.68	6.03	188.00	32.45	9.97	0.79
Non enzyme juice	6	2	3.72	11.12	15.29	0.83	0.50	2.21	4.35	5.23	167.82	36.82	10.33	0.86
Enzyme juice	7	3	3.49	13.93	12.56	7.59	0.59	3.11	4.43	6.76	2363.75	77.86	10.98	2.26
Enzyme juice	8	3	3.53	14.21	12.13	9.09	0.64	2.97	5.10	6.13	2267.08	75.37	11.56	1.94
Enzyme juice	9	3	3.52	14.19	13.04	8.67	0.65	2.40	3.39	7.30	2352.43	75.98	11.79	1.91
Non enzyme juice porridge	10	4	5.84	2.68	63.12	0.00	0.00	1.04	1.98	2.51	832.34	23.35	6.34	1.14
Non enzyme juice porridge	11	4	5.85	2.67	62.31	0.00	0.09	1.11	1.88	2.27	856.78	23.13	5.20	1.12
Non enzyme juice porridge	12	4	5.89	2.72	61.86	0.00	0.13	0.57	1.89	2.15	798.33	21.99	5.74	1.08
Enzyme juice porridge	13	5	5.83	2.58	62.12	0.00	0.10	1.56	1.97	2.97	1196.51	44.34	5.21	0.61
Enzyme juice porridge	14	5	5.81	2.66	59.98	0.45	0.13	1.45	2.55	2.87	1260.56	42.69	4.43	0.63
Enzyme juice porridge	15	5	5.82	2.71	61.02	1.60	0.11	1.16	2.47	2.79	1029.29	43.30	4.47	0.55
Plain porridge	16	6	5.93	1.95	60.56	0.00	0.10	0.00	0.00	0.00	43.13	2.30	1.67	0.34
Plain porridge	17	6	6.12	1.79	58.30	0.00	0.04	0.13	0.00	0.00	45.67	2.16	1.83	0.28
Plain porridge	18	6	6.16	1.88	59.53	0.00	0.00	0.00	0.00	0.00	80.23	1.85	1.77	0.29

Zone of inhibition of methanolic extracts (mm)

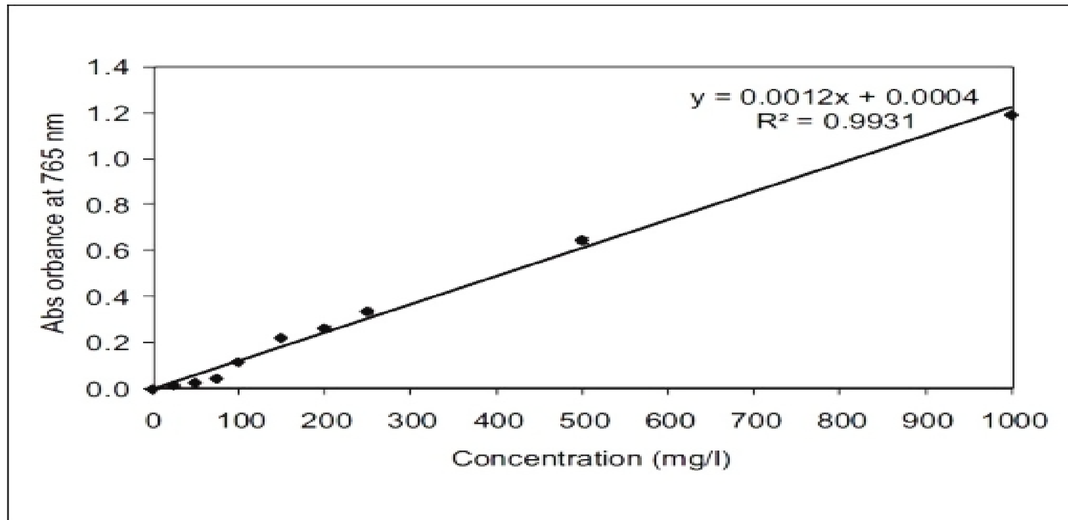
	Sample	Treatment	*S.aureus*	*Paeruginosa*	*E.coli*	*Salmonellaspp.*	*C.albicans*	*A.niger*
Unprocessedpulp	1	1	18	18	16	9	2	3
Unprocessedpulp	2	1	17	19	15	8	2	2
Unprocessedpulp	3	1	17	21	17	7	3	2
Nonenzymejuice	4	2	3	2	3	0	1	0
Nonenzymejuice	5	2	4	2	2	0	2	0
Nonenzymejuice	6	2	3	1	1	0	1	0
Enzymejuice	7	3	21	16	22	12	4	5
Enzymejuice	8	3	20	17	23	13	3	6
Enzymejuice	9	3	22	19	23	12	3	5

	sample	treatment	Vit. C mg/100g	TPC GAE mg/100g	AOA %	Iron mg/100g	Zinc mg/100g
NEJ Undigested	1	1	0.00	1340.89	37.89	10.45	0.81
NEJ Undigested	2	1	0.56	1388.00	32.45	9.97	0.79
NEJ Undigested	3	1	0.83	1367.82	36.82	10.33	0.86
EJ Undigested	4	2	8.59	3363.75	77.86	10.98	2.26
EJ Undigested	5	2	10.09	3267.08	75.37	11.56	1.94
EJ Undigested	6	2	9.67	3352.43	75.98	11.79	1.91
NEJP Undigested	7	3	0.00	832.34	23.35	6.34	1.14
NEJP Undigested	8	3	0.00	856.78	23.13	5.20	1.12
NEJP Undigested	9	3	0.00	798.33	21.99	5.74	1.08
EJP Undigested	10	4	0.98	1196.51	44.34	5.21	0.61
EJP Undigested	11	4	0.45	1260.56	42.69	4.43	0.63
EJP Undigested	12	4	1.60	1029.29	43.30	4.47	0.55
NEJ gastric digest	13	5	0.00	1498.22	40.02		
NEJ gastric digest	14	5	0.35	1507.11	39.88		
NEJ gastric digest	15	5	0.69	1489.33	37.53		
EJ gastric digest	16	6	6.31	3472.93	79.80		
EJ gastric digest	17	6	7.95	3366.01	78.32		
EJ gastric digest	18	6	7.84	3393.22	77.99		
NEJP gastric digest	19	7	0.00	793.38	22.89		
NEJP gastric digest	20	7	0.00	788.35	21.56		
NEJP gastric digest	21	7	0.00	777.36	21.01		
EJP gastric digest	22	8	0.65	997.23	39.52		
EJP gastric digest	23	8	0.27	1003.56	40.49		
EJP gastric digest	24	8	0.88	945.27	38.97		
NEJ intestinal digest	25	9	0.00	398.58	24.65	6.04	0.23
NEJ intestinal digest	26	9	0.09	501.67	22.01	4.98	0.27
NEJ intestinal digest	27	9	0.15	478.50	20.33	5.93	0.32
EJ intestinal digest	28	10	3.81	493.65	56.09	6.53	0.72
EJ intestinal digest	29	10	3.98	553.06	54.23	6.34	0.69
EJ intestinal digest	30	10	2.95	521.98	54.93	5.99	0.58
NEJP intestinal digest	31	11	0.00	231.44	18.65	2.94	0.49
NEJP intestinal digest	32	11	0.00	188.54	17.87	2.68	0.44

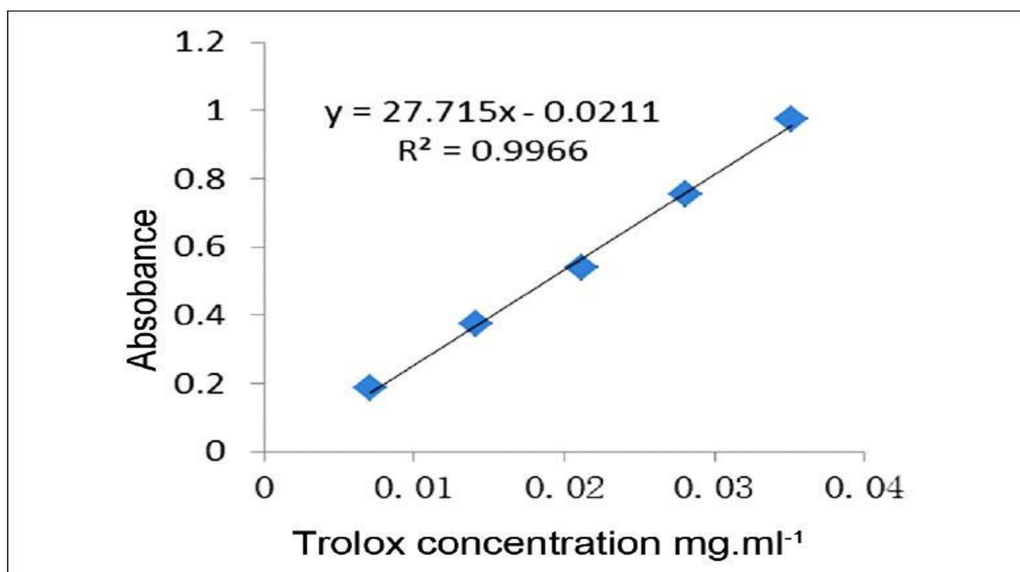
NEJP intestinal digest	33	11	0.00	190.56	18.05	2.87	0.38
EJP intestinal digest	34	12	0.39	200.98	29.07	3.07	0.11
EJP intestinal digest	35	12	0.00	289.56	31.21	1.94	0.17
EJP intestinal digest	36	12	0.53	245.00	28.83	2.33	0.19

Appendix 4: Calibration curves

Calibration curve for gallic acid



Calibration curve for trolox



Appendix 5: Statistical Analysis Tables

pH

ANOVA

Juice pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.039	2	.020	22.948	.002
Within Groups	.005	6	.001		
Total	.044	8			

Post Hoc Tests

Multiple Comparisons

Juice pH

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Unprocessed pulp	Non enzyme juice	-.04333	.02388	.120	-.1018	.0151
	Enzyme juice	.11333*	.02388	.003	.0549	.1718
Non enzyme juice	Unprocessed pulp	.04333	.02388	.120	-.0151	.1018
	Enzyme juice	.15667*	.02388	.001	.0982	.2151
Enzyme juice	Unprocessed pulp	-.11333*	.02388	.003	-.1718	-.0549
	Non enzyme juice	-.15667*	.02388	.001	-.2151	-.0982

*. The mean difference is significant at the 0.05 level.

ANOVA

Porridge pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.108	2	.054	10.208	.012
Within Groups	.032	6	.005		
Total	.140	8			

Post Hoc Tests

Multiple Comparisons

Porridge pH

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval

					Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	.04000	.05944	.526	-.1054	.1854
	Plain porridge	-.21000*	.05944	.012	-.3554	-.0646
Enzyme porridge	Non enzyme porridge	-.04000	.05944	.526	-.1854	.1054
	Plain porridge	-.25000*	.05944	.006	-.3954	-.1046
Plain porridge	Non enzyme porridge	.21000*	.05944	.012	.0646	.3554
	Enzyme porridge	.25000*	.05944	.006	.1046	.3954

*. The mean difference is significant at the 0.05 level.

ANOVA

Porridge Brix (TSS %)	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.272	2	.636	166.848	.000
Within Groups	.023	6	.004		
Total	1.295	8			

Post Hoc Tests

Multiple Comparisons

Porridge Brix (TSS %)

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	.04000	.05041	.458	-.0833	.1633
	Plain porridge	.81667*	.05041	.000	.6933	.9400
Enzyme porridge	Non enzyme porridge	-.04000	.05041	.458	-.1633	.0833
	Plain porridge	.77667*	.05041	.000	.6533	.9000
Plain porridge	Non enzyme porridge	-.81667*	.05041	.000	-.9400	-.6933
	Enzyme porridge	-.77667*	.05041	.000	-.9000	-.6533

*. The mean difference is significant at the 0.05 level.

ANOVA

Porridge Dry matter %	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	13.219	2	6.610	6.999	.027
Within Groups	5.666	6	.944		
Total	18.885	8			

Post Hoc Tests

Multiple Comparisons

Porridge Dry matter %

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	1.39000	.79346	.130	-.5515	3.3315
	Plain porridge	2.96667*	.79346	.010	1.0251	4.9082
Enzyme porridge	Non enzyme porridge	-1.39000	.79346	.130	-3.3315	.5515
	Plain porridge	1.57667	.79346	.094	-.3649	3.5182
Plain porridge	Non enzyme porridge	-2.96667*	.79346	.010	-4.9082	-1.0251
	Enzyme porridge	-1.57667	.79346	.094	-3.5182	.3649

*. The mean difference is significant at the 0.05 level.

ANOVA

Porridge Vitamin C mg/100g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.609	2	1.805	51.739	.000
Within Groups	.209	6	.035		
Total	3.818	8			

Post Hoc Tests

Multiple Comparisons

Porridge Vitamin C mg/100g

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	-1.34333*	.15249	.000	-1.7165	-.9702
	Plain porridge	.00000	.15249	1.000	-.3731	.3731
Enzyme porridge	Non enzyme porridge	1.34333*	.15249	.000	.9702	1.7165
	Plain porridge	1.34333*	.15249	.000	.9702	1.7165

Plain porridge	Non enzyme porridge	.00000	.15249	1.000	-.3731	.3731
	Enzyme porridge	-1.34333*	.15249	.000	-1.7165	-.9702

*. The mean difference is significant at the 0.05 level.

ANOVA

Porridge Protein g/100g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.677	2	.338	123.809	.000
Within Groups	.016	6	.003		
Total	.693	8			

Post Hoc Tests

Multiple Comparisons

Protein g/100g

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	-.49667*	.04269	.000	-.6011	-.3922
	Plain porridge	.14333*	.04269	.015	.0389	.2478
Enzyme porridge	Non enzyme porridge	.49667*	.04269	.000	.3922	.6011
	Plain porridge	.64000*	.04269	.000	.5355	.7445
Plain porridge	Non enzyme porridge	-.14333*	.04269	.015	-.2478	-.0389
	Enzyme porridge	-.64000*	.04269	.000	-.7445	-.5355

*. The mean difference is significant at the 0.05 level.

ANOVA

	Porridge	Sum of Squares	df	Mean Square	F	Sig.
Glucose g/100g	Between Groups	2.792	2	1.396	31.127	.001
	Within Groups	.269	6	.045		
	Total	3.062	8			
Sucose g/100g	Between Groups	9.273	2	4.637	136.596	.000
	Within Groups	.204	6	.034		
	Total	9.477	8			
Fructose g/100g	Between Groups	13.932	2	6.966	500.766	.000
	Within Groups	.083	6	.014		

ANOVA

	Porridge	Sum of Squares	df	Mean Square	F	Sig.
Glucose g/100g	Between Groups	2.792	2	1.396	31.127	.001
	Within Groups	.269	6	.045		
	Total	3.062	8			
Sucose g/100g	Between Groups	9.273	2	4.637	136.596	.000
	Within Groups	.204	6	.034		
	Total	9.477	8			
Fructose g/100g	Between Groups	13.932	2	6.966	500.766	.000
	Within Groups	.083	6	.014		
	Total	14.016	8			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Glucose g/100g	Non enzyme porridge	Enzyme porridge	-.48333*	.17293	.031	-.9065	-.0602
		Plain porridge	.86333*	.17293	.002	.4402	1.2865
	Enzyme porridge	Non enzyme porridge	.48333*	.17293	.031	.0602	.9065
		Plain porridge	1.34667*	.17293	.000	.9235	1.7698
	Plain porridge	Non enzyme porridge	-.86333*	.17293	.002	-1.2865	-.4402
		Enzyme porridge	-1.34667*	.17293	.000	-1.7698	-.9235
Sucose g/100g	Non enzyme porridge	Enzyme porridge	-.41333*	.15043	.033	-.7814	-.0452
		Plain porridge	1.91667*	.15043	.000	1.5486	2.2848
	Enzyme porridge	Non enzyme porridge	.41333*	.15043	.033	.0452	.7814
		Plain porridge	2.33000*	.15043	.000	1.9619	2.6981
	Plain porridge	Non enzyme porridge	-1.91667*	.15043	.000	-2.2848	-1.5486
		Enzyme porridge	-2.33000*	.15043	.000	-2.6981	-1.9619
Fructose	Non enzyme	Enzyme porridge	-.56667*	.09630	.001	-.8023	-.3310

g/100g	porridge	Plain porridge	2.31000*	.09630	.000	2.0744	2.5456
	Enzyme porridge	Non enzyme porridge	.56667*	.09630	.001	.3310	.8023
		Plain porridge	2.87667*	.09630	.000	2.6410	3.1123
	Plain porridge	Non enzyme porridge	-2.31000*	.09630	.000	-2.5456	-2.0744
		Enzyme porridge	-2.87667*	.09630	.000	-3.1123	-2.6410

*. The mean difference is significant at the 0.05 level.

ANOVA

porridge Total Phenol Content GAE mg/100g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1930841.201	2	965420.601	186.258	.000
Within Groups	31099.465	6	5183.244		
Total	1961940.667	8			

Post Hoc Tests

Multiple Comparisons

Porridge Total Phenol Content GAE mg/100g

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	-332.97000*	58.78347	.001	-476.8080	-189.1320
	Plain porridge	772.80667*	58.78347	.000	628.9687	916.6446
Enzyme porridge	Non enzyme porridge	332.97000*	58.78347	.001	189.1320	476.8080
	Plain porridge	1105.77667*	58.78347	.000	961.9387	1249.6146
Plain porridge	Non enzyme porridge	-772.80667*	58.78347	.000	-916.6446	-628.9687
	Enzyme porridge	-1105.77667*	58.78347	.000	-1249.6146	-961.9387

*. The mean difference is significant at the 0.05 level.

ANOVA

Porridge Free Radical Scavanging %

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2563.498	2	1281.749	2.999E3	.000
Within Groups	2.564	6	.427		

ANOVA

Porridge Free Radical Scavanging %

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2563.498	2	1281.749	2.999E3	.000
Within Groups	2.564	6	.427		
Total	2566.062	8			

Post Hoc Tests

Multiple Comparisons

Porridge Free Radical Scavanging %

LSD

					95% Confidence Interval	
(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Non enzyme porridge	Enzyme porridge	-20.62000*	.53375	.000	-21.9260	-19.3140
	Plain porridge	20.72000*	.53375	.000	19.4140	22.0260
Enzyme porridge	Non enzyme porridge	20.62000*	.53375	.000	19.3140	21.9260
	Plain porridge	41.34000*	.53375	.000	40.0340	42.6460
Plain porridge	Non enzyme porridge	-20.72000*	.53375	.000	-22.0260	-19.4140
	Enzyme porridge	-41.34000*	.53375	.000	-42.6460	-40.0340

*. The mean difference is significant at the 0.05 level.

ANOVA

porridge		Sum of Squares	df	Mean Square	F	Sig.
Iron mg/100g	Between Groups	25.826	2	12.913	73.836	.000
	Within Groups	1.049	6	.175		
	Total	26.875	8			
Zinc mg/100g	Between Groups	1.009	2	.505	409.090	.000
	Within Groups	.007	6	.001		
	Total	1.016	8			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Iron mg/100g	Non enzyme porridge	Enzyme porridge	1.05667*	.34146	.021	.2212	1.8922
		Plain porridge	4.00333*	.34146	.000	3.1678	4.8388
	Enzyme porridge	Non enzyme porridge	-1.05667*	.34146	.021	-1.8922	-.2212
		Plain porridge	2.94667*	.34146	.000	2.1112	3.7822
	Plain porridge	Non enzyme porridge	-4.00333*	.34146	.000	-4.8388	-3.1678
		Enzyme porridge	-2.94667*	.34146	.000	-3.7822	-2.1112
Zinc mg/100g	Non enzyme porridge	Enzyme porridge	.51667*	.02867	.000	.4465	.5868
		Plain porridge	.81000*	.02867	.000	.7398	.8802
	Enzyme porridge	Non enzyme porridge	-.51667*	.02867	.000	-.5868	-.4465
		Plain porridge	.29333*	.02867	.000	.2232	.3635
	Plain porridge	Non enzyme porridge	-.81000*	.02867	.000	-.8802	-.7398
		Enzyme porridge	-.29333*	.02867	.000	-.3635	-.2232

*. The mean difference is significant at the 0.05 level.

ANOVA

Brix (TSS %)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.728	2	14.364	161.780	.000
Within Groups	.533	6	.089		
Total	29.261	8			

Post Hoc Tests

Multiple Comparisons

Brix (TSS %)

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound

Unprocessed pulp	Non enzyme juice	-1.63667*	.24330	.001	-2.2320	-1.0413
	Enzyme juice	-4.33333*	.24330	.000	-4.9287	-3.7380
Non enzyme juice	Unprocessed pulp	1.63667*	.24330	.001	1.0413	2.2320
	Enzyme juice	-2.69667*	.24330	.000	-3.2920	-2.1013
Enzyme juice	Unprocessed pulp	4.33333*	.24330	.000	3.7380	4.9287
	Non enzyme juice	2.69667*	.24330	.000	2.1013	3.2920

*. The mean difference is significant at the 0.05 level.

ANOVA

Dry matter %

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	104.957	2	52.478	67.085	.000
Within Groups	4.694	6	.782		
Total	109.650	8			

Post Hoc Tests

Multiple Comparisons

Dry matter %

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Unprocessed pulp	Non enzyme juice	5.80000*	.72216	.000	4.0329	7.5671
	Enzyme juice	8.12000*	.72216	.000	6.3529	9.8871
Non enzyme juice	Unprocessed pulp	-5.80000*	.72216	.000	-7.5671	-4.0329
	Enzyme juice	2.32000*	.72216	.018	.5529	4.0871
Enzyme juice	Unprocessed pulp	-8.12000*	.72216	.000	-9.8871	-6.3529
	Non enzyme juice	-2.32000*	.72216	.018	-4.0871	-.5529

*. The mean difference is significant at the 0.05 level.

ANOVA

Vitamin C mg/100g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	306.486	2	153.243	133.090	.000
Within Groups	6.909	6	1.151		
Total	313.394	8			

Post Hoc Tests

Multiple Comparisons

Vitamin C mg/100g

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Unprocessed pulp	Non enzyme juice	14.12000*	.87614	.000	11.9762	16.2638
	Enzyme juice	5.13333*	.87614	.001	2.9895	7.2772
Non enzyme juice	Unprocessed pulp	-14.12000*	.87614	.000	-16.2638	-11.9762
	Enzyme juice	-8.98667*	.87614	.000	-11.1305	-6.8428
Enzyme juice	Unprocessed pulp	-5.13333*	.87614	.001	-7.2772	-2.9895
	Non enzyme juice	8.98667*	.87614	.000	6.8428	11.1305

*. The mean difference is significant at the 0.05 level.

ANOVA

Protein g/100g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.691	2	.845	130.948	.000
Within Groups	.039	6	.006		
Total	1.729	8			

Post Hoc Tests

Multiple Comparisons

Protein g/100g

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Unprocessed pulp	Non enzyme juice	1.03667*	.06560	.000	.8761	1.1972
	Enzyme juice	.71667*	.06560	.000	.5561	.8772
Non enzyme juice	Unprocessed pulp	-1.03667*	.06560	.000	-1.1972	-.8761
	Enzyme juice	-.32000*	.06560	.003	-.4805	-.1595
Enzyme juice	Unprocessed pulp	-.71667*	.06560	.000	-.8772	-.5561
	Non enzyme juice	.32000*	.06560	.003	.1595	.4805

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Glucose g/100g	Between Groups	.832	2	.416	6.939	.028
	Within Groups	.360	6	.060		
	Total	1.191	8			
Sucose g/100g	Between Groups	4.034	2	2.017	3.032	.123
	Within Groups	3.992	6	.665		
	Total	8.026	8			
Fructose g/100g	Between Groups	3.318	2	1.659	8.447	.018
	Within Groups	1.179	6	.196		
	Total	4.497	8			

Post Hoc

Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Glucose g/100g	Unprocessed pulp	Non enzyme juice	.41000	.19989	.086	-.0791	.8991
		Enzyme juice	-.33333	.19989	.146	-.8224	.1558

	Non enzyme juice	Unprocessed pulp	-.41000	.19989	.086	-.8991	.0791
		Enzyme juice	-.74333*	.19989	.010	-1.2324	-.2542
	Enzyme juice	Unprocessed pulp	.33333	.19989	.146	-.1558	.8224
		Non enzyme juice	.74333*	.19989	.010	.2542	1.2324
Sucose g/100g	Unprocessed pulp	Non enzyme juice	1.40333	.66599	.080	-.2263	3.0330
		Enzyme juice	1.43667	.66599	.074	-.1930	3.0663
	Non enzyme juice	Unprocessed pulp	-1.40333	.66599	.080	-3.0330	.2263
		Enzyme juice	.03333	.66599	.962	-1.5963	1.6630
	Enzyme juice	Unprocessed pulp	-1.43667	.66599	.074	-3.0663	.1930
		Non enzyme juice	-.03333	.66599	.962	-1.6630	1.5963
Fructose g/100g	Unprocessed pulp	Non enzyme juice	1.42000*	.36187	.008	.5345	2.3055
		Enzyme juice	.32667	.36187	.401	-.5588	1.2121
	Non enzyme juice	Unprocessed pulp	-1.42000*	.36187	.008	-2.3055	-.5345
		Enzyme juice	-1.09333*	.36187	.023	-1.9788	-.2079
	Enzyme juice	Unprocessed pulp	-.32667	.36187	.401	-1.2121	.5588
		Non enzyme juice	1.09333*	.36187	.023	.2079	1.9788

*. The mean difference is significant at the 0.05 level.

ANOVA

Total Phenol Content GAE mg/100g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6156819.071	2	3078409.535	2.444E3	.000
Within Groups	7556.322	6	1259.387		
Total	6164375.393	8			

Post Hoc Tests

Multiple Comparisons

Total Phenol Content GAE mg/100g

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Unprocessed pulp	Non enzyme juice	1417.88333*	28.97570	.000	1346.9823	1488.7843
	Enzyme juice	-544.30000*	28.97570	.000	-615.2010	-473.3990
Non enzyme juice	Unprocessed pulp	-1417.88333*	28.97570	.000	-1488.7843	-1346.9823
	Enzyme juice	-1962.18333*	28.97570	.000	-2033.0843	-1891.2823

Enzyme juice	Unprocessed pulp	544.30000*	28.97570	.000	473.3990	615.2010
	Non enzyme juice	1962.18333*	28.97570	.000	1891.2823	2033.0843

*. The mean difference is significant at the 0.05 level.

ANOVA

Free Radical Scavanging %

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2714.327	2	1357.164	105.010	.000
Within Groups	77.545	6	12.924		
Total	2791.872	8			

Post Hoc Tests

Multiple Comparisons

Free Radical Scavanging %

LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Unprocessed pulp	Non enzyme juice	31.10333*	2.93532	.000	23.9209	38.2858
	Enzyme juice	-9.58000*	2.93532	.017	-16.7625	-2.3975
Non enzyme juice	Unprocessed pulp	-31.10333*	2.93532	.000	-38.2858	-23.9209
	Enzyme juice	-40.68333*	2.93532	.000	-47.8658	-33.5009
Enzyme juice	Unprocessed pulp	9.58000*	2.93532	.017	2.3975	16.7625
	Non enzyme juice	40.68333*	2.93532	.000	33.5009	47.8658

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Iron mg/100g	Between Groups	12.472	2	6.236	38.500	.000
	Within Groups	.972	6	.162		
	Total	13.444	8			
Zinc mg/100g	Between Groups	2.961	2	1.480	65.920	.000
	Within Groups	.135	6	.022		
	Total	3.095	8			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Iron mg/100g	Unprocessed pulp	Non enzyme juice	2.87000*	.32861	.000	2.0659	3.6741
		Enzyme juice	1.67667*	.32861	.002	.8726	2.4807
	Non enzyme juice	Unprocessed pulp	-2.87000*	.32861	.000	-3.6741	-2.0659
		Enzyme juice	-1.19333*	.32861	.011	-1.9974	-.3893
	Enzyme juice	Unprocessed pulp	-1.67667*	.32861	.002	-2.4807	-.8726
		Non enzyme juice	1.19333*	.32861	.011	.3893	1.9974
Zinc mg/100g	Unprocessed pulp	Non enzyme juice	1.21667*	.12235	.000	.9173	1.5161
		Enzyme juice	.00000	.12235	1.000	-.2994	.2994
	Non enzyme juice	Unprocessed pulp	-1.21667*	.12235	.000	-1.5161	-.9173
		Enzyme juice	-1.21667*	.12235	.000	-1.5161	-.9173
	Enzyme juice	Unprocessed pulp	.00000	.12235	1.000	-.2994	.2994
		Non enzyme juice	1.21667*	.12235	.000	.9173	1.5161

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
S. aureus	Between Groups	521.556	2	260.778	469.400	.000
	Within Groups	3.333	6	.556		
	Total	524.889	8			
P. aerugonisa	Between Groups	561.556	2	280.778	168.467	.000
	Within Groups	10.000	6	1.667		
	Total	571.556	8			
E. coli	Between Groups	667.556	2	333.778	429.143	.000
	Within Groups	4.667	6	.778		
	Total	672.222	8			

Salmonella spp.	Between Groups	234.889	2	117.444	264.250	.000
	Within Groups	2.667	6	.444		
	Total	237.556	8			
C. albicans	Between Groups	6.000	2	3.000	9.000	.016
	Within Groups	2.000	6	.333		
	Total	8.000	8			
A. niger	Between Groups	42.889	2	21.444	96.500	.000
	Within Groups	1.333	6	.222		
	Total	44.222	8			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
S. aureus	Unprocessed Pulp	Non enzyme juice	14.00000*	.60858	.000	12.5109	15.4891
		Enzyme juice	-3.66667*	.60858	.001	-5.1558	-2.1775
	Non enzyme juice	Unprocessed Pulp	-14.00000*	.60858	.000	-15.4891	-12.5109
		Enzyme juice	-17.66667*	.60858	.000	-19.1558	-16.1775
	Enzyme juice	Unprocessed Pulp	3.66667*	.60858	.001	2.1775	5.1558
		Non enzyme juice	17.66667*	.60858	.000	16.1775	19.1558
P. aerugonisa	Unprocessed Pulp	Non enzyme juice	17.66667*	1.05409	.000	15.0874	20.2459
		Enzyme juice	2.00000	1.05409	.107	-.5793	4.5793
	Non enzyme juice	Unprocessed Pulp	-17.66667*	1.05409	.000	-20.2459	-15.0874
		Enzyme juice	-15.66667*	1.05409	.000	-18.2459	-13.0874
	Enzyme juice	Unprocessed Pulp	-2.00000	1.05409	.107	-4.5793	.5793
		Non enzyme juice	15.66667*	1.05409	.000	13.0874	18.2459
E. coli	Unprocessed Pulp	Non enzyme juice	14.00000*	.72008	.000	12.2380	15.7620
		Enzyme juice	-6.66667*	.72008	.000	-8.4286	-4.9047
	Non	Unprocessed Pulp	-14.00000*	.72008	.000	-15.7620	-12.2380

	enzyme juice	Enzyme juice	-20.66667*	.72008	.000	-22.4286	-18.9047
	Enzyme juice	Unprocessed Pulp	6.66667*	.72008	.000	4.9047	8.4286
		Non enzyme juice	20.66667*	.72008	.000	18.9047	22.4286
Salmonella spp.	Unprocessed Pulp	Non enzyme juice	8.00000*	.54433	.000	6.6681	9.3319
		Enzyme juice	-4.33333*	.54433	.000	-5.6653	-3.0014
	Non enzyme juice	Unprocessed Pulp	-8.00000*	.54433	.000	-9.3319	-6.6681
		Enzyme juice	-12.33333*	.54433	.000	-13.6653	-11.0014
	Enzyme juice	Unprocessed Pulp	4.33333*	.54433	.000	3.0014	5.6653
		Non enzyme juice	12.33333*	.54433	.000	11.0014	13.6653
C. albicans	Unprocessed Pulp	Non enzyme juice	1.00000	.47140	.078	-.1535	2.1535
		Enzyme juice	-1.00000	.47140	.078	-2.1535	.1535
	Non enzyme juice	Unprocessed Pulp	-1.00000	.47140	.078	-2.1535	.1535
		Enzyme juice	-2.00000*	.47140	.005	-3.1535	-.8465
	Enzyme juice	Unprocessed Pulp	1.00000	.47140	.078	-.1535	2.1535
		Non enzyme juice	2.00000*	.47140	.005	.8465	3.1535
A. niger	Unprocessed Pulp	Non enzyme juice	2.33333*	.38490	.001	1.3915	3.2752
		Enzyme juice	-3.00000*	.38490	.000	-3.9418	-2.0582
	Non enzyme juice	Unprocessed Pulp	-2.33333*	.38490	.001	-3.2752	-1.3915
		Enzyme juice	-5.33333*	.38490	.000	-6.2752	-4.3915
	Enzyme juice	Unprocessed Pulp	3.00000*	.38490	.000	2.0582	3.9418
		Non enzyme juice	5.33333*	.38490	.000	4.3915	6.2752

*. The mean difference is significant at the 0.05 level.

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	6.2	4.613333333
Variance	0.554286	0.26552381

Observations	15	15
Pearson Correlation	0.87258	
Hypothesized Mean Difference	0	
df	14	
t Stat	8.234217	
P(T<=t) one-tail	4.89E-07	
t Critical one-tail	1.76131	
P(T<=t) two-tail	9.77E-07	
t Critical two-tail	2.144787	