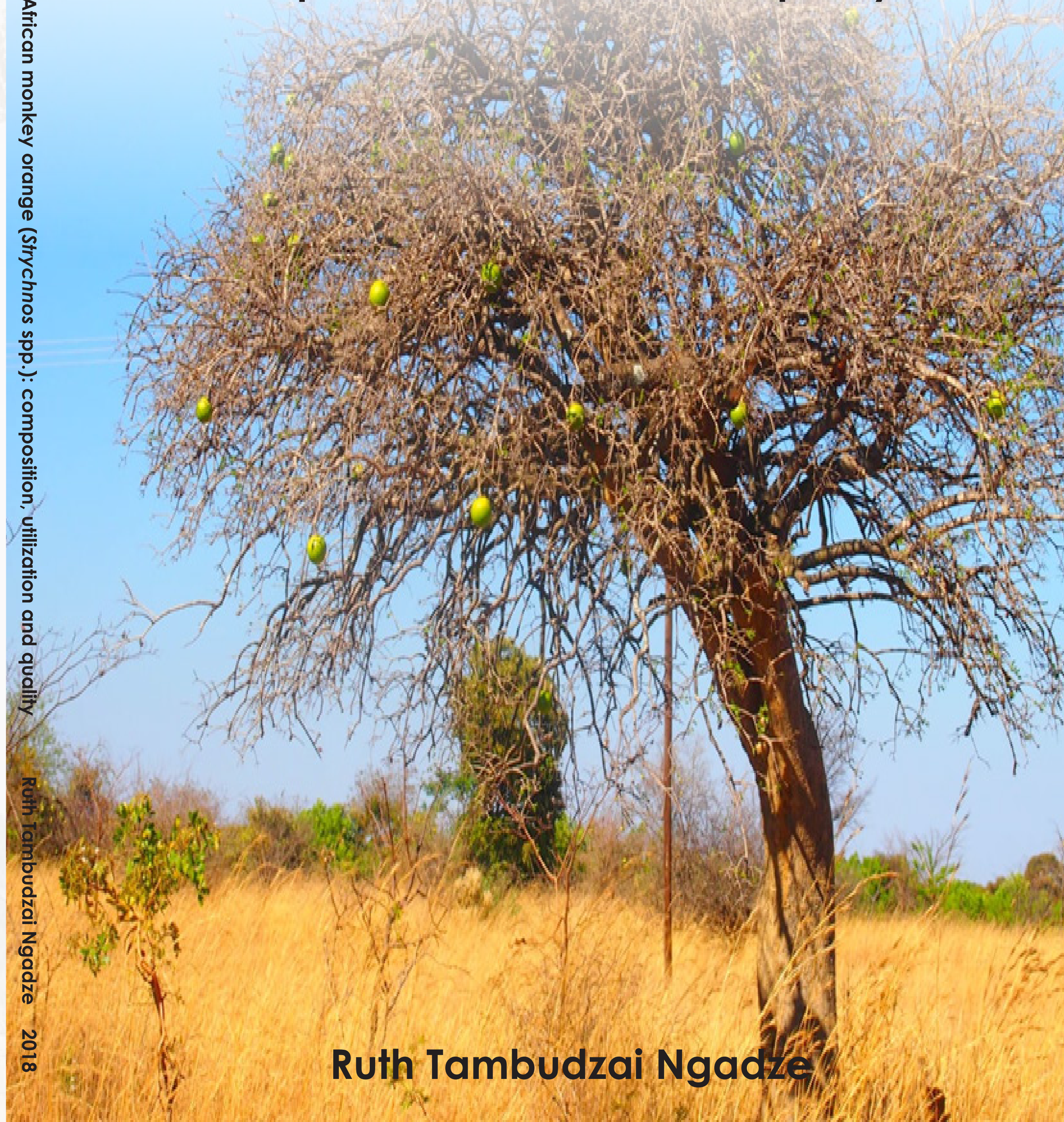
Value addition of Southern African monkey orange (*Strychnos* spp.): composition, utilization and quality



Ruth Tambudzai Ngadze

Value addition of Southern African monkey orange (*Strychnos* spp.): composition, utilization and quality Ruth Tambudzai Ngadze 2018

Propositions

1. Food nutrition security can be improved by making use of indigenous fruits that are presently wasted, such as monkey orange.
   (this thesis)

2. Bioaccessibility of micronutrients in maize-based staple foods increases by complementation with *Strychnos cocculoides.*
   (this thesis)

3. The conclusion from Baker and Oswald (2010) that social media improve connections, neglects the fact that it concomitantly promotes solitude.
   (*Journal of Social and Personal Relationships* 27:7, 873–889)

4. Sustainable agriculture in developed countries can be achieved by mimicking third world small-holder agrarian systems.

5. Like first time parenting, there is no real set of instructions to prepare for the PhD journey.

6. Undertaking a sandwich PhD is like participating in a survival reality show.

Propositions belonging to the thesis, entitled:

Value addition of Southern African monkey orange (*Strychnos* spp.): composition, utilization and quality

Ruth T. Ngadze

Wageningen, October 10, 2018

Value addition of Southern African monkey orange (*Strychnos* spp.): composition, utilization and quality

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Value addition of Southern African monkey orange (*Strychnos* spp.): composition, utilization and quality

Ruth Tambudzai Ngadze

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Chapter 1

General introduction

1.1 Importance of indigenous fruits in sub-Saharan Africa

About 815 million people had insufficient food and were affected by hunger in 2016, with the majority of these people living in developing countries (FAO et al., 2017). The situation in sub-Saharan Africa is worsened by the multiple burden of malnutrition: undernutrition, overweight / obesity and micronutrient (i.e. vitamin A and the minerals Fe, I and Zn) deficiencies, referred to as hidden hunger (FAO, 2017). In 2016, the prevalence rate of undernutrition in the sub-Saharan Africa region was 22.7 %. Iron deficiency, commonly known as anaemia, affected 39 % of women in the child-bearing age, while one in three children under the age of five was stunted and 11.8 % were overweight in Southern Africa (FAO, 2017; FAO et al., 2017). Mineral malnutrition is rife in rural communities because households rely heavily on cereals, roots and tubers as staples leading to diets that lack nutritional diversity (Allen et al., 2006). Simultaneously, the high starch diets also contribute to overweight and obesity due to the imbalance between excessive calorie/energy intake and expenditure (Joint FAO and WHO, 2005). Various studies have been conducted that ascertain non-timber forest products (NTFPs) as a means of diet diversification and nutrient source (FAO, 1996). NTFPs already play a traditional role as food sources throughout the tropics and add to food security in dry periods. Among NTFPs, indigenous edible fruits are occasionally used to meet food shortages and they remain a major option for coping with micronutrient deficiencies in diets of rural households in the semi-arid areas of Southern Africa during vulnerable times (Laverdière & Mateke, 2003). Indigenous edible fruits have also been adapted over time to food needs, habits and tastes of traditional societies in their own localities (Gomez, 1988).

International recognition and trade of wild fruits have increased in the past decades for fruits such as Araca-pera (*Psidium acutangulum*), cherimoya fruits (*Annona cherimola*) and especially acai berry (*Euterpe oleracea*) from the Amazon forest. These fruits have been reported to have antioxidant activity comparable to some reported so-called super fruits (Gruenwald, 2009; Li et al., 2016). Similar fruits exist in the semi-arid areas of Africa and have been accepted, cultivated and marketed internationally, especially in Europe, Japan, New Zealand and the USA for their high nutrient contents and specific ingredient uses, e.g. horned melon (*Cucumis metulifer*) trade marketed as Kiwano, melon (*Cucumis melo*), marula (*Sclerocarya birrea*) and tamarind (*Tamarindus indica)* (National Research Council, 2008). Though not yet cultivated commercially, baobab (*Adansonia digitata*) has also recently entered the European market as a food ingredient because of, among other factors, its high vitamin C and antioxidant content (Gruenwald, 2009). These developments have created a new impetus to the potential role of unexploited wild indigenous fruits for use as ingredients in the production of functional foods for local communities with promising prospects for global recognition and trade.

In Zimbabwe, over 180 wild food plants were inventoried, of which indigenous fruit trees comprise about 20 % (Akinnifesi et al., 2006; Tredgold, 1986). From these, a number of indigenous fruits have been prioritized for their potential to improve malnutrition problems, especially for rural communities in Zimbabwe. The International Centre for Underutilised Crops (ICUC), nowadays called Crops for the Future, in partnership with The World Agroforestry Centre and the International Plant Genetic Resources Institute through their ‘Fruits for the Future’ program initiated domestication of indigenous fruit trees (IFTs) and knowledge dissemination programs. The premise of the program was that the health and livelihood of communities could be improved by reduced dependency on expensive exotic fruit imports, through substitution with locally available, cheaper indigenous fruits that the community ranks as important. Indigenous fruits were therefore selected based on farmers’ preferences for domestication, ease of access, social and economic importance (Laverdière & Mateke, 2003).

1.1.1 Nutritional relevance of indigenous fruits

Fruits are widely recommended sources of minerals, vitamins and phytochemicals, which confer health benefits and reduction in disease risks. Minerals have important physiological roles in human health. Generally minerals have important physiological roles in cognitive skills, learning abilities, body growth and bone formation, essential for proper development of infants and children. Iron and Zn mineral deficiencies, which are very common in sub-Saharan Africa, can result in deleterious effects on the normal functioning of the human body related to the risk of a number of health consequences such as child stunting, anaemia and depressed functioning of the immune system, which are borne disproportionately by children and women. Consequently, malnourished pregnant women deliver children likely to suffer from suboptimal growth or development (Caulfield et al., 2006; Kennedy et al., 2003). Most African indigenous fruits can provide the essential minerals Fe and Zn as shown with a few examples in table 1. The mineral contribution of marula to the recommended daily intake (RDI) is estimated by calculation of the highest content in literature to be 9 % of Fe and 3 % of Zn and for baobab these figures are 39 % of Fe and 29 % of Zn when 100 g of fruit pulp is consumed. Monkey orange is assumed to meet the RDI for Zn and Fe for pregnant females (19-50 years) when 100 g of pulp/ fruit is consumed using the RDI values of Rosenberg et al. (2004). Epidemiological research links regular consumption of fruits directly to long term prevention of numerous chronic diseases such as cardiovascular diseases (Hung et al., 2004) and some cancers (Block et al., 1992; Jansen et al., 2004). African indigenous fruits provide bioactive compounds (table 1.1). The compounds associated with these health properties are phytonutrients (Eichholzer et al., 2001), of which the phenolic compounds generally account for these benefits (Saura-Calixto et al., 2007). Phenolic compounds are secondary plant metabolites, which widely occur in fruits. They have a radical scavenging ability that maintains non-enzymatic and enzymatic antioxidant defence systems in the human body as well as antiviral, antimicrobial and anti-tumour activities (Haminiuk et al., 2012). Their overall health benefits are believed to arise from the synergistic effect with other dietary compounds in the food matrix (Rimm, 2002). The phenolic compounds comprise various compound classes with different physicochemical properties and stability to processing conditions and storage – temperature, pH or whether they are substrate to endogenous polyphenol oxidase.

Table 1.1 Nutritional composition of common indigenous fruits with bioactive compounds

Fruit pulp	Fe	Zn	Ca	K	Cu	Mg	Vitamin C	Reference
	mg/100 g						g / 100 g	
*Strychnos spp. (monkey orange) [*]	140	29	105	2867	2.4	206	88	Ngadze et al., 2017
Irvingia gabonensis (African mango)							56	Stadlmayr et al., 2013
Tamarindus indica (tamarind)	5	3.1	217	970	0.42	94	20	
Sclerocarya birrea (marula)	2.5	0.34	480	548	0.04	310	180	Bille et al., 2003; Hiwilepo-van Hal et al., 2014
Adansonia digitata (baobab) [*]	10.4	3.2	701	3272	1.8	300	360	Chadare et al., 2008
Actinidia deliciosa (kiwi)	0.31		34	312	0.13	17	250	Roussos, 2016
Citrus sinensis (orange)	2.1	1.0	370	1200	0.55	100	80	
Fragaria × ananassa (strawberry)	0.41	0.14	16	153	0.048	13	59	Giampieri et al., 2012

Values taken from highest content reported in nutritional review literature

[*]Shows values based on DW

1.1.2 Use of indigenous fruits in food dishes

Indigenous fruits are harvested at different times of the year and are highly appreciated and used in the processing of a range of food products, which are consumed by local communities. Table 1.2 shows some indigenous fruits of Africa that are used as ingredients in cereal-based porridges/ gruels, cakes or as condiments to improve taste, colour, flavour and nutritional composition in staple foods and meat gravies. Fruits as ingredients, used with water or solely, add a substantial amount of vitamins, minerals and bioactive compounds to the starch-based staple cereal flours (maize, sorghum, wheat and millet), whose principal components are starch, protein and fat. Enhancement with fruit adds to the complexity of the food matrix by nature of dietary compound interactions, which may affect the food product's nutritional composition and physical properties (macro and microstructure) with respect to nutrients, health and aroma compound release and sensory perception. Additionally, the end product may be consumed concomitantly with other foods (e.g. fruit chutney served with a staple meal or meat reduces product pH and increases total soluble solids), thereby enhancing or inhibiting nutrient bioaccessibility and bioavailability. The "meat factor" also improves the bioaccessibility of essential minerals such as Fe from fruits, which otherwise would have reduced bioaccessibility in the absence of meat (Hurrell et al., 2006). Incorporation of fruits in meals adds health and nutrition benefits to the diet of the consumers, including but not limited to health beneficial phytochemicals, promoter factors for micronutrient digestion, reduced starch digestion and overall functional properties (Cilla et al., 2009). Thus, improvement of practices used for processing traditional fruits has potential for improving consumer sustenance, utilization of available raw materials as well as social and economic advancement of local communities.

1.2 Strychnos species

Strychnos spp. is among the top priority fruit species in Southern Africa as ranked by farmers on basis of nutritional, sensorial and economic importance, making it an important food source for rural populations in sub-Saharan Africa and particularly Zimbabwe. The fruit is eaten fresh or processed into several food products that are commonly consumed in the resource-limited communities of these countries. Monkey orange fruit characteristics differ between species. Generally they are orange-sized fruits with a hard woody yellow-orange shell, which has to be cracked open to obtain the fruit flesh. The fruit contains numerous hard seeds embedded in a fleshy pulp, which partly liquefies upon ripening.

Table 1.2 Some local African products made from indigenous fruits

Fruit	Main ingredients	Served as or with	Reference
Strychnos spp. (monkey orange)	pulp/ maize meal pulp /wheat flour	porridge cakes mahewu	Ngadze et al., 2017
Musa (plantain)	fruit flour/water fruit flour, beans, amaranth leaves and olive oil	porridge porridge	Ekesa et al., 2012
Tamarindus indica (tamarind)	pulp pulp/sorghum/ millet	condiment: chutney porridge/gruel	Leakey, 1999 National Research Council, 2008
Uapaca kirkiana (sugar plum)	pulp/millet or sorghum flour	porridge cakes	Gomez, 1988
Cucumis metuliferus (horned melon)	Pulp	condiment: chutney	Mabaya et al., 2014
Citrullus lanatus (Kalahari melon)	pulp/ maize meal	porridge	
Dovyalis caffra (kei apple)	pulp/wheat flour	cake	Directorate Plant Protection S.A, 2013
Elaeis guineensis (African palm oil)	Pulp	thick gravy soup with starch dish *	*Personal communication*
Diospyros mespiliformis (jackal berry)	pulp/ cereal flour	porridge	National Research Council, 2008
Garcinia livingstonei (imbe)	pulp/ cereal flour	porridge	
Vangueria infausta (medlar)	pulp/ maize meal	fermented porridge thick gravy with starch dish*	
Bequaertiodendron magalismontanum (milk plum)	pulp/ maize meal	porridge	
Balanites aegyptiaca (desert date)	pulp/ cereal	porridge	
Adansonia digitata (baobab)	pulp/ cereal	porridge hot dish	
Carissa macrocarpa (carissa)	pulp/cereal	porridge	
Sclerocarya birrea (marula)	pulp/cereal	porridge	
Cucumis melo (melon)	dried pulp/peanut/meat	millet bread*	
Blighia sapida (ackee apple)	pulp/ salt fish	ackee and saltfish dish	*Personal communication*

*Served with stated food product as a side dish during a meal

1.2.1 Ecology, agronomy and distribution

Among the common monkey orange species found in Southern Africa, three were designated as important in Zimbabwe. To differentiate these species, their characteristics are given in table 1.3. *Strychnos* spp., belonging to the Loganiaceae family, is a deciduous tree and comprises 75 species (Bisset, 1970). *Strychnos* spp. has a wide distribution in sub-Saharan Africa, in particular in the drought-prone areas and semi-arid Kalahari Sandvelds with 600 – 1500 mm rainfall; the tree is more frequent where demanding trees fail to grow (SCUC, 2006b). Generally *Strychnos* spp. grow well in deep sandy soils on well-drained slopes, clays and red loamy sands derived from limestone (Mwamba, 2006). *Strychnos* spp. trees are light demanding, tolerate hot/dry summers and are deciduous. The trees grow naturally and are selectively conserved in natural forests and woodlands or serve as boundaries/ fence lines for homesteads and farms. They are communally owned and rural inhabitants have free access, making them easily available for trading purposes or inclusion into the diet. The domestication of the *Strychnos* spp. is still at experimental stages. Propagation is by seeds or by grafting and budding techniques, with fruit bearing starting 3-5 years after transplanting. Experimentation orchards have been reported for *S. spinosa* species planted in Besor Research Farm, in the Western Israeli Negev desert (Mizrahi et al., 2002). Adaptability tests were conducted in the USA (Florida) (Mwamba, 2006). A European Commission project initiated studies on the production potential of *S. cocculoides* in Namibia and Botswana during the last decade (Mwamba, 2006).

1.2.2 Harvesting and fruit characteristics

S. cocculoides flowers during the rainy season. It takes 8-9 months from flower fertilization to fruit ripening in the dry season (Orwa et al., 2009). In Zimbabwe, harvesting is between August and December (Akinnifesi et al., 2007a), the so-called “lean season”, during which the fruits supplement staple crops before the new harvest. In this season less than three meals per day are consumed and the diet is deficient in minerals and vitamins with an over dependency on starchy staple foods. A single fruit weighs 145-385 g and superior trees produce up to 500 fruits with an average of 25-100 fruits per mature tree (Laverdière & Mateke, 2003; Mkonda et al., 2002). The fruits have robust shells and stay edible in the tropical heat for up to 3 months after maturity, hence making it a favourable fruit in marginal areas without sufficient infrastructure. However, fruit quality deteriorates faster in the commonly used storage facilities due to high humidity, temperature and the presence of moulds. When fruits are not washed or washed and not air dried sufficiently before storage, their spoilage is high due to powdery mildew attack. Generally fruits from *Strychnos* spp. have a characteristic sweet - sour taste / acidic taste (pH 3.6) and flavour that is described as encompassing a variety of exotic fruits (Sitrit et al., 2003). The seeds and pulp do not adhere to the shell and can be removed with ease. Variability is in the taste, colour, texture and flavour, which exists between and within species. Moreover, monkey orange species differ in their proximate composition, gelling properties and specific optimum processing conditions, thus affecting the standardization of their food products.

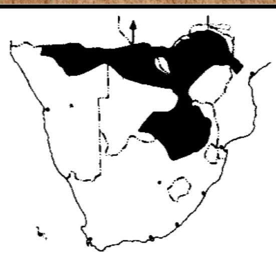
a b

Figure 1.1 *Strychnos cocculoides* trees in Zimbabwe with one trunk (a) and several trunks (b)

1.2.3 Nutritional value of edible Strychnos spp. of Southern Africa

Edible *Strychnos* spp. have high mineral contents, namely Fe 70 -140 mg/ 100 g DW (Arnold et al., 1985; Malaisse & Parent, 1985) and Zn 29 mg/ 100 g DW (Hassan et al., 2014), and a vitamin C content of 34 - 88 mg/ 100 g DW (Amarteifio & Mosase, 2006; Arnold et al., 1985). The nutritional composition of monkey orange is comparable to or better than that of other common indigenous fruits of sub-Saharan Africa and more diffuse exotic fruits, thus making it a good local source of nutrients (table 1.1). Thus, monkey orange is an important food, especially so for rural communities as these often cannot afford to augment dietary staple foods. Additionally, monkey orange has phenolic compounds with antioxidant activity equivalent to baobab (Nhukarume et al., 2010), giving it added health-supporting benefits. Although it is known that monkey orange has antioxidant activity, information with regard to particular phytonutrients, their content in pulp and products is lacking.

Table 1.3 Description and distribution patterns of *Strychnos* spp. from Southern Africa[1]

Strychnos spp.	*S. cocculoides* (corky monkey orange)	*S. spinosa* (spiny monkey orange)	*S. madagascariensis* (black monkey orange)
Tree description	Height 1-8 m with one or several trunks. Trunk diameter 25 cm. Spreading branches and a rounded crown. Bark is pale grey to creamy-brown with thick, corky ridges and spines. Leaves dark green, with or without rough hairs, broadly ovate-oblong to almost circular.	Height 1-7 m. Trunk diameter 15 cm. Bark fluted hard, grey-whitish. Branches are recurved or straight spines. Branchlets end in a straight spine. Leaves light to dark green. Glossy above, dull and paler below in pairs or 3s, elliptic, ovulate to almost circular.	Height up to 6-15 m. Trunk diameter up to 40 cm. Bark light grey smooth and unarmed. Branchlets knobbly dwarf. Leaves green above and pale green below, leathery, velvety clustered towards end of branchlets, elliptic to circular.
Fruit description	Fruit diameter 6-12 cm. Unripe fruit is dark green with paler mottling. Ripe fruit is orange or yellow.	Fruit diameter 12-15 cm. Unripe fruit is yellow-green. Ripe fruit is yellow.	Fruit diameter 7.5 cm. Unripe fruit is bluish-green. Ripe fruit is orange or yellow.
Ripe fruit*			
Distribution pattern in Southern Africa			

Description and distribution (Bisset, 1970; Orwa et al., 2009; Palgrave & Palgrave, 2002; SCUC, 2006b)
**Fruit pictures taken in Zimbabwe 2014 - 2016 fruiting seasons*

1.3 *Strychnos spp.* fruit use in food production

Strychnos spp. fruits have several perceived health benefits, such as an improved immune system for the young and elderly, according to information passed on from one generation to the next and serve as a source of income to support livelihoods (Ngadze et al., 2017b). When in season, the fruits are consumed either fresh, processed into other food products or added to various dishes as a flavour enhancer to increase taste and palatability (figure 1.2). The fruit's taste makes it a fruit of choice among local rural populations. Previous studies reported that monkey orange products were preferred to mango and baobab products, mainly because of their taste (Saka et al., 2007). The pulp is commonly used as an alternative to milk or water in culturally embedded foods such as maize porridge. Maize porridge is made by cooking maize meal in boiling pulp or water for several minutes (figure 1.2a). Pulp is also used for confectionery products and processing alcoholic and non-alcoholic beverages (called *mahewu* in local Shona language), jams and juices (Chirwa & Akinnifesi, 2008). During jam processing, no pectin is added as the fruit has good setting characteristics (Saka et al., 2007). When the product is not sour enough as a juice, processors subject the product to spontaneous fermentation to provide sensory appeal. Other than using the juice as an ingredient, the flesh (seed + pulp) can also be dried and then pulverised to separate the fruit particles from the seeds to obtain a powder or fruit-derived leather for consumption as a snack between meals or a flavouring ingredient in starch-based foods (figure 1.2d).

Though monkey orange pulp liquefies during ripening, the pulp does not liquefy completely and some pulp remains attached to the seed. Factors affecting liquefaction are maturity level, storage conditions and the applied maceration treatment. Seed- pulp separation is an important processing constraint for monkey oranges, which leads to its underutilization. To date little is known about the improvement of its yield and effect on quality. When processing the fruit at home or at a small scale, juice is obtained by maceration in water (hot or cold) and occasional manual stirring to disrupt the pulp and detach it from the seed. This practice varies from home to home, ranging from 30 min to 2 h on or off heated wood charcoal. In all cases, some pulp still remains attached to the seed and is discarded as waste. Juice that is obtained after thermal treatment will have suffered damage to thermally sensible compounds such as bioactive compounds with antioxidant activity (Sun et al., 2014). On the other hand, some phenolic compounds may become more available due to cell wall destruction of the plant material. The outcome of this process depends on other factors such as the pH of the product and the heat stability of the compounds. Addition of water for maceration and heat treatment cause flavour and or nutrient losses due to the additive effect of dilution of the juice and loss of volatiles, respectively, thereby potentially reducing sensory appeal and nutritional quality. It is therefore important to use processing conditions that retain nutrients, bioactive compounds and improve sensory quality of the product. An option to increase juice yield, improve physicochemical characteristics such as clarity, and maintain health beneficial compounds is the use of a pectinase during the maceration phase (Joshi et al., 2012).

To make maize porridge, which can be enhanced by monkey orange, processors commonly use refined white maize meal. The meal is obtained by de-germing and dry milling maize kernels for size reduction, after which the bran and fibre are removed by sifting, sieving, screening or aspiration. This white refined maize meal is preferred by consumers for making thin porridge and thick gruel (sadza- in local Shona language), rather than whole grain maize meal. However, refined maize meal has reduced micronutrients, phenolic compounds and fibre because of the milling method applied (Gwirtz & Garcia-Casal, 2014; Kandil et al., 2012). White maize porridge is a starchy, high glycaemic index (GI) food. When eaten as such, the porridge is deficient in micronutrients and can increase postprandial blood glucose, which is linked to diabetes and obesity as a result of increased conversion of the cooked starch to glucose. Consequently, the nature of the food matrix in combination with thermal processing has an effect on nutrient content and digestibility. When polyphenols or a polyphenol-rich food are added to a food that mainly consists of carbohydrates, starch digestion is reduced, thereby reducing the risk of postprandial glycaemia (Coe et al., 2013; Kandil et al., 2012; Karim et al., 2017).

1.3.1 Other uses of monkey orange

During the lean season when the pastures are scarce, livestock also feeds on the monkey orange seed waste and green leaves of the fruits. The fruit shell is used to make musical rattles, spice dispensers or household ornaments. The unripe fruit and fruit parts (bark, roots and leaves) have a long history of use as folk medicine for improving health conditions such as coughs, stomach aches and wounds (Motlhanka et al., 2008; Mwamba, 2006). The unripe fruit can also be used to make dye for colouring food trays and soap for washing clothes due to its saponin content. *S. cocculoides* seed produces a reddish oil, which is considered for use in the cosmetic industry by PhytoTrade Africa. *S. innocua* seeds contain galactomannan, a polysaccharide with industrial applications (Mwamba, 2006). The wood is used in making posts and tool handles as well as for firewood.

Figure 1.2 Food products from *Strychnos* spp.: **a**. maize porridge; **b.** wheat flour cake; **c.** solar dried snack; **d.** sundried snack

1.4 Implications of *S. cocculoides* use

To date, monkey orange fruits are still underutilized and losses of the ripe fruit have been observed in their natural habitat. There are no current data on tree densities and fruit losses in the different regions in Zimbabwe, though sightings of losses are prevalent (figure 1.3). Underutilization is common when the harvest is bountiful and when other food sources are available. Furthermore, underutilization is attributed to difficulties in manual extraction of the pulp from the seed. Previous studies on monkey orange have not targeted processing constraints and the fruits' potential to solve food-related problems that are crucial to improving human health and nutrition. The fruits are sold and traded in Botswana for US $ 0.45 per fruit, generating benefits from fruit sales for many rural households (Mwamba, 2006). Additionally, children are accustomed to the taste of the fresh fruits and like the foods produced with the characteristic flavour of monkey orange. The processed product has potential for sale at both small scale and large scale giving opportunities for commercialization. However, regardless of the fruits' nutritional and economic potential, the poor seed - pulp separation has some practical consequences such as:

- restriction of fruit consumption to periods of critical food shortages
- reduced inclination to consume and process the fruit when other less "cumbersome" indigenous fruits are available
- erosion of processing knowledge passed on orally from generation to generation
- reduced diet diversification

Figure 1.3 *Strychnos* spp. losses

1.5 Objectives and research questions

The general objective of this thesis was to optimize indigenous processing of *S. cocculoides* to reduce fruit losses and contribute to food security and livelihoods of vulnerable rural communities in the production areas. In order to attain this goal, the specific objectives were:

a. To critically review existing literature on commonly consumed *Strychno*s spp. with a focus on the nutritional composition, sensory properties and effects of processing on product quality;
b. To document and evaluate indigenous processing knowledge and constraints associated with *Strychnos* spp. processing in Zimbabwe;
c. To characterize phenolic compounds of commonly consumed primary products of *S. cocculoides* and determine the effects of maceration techniques on juice yield and quality;
d. To determine the digestibility of nutrients in maize porridge enhanced with *S. cocculoides* juice.

The research questions that were formulated to address these objectives are schematically presented in figure 1.4.

1.6 Thesis outline

This general introduction provides information on relevance of indigenous fruits to nutrition and food production with emphasis on the commonly consumed *Strychnos* spp. in Southern Africa, their importance to the communities in which they are consumed and the research issues that this thesis addresses. Chapter 2 presents a review of the uses, nutritional composition, sensory characteristics and effects of processing on the product quality of the most common varieties of monkey orange. In chapter 3, consumption patterns, processing methods and constraints in different ecological regions of Zimbabwe are described on the basis of indigenous knowledge. The chapter identifies areas of future research, which serve as a base for chapter 4 and 5. Chapter 4 gives an overview of identified health-supporting compounds in *S. cocculoides* juice, a product that was identified as an important primary ingredient of several other *S. cocculoides* products. Polyphenol content and juice yield of traditional or enzyme- extracted juice and the effect of the juice extraction method on the identified phenolic compounds were determined. Subsequently, chapter 5 studies the digestibility of polyphenols, minerals and starch hydrolysis when *S. cocculoides* juice is used as an ingredient in maize porridge. Finally, chapter 6 presents the general discussion of the core findings, concluding remarks and recommendations for future research.

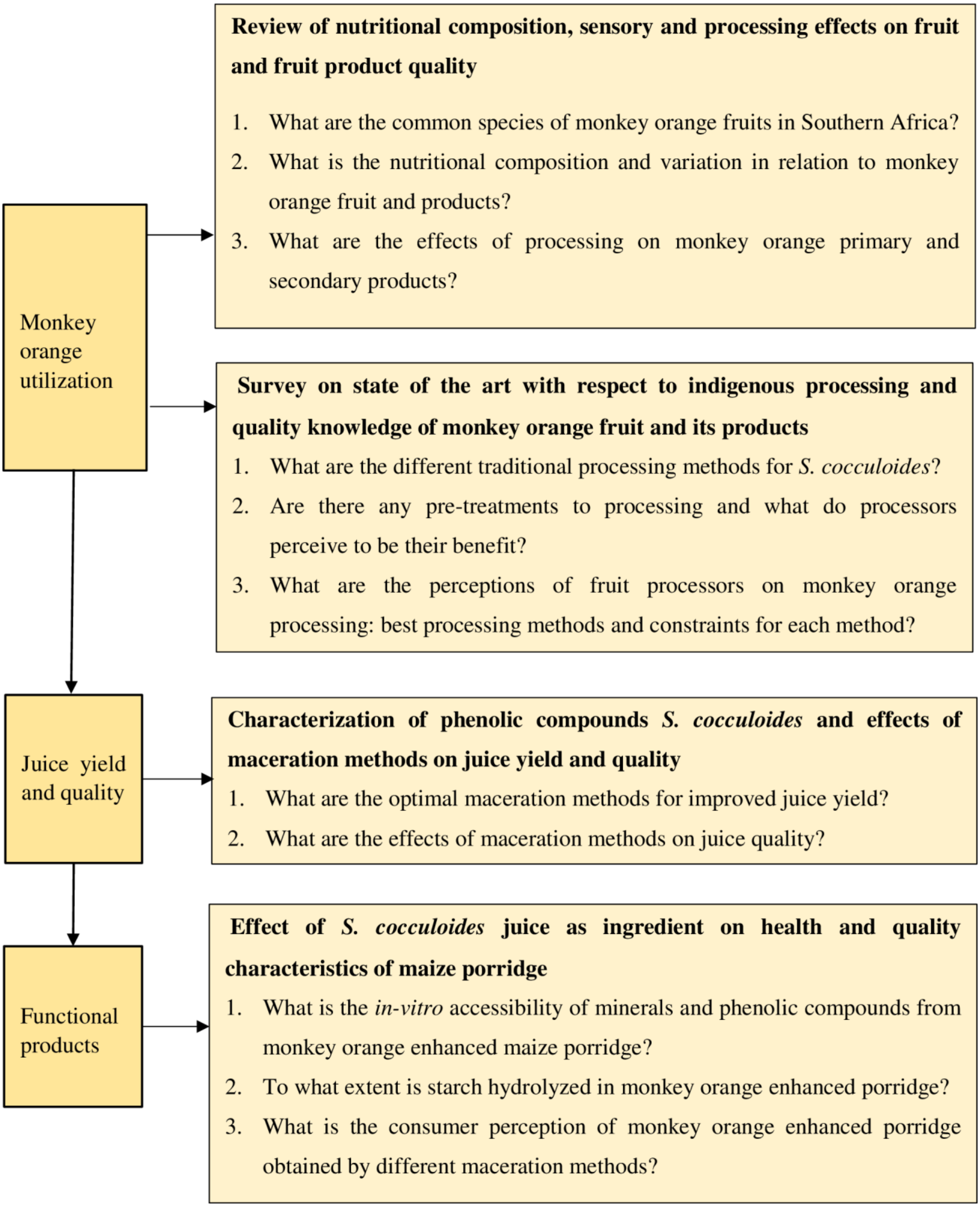


Figure 1.4 Research design for the optimization of *S. cocculoides* fruit processing

Chapter 2

Local processing and nutritional composition of indigenous fruits: the case of monkey orange (*Strychnos* spp.) from Southern Africa

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Abstract

Monkey orange (*Strychnos* spp.) is a widely distributed fruit species in Southern Africa commonly consumed by the local population. It has potential to improve the nutritional status of rural populations, being a precious food source in areas with periodic shortages, since it is rich in vitamin C, Zn and Fe. To improve the availability of this food outside its production season, processing and preservation techniques used at household level need upgrading as they are unreliable and their effects on nutritional quality are unknown. Based on this review, we recommend better indigenous fruit production as a sustainable solution to malnutrition in rural areas in transition countries.

Keywords: *Strychnos cocculoides*, *Strychnos innocua*, *Strychnos spinosa* and *Strychnos pungens*, sensory properties.

2.1 Introduction

Monkey orange (*Strychnos* spp.) is a member of the Loganiaceae family, indigenous to tropical and subtropical Africa (Bisset, 1970). Up to 75 *Strychnos* species have been recognized in Africa, of which twenty species produce edible fruits in Central and Southern tropical Africa, drought prone areas and semi-arid regions (SCUC, 2006a), where the tree remains dormant when water is unavailable (Mwamba, 2006). Most commonly consumed species, which are prevalent in woodlands of Southern Africa, are *S. innocua* (synonym: *S. madagascariensis*), (Mwamba, 2006) *S. cocculoides*, *S. pungens*, and *S. spinosa* (Bisset, 1970).

Generally, fruits of the four monkey orange species under review are indehiscent, oval shaped, yellow or orange coloured, possessing a thick woody shell (Coates Palgrave et al., 2002). The pulp is bright yellow or brown, juicy, sweet and or sour, with few to numerous hard seeds imbedded in the fleshy pulp (Coates Palgrave et al., 2002; Mbiyangandu, 1985). Whole fruit weighs from 145 g to 383 g (Mkonda et al., 2002) and a single tree produces 300 to 700 fruits, which translates to approximately 40 to 100 kg fruit weight per tree (SCUC, 2006a). Monkey oranges are characterized by their seasonality, being harvested between August and December (Akinnifesi et al., 2007a), the so-called "lean season"– a time of cultivated food shortages in Zimbabwe. Fresh monkey oranges are consumed immediately after cracking and become inedible when left exposed to air due to both microbial spoilage and physico-chemical reactions that cause off flavours and enzymatic browning. The intact fruits have been reported to stay fresh and edible for three months, enabling their trade between countries in Southern Africa (Mkonda et al., 2002; Ruffo et al., 2002). During prolonged storage, the fruits are susceptible to deterioration because of high temperature, rainfall, disease, poor handling, and storage conditions that impact physiological and biochemical changes. Reduction of post-harvest losses and increase in shelf life have become an urgent need to promote better use of monkey oranges. To prolong shelf life and maintain quality, the fruit is sometimes domestically processed to jams, juices, wine, beer, fritters, muffins or dried fruit leathers (Bille et al., 2013; McGregor, 1995; Orwa et al., 2009; Rampedi & Olivier, 2013; Zinyama et al., 1990), but still most of the product is lost if not consumed fresh.

Monkey oranges are widely consumed by rural communities, particularly women and children in Southern Africa, because of their pleasant taste and flavour (Amarteifio & Mosase, 2006). Nutritionally, the fruits possess potential health benefits attributable to high energy, fiber, minerals (Fe and Zn) and vitamin C (Bello et al., 2008; Saka & Msonthi, 1994). In Zimbabwe, 30 % of pregnant and lactating women are assumed to be Fe deficient (Banziger, 2000) while maternal anemia is a common cause of maternal and neonatal mortality (Gadaga et al., 2009). Thirty three percent of children six to 59 months old were stunted (FNC, 2010) and 56 % of the same age group suffer some degree of anemia (ZIMSTAT & ICF International, 2012). *Strychnos* spp. depending with species have an Fe content up to 140 mg/ 100 g, (Malaisse & Parent, 1985) giving potential to deliver Fe when used as a food source by pregnant or lactating women and children. *Strychnos* spp. have been described to be among the indigenous fruits in many parts of Southern Africa that have commercial potential and contribution to trade (Van Wyk, 2011). Potential benefits of improved livelihood security through income generation by sale of fruit and fruit products for rural communities also exist.

In Southern Africa identification of superior genotypes, collection of germplasm (Mwamba, 2006), and cultivation of *S. spinosa* and *S. cocculoides* for consistent high yields of large, quality fruits are ongoing. On cultivation positive responses from seedlings and grafting were obtained taking three years and four to five years to bear fruit respectively (National Research Council, 2008).

Previous reviews of *Strychnos* spp. by Mwamba, 2006 and Orwa et al., 2009, were directed towards taxonomy, physical properties, ecology, agronomy, reproduction, harvest, selection and genetic resources. The current review provides on overview of nutritional composition, sensory properties, food uses, and impact of processing techniques on nutritional and sensorial quality of the four common *Strychnos* spp. in Southern Africa. Thereafter, recommendations for future studies pertaining to processing and storage of monkey orange in order to maintain year-round availability, nutrient retention and sensory quality are described.

2.2 Sensory properties

2.2.1 Taste and texture

Overall, the ripe monkey orange species has fleshy (Malaisse & Parent, 1985), sweet (Amarteifio & Mosase, 2006), yellow, very aromatic pulp (Mbiyangandu, 1985; Tanton & Haq, 2007) and contains numerous hard brown seeds (Sitrit et al., 2003). There is wide variability in the general description of taste, colour, texture and flavour between and within species (table 2.1).

Fruit sweetness depends highly on sugar composition (Lee et al., 2013); Sitrit et al., 2003 report accumulation of sugars and organic acids during ripening and sucrose conversion to glucose and fructose at the onset of ripening for *S. spinosa*. Total sugars were 28.2 g/100 g dw and the most abundant sugar was sucrose (12.9 g/100 g dw), a disaccharide, followed by the monosaccharides glucose (4.6 g/100 g dw) and fructose (1.9 g/100 g dw) (Sitrit et al., 2003). It was shown that the degree of monkey orange ripeness had an effect on sugar composition, thus taste is dependent on the stage of ripening in addition to environmental factors like soil, geographical location and climatic differences.

Organic acids in fruits originate from biochemical processes or from the activity of some microorganisms such as yeasts and bacteria (Lee et al., 2013). The presence of organic acids (citric, malic and succinic acids) explains the acidic component that blends with sugars and results in the species characteristic blended acid-sweet taste. Sirit et al., 2003 found that *S. spinosa* contained malic acid 1.9 g/ 100 g dw, succinic acid 0.5 g/ 100 g dw and citric acid levels of 2.4 g/ 100 g dw. Amarteifio and Mosase, 2006 reported a citric acid content of 0.77 g/100 g dw for *S. spinosa*, indicating the acidic nature of the species. Citric acid concentrations of 3.8 to 4.9 g/100 g, found in a sour variety of *Ziziphus mauritiana* fruit (Nyanga et al., 2008), were higher than that found in *S. spinosa*. Hence, in comparison with other indigenous sour fruits, *S. spinosa* contains less citric acid, thus exhibiting a more palatable and less sour taste. However, *S. innocua* has a bitter taste that can be attributed to the presence of tannins (1.01 ± 0.01 mg/g), (Bello et al., 2008), though this can vary between and among trees of the same provenance.

Saka et al., 2007 reported a mean acidity of 1.13 % for *S. cocculoides* processed juice. The relatively high acidity of *Strychnos* spp. may contribute to the long shelf life of fresh fruits when compared to other fruits. The pH of *S. spinosa* was 3.2 (Amarteifio & Mosase, 2006) and 2.8 (Sitrit et al., 2003) while that of processed *S. cocculoides* juice was 3.5 (Saka et al., 2007).

Partial solubilization of pectin and cellulose during ripening by the endogenous plant enzymes polygalacturonase (PG), pectinmethylesterase (PME), lyase, and rhamnogalacturonase, (Prasanna et al., 2007) contributes to changes in texture and juiciness of fruits. Though consistency differs among species and level of ripeness, monkey orange fruits exhibit a thick gel or juicy texture. The degree of pectin solubilization determines texture characteristics: the higher the hydrolysis, the juicer the fruit.

Table 2.1 Sensory properties of the four main species of monkey orange fruit

Monkey orange species	**Taste**	**Aroma volatiles**	**Aroma description**	**Texture**	**Colour**	**Reference s**
S. cocculoides Bak.	Sweet	isobutyl acetate, 2-methylbutyl acetate, ethyl-2-methylbutyrate, 2, 6-ditetrabutyl-4-methyl-phenol, butyl-2-methyl butyrate, and geranyl acetate,	Fruity sweet	Jelly-like Juicy	yellow	(Mwamba, 2006; Palgrave et al., 2002; Shoko et al., 2013; Bicas et al., 2011)
S. spinosa Lam.	Tarty/ fermented Acid-sweet	Phenylpropanoids, trans-isoeugenol	Clove	ND*	yellow	(Mwamba, 2006; Palgrave et al., 2002; Sitrit et al., 2003)
S. innocua Del.	Sweet Bitter	ND	ND	ND	deep yellow to brown yellow	(Bisset, 1970; Palgrave et al., 2002)
S. pungens Sol.	Tarty/ fermented Pleasant tasting	ND	ND	Juicy	yellow	(Palgrave et al., 2002)

*ND: No Data

Saka et al., 2007 compared the sensory properties of sugar plum (*Uapaca kirkiana*), baobab (*Adansonia digitata*), mango (*Mangifera indica*), and *S. cocculoides* juice, where the monkey orange juice was the most preferred by sensory panelists. These findings concurred with the study of Bille et al., 2013 where diluted *S. cocculoides* juice resulted in high consumer acceptance, especially with improved liquefaction and clarification. *S. cocculoides* jam was highly scored by consumers, also because of its taste (Tumeo et al., 2008) and in general consumer acceptance of the other monkey orange fruit products was high in literature surveyed (Bille et al., 2013; Saka et al., 2007; Tumeo et al., 2008). All in all, the sensory studies indicated the potential for product development and commercialization of the species.

2.2.2 Volatiles

Fresh monkey orange fruit emits a distinct and delicate mixture of complex aroma volatiles, which are perceived by consumers as a mixture of pineapple, apricot, melon, clove, and citrus (Sitrit et al., 2003). According to data of surveys in Zimbabwe, *S. cocculoides* juice is added to cereal porridge (a thin maize slurry) for sour flavour enhancement and vitamin enrichment (Akinnifesi et al., 2007b). The most abundant volatiles of ripe *S. cocculoides* pulp of Malawian provenances were acetate and butyrate esters,(Shoko et al., 2013) which exhibit a fruity sweet flavour (Bicas et al., 2011), this concurs with consumer descriptions of fruit flavour. The main volatile flavour compounds found in the peel of ripe *S. spinosa* fruits were trans-isoeugenol and eugenol, which have a pungent clove aroma, and p-transanol [4-(1-propenyl)-phenol], while the unripe fruit lacks volatile compounds (Sitrit et al., 2003). Volatile compounds of *S. spinosa* and *S. cocculoides* found in literature are shown in figure 2.1, while no data for volatiles of *S. pungens* and *S. innocua* were found.

2.3 Nutritional composition of fruit flesh

Different units to express nutritional content were used in the literature collected for this review; thus, all data were converted to g/100 g for macronutrients, mg/100 g for micronutrients and dry matter basis where possible to enable data comparison. In some cases, data had to be omitted due to missing water content or dry matter content and when it was not clear whether dry or wet basis was used. In other cases, it was not stated whether the flesh, juice or pulp was used in analysis and therefore data are presented in general terms as the edible portion of the fruit. Where data for a particular species were found from one source, it is assumed to be the mean value.

2.3.1 Macronutrients

The reported minimum and maximum macronutrient composition of monkey orange species varies between and within species (table 2.2). There was large variation between the minimum and the maximum carbohydrate content within species of monkey orange. *S. innocua* had the highest total carbohydrate content variation: 15.4 g/100 g dw (Bello et al., 2008) to 61 g/100 g dw (Saka & Msonthi, 1994). This appears to result from inaccuracies due to the applied methodology as carbohydrates were determined by an indirect method, namely the difference method. Also, the reported protein range for *S. innocua* was remarkably wide, namely from 0.3 g/100 g dw (Malaisse & Parent, 1985) to 12.8 g/100 g dw (Arnold et al., 1985) where outlier values may be due to small sample sizes in the studies. All authors used the Kjeldahl analysis with a conversion factor of 6.25 for protein determination; hence, the methodology cannot be the cause of the observed variation in data. Of the studies under review, no authors analyzed for amino acids.

In the literature, there is also a notable variability in fat content for all the species ranging from 0.3 g/100 g dw (Malaisse & Parent, 1985) to 20 g/100 g dw (Hassan et al., 2014). Saka and Msonthi, 1994 reported a fat content of 31.2 g/100 g dw for *S. spinosa*, which was the maximum value for fruits of the same species and other reported species. The high value is an outlier and can be the result of irregularities in the methodology where sample size was n=1. The large variation in fat content between and within *S. spinosa* and other species can thus be attributed to sample size and methodology as in some cases authors did not describe the method of analysis.

Reported energy values range from 1315.4 kJ/100 g (Arnold et al., 1985) to 2083.6 kJ/100 g (Hassan et al., 2014) for all the four species. The differences in values between studies and from species to species could result from different coefficients used to compute energy values. In a comparative study, *S. spinosa* ranked superior to other indigenous fruits; the energy value for *S. spinosa* was 1923 kJ/100 g, which was higher than for ber (*Ziziphus mauritiana*), with an energy value of 1588 kJ/100 g, and baobab (*Adansonia digitata*), with an energy value of 1480 kJ/100 g (Saka & Msonthi, 1994). Generally, the crude fiber content ranged from 2.5 g/100 g dw (Malaisse & Parent, 1985) to 22.2 g/100 g dw (Arnold et al., 1985) for monkey orange species reviewed. The fiber content of *S. spinosa* (17.6 g/100 g dw) was similar to that of *S. innocua* (17.9 g/100 g dw), as reported by Saka and Msonthi, 1994. Food high in fiber is often richer in micronutrient (Dhingra et al., 2012), thus this might explain the high micronutrient content of *S. innocua* and *S. spinosa* (table 2.2). The ash content of the four monkey orange species was between 0.5 g/100 g for *S. innocua* and *S. cocculoides* (Arnold et al., 1985; Hassan et al., 2014) to 4.7 g/100 g for *S. innocua* (Bello et al., 2008). Arnold et al., et al 1985 reported low ash contents for *S. spinosa* (1.8 g/ 100 g dw), *S. cocculoides* (0.5 g/ 100 g dw) and *S. pungens* (1 g/100 g dw); these low contents could be related to soil chemistry and micro climate of sampling locality. The reported variation in moisture content was high for *S. innocua*: 60 % (Hassan et al., 2014) to 91 % (Bello et al., 2008). The information on sample preparation was not documented and the variation may also be caused by the difficulty to obtain juice and or flesh due to the stickiness of the mesocarp to the endocarp. Thus overall high fiber and fat content can be attributed to and explained by contamination of the edible portion with seed material. Though water content variation was lower within the other three species than between the species, the high water quantities can affect the shelf life when fruits are not appropriately stored.

Table 2.2 Macronutrient composition[1] of the edible portion of monkey orange species *S. cocculoides, S. spinosa, S innocua and S. pungens*

	S. spinosa				***S. cocculoides***				***S. innocua***				***S. pungens***			
	Mean	Min	Max	Reference	Mean	Min	Max	Reference	Mean	Min	Max	References	Mean	Min	Max	References
CHO* g/100 g	28.7	15.2	42.1	Arnold et al., 1985; Saka & Msonthi, 1994	18.2	16.8	19.6	Malaise & Parent 1985; Arnold et al., 1985	32.5	15.4	61	Malaise & Parent 1985; Saka & Msonthi, 1994; Bello et al., 2008; Hassan et al., 2014	21.3	18.9	23.6	Malaise & Parent 1985; Arnold et al., 1985
Protein g/100 g	9.0	5.4	12.8		3.5	0.3	6.6		5.9	0.3	11.5		4.3	3.9	4.6	
Moisture %	78.8	ND*	ND	Arnold et al., 1985	78.2	76	80.4	Malaise & Parent 1985; Arnold et al., 1985	74.1	60.2	91	Bello et al., 2008; Malaise & Parent 1985; Hassan et al., 2014	68.2	64.3	72.1	Malaise & Parent 1985; Arnold et al., 1985
Fat g/100 g	15.8	0.47	31.2	Arnold et al., 1985; Saka & Msonthi, 1994	0.4	0.3	0.5		6.9	0.7	20	Saka & Msonthi, 1994; Bello et al., 2008; Malaise & Parent 1985; Hassan et al., 2014	1.8	0.7	2.9	
Fibre g/100 g	9.2	3.3	17.6	Amarteifio & Mosase, 2006; Arnold et al., 1985; Saka & Msonthi, 1994	4.6	4.59	4.6		9.4	2.5	17.9	Bello et al., 2008, Saka and Msonthi, 1994,Malaise & Parent 1985	13	3.8	22.2	
Ash g/100 g	3.5	1.8	4.6	Amarteifio & Mosase, 2006; Arnold et al., 1985; Saka & Msonthi, 1994	2.2	0.5	3.8		3.3	0.5	4.7	Saka & Msonthi, 1994; Bello et al., 2008; Malaise & Parent 1985, Hassan et al., 2014	2	1	3	
Energy kJ/100 g dw	1681	1439	1923	Arnold et al., 1985; Saka & Msonthi, 1994	1477	1382	1571		1628	1390	2084	Saka & Msonthi, 1994; Malaise & Parent 1985, Hassan et al., 2014	1363	1315	1411	

ND: No Data; CHO*: Carbohydrate*

[1]Different units to express nutritional content were used in the literature collected for this review, thus all data were converted to g/100 g for macronutrients kJ/100J for energy and dry matter basis where possible to enable data comparison. In some cases data had to be omitted due to a missing water content or dry matter content and when it was not clear whether dry or wet basis was used. In other cases it was not stated whether the flesh, juice or pulp was used in analysis and therefore data are presented in general terms as the edible portion of the fruit. Where data for a particular species was found from one source it is assumed to be the mean value.

2.3.2 Minerals and vitamins

The reported mean mineral contents of monkey orange show *S. innocua* as having the highest mean content of Cu (2.4 mg/100 g), Na (99.6 mg/100 g) and Zn (28.7 mg/100 g) (table 2.3), while *S. cocculoides* had highest mean content of Fe (70.5 mg/100 g) of the four monkey orange species. However, the large variation in mineral content that exists between species and within species may result from phenotypic variations, climatic differences and soil chemistry, which affect ion availability and uptake. Some reported mineral contents were obtained from a single literature source such as for *S. cocculoides* (Mg, Na, Zn, K and Cu), *S. innocua* (Zn and Cu) and *S. pungens* (Mg, Na, Zn, K and Cu). The data were insufficient for effective comparison of mineral contents between species. When compared to the other indigenous fruits, baobab (*Adansonia digitata*), marula (*Sclerocarya birrea*), and the medlar (*Vangueria infausta*), the highest source of Fe and Zn was from *S. spinosa* (Amarteifio & Mosase, 2006). Though mineral contents show extreme variations in minimum and maximum values, and data are sometimes only from one source without a methodology description, the available data suggest *S. innocua* and *S. cocculoides* to be important sources for Zn and Fe, respectively. This warrants further research on validation of mineral contents and bio-accessibility studies of mineral contents of *S. innocua* and *S. cocculoides* as they appear to have potential to complement local diets that are mineral deficient. Thereafter, research on bioavailability of Fe and Zn is needed to determine to what extent the species contribute to improving human nutrition.

The vitamin C content of monkey orange fruits ranged from 34.2 mg/100 g dw (Arnold et al., 1985) to 88 mg/100 g dw (Amarteifio & Mosase, 2006). In comparison with other fruits, the reported maximum vitamin C content for *S. spinosa* is comparable to marula (*Sclerocarya birrea*) (128.3 mg/100g), baobab (*Adansonia digitata*) (141.3 mg/ 100g), (Amarteifio and Mosase, 2006), oranges (50 mg/100 g) and strawberries (59 mg/100 g) (Eromosele et al., 1991). Mean vitamin C content of *S. spinosa* was higher than for the other species. Other vitamins assayed were thiamine (vitamin B1) and riboflavin (vitamin B2). Wehmeyer, 1966 found large differences between thiamine and riboflavin contents of *S. pungens* flesh adjacent to the shell on the one hand, and adjacent to the seed on the other hand. A thiamine content of 2.74 mg/100 g dw and a riboflavin content of 1.85 mg/100 g dw were reported for flesh inside the shell, while a thiamine content of 0.10 mg/100 g dw and a riboflavin content of 0.74 mg/ 100 g dw were reported for flesh surrounding the seeds (Wehmeyer, 1966). It is not clear why the demarcation was made between flesh around the seed and flesh around the shell for thiamine and riboflavin determinations. However, lower thiamine contents were found for pulp of *S. cocculoides*: 0.03 mg/100 g dw, *S. pungens*: 0.05 mg/100 g dw and *S. spinosa*: 0.23 mg/100 g dw by Arnold et al., 1985. No data were found for the riboflavin content of the other three species.

2.3.3 Phytochemicals

Phytochemicals are non-nutritive, biologically active compounds, for example phenolic acids, flavonoids and carotenoids (Alasalvar & Shahidi, 2012; Fernandes et al., 2011), to which health protective properties are ascribed, such as preventive actions against aging, inflammation and certain cancers (Shofian et al., 2011). Protective effects of phytochemicals are because of their properties as free radical scavengers, hydrogen – donating compounds, singlet oxygen quenchers and or metal chelators (Ikram et al., 2009). Nhukarume et al., 2010 found *S. spinosa* had phenolic content radical quenching ability trends and flavonoids, expressed as catechin equivalence comparatively similar to baobab nectar. Proanthocyanidins, expressed as % leucocyanidin equivalence, were comparatively similar to mobola plum (*Parinari curatellifolia*). A high colour intensity is commonly related to a high total antioxidant capacity of a product (Kalt, 2005; Wicklund et al., 2005), a relation which warrants further investigation for the monkey orange species as they have bright orange (*S. innocua*) and orange - brown colours (*S. cocculoides*, *S. spinosa* and *S. pungens*), which promise the fruits to be high in phytochemicals. Quantification and classification of the phenols needs to be carried out in further research.

2.3.4 Anti-nutritional and toxic compounds

Anti-nutritional compounds have a negative effect on the digestibility of protein and carbohydrates, and decrease bioavailability of minerals such as Fe, Zn, P, Ca and or Mn. Anti-nutritional compounds such as tannins (1.01 mg/g), trypsin inhibitors (10.12 mg/g), (Bello et al., 2008) phytates (1.57 mg/g) and oxalates (0.83 mg/g) were found in *S. innocua* juice (Bello et al., 2008; Hassan et al., 2014). In separate studies of other indigenous fruits Umaru et al., 2007, found for baobab: tannin (2.22 ± 0.32 mg/g), phytates (0.69 ± 1.5 mg/g) and oxalate (9.5 ± 0.42 mg/g) and for marula (*Sclerocarya birrea*): tannin (2.04 ± 0.30 mg/g), phytates (3.56 ± 0.54 mg/g) and oxalate (4.9 ± 1.70 mg/g). Tannin, oxalates and phytates values of marula are higher than those reported for *S. spinosa.*

Table 2.3 Mineral and vitamin C composition[2] of the edible portion of monkey orange species *S. cocculoides, S. spinosa, S innocua* and *S. pungens*

mg/100g	*S. spinosa*				*S. cocculoides*				*S. innocua*				*S. pungens*			
	Mean	Min	Max	References	Mean	Min	Max	References	Mean	Min	Max	References	Mean	Min	Max	References
Fe	3.3	0.1	13.6	Amarteifio and Mosase, 2006,Saka and Msonthi, 1994,Sitrit et al, 2003, Arnold et al., 1985	70.5	0.9	140	Malaise and Parent 1985; Arnold et al., 1985	11.4	6	30	Saka and Msonthi, 1994, Malaisse and Parent, 1985, Hassan et al., 2014	6.1	2.2	10	Malaisse and Parent 1985; Arnold et al., 1985
P	60.3	26.3	108.1		116.5	103	130		77.7	2.6	210.6		133.6	97.1	170	
Ca	73.1	14.9	216		46.5	45	48		22.6	6	55		67.5	30	105.2	
Mg	81.2	43	205.7	Amarteifio and Mosase, 2006,Saka and Msonthi, 1994, Sitrit et al, 2003, Arnold et al., 1985	137.2	ND*	ND	Arnold et al., 1985	87	10.7	163.3	Saka and Msonthi, 1994, Hassan et al., 2014	136.6	ND	ND	Arnold et al., 1985
Na	23.3	13.2	36.2		4.5	ND	ND		99.6	45.9	153.3		7.2	ND	ND	
Zn	0.5	0.2	0.6		0.4	ND	ND		28.7	ND	ND		1.22	ND	ND	
K	1342.4	923.3	1968.3		959.2	ND	ND		1561.7	256.3	2867		1718	ND	ND	
Cu	0.62	0.09	2.2	Sitrit et al, 2003, Arnold et al., 1985	0.36	ND	ND	Arnold et al., 1985	2.4	ND	ND	Hassan et al., 2014	1.28	ND	ND	Arnold et al., 1985
Vitamin C	69	50	88	Amarteifio and Mosase, 2006, Arnold et al., 1985	34.2	ND	ND		45.2	ND	ND		38.6	ND	ND	

[2]Different units to express nutritional content were used in the literature collected for this review, thus all data were converted mg/100 g for micronutrients, vitamin C and dry matter basis where possible to enable data comparison. In some cases data had to be omitted due to a missing water content or dry matter content and when it was not clear whether dry or wet basis was used. In other cases it was not stated whether the flesh, juice or pulp was used in analysis and therefore data are presented in general terms as the edible portion of the fruit. Where data for a particular species was found from one source it is assumed to be the mean value.

In the study by Bello et al., 2008 the reported anti-nutrient values of *S. spinosa* were too low to be of any nutritional importance as they were not high enough to bind a significant amount of minerals and were below the established toxic level. However, Hassan et al., 2014 concluded from their study that the calculated molar ratios of [Phytates]/[Ca] and [Phytates]/[Fe] were above the critical levels that affect bioavailability of Ca and Fe. These results however need to be supported by in vivo bioavailability studies.

A potentially toxic natural secoiridoid, namely kingiside aglucone, was isolated from unripe bitter *S. spinosa* pulp (Msonthi et al., 1985). Toxic effects of kingiside aglucone in mice were reported for high dosage of unripe pulp of *S. spinosa*; however, specific searches with extracted ion chromatograms for strychnine, brucine and associated compounds found no terpene indole alkaloids in unripe *S. spinosa* pulp (Emmanuel et al., 2012). The fruit extracts of unripe *S. spinosa* pulp were also found to be acaricidal on cattle ticks, though the extracts did not show the classical dose dependence that occurs with conventional insecticides (Madzimure et al., 2013) and no toxic effects were elicited in guinea pig and mice (Watt & Breyer-Brandwijk, 1962). For these studies, an extrapolation to human toxicity was not done. Seeds of monkey orange fruits reportedly contain strychnine and are bitter tasting. When swallowed they pass the body safely but if chewed may produce vomiting and headaches (National Research Council, 2008). Thus, according to the studies reviewed, the toxic alkaloids are present in the seeds and unripe pulp of *S. spinosa*. From the literature, there is no scientific evidence that ripe *S. spinosa, S. cocculoides, S. innocua* and *S. pungens* pulp contain alkaloids, neither have the fruits been reported to be toxic in their ripe state. Therefore, the loss of toxic alkaloids after fruit ripening may be caused by alkaloid degradation at maturation that renders the fruit consumable by both animals and human as sensory properties, sugar, colour and flavour contents also increase. To the best of our knowledge, there is no research on the toxicity of ripe and unripe pulp and seed of *S. pungens* and *S. cocculoides*.

2.4 Post-harvest handling and storage

Monkey orange fruits are mainly harvested as mature, fully developed fruit by plucking, knocking, and shaking trees or collecting ripe fallen fruit. When fully developed though unripe fruits are harvested, they are subsequently buried under a thin layer of sand for several months until ripe (or until they liquefy) to protect them from other fruit hunters and animals (Motlhanka et al., 2008; Rampedi & Olivier, 2013; SCUC, 2006a; Sitrit et al., 2003) and to prevent postharvest losses (Kalaba et al., 2009a). After storage, the fruit pulp changes from a dry texture with a pale cream colour to a golden colour (yellow or brown and juicy depending on species) ready to be consumed (figure 2.1) (Mwamba, 2006).

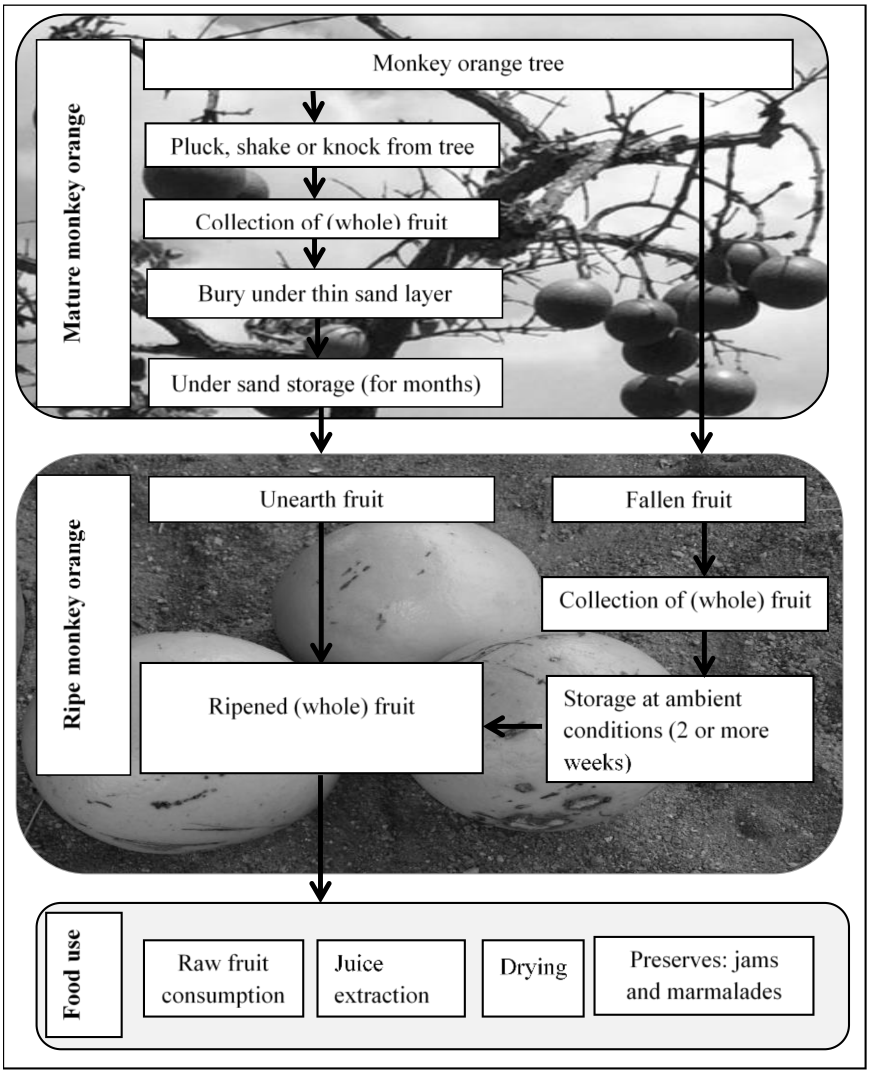

Figure 2.1 Household post-harvest handling storage and food use of *Strychnos* spp.

Burying of the fruits may provide temperatures adequate for increased enzyme activity to degrade pectin, which is catalyzed by pectinases, such as PME and PG, to bring about the ripe texture of the fruits. Like other climacteric fruits during storage and ripening, monkey oranges increase in total soluble solids content (TSS) with accumulation of sucrose, glucose and fructose as well as citric and malic acids as realized for *S. spinosa* cultivated in Israel (Sitrit et al., 2003).

When picked, ripe monkey orange can be stored for up to two weeks under ambient conditions -mainly in shade- before they spoil (Mkonda et al., 2002; Ruffo et al., 2002; SCUC, 2006a). Slow spoilage can be attributed to the hard shell of the fruit, which also resists fungi and fruit flies (National Research Council, 2008). However, generally fresh fruits with a high moisture content like monkey orange undergo direct or indirect nutrient and quality losses upon storage. Limited knowledge of fruit handling and marketing contribute to quality losses (Saka et al., 2007). Data for sensory and nutrient composition during storage (from maturity to ripening) for *S. cocculoides*, *S. innocua* and *S. pungens* are not available. Once such data become available, post-harvest handling techniques and storage methods will need to be developed in order to maintain and improve the shelf life of the fruit and fruit products.

2.5 Food uses of Strychnos spp.

During prioritization studies of indigenous fruits, individual species were ranked according to their role in food security, commercialization potential, taste, and abundance (Akinnifesi et al., 2007b). From surveys on domestication of the 50 most common indigenous fruit species consumed in Southern African countries (Malawi, Zambia, Zimbabwe, Tanzania and Mozambique), the monkey orange fruit, namely *S. cocculoides*, ranked third overall (Mkonda et al., 2002; Saka et al., 2004). In country specific results, *S. cocculoides* was ranked first in Zimbabwe and Tanzania, where interviewed households preferred the fruit tree for domestication as a food source (Akinnifesi et al., 2002).

2.5.1 Consumption of raw fruits

In households, monkey orange fruit is consumed fresh and unprocessed, (Farinu, 1986; Gomez, 1988; Watt & Breyer-Brandwijk, 1962) immediately after cracking the shell as it is a traditional belief that unprocessed pulp that is left exposed to atmospheric conditions cannot be consumed later. Depending on the species, monkey orange pulp can be processed to food products and is often cooked with maize meal to a sweet porridge, dissolved in water to create sweet and tasty drinks, dried for later consumption or made into jams and marmalades (Mwamba, 2006; National Research Council, 2008; Zinyama et al., 1990).

2.5.2 Processing

Indigenous fruit is processed into value added products to improve digestion, metabolism, and preservation (Nayak et al., 2015; Ruffo et al., 2002; Saka et al., 2004). Monkey oranges are processed domestically and at small scale to dried products, juices and preserves where generally the preparation methods and conditions vary from place to place. Post-harvest processing such as maceration, drying, juicing, cooking, and storage is expected to have an effect on nutrient content, bioavailability, anti-oxidant activity of bioactive compounds and physical properties (Chong et al., 2013; Nicoli et al., 1999). For monkey oranges, thermal processing is of utmost importance as all its known products undergo some form of thermal treatment.

2.5.3 Drying

Drying reduces water activity, which diminishes the bacterial activity in the dehydrated food, increases shelf life, reduces the product's mass and adds value (Chong et al., 2008; Nayak et al., 2015). In Southern Africa, monkey orange fruits are commonly dried by fire and direct sun to fruit rolls, leathers, pound into flour (*S. cocculoides*) used to cook porridge, referred to locally as 'bozo' in Mozambique (Akinnifesi et al., 2007b) or re-cooked as a sauce (Mizrahi et al., 2002). Sun dried monkey orange pulp can be stored from two months to five years (Shackleton et al., 2000), which makes thermal drying an ideal preservation method due to its affordability for rural communities and as a means to secure continuous fruit availability into the next season.

The moisture content of monkey orange ranges from 60 % (Malaisse & Parent, 1985) to 91 %, (Bello et al., 2008) of which, depending on the method and degree of heating, a large proportion is removed by the drying process. Sufficiently dried fruit products generally have a residual water content of 18 % to 24 %, and with proper packaging have a long shelf life because of their high-sugar concentration and reduced water activity (0.72 to 0.75) (Pátkai, 2012). Due to the characteristics of conventional drying processes, nutrients and compounds sensible to heat, light, and oxygen, such as vitamin C, might be degraded (Santos & Silva, 2008). Other studies have shown that dried fruits are rich and shelf stable sources of dietary polyphenolic and anti-oxidants (Bennett et al., 2011). The elevated antioxidant activity might be due to the concentration of polyphenolic compounds during drying and the formation of Maillard products, (Capanoglu, 2013) which have an anti-oxidant activity attributable to the high molecular weight of brown pigments formed in the advanced stages of the reaction (Nicoli et al., 1999). Overall the changes in anti-oxidant properties in foods can be attributed to the sum of different or opposite events that retain or decrease anti-oxidant activity in dried products. Thus, further studies measuring anti-oxidant activity and phenol content in dried monkey oranges need to be performed to determine their role in human health.

Moisture content maintains the equilibrium media for the stability of fruit pigments and anti-oxidants such as anthocyanins (Nayak et al., 2015). Loss of moisture through heat reduces pigment stability and degrades pigments, which results in discolouration of the product (Wicklund et al., 2005). Total colour change (ΔE) is an important physical property of dehydrated fruit, in which high thermal drying temperatures and time can result in an extremely hard, burnt, off-flavour and tasteless product (Chong et al., 2013). Because of their high sugar content, monkey oranges are sticky and difficult to handle in dryers and have a potential for caramelization, which can change fruit to a brown or darker colour and has a negative impact on sensory quality attributes. Thus adequate drying procedures are required to obtain a product of sufficient sensory attributes for the consumer.

The dried products are now mainly produced for home consumption and have not been sold commercially. To the best of our knowledge there is scanty scientific information about the suitability of drying techniques for monkey orange fruit. Retention of nutrients and sensory appeal in dried products is an important quality aspect of food preservation (Chong et al., 2013; Fernandes et al., 2011). Thus, studies on the species' suitability for dried products, the drying kinetics and characteristics of the commonly consumed monkey oranges need to be conducted to avail suitable drying techniques that fit local conditions and offer the possibility of commercialization.

2.5.4 Juice extraction

Juice is extracted at small scale by initially soaking the pulp to soften the tissue around the seed for efficient extraction. Due to their water solubility, vitamin C and other phenolic anti-oxidants can be leached from the fruit tissue to the processing water, while carotenoids, thiamine, folate and niacin increase during processing as a result of dissociation from plant matrix materials (Hotz & Gibson, 2007; Kalt, 2005). When monkey orange juice is extracted, processing is manually done by mashing, using a hand held whisk or wooden spoon. The pulp is diluted with water, then heated to 92 °C for 3 minutes to initiate precipitation of colloidal substances, which later on can be removed by filtration (Mbiyangandu, 1985). The filtrate is obtained by sieving pulp with a muslin cloth and used in processing juice, while the residue remains for jam making (Saka et al., 2007). Hence, the juice is expected to retain a significant amount of anti-oxidants after processing.

Clear monkey orange juice was preferred by consumers in comparison to other indigenous fruit juices based on taste, mouth feel, flavour, and sweetness (Bille et al., 2013; Saka et al., 2007; Tumeo et al., 2008). During processing, the natural monkey orange colour turns dark brown. To enhance the natural juice colour, food grade egg yellow or lemon juice as an anti-browning agent is added (Bille et al., 2013; Saka et al., 2007). To increase the shelf life, processed juice of the fruit can be preserved by using benzoic acid and sodium sulphate additives (Mwamba, 2006). In orange juice processing, high mechanical extraction pressure application, de-pulping and aeration conditions can reduce the volatile components and alter the flavour of the resultant juice (Perez-Cacho & Rouseff, 2008). Since traditional monkey orange juice extraction is mild due to equipment constraints and processing steps are limited to heating and hand extraction, the extent of aroma alteration and odor development by processing is hypothesized to be minimal though there are no studies to this effect. Saka et al., 2007, identified a reduction in the mineral elements Ca and Mg from fresh to processed juice of *S. cocculoides*. To date, few studies have been done on nutritional and sensorial characterization of fresh and pasteurized monkey orange juice, thus more work is recommended.

2.5.5 Preserves; jams and marmalades

In fruit processing, preserves such as jams, sauces, pickles and chutneys play a significant role in reducing the loss of fresh produce (Singh et al., 2009). Traditionally and at small scale, jams and marmalades are processed from monkey orange fruits, whereby the type of preserve depends on species (Mwamba, 2006; Van Wyk, 2011). From literature reviewed, jam processes vary from author to author. However, a consensus in the applied methodologies is the use of pulp alone or a mixture of pulp and juice with heating to reach the required soluble solids content and as a pasteurization step before packaging and setting (Bille et al., 2013; Saka et al., 2007). No pectin is added as monkey orange pectin depolymerisation allows jams to set and spread well, a component that contributes to the sensorial quality of monkey orange jam. Contrary to the loss of water soluble anti-oxidants, the removal of water concentrates minerals as shown in the study of Saka et al., 2007 where Ca, Mg, K, Na, Zn and P contents were higher in jam than in the juice (filtrate) from which the residue used for jam processing was obtained. When comparing with other indigenous jams, consumers in the studies of Bille et al.,2013 and Saka et al.,2007 preferred the monkey orange jam because of its sweet taste and delicate flavour. However, due to thermal processing of fruits, possible undesirable changes in sensorial, functional and nutritional value by destruction of phenolic antioxidants occur (Igual et al., 2013; Nayak et al., 2015). As previously alluded, thermal processing can lead to the release of bound phenolic compounds, hence the total phenolic content can be attributed to both free and bound polyphenolic compounds (Lee et al., 2013). Thus, the retention or loss of phenolic compounds, nutrients and organoleptic characteristics during jam processing needs to be further investigated.

2.6 Overall discussion

Information on the contents of micronutrients of all four species was limited in the literature surveyed, as research emphasis on fruit species was on macronutrients. More research needs to be conducted on micronutrient composition, especially with respect to *S. innocua, S. spinosa* and *S. cocculoides*, as their nutritional composition from available literature is exceptional.

In the available literature large variations in nutritional and sensorial attributes were reported, which may have been caused by several factors. Fruit maturation and ripeness at time of analysis was not clarified, and therefore could have promoted the variable data on nutritional composition and sensorial quality. Other factors, such as different agronomic and environmental factors (soil types and climate), contribute to variation as samples spanned from different regions in Southern Africa and as far as Israel. Agro-technical conditions (including irrigation and fertilization) might affect ion uptake, thus resulting in mineral variation within and between species (Sitrit et al., 2003). Methodological differences in conditions and types of assays contributed to variation, where in some cases absence of methodology descriptions made evaluating results and drawing conclusive remarks on nutritional composition difficult. Overall, the data set used was small and thus more work needs to be done by sound sampling plans and reliable food analysis procedures of each monkey orange fruit species.

Processing techniques presented in this paper largely require thermal treatments that have negative and or positive consequences on the nutritional and sensorial quality of products and a beneficial role in preservation. However, information on the extent of the effects of processing methods is lacking. The presence, possible release and quantities of toxic alkaloids into the processed products also warrants further investigation for quality control purposes during processing.

Fruit weights for provenances from Tanzania, Zambia and Zimbabwe range from 145 to 383 g (Mkonda et al., 2002). Pulp contents of large fruits are between 34 to 48 %, (Mkonda et al., 2002) so the amount of consumed pulp is 130.2 to 183.4 g, and for small fruits these figures are 50 to 57 %, (Mkonda et al., 2002) and 72.5 to 82.7 g, respectively. Based on these data, an estimate of contribution to the recommended daily intake can be calculated per consumption of 100 g serving of fruit based on Dietary Reference Intakes of Rosenberg et al., 2004. On a per serving basis (100 g), *S. innocua* delivers more than 100 % of the RDI for Zn, while *S. cocculoides* delivers more than 100 % of the RDI for Fe and *S. spinosa* delivers more than 100 % of the RDI for vitamin C (table 2.4). Overall the monkey orange species serve as a rich source of carbohydrates, protein, fiber, Zn, Fe and vitamin C. It is therefore paramount that research clearly assesses the potential beneficial contribution to human health of ingestion of monkey orange as well as the bio- accessibility and bio-availability of its minerals to contribute to micronutrient deficient diets.

Table 2.4 Monkey orange composition with RDI * for children 4-8, pregnant females 19-50, adult males 19-50 and adult females 19-50 years (Species*: S. spinosa*, *S. cocculoides*, *S. innocua* and *S. pungens*)

	CHO	Protein	Fiber	Zn	Fe	Vit. C
	g/day	g/day	mg/day	mg/day	mg/day	mg/day
RDI children 4-8 years	130	19	25	5	10	25
S. spinosa pulp composition	28.7	9.1	9.2	0.5	3.3	69
% contribution	22	48	37	10	33	276
S. cocculoides pulp composition	18.2	3.5	4.6	0.4	70.5	34.2
% contribution	14	18	18	8	705	137
S. innocua pulp composition	32.5	5.9	9.4	28.7	11.4	45.2
% contribution	25	31	38	574	114	181
S. pungens pulp composition	21.3	4.3	13	1.22	6.1	38.4
% contribution	16.4	23	52	24	61	154
RDI Pregnant female 19-50 years	175	71	28	11	27	85
S. spinosa pulp composition	28.7	9.1	9.2	0.5	3.3	69
% contribution	16.4	13	33	5	12	81
S. cocculoides pulp composition	18.2	3.5	4.6	0.4	70.5	34.2
% contribution	10.4	5	16	4	261	40
S. innocua pulp composition	32.5	5.9	9.4	28.7	11.4	45.2
% contribution	18.6	8	34	266	42	53
S. pungens pulp composition	21.3	4.3	13	1.2	6.1	38.4
% contribution	12.2	6	46	11	23	45
RDI adult male 19-50 years	130	56	38	11	8	90
S. spinosa pulp composition	28.7	9.1	9.2	0.5	3.3	69
% contribution	22.1	16	24	5	41	77
S. cocculoides pulp composition	18.2	3.5	4.6	0.4	70.5	34.2
% contribution	14	6	12	4	881	38
S. innocua pulp composition	32.5	5.9	9.4	28.7	11.4	45.2
% contribution	25	11	25	261	143	51
S. pungens pulp composition	21.3	4.3	13	1.22	6.1	38.4
% contribution	16.4	8	34	11	76	43
RDI adult female 19-50 years	130	46	25	8	18	75
S. spinosa pulp composition	28.7	9.1	9.2	0.5	3.3	69
% contribution	22.1	20	37	6	18	92
S. cocculoides pulp composition	18.2	3.5	4.6	0.4	70.5	34.2
% contribution	14	8	18	5	392	46
S .innocua pulp composition	32.5	5.9	9.4	28.7	11.4	45.2
% contribution	25	13	38	359	63	60
S. pungens pulp composition	21.3	4.3	13	1.22	6.1	38.4
% contribution	16.4	9	52	15	34	51

* (Rosenberg et al., 2004)

2.7 Conclusions and research priorities

Monkey orange fruits have potential to impart health benefits and improve nutritional status of the rural population thanks to the vitamin C, Zn and Fe content. With regard to nutritional and sensorial quality, several recommendations for future research were identified as summarized in figure 2.2.

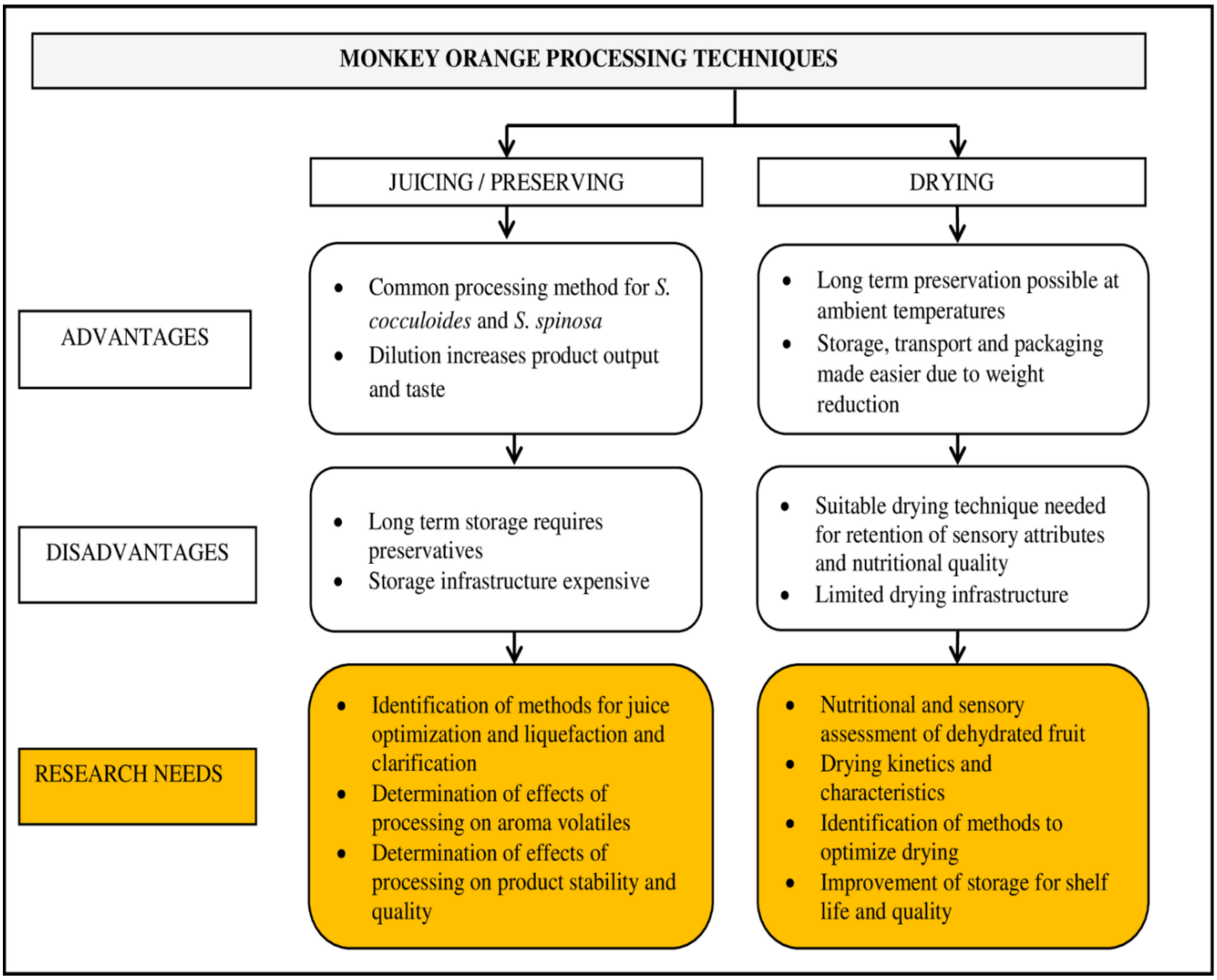

Figure 2.2 *Strychnos* spp. main processing techniques, advantages, challenges and research needs

Previous studies concur that monkey orange fruit has desirable sensory properties and its nutritional composition is comparable to and in some cases better than that of its exotic and indigenous counterparts. However, compared to many exotic fruit species, very little research work has been done on processing for value addition of monkey orange fruits in Southern Africa. Minimizing loss of nutrients, anti-oxidants, organoleptic properties and reducing levels of potential toxic alkaloids to minimum during processing is important to obtain a nutritious fruit product as processing has an influence on the overall quality of fruit products. The evidence collected in this review shows that the impact of processing is not well documented, and that the assessment of the contribution by monkey orange to nutrient intake of regular consumers is not accurate. Thus, optimization of the state of the art with respect to processing techniques and assays of the nutritional and sensorial quality of monkey orange products is essential for the implementation of preservation procedures and the consequent promotion of monkey orange consumption. Figure 2.2 gives an illustration of the advantages, disadvantages and recommendations for monkey orange processing as concluded from this review.In Africa indigenous fruit trees supplement the diet of many rural families by providing essential micronutrients and health benefits as well as serve as an alternative for cash and income, especially in times of famine (Bille et al., 2013; Legwaila et al., 2011; Mithöfer & Waibel, 2003; Packham, 1993). The wide distribution of the monkey orange trees in drought prone areas and semi-arid regions, coupled with the fruit nutritional quality renders the fruit an important food source for particularly children and pregnant women. Thus, improved manufacturing of processed foods through optimization of preservation techniques as a sustainable solution to malnutrition in rural areas of transition countries needs to be determined.

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Chapter 3

Improvement of traditional processing of local monkey orange (*Strychnos* spp.) fruits to enhance nutrition security in Zimbabwe

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Abstract

Although the monkey orange (*Strychnos* spp.) tree fruit is widely distributed in Southern Africa and particularly in Zimbabwe, it is underutilized and little attention has been given to its potential commercialisation due to limited knowledge and information. Most of the fruits and their products are wasted because of limited harvest time, process control and storage conditions, leading to variability in shelf life and sensory quality, thereby impacting nutritional quality. Traditional processing techniques make insufficient use of this food resource within rural communities. This study aimed at identifying the existing bottlenecks by means of a survey among 102 smallholder farming respondents in the wet and dry regions of Zimbabwe. Results revealed that *S. cocculoides* and *S. spinosa* were used by 48 % of respondents as a functional ingredient in porridge, by 25 % in fermented *mahewu* drink and by 15 % of respondents as a non-alcoholic juice. The fruits of *S. innocua* and *S. madagascariensis* are preferably processed into dried products. Taste, flavour and colour were the important quality characteristics for all processed products, and constraints to be solved are seed-flesh separation, long processing times, separation of juice and pulp during storage as well as pulp viscosity. Respondents reported monkey orange products to have health benefits for children and immune-compromised people, who on regular consumption have reportedly increased weight and resistance to disease. The positive perception about the processed products of *Strychnos* spp. offer a good opportunity to improve nutrition security by capitalizing on these not-yet-fully-exploited resources, but technological solutions to improve sensory quality and shelf life must be developed.

Keywords: Traditional processing · Monkey orange · *Strychnos cocculoides* · *Strychnos spinosa* · *Strychnos innocua* · *Strychnos madagascariensis*

3.1 Introduction

Indigenous fruit trees in Africa supplement the diet of many rural families by providing essential nutrients (Bille et al., 2013; Nhukarume et al., 2010) and serve as a livelihood source, especially in times of famine (Legwaila et al., 2011; Mithöfer & Waibel, 2003a; Packham, 1993a). Hundreds of indigenous fruit species exist in Africa that are locally significant, although they might be unknown in global markets (Jamnadass et al., 2011). These locally important species are, however, frequently underutilised, leading to erosion of their usefulness and restricting development options for poor communities (Ekué et al., 2010). Some studies have been conducted to assess knowledge by local communities in Zimbabwe on processed products of indigenous edible fruits such as beverages derived from sand apple (*Parinari curatellifolia*), marula (*Sclerocarya caffra*) (Gadaga et al., 1999), baobab (*Adansonia digitata*) (Mpofu et al., 2014), and ber (*Ziziphus mauritiana*) (Nyanga et al., 2008).

Strychnos spp. (monkey orange) has been identified among the top priority fruit species in Southern Africa through ethnobotanical surveys (Mkonda et al., 2002; Saka et al., 2004), particularly in dry areas of Zimbabwe such as Binga (Mpofu et al., 2014). This fruit tree proliferates in areas with a prolonged dry season, remains dormant when water is unavailable and bears fruit in abundance (Mwamba, 2006; National Research Council, 2008). The excess production of the fruit leads to its underutilization and this can be seen in the veld around Zimbabwe where fruit remains littered and unpicked when in season. Five common *Strychnos* species grow wildly across the agro–ecological regions of the country, with wide distribution patterns. *S. cocculoides* is found in the north, west, central and south, *S. spinosa* in the north, west, central, east and south, *S. innocua* in the north, central, east, and south, *S. madagascariensis* in the north, west, central, east and south, and *S. pungens* in the north, west and central parts of Zimbabwe (Mapaura & Timberlake, 2004; Van Wyk & Van Wyk, 1997). Though the *Strychnos* spp. fruit is widely distributed in Zimbabwe, it is underutilized and little attention has been given to its potential commercialisation due to limited knowledge and dissemination of information about propagation, agronomic practices and product processing techniques.

Strychnos spp. fruits ripen and are harvested from September to December (Akinnifesi et al., 2007a), a time when intense agricultural labour coincides with low maize stocks and the unavailability of freshly-gathered vegetables (McGregor, 1995). The season of prolonged food scarcity often increases reliance on consumption of indigenous fruits by the entire family (Shackleton et al., 2000). In addition to the consumption of fresh fruit, traditional fruit processing is most common in drier climatic areas for the supplementation of food requirements (Saka et al., 2004). An estimated 46 % of rural households have reported processing indigenous fruit into juices and/or porridges (Kalaba et al., 2009b), along with several other food products that can be used as a complement and substitute for the local cereal-based staple foods.

Strychnos spp. has been identified to contribute more than 100 % of the recommended daily intake for vitamin C, Fe and Zn; especially for children between four and eight years old and for pregnant women (Ngadze et al., 2016). Gadaga et al. (2009) noted that over 65 % of Zimbabweans live in rural areas and are food insecure, especially during prolonged dry periods, while malnutrition problems through vitamin and mineral deficiencies have public health significance in the country. Because of their wide availability and nutritional composition, monkey oranges have potential for contributing to the alleviation of vitamin and micronutrient deficiencies of the vulnerable rural population, particularly children and women, by complementing the monotonous staple food diet. In Zimbabwe, extension services of government and Non-Government Organizations (NGO's) are a bridge for the transfer of information on the contribution of indigenous fruit species to health and nutrition to the end user, through training of women groups and cooperatives in new processing possibilities.

To date, indigenous knowledge on the collection and processing of *Strychnos* spp. products in Zimbabwe has received limited attention. Usually indigenous fruits are considered to be a "poor man's food" or famine food. Erosion of cultural norms through urbanization and increased cultivation of exotic fruits and their marketing have led to further reduction of the use of indigenous fruits. The lack of awareness of the potential health benefits and standardized processing of these fruits keep them away from most diets and adversely affect the utilization of the fruits and nutrition security.

Thus, our present study aimed to assess and document specific traditional processing techniques and their bottlenecks in identified rural communities and regions in Zimbabwe with the objective to determine which food technological improvements would support the role of indigenous monkey orange fruits as a way to improve nutrition security. *Strychnos* spp. has the potential to generate income for actors in the value chain within local and regional markets, as in the case of baobab fruit and other African indigenous fruits that reportedly reduce poverty by 33 % during the critical period of the year (Chadare et al., 2008a; Mithöfer & Waibel, 2003a). Aspects explored in our study include processed products, processing steps, constraints and consumption patterns of the fresh fruits and their products. The study also provides information on local food uses of *Strychnos* species, which is important for further research on the implications of processing and storage for nutritional health benefits and sensory quality.

3.2 Methodology

3.2.1 Study area and sample size

The study areas (figure 3.1) were chosen on the basis of the abundance of *Strychnos* spp. fruits, in wet and dry regions of Zimbabwe. For exhaustive data collection on the different species, areas that cover the five agro ecological regions of Zimbabwe (Ovincent et al., 1961), were chosen carefully using information gathered initially with assistance from NGO extension services on the basis of community utilization of *Strychnos* spp. The objective of this research was primarily based on indigenous knowledge, where a limited number of people served as primary data sources. Thus, holders of information about indigenous fruit processing and about village chiefs were identified and purposively selected on account of their experience and past participation in other surveys. Villages were visited prior to the survey for familiarization with the community. Consent from the village chief was obtained after clear demonstration of research intentions and protocols. Monkey oranges are underutilized, not commercialised and commonly consumed and processed locally in rural homesteads. Thus, often the actors that process the fruits are concealed leading to the 'snowballing method' as the best method to uncover that population. According to Katz (2006), “Snowball sampling is a special nonprobability method for developing a research sample where existing study subjects recruit future subjects from among their acquaintances and where a sampling frame is hard to establish". Though snowballing is not statistically representative, the sampling technique is suitable when the human populations being surveyed are difficult to pinpoint and locate because of their limited numbers. Village chiefs, elders and leaders of women's groups suggested the initial participants for the survey, then the identified respondents suggested other households, where selection was based on their knowledge of processing *Strychnos* spp. Informed consent was obtained from all individuals in each participating household in this study. With an estimated average size of 150 inhabitants per village, at least five % of the population of the village were interviewed as a representative sample (Mpofu et al., 2014). A total of 102 respondents were interviewed, where 20–40 informants were recruited in each community.

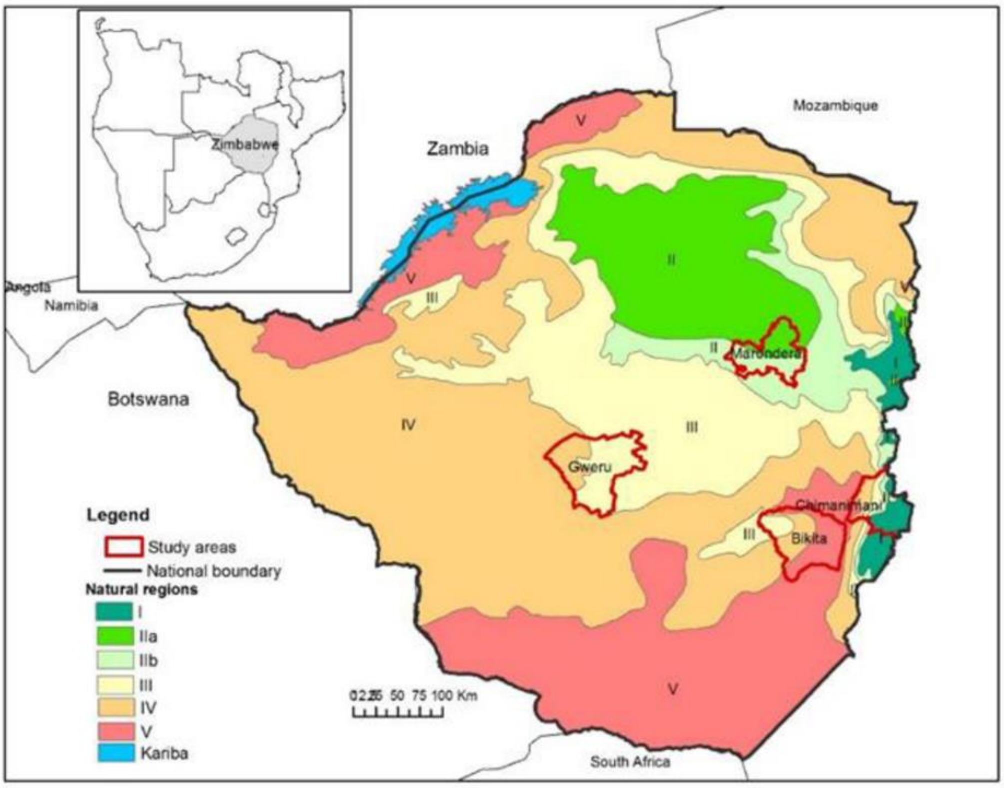

Figure 3.1 Study areas surveyed for indigenous knowledge of *Strychnos* spp. fruit collection and product processing in Zimbabwe (Annual rainfall region I >1000 mm, II 700-1050, III 500-800, IV 450 -650 and region V <450)

3.2.2 Data collection

Data were obtained during field visits using questionnaires administered through face-to-face interviews, in detailed focus group discussions and by observations during the monkey orange season from September through December 2014. Observations during the harvesting and processing of monkey oranges were made as follow-ups to the information obtained from the respondents. The interviews were either with individuals or in group discussions in the local Shona and Ndebele languages (with the assistance of local translators), which were well understood by all respondents, so as to obtain in-depth information. Detailed discussions were used to generate data from groups of between 10 to 15 participants in selected communities. A relevant leader of the community, who was believed to have more experience and in-depth knowledge of the fruit and area, was identified for each focus group. The focus group dynamics facilitated the generation of forgotten information. The ages of respondents ranged from 20 to above 70 years. In each locality, information was gathered by answering questions based on the following parameters: (1) fruit harvesting and gathering, (2) processing methods, (3) consumption patterns, (4) storage practices of fruit and fruit products, and (5) processing and storage constraints. Specific questions were developed for each actor and were subdivided as follows:

- At the collector's level: Fruit species, collection period, distance covered for fruit collection, fruit quality characteristics (colour, taste, maturity and size), method of collection, fruit storage and ripening, collection constraints, customers for fruits, selling price, end uses of fruit.
- At the processor level: Processed products, quality of processed products (colour, taste, odour), frequency of processing, end consumer (household use or trade), quantity and price of products and customer preferences.
- At the consumer level: Procurement of product, quality characteristics, storage of product, consumption time and quantities, quality perception of consumed product (colour, taste, odour), further processing including the use of product as an ingredient.

Focus group discussions were focused on obtaining information about harvesting methods, product processing and quality constraints, processing steps and the uses of product as an ingredient. Observations during the harvesting and processing steps were conducted as a follow up to the information obtained from the farming respondents.

3.2.3 Data analysis

The data were analysed using SPSS 22.0 for Windows (Apache Software Foundation, USA) and Microsoft Excel 2013. Data were subjected to descriptive statistics from coded questions asked in the individual interviews.

3.3 Results and discussion

3.3.1 Gathering and selection of monkey orange fruits and food products

a. *b.* *c.*

Figure 3.2 Pulp of a. *Strychnos spinosa*, b. *Strychnos cocculoides* and c. *Strychnos innocua*

Figure 3.2 shows photographs of the pulp of fruits from three *Strychnos* species found in Zimbabwe. Data collection for fruits of the same species may be imprecise (Termote et al., 2010). “Under-differentiation” of a species is encountered when a single vernacular name can be used for species of morphological similarity, or “over-differentiation” when several names are used for one species (Rampedi & Olivier, 2013). Thus, to gather reliable and reproducible information, the species were grouped into two major groups: group A (comprising *S. cocculoides* and *S. spinosa*) and group B (*S. innocua* and *S. madagascariensis*). Within either group, the species’ phenotypic and sensory characteristics are similar and hence traditionally similar vernacular names are used (table 3.2).

In all the areas visited, *Strychnos* spp. are harvested from September to December, i.e. the warm summer period marking the end of the dry season and the beginning of the rainy season. Citation of fruit species in the localities we visited is illustrated in table 3.1. The most frequently cited species in Lower Gwelo and Marondera were *S. cocculoides* and *S. spinosa*, while Bikita and Chimanimani respondents cited mainly *S. innocua* and *S. madagascariensis*. *S. pungens* is not included in the description of the processing results because respondents did not process fruits of this species due to its limited availability. Each informant cited on average three species known to them and identified two species found in their locality.

Table 3.1 Inventory of *Strychnos* spp. identified and collected in Zimbabwe

Species group	Vernacular name (Shona)	Common name	Botanical name	Citation in locality (%) Wet region Chimanimani	Marondera	Dry region Lower Gwelo	Bikita
A	Matamba Mazhumwi	Corky-bark monkey orange	*Strychnos cocculoides*	55	95	96	50
	Matamba Mun'ono	Spine-leaved monkey orange	*Strychnos spinosa*	30	93	64	65
B	Hwakwa Makwakwa Mudo	Dull-leaf monkey orange	*Strychnos innocua*	100	28	36	100
	Hwakwa Makwakwa	Hairy-leaved monkey orange	*Strychnos madagascariensis*	100	N/A	N/A	100
	Mutamba-usiku	Green monkey orange	*Strychnos pungens*	5	48	N/A	100

Gathering and fruit selection are principally activities conducted by women during their daily chores when they tend the field or collect firewood, and by children going to and from school or as they herd cattle. Females made up the majority of the respondents (83 %) (table 3.2). Females are usually identified as the majority of respondents in studies such as ours because they are regarded as the custodians of traditional knowledge (Dovie et al., 2008).

Table 3.2 Demographic profile of respondents in the districts visited during a survey for indigenous knowledge of *Strychnos* spp. fruit collection and product processing in Zimbabwe

Region	District	N	Gender		Age				
			Female	Male	20	21-35	36-50	51-70	Above 70
Wet-semi wet region	Chimanimani (Region I and II)	20	16	4	6	1	7	3	3
	Marondera (Region II)	40	32	8	1	5	13	14	7
Dry region	Lower Gwelo(Region IV and V)	22	18	4	0	2	9	7	4
	Bikita(Region III, IV and V)	20	19	1	0	6	8	5	1
	Total	102	85	17	7	14	37	29	15

In this study we found that *Strychnos* spp. trees were often confined to another individual's homestead or field and respondents freely collect the fruits without seeking authority since monkey orange trees and fruits continue to be regarded as communally-owned in all areas visited. Because the fruits are found within walking distance from homesteads and are communally owned, they are accessible and free to harvest, and available in times of seasonal imbalances, especially for the rural poor. In Zimbabwe, indigenous fruit trees are generally located in forests, cropped fields, grazing parks and around homesteads (Akinnifesi et al., 2002).

The fruits are harvested by picking fallen fruits, or by climbing trees and plucking fruit from branches. Other harvesting practices such as shaking trees, or hitting them with stones or tree branches are also used. These modes of harvesting may cause damage to the hard shell of the fruit, which is a protective barrier to insect infestation and microbial contamination, thus potentially causing loss of sensory, nutritional and physical properties of the fruit.

For all species, major attributes for fruit maturity and harvesting were colour (89 % of respondents), liquefaction (72 %) and taste (49 %). Fruit colour changes from green to bright yellow or orange (colour intensity depending on species) at the onset of ripening. Specifically, for *S. cocculoides* and *S. spinosa* liquefaction is a common maturity indicator used by respondents, identified by holding the fruit to the ear and shaking to listen for the sound of sloshing pulp and loose seeds. The best tasting fruits are known from past experience and respondents know the location of the trees that bear the most delicious fruits. These results concur with those of Akinnifesi et al. (2007a), where they concluded that traditionally in Southern Africa the harvesting of indigenous fruits depends largely on past knowledge and observations within the community.

We noted that in all areas there is variation in taste between species and within species, from bitter, astringent to sweet. Taste and flesh consistency are also used to distinguish between species' groups. *S. cocculoides* and *S. spinosa* were described as sweet or sweet and sour, with a brown juicy flesh when ripe. The sweet taste of fruit from *S. cocculoides* explains why it is the most preferred of the monkey orange species (Mwamba, 2006). Citric, malic and succinic acids found in *S. spinosa* species may contribute to the sourness perceived by consumers. *S. madagascariensis* and *S. innocua* fruits were described as containing bright yellow firm flesh, with a sweet to bitter taste, which has been attributed to the presence of tannins in *S. innocua* flesh (Bello et al., 2008). Fruit colour, liquefaction and taste superseded other collection and selection criteria such as fruit size (21 %) and weight (17 %).

3.3.2 Fruit storage practices and constraints

Of the four monkey orange species, *S. cocculoides* was the only species that was picked unripe and stored for ripening; the other three species were only picked when ripe. In total, from all the regions surveyed for *S. cocculoides*, 89 % percent of the respondents picked fallen ripe fruits, and 70 % picked mature fruits from the tree. Twenty seven percent of respondents picked light green mature fruit for ripening during storage. According to respondents, the degree of maturity was not a constraint as through experience they know the ideal stage of maturity that allows the fruit to ripen in storage. Because of their hard shell and acidic nature with a pH of around 3.5 (Saka et al., 2007), *Strychnos* spp. can tolerate relatively high temperatures and long storage times before deteriorating in quality. Ripening could be either accelerated or reduced by different storage methods. Methods used to accelerate ripening are the mimicking of dark, air tight conditions, such as by burying fruits underground in sand, in sacks with dry hay, dry chicken manure, mealie meal or fine wood ash. Thus, the fruits are kept in the buried environments in order to control the atmospheric conditions that allow the concentration of the ethylene plant hormone that consequently hastens the ripening period, signifying the climacteric nature of the fruit (Barry & Giovannoni, 2007; Sitrit et al., 2003). Respondents indicated the storage treatment and estimated the storage time of the fruit based on their experience. They indicated that picking of mature unripe fruits for storage in burial places also reduces competition from other fruit collectors, especially of a well-known, sweet tasting variety, which is in line with findings of Motlhanka et al. (2008) and Rampedi and Olivier (2013). Unripe mature fruits can be stored in this manner for more than two months (according to 18 % of the respondents). To delay ripening, mature fruits are kept cool by storing them in ventilated grain sacks, under (or in) granaries and in closed clay pots. Ripe fruits can be stored for one month (59 % of the respondents), two months (9 % of respondents) and more than two months (1 % of respondents), before they rot. Thirty percent of respondents said they immediately consumed or processed the fruit and did not store the fruit at any stage of collection. The constraints identified by the respondents were a shortage of fruits of the sweet tasting variety, and seasonality, where respondents desired a longer storage time and extended availability of fruits throughout the year.

3.3.3 Indigenous processing knowledge

According to our survey, fruits of *Strychnos* spp. are harvested for a period of between three to four months and in excess of what is consumable during the harvest season; and thus some fruits are processed. *Strychnos* spp. products were broadly classified as products for either future use or immediate use, for the purposes of this survey. For all processed products of *Strychnos* spp. the first step was to wash fruits with clean water to remove dirt and dust particles. The flesh is exposed by pounding and cracking the shell on a hard surface or with a stone, after which specific processing is done. An outline of the processing methods used is shown in figure 3.3 and details of the processing of each product are given in the following sections

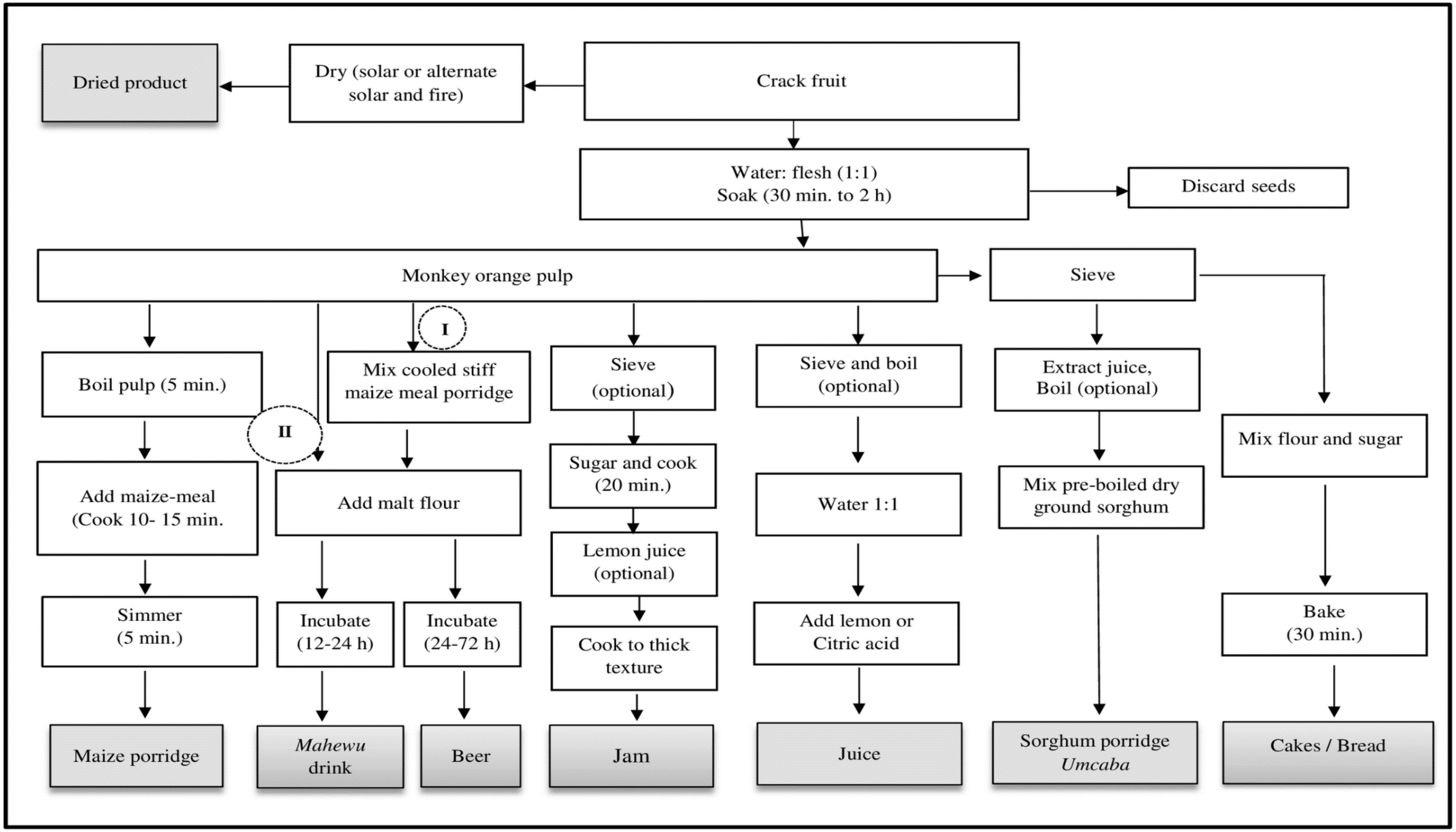

Figure 3.3 Overview of processing methods of *Strychnos* spp. fruit in Zimbabwe

Food products for prolonged storage

Drying of S. innocua and S. madagascariensis

In Bikita district, high temperatures and low rainfall often result in poor harvests of staple food crops and fresh vegetables. This has led to reliance on the processing of indigenous fruit, particularly drying of *S. innocua* and *S. madagascariensis* (typical to dry regions in Zimbabwe such as Bikita), so as to reduce fruit moisture content for prolonged preservation. All respondents in Bikita district use alternate sun and fire drying due to a lack of electricity for refrigeration and mechanized equipment. The alternate use of the two drying methods was done primarily to reduce drying time as the sugar content of *S. innocua* necessitates prolonged drying. In contrast, there was no drying practiced with the species in Chimanimani district, a wetter area in Zimbabwe. It was observed that though abundant in Chimanimani district during the fruiting season, *S. innocua* and *S. madagascariensis* fruits are heavily littered, indicating underutilization even for consumption as fresh fruits. This was attributed to the fact that the region's high rainfall and fertile soils are appropriate for other agricultural cropping practices, and hence other food sources are available and affordable.

Prior to drying, fruit flesh is pre-treated with either fine salt, crystalline sugar or both, depending on consumer preference. The flesh / seed is spread and pressed to stick to the surface of hand-plaited reeds interlocked with strips of palm leaves. By midmorning, the rack is exposed to the sun on a traditional dish rack (called *dara*) or on bundles of firewood. The rack's position is changed throughout the day for maximum exposure to the sun. At dawn of the following day, the rack is fire-dried with wood as the fire source, or (when wood is unavailable) corn cobs or stalks (figure 3.4). Fire drying is done for at least four hours, and thereafter the product is left uncovered on the kitchen floor overnight. A layer of fresh fruit flesh is added the next morning and the consecutive sun and fire drying steps follow as the previous day. The alternate sun and fire drying steps are continued for five to seven consecutive days, where the length of processing depends on the desired thickness of product, household size and consumer preference. The product obtained can be stored for more than three months according to 75 % of respondents, while 25 % of respondents said they consumed the product soon after processing and did not store for later consumption. Most consumers of the product said they had a sensory preference for the smoky flavour of the product. Wood smoke and fire-charred wood produce chemical components such as lactones, alcohols, carbonyls, esters, furans, and phenols, which exhibit several flavours, including pungent, bitter, spicy, burnt sugary, or smoky flavours (Maga, 1987). Without the smoky flavour the product was reported as not well accepted by consumers.

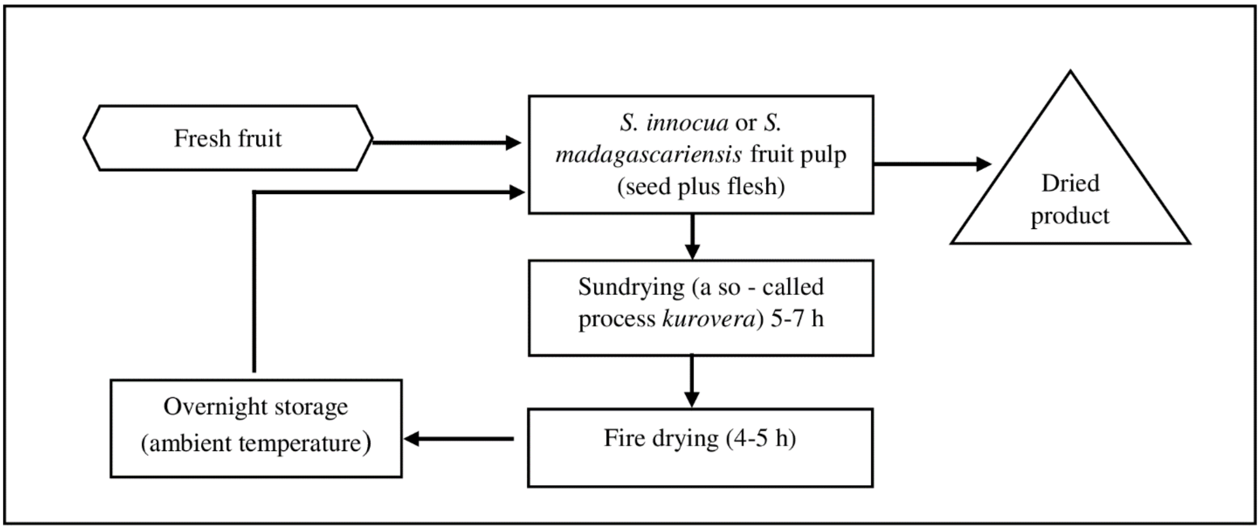

Figure 3.4 Drying process of *S. innocua* and *S. madagascariensis* in Bikita district, Zimbabwe

Sun drying of S. cocculoides and S. spinosa

Solar drying of *S. cocculoides* and *S. spinosa* was commonly done in Lower Gwelo (68 %) district. During hot summer days, monkey orange flesh is either spread over a flat surface with maximum heat absorbance or in traditionally-made solar driers covered with transparent or black plastic. The solar dryers use convective air for heat that subsequently dries the product. Drying takes between five days and two weeks, depending on prevailing weather conditions and whether the desired moisture content is reached. The determination of the moisture content is subjective, and physical tests, such as stickiness of the product, are reported often used to evaluate if the product is dry enough. When dried sufficiently, the product is stored at room temperature in perforated sacks. The dried product is the starting material to prepare sweet and snack products or for inclusion as an ingredient in different preparations such as juice, porridge or beverages.

Food products for immediate consumption

S. cocculoides and *S. spinosa* pulp is combined with other ingredients to make porridge, alcoholic and non- alcoholic beverages and, less frequently, jam and confectionary for immediate consumption as in table 3.3. Immediate consumption in this context implies consumption from the time of processing until no more than two to three days later.

Porridge

Two variations of cereal-based porridge meals made with *S. cocculoides* or *S. spinosa* (group A) pulp were encountered in the survey. The first porridge variant was a maize-based porridge (called *mutandabota*) prepared in 15 % of the households in Bikita, 30 % of those in Chimanimani, 55 % in Marondera and 82 % of households in Lower Gwelo. Maize porridge is the most commonly processed product using group A spp. The second porridge variant was a sorghum-based porridge (called *umcaba*), as identified by a focus group in Lower Gwelo area.

For the maize-based porridge, pulp is macerated in water for 30 min. to two h, either standing or with whisking using a wooden spoon to detach flesh material from the seeds. The resulting juice or pulp is subjected to heat, and upon boiling, white maize meal (*Zea mays*) to water 1:4 (w/v) is added with stirring until a lump free, gelatinized and uniform slurry is obtained; the maize-based porridge. For the sorghum-based variant, untreated monkey orange pulp or juice is mixed with boiled, solar-dried ground sorghum, and then consumed immediately. In both processing methods, the porridge products are generally thin, flavoured with peanut butter or sweetened with crystalline sugar. Texture of the maize meal variant depends on the weight to volume ratio of maize meal flour to monkey orange pulp. The maize porridge is usually consumed immediately while slightly hot or warm as a breakfast food or snack. It can also be stored in closed clay pots and in cool conditions for a maximum of two days for re-warming prior to consumption.

Fermented beverage

Thirty five percent of the households interviewed in Marondera and 41 % of respondents in Lower Gwelo fermented *S. cocculoides* and *S. spinosa* pulp to make *mahewu*, a sweet sour cereal-based fermented non-alcoholic beverage containing fresh monkey orange pulp. Two variations were encountered in the preparation of a fermented beverage. In processing method I, the pulp was mashed with cool stiff maize meal porridge (*sadza*) (figure 3.3). Either maize, sorghum or millet flour was added to the mashed product as the fermentation substrate. In processing method II (figure 3.3), sorghum or millet flour was added to the fruit pulp without mashed stiff maize porridge as an initial ingredient. For either variant, the resultant mixture is incubated for 12-24 hours to facilitate fermentation at ambient temperatures and both are referred to as *mahewu*. *Saccharomyces* yeasts, inherent to the cereals, contribute to flavour development of *mahewu* (Blandino et al., 2003).

Respondents in Marondera (10 %) and Lower Gwelo (14 %) added yeast and prolonged the fermentation time to produce an alcoholic brewed beer. When an alcoholic beverage is to be prepared, yeast is added to the product of process I, set near a cooking fire (to maintain temperatures above 30 °C) and allowed to ferment naturally over a period of three to four days.

Unfermented beverage

The whole fruit pulp or sieved monkey orange juice may be used to prepare juice. Pulp is sieved or strained with a mesh sieve substituted traditionally with a woven high-density polyethylene (HDPE) fruit sack. Water is added in a 1:1 ratio and lemon fruit juice or citric acid (depending on availability) is added as a preservative for the juice. The juice is boiled and crystalline sugar added, again depending on consumer preference and intended storage time. The product was consumed within three days. This type of juice was prepared by 5 % of the households in Chimanimani, 15 % in Marondera and 41 % in Lower Gwelo.

3.4 Product consumption

Monkey orange products and their consumption patterns among respondents are shown in table 3.3. Dried *S. innocua* and *S. cocculoides* were mainly consumed as a sweet snack at lunch time or between meals during time of chores by women and children. Fermented beverage (*mahewu*) was consumed fresh, though some respondents stored the product for a maximum of two days in a cool environment. Beer was consumed fresh and up to two to three days after preparation as a leisure drink during traditional ceremonies and social gatherings.

Table 3.3 Meal times of specific monkey orange-based products in study regions

Species	**Product**	[3]**Meal times (N)**			
		Breakfast	**Lunch**	**Between meals**	**Supper**
S. innocua and *S. madagascariensis*	Dried	3	5	17	0
S. cocculoides and *S. spinosa*	Porridge	49	1	2	0
	Dried	1	4	13	0
	Juice	1	4	10	0
	Mahewu	2	6	16	0
	Beer	0	1	5	0

3.5 General utilization

In the dry region, consumption of monkey orange fruits was 100 % for group B spp. and 74 % for group A spp. In the wet region consumption was 82 % for group A. The high values obtained were because the fruit was consumed in its unprocessed fresh state, hence the high general use for both groups of species as a pulp. Fruits from the species

[3] Respondents answered to meal times for all the products consumed in the household, where each respondent may have answered consumption times for more than one product.

groups were used to process the same products, though *S. innocua* and S. *madagascariensis* fruits were not used as a dried product in the wet region. The proportion in which a particular product was used only significantly varied ($p < 0.05$) between the two regions for both groups of species for the dried product of group A and for fresh pulp of group B (Figure 3.5).

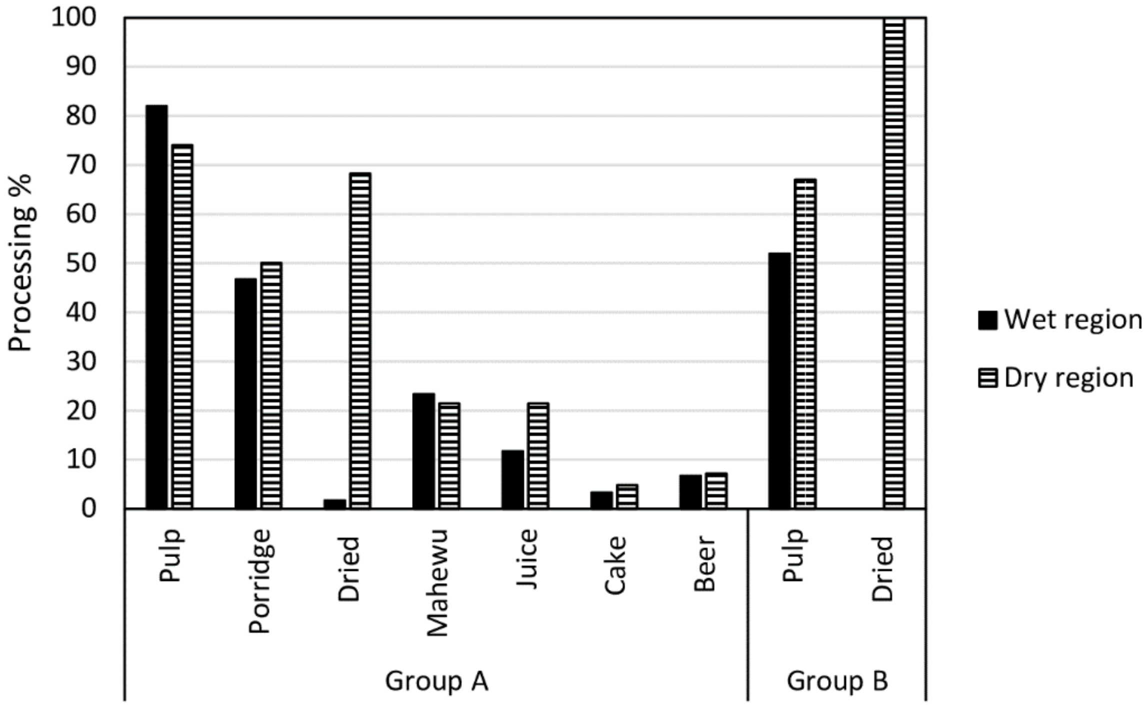

Figure 3.5 Processing frequency of *Strychnos* spp. among study regions

There was a marked difference in uses between the two groups of *Strychnos* spp. Group A species had more uses and were processed into more products than group B species in both regions. Uses for group B species was higher in the dry region than in the wet region. Respondents from the wet region only consumed the fresh fruit of *S. innocua* or *S. madagascariensis*. These findings and observations suggest that the poorest of the resource limited communities (in the dry region) better utilize their indigenous fruit by processing than rural communities that are better off. Communities, however, do not have proper preservation techniques to allow for long term use and storage when fruits are out of season, leading to loss of product by rotting and underutilization. We found in this study that, in addition of their food value, monkey orange fruits and products are marketable. In general, selling was done in the drier and poorer regions, indicating the possibility to supplement income in these marginal areas (Mithöfer,2003). Dried *S. innocua* or *S. madagascariensis* was mainly sold informally at rural growth points (small business centres in rural communities where basic essential services and markets are concentrated) and from home-to-home. A stack of dried fruits about 100 g in weight was sold for US $1 with its price doubling as the fruit drew out of season. Juice was sold at US $1 for 750 ml at gatherings. None of the products was reportedly sold in the formal markets at the time of the survey, demonstrating underutilization of monkey orange despite its potential for trade as has been indicated in other studies where monkey oranges are traded between Southern African countries (Mkonda et al., 2002; Ruffo et al., 2002; Southampton Centre for Underutilised Crops, 2006). Women, being the custodians of neglected and underutilized fruits, may be empowered by a change in this situation.

3.6 Product processing constraints and quality criteria

Figure 3.6 presents an overview of processing operations, constraints and sensory quality characteristics of products from *Strychnos* spp. in the study areas.

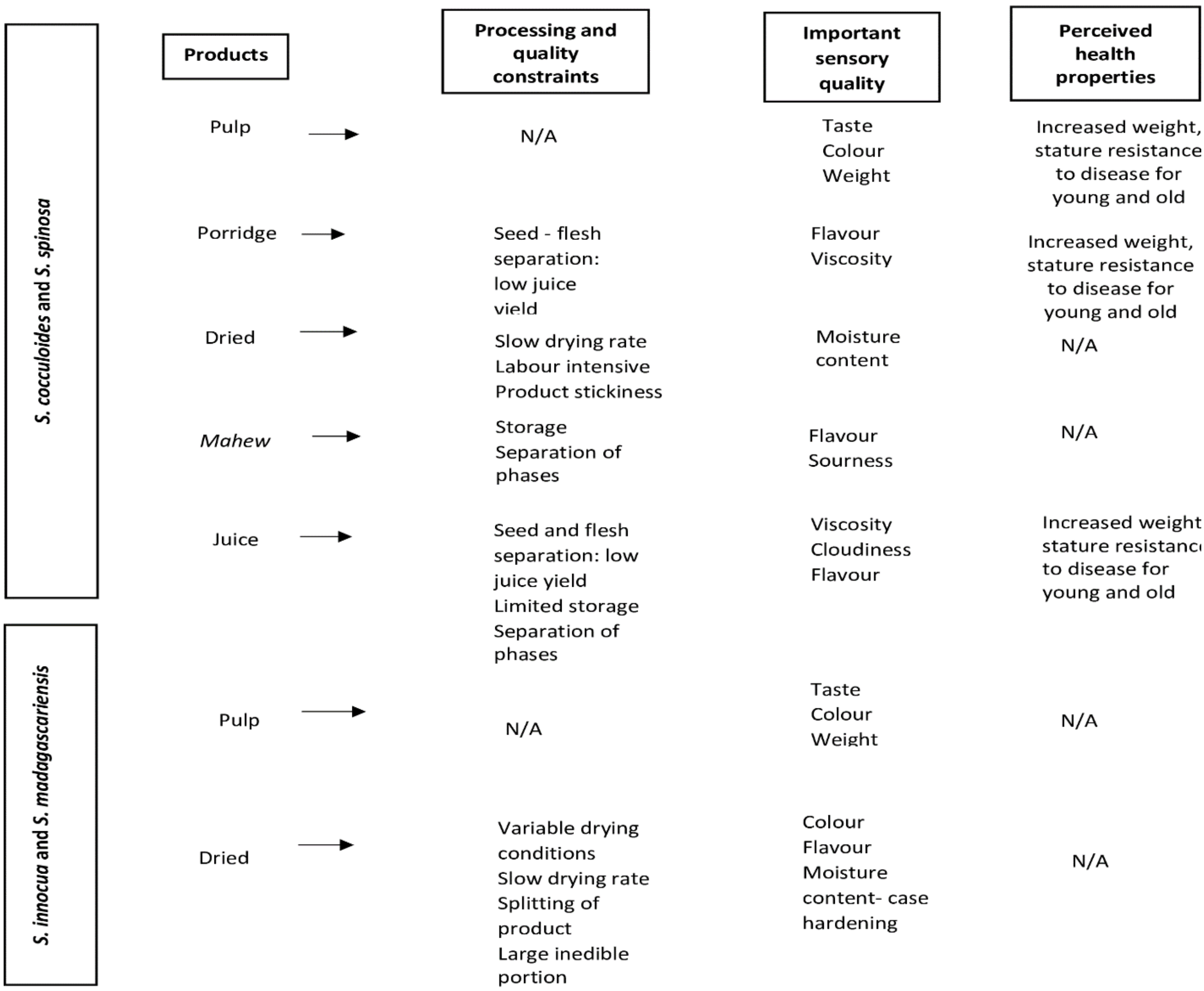

Figure 3.6 Process constraints and quality criteria identified by respondents in study regions

Solar drying of *S. cocculoides* and *S. spinosa* is highly dependent on environmental temperatures and conditions. Fruits are harvested in the rainy season, a period when drying conditions are indefinite, usually resulting in extended drying times and added demand for labour. The dried products are also highly hygroscopic, and require storage in dry conditions. To prevent the loss of quality by an ingress of moisture, the processed product was stored in plastic sacks or tins with tight closing lids and re-dried to extend its shelf life. The absence of efficient drying and storage facilities for monkey oranges was identified as a prominent difficulty, especially when the rainy season begins. These processing and quality constraints were highlighted as highly problematic in the focus group discussion. Other quality constraints observed during drying were dust, and animal and mould contamination, especially during humid weather. When solar driers were used, poor hygienic standards were also noted as a factor of equal importance through the inclusion of seeds and vegetables from previous drying processes. This may also account for the degradation of product quality.

Important quality characteristics of dried *S. innocua* and *S. madagascariensis* product are colour, a smoked flavour and low moisture content, though they vary from one processor to another and from time to time. Respondents appreciated a golden to brown colour; a darker colour than this was perceived as charred and likely to have a characteristic burnt taste and flavour. Though fruit is spread uniformly on the drying rack, the desired colour is difficult to achieve due to variations in fire and solar temperatures because heat and air flow are not controlled or uniformly distributed on the drying rack during the drying period. Therefore processors have to continuously change the rack position during both fire and solar drying. Typical wood smoke flavour is induced by factors such as wood moisture content, air temperature, intermediate or stable volatile compounds and chemical reactions with other food components (Maga, 1987). These are not controllable at a household level. The type of wood used in fire-drying is based on availability, therefore differences in product sensory quality are encountered. The fruit may be pre-treated with sugar or salt before drying. Respondents indicated that crystalline sugar facilitates rapid drying, prevents the compacting of the dried product on the rack and eases separation on handling and consumption, while salt maintains colour and texture of the fruit. Pre-treatment with sugars (i.e. osmotic dehydration) has been used in the preservation of other fruit to hasten drying, reduce browning, increase volatile retention, and reinforce a sweet taste (Osorio et al., 2007). In some cases in the areas visited, pre-treatment was immediately followed by sun drying with no incubation leading to the assumption that some of the pre-treatment effects may not reach the maximum due to limited exposure to the osmotic agents, the sugar or salt.

Respondents mentioned increased flavour, sweetness, clarity and low viscosity as good quality indicators of monkey orange juice. Colloids such as polysaccharides are known to be responsible for the viscosity of fruit juice and cloudiness in other fruit juices such as lemon juice (Uçan et al., 2014). *S. cocculoides* and *S. spinosa* juice is viscous and the viscosity affects the appearance of the product and the overall acceptance. Processors add a significant amount of water until the juice is sufficiently liquefied. The dilution and resultant consistency depend on consumer preference, as found in the study of Bille et al. (2013), where panellists also preferred diluted juice. However, on the contrary, respondents did agree that although viscosity is reduced by dilution, the major drawback was a reduced *Strychnos* spp. flavour. During processing the separation of seed and flesh is low, giving low juice yield during processing.

Porridge is mainly prepared for and consumed by the whole family and usually introduced as a complementary food to infants from as early as four to six months. Porridge that had simmered longer lost the characteristic monkey orange flavour. Thermal treatments can inactivate volatile-forming enzymes as well as cause the loss of the volatile compound precursors (Nijhuis et al., 1998). Cooking time and ratios of addition of monkey orange juice or pulp also may have a bearing on the resultant flavour.

The most important sensory quality determinants of monkey orange fruits were colour and taste (sweetness). Fruits are picked before maturity for ripening during storage. Respondents use external visual attributes such as colour, size and surface structure to predict the taste of the fruit. Selection of this nature is however a subjective method (Reid, 2002). There are currently no objective minimum indices for the determination of maturation at the household level in rural areas, which results in variation in fruit selection. All in all, fruit harvesting and processing conditions, and parameters, are not standardized and therefore variations in product quality are prominent for all the products we identified.

3.7 Conclusion and recommendations

Collection, processing and consumption of the monkey orange fruit is a common activity in the dry and wet regions we assessed in Zimbabwe. Health benefits, value addition, market access, and education about its cultivation and propagation have an effect on proper utilization of the fruits in both regions for food and nutrition security.

Respondents in farming communities believe the fruit products, particularly porridge, have health benefits. The chemical compounds involved and mechanisms for nutritional and health benefits are unknown, as these appear to be new findings that require further scientific studies for substantiation. Exploration of strategies through scientific research for value addition and market access by small-scale fruit processing units through community cooperatives are an important contribution to curbing the challenge of food and nutrition security for households and individuals in Zimbabwe. Increasing education on the cultivation and propagation of monkey orange trees through information and planting material available at different centres in Southern Africa, such as Veld Products Research and Development, Botswana, and World Agroforestry Centre, Zimbabwe (Southampton Centre for Underutilised Crops, 2006), are also important for sustainability of these indigenous fruits. We recommend to put in place a research and development program targeted at monkey orange and other underutilised fruits under the custodianship of stakeholders from government, research institutes and processors. The results of improved technologies and know-how, which will benefit women cooperatives and small-scale processors by expanding what they sell and promote their incomes, can be transferred by government extension services and NGO’s.

Constraints for *Strychnos* spp. processing (i.e. drying rate, product splitting, flesh/seed separation, juice yield, phase separation), shelf life and sensory quality characteristics (colour, flavour, and viscosity) were increased by mainly un-standardized processing conditions and treatments. These constraints illustrate how generic some food technological problems and solutions can be, and yet how product-specific some problems and solutions are in relation to the different fruits of the same species; a concept that can be translated to various types of indigenous fruits in Southern African countries.

In scientific literature, *Strychnos* spp. have been labelled (amongst other indigenous fruits) as “lost fruit” (National Research Council, 2008) and as neglected and underutilized species that are being lost at an extraordinary rate before they can be completely characterized, researched and promoted (Padulosi et al., 2013). Food and nutrition security is multi-faceted due to its interrelated complex dimensions. To facilitate the achievement of food and nutrition security, every contribution from all disciplines is worthwhile. Therefore, as a follow-up to this survey, it is paramount from a food quality and design perspective to conduct experimental research to assess product quality characteristics and the contribution to nutrition of traditionally-processed products from *Strychnos* spp.

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Conflict of interest. The authors declare that they have no conflict of interest.

Chapter 4

Effect of heat and pectinase maceration on phenolic compounds and physicochemical quality of *Strychnos cocculoides* juice

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Abstract

Strychnos cocculoides fruit is an important food source for rural populations in Zimbabwe in times of scarcity. Its thick pulp tightly adheres to its seeds, causing pulp extraction constraints and waste during processing, leading to underutilisation. Therefore, pectinase maceration combined with heat treatments was studied to improve juice yield and juice quality. Metabolite profiling according to the heat map, FancyTile chromatic scale approach and phenolic compound content were used to compare the identified compounds. Prior to treatments, 16 known phenolic compounds, predominantly belonging to the phenolic acids, flavonoids and iridoid glucoside classes, were tentatively characterized for the first time in *S. cocculoides* using High Resolution Mass Spectrometry and LC/MS/MS. Overall, results showed that enzymatic treatments increased pulp yield (by 26%), physicochemical quality (38% increase in juice clarity), content of phenolic compounds (predominantly kaempferol, quercetin, caffeic acid, protocatechuic acid, iridoids) and antioxidant activity.The improved extraction of *S. cocculoides* pulp increases juice yield as well as juice quality by supplying larger amounts of phenolic compounds that have potential health benefits and act as dietary sources of antioxidants for the prevention of diseases caused by oxidative stress.

Keywords: *S. cocculoides*, pulp yield, phenolic acids, flavonoids, iridoid glucosides

4.1 Introduction

Monkey orange species (e.g *S. cocculoides*) belonging to the Loganiaceae family are characterised by viscous flesh tightly adhering to hard seeds (1:2 w/w ratio pulp and seed) fresh weight (FW), hampering pulp extraction by simple pressing. The fruit is either consumed fresh or macerated in water with/without heat to enhance pulp extraction for use as an ingredient in other food products (Ngadze et al., 2017a). The limited seed – pulp separation reduces pulp yield with a subsequent increase in waste and loss of sensory and nutritional quality of the end product. Generally three extraction methods are used to increase the yield of pulp and juice viz., hot, cold or enzymatic pre-treatments (Sharma et al., 2015). Pre-treatment with pectinolytic enzymes in fruit pulp has been used widely and successfully since the 1930's to soften tissues, increase juice yield, production efficiency and ease separation of clean seeds from the pulp (Ramadan & Moersel, 2007; Ribeiro et al., 2010). Enzymes hydrolyse glucoside bonds of the main chains of polygalacturonic acid in cell walls for increased recovery of soluble fruit components and sensory quality (Joshi et al., 2012; Puri et al., 2012). An enzymatic maceration treatment using suitable conditions for the *S. cocculoides* matrix is expected to improve yield and quality of the pulp.

S. cocculoides fruit features considerable amounts of nutrients important to human health such as minerals, fibre and vitamin C (Bello et al., 2008; Saka & Msonthi, 1994). Favourable biological activities have also been reported for *S. spinosa* species which have closely similar genotypic and phenotypic characteristics as *S. cocculoides* (Nhukarume et al., 2010). Tropical and exotic fruits are rich in bioactive phenolic compounds that occur as different classes of secondary metabolites. The phenolic compounds reportedly have increased health benefits beyond basic nutrition and contribute to anti-oxidant activity actions within the human body together with some vitamins and the endogenous defence system (Haminiuk et al., 2012; López-Cobo et al., 2015; Mattila et al., 2006). Phenolic compounds also contribute to fruit characteristics such as colour, flavour, bitterness, astringency, and together with polysaccharides add to chemical stability and sensory perception of food products (Fernandez de Simon et al., 1992; Oliveira et al., 2014). Other phytochemicals such as, iridoid glycosides (IGs), which have many potential biological activities (West et al., 2016) were reported in *S. cocculoides* bark and *S. spinosa* branches (Itoh et al., 2005; Sunghwa & Koketsu, 2009).

S. cocculoides fruit trees thrive during the dry season and remain dormant when water is unavailable. Abiotic stress can elicit the presence and lead to high concentration of particular phenolic compounds (Russell et al., 2009), providing a potential source of healthy and nutritious food useful in drought periods. *S. cocculoides* products are poorly exploited and hence there is a need to provide technological solutions to this challenge. The current study identified the phenolic compounds and assessed the effect of maceration treatments on phenolic compounds content using a novel representation by combination of heatmap, hierarchical clustering and FancyTile approach was used. The FancyTile approach uses a targeted metabolite profiling of different class metabolites by High Resolution Mass Spectrometry (HRMS) with a chromatic representation of the variations in different food samples (Troise et al., 2014). In this study, original data regarding phenolic compounds in *S. cocculoides* are reported and processes to improve the pulp yield and health beneficial compounds are proposed.

4.2 Materials and Methods

4.2.1 Standards and Chemicals

Cynarine (1,3-dicaffeoylquinic acid), caffeic acid, syringic acid, ferulic acid, chlorogenic acid, protocatechuic acid, rutin, quercetin, quercitrin, isoquercitrin, myricetin, kaempferol, naringenin 7-O-glucoside, naringin, catechin standards were purchased from Sigma (Italy), naringenin from Aldrich (Italy), loganic acid was purchased from Extrasynthese (France) . Methanol, acetonitrile, formic acid and water HPLC grade were obtained from Merck (Darmstadt, Germany). Commercial pectinase enzymes from Aspergillus niger were obtained from Sigma Chemicals GmbH stored at 4 °C.

4.2.2 Preliminary enzyme treatment and pulp extraction

Ripe *Strychnos cocculoides (S. cocculoides)* fruits selected according to degree of ripeness (having a bright orange colour) without any visual defects were collected from Lower Gwelo, Zimbabwe with the help of local processors. The fruits were transported at 4 °C to Wageningen University & Research (The Netherlands). Fruits were shelled by hammering, the fruit flesh blended and stored at -20 °C until further analysis. Preliminary enzymatic experiments of monkey orange pulp were performed at varying pectinase range 0.5 to 2 % (w/v) and incubation time 120 to 300 minutes. Scheme of enzymatic and non-enzymatic treatments is shown in figure 4.1 for HNP: Hot water extraction without pectinase, CNP: Cold water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase. As monkey orange pulp is very difficult to disintegrate from the seed, higher enzyme dosage and time than typically used for other fruits was applied. Pulp yield was determined by the following expression : Yield (%) = (A/B) x 100. Where Y is the % pulp yield, A is the detached pulp weight (g), B is the initial fruit weight (g). The variables which produced the highest pulp yield and dry matter were used as optimal treatments. Figure 4.1 depicts the enzyme treatment process and the resultant pulp used in further analysis. For further analysis the pulp/juice mixture was further centrifuged at 4 700 rpm for 15 minutes to remove coarse pulp particles and the supernatant collected was considered as clear juice. Dry matter was determined by drying 2 g pulp at 105 °C overnight to constant weights (AOAC, 1995).

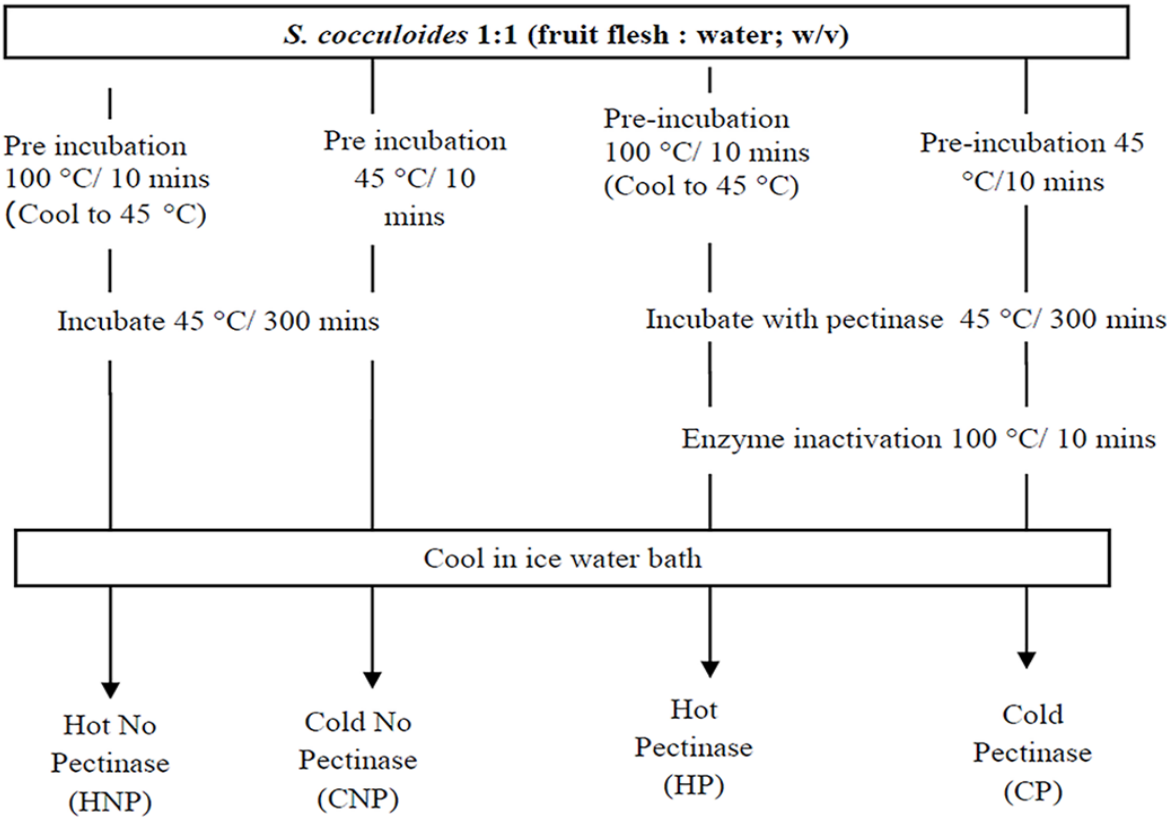

Figure 4.1 *Strychnos cocculoides* fruit treatments (HNP, CNP,HP and CP)
HNP: Hot water extraction without pectinase, CNP: Cold water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase.

4.2.3 Physicochemical properties

The pH was measured using pH meter (pH1002 VWR phenomenal). Soluble solids determined using a refractometer and units specified in °Brix. Clarity was determined by measuring the % transmittance at 660 nm using a Cary 50 Bio UV-vis spectrophotometer with distilled water serving as a blank (Sreenath et al., 1994). Colour measurements CieLab coordinates (L*, a*, b*) were directly read with Hunter ColorFex LAB (Escolab Netherlands) apparatus, calibrated with a black, green and white tile. The L* value is a measure of lightness, ranging from 0 (black) to 100, the a* value ranges from -100 (green) to +100 (red) and the b* value ranges from -100 blue (purple) to +100 (yellow). Colour differences (delta) was done so as to have one colour value and expressed as $\Delta E^* = [(\Delta L^{*2}) + (\Delta a^{*2}) + (\Delta b^{*2})]^{1/2}$ (Pathare et al., 2013). The sample with the lightest colour was used as reference.

4.2.4 Sugars analysis

Samples were centrifuged at 4700 rpm for 15 min at 20 °C, after which 1 ml of residue was diluted up to 20 times with distilled water. External standards for sucrose, glucose and fructose in the range of 2.5 – 0.078 mg/ml were used for extract quantification. Standards and extracts were filtered through a 0.45 µm filter for high performance liquid chromatography (HPLC) analysis. Individual sugars were separated by HLPC using a Thermo Separation Products model with P-2000 Pump and ELSD-2100 polymer lab detector. Separation was conducted on a Grace prevail carbohydrates column, 5µm, (250 mm x 4.6 mm) with an evaporator temperature of 90 °C, nebuliser temperature of 50 °C and gas 1.6 arbitrary units. Running time was 30 min with a flow rate of 0.8 ml/min on isocratic 75/25% acetonitrile/water. Extract peaks were identified by comparing retention times (RT) with sugars standards.

4.2.5 Characterization of phenolic compounds in S.cocculoides juice

Freeze-dried samples (1.0 g) were solved in 20 mL of methanol/water (70:30, *v/v*) and homogenized with an Ultra-Turrax T25 digital (Ika, Germany) for 2 min, ultrasonically extracted for 30 min, and then centrifuged at 14800 rpm for 10 min. The supernatants were used for HRMS analysis. LC-MS data were acquired on an Accela U-HPLC system coupled to an Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Chromatographic conditions were used as previously described by Zhu et al. (2014) with some modifications: a Kinetex 2.6 µ C18 100 A column (100 mm × 2.1 mm) (Phenomenex, Torrance, CA) thermostated at 40 °C was used, the mobile phase consisted of 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B). Gradient elution was linearly programmed as follows: 2 % B (0.5 min), 2–10 % B (0.5-6) min, 10–33 % B (6-20 min), 33-90 % B (20-23 min). The flow rate was set to 300 µL/min and the injection volume was 10 µL. The U-HPLC was directly interfaced to an Exactive Orbitrap MS equipped with a heated electrospray interface (HESI). Acquisition was performed in both positive and negative ionization modes, in the mass range of *m/z* 100–1500. Chromatographic data acquisition and peak integration were performed using Xcalibur software (Thermo Fisher Scientific, San Jose, USA). Calibrations curves of flavonoids were constructed in the linearity range 10-5000 ng/mL, phenolic acids and loganic acid were constructed in the linearity range 100-5000 ng/mL. All iridoids were expressed as equivalents of loganic acid, kelampayoside A was expressed as equivalents of rutin, 2-hydroxy-3-*O*-β-D-glucopyranosyl-benzoic acid was expressed as equivalents of protocatechuic acid. Identification of iridoids: sweroside and secoxyloganin or secologanoside-7-methyl ester was based on MS/MS analysis performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) comparing fragmentation pattern with literature data (Qi et al., 2009).

4.2.6 Anti-oxidant activity determination by DPPH assay

The antioxidant activity was studied by evaluating the free- radical scavenging effect on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The method used was as described by Brand-Williams et al. (1995) with some modification. Pulp extract was mixed with 100 % aqueous methanol, the suspension was incubated for 30 minutes at room temperature while vortexing at 1620 rpm with a Heidolph multi tube mixer then ultrasonically extracted for 30 minutes at 35 °C and centrifuged for 10 minutes at 4000 rpm at 4 °C. Dilutions, incubation and absorbance measurement and Trolox equivalent value was determined as described by Hiwilepo-van Hal et al. (2013).

4.2.7 Statistical Analysis

All results were given as means ± standard deviation of three independent determinations of each parameter. One-way analysis of variance with Tukey test was used to compare significant differences between means. Pearson correlation coefficients was used to determine relationships among variables, differences were considered to be significant ($p < 0.05$). Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS v23.0) software.

4.2.8 Multivariate analysis

Multivariate data analysis was divided into three steps according to the concentration of polyphenols in the four sample treatments. The heatmap was calculated using the function OMICs in XLSTAT environment (Addinsoft, NY v 2014.5.03). The dendrograms were constructed by using agglomerative hierarchical clustering algorithm proposed by Ward. This method aggregates two groups in order that within-group inertia increases as little as possible to keep the clusters homogeneous. Finally, according to the clusters, a FancyTiles chromatic scale was conducted as described by Troise et al. (2014) by considering fixed range among the four treatments. A summary of phenolic compounds retention time, chemical formula, selected ion and theoretical mass are reported in Supplementary data section.

4.3 Results and discussion

4.3.1 Pulp yield

Maximum pulp yield and dry matter were obtained at 0.5 % (w/v) pectinase and incubation of 300 minutes, further increase in pectinase concentration had no significant effect on pulp yield (data not shown). The results showed that the pulp yield of CNP was and HNP was 59 and 60 % respectively while HP and CP gave an 85 % pulp yield (26 % increase), a significant increase compared to CNP and HNP pulp yield. Application of enzyme resulted in a general increase in pulp yield. Enzyme treatment proved to be advantageous in releasing cellular contents that increased the total solids, high pulp recovery and dry matter (12.5 %) in the HP treatment hence reducing processing wastage. With the addition of pectinase to *S. cocculoides* the tissue of the pulp disintegrated and is better detached from the seed. Pectinase is a crude enzyme preparation having multiple enzyme activities: polygalaturonase, pectate lyases and pectin methylesterase that disrupt hydrophilic bonds between water and pectin facilitating the increased extraction yield (López-Cobo et al., 2015). Heating prior to enzymatic extraction further increases extract recovery significantly by depolymerizing lignin components and softening fruit tissue (Sharma et al., 2015).

4.3.2 Effect of enzymatic treatments on physicochemical quality

Brix, pH, clarity and colour data measured in response to the maceration treatments are reported in table 4.1. The highest brix value (10.13) and the lowest pH (3.3) were obtained using HP treatment. Among the four treatments, there was significant higher clarity of juice from enzyme treatments than non- enzyme treatments. A small difference between enzyme treated samples and the non-enzyme samples, $\Delta E^* < 1.8$ and $\Delta E^* < 0.5$ between CP and HP were obtained making colour perceptually insignificant. High brix with enzyme extraction is related to increased tissue breakdown during degradation of cell wall polymers releasing sugars which contribute to soluble solids. Similar to results of *S. cocculoides* enzymatic treatment, a drop in pH was observed with date (*Deglet Nour, Allig* and *Kentichi*) syrup (Abbès et al., 2011). Low pH during HP is explained by the release of carboxylic acids and galacturonic acid during enzyme treatments (Bille et al., 2013; Ramadan & Moersel, 2007). Carboxylic acids: succinic and citric acid have been found in edible *S. spinosa* at high contents (Amarteifio & Mosase, 2006). Lower pH has a positive impact on the keeping quality of the juice as a mild preservative and on the sensory quality for flavour development in maintaining sugar/acid ratios. Pectinase hydrolyses pectin causing pectin- protein complexes to aggregate into larger particles that settle out and improve juice clarity (Landbo & Meyer, 2004; Ni et al., 2014; Shah et al., 2015), our data are in agreement with these findings. The highest clarity obtained from HP treatment (92.1 %) is comparable to clear filtered enzyme treated apple juice (94.1 %) and pomegranate juice (95. 5 %) flocculated with gelatine and bentonite (Llorach et al., 2002; West et al., 2016) hence no further clarification of *S. cocculoides* juice is required after pectinase treatment. The large pulp fragments in non- enzyme treated samples were subsequently removed by centrifugation though smaller fragments, undissolved tissues, insoluble calcium pectate (Shah et al., 2015), remained that lead to visible poor clarity of juice.

Table 4.1 Physicochemical properties of *S. cocculoides* juice

Treatment	Total soluble solids (°Brix)	pH	Clarity (% transmission at 660 nm)	Colour		
				L^*	a^*	b^*
CNP	9.3 ± 0.23[b]	3.6 ± 0.00[a]	56.1 ± 2.33[b]	36.7	-1.11	7.76
HNP	9.6 ± 0.35[a b]	3.43 ± 0.58[b]	53.0 ± 0.23[c]	36.5	-1.01	7.71
HP	10.1 ± 0.12[a]	3.33 ± 0.1[c]	92.1 ± 0.18[a]	37.9	-1.32	6.43
CP	9.9 ± 0.27[a b]	3.53 ± 0.58[a]	90.8 ± 0.13[a]	37.6	-1.25	6.03

Different letters in the same column indicate significant differences at $p<0.05$. Data are reported as mean ± standard deviation (n=3).CNP: Cold water extraction without pectinase, HNP: Hot water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase

The orange-brown colour is an essential characteristic of *S. cocculoides* food products (Ngadze et al., 2017b), thus either enzymatic or non-enzymatic maceration treatments gives this characteristic. The orange-brown discolouration which occurs in cold extractions can be related to enzymatic browning by naturally occurring polyphenolases occurring polyphenol oxidases in the fruit, which catalyse phenol oxidation to form quinones which then polymerize to form melanoidins (Shah et al., 2015). The expectation was enzyme bleaching by hot treatments would result in a lighter product through inactivation of the endogenous enzymes by the pre incubation step (100 °C for 10 minutes), however this was not the case. During hot extractions other mechanisms such as non-enzymatic browning would have occurred such as phenolic compounds metal ion complexes (mainly Fe and Cu), ascorbic acid degradation (Robards et al., 1999), or Maillard reaction (Manzocco et al., 2000). These reactions in the separate treatments promote browning resulting in the mixed effects on colour that occurred. Such insignificant differences in colour are beneficial in that they do not cause any undesirable effects to the product by maintaining the preferred orange- brown colour. However, unwanted flavour changes may arise from the additive effects of pre-treatment incubation and inactivation of pectinase enzyme after treatment (100 °C for 10 minutes) leading to flavour losses or development of cooked flavours, effects which need to be investigated.

4.3.3 Effect of enzymatic treatment on individual sugars

Soluble sugars identified post maceration were fructose: 20 – 24 g/100 g DW and glucose: 17 – 25 g/ 100 g DW. Sucrose was only detected in the untreated fruit juice (4.4 g/100 g DW) and was unquantifiable in the four treatment extracts by the assay used mainly due to effect of maceration dilution(1:1) and assay dilution (x 20). Though not significantly different higher fructose and glucose content were obtained in cold extractions (CP and CNP) than hot extractions (HNP and HP) treatments. Sitrit et al. (2003), reported a 2-fold increase in the conversion of sucrose to glucose and fructose during ripening of *S. spinosa*. The low sucrose content from our findings show that sucrose was converted into its monomeric sugars during ripening and storage of *S. cocculoides*, a characteristic which is common with other climacteric fruits (Adão & Glória, 2005). High sugars level during cold extractions are attributed to soluble or cell wall bound invertase activity (optimum temperature 40 - 50 °C) (Ranwala et al., 1992) enhanced by the lower pre-incubation temperatures. The sugars results provide useful information for investigation of use of macerated *S. cocculoides* as source of rapid energy and sensory appeal and as an ingredient in confectionery, base in juice or candy sweetening.

4.3.4 Phenolic compounds

A total of 16 phenolic compounds were tentatively identified in *S. cocculoides* juice by HRMS (figure 4.2). This is the first complete description of the polyphenol profile reported for edible portion of *S. cocculoides*. The fruit, *S. cocculoides* presents a significant source of hydroxycinnamic acids (HCA), which were found to be the predominant phenolic acids. The principal phenolic acids in *S. cocculoides*: Caffeoylquinic acid (CQA) - an ester of caffeic acid and quinic acid and diCQA (cynarine) are the most ubiquitous and sometimes the only phenolic compounds in fruits (Scalbert & Williamson, 2000). Hydroxybenzoic acid (HBA): 2-hydroxy-3-O-β-D-glucopyranosyl-benzoic acid and protocatechuic acid were also identified in *S. cocculoides*. Previously 2-hydroxy-3-*O*-*β*-D-glucopyranosyl-benzoic acid was identified in the root and stem bark of *S. cocculoides* (Sunghwa & Koketsu, 2009) and in *Geniostoma antherotrichum* a plant of Loganiaceae family that possesses medicinal properties (Rashid et al., 1996). Subclasses of flavonoids found in *S. cocculoides* are: flavonols (quercetin, kaempferol), which are predominant in apricots, plums, peaches (Robards et al., 1999) and flavonone (naringenin glucoside isomers) which is principally present in citrus fruits, (Gebhardt et al., 2002; Oliveira et al., 2014). These findings are in agreement with perceived apricot and citrus flavour by consumers (Sitrit et al., 2003). IGs (morroniside) and kelampayoside A (a phenolic apioglucoside) were previously identified in roots and stem bark of *S. cocculoides* (Sunghwa & Koketsu, 2009). Sweroside and secoxyloganin were reported in branches of *S. spinosa* (Itoh et al., 2005). IGs are not uncommon in fruits and have been identified in substantial amounts in fruit pulps of noni (*Morinda citrifolia*), genipap (*Genipa americana L.*), European cornelian cherry (*Cornus mas*) and cranberries (*Vaccinium macrocarpon)* (Bentes & Mercadante, 2014; Su et al., 2005; West et al., 2016).

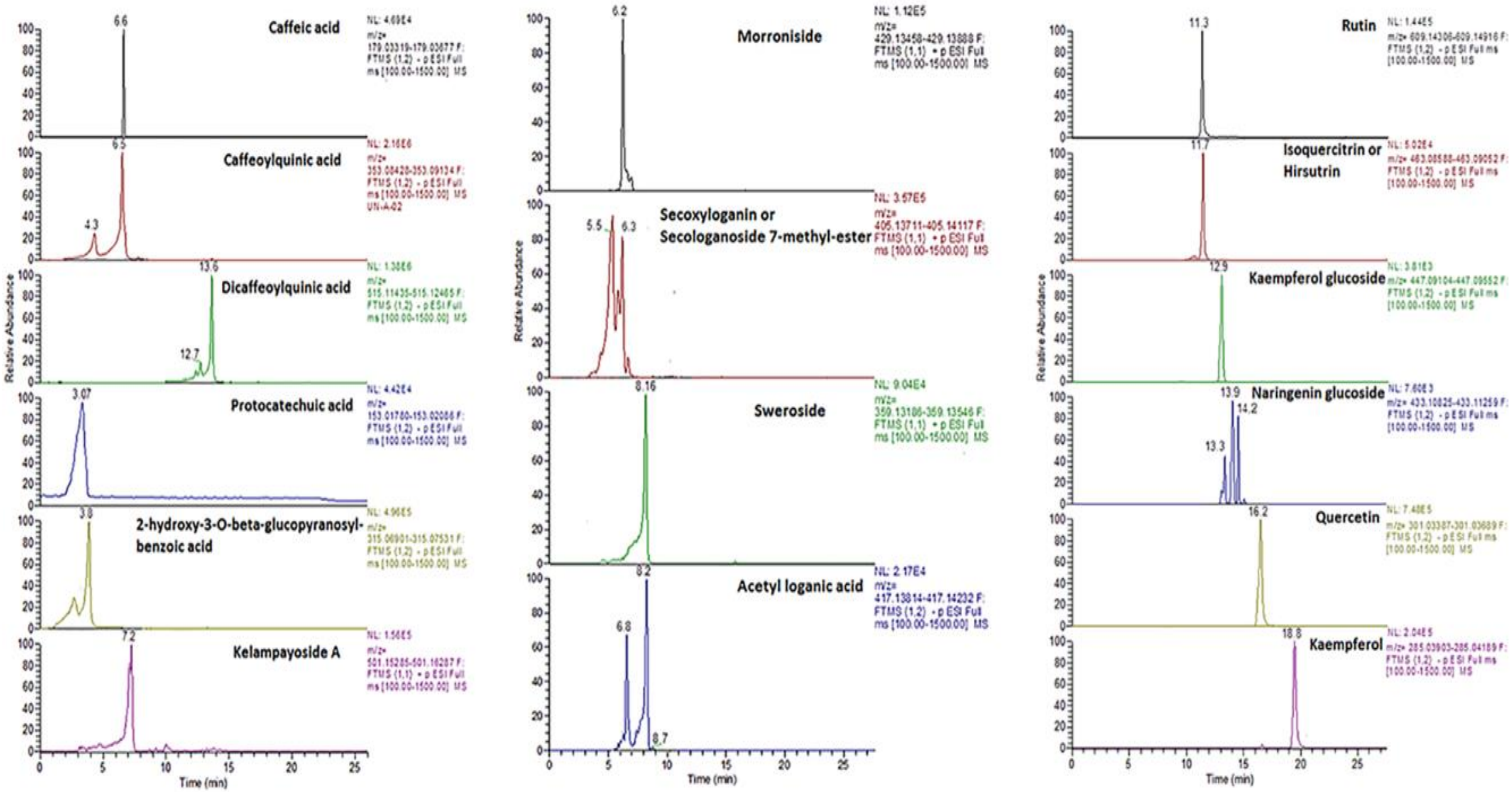

Figure 4.2 Extracted ion chromatograms of a: phenolic acids and kelampayoside A (phenolic apioglucoside), b: IGs and c: flavonoids in a representative extract of *Strychnos cocculoides* juice

4.4.1 Effect of enzymatic treatments on phenolic compounds content

Phenolic acids

Results did show no significant difference in the total phenolic acids between treatments (figure 4.3A). Pectinase treatments gave a slight reduced content of caffeoylquinic acid (chlorogenic acid) though not significantly different ($p>0.05$) to that of the no pectinase treatment. Enzyme and heat treatments gave a decrease in caffeoylquinic acid (chlorogenic acid) content, complementary to an increase in caffeic acid ($R = -0.893$). Caffeic acid is uncommonly found as a free acid in unprocessed plant material, increasing after deconjugation or hydrolysis of caffeoylquinic acid (Clifford, 2000), hence this inverse relationship after treatment processes in our study. HCA exhibits geometric isomerism (Robards et al., 1999), and these artefactual changes during extraction resulting in the different isomers identified. The contents of 2-hydroxy-3-*O*-*β*-D-glucopyranosyl-benzoic acid followed the trend of chlorogenic acid with treatment type. Protocatechuic acid (PCA) had minute concentration in all four treatments. PCA is reportedly sparingly soluble in water with a high melting point (Kakkar & Bais, 2014) thus we attribute the low content obtained to sedimentation of PCA into the pellet after centrifugation.

Iridoids and phenolic apioglucoside

In general, heat extractions gave higher contents of IGs than the cold extractions in the order HNP>HP>CP>CNP (figure 4.3B). HNP contained the largest amount of sweroside, acetylloganic acid isomers, and secoxyloganin than the other treatments. HNP extractions and HP had a higher overall contribution to IGs content 35 % and 29 % respectively. Only morroniside did not show any significant differences in concentration with all four treatments. The only phenolic apoiglucoside characterised for *S. cocculoides* : kelampayoside A contents presented in figure 4.3C had higher contents at HNP and HP treatments. The high contents of IGs and kelampayoside A after heat treatment present an added advantage in the maintenance of health beneficial compounds during the design of thermally processed foods of *S. cocculoides*.

Flavonoids

Six flavonoids were identified in *S. cocculoides* juice with rutin (a quercetin glucoside) as the predominant flavonoid during CNP treatment (figure 4.3D). Similar to caffeoylquinic acid, heat with or without enzyme in the aqueous environment had a detrimental effect on the content of rutin. In contrast, quercetin galactoside, quercetin and kaempferol showed no significant difference between CP and HP. For naringenin glucoside isomers HP, HNP and CP treatments no significant difference was observed ($p > 0.05$) and exhibited higher contents than CNP extractions. Upon pectinase hydrolysis (CP and HP), rutin is degraded to more stable aglycone quercetin (deglycosylation) and the disaccharide rutinose, thus a strong negative correlation with quercetin 3-O-rutinoside and quercetin ($R= -0.84$) was realised. Commercial pectinase preparations display secondary enzymatic activities such as glycosidases that can change the profile of phenolic compounds (Sandri et al., 2014), hence the degradation of rutin to quercetin is most probably due to the presence of glycosidase in the enzyme preparation. Additionally rutin is degraded by the heat in aqueous conditions because of the reactive oxygen species (ROS): ($^{\cdot}O^{-}_{2}$, $HO^{-}_{2}{}^{\cdot}$ and $HO^{\cdot-}$) (da Costa et al., 2002; Deng et al., 2011), which were also probably increased by the surface area exposed to water and oxygen. Overall, we expect the biological effect of flavonoids aglycones is more relevant when thermal processing of *S. cocculoides* juice is conducted due to their stability with heat treatments.

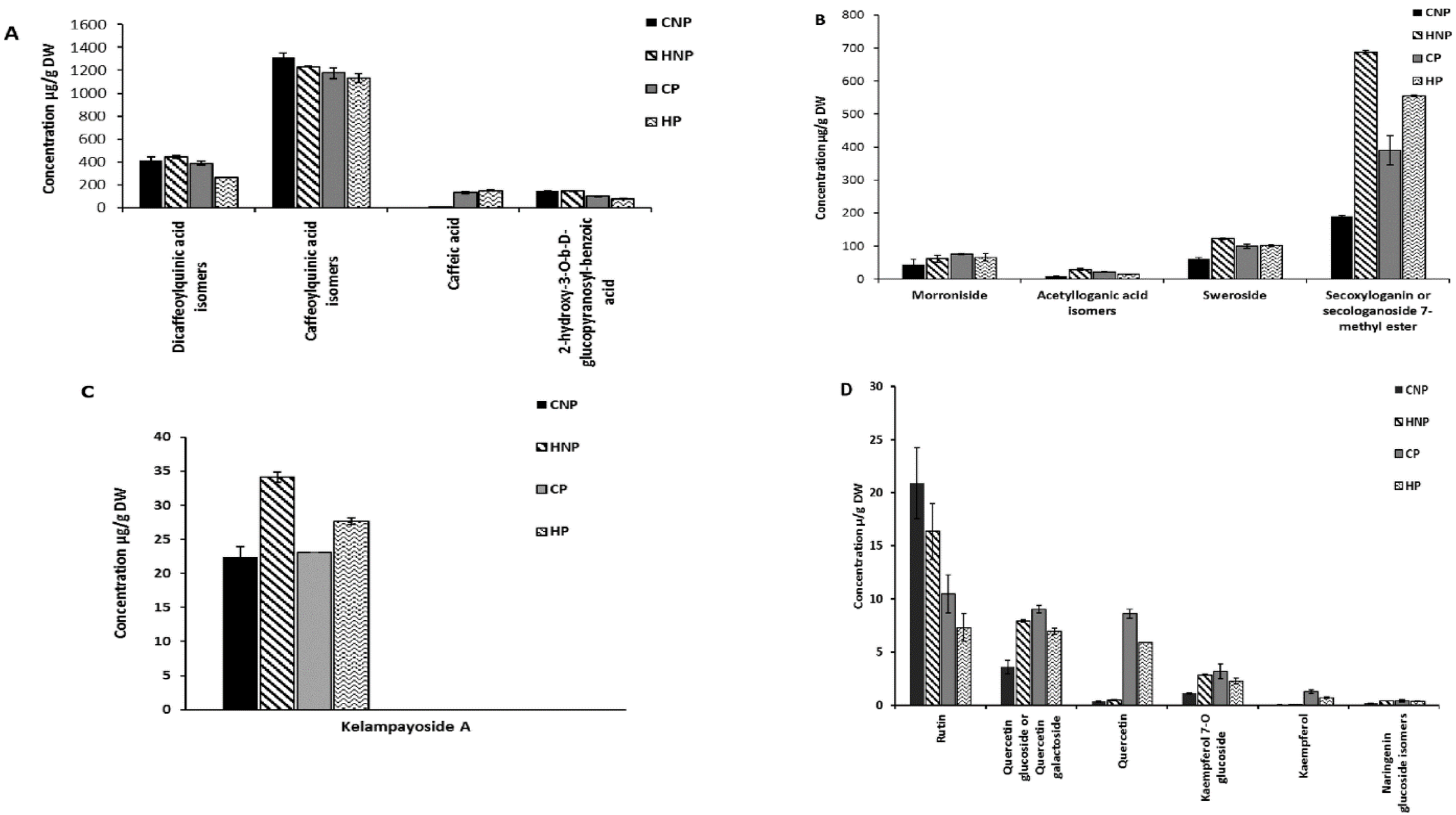

Figure 4.3 Phenolic compounds content of A: Phenolic acids, B: IGs, C: Kelampayoside A, D: Flavonoids, of *Strychnos cocculoides* pulp. Data are expressed as µg/ g of dry weight ± standard deviation with n = 3.

4.3.5 Multivariate data analysis

In figure 4.4 heat map exploratory data analysis tool was outlined to simultaneously analyze variables and sample clustering in a synthetic way. To examine the multivariate distances between the compounds, the values were scaled and a hierarchical cluster based on Ward's method was performed. The cluster analysis quantified the degree of similarity between the polyphenols as well as the four treatments, respectively, by calculating the distance between the possible pairs of molecules and samples (Ward, 1963). The resulting dendrogram highlighted that the closer the polyphenols are to each other, the smaller the differences in their concentration according to the treatment performed. The hierarchical cluster grouped the treatments into two clusters T1 and T2 corresponding to the group with and without pectinase, while the polyphenols were clustered into two principal groups (P1 and P2). Cluster P1 included esterified compounds as caffeoylquinic acid isomers, dicaffeoylquinic acid isomers, rutin and 2-hydroxy-3- *O*-*β*-D -glucopyranosyl benzoic acid. These compounds were efficiently extracted in non- pectinase treatments, while the presence of pectinase caused degradation of the ester linkages resulting in overall lower concentration of the initially characterized compounds. Interestingly, the hot water further degraded rutin and caffeoylquinic acid isomers, while 2-hydroxy-3- *O*-*β*-D -glucopyranosyl benzoic acid and dicaffeoylquinic acid isomers were scarcely influenced by temperature. Cluster P2 was grouped into three subgroups: (P2C) IGs and kelampayoside; (P2B) glycosides and morroniside (P2A) flavonoid aglycones and caffeic acid. The last two groups P2B and P2C defined the molecules positively influenced by the presence of pectinase. The enzyme was able to promote the extraction of kaempferol , quercetin, caffeic acid and the glycosides. On the contrary, the treatment without pectinase at low temperature was not sufficient to obtain a good recovery of kaempferol glucoside, naringenin glucoside and quercetin glucoside. A well-defined behavior was outlined also for IGs: the best performances were obtained at higher temperature without pectinase with the exception of morroniside that was efficiently extracted with cold pectinase and for this reason it was very close to glycosides.

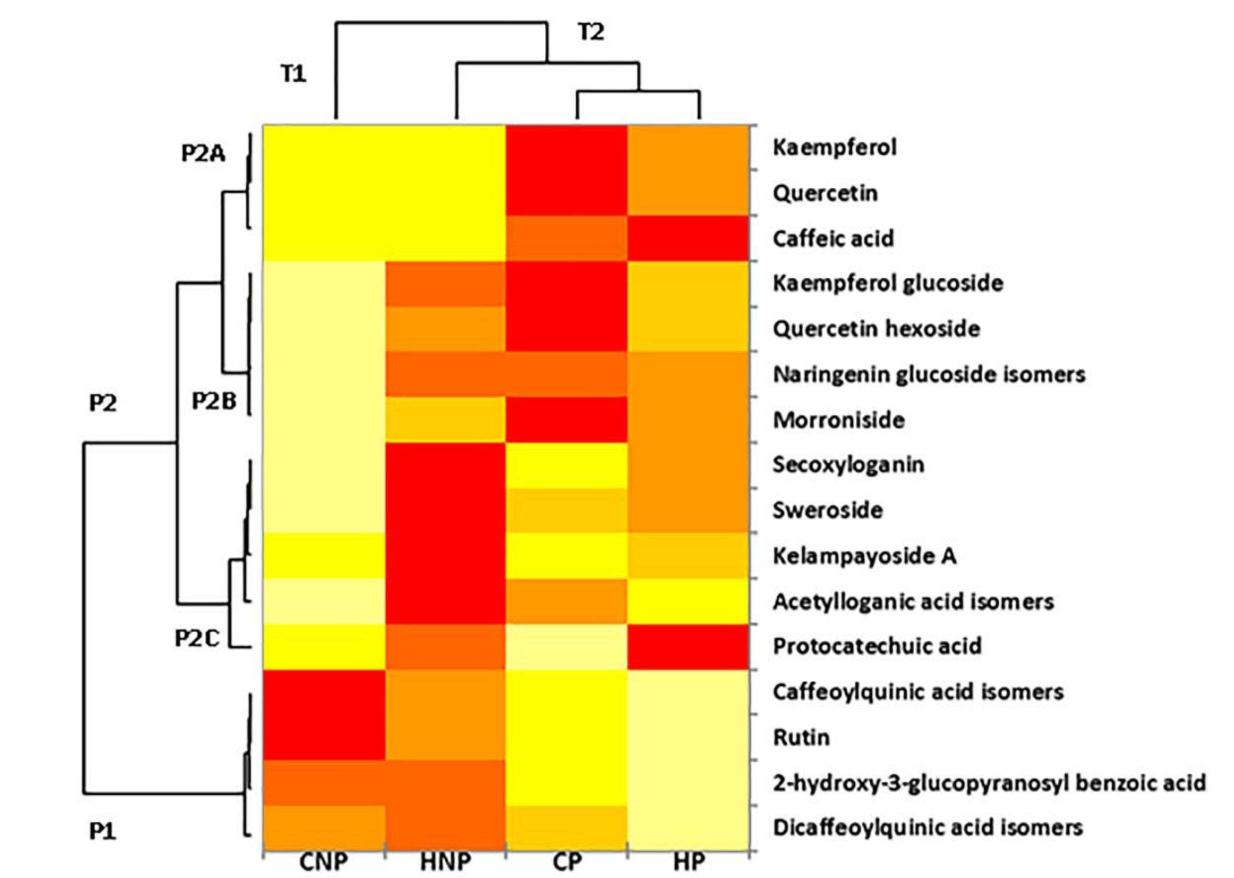

Figure 4.4 Heatmap calculated from quantitative data of polyphenol concentrations in the four different treatments (CNP, HNP, CP and HP). The colour scale moves from yellow to red with increased content. The clusters P1, P2, T1 and T2 refer to the polyphenol classes and to the technological treatments, respectively. CNP: Cold water extraction without pectinase, HNP: Hot water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase

The cluster behaviour was used for the Fancy Tiles, as highlighted in figure 4.5. This approach resumed in a spectrum inverted chromatic scale the main quantitative differences among the four treatments achieve an immediate snapshot of the effects of temperature and pectinase on the concentration of polyphenols. The use of FancyTiles combined with the quantification of the target analytes simplified the visualization of the data obtained outlining the key differences among the four treatments. Despite the use of logarithmic scale, the quantitative tiles were already shown by previous authors for amino acids in milk and tea seed oils combining the simplified outputs with the mass spectrometry quantification (Troise et al., 2016; Wang et al., 2017). Nine compounds were divided into three different categories (P2A, P2C and P1) according to the hierarchical clustering in figure 4.4 and for each treatment a specific tile was created by using fixed ranges. For the aglycones (P2A) the colour moved to blue to yellow and green respectively in presence of the enzymes for caffeic acid and quercetin, respectively, while kaempferol remained close to blue colour. The presence of pectinase influenced the hydrolysis of esterified compounds group P1 that moved from red/orange tonality to yellow green. Regarding group P2C the presence of higher temperature without enzyme promoted the efficient extraction of secoxyloganin and sweroside that showed red and yellow tiles, from the orange and green tiles in the other two treatments.

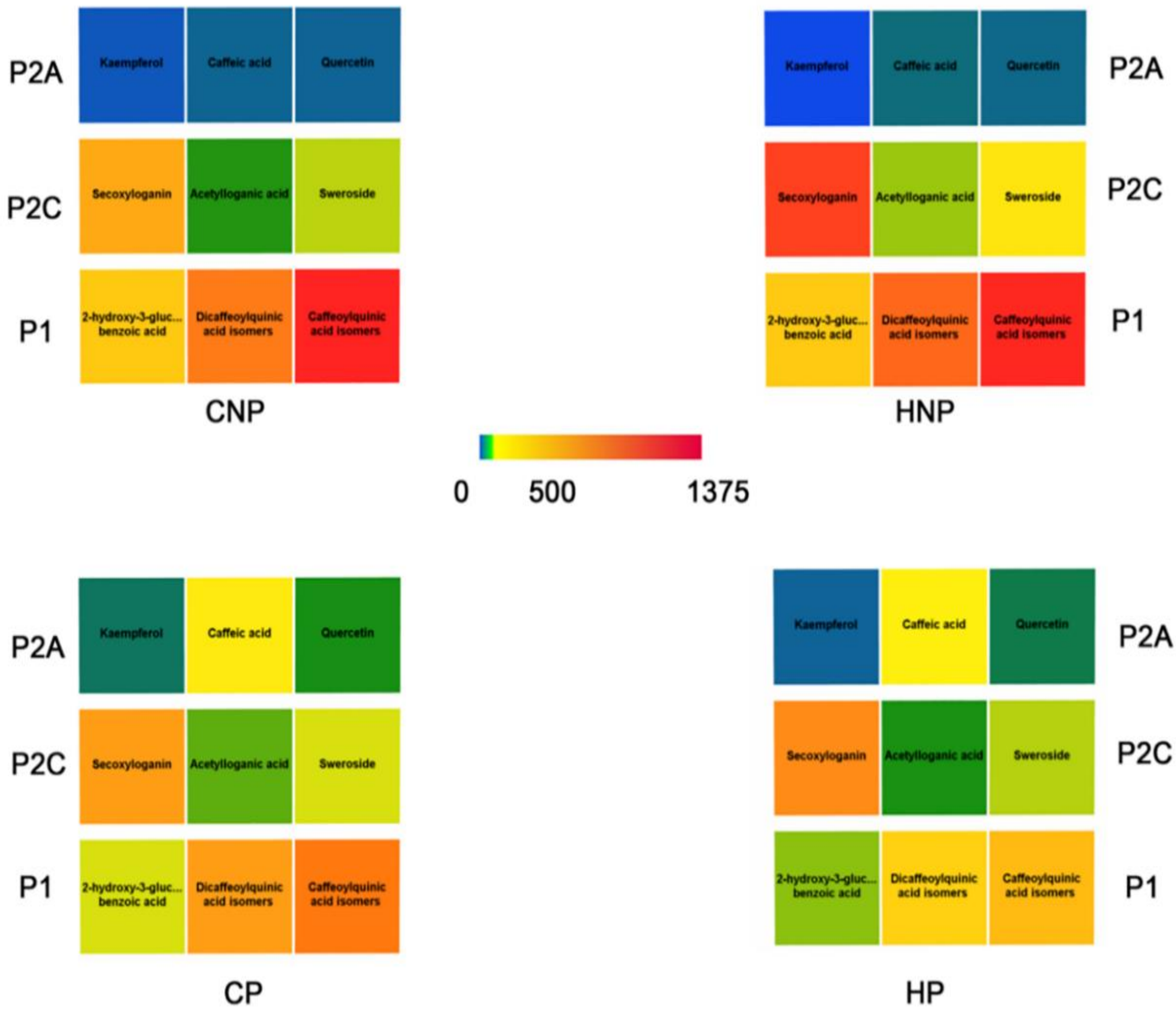

Figure 4.5 FancyTiles schema for the four extraction methods of *S. cocculoides*. The values of the scale are reported in µg/g DW while P2A, P2C and P1 report the clustering highlighted by the dendrogram in figure 4.4 CNP: Cold water extraction without pectinase, HNP: Hot water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase

4.3.6 Anti-oxidant activity

Results of the antioxidant activity are shown in figure 4.6. Enzymatic treatments had higher anti-oxidant activity (AOA) than the non-enzyme treatments due to the enhanced solubility of bioactive compounds into the matrix. Statistically significant correlation was found between caffeic acid and AOA (*R* = 0.989; p = 0.01) in the different maceration treatments. Positive correlations for quercetin and AOA (*R* = 0.885) and kaempferol and AOA (*R* = 0.802) were also realized. Inverse relationships (negative correlation) for 2-hydroxy-3-*O*-*β*-D-glucopyranosyl-benzoic acid (*R*= - 0.997; p=0.003), rutin (*R*= - 0.975; p= 0.02), dicaffeoylqinic acid isomers (*R* = - 0.85) and caffeoylqunic acid isomers (*R*= - 0.938) with AOA were observed.

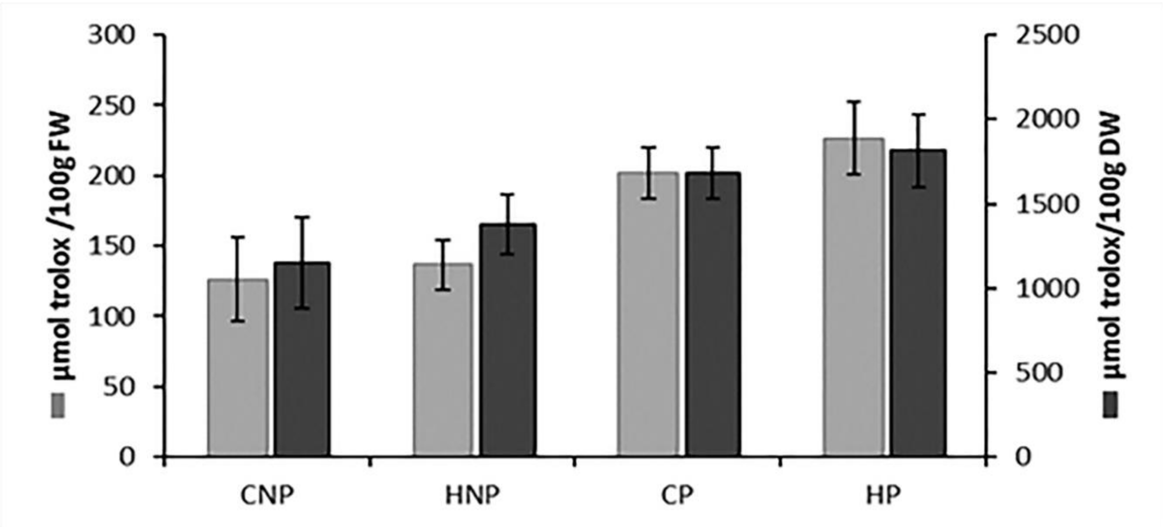

Figure 4.6 TEAC of *S. cocculoides* juice. Data are expressed as µmol trolox/ 100 g ± standard error CNP: Cold water extraction without pectinase, HNP: Hot water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase.

The obtained correlations corroborate similar effects on the phenolic compounds contents of each treatment and the contribution to AOA at different treatments. Caffeic esters at higher concentrations are susceptible to oxidation (Robards et al., 1999), thus a negative correlation with AOA follows as was expected at HP. Low AOA by CNP was probably caused by release and action of cytoplasmic polyphenol oxidases (PPO) responsible for enzymatic oxidation of phenolic compounds (Lima et al., 2015; Robards et al., 1999). Compounds not detected by MS/ MS have been reported when treatments at high temperatures and exposure times form antioxidant active nonphenolic compounds and phenol derivative compounds of higher AOA than their parent phenolic compounds, (Ferracane et al., 2008; Ioannou et al., 2012; Vergara-Salinas et al., 2012). Additionally the radical scavenging capacity test is also not only sensitive to phenolic compounds but also other compounds such as organic acids, reducing sugars and other synergistic effects between phenolics (Li et al., 2013). From the current findings the presence of phenolic compounds such as quercetin and kaempferol that were relatively stable at hot enzyme extractions and their strong positive correlation with AOA, would warrant these compounds as contributory compounds to the increased AOA at high temperatures. However, we cannot attribute AOA wholly to a single or class of phenolic compounds we thus propose investigation on the synergistic effect with other different antioxidants existing in *S. cocculoides* pulp.

4.4 Conclusion and recommendations

Overall, the pectinase treatments have significant effect on increased pulp yield, physicochemical, phenolic compounds (kaempferol, quercetin and their glycosides, caffeic acid, protocatechuic acid, IGs), and AOA of *S. cocculoides*. Enzymes were able to macerate plant cell wall and detach pulp from the outer endocarp of the fruit. Therefore, results indicates pectinase treatments can be used as a valuable tool to increase pulp yield together with reduced waste from the view point of consumer utilization and ecology while giving products of higher physicochemical and nutritional quality. It is to the best of our knowledge that this is the first time where a thorough characterisation of phenolic compounds of ripe *S. cocculoides* was conducted and useful information between treatments conducted obtained by FancyTile approach. The phenolic acids, flavonoids and IGs reported in *S. cocculoides* provide pharmacological properties: anti-inflammation, anti-cancer, antiviral and antimicrobial properties that benefit human health (Bentes & Mercadante, 2014; Whitehead et al., 2016), improve shelf life and safety of food products from which they are processed. The effect of treatments can thus provide basis for improved standardized processing techniques and provides an indication of natural sources of physiologically valuable compounds that can attract interest for functional food exploration. The current findings also aim at recommendation on increased utilization and value addition of *S. cocculoides* pulp in use as a starting ingredient for products consumed in localities where the fruit proliferates for overall food security. Theoretically, enzymatic treatments make a better choice for value addition due to the pulp yield, physiochemical quality, phenolic compounds and AOA. However, considerations have to be made to suite consumer preference, taste and other intrinsic features such as endogenous enzymes and pectin content that could interfere with the outcome of enzymatic treatments still warrants further experimental work.

Acknowledgements

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Chapter 5

Monkey orange fruit juice improves the nutritional quality of a maize-based diet

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Abstract

Addition of fruit juice from indigenous species to cereal-based staple foods can improve the nutritional quality of the nutriments such as Fe and Zn. This paper illustrates this for the use of juice from *Strychnos cocculoides* (monkey orange) in a maize-based porridge. Monkey orange juice is traditionally used to supplement maize porridge - a staple breakfast cereal especially for vulnerable groups. Monkey orange fruits contain high amounts of micronutrients and phenolic compounds and are widely distributed throughout sub-Saharan Africa. The valuable components can be efficiently extracted by traditional and pectinase maceration techniques. The bioaccessibility of minerals and main phenolic compounds in maize porridge (5 g maize meal) supplemented by monkey orange juice (100 ml) were assessed after *in-vitro* digestion together with the kinetics of starch degradation. Caffeic and protocatechuic acids exceeded 100 %, and chlorogenic acid 81 % bioaccessibility after simulated intestinal digestion. Rutin was undetected after the simulated intestinal phase due to precipitation in the pellet. *In-vitro* bioaccessibility of minerals ranged from 12 – 62 % in monkey orange enriched porridge. A 50-70% decrease of starch hydrolysis was observed at the end of the simulated intestinal digestion of monkey orange maize porridge confirming the known potential of phenolic compounds to decrease the glycaemic index of starch-rich foods. Consequently monkey orange juice appeared a suitable ingredient to enrich staple maize porridge thanks to its micronutrients and health benefit potential. Similar relationships of other fruits and starchy foods warrant study as a means to improve the nutritional quality of the diets of malnourished populations.

Keywords: *S. cocculoides*, bioaccessibility, maize porridge, glycaemic index, phenolic compounds, minerals, *in-vitro* solubility

5.1 Introduction

Sub-Saharan Africa experiences food insecurity and the multiple burden of malnutrition: undernutrition, micronutrient deficiencies, child and adult obesity (FAO, 2017b). In that region several fruit species have potential for alleviation of these deficiencies due to their high nutrient content. Though abundant they remain underutilized (Akinnifesi et al., 2006). Among these is *Strychnos cocculoides* (monkey orange), a fruit that is highly appreciated because of its perceived health benefits, taste and resistance to drought (Saka et al., 2004). Monkey orange pulp has a typical sweet- sour flavour (Amarteifio & Mosase, 2006), which contributes to consumer acceptance. Moreover, the fruit has plenty of fibre (4 g/ 100 g) and a high micronutrient content: Fe 70 - 140 mg / 100 g, (Arnold et al., 1985; Malaisse & Parent, 1985), and vitamin C 34 mg / 100g content (Arnold et al., 1985). Micronutrients have physiological importance to human health for their role in growth, bone formation, enzyme activity and energy metabolism, among several others (Martínez-Ballesta et al., 2010; Pereira et al., 2016). Previous studies have identified phenolic compounds in monkey orange fruit pulp, which are effectively extracted by maceration with pectinase enzymes (Ngadze et al., 2018). The phenolic compounds identified belong to phenolic acid, flavonoid, iridoid and phenolic apioglucoside fractions. Among these secondary metabolites chlorogenic acid, caffeic acid, rutin and secoxyloganin are present in high concentrations. Fruit and vegetable secondary metabolite compounds are linked to health benefits such as reduced risk of non-communicable chronic diseases: cardiovascular, neurodegenerative diseases and certain cancers (Egea et al., 2010). Iridoid glucosides have wide distribution in medicinal plants and fruits such as noni (*Morinda citrifolia*), olive (*Olea europaea*), cornelian cherry (*Cornus mas*) and have been associated with biological activities such as reduction of postprandial blood sugar, inhibition of advanced glycation end products, improvement of short term memory capacity, anti - inflammatory activity, and anti-fungal growth in fruits (Bentes & Mercadante, 2014; West et al., 2016; Whitehead et al., 2016).

Traditionally monkey orange juice and pulp are used as ingredients for maize porridge, the most common processed food product of this fruit, consumed mostly by children and other vulnerable adults (Ngadze et al., 2017b). Given the importance of the health beneficial compounds associated with monkey orange fruit, a mixed food matrix such as this possibly has added nutritional value and health benefits. However, when maize porridge is consumed, potential antagonistic effects to human health also exist, as maize contains rapidly digested starch with a characteristically high glycaemic index (GI) (Warren et al., 2015), which causes a sharp increase of the postprandial blood glucose concentration linked to obesity. In *in-vitro* and *in-vivo* digestion studies phenolic compounds have been found to reduce starch breakdown in carbohydrate-rich foods such as white bread (Coe et al., 2013). On the other hand, the presence of micronutrients and bioactive compounds in food is not in itself enough to ensure their bioavailability. The absorption efficacy depends on many factors such as the structure of the food matrix, the stability, the intra / inter-actions among the food components and the modifications of nutrients by intestinal and hepatic enzymes during digestion (Cilla et al., 2009; Kamiloglu et al., 2015; Manach et al., 2004). Currently, information is lacking on the biological function of the nutrients and compounds of the most important and consumed product of monkey orange: the maize porridge enhanced with fruit juice.

The objective of this study was therefore to determine the digestion of phenolic compounds, minerals and starch of maize porridge enhanced with *S. cocculoides* juice. For this, monkey orange enhanced porridge and maize porridge made with water as control were digested *in-vitro* and the effect on bioaccessibility of compounds was determined. Additionally, since different juice extraction techniques may give different effects on colour, texture and flavour, sensory tests were conducted to ensure compatibility of research findings with consumer preference and acceptance. The data obtained are anticipated to support the value addition and improved use of *S. cocculoides* as a functional ingredient in food formulations.

5.2 Materials and Methods

5.2.1 Standards and reagents

Caffeic acid (CA), chlorogenic acid, protocatechuic acid (PCA), rutin and naringenin standards were purchased from SigmaAldrich (Germany). Total starch and D-glucose (glucose oxidase/peroxidase; GOPOD) assay kits were purchased from Megazyme

(Ireland). Commercial pectinase enzymes from *Aspergillus niger*, porcine pepsin, bile, pancreatin and amyloglucosidase were obtained from SigmaAldrich (Germany).

5.2.2 Sample preparation

Hulled, finely milled white maize meal (as is typically used in maize porridge preparation) was used. Porridge preparation is presented in figure 5.1. Juice preparation was as described by Ngadze et al.,2018. The final product was a homogenous slurry, free from lumps. Samples were kept in a water bath at 37 ºC and immediately used for further analysis to prevent starch retrogradation.

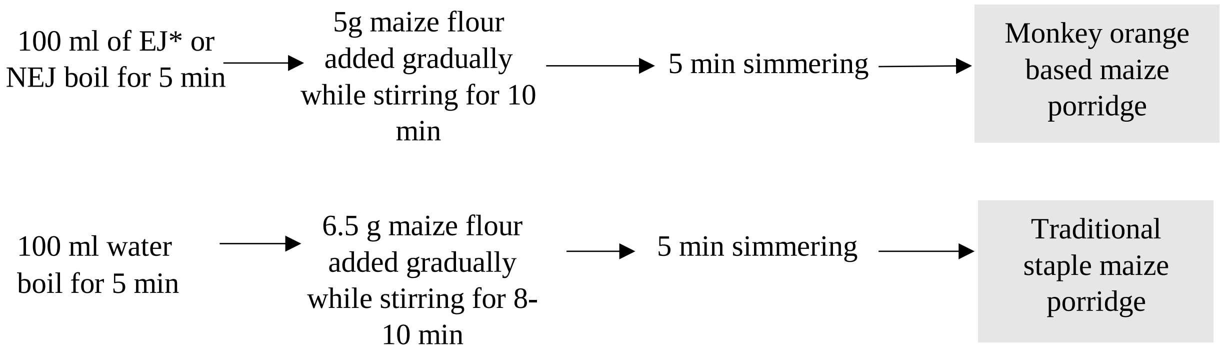

Figure 5.1 Scheme of maize porridge preparation
* EJ: enzyme macerated *S. cocculoides* juice; NEJ: traditional macerated *S. cocculoides* juice.

5.2.3 Organic acids determination

Samples were centrifuged at 10,000 *g* for 10 min, after which they were diluted with 0.1 M KH_2PO_4 (pH 2.5) eluent. Samples were further filtered through a 0.2 µm filter. Individual organic acids were separated by HLPC (Thermo Scientific Dionex Ultimate 3000) equipped with a photodiode array detector. Separation was carried out on a Prevail Organic acids column (4.6 x 250 mm). Detected peaks were scanned between 190-600 nm. Flow rate was set at 1.0 mL/min, run time 25 min. Sample injection volume was set at 20 µL. Individual organic acids were identified based on retention time and comparison of UV/visible spectra of external standards and quantified from calibration curves with standard solutions.

5.2.4 Simulated in-vitro gastro intestinal digestion

Simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used to carry out a three phase simulated *in-vitro* digestion according to Minekus et al. (2014). The whole *in-vitro* experiment from oral, gastric to intestinal phase took approximately 4 h (fig. 5.2). Digestion experiments were conducted inside an incubator to keep the temperature constant. Samples were mixed with the use of a laboratory rotator at constant speed (20 rpm). The subsequent analysis of phenolic compounds, mineral analysis and starch were carried out on samples collected at the end of incubation for both simulated gastric phase (SGP) and simulated intestinal phase (SIP). Digested starch analysis was determined at different time points throughout digestion. The SGP and SIP were stopped by snap freezing the samples in liquid nitrogen. Samples were stored at -20 ºC until further analysis. A blank consisting of 5 ml distilled water was digested as in figure 5.2 (for mineral content correction). Digestion was performed in triplicate.

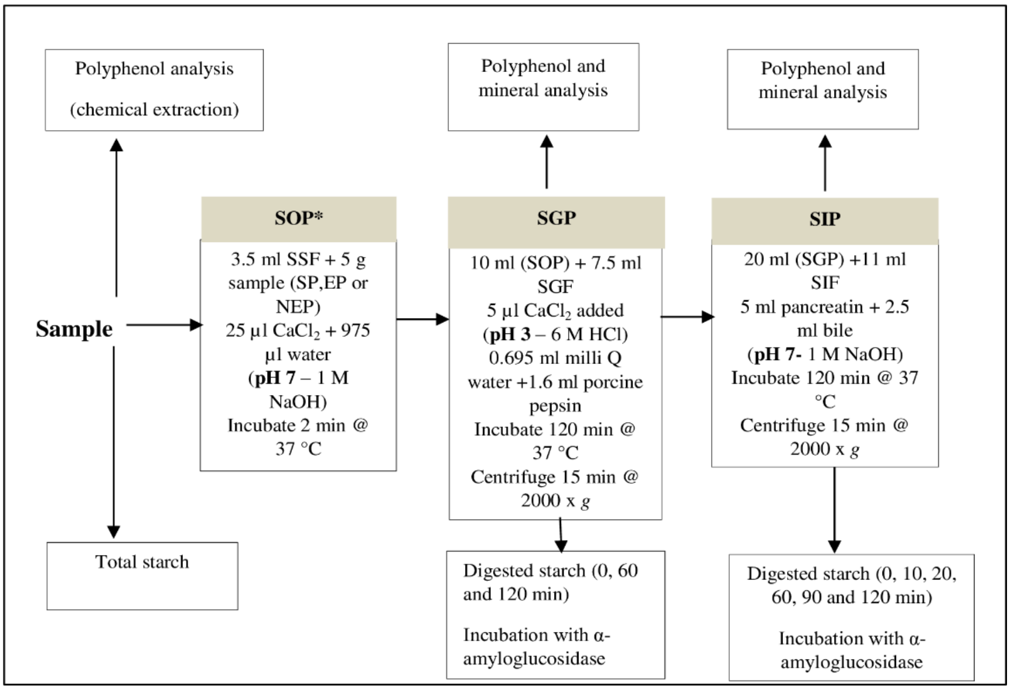

Figure 5.2 Simulated *in-vitro* digestion of SP: staple maize porridge in water; EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice and NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice * SOP: simulated oral phase, SGP: simulated gastric phase, SIP: simulated intestinal phase according to Minekus et al. (2014)

5.4.1 In-vitro digestion of traditional and monkey orange maize porridge starch

Total starch of undigested samples was first determined by the amyloglucosidase / α-amylase method K-TSTA-50A/K-TSTA-100A 02/17. To measure the degree of starch hydrolysis by *in-vitro* digestion, glucose release at different time points was measured. Samples taken at each time point were further incubated with α-amyloglucosidase for complete hydrolysis as described by Rovalino-Córdova et al. (2018). The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times during *in-vitro* digestion.

5.2.5 Analysis of phenolic compounds

Undigested samples were dissolved in methanol in a 1:1 ratio. The suspension was incubated for 30 min at room temperature while vortexing with a Heidolph multi tube mixer at 120 rpm. Ultrasonic extraction was applied to the mixed suspension for 30 min at 35 °C, then centrifuged for 10 min at 4000 rpm at 4 °C. The supernatant was filtered with 0.20 µM filters, after which the filtrate was used for HPLC analysis. Individual phenolic compounds were separated by HPLC (Thermo Scientific Dionex Ultimate 3000) equipped with a photodiode array detector. Separation was carried out on a Varian Polaris 5 C18-A column (4.6x150 mm). Mobile phase (A) was aqueous trifluoroacetic acid (pH 2.5) and mobile phase (B) acetonitrile. Flow rate was set at 1.0 mL/min and gradient elution was linearly programmed as follows: 0 to 20 min 42 % B, followed by holding for 5 min, at 26 to 31 min the mobile phase B was set back from 42 % to 0 %. Sample injection volume was set at 20 µL. Detected peaks were scanned between 220 and 550 nm. Individual phenolic compounds were identified based on their retention time and comparison of UV/visible spectra of external standards. Concentrations were quantified from calibration curves of external standards. Bioaccessibility of phenolic compounds was determined as the ratio of the phenolic compound content in the supernatant as compared to the non-digested sample.

5.2.6 Mineral determination

Fruit, maize porridge in water – staple maize porridge (SP), maize porridge enhanced with enzyme extracted juice (EP) and maize porridge enhanced with traditionally extracted juice (NEP) were digested using hydrofluoric acid and concentrated nitric acid. Nitrous vapors were removed by adding hydrogen peroxide (SWV E1014). Concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn were determined by an inductively coupled plasma–optical emission spectrometer (ICP-OES): Thermo, iCAP 6500 duo according to SWV E1362. For all minerals, samples were corrected for added reagents and water by subtracting each mineral content of the blank from the supernatant. All measurements were performed in duplicate.

5.2.7 Consumer preference and acceptance of porridge enriched with monkey orange juice

Eighty-eight regular consumers of porridge enhanced with monkey orange from Lower Gweru district in Zimbabwe consented to participate in sensory tests. Oral instructions on testing and responding were first given to the participants in their local language before the tests commenced. The porridge was cooked in the regular aluminum pots on wood fuel, and kept at approximately 38 – 40 ºC during serving. About 15 g of sample were served in Styrofoam cups with plastic spoons. Porridge serving was done at mid-morning, the time that monkey orange porridge / regular staple maize porridge is consumed in this community. Water was provided to refresh the palate between sample tasting.

Consumer preference

The paired comparison test was used as an affective test to measure relative preference to EP and NEP porridge. The panelists were presented with two coded samples, in a randomized order (for each panelist). One sample (EP) porridge was presented to the first half of panelists and the other (NEP) was presented to the second half of panelists first. Consumers were asked to provide a written description of taste on their choice. Results were based on the minimum number of agreeing judgements necessary to establish significance ($p<0.05$).

Triangle taste test

Both EP and NEP were subjected to a triangle taste test to determine if the maceration treatment resulted in a detectable difference between the two products. Each panelist received three coded samples and had to identify one odd sample. One half of the panelists tasted two samples of NEP and one of EP while the other half tested one sample of NEP and two samples of EP. The tasting of the porridge by the panelists was randomized and the order of tasting (left to right) was indicated on the score sheet. Results were based on the minimum number of correct responses required to give correct judgement ($p<0.05$).

5.2.8 Statistical analysis

Data was presented as means ± standard deviations of triplicate observations and evaluated by one-way analysis of variance (ANOVA) using SPSS 23.0 Statistics (SPSS Inc., Chicago, IL USA). Turkey post hoc analyses were performed to assess the significance of difference at 95% significance level ($p<0.05$). For sensory analysis, tables giving the number of judgements required for significance for preference and triangle tests were used as prepared by Roessler et al. (1978).

5.3 Results and discussion

5.3.1 Organic acids of maize porridges

The organic acid contents in table 5.1 show monkey orange porridges to contain higher amounts of organic acids and to have lower pH than staple maize porridge. Malic acid was the predominant organic acid in *S. cocculoides* juice and subsequently in its porridges, EP and NEP. Besides malic acid, other acids, namely fumaric acid, citric acid and formic acid, were found. Fumaric acid was not detected in EP. NEP had higher contents of all the acids and a correspondingly lower pH than EP and SP, whereas the higher pH in SP corresponded to the lower total organic acid content. Sitrit et al. (2003) previously identified citric acid and malic acid in *S. spinosa* species. As far as we know, the organic acids were not previously reported in *S. cocculoides* species.

Table 5.1 Organic acid content of maize porridge samples

	Organic acids (mg/100 g FW)				
	Fumaric acid	Citric acid	Malic acid	Formic acid	pH
SP**	8.1 ± 0.9	0.05 ± 0.00	nd*	0.03 ± 0.02	6.6 ± 0.0
EP	nd	0.73 ± 0.05	49.4 ± 3.3	0.05 ± 0.00	4.0 ± 0.1
NEP	23.1 ± 7.7	0.90 ± 0.0	54.7 ± 3.3	0.10 ± 0.0	3.6 ± 0.0

*nd=not detected
** SP: staple maize porridge in water; EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice; NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice. For each measurement means ± standard deviation (n=3).

5.3.2 In-vitro bioaccessibility of phenolic compounds

The phenolic compounds with the highest concentrations identified in previous studies were analyzed during *in vitro* digestion, i.e. SGP and SIP, and compared to the contents present before digestion assessed by methanol extraction in maize porridge. Bioaccessibility of digested compounds was expressed as a percentage of the total amount of each compound extracted from the undigested sample. The concentrations of chlorogenic acid, caffeic acid, protocatechuic acid and rutin from monkey orange enhanced maize porridge at different digestion phases is presented in figure 5.3.

The studied compounds were not found in undigested and digested SP, thus phenolic compounds identified in monkey orange maize porridge are assumed to be solely from monkey orange fruit. There was no significant difference between both samples with regard to chlorogenic and caffeic acid at all digestive phases. No protocatechuic acid was detected at the SIP of EP. Contents at SIP were higher and significantly different from BD and SGP. There was no significant difference of rutin BD for EP and NEP. A significant difference for rutin was realized at SGP with EP having a higher content and no rutin detected at SIP for both samples. Monkey orange enhanced maize porridge samples showed a bioaccessibility of phenolic compounds exceeding 100 %, with the exception of chlorogenic acid at SGP and SIP digestion of EP (81 and 84 % respectively) (table 5.2).

Chlorogenic acid was the major phenolic compound found in monkey orange porridges. This was expected as in previous studies we found that chlorogenic acid (an ester of caffeic acid and quinic acid) accounts for 65 % of the phenolic compounds identified in monkey orange juice (Ngadze et al,. 2018). A small loss of chlorogenic acid may indicate its good digestion and recovery in the small intestines. The hydrolysis of chlorogenic to caffeic acid is also highly likely in our study as the decrease at SGP in EP gave a corresponding increase in caffeic acid at SIP. This is in contrast to other reports that suggest that the only significant mechanism for chlorogenic metabolism is by enzymes of colonic microflora (Scalbert & Williamson, 2000). Our findings are in agreement with studies by Farah et al. (2006) and Kahle et al. (2011) that also showed chlorogenic acid hydrolysis in simulated and human digestive fluids.

A 3-fold increase of protocatechuic acid was observed at the intestinal phase of NEP. Reportedly *p* - hydroxybenzoic acid can be hydroxylated to protocatechuic acid (Heleno et al., 2015). This suggests that the interaction of *p* - hydroxybenzoic with other organic compounds in the food matrix, which includes organic acids, may cause an increase in protocatechuic acid at SIP. In contrast, degradation of protocatechuic acid in the SIP of EP could have been caused by biosynthesis to other derivative compounds not measured in this study. A similar trend in starchy food was reported by Coe et al. (2013), where baobab fruit extract baked into white wheat bread resulted in an increase in total polyphenol content through the gastro intestinal phases. Several other studies on fruit also obtained increased polyphenol contents during *in-vitro* digestion: Bermúdez-Soto et al. (2007) in chokeberry juice, grape (Tagliazucchi et al., 2010) and in a fruit juice-milk blend (Rodríguez-Roque et al., 2014).

Rutin was present in low concentrations in the gastro intestinal phases and was no longer detected after SIP. The low concentrations were probably due to the low amount of rutin in monkey orange juice (0.05 µg/g) and enriched porridge (0.02 µg/g). Though rutin by nature is stable at high pH, it is insoluble in aqueous solutions (Friedman & Jürgens, 2000; Zhu, 2015) and precipitates into the pellet during SIP to reach the colon for degradation by colonic flora (Scalbert & Williamson, 2000). The amount that was obtained in the pellet (0.016 µg/g), cannot account for the diminished content from the SIP. The probable explanation for our findings is the interaction of rutin with other dietary constituents rendering them non – extractable by the formation of irreversible complexes since flavonoid glycosides do not form corresponding aglycones during gastric digestion (Manach et al., 2004).

There were no notable differences in chlorogenic acid, caffeic acid and protocatechuic acid BD and SGP showing that the process did not alter the stability of the compounds in both EP and NEP. These results are in agreement with Bermúdez-Soto et al. (2007) and Tagliazucchi et al. (2010) where compound stability was demonstrated during digestion. In our previous study esterified phenolic compounds represented a major fraction. Esterified compounds, after consumption and metabolism, are likely to be bio-available and possibly bio-active in the gastrointestinal tract (Russell et al., 2009). From the findings it appears that the increase of free phenolic compounds during digestion could be attributed to the hydrolysis of polyphenol glycosides or their release from other dietary compounds by intestinal enzymes or endogenous glycosidase. The extent of the release of free phenolic compounds also depends on the level of polymerization, type of sugar molecule attached, hydrophobicity and extent of binding with the food matrix (Manach et al., 2005; Scalbert & Williamson, 2000). The changes that occurred, showed the manifold changes that occur during digestion that have an impact on bioaccessibility and possible bioavailability of phenolic compounds. Though some of the polyphenol aglycones are conjugated in the liver, 48 % are reported to be absorbed in the small intestines (D’Archivio et al., 2010; Saura-Calixto et al., 2007). Thus the results show that substantial amounts of phenolic compounds from the consumed product are bioaccessible for absorption, even though we cannot exclude the static experimental conditions used.

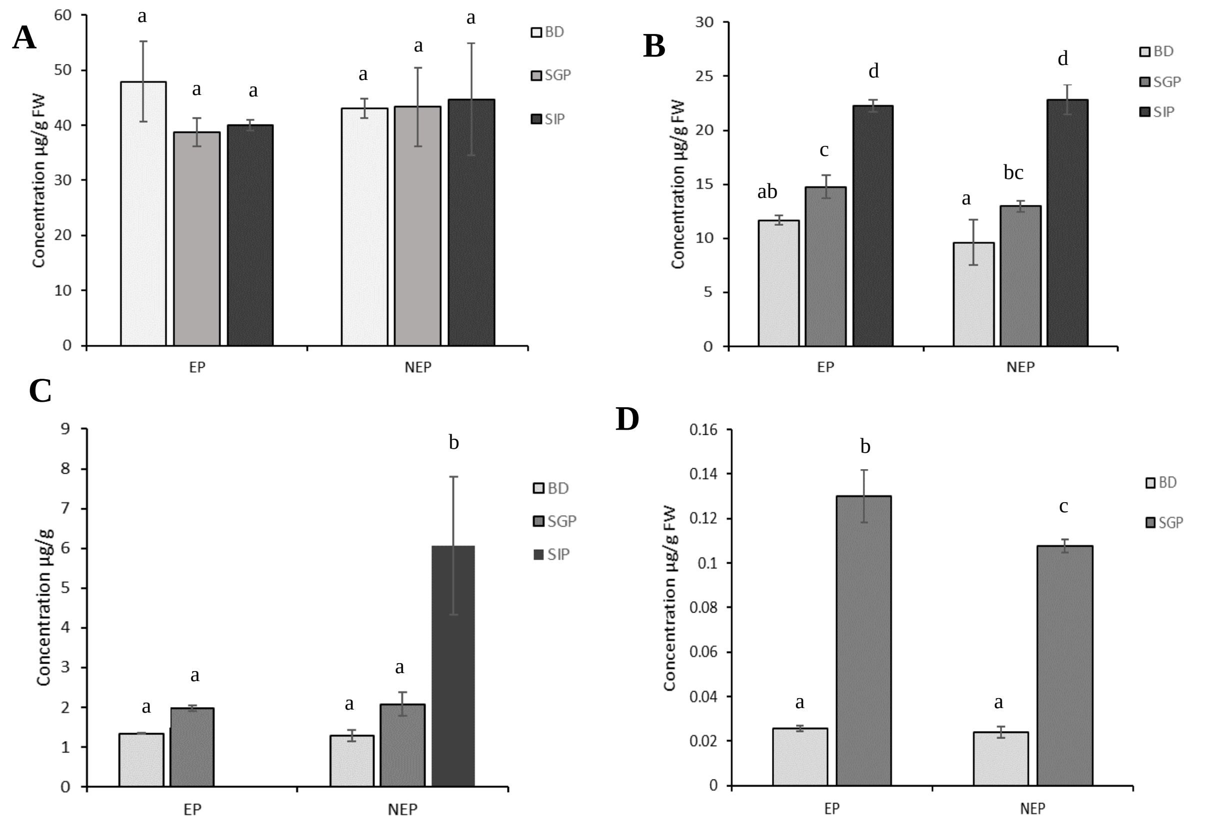

Fig 5.3 Comparison of concentrations of A. chlorogenic acid, B. caffeic acid, C. protocatechuic acid D. rutin in maize porridge at the end of different digestion stages

EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice; NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice; BD: before digestion; SGP: simulated gastric phase; SIP: simulated intestinal phase; Data represents average values ± standard deviation of three independent samples. Bars with different letters for each phenolic compound indicate statistically significantly differences ($p < 0.05$).

Table 5.2 Phenolic compounds bioaccessibility of *S. cocculoides* enriched porridge

	Bioaccessibility (%)			
	Chlorogenic acid	Caffeic acid	Protocatechuic acid	Rutin
NEP (SGP)	101	135	162	447
NEP (SIP)	103	238	492	nd
EP (SGP)	81*	126	148	506
EP (SIP)	84*	190	nd	nd

NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice; EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice: SGP, is simulated gastric phase; SIP, is simulated intestinal phase and nd: not determined.* loss in phenolic compound.

5.3.3 In-vitro starch digestibility

Starch hydrolysis during *in-vitro* digestion is presented in figure 5.4. Rate of starch hydrolysis was constant during SGP for all samples and lower for SP (less than 5 % at 120 min) than EP and NEP (both at 15 % at 120 min). As digestion proceeded to SIP, there was a rapid increase in the rate of hydrolysis for all samples during the first 20 min. Interestingly, a significant reduction in starch hydrolysis was observed in EP and NEP samples at the beginning of SIP and at the end of SIP as compared to the control porridge. There was a significant difference ($p<0.05$) at the end of SIP between EP and NEP where the latter had a lower percentage of starch hydrolysis. The observed starch hydrolysis at the start of SGP for all porridge samples could have been due to a prolonged transition from the SOP to SGP during pH adjustment resulting in continuous α-amylase activity. Additionally, phenolic compounds, when added to starch systems, alter the functional properties of starch (Xiao et al., 2013). This, together with the effect of boiling maize meal in excess water/ monkey orange juice at approximately 1:20 w/v, resulted in gelatinization and solubilisation, which increased susceptibility to amylolytic attack (Dartois et al., 2010; Dhital et al., 2016). However, glucose release did not increase during SGP, probably due to inactivation of salivary α-amylase by the low gastric pH (Stuknytė et al., 2014). During digestion of both monkey orange porridges, bioaccessibility of chlorogenic acid, caffeic acid and protocatechuic acid was high. Of these, chlorogenic acid and caffeic acid have been classed as potent inhibitors of porcine pancreatic alpha amylase (PPAA) (Karim et al., 2017). Several researchers have reported phenolic compounds such as chlorogenic acid and caffeic acid to inhibit enzyme activity by reducing the number of binding sites for enzymes to different extents during starch digestion (Kandil et al., 2012; Karim et al., 2017; Oboh et al., 2015; Sun et al., 2016). On the contrary, other phenolic compounds have limited inhibitory activity against PPAA, for example, rutin (a quercetin derivative) because of its stereoscopic spatial structure that limits inhibition at enzyme active sites (Liu et al., 2014). NEP had lower starch hydrolysis than EP, probably because of the additive effect of organic acids and iridoid glucosides, which had a higher starting content in NEJ than EJ (findings from previous research). Modification of starch with organic acids has been found to increase resistant starch levels in maize starch (Kim & Shin, 2011; Wepner et al., 1999), thereby reducing available starch for hydrolysis.

Previous reports have shown iridoid glycosides from products of Japanese cornelian cherry (*Cornus officinalis*) to have α-glucosidase inhibitory effects as efficient as acarbose *in - vitro* and *in – vivo*, which are attributed to reducing postprandial hyperglycaemia (He, 2011). In diabetes induced animal studies, iridoid glucosides attenuated elevation in serum and hepatic glucose through inhibiting diabetic oxidative stress (Park et al., 2011; Yamabe et al., 2007). Thus, iridoid glucosides identified in *S. cocculoides* juice in high amounts from previous studies -if present in the monkey orange enriched maize porridge- may have a contributory effect in the decrease of starch hydrolysis by inhibition of α-amylase / α-glucosidase or by interaction with starch. This hypothesis warrants further research to underpin the findings.

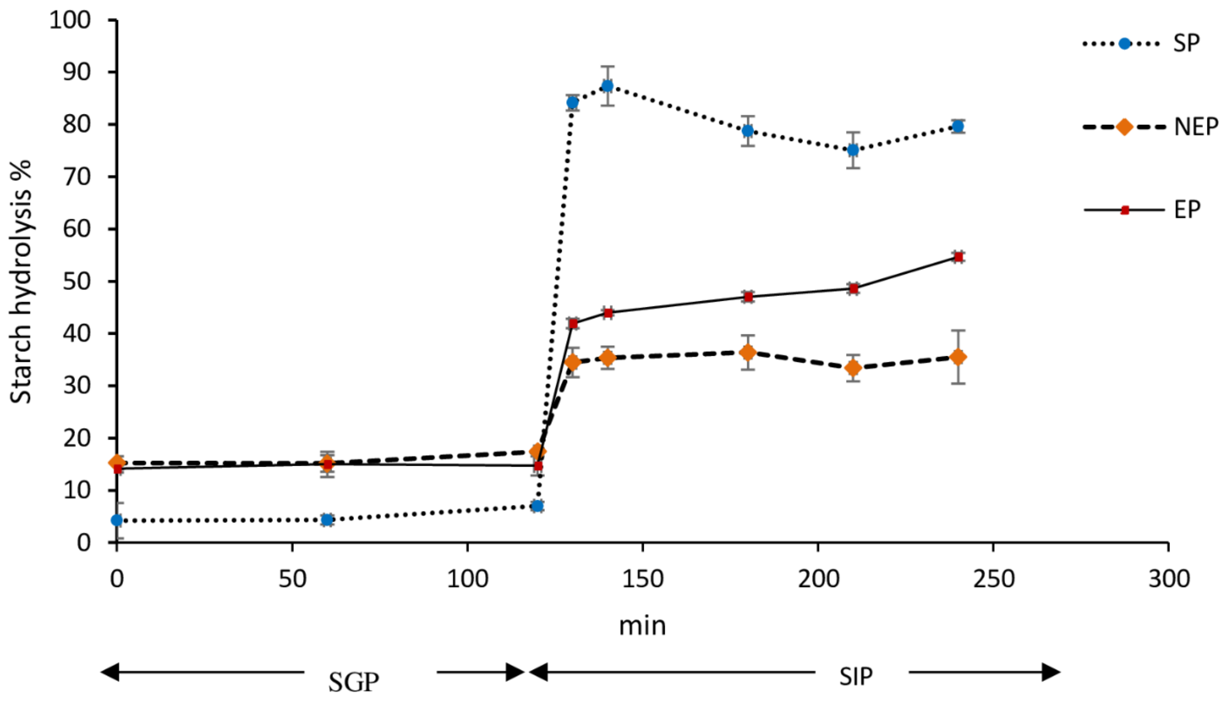

Figure 5.4 Starch digestibility (%) of the maize porridge samples
NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice; EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice; PW: control sample maize porridge made with water.

5.3.4 In-vitro mineral bioaccessibility

In-vitro bioaccessibility of nine minerals in the three maize porridge samples is presented in figure 5.5. SP had significantly ($p<0.05$) higher Fe, Zn, Al, Cu and P contents before digestion as compared to EP and NEP. However, EP and NEP had higher digestibility of the all other minerals except Mn. The higher *in-vitro* bioaccessibility of

Fe in EP and NEP is linked to the acidic conditions of the *S. cocculoides* enhanced matrix due to presence of organic acids and phenolic compounds. Organic acids enhance mineral solubility by optimizing pH conditions for activity of phytase or by the formation of soluble ligands with minerals (Gibson et al., 2006). Zinc digestibility yielded low percentages and was soluble only in the EP sample with a 3 % retention realised, which is consistent with those of de Lima et al. (2014), who found 3.7 % bioaccessibility for Zn in cashew apple. For NEP and SP the bioaccessibility of Zn was below the detection limit, though it was present before digestion in both samples. Low bioaccessibility can be attributed to the combined effect of anti-nutrients and retention of (NEP and SP) Zn in the undigested pellet. Co-precipitation of Zn with Ca as a Zn-Ca-phytate complex occurs in food matrices with a high Ca content (Sandström, 2001). In the study high Ca contents were realised after digestion in the samples, thereby probably reducing Zn digestibility. On the other hand, Zn bioaccessibility for fruit pulp increased from below detection to 0.7 mg/ 100 g (results not shown) after digestion. This indicates that Zn was released from the food matrix under digestive conditions. The limitation of a high bioaccessibility of Mn impairs Fe absorption (Sandström, 2001). This may impose a risk of reduced Fe utilization when available in high contents such as in the SP sample. However, the consequences of these mineral interactions will depend on the amount that is released from the food matrix. Though Al has potential toxicity at trace levels, its content after digestion for EP or NEP was below the provisional tolerable weekly intake of 2 mg / kg body weight (Peixoto et al., 2016), thus its content in the monkey orange enhanced maize samples does not present a risk.

Despite the fact that there was no significant difference in the Cu, Fe, Ca, and P contents between SP, EP and NEP after digestion, the bioaccessibility percentage was higher in EP and NEP. Bioaccessibility was determined as a ratio of the initial content, thus when a significant drop in content is realised, the proportion caused a lower calculated solubility percentage. The reduction in mineral content in SP can be a result of an increase in mineral and insoluble compound complexes that remain in the pellet or the chelation with phytic acid. In general the results show that mineral digestion may be affected by the sample matrix, processing factors and mineral availability in the food (Cilla et al., 2009), thus bioaccessibility differs for each mineral and matrix.

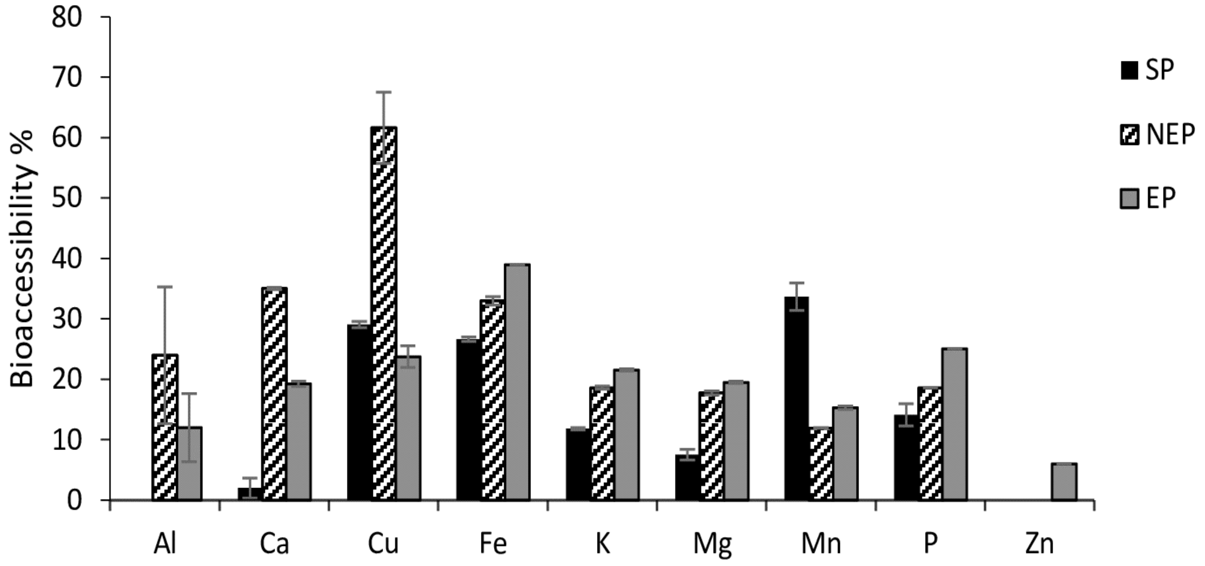

Figure 5.5 Bioaccessibility of individual minerals

SP: staple maize porridge in water; NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice; EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice

5.3.5 Consumer preference and acceptance of monkey orange enriched porridge

Preference tests

Forty-nine of the panelists preferred EP while 39 of the panelists preferred NEP, indicating no significant difference ($p = 0.05$) in liking between the two juices when used in maize porridge preparation. Monkey orange juice/ pulp is commonly used in maize porridge preparation as a substitute for water or milk in order to improve sensorial and nutritional properties. The consumers described differences in taste, flavour and product consistency. This is an important finding as the goal is for the consumer to like the product that gives the most health benefits.

Triangle tests

The triangle tests determined if a significant difference existed between maize porridge made from traditional and enzyme macerated juice. From the results 65 assessors correctly identified the ‘odd’ sample, indicating a significant difference ($p = 0.05$)

between the two types of porridges enhanced with the different juices. The difference observed by the panelists was largely due to the maceration method. Traditional maceration does not deploy enzymes. Therefore some monkey orange components, such as sugars and acids, were not released resulting in perceived flavour differences. Enzymatic maceration increased the recovery of soluble fruit components that have an effect on sensory quality reducing sugars and colour (Sharma et al., 2015).

Consumer description of product choice

Taste descriptions of the maize porridge samples that were given as the reason for product choice are presented in figure 5.6. Panelists described the product they preferred, and for consistency they assessed which product was more viscous than the other. The EP was described by panelists as more sour and with a more pronounced characteristic monkey orange flavour. This is contrary to the expectation that NEP would present a more intense sourness arising from the high molecular weight organic acids, such as fumaric acid, present in NEP next to citric and malic acid. The molecular weight, chemical structure and polarity of acids have been reported as important factors in perceived sourness intensity (Hartwig & McDaniel, 1995). Since this is not the case, the perceived sour taste described by the panelists for EP is attributed to the higher content of free phenolic compounds, which also contribute to sourness, astringency and bitterness of a food product (Sharma et al., 2015). The results also show that the volatile compounds are unaffected by either maceration treatments as both porridges were described as having a specific monkey orange flavour. The sugars from the fruit were released by tissue breakdown by the enzymatic maceration treatments, hence giving EP a sweeter taste. Consumers also described the EP as having a thinner consistency. Phenolic compounds have been reported to increase viscosity of starch foods such as wheat (Zhu et al., 2008), while in contrast the addition of organic acids to starch, reduced starch viscosity by hydrolyzing starch amylose and amylopectin at low pH (Majzoobi & Beparva, 2014). The net effect of these compounds in the food product has an effect on the quality and as ingredients for a functional food product. These sensory attributes require further investigation as they are particularly important when feeding vulnerable groups such as children, who generally tend to like flavourful and easy to swallow food products.

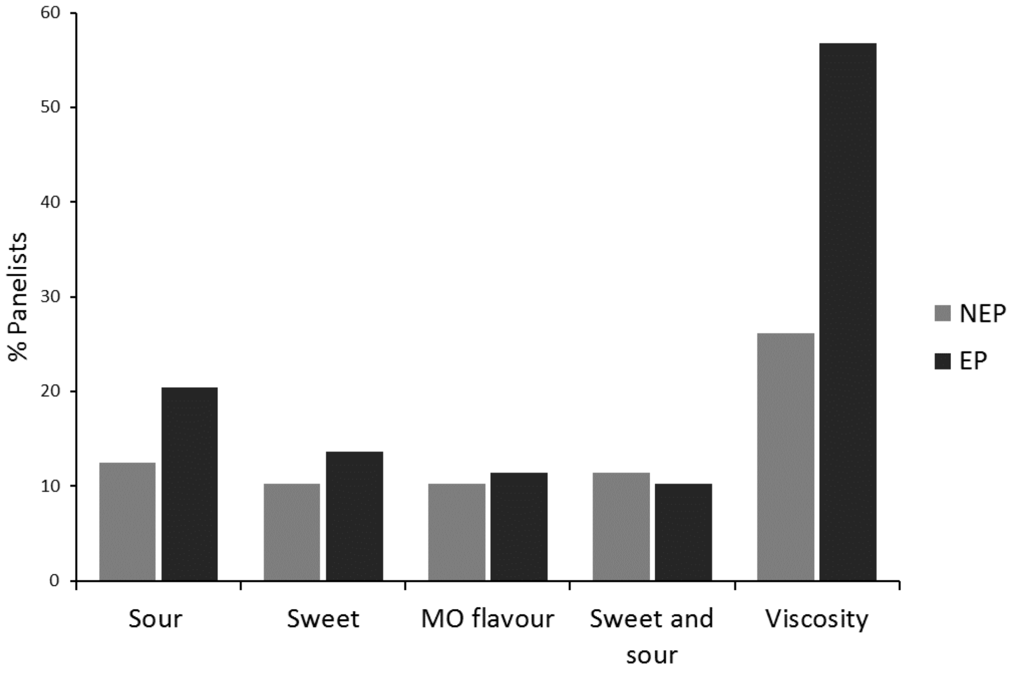

Figure 5.6 Consumer description of *S. cocculoides* enriched maize porridge

NEP: maize porridge enriched with traditional macerated *S. cocculoides* juice; EP: maize porridge enriched with enzyme extracted *S. cocculoides* juice

5.4 Conclusions

This study demonstrates that enhancing maize porridge with monkey orange juice not only enhances the bioactive compounds and mineral bioaccessibility but also reduces starch hydrolysis significantly *in-vitro*. Under SIP conditions, the bioaccessibility of phenolic acids and minerals was improved by the addition of monkey orange juice (regardless of maceration treatment). Overall, bioaccessibility of minerals is improved by the addition of monkey orange juice in maize porridge. These findings are crucial, as minerals are important in human physiological functions and severe deficiencies for some of them have been reported in sub-Saharan countries. Additionally, the study demonstrated that consumers were able to detect a difference in taste between the two porridges but showed no difference in preference between the porridges made with enzyme-treated or traditional macerated juice. As a result, monkey orange porridge can be considered a good option for food products with functional properties by combining health properties of the fruit and the potential to reduce starch digestibility. We recommend to use these findings for the identification of other suitable combinations of indigenous fruit juices and cereal-based staple foods to improve the diets of resource-poor, malnourished communities.

Acknowledgements

We are grateful for the financial support by the Netherlands Fellowship Programme (grant award CF151/2013).

Chapter 6

General discussion

6.1 Introduction

Monkey orange is among the key indigenous fruits consumed during times of sudden socio-economic or environmental changes such as droughts or before the harvest of staple crops when fresh gathered vegetables are not available (Akinnifesi et al., 2002). Monkey oranges have the characteristics of a successful crop with a high nutrient content, consumer preference, good keeping quality and economic returns (National Research Council, 2008; SCUC, 2006b). These are crucial characteristics, especially for the rural communities where the fruit is processed and consumed to contribute to nutrition. When the fruit is processed into a food product, the juice is obtained traditionally by macerating in water - an arduous process, which makes processing unfavourable. Moreover, the pre-treatment step results in limited juice yield and high pulp losses (i.e. that which remains attached to the seed), which is exacerbated by resulting poor quality pulp such that the potential health benefits of the fruit are not realised for the consumer.

The traditional foods made of indigenous forest trees (IFTs), and particularly monkey orange, receive limited attention even when they contribute to the diet of rural households. Monkey orange juice in food production is studied in this thesis with the targeted end product being an enhanced maize porridge. Maize, being the staple food in Southern Africa and Zimbabwe, is the main source of energy. When in season monkey orange is commonly used as an ingredient in producing maize porridge (gruel), which is consumed as a breakfast cereal. Maize porridge enhanced by monkey orange is consumed by all age groups and traditionally as food for infants (regular or weaning food), school going children and immune compromised individuals. The monkey orange maize porridge is used as a flavour enhancer and also provides micronutrients and bioactive compounds, which provide health and nutritional benefits.

Bioactive compounds, such as phenolic compounds, are important in antioxidant activity, maintaining health (Steinmetz & Potter, 1996) and largely account for the bioactivity in fruit juice as suggested by scientific evidence that polyphenols possess higher *in-vitro* antioxidant capacity than vitamins and carotenoids (Saura-Calixto et al., 2007). Minerals play a central role in metabolic processes involving oxygen transport and storage as well as the oxidative metabolism, cellular growth, enzymatic reactions, macronutrient synthesis and physiological processes- important for cognitive function, growth and bone formation in the development of children (Pereira et al., 2016). Therefore the use of monkey orange juice in staple food preparations presents a potential option for improving the nutritional status of people in Southern Africa, whose diets are usually deprived of micronutrients and bioactive compounds due to economic restraints.

In summary, this study investigated the quality of monkey orange pulp and its commonly consumed product with the aim to improve indigenous fruit processing and determine the subsequent effects on product quality so as to help alleviating nutritional problems. In order to attain this goal, existing literature on commonly consumed *Strychno*s spp. was reviewed with respect to nutritional composition, sensory properties and effects of processing on quality. Thereafter, indigenous knowledge on important aspects of sensory quality, perceived health benefits, processing and quality constraints associated with *Strychnos* spp. was explored. Next, phenolic compounds of the pulp of common species of monkey orange *S. cocculoides* were characterized and the effects of improved maceration techniques on juice yield and quality were determined. Lastly, biological functions (i.e. starch hydrolysis, mineral and polyphenol *in-vitro* solubility) of maize porridge enriched with *S. cocculoides* were investigated, concurrently with consumer acceptance of the porridge. Consumer tests were done to determine the influence of maceration on sensory properties.

These research objectives are summarized from a product chain perspective as that takes the entire chain from raw material to the consumer into account (figure 6.1). The approach used in this thesis gives insights on state of the art with respect to harvesting, storage, processing and consumption. This develops a consumer guided solution by product priority research setting for improved food processing that suits local conditions with the aim of determining the effect of changes that occur during processing on health potential of the end product.

The main findings of the research are recapitulated in this chapter and the extent to which the objectives were reached and possibilities for further research are discussed using an integrative perspective.

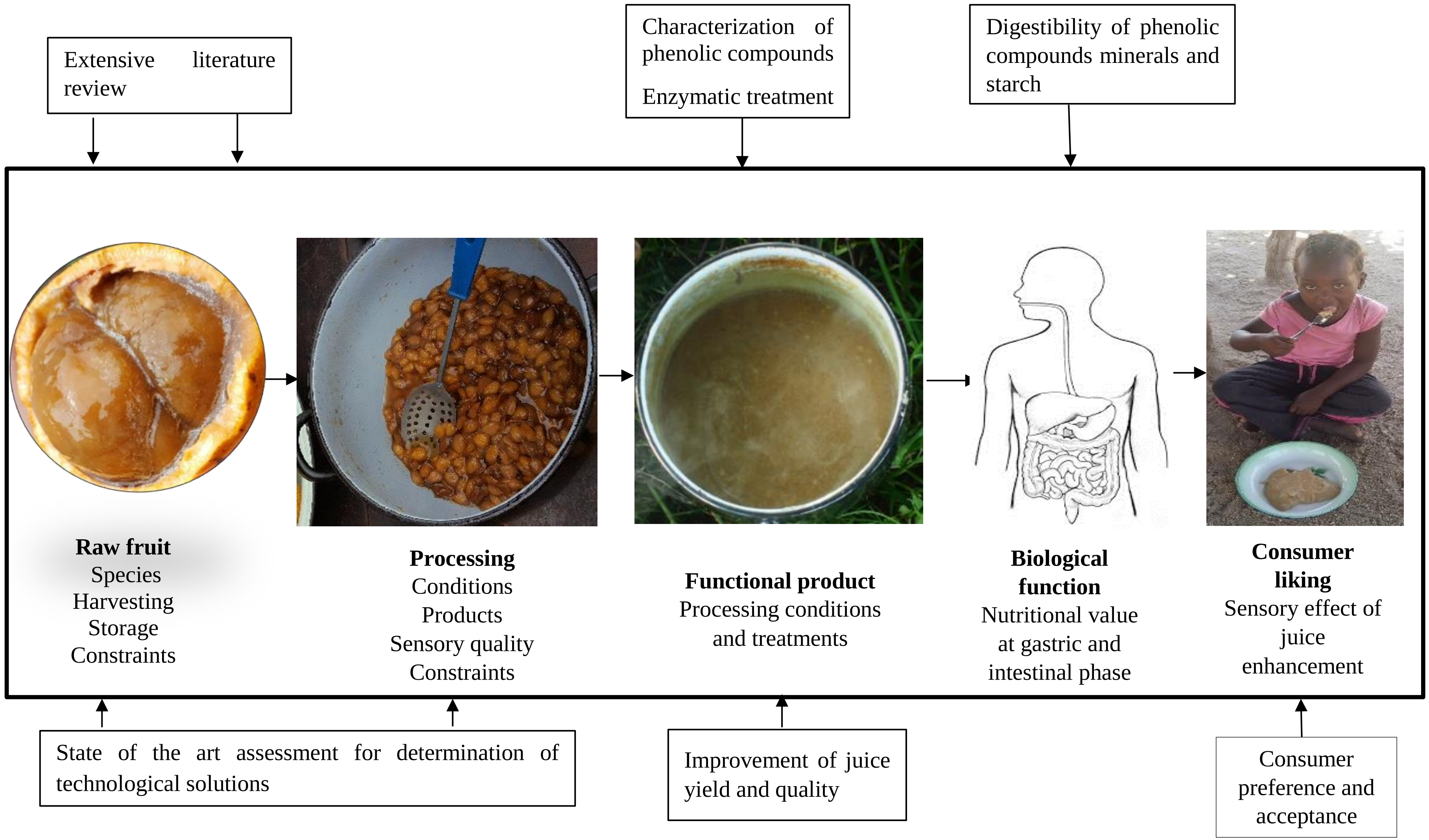

Figure 6.1 Research objectives from a *Strychnos* spp. product chain perspective

6.2 Variability of quality and nutrient content data

Strychnos spp. were reported to have high vitamin C, Fe and Zn contents (higher than its indigenous fruit counterparts), which is essential to the diet of rural populations, especially in drought conditions when there is limited access to other sources of micronutrients. The taste and flavour of the fruit varies within and between species. Overall the sweet - sour taste deems it a favoured food ingredient. The fruit is mainly consumed as such and the existing traditional processing steps and their effect on quality for the various derived products are not well documented. Assumptions were made based on scientific reports on how processing may affect nutritional content and sensory quality by extrapolating from known processing effects on product quality. As a conclusion, we recommended to focus on the effect of processing on the food product's nutritional and organoleptic quality.

There is scanty information on volatiles and bioactive compounds of the fruit. Nevertheless, limited data on nutritional composition, particularly micronutrients, bioactive and volatile compounds, exist. However, when these data are available, large variations occur. The variations in data arise from an array of factors such as methodology- sample collection, maturity (from the market or freshly picked), analytical methods and sample variability as a result of agronomic environmental factors, which include sample origin. Variations themselves often start at sample identification due to the close similarity of the phenotypic characteristics of the species, i.e. *S. cocculoides* ~ *S. spinosa* and *S. innocua* ~ *S. madagascariensis* ~ *S. pungens*. Moreover, in most cases there was no clear indication of the fruit parts that were analyzed, with varying descriptions such as flesh around the seed/ shell, juice, pulp and edible portion, making comparisons for a group of species difficult. Agro- ecological (natural) and agro-technical conditions (irrigation, fertilizer and growth of superior germplasm) are, in regard to the former, beyond control due to uncontrolled natural settings. Soil conditions such as acidity have an effect on mineral concentration, particularly Fe and Al. Because of limited reports, some data were compared for fruits grown in different environments, implying added complexity when determining whether the disparities were a result of biological variation or experimental uncertainty. Experimental conditions such as degree of precision and differences in analytical methods also contributed to the observed variation. We recommend care in sample collection, specificity in the description of the fruit parts used, standardization of methods used for analysis and unambiguous descriptions of research protocols. Even when standardized, we also recommend precision in the analytical methods used. Furthermore, sample identification and verification needs to be done before analysis by horticultural botanists with taxonomic expertise.

6.3 Traditional processing practices

In records of indigenous knowledge, the end uses of monkey orange were determined by broad species classification and grouping. The main differentiation criteria were flesh consistency and colour. One group was characterised by its brown liquefied texture (**group A**: *S. cocculoides* and *S. spinosa*), and the other by its orange firmer texture when ripe (**group B**: *S. madagascariensis* and *S. innocua*). Consequently, the end uses were similar in all localities for each group and the resulting constraints depended mainly on the grouping rather than the locality. Similar constraints to processing in the different localities were translated to a common problem for the communities. Monkey orange (*S. cocculoides*) enhanced maize porridge was the most important product consumed (on a daily basis) when the fruit is in season. The monkey orange porridge was perceived as a health beneficial food and its consumption was encouraged during infant weaning to improve child stature and for immuno- compromised persons, where regular consumption reportedly improved their health status. Seed - pulp separation was the main constraint in obtaining juice for use in further processing. Pulp maceration was done manually with warm or cold water without any enzyme. In conclusion, when considering the processing constraints, the development of standardized processing, appropriate infrastructure and processing devices will be beneficial for getting quality monkey orange products.

The survey was conducted from September through December, when the fruit is in season, thus confirmation of the species in question and recall of processing was highly reliable. However, the disadvantage was that questions related to off season aspects may not have been accurately provided due to memory inaccuracies. Owing to fruit underutilization and cultural dilution, the survey was not anticipated to fully represent the population of the area but was intended to give insight on the products, processing and quality constraints, sensory quality and perceived health properties. It became eminent to obtain a representative sample of the respondents who had the knowhow and regular experience in processing monkey orange fruit. The final selection of respondents was based on snowballing to make the data obtained as reliable as possible. Respondents were handpicked based on having some form of knowledge on processing or a general understanding of processing methods regardless of specific factors. During the survey women proved to have a pivotal role in processing, and were more available and knowledgeable about product and processing techniques. Several of the cooperative groups in the focus groups were dominated and spearheaded by women. Given the importance of women in rural community social networks, we suggest the prioritization of their role in technological interventions regarding processing, including training.

As a spin-off for this thesis, the species used in further research was based on the results of the literature review and survey with consideration of consumer preference, distribution and availability to the general population. Further research was recommended for improving traditional processing by increasing juice/ pulp yield while maintaining or improving the physicochemical characteristics and on investigating the effect of processing on quality aspects.

6.4 Quality aspects and production of healthy juice

Enzyme treatments increased juice yield as shown in figure 6.2, which shows a pictorial presentation of the detachment of pulp and seed. The physicochemical properties (brix, pH, colour, and clarity- all properties beneficial for sensory appeal as desired by consumers), fructose and glucose, improved by both thermal and non- thermal enzymatic pre-treatments. The product colour and taste are important sensory quality criteria for the consumer. However, the product was not as light as expected after blanching and enzymatic treatment. In order to improve the physicochemical properties further processing solutions are required. For instance, to improve the clarity and colour while at the same time improving the yield, the recommendation is to use clarifying agents or ultrafiltration and anti-browning agents such as L-cysteine or cinnamic acid as an additional step during processing. This also depends on consumer preference.

Sixteen phenolic compounds were tentatively characterized for the first time in *S. cocculoides* pulp, namely *phenolic acids*: caffeoylquinic acid isomers (chlorogenic acid), di caffeoylquinic acid, protocatechuic acid, caffeic acid; *flavonoids*: quercetin, kaempferol, rutin and naringenin glucoside isomers; *iridoid glucosides*: morrinoside, secoxyloganin, acetylloganic acid isomers, sweroside, and *phenolic apioglucoside:* kelampayoside A. There was large variability in phenolic contents between the treated (traditional or enzymatic) and untreated fruit pulp (fresh fruit). Fruit pulp had lower phenolic compounds in the four identified groups as shown in figure 6.3. The maceration conditions subjected to the fruit pulp allow for cell wall polysaccharide hydrolysis (when enzyme is added) or cellular decompartmentalization of the cell structure, which released phenolic compounds. The most thermal-sensitive compounds were quercetin and quercetin glycosides, whereas phenolic acids (chlorogenic acid, 65 % of total phenolic compounds), iridoid glucosides (secoxyloganin and sweroside, 23 % of total phenolic compounds) and kelampayoside A were more stable in thermal pre-treatment conditions. Though chlorogenic acid is known to be stable to heat and acid pH (Friedman & Jürgens, 2000), the actual product temperature could have been lower than that of the water in which it was incubated, thus limiting degradation.

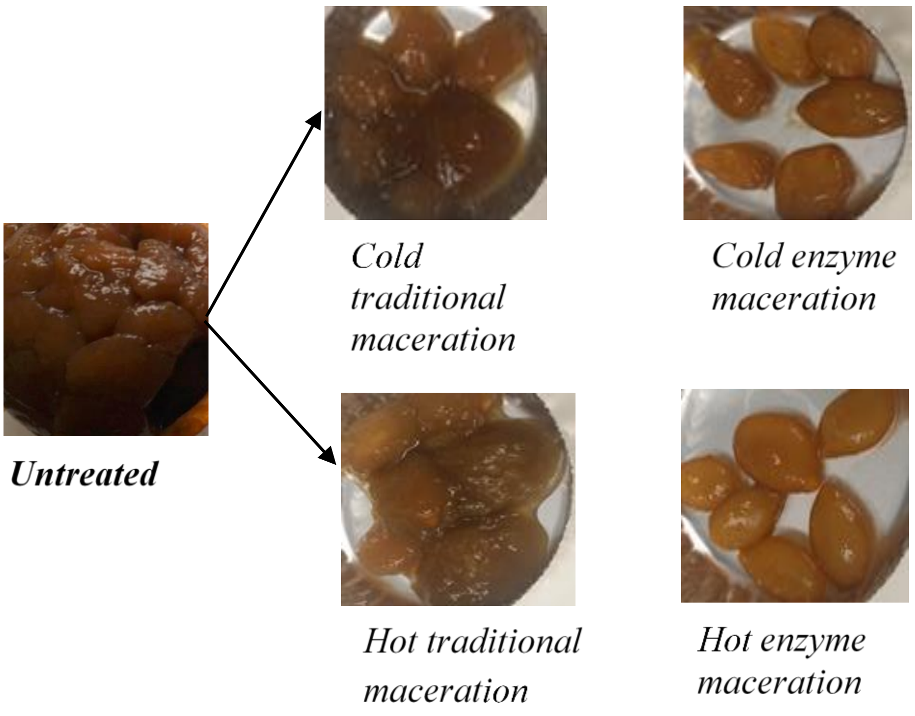

Figure 6.2 Detachment of seed-pulp during different maceration conditions

Hot pre-treatment and enzymatic incubation were shown to be effective to improve yield, physicochemical properties and amount of phenolic compounds of monkey orange juice. Based on these findings, processors are advised to subject the fruit pulp to thermal pre-treatment conditions to reduce losses by poor seed-pulp separation, increase health beneficial compounds and safety. The thermal pre-treatment a form of blanching, which can also act as an affordable pasteurization step to inactivate heat sensitive microorganisms and yeasts, which may be present during fruit preparation and handling. Given the low pH and high acidity of monkey orange fruit, we expect the microbial deterioration of the juice to be low, though biochemical changes may emanate from yeasts or acetotolerant microorganisms. Iridoid glucosides are monoterpenes and are widely distributed in medicinal plants (Kucharska et al., 2015). Their presence also has potential to improve product safety because of the reported anti-fungal, anti-inflammatory and anti-microbial activities (Bentes & Mercadante, 2014; Whitehead et al., 2016). Iridoid glucosides are not uncommon in fruits and have been identified in substantial amounts in fruit pulp of noni (*Morinda citrifolia*), genipap (*Genipa americana* L.), European cornelian cherry (*Cornus mas*) and cranberries (*Vaccinium macrocarpon)* (Bentes & Mercadante, 2014; Su et al., 2005; West et al., 2016). Sweroside and secoxyloganin were reported in branches of *S. spinosa* (Itoh et al., 2005), while morroniside and kelampayoside A were previously identified in roots and stem bark of *S. cocculoides* (Sunghwa & Koketsu, 2009). Iridoids have the ability to inhibit advanced glycation end products and elevations of serum and hepatic glucose (West et al., 2016), which when further investigated in monkey orange can be an appropriate means of controlling diabetes and ailments associated with aging.

Chlorogenic acid, an ester of caffeic acid and quinic acid, is the most ubiquitous and sometimes the only phenolic compound in fruits (Scalbert & Williamson, 2000). Previously 2-hydroxy-3-*O*-β-D-glucopyranosyl-benzoic acid was identified in the root and stem bark of *S. cocculoides* (Sunghwa & Koketsu, 2009) and in *Geniostoma antherotrichum*, a plant of the Loganiaceae family that possesses medicinal properties (Rashid et al., 1996). Quercetin and kaempferol are predominant flavanols in apricots, plums and peaches (Robards et al., 1999), while naringenin glucoside isomers are principally present in citrus fruits (Gebhardt et al., 2002; Oliveira et al., 2014). These findings confirm the perceived apricot and citrus flavour in monkey orange as described by consumers (Sitrit et al., 2003).

Antioxidant activity was in the order HP > CP > HNP > CNP (HNP: Hot water extraction without pectinase, CNP: Cold water extraction without pectinase, HP: Hot water extraction with pectinase, CP: Cold water extraction with pectinase). However, when the total concentration of identified phenolic compounds is computed, the highest is HNP while HP and CP maceration were equal and CNP the lowest. The expectation was that the anti-oxidant activity would be higher at HP because samples had a lower pH, which stabilizes phenolic acids in the juice. HP had a higher concentration of phenolic compounds that exhibit a higher anti-oxidant activity than other compounds, and additionally other compounds with anti-oxidant activity were released that were not measured in the study. Moreover, since oxygen was not excluded in the analysis, there could be compounds that were oxidised faster in HNP or anti-oxidant compounds could have formed complexes with other compounds during maceration conditions, hence giving a reduced effect on the anti-oxidant activity. Other effects, including the methodology used, may also have had an effect as anti-oxidant activity from other compounds could have been revealed by using a different analysis.

As the anti-oxidant content increased by enzymatic treatments next to an increased yield, processers are able to optimize yield as well as have a product high in phenolic compounds and anti-oxidant activity. By blanching and macerating with enzymes, the problem of low yields can be solved. As a startup, small-scale central processing in villages would be needed for training of temperature control and enzymatic maceration. These findings need to be disseminated via reliable government or Non-Governmental Organizations (NGO). However, more product would have to be sold to obtain funds to procure the enzyme, which requires improved marketing efforts. In cases where enzymes are unavailable, heat treatment alone can be used as a means of increasing the amount of health beneficial compounds with the concomitant possibility of storing the juice for longer periods. In conclusion, the blanched juice product has the added benefit of a high content of phenolic compounds, is a useful component of strategies intended to improve health and is more applicable for improved yield.

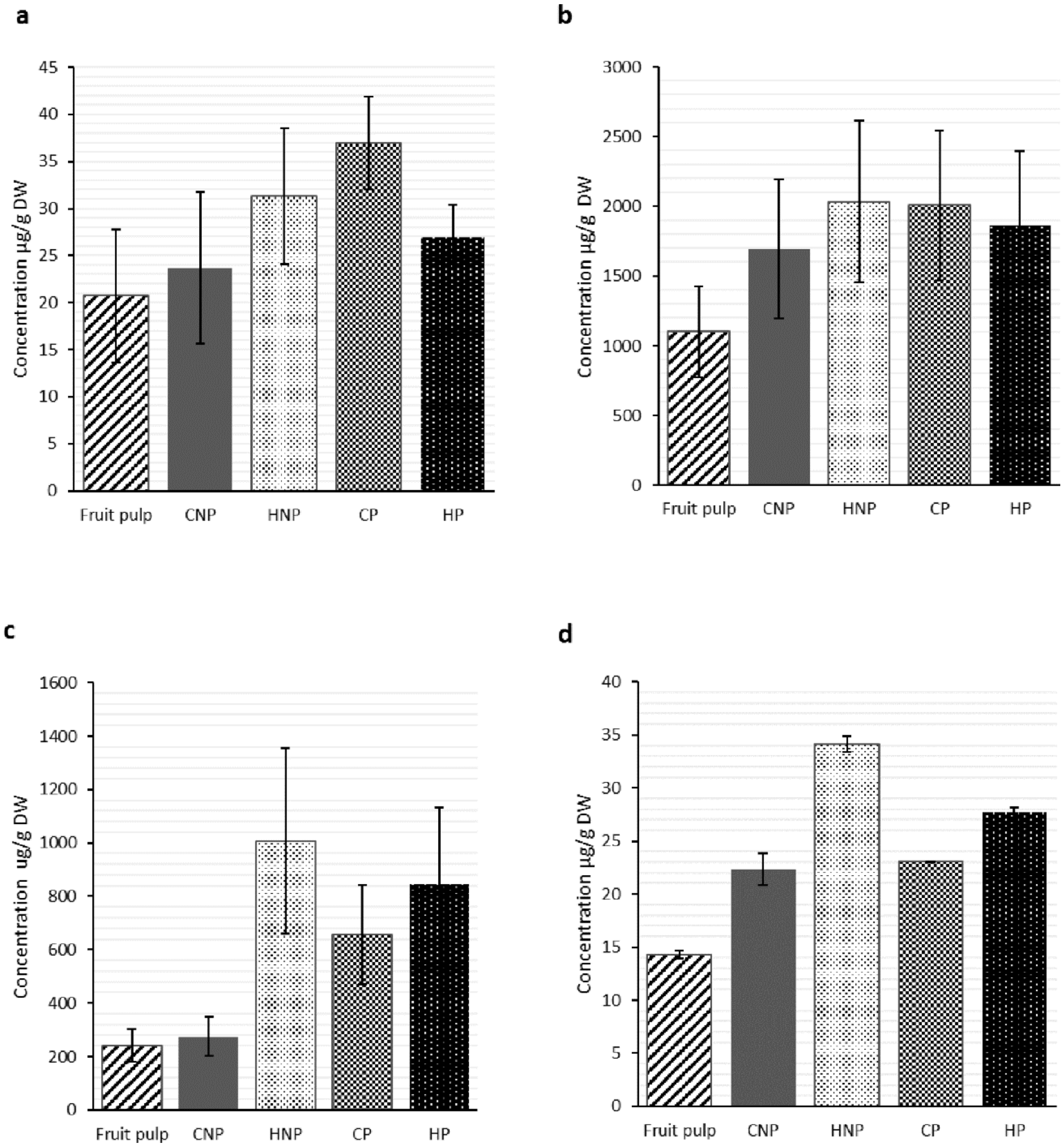

Fig 6.3 Classes of phenolic compounds per treatment; CNP: Cold no-enzyme juice; HNP: Hot no-enzyme macerated juice; CP: Cold enzymatically macerated juice; HP: Hot enzymatically macerated juice a: Flavonoids, b: Phenolic acids, c: Iridoid glucosides d: Kelampayoside A
.

Limitations and recommendations:

- Fruit maturity was based on objective methods, but even when fruits of the same maturity were used, sample preparation challenges were encountered. Individual fruits were not homogenous with regard to liquefaction, seed size and content. The seeds used were weighed and measured to be as similar as possible. This showed the difficulty of not having a standardized maturity index and sample preparation method for heterogeneous fruits. This could also be the practical reason that led to the variable results on nutritional composition found in literature. Standardized measurements of samples are required, as overall it is necessary to study such foods to gain understanding of their quality aspects.

- Pectinase was used as the enzyme for improving juice yield as it is used widely for juicing and extraction processes at small-scale and in industry. It is recommended to use a mixture of pectinolytic and cellulolytic enzymes to increase dry matter and to test the effect on quality.
- Monkey orange contains other compounds that were not further analyzed in this study. Preliminary experiments identified the presence of vitamin C. The presence of vitamin C was verified by spiking with an ascorbic acid standard. The UV-Vis spectra and retention time showed the presence of vitamin C. However, it co-eluted with an unknown compound. Moreover, several other peaks of unknown compounds that belong to monkey orange were observed. To learn more about these, we recommend purification and structure determination by Nuclear Magnetic Resonance (NMR).

6.5 Performance of monkey orange as a functional ingredient

In line with outcomes of the survey: the most important food product option that is beneficial to the rural population, was identified as a maize porridge made from *S. cocculoides* (*vis* – maize porridge cooked with water, maize porridge enhanced with enzyme extracted *S. cocculoides*, maize porridge enhanced with traditional extracted *S. cocculoides*). Organic acids in porridge enhanced with *S. cocculoides* were fumaric acid, citric acid, malic acid and formic acid. Other organic acids were present in maize porridge prepared with water except for malic acid. The substitution of water with monkey orange juice increased phenolic compounds and mineral solubility *vis* Fe, Zn, Ca and Mg, while starch hydrolysis was reduced. The presence of phenolic compounds and organic acids (malic acid and citric acid) was attributed as the contributory factors to reduced starch hydrolysis and improved mineral solubility. Reducing starch hydrolysis is important because it helps lower caloric intake, providing benefits against obesity and type 2 diabetes (Barros et al., 2012).

Adding monkey orange to maize porridge gave a more nutrient dense product with a product that had an *in-vitro* solubility >100 % for K, Mg, Mn and P. In the light of the benefits of these components in the diet, assuming all *in-vitro* soluble (IVS) minerals analysed are available, the percentage contribution to recommended daily intakes (RDI) were estimated (table 6.1). These estimations indicate that monkey orange is a rich source of minerals. Consuming its juice alone will not give 100 % of the RDI, but the results show that consuming monkey orange porridge can deliver improved benefits to a diet that is deficient of minerals and bioactive compounds. Moreover, the porridge has a high *in-vitro* solubility of phenolic compounds, that confer nutritional benefits to consumers. As there are no data on the RDI levels above which adverse effects can occur, estimations to RDI were not done. Overall, consumers will have to consume other sources of these minerals in order to meet their daily requirements.

Table 6.1 Percentage contribution of monkey orange intake to RDI of minerals[1]

	Fe (mg/day)	Zn (mg/day)	Ca (mg/day)	Mg (mg/day)	Mn (mg/day)	Cu (µg/day)	P (mg/day)
RDI children (4-8 years)	10	5	800	130	1.5	440	500
IVS fruit pulp	0.4	0.72	6.5	31	1.1	30	23
% contribution	10	36	2	60	73	7	5
Pregnant females (19-50 years)	27	11	1000	350	2	1000	700
IVS fruit pulp	0.4	0.72	6.5	31	1.1	30	23
% contribution	4	16	1	9	55	3	3
Lactation (19-50 years)	9	12	1000	310	2.6	1300	700
IVS fruit pulp	0.4	0.72	6.5	31	1.1	30	23
% contribution	11	15	2	25	106	6	8
Males above 70 years	8	11	1200	420	2.3	900	700
IVS fruit pulp	0.4	0.72	6.5	31	1.1	30	23
% contribution	13	16	1	19	120	8	8
Females above 70 years	8	8	1200	320	1.8	900	700
IVS fruit pulp	0.4	0.72	6.5	31	1.1	30	23
% contribution	13	23	1	24	153	8	8

*IVS : *in-vitro* solubility mg/ 100g FW
% contribution based on consumption of 250 g pulp per day (all values were rounded to the nearest whole number)
[1] (Rosenberg et al., 2004)

The contents of micronutrients and phenolic compounds of maize are higher in the bran than the maize starch, and processes such as hammer-milling and sifting reduce the mineral content (Gwirtz & Garcia-Casal, 2014; Kandil et al., 2012). In contrast, milling can enhance mineral solubility as it reduces the phytate content, thereby giving an opposite effect. Several other dietary factors may have an inhibitory effect on the mineral bioaccessibility and bioavailability, such as the food matrix, mineral interactions with Ca, phytic acid, fibre, tannins, polyphenols and pre-treatment of the food during processing (Gibson et al., 2006). Phytates in grain have strong binding affinity to divalent cations such as Zn^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (da Silva et al., 2013). We recommend phytate removal in maize meal for improved mineral *in-vitro* solubility. Several strategies are commonly employed in Zimbabwe to remove phytates, such as soaking and fermentation of maize meal for porridge use. Other strategies such as village based or home pounding, germination and boiling of grain have been reported to counteract the inhibiting factors by reducing phytate and oxalate content (Gibson et al., 2006). Additionally, to counter mineral inhibitory effects, incorporating promoter factors such as acidulants like ascorbic acid, organic acids, protein or fat into the diet or meal is suggested (Cilla et al., 2009). Iron bioavailability of botanic origin is low because of the low concentration of amino acids such as cysteine. Heme-Fe is more bioaccessible and its absorption is independent of the food composition (da Silva et al., 2013). Protein components in meat form an Fe - peptide complex that prevents Fe from binding to other compounds such as phytate and phenolic acids present in the food known as the "meat factor" (Hurrell et al., 2006). To enhance the bioavailability of non-heme-Fe, the consumption of meat and inclusion of fat in the diet is generally recommended. Consequently, given that poor communities may not have access to meat products, it is imperative to fund rearing projects such as poultry and pig farming for occasional consumption in rural communities. Other alternate sources for increasing mineral content and *in-vitro* solubility in the food matric could be by complementation with other products of high mineral content such as ground insect flour. Latunde-Dada et al. (2016) demonstrated in a study involving comparison between composite insect/whole-wheat flour and sirloin/whole-wheat flour that Fe bioavailability from wheat/buffalo worm compared favourably with sirloin composite. This is a product design recommendation that requires in-depth optimization and sensory studies.

Minor phenolic compounds of monkey orange and maize porridge were analysed but not quantified and based on peak area, milli absorption units (mAU) (table 6.2). The results indicate that only tannic acid was higher in maize porridge while naringenin, myricetin, quercetin and kaempferol were equally higher in the monkey orange enhanced porridge. Tannic acid has an inhibitory effect on mineral solubility, especially Fe (Chung et al., 1998). Since it is the major phenolic compound in unenhanced maize porridge, we postulate that it significantly contributed to the decreased *in-vitro* solubility of minerals. Naringenin, ferulic acid, myricetin and kaempferol increased at the end of gastric and intestinal digestion. Free phenolic compounds can be released during digestion conditions because phenolic compounds are known to exist as other complexes linked to organic acids, amines, lipids, carbohydrates and other phenols (Liu, 2007). The compound release could also be related to the enzymatic hydrolysis achieved from esterase activity during digestion and that of phenolic compounds stability at gastro intestinal pH.

S. cocculoides showed the ability to reduce starch hydrolysis in maize porridge. This is mainly linked to the presence of phenolic acids and organic acids of the fruit. *In-vitro* studies suggest that polyphenol-rich fruits may reduce starch hydrolysis and absorption of starch in-vivo thereby suppressing postprandial glycaemia (Törrönen et al., 2013). Organic acids have the added effect of increasing resistant starch levels, which has properties similar to dietary fibers, in that it is not digested in the small intestines, which control the glycemic index (GI) (Kim & Shin, 2011). The conclusion was that the combined effect of all the phenolic compounds (including those not quantified) and organic acids present caused the exhibited reduction in starch hydrolysis. The enhancement of the high GI maize porridge with *S. cocculoides* to increase the release of health-supporting components and reduce starch hydrolysis appears to be a sound strategy in the development of indigenous fruit-based functional ingredients and foods.

Table 6.2 Area (mAU*min) of other identified phenolic acids in maize porridge samples

		Naringenin	Tannic acid	Ferulic acid	Myricetin	Quercetin	Kaempferol
Maize porridge enriched with enzyme-extracted *S. cocculoides* juice	BD[1]	0.02	5.0	0	0.09	0.8	0.002
	GP	0.01	1.3	0.03	0.02	0.4	0.001
	IP	0.22	1.0	0.5	0.01	0.06	0.008
Maize porridge enriched with traditional macerated *S. cocculoides* juice	BD	0.02	5.4	0.2	0.1	0.8	0
	GP	0.01	0.6	0.5	0.1	0.15	0.001
	IP	0.1	0.5	0.04	0.8	0.06	0.006
Maize porridge in water	BD	0.006	6.5	0.01	0.02	0.4	0.002
	GP	0.003	0.06	0.5	0.003	0.06	0.002
	IP	0.24	0.01	0.3	0.96	0.02	0.007

[1] BD**:** before digestion; GP: after 120 min gastric phase; IP: after 120 min intestinal phase.

Limitations and recommendations:

- Equipment and conditions used for the maceration treatment and porridge preparation do not correspond to reality. In Zimbabwe cooking of maize porridge is done on wood fire and maintenance of heat on wood charcoal. The results obtained should be further elaborated in a real practice context at various times and temperatures.
- Other than dietary factors, starch digestion is also influenced considerably by several other factors such as the botanical source of the starch, structure, presence of other food components such as proteins, lipids and non-starch polysaccharides, as well as changes and interactions occurring during food processing (Dona et al., 2010; Singh et al., 2010). Thus, it will be valuable to further study the influence of maize properties and cooking practices on starch digestibility. Consumption studies, when

available, should not be ignored as these will determine if an adequate amount of food is consumed for absorption of a single nutrient.

- The main characteristics of phenolic compounds linked to disease prevention is their bioactivity such as antioxidant activity. After having found the phenolic compound profiles *in-vitro*, it is imperative to evaluate the stability of the antioxidant capacity after digestion so as to underpin potential health benefits due to the high *in-vitro* solubility of phenolic compounds.

6.5.1 Consumer preferences concerning S. cocculoides enriched porridge

Indigenous fruits have long been used to improve taste and palatability of staple foods that are mainly starch based. The design of a new product such as enzyme macerated monkey orange enhanced porridge is an example of a reformulated product, where ingredients are changed for improved quality. Due to changing consumer perceptions, the success of any type of product development, including a reformulation, depends largely on consumer needs and wishes (Linnemann et al., 2006). Consumers were able to detect the difference in taste for the enzyme and non-enzyme porridge in triangle tests, although there was no statistically significant difference for the two enhanced porridges. The results give an indication that the reformulated product can be accepted by the consumers as the consumers do not perceive the difference. The reason for some consumers to detect the difference is possibly because of volatile compounds present in the enzyme maceration treatment porridge. This is probably related to more organic acids and phenolic compounds that could have liberated a sour taste in the enzyme macerated enriched porridge. Phenolic acids and iridoid glucosides contribute to sensory attributes such as colour, astringency, bitterness and flavour (Fernandez de Simon et al., 1992; Mander & Liu, 2010). Organic acids found in the monkey orange porridges are important in enhancing flavour and pH control, while having the added advantage of acting as synergist for anti-oxidants (Hartwig & McDaniel, 1995) and contributing to food preservation due to a low pH, which presents potential safety, sensory and health benefits for the consumers. In addition to a taste influence, *S. cocculoides* tended to have an effect on the viscosity of maize porridge, whereby consumers perceived a reduced thickness of the final product. It is reported that highly viscous complementary foods are excessively diluted with water, leading to reduced energy and nutrient densities

(Amagloh et al., 2013). Monkey orange maize porridge has the benefit of fortification with nutrients from the fruit without further loss of nutrients by water dilution. Though the sour taste implicated by organic acids is not preferred by some of the consumers, they are important compounds as they are attributed to the promoter effect on mineral solubility and starch digestibility in the monkey orange porridge as realised from the results of the thesis.

Limitations and recommendations:

- The demographic profile of the sensory panellists omitted children below 12 years of age. This group is important in determining differences and preference of the product as they are a group of critical concern with issues related to micronutrient deficiencies. Given these considerations, the monkey orange porridge from different pre-treatments needs to be validated by this group in future sensory studies.
- Consumers prepare maize porridge from different dry maize milling techniques (hammer –milled, dehulled or sifted), which have an effect on particle size. The physical properties of maize meal may have an effect on the attributes such as texture and viscosity of the porridge. Analyses of physical functional properties are required for a better understanding of the effect of biological functions and consumer preferences.

6.6 Implications and recommendations

From the findings of this study, several research areas were identified for further exploration, namely:

- With regard to health promoting compounds, the identification of iridoid glucosides in monkey orange presents an interesting area that needs to be explored further. Given the potential of these compounds on anti-fungal and antibacterial effects, their presence in monkey orange will be beneficial particularly to rural communities due to the inadequate sanitary conditions, clean water supply and infrastructure. Iridoids have also been suggested to be natural glycation inhibitors and they confer health benefits because of their susceptibility to intestinal absorption (West et al., 2016).

- Studies on volatile flavour profiles and compound stability during maceration pre-treatments for juice yield and preparation of maize porridge are required. Additionally further studies on functional properties such as texture, viscosity and gelatinization temperatures are required in order to correlate the findings to more specific consumer sensory tests.

- Building kinetic models to make predictions of phenolic compound concentrations and antioxidant activity of extracted monkey orange juice during ambient or refrigerated storage would be beneficial for shelf life studies and as quality predictors for processors. This will allow proper storage conditions that give good product quality for a longer period of time.

- Investigation on other mineral solubility enhancers to the diet such as meat and insects or the addition of fat as an ingredient to monkey orange porridge during cooking are recommended to determine if mineral digestibility is improved for combating micronutrient deficiencies in poor communities. The application of household processing methods to enhance micronutrient contents and bioavailability should be studied in detail.

- The most abundant phenolic compounds in the diet or *in-vitro* do not necessarily produce the highest concentration *in-vivo*. The bioactivity of the phenolic compounds and their breakdown compounds would be an important aspect to explore as it remains unclear to what extent the phenolic compounds are available *in-vivo*.

- The staple foods of most countries in sub-Saharan Africa are rich in starch. The baseline findings that phenolic compounds from a fruit such as monkey orange have an effect in reducing starch hydrolysis, present an advantage on human health by lowering caloric density and further potentially preventing diabetes and obesity. Studies to investigate the interactions and mechanisms of phenolic compounds, particularly iridoids, with maize flour and the effects of *in-vitro* and *in-vivo* starch digestibility are recommended. Furthermore, the relationships between the complex starch system, phenolic compounds and digestive enzymes need to be established.

- Studies on identification and isolation of local sources of pectinolytic enzymes that are affordable and easily accessible to rural communities are also recommended. Biotechnology industries are encouraged to take this course of action on the production of enzymes for juice extraction that can be used for a broad range of IFTs. This leads to added benefits of community empowerment, knowledge transfer and capacity building.

6.7 Concluding remarks

The composition of nutritional compounds from monkey orange varies within region and between species. Despite these differences the amounts of micronutrients and macronutrients of monkey orange remain comparatively high. The goal of this thesis was to improve value addition of an underutilized indigenous fruit by using results from scientific work dovetailing with local state of the art production circumstances. We found that monkey orange seed - pulp separation is a common constraint that leads to fruit underutilization in localities where the fruit species is dense. The juice yield can be significantly improved by pectinase maceration. The use of enzymes should be encouraged as a means of reducing pulp loss with increase in yield and physicochemical characteristics and quality. Moreover, monkey orange is a source of health promoting compounds, which have anti-oxidant activity properties: phenolic acids (chlorogenic acid, caffeic acids, protocatechuic acid), flavonoids (rutin, quercetin, kaempferol, naringenin glucosides), iridoids (sweroside, acetylloganic acid, secoxyloganin, morroniside) and phenolic apioglucoside: kelampayoside A. Not all compounds identified are stable to the same extent; overall, results of the major compounds showed that thermal treatments with or without enzymes also improved extraction of the major phenolic compounds and overall antioxidant activity. Processors can use heat pre-treatment (100 ºC for 10 min) and enzyme maceration (0.5 % pectinase enzymes for 300 min at 37 ºC) without loss of the phenolic compounds while at the same time providing a means of pasteurization of the juice product. Furthermore, these compounds together with minerals are present when the juice undergoes processing to the monkey orange enhanced maize porridge. Together they are soluble *in-vitro* and cause a reduction in starch hydrolysis in the intestinal phase of digestion as compared to a staple porridge made from water and maize meal. Sensory tests with lead users show that the characteristic taste of monkey orange enhanced porridge is improved and viscosity reduced in enzyme enhanced juice. It is advisable for consumers to incorporate monkey orange juice when processing maize porridge. This will improve the nutritional value, bioactive compounds, reduce glucose release and improve the bland taste of maize porridge, thereby encouraging consumption.

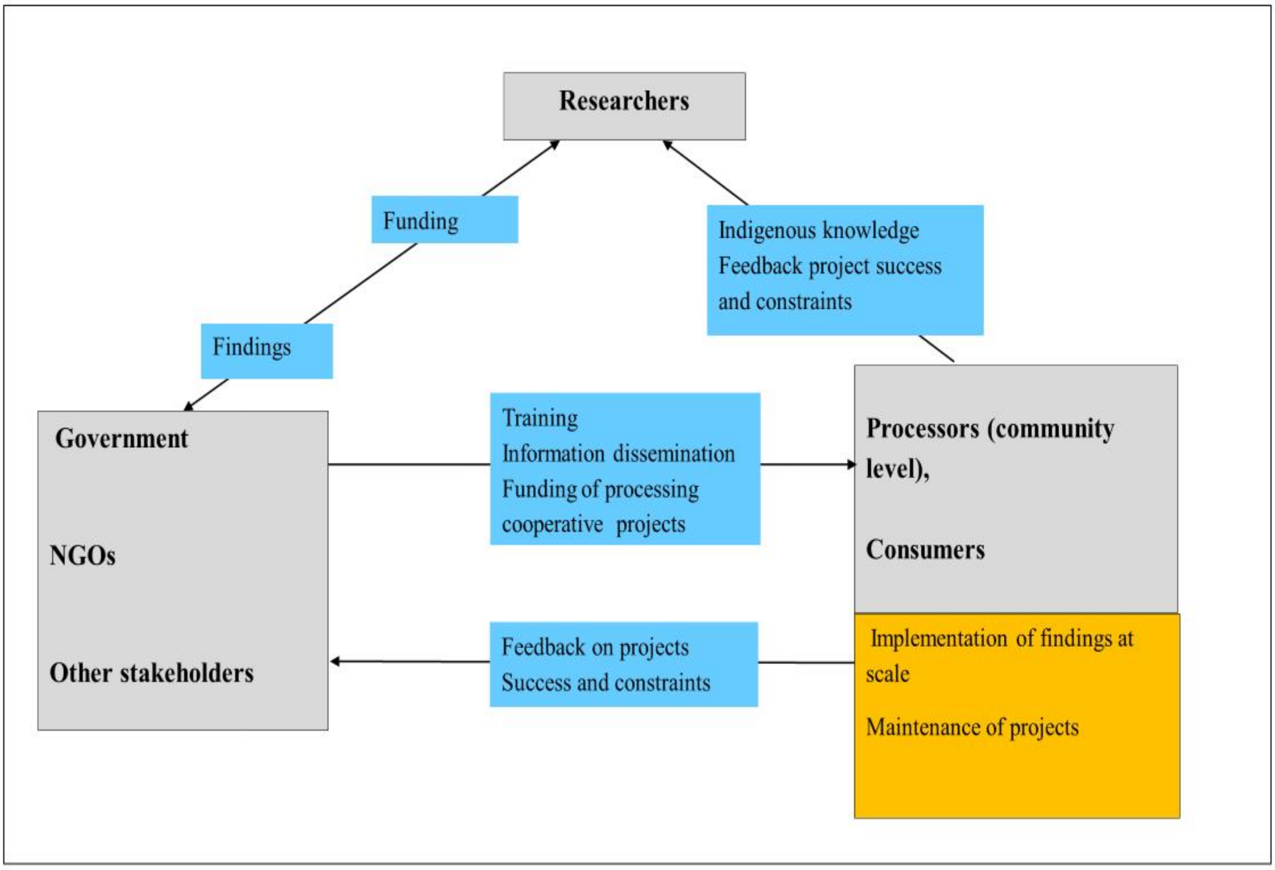

Figure 6.4 Recommendations for dissemination of research findings between relevant stakeholders

Figure 6.4 illustrates the recommendations for information dissemination and how scientific findings and activities can be improved with the inclusion of interested stakeholders. The research findings can be disseminated to the rural and urban communities in a simplified way that is understood by the ordinary consumers, through local NGOs and other relevant stakeholders such as the Agricultural Extension Services to promote utilization. Programs that address food security and reduce poverty have been implemented by the Zimbabwean government, such as Feed the Future Zimbabwe Crop Development Programme, under the Zimbabwe Agenda for Sustainable Socio-economic Transformation (Zim-Asset), whose key focal points are increasing income, food security and creating employment through agricultural production of cash crops. Under these clusters, IFTs are not yet addressed in-depth or included in their activities. We recommend these stakeholders to make an improved effort in research, training and dissemination of information that is related to IFT processing nutrition and health.

Monkey orange juice processing has been done traditionally at household and small-scale rural level in Zimbabwe for decades. Improved utilization and local processing of monkey orange juice is feasible. The main barrier to obtaining a high juice yield is the lack of infrastructure and resources at these levels. Financial support, subsidies and / or credit facilities from government and donors need to be introduced for the procurement of enzyme, equipment and simple measurement tools (temperature/ time) to enable success at local small and medium scale via women cooperative groups in rural communities. With financial support ultimately processors can acquire equipment and enzymes when products are commercialized. It is noteworthy that certain research institutes have realised the potential of food products from IFTs, such as The World Agroforestry Centre (ICRAF) in partnership with CPWild, whose aim is encouraging the establishment of indigenous fruit processing enterprises for commercialization. Additionally, women cooperatives have received support for activities in value addition and product innovation, which will ultimately improve indigenous fruit utilization by organisations such as PhytoTrade. However, in the past these projects have focused on a limited number of IFTs such as baobab, marula and Kalahari melon. This restricts the usefulness of the programs on addressing food security in a broader perspective. We recommend financial support activities for commonly consumed and preferred IFTs to be included in such programs to have a wider contribution to health and nutrition.

The fact that monkey oranges grow in the wild, gives high variation in fruit quality. Moreover, their abundance may be reduced by over harvesting or poor harvesting techniques due to anticipated increased demand. We recommend increasing horticultural attention and sustainability initiatives to meet demand and mitigate environmental degradation by the governmental department of research and specialist services (DRSS) and forestry research institutes. Systematic harvesting methods and central collection points headed by village chiefs together with standardised agro techniques for propagation of superior germplasm and provision of buds or suckers to rural communities will increase density and productivity of fruits. This will result in products of high quality during fruit season that are marketable and provide a source of livelihood for the communities. Currently the agro techniques remain at prototype stages for relevant stakeholders as monkey oranges are difficult to grow and have a slow rate of natural regeneration. Examples can be taken from the orchard plantations of *S. spinosa* at Besor Experimental Station in the western Negev Desert, Israel, which are under trial (Mizrahi et al., 2002; Sitrit et al., 2003).

Collection and dissemination of information, training and education using scientific findings is the objective of a PhD such as this one. Our work was focused on finding and tackling a problem with indigenous fruit processing by using scientific skills so as to ultimately improve utilization. The findings of this research have solved some parts of the product chain constraints that have had a major influence on its underutilization. Importantly scientific knowledge has been increased by this research by providing insights in the characterisation of bioactive phenolic compounds and the effect of improved juice yield with retention of physiochemical characteristics and health promoting compounds. The biological function of the common consumed maize porridge product on minerals, phenolic compounds and starch were also shown. Furthermore, incorporation of monkey orange into maize porridge enhanced the characteristic fruit flavour. The findings also give a basis for further investigations aimed at the improvement of monkey orange processing. Improving processing can enhance the nutritional value and gives possibilities for commercialization when marketing efforts are increased. Areas for research were identified and can be investigated further. However, these findings are not useful only to monkey orange but show the much needed intervention in research of other indigenous fruits that have been underutilized and may be “forgotten” by future generations.

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Summary

Background and objective

In sub-Saharan Africa food insufficiency is worsened by the multiple burden of malnutrition: undernutrition, overweight / obesity and micronutrient deficiency particularly Fe and Zn. The local communities in these areas rely on edible fruits harvested from the wild as a food source and food supplement. Among the preferred and commonly harvested fruits is monkey orange (*Strychnos* spp. namely – *S. cocculoides, S. spinosa*, *S. innocua*, *S.madagascariensis* and *S. pungens*), that is widely consumed and processed by locals for food and medicine. Monkey orange is rich in nutrients and is an extensively distributed fruit species in Southern Africa (Zimbabwe, Namibia, Botswana, South Africa, Mozambique and Malawi) and is consumed as fresh or processed as an ingredient to other food products by the local population. Although the monkey orange fruit is widely distributed in Zimbabwe, it is underutilized and little attention has been given to its potential commercialisation due to limited knowledge and information. Rural communities use traditional processing techniques which make insufficient use of monkey orange within communities though potential to improve the nutritional status of rural populations exists as it is a precious food source in areas with periodic shortages. To date, limited studies have been undertaken on the processing of the monkey orange fruits to value added products as a food and nutrition security resource.

The present thesis focused on optimization of indigenous processing of *S. cocculoides* to reduce fruit losses and contribute to food security and livelihoods of vulnerable rural communities in the production areas. In order to attain this goal, the specific objectives were: (i) to critically review existing literature on commonly consumed *Strychno*s spp. with a focus on the nutritional composition, sensory properties and effects of processing on product quality; (ii) to document and evaluate indigenous processing knowledge and constraints associated with *Strychnos* spp. processing in Zimbabwe; (iii) to characterize phenolic compounds of commonly consumed primary products of *S. cocculoides* and determine the effects of maceration techniques on juice yield and quality; and (iv) to determine the digestibility of nutrients in maize porridge enhanced with *S. cocculoides* juice.

Main findings

In **Chapter 2**, a literature review of Monkey orange (*Strychnos* spp.) showed that the fruit pulp/ juice is rich in vitamin C (88 mg/ 100 g), Zn (28 mg/100 g) and Fe (140 mg/100g). The reported data on nutritional composition are however variable. This variation could have risen from several factors such as agro-ecological and agro-technical conditions that influences the growing conditions (soil type, rainfall and sunlight intensity), sample type, maturity level, species differentiation and variable methods of analysis. The review showed that analysis was not standardised hence to obtain more reliable results more attention is to be paid from steps of sample collection to analysis. To improve the availability of monkey orange food outside its production season, processing and preservation techniques used at household level need upgrading as they are unreliable and their effects on nutritional quality are unknown. Based on the review, priorities for research were concluded: 1. increase and improve research on micronutrient composition assays of the consumed species of monkey orange, 2. quantification and classification of phenols needs to be carried out 3. research about contribution of monkey orange to human health through bio-accessibility and bio-availability studies of micronutrients, 4. optimization of processing techniques on nutritional value and quality of most commonly consumed monkey orange products needs to be assessed.

The survey conducted as described in **chapter 3** showed that most of the fruits and their products are wasted because of limited harvest time, process control and storage conditions, leading to variability in shelf life and sensory quality, thereby impacting nutritional quality. *S. cocculoides* and *S. spinosa* were used by the majority of respondents as a functional ingredient in porridge, followed by fermented *mahewu* drink and as a non-alcoholic juice while fruits of *S. innocua* and *S. madagascariensis* are preferably processed into dried products. The survey showed that monkey orange products to have health benefits for children and immune-compromised people, who on regular consumption have reportedly increased weight and resistance to disease. Taste, flavour and colour were the important quality characteristics for all processed products. The major constraint to be solved are seed-flesh separation together with long processing times, separation of juice and pulp during storage as well as pulp viscosity. For dried products constraints were indicated as variable drying conditions, slow drying rate, splitting of product and large inedible portion. Seed –pulp separation of *S. cocculoides* and *S. spinosa*, is a manual operation which leads to a large amount of pulp loss that remains attached to seed when extracting the juice or pulp. Based on the most commonly processed products and ingredient use, the recommendation for optimization of juice extraction was undertaken in Chapter 4.

Chapter 4, shows phenolic compounds characterization in *S. cocculoides* and proposes processes to improve pulp yield, quality and the health beneficial compounds. The most frequently processed species **-** *S. cocculoides* as recognized from the survey was evaluated. Prior to treatments, a total of 16 known phenolic compounds predominantly belonging to the phenolic acids, flavonoids and iridoid glucosides classes were tentatively characterized for the first time in *S. cocculoides* using High Resolution Mass Spectrometry and LC/MS/MS to confirm iridoid presence. Metabolite profiling according to the heat map, FancyTile chromatic scale approach and phenolic compound content were used to compare the identified compounds between treatments. Overall, the results showed that enzymatic treatments increased pulp yield by 26 % , physicochemical quality (38 % increase in juice clarity), content of phenolic compounds (kaempferol, quercetin, caffeic acid, protocatechuic acid, IGs) and antioxidant activity.

Chapter 5 focused on further analysis of the maize porridge- a staple breakfast cereal. The effect of using juice macerated with and without enzyme on the bioaccessibility of main phenolic compounds and micronutrients was assessed during *in-vitro* digestion together with the kinetic of starch degradation in the maize porridge. Chlorogenic, caffeic and protocatechuic acids had more than 100 % after simulated intestinal digestion phase. Rutin was undetected after the simulated intestinal phase owing to precipitation in the pellet. *In vitro* bioaccessibility of minerals ranged from 12 – 62 % in monkey orange enriched porridge. A 50-70% decrease of starch hydrolysis was observed at the end of simulated intestinal digestion of monkey orange maize porridge confirming the potential of phenolic compounds to decrease the glycaemic index of starch rich foods. Results suggested monkey orange juice as a suitable ingredient to enrich staple maize porridge thanks to its micronutrients value and the health benefits potential. In addition consumers did recognise the difference in ingredients used for porridge processing though products were equally liked in preference tests.

The results were integrated and discussed in **chapter 6**. The core of the discussion was the role of improved monkey orange juice extraction in relation to the yield, nutrition, physicochemical properties and preferences of rural populations in Zimbabwe.

Conclusions

The optimization of juice extraction is essential for reduced juice and pulp losses as well as the bioaccessibility of nutrients and phenolic compounds when used as an ingredient in food products. The improved processing can be beneficial as a means of income generation by women cooperative groups and production of more monkey orange products. Furthermore, the use of monkey orange fruit in starch based product presents a new direction in future work on reduction of starch hydrolysis for staple food products commonly consumed in Africa. The work from this thesis shows areas of further research on monkey orange potential to improve human health and nutritional problems in Zimbabwe. Thus, improving food use for food security as well as provision of some solutions to nutritional problems in sub-Saharan Africa.

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About the author

Curriculum vitae

Ruth Tambudzai Ngadze was born in Hwange, Zimbabwe, on 08 June 1981. Ruth completed her Advanced level education at Visitation Makumbi High School, Domboshawa in Zimbabwe and graduated from the University of Zimbabwe with a Bsc Food Science and Technology degree 2004. Thereafter, she was employed as a tutor at the University of Zimbabwe from 2005-2006. She left the University of Zimbabwe in September 2006 after being awarded a NUFFIC scholarship by the Netherlands Government for an Msc degree in Food Quality Management at Wageningen University and Research in the Netherlands. After completing her Msc, Ruth joined Chinhoyi University of Technology in April 2011 and is employed there to date, as a lecturer in the Department of Food Science and Technology. In 2013 she started her sandwich PhD with the department of Food Quality and Design, funded by NUFFIC (grant award CF151/2013). She carried out her research in Netherlands and Zimbabwe between 2013 and 2018. This thesis presents the results of the scientific research, which have been published in peer-reviewed journals.

Overview of completed training activities

Discipline specific activities		
Courses	Graduate school/Institute	Year
Genetics and Physiology of Food-associated Microorganisms	VLAG, Wageningen	2013
Sensory Perceptions and Food Preference	VLAG, Wageningen	2013
3rd International Conference on Food Digestion	Cost Action FA 1005, Wageningen	2014
Role of SME's in food security in Africa, inclusive business by African entrepreneurs	KLV, Wageningen	2014
Advanced Food Analysis	VLAG,Wageningen	2014
Enhancing nutrition and food security through improved capacity of agriculture higher education institutions in ESA	Edulink II Project: FED 2013/320-148 Chinhoyi, Zimbabwe	2014
Reaction kinetics in food science	VLAG, Wageningen	2016
Conference on Food safety and Food security (FSaS)	University of Johannesburg South Africa	2016
Chemometrics	VLAG, Wageningen	2017
Healthy and sustainable diets: synergies and trade offs	VLAG, Wageningen	2017

General courses and workshops		
VLAG PhD week	VLAG, Baarlo	2013
How to write a world class paper	WGS, Wageningen	2013
Philosophy and ethics of Food Science and Technology	WGS, Wageningen	2014

Information literacy for PhD including Endnote Introduction	WGS, Wageningen	2014
Techniques for Writing and Presenting a Scientific Paper (TWP	WGS, Wageningen	2014
Project and time management	WGS, Wageningen	2015
PhD Workshop Carousel	WGS, Wageningen	2015
Introduction to R	VLAG, Wageningen	2017
Reviewing a scientific paper	WGS, Wageningen	2017
Applied Statistics	VLAG, Wageningen	2017
Optional courses		
Preparation of research proposal	FQD, Wageningen	2013/2014
PhD Colloquia	FQD, Wageningen	2013-2017
Organizing supervisor trip to Zimbabwe	FQD and Harare Zimbabwe	2016
Scaling Up Nutrition Country Joint Self-Assessment	Harare, Zimbabwe	2018

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