Sensory and physico-chemical analysis of roasted marama beans [*Tylosema esculentum* (Burchell) A. Schreiber] with specific focus on compounds that may contribute to bitterness

by

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education

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Patricia Nyembwe

ABSTRACT

Sensory and physico-chemical analysis of roasted marama beans [*Tylosema esculentum* (Burchell) A. Schreiber] with specific focus on compounds that may contribute to bitterness

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MSc Food Science

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Marama bean [*Tylosema esculentum* (Burchell) A. Schreiber] is an underutilized legume which grows wild in selected part of southern Africa and forms part of the diet for the indigenous population in this area. Marama bean seeds are not eaten raw but after roasting they have a delicious nutty flavour. A bitter taste develops depending on the extent of heating. Heat treatment by roasting marama beans at 150 °C for 20 min inactivates anti-nutritional factors particularly trypsin inhibitor. The bitter taste can limit the utilisation and consumption of this pulse. Research is lacking on the compounds that could be responsible for the bitter taste in marama beans. Different chemicals including phenolic compounds and saponins contribute to bitterness in many edible legumes. An increase in the quantity of the bitter substances in roasted marama bean might contribute to more bitter taste. In this study sensory profiles and potential bitter substances of marama bean cotyledons were investigated as a function of roasting time.

Marama beans were roasted at 150 °C for different time periods 20, 25 or 30 min. Three pastes were prepared from the roasted cotyledons; paste^{20}, paste^{25} and paste ^{30}. Eight samples of water extracts were prepared from full fat and defatted flours from roasted and unroasted marama cotyledons. Descriptive sensory evaluation was conducted with a trained panel to rate the intensity of aroma and flavour attributes of marama pastes and bitterness of water extracts. Marama pastes were subjected to colour measurement, lightness (L*), red/green characteristics (a*) and blue/yellow characteristics (b*). The total phenolic and saponin contents of marama water

extracts were determined. The phenolic profile were analysed by reverse phase-high performance liquid chromatography (RP-HPLC).

The paste^{20} was lighter and the least bitter while paste^{30} was dark-coloured and the most bitter. The water extracts prepared from unroasted flours were rated the least bitter while the water extract prepared from defatted flour made from marama bean roasted for 30 min was the most bitter. Roasting of marama seeds resulted in significant increases in its water soluble phenolic content. The concentration of total phenolics in the seeds roasted for longer time periods was higher. The major extractable phenolic acids present in marama water extracts were found to be gallic and protocatechuic acids, the concentrations of which increased as a function of roasting time and may be the cause of more intense bitterness. Marama beans have saponins content varying from 84.4 mg/100 g in raw flour to 94.9 mg/100 g in roasted flour. The different concentrations of saponins in water extracts prepared from roasted and unroasted marama flour (55-62 mg/L) were higher than the reported bitter threshold value for saponins in water (2-12 mg/L). Thus, saponins in marama beans possibly also contributed to more bitterness. Roasting time and temperature combinations shoud be considered when processing marama beans for food products. At 150 ˚C roasting temperature, marama beans should not be roasted for more than 20 min to avoid bitterness, colour darkening as well as development of a burnt flavour.

Roasted marama beans

DEDICATION

I dedicate this dissertation to my wonderful family. Particularly to my understanding and patient husband, Serge, and to our precious children, Emmanuel, Gabriel, Abigael and Dora who are the joy of our lives. I must also thank my loving mother and my family who have helped so much with baby-sitting and have given me their fullest support. Finally, I dedicate this work to my late father, Prof. Patrice Nyembwe of whom believed in diligence, science, and the pursuit of academic excellence.

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave me the possibility to complete this dissertation. I sincerely thank Prof. Henriette L. de Kock, Prof. Amanda Minnaar and Prof. Gyebi Duodu for their guidance, support and patience throughout this project. It was a great privilege for me to work under your supervision.

Thanks to the staff and students, Department of Food Science, University of Pretoria, for all their help, support, interest and valuable hints.

Especially, I would like to give my special thanks to my husband Serge whose patient love enabled me to complete this work.

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1. INTRODUCTION AND PROBLEM STATEMENT

Marama bean [*Tylosema esculentum* (Burchell) A. Schreiber] is an underutilized legume which grows wild in parts of southern Africa and forms part of the diet for the indigenous population in this area (Jackson, Duodu, Holse, Lima de Faria, Jordaan, Chingwaru, Hansen, Cencic, Kandawa-Schultz, Mpotokwane, Chimwamurombe, de Kock & Minnaar, 2010). The seeds are rich in lipid and protein, and have the potential to improve nutrition and increase food security for people living in these rural areas (Holse, Husted & Hansen, 2010).

The protein content (29-38%) (Holse *et al*., 2010) is comparable to oilseed legumes such as soya bean (36-43%) (Vaidehi & Kadam, 1989; Stauffer, 2005). The oil content of marama bean (32-42%) (Holse *et al*., 2010) is much higher than that of soya bean (20-24%) (Salunkle & Kadam, 1989), and compares favourably with oilseeds such as groundnuts (45-55%), sunflower seeds (22-36%), rapeseed (22-49%) and canola (43%) (Salunkle & Kadam, 1989). However, the use of marama beans remains mainly domestic.

Marama bean seeds are not eaten raw as they are reportedly tasteless with an unpleasant slimy texture, but after heating they have a delicious nutty flavour, resembling roasted peanuts (Mmonatau, 2005). The nutty flavour has been suggested to be caused by the formation and release of flavour compounds due to Maillard reaction as a result of heating (Kayitesi, 2009). A further reason for heat treating marama beans is to inactivate anti-nutritional factors. Maruatona (2008) reported that roasting marama beans at 150 °C for 20 min is required to inactivate the trypsin inhibitor. This time/temperature combination is therefore regarded as a minimum requirement for consumption purposes. Depending on the extent of heating, marama beans are perceived to develop a bitter taste. Mmonatau (2005) reported an undesirable bitter taste in roasted marama bean when heating at 150 °C for 30 min. Furthermore, Kayitesi (2009) also reported a slight bitter taste in sorghum porridges composited with defatted marama flour. The bitterness is more pronounced in roasted marama bean than in raw seed (personal observation). The perceived bitter taste can limit the utilisation and consumption of this pulse. However, no research has been

published on the compounds that could be responsible for a bitter taste in marama beans. The bitterness of marama beans has not been studied and/or quantified.

Troszynska (2004), reviewed phenolic compounds and reported that phenolic acids, flavonoids and tannins are substances responsible for bitterness in plant foods. In addition, other chemical compounds such as alkaloids and saponins are believed to impart a high level of bitter taste in many edible legumes (Rochfort & Panozzo 2007). These include lupins, lentils, chickpeas, soybean, various beans and peas (Rochfort & Panozzo 2007). The alkaloid and saponin content of marama bean is not known.

Marama bean cotyledons contain gallic acid, protocatechuic acid, caffeic acid sinapic acid, vanillic acid, ferulic acid, naringin and quercertin (van Zyl, 2007). These phenolics are described as bitter tasting (Peleg & Noble, 1995) and some may increase in extractable quantities upon heat treatment (Dvorak, Pechova, Pavlata, Filipek, Dostalova, Reblova, Klejdus & Kovarcik, 2005). Increase in quantity of the bitter substances in roasted marama bean may also contribute to more perception of the bitter taste. The aim of this study was to determine the different possible substances that may cause bitterness in marama bean cotyledon and quantify them. The presence of bitter compounds will be related to heat treatment and to the sensory profiles of roasted marama beans.

2. LITERATURE REVIEW

This chapter is divided into three parts. The first part is a review of the marama bean. It mainly discusses the habitat of the plant and morphology of the seed followed by a review of processing and utilisation of marama beans. The second part reviews the effect of heat treatment on sensory and chemical properties of marama beans. The third part provides a review of the different compounds that contribute to bitterness in legumes and plant foods followed by a discussion of human perception of bitterness and briefly consider methods of bitterness evaluation. In this literature study marama bean will be compared to other oilseed legumes such as soybean and also to other plant foods such as almond, olive etc. This is because limited published information is available on marama beans, particularly in the area of bitter compounds and sensory profile of the roasted bean.

2.1 .1 Habitat and morphology of marama bean

Marama bean also known as morama bean is a long perennial legume native to the Kalahari dessert (Maruatona, Duodu & Minnaar, 2010). It is commonly found and consumed in rural areas of some southern African countries namely Namibia, Botswana particularly where the marama bean plant grows in natural abundance (figure 2.1) (Müseler & Schönfeldt, 2006) and to a lesser extent in the Limpopo provinces and North-West provinces of South Africa (Mmonatau, 2005). Marama beans belong to the family Fabaceae; subfamily Ceasalpinoidae and genus *Tylosema* (Hartley, Tshamekang & Thomas, 2002). It is native to dry areas with little seasonal rainfall and, being a legume, particularly important in subsistence agriculture (Müseler & Schönfeldt, 2006). Usually accepted by the local population and better adapted to existing environmental conditions. Marama plant grows at altitudes of between 1 000 and 1 500 m with 300 to 700 mm rainfall, and at a minimum temperature above 15 °C and a maximum of approximately 33 °C (Müseler & Schönfeldt, 2006). It is dormant in winter and regrows from the tuber in spring. The plant grows in well-drained, fine, generally calcareous sands, but also in regions of harder calcareous conglomerates, at pH 6 to 8, with very little organic matter, nitrate or phosphate (Jackson *et al*., 2010).

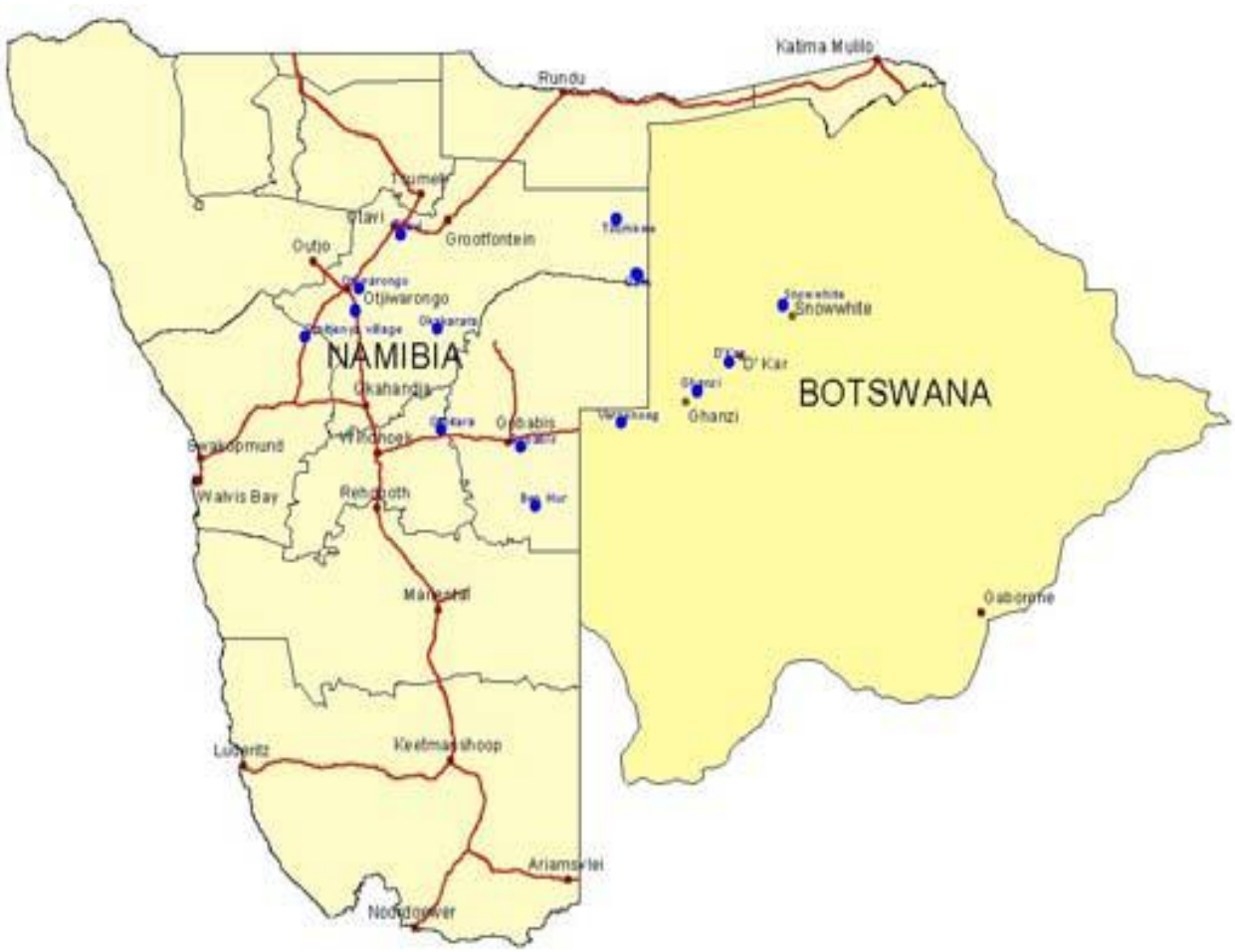


Figure 2.1: Map of Bostwana and Namibia showing the geographic distribution of marama bean plants, where the marama bean plant grows in natural abundance (Agricultural Laboratory; Ministry of Agriculture, water, and Forestry, 2004 as cited by Müseler & Schönfeldt , 2006)

Marama bean is a creeper plant which has branches that grow up to six metres long with bi-lobed leaves (Kapewangolo, 2010). The plant produces golden yellow clusters of flowers (Figure 2.2A) during spring to summer in the region and the fruit ripens in autumn (Jackson *et al*., 2010). It takes three to four years after seeding before new seeds can be harvested in considerable amounts (Holse *et al*., 2010). The pod is ovate to ovate-oblong, sometimes spherical with length and width varying from 1.3-1.8 cm and 1.2-1.5 cm respectively (Amonsou, 2010). Usually the pod contains two seeds but can produce as many as six seeds each weighing approximately 2.4 g when mature (Kayitesi, 2009).

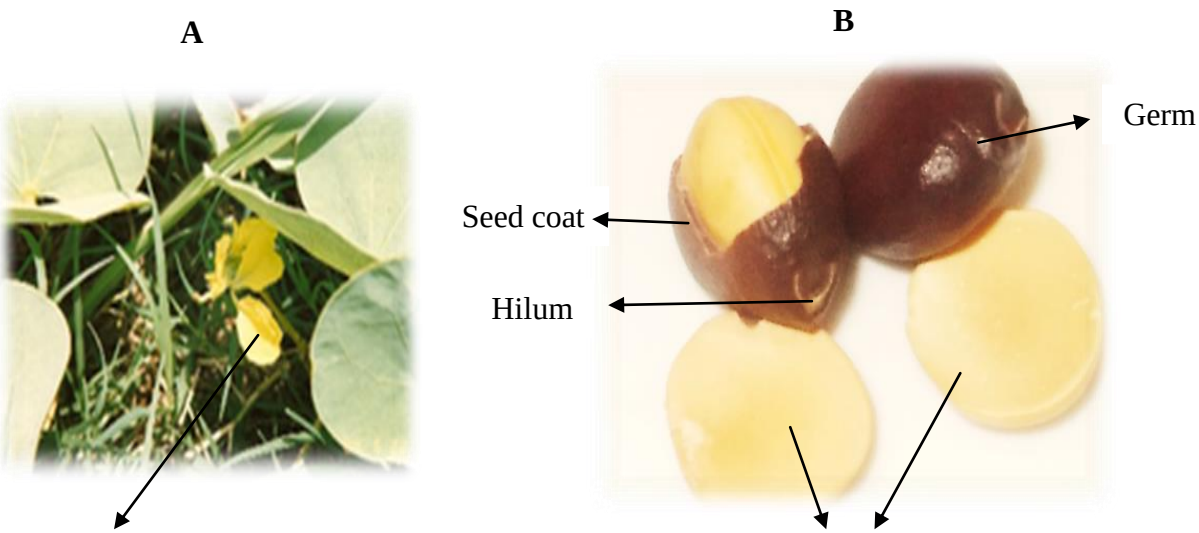


Figure 2.2: A) picture of marama flower (Wikipedia, 2013); B) Marama bean seeds showing major anatomical features

Marama bean is a dicotyledon (Maruatona *et al*., 2010) consisting of three major parts as shown in Figure 2.2B. The seed coat (hull) which is dark brown with a seed scar or hilium, the cotyledons and the germ. The ripe seeds are chestnut spherical comprising two cotyledons that are firm and cream coloured (Mmonatau, 2005). Removal of the seed coat causes the inner flesh to separate in two cotyledons. The seeds have very low water content and can be stored for months (Holse *et al*., 2010).

2.1.2 Processing and utilisation of marama bean

Marama bean is classified as an underutilized crop as it has not been fully exploited in terms of its potential use as a food and/or non-food product (Takundwa, 2010). The green beans (immature) are boiled and consumed as green vegetables or in soup. The dry ripe beans still in the seed coat are roasted in a pot of sand on fire and eaten as a snack (Holse *et al*., 2010). The beans are considered ready when they pop during the roasting process. Traditionally, one or two seeds are cracked to see if they are ready, then roasted marama beans are cooled in the sand on the floor (Holse *et al*., 2010). The sand is used to distribute the heat evenly and prevent the seed coat from breaking (Kapewangolo, 2010). Because marama bean seed coats are hard and not consumed, the cotyledons need first to be separated. The seed coats are more easily removed from the bean after roasting than when the seeds are raw (Kapewangolo, 2010).

Usually indigenous people use a stone to remove the seed coat from the bean (Kapewangolo, 2010). On a laboratory scale, marama beans can be roasted in a forced convection continuous tumble roaster (Marautona, 2008). Hot air is forced through the product during the roasting process, creating a very even roasted product. The product is tumbled about 45 times before it leaves the rotor (Kapewangolo, 2010). The temperature can be set between 0 to 400ºC and the speed controller can also be adjusted. The seed coats are removed from the bean using a cracker (Marautona, 2008). Local people also boil marama bean with cornmeal (maize meal) or pound them into powder that is boiled in water to produce either a cocoa-like beverage or porridge (Kapewangolo, 2010). Marama oil can be collected on the water surface after cooking of green beans (Holse *et al*., 2010). The marama oil can be used for frying and for skin care. Due to its high protein content (Holse *et al*., 2010), marama bean can be used to increase the protein quality of cereal-based foods (Kayitesi, 2009), or can be treated for extraction of bioactive phytonutrients for use as nutraceutical products (Van Zyl 2007). In addition, defatted flour from unheated marama beans could be useful in food systems requiring high emulsifying capacity e.g bakery (Maruatona, 2008).

2.2 Effect of heat treatment on sensory and chemical properties of marama bean

Raw marama bean seeds are hard, oily, and tasteless with an unpleasant slimy texture (Mmonatau, 2005). Roasting is used to make the seed more palatable by increasing the sensory properties of the beans (Mmonatau, 2005; Kayitesi, 2009). Usually, the Maillard reaction between reducing sugars and amino acids is a common reaction in foods which undergo thermal processing such as roasting, and cause the occurrence of browning phenomena, and flavour development that could be desirable or undesirable depending on the extent of heating. Previously, roasted marama beans ground into flour were described as having a delicious nutty flavour while a bitter taste was reported in the flour prepared from the beans overheated at 150 ºC for 30 min (Mmonatau, 2005). Kayitesi (2009) suggested that the nutty flavour was due to the development of flavour compounds via Maillard reaction, caramelisation, and lipid-Maillard product interaction. In addition, the author related the bitter taste to off-flavour development compounds from Maillard reactions such as furan and caramel compounds when heating marama beans for longer time period or to the presence of

phenolic compounds in the marama cotyledons. The final products of the Maillard reaction are melanoidins known as brown nitrogenous polymers and co-polymers (Martins, Jongen & Van Boeckel, 2001). Melanoidins may result in desirable flavour (caramel like) or undesirable flavours (bitterness), and are also responsible for the brown colour development (Martins, Jongen & Van Boeckel, 2001).

Dry heating marama beans prior to flour processing increased total analyzable phenolic content and antioxidant activity of the flours (Kayitesi, 2009). This was attributed to the disruption of the cell wall through heating or the release of insoluble bound phenolic compounds which led to better extractability of these compounds (Kayitesi, 2009). A study by Dewanto, Wu and Liu (2002) reported that thermal processing may release bound phenolics by breakdown of cellular constituents. Dvorak *et al.* (2005) also reported an increase of phenolic acids such as protocatechuic acid, sinapic acid, vanillic acid, ferulic acid, except caffeic acid in peas after heat treatment by steam vapour. The treatment increased the extractability of phenolic acids by disturbance of cell walls and cell organelles.

Heat processing is widely accepted as an effective means of inactivating the thermo-labile antinutritional factors particularly trypsin inhibitors in marama beans (Maruatona, 2008). The nutritive quality is also notably improved by roasting. The protein quality improves by inactivating trypsin inhibitors and by unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes.

2.3 Bitter compounds in legumes and food plants

This section is an overview of natural bioactive non- nutrient substances causing bitter taste in food products. The compounds are discussed following their general division into three classes: phenolic compounds (phenolic acids, flavonoids, tannin polymers), terpenoids (saponins) and nitrogen compounds (alkaloids).

2.3.1 Phenolic compounds

Phenolic compounds are a class of low molecular weight secondary plant metabolites found in most plants (Nandutu, Clifford & Howell, 2007). Chemically they are substances possessing an aromatic ring bearing one or more hydroxyl (OH) group. The legumes with highest phenolic content are dark and highly pigmented varieties (Campos-Vega, Loarca-pina & Oomah, 2009). Phenolic compounds may be generally divided into phenolic acids, flavonoids and highly-polymerised compounds (tannins). Each of the groups mentioned contains substances responsible for introducing bitterness in plant foods (Troszynska, 2004).

Marama beans seed coat and cotyledons have been reported to contain phenolic compounds [van Zyl, 2007; Kayitesi, 2009; Shelembe, Cromarty, Bester, Minnaar & Duodu (2012)]. There is a significant level of phenolic acids and flavonoids in marama bean, especially in the seed coat. The total phenolic content in marama bean cotyledon was reported as 2.8 % (van Zyl, 2007) to 3.0 % (Kayitesi, 2009). It was also reported that marama bean cotyledon contains high levels of the amino acid tyrosine (Maruatona *et al*., 2010) which is phenolic in nature and can therefore contribute to the total phenolic content of the cotyledon as determined with the Folin-Ciocalteu assay.

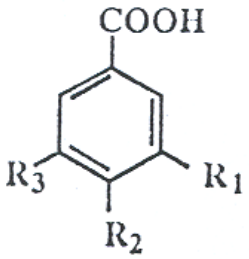
2.3.1.1 Phenolic acids

Phenolic acids contain hydroxyl (OH) or methoxy (OCH_3) groups substituted at various positions on the aromatic ring (Caballero, Trugo & Finglas, 2003). In plants phenolic acids may occur in free or bound form. Bound phenolic acids are those that are complexed or form conjugates (i.e. esterified) with sugars, proteins or cell wall polysaccharides. Phenolic acids are mainly benzoic and cinnamic acid derivatives and their chemical structure are shown in Figure 2.3 (Shahidi & Nacsk, 2004). Marama bean cotyledon contains gallic acid (23.4mg/100g), protocatechuic acid (152mg/100g), sinapic acid (20.5mg/100g), caffeic acid (38.9mg/100g) vanillic acid (59.2mg/100g) and ferulic acid (8.9mg/100g) (van Zyl 2007).

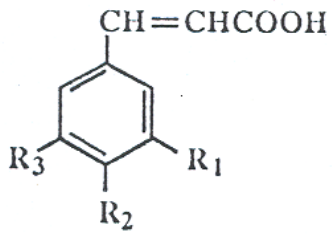
According to Drewnowski and Gomez-Carmeros (2000), low-molecular weight phenolic compounds tend to be bitter. Peleg and Noble (1995) studied the sensory properties of phenolic acids commonly found in fruits, vegetables, grains and spices. These included salicylic acid (2-hydroxy benzoic acid), *m*-hydroxy benzoic acid (3-hydroxy benzoic acid), gentisic acid (2, 5-hydroxy benzoic acid), protocatechuic acid (3, 4-hydroxy benzoic acid) and gallic acid (3, 4, 5-trihydroxy benzoic acid) in water. Each of these compounds elicited multiple sensations including sweetness, sourness, astringency, bitterness and prickling. Astringency is defined as a "drying or puckering mouth feel detectable throughout the oral cavity". Astringency may be due to a complexing reaction between dietary polyphenols and proteins of the mouth and saliva, causing friction on the mouth surface (Drewnowski & Gomez-Carmeros, 2000). Although the compounds were structurally similar, their sensory properties differed qualitatively and quantitatively. Gentisic acid was most sour, benzoic acid was highest in pricking sensation, salicylic acid was most astringent, *m*-hydroxyl benzoic acid was the sweetest, protocatechuic acid, gallic acid and gentisic acid were most bitter. The latter are of particular importance because marama bean cotyledons contain protocatechuic acid and gallic acid (van Zyl, 2007). However in other plant foods such as olive oil, phenolic acids including *p*-cumaric acid, caffeic acid, sinapic acid, and ferulic acid have also been reported as contributing to bitterness (Bruhl, Matthaus, Fehling, Wiege, Lehmann, Luftmann, Bergander, Quiroga, Scheipers, Frank, & Hofmann, 2007).

Sensory attributes of food products are determined to a large extent by the derivatives of cinnamic acid than by those of benzoic acid (Troszynska, 2004). According to this author, caffeic acid forms chlorogenic acid with quinone. The concentration of chlorogenic acid affects considerably the sensory quality of food, since it contribute to enzymatic browning of food products, thus evoking their astringency. Sinapic, ferulic and coumaric acids can form esters with choline, which in turn generate bitterness, astringency, pungent taste and irritating flavour in some products made from vegetables and seeds of the *Cruciferae* family.

Benzoic acid



Cinnamic acid



Acid	R1	R2	R3	Acid	R1	R2	R3
p- Hydroxybenzoic	H	OH	H	*p*- Coumaric	H	OH	H
Protocatechuic	OH	OH	H	Caffeic	OH	OH	H
Vanillic	OCH_3	OH	H	Ferulic	OCH_3	OH	H
Syringic	OCH_3	OH	OCH_3	Sinapic	OCH_3	OH	OCH_3
Gallic	OH	OH	OH				

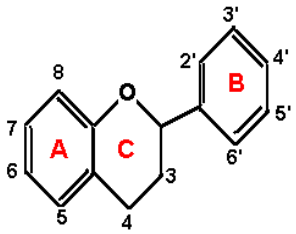
Figure 2.3: Chemical structure of phenolic acids indicating the benzoic acids and cinnamic acids derivatives (Shahidi & Naczk, 2004)

2.3.1.2 Flavonoids

Flavonoids, the largest group of phenolic compounds (Drewnowski & Gomez-Carmeros, 2000) contain a benzopyran nucleus with aromatic substituent at carbon 2 of C ring (Figure 2.4) (Shahidi & Naczk, 2004). Flavonoids are found in plants either as glycosides (esterified to sugar molecules) or aglycones (not esterified to a sugar molecule). Flavonoids have a C6-C3-C6 structural skeleton (Shahidi & Naczk, 2004).

Flavonoid sub-groups are classified based on substitution pattern of the C ring and position of the B ring. The flavonoid sub-groups include flavonol, flavone, flavonone, flavanol, anthocyanidin and isoflavone. The flavonoids quantified from marama bean cotyledon are fisetin (39.2 mg/100g), rutin (25.7 mg/100g), quercetin (23.5 mg/100g), kaempferol (20.0 mg/100g), myricetin (19.1mg/100g), naringin (9.9mg/100), catechin (11.5 mg/100g) and hesperidin (8.9 mg/100g) (van Zyl, 2007).

The sensations of astringency and bitterness elicited by flavonoid compounds have been reported more for fruits and beverages than for legumes. However, the flavan-3-ol monomers (catechin, epicatechin, epigallocatechin, epicatechin and gallate), their oligomers and polymers, which are called proanthocyanidins or condensed tannins, are the most abundant in wine and tea (Czochanska, Foo & Porter, 1979). Both procyanidins (polymers of epicatechin and catechin) and prodelphinidins (polymers of epigallocatechin) have been detected in grapes (Czochanska *et al*., 1979; Troszynska, 2004). Variation in phenol composition such as molecular size or chain length (monomer, dimer, trimer), extent of galloylation, small differences in configurations such as stereochemistry of sub-units (catechin or epicatechin) and site of linkage between the sub-units (C4→C6 or C4→C8) produce significant differences in the intensity and duration of the bitterness and astringency of phenolic compounds (Peleg, Gacon, Schilch & Noble, 1999).



Kaempferol: 3, 5, 7, 4': OH

Quercetin: 3, 5, 7, 3', 4': OH

Catechin: 3, 5, 7, 3', 4': OH

Figure 2.4: General structure of Flavonoids (Shahidi & Naczk, 2004)

According to Troszynska (2004), the threshold recognition values of bitter taste established in water for catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate are 600; 600; 350, 200; and 300 mg/kg, respectively. Whereas the threshold recognition values of epicatechin gallate and epigallocatechin gallate for astringency are 500 and 600 mg/kg, respectively. Flavonols manifesting bitter taste include quercertin (Troszynska 2004, Drewnowski & Gomez-Carmeros 2000), which is an aglycone of quercitrin, rutin and other glycosides. The recognition threshold of quercetin in 5% aqueous ethanol and in beer is low and accounts for 10 mg/kg, whereas the detection threshold value of quercitrin (quercetin 3-rhamnoside) in beer is considerably higher and reaches 250 mg/kg (Troszynska, 2004). Another intensively bitter compound belonging to the flavanones is naringin which is responsible, among others, for the bitter taste of grapefruits as well as tomatoes and

their juices (Troszynska, 2004). The recognition threshold value of naringin causing bitterness is 25 mg/kg (Troszynska, 2004).

2.3.1.3 Isoflavones

Isoflavones are commonly known as phytoestrogens. Chemically they contain a benzopyran nucleus with aromatic substituent at carbon 3 of the C ring (Figure 2.5). Genistein, a bitter and astringent isoflavone glucoside, is thought to be responsible for the objectionable taste of soybean (Chang, Huan, & Ho, 1990). They also impart the characteristic taste to the secondary products miso, soybean paste, and soy sauce. According to Holse *et al.* (2010), no isoflavones were found in marama bean, contrary to soya bean which is a rich source of isoflavones.

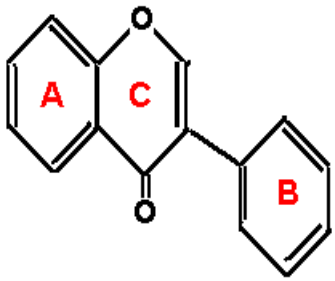


Figure 2.5: General structure of isoflavone (Shahidi & Naczk, 2004)

2.3.1.4 Tannins

Tannins are divided into two main classes: hydrolysable tannins and condensed tannins (Shahidi & Naczk, 2004). Hydrolysable tannins (Figure 2.6) are phenolic carboxylic acids (mainly gallic acid) esterified to sugars such as glucose. They break down into sugars and a phenolic acid (gallic acid) upon hydrolysis with acid, alkali or hydrolytic enzymes. Condensed tannins (Figure 2.6) are polymers of flavan-3-ol units. They yield anthocyanins upon heating in acidic media and so are called proanthocyanins or (proanthocyanidins) (Shahidi & Naczk, 2004).

Polyphenols of high molecular weight such as condensed tannins are predominantly bitter and astringent (Drewnowski & Gomez-Carmeros, 2000). Shelembe *et al.* (2012) reported the presence of condensed tannins in marama beans seed coats,

however no research has been publish on whether or not marama bean cotyledons contain condensed tannins.

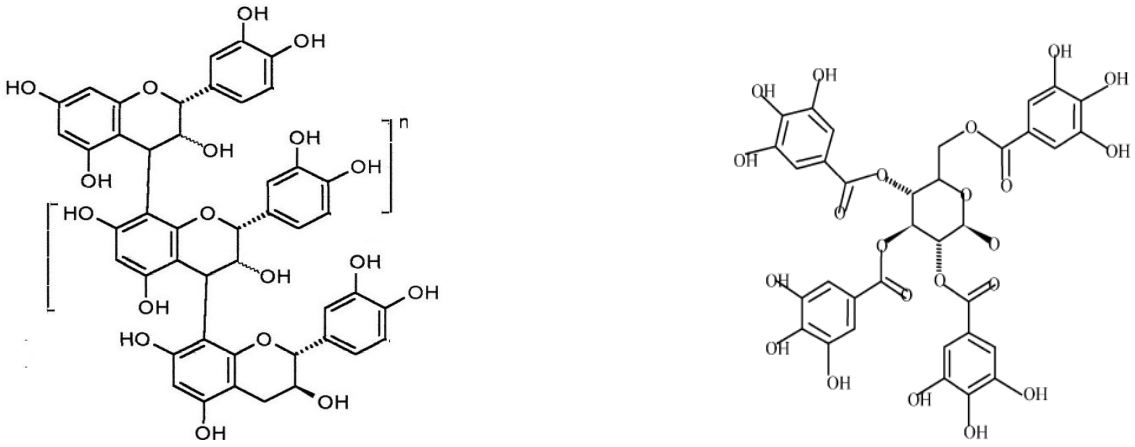


Figure 2.6: Structure of condensed tannins(n=1 to>10) and hydrolysable tannins (Shahidi & Naczk, 2004)

2.3.2 Saponins in legumes

Saponins are secondary metabolites that occur in a wide variety of legume seeds, such as peas, soybeans, lentils and lupins (Heng, Vincken, van Koningsveld, Legger, Gruppen, van Boekel, Roozen & Voragen, 2006). They are known to have a bitter taste and possess health-beneficial effects, such as lowering of cholesterol levels (Heng *et al.*, 2006). Saponins are glycosides; that is, they are composed of a carbohydrate and non carbohydrate or aglycone components (Rochfort & Panozzo, 2007). The aglycones are often referred to as sapogenin. The sapogenin nucleus may be either of steroid or triterpenoid structure (Shi, Arunasalam, Yeung, Kakuda, Mittal, & Jiang, 2004). The aglycone (or sapogenin) is linked to one, two or three saccharide chains of varying size and complexity via ester and or ether linkages. The most common linked sugars are galactose, arabinose, xylose and glucose (Khokhar, & Apenten, 2003). Saponins are non-volatile, amphiphilic and surface-active (Khokhar & Apenten, 2003; Rochfort & Panozzo, 2007). The amphiphilic behaviour of saponins is a result of opposing lipophilic and lipophobic characteristics of the carbohydrate and aglycone moieties; thus the structure affects its chemical behavior and leads to the expression of many of its biological properties (Khokhar & Apenten, 2003). The amphiphilic character also makes them natural surfactants because the carbohydrate portion of the molecule is water-soluble while the sapogenin is fat-soluble. This detergent property leads to considerable foaming of aqueous saponin

solutions. The characteristic honey-comb froth produced is often used in plant-screening work as presumptive evidence of the presence of saponins in the plant (Khokhar & Apenten, 2003).

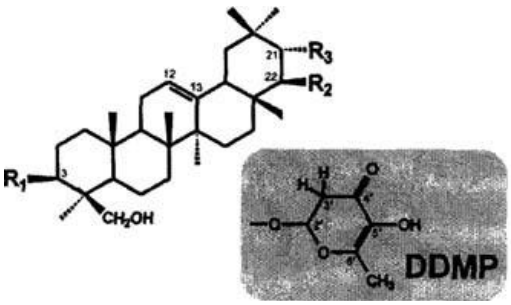
Saponins are generally categorized into three main groups, on the basis of their aglycone (soyasapogenol) structures: groups A, B and E (Figure 2.7) (Campos-Vega *et al.*, 2010; Heng *et al.*, 2006). The aglycones of group A saponins have a hydroxyl group at the C-21 position, whereas those of group B saponins have a hydrogen atom. Group E saponins differ from group B saponins in that their aglycones have a carbonyl group at C-22 (Heng *et al*., 2006). Group A saponins are bisdesmoside saponins, having sugar chains at the C-3 and the C-22 positions of their aglycones (soyasapogenol A), whereas Groups B and E saponins are monodesmoside saponins, having a sugar chain linked to only the C-3 position of their aglycones (soyasapogenol B and soyasapogenol E, respectively) (Heng *et al.*, 2006).

Group A saponins may contain acetyl groups attached to the terminal sugar residue of the C22 oligosaccharide chain (Heng *et al*., 2006). Group B saponins may contain a DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) moiety at C-22, which, upon heating, is released as maltol (Heng *et al.*, 2006). Saponin B (soyasaponin I) has been reported as the main saponin component in green peas. However, it was not until recently that DDMP saponin (soyasaponin) was also identified in peas (Heng *et al.*, 2006). DDMP saponin is widely distributed in legumes and is the predominating saponin in soybean (Heng *et al.*, 2006).

Saponin B is the major saponin in processed soy products and heated pea products, presumably due to the conversion of DDMP saponin to saponin B during extraction and processing. In addition, reports have shown that DDMP saponin is converted to saponin B in acidic or basic solutions (Heng *et al.*, 2006).

Saponin content may be variable, even among the same species of edible beans, because of variations in cultivars, varieties, locations, irrigation condition, type of soil, climatic conditions, and year during which they are grown (Khokhar & Apenten, 2003). The saponins content of some legume seed are given in Table 1. Cooking and processing can have a significant effect on the levels of available saponins in legumes.

Interestingly, the results are not necessarily the same for all legumes. Soaking and cooking studies on chickpeas and lentils suggest that 2-5% of saponins can be lost from chickpeas during cooking, but much greater 6-14% can be lost from lentils (Rochfort & Panozzo, 2007). The method of cooking has significant effects on saponins loss, with autoclaving having a large effect. Some saponins are thermolabile and may interconvert or degrade (Rochfort & Panozzo, 2007).



saponin type	R_1	R_2	R_3
A	$-O-(Gly)_m$	$-O-Gly-[Gly-(OAc)_n]$	–OH
B	$-O-(Gly)_m$	–OH –O–DDMP	–H
E	$-O-(Gly)_m$	=O	–H

Figure 2.7: Structure of saponins (Heng *et al.*, 2006)

Table1: Saponins content of some edible legume (**Khokhar & Apenten, 2003)**

Source	Saponins content (% dry weight)
Soybeans (*Glycine max*)	5.6
Chickpea (*Cicer arietinum L.*)	3.6
Lucerne(*Medicago sativa*)	2.5
Lupine (*Lupinus angustifolus*)	1.5

2.3.3 Alkaloids in legumes

Alkaloids are groups of secondary metabolites, which contain nitrogen-bearing molecules. Many alkaloids are physiologically active and can be used in small quantities as medicines, but if taken in larger doses they can be extremely poisonous. (Australia New Zealand Food Authority, 2001). In general, the majority of alkaloids from edible legumes have been reported from lupins. Lupins are often referred to as either bitter or sweet. Bitter lupins, such as Lupini beans have high concentration of alkaloids (mainly sparteine), which make them bitter to the taste (Australia New

Zealand Food Authority, 2001). The alkaloid trigonelline was also reported in peas, chickpeas, and lentils. Although there may be trace amounts of alkaloids present in legumes cultivated for human consumption, preparation often removes these chemicals (Rochfort and Panozzo, 2007). Soaking prior to cooking also removes a significant proportion of alkaloids in lupin seed (Australia New Zealand Food Authority, 2001). However no scientific reports exist on whether marama bean contains alkaloids or not.

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2.3.4 Cyanogenic glycosides

Cyanogenic glycosides (CNglcs) belong to those secondary metabolites which are classified as phytoanticipins. When plant tissue containing CNglcs is disrupted, the CNglcs are brought into contact with degrading enzymes which cause release of toxic hydrogen cyanide (HCN), an aldehyde or ketone and glucose. However, they are described as having a bitter taste (Gosselin, Hodge, Smith & Gleason, 1976). Bitter almonds oil contains amygdalin, which may be decomposed by digestive enzymes to form hydrocyanic acid (HCN), glucose and benzaldehyde (Gosselin *et al*, 1976). According to Holse *et al*. (2010), marama beans do not contain either cyanogenic glycosides or the enzymes that break these down to hydrogen cyanide.

2.3.5 Lipid oxidation products

According to Sessa, Warner & Rackis (1976), legumes contain unsaturated lipids that are susceptible to oxidative deterioration. Enzymic and non enzymic deterioration of these lipids results in the development of off-flavours. In soya bean, the interaction of lipid breakdown products with proteins, carbohydrates, and other constituents can affect flavour characteristics and also increase the problems of their removal from soy protein products. Protein-bound lipids are responsible to a large extent for the bitter, astringent, and rancid flavours of protein products prepared from raw soybean meal (Rackis, Sessa & Honig, 1979).

Lipoxygenase is a major contributor to the off-flavours in legume protein products. Lipoxygenase mediates conversion of polyunsaturated fatty acids to aldehydes and alcohols (Sessa, Warner & Honig, 1974). Development of off-flavors can be readily

controlled by rapid inactivation of lipoxygenase with heat, alcohol, acid, or antioxidants treatment. A novel 5-substituted-2-furaldehyde from linolenic acid decomposition is released from bitter tasting soy phospholipids. Non-volatile oxygenated fatty acids are also generated in model systems with soybean lipoxygenase and linoleic acid or its hydroperoxide in the presence of electron donors. Similar fatty acids can also arise from action of cysteine-Fe^{3+} on linoleic acid hydroperoxides, are found on bitter-tasting soy phosphatidylcholines (SPC), and are produced by soy lipoxygenases acting on purified SPC substrates.

A bitter taste whose intensity increases during maturation contributes to the flavour of soybeans (Rackis *et al*., 1979). In stored soybeans Sessa *et al*. (1974, 1976) localized the bitter taste to one lysolecithin and two lecithin fractions, which together represented at least 0.08% of the defatted flour. The authors found that autoxidized soy lecithins are extremely bitter with thresholds in the range of 0.006%. Further experiments suggested that it was not the phosphocholine moiety of the lecithin molecule but rather a bound oxidized fatty acid that was responsible for the bitter taste. Enzymatic oxidation of linoleic acid by use of a protein preparation of soybeans with lipoxygenase and peroxidase activities generates fatty acids, which after emulsification with a sugar ester taste bitter (Sessa *et al*., 1974, 1976). The main bitter substance was identified as a mixture of 9,12,13- trihydroxyoctadec trans-10-enoic acid and 9,10,13-trihydroxyoctadec-trans-11-enoic acid. These trihydroxy compounds (Tri-OH) exhibit a bitter threshold of 0.64.9 pmol/mL that increases threefold when the double bond is hydrogenated (Sessa *et al*., 1976). The occurrence of Tri-OH in bitter-tasting soybean flakes was established by Sessa *et al*. (1976).

Raw peanut contains active enzymes that can adversely affect flavour. In roasted peanuts, metalloproteins can initiate the formation of off-flavours derived from lipid oxidation (Rackis *et al.*, 1979). Raw peanut has a grassy flavour that can be eliminated by steaming; however, depending upon time and temperature of steaming, a bitter taste will develop (Rackis *et al*., 1979).

Marama beans are rich in unsaturated fatty acids such as oleic acid (48.5%) which is the most abundant fatty acid in marama bean, followed by linoleic acid (19.2%), linolenic (2%) and palmitoleic (1.7%) (Kayitesi, 2009). From a food oxidation point

of view, lipids with high levels of unsaturated fatty acids are important to take into consideration because they are good substrates for lipoxygenase. However, unlike soy bean, marama beans do not contain lipoxygenase (Maruatona, 2008). This will then prevent the conversion of unsaturated fatty acids to hydroperoxides, which undergo rapid degradation to give a range of compounds such as aldehydes, ketones and alcohols that may lead to development of off flavours including bitterness in marama bean.

2.3.6 Amino acids and peptides

Free amino acids can contribute to the flavour including bitter taste of protein-rich foods such as soya beans (Dajanta, Apichartsrangkoon, Chukeatirote & Frazier, 2011), soya sauce, miso, cheese (Kirimura, Shimizu, Kimizuka, Ninomiya & Katsuya, 1969), fish and meat (Belistz, Grosch, Schieberle, 2009). Studies of sensory charateristics of pure amino acids in powder form or in aqueous solution show that various amino acids can be described as being sweet, sour, salty, bitter, monosodium L-glutamate- like (umami), or otherwise (Kirimura *et al*., 1969). Arginine has a bitter taste accompanied by slight sweetness, whereas serine has a sweet taste accompanied by sourness and a monosodium glutamate (MSG)-like taste. Glutamic acid is a combination of the sour taste with a taste like MSG. In alanine the sweet taste is combined with a slight taste like MSG. In general, the tastes of the individual amino acids are complex and need to be described by more than one taste characteristic. Of the amino acids tested, the taste intensities of alanine, arginine, glutamic acid, serine, and threonine increased as their concentrations were increased. The author concluded that contribution of amino acids to flavour of foods often exceeds the taste properties of the pure compounds. Belistz *et al.* (2009) reported that taste quality of an amino acid is influenced by the molecular configuration and the taste intensity is influenced by hydrophobicity of the side chains. Bitter amino acids are generally within the L-series whereas sweet amino acids are primarily found among members of the D-series. Table 2 provides the taste quality and taste intensity of some amino acids. L-tryptophan and L-tyrosine are the most bitter amino acids with a threshold value of 4-6 mmol/l. D-tryptophan is the sweetest amino acid with a threshold value of 0.2-0.4 mmol/l. The bitter taste of L-amino acids can interfere with the utilization of these acids, e.g. in defined diets (Belistz *et al*., 2009). Moreover L-phenylalanine in soy

products has been described as phenol-like, bitter, astringent, or sour (Drewnowski & Gomez-Carmeros, 2000). Marama cotyledons contain arginine (4.02 g/100 g), phenylalanine (2.65 g/100 g), histidine (1.57 g/100 g), methionine (0.46 g/100 g), valine (2.72 g/100 g), isoleucine (2.46 g/100 g), leucine (3.64 g/100 g) and tyrosine (7.70g/100g) (Maruatona, 2008). However, the possible significance of amino acids in marama flavour particularly bitterness has not been given much attention.

Peptides are formed by binding amino acids together through an amide linkage (Belistz *et al.*, 2009). While the taste quality of amino acids does depend on configuration, peptides except for the sweet dipeptide esters of aspartic acid, are neutral or bitter in taste with no relationship to configuration (Belistz *et al.*, 2009). As with amino acids, the taste intensity is influenced by hydrophobicity of the side chains. The taste intensity does not appear to be dependent on amino acid sequence (Belistz *et al.*, 2009). Bitter tasting peptides can occur in food after proteolytic reactions. For example, the bitter taste of cheese is a consequence of faulty ripening. On the other hand Kirimura *et al.* (1969) reported that the taste characteristics of peptides are complex, but can be classified into three groups: Compounds in Group 1 have a sour taste; those in Group 2 have a bitter taste; and those in Group 3 have little or no taste. Peptides such as L-Ala-L-Asp, L-Glu-L-Glu, and Gly-L-Asp-L-Ser-Gly are sour, peptides such as L-Leu-L-Leu, L-Arg-L-Pro, and L-Val- L-Val-L-Val are bitter, and peptides such as L-Lys-Glu, L-Phe-L-Phe, and Gly-Gly-Gly-Gly have little or no taste.

According to Kirimura *et al.* (1969), it appears that a relationship exists between the patterns of component amino acids and the taste of dipeptides. Dipeptides which tasted sour contained two acidic amino acids, and neutral-amino acids, or acidic- and aromatic-amino acids. It is presumed that the sour taste is caused by hydrogen ions arising from the dissociation of dipeptides (Kirimura *et al*., 1969). Dipeptides which tasted bitter contained neutral amino acids with either large alkyl groups or a combination of large and small alkyl groups, neutral and aromatic amino acids, or neutral and basic amino acids. Dipeptides having little or no taste were composed of two amino acids with small alkyl groups, acidic and basic amino acids, or two aromatic amino acids**.** It is interesting that dipeptides composed of sweet amino acids, Gly-Gly, Gly-L-Ala, and Gly-L-Pro, are almost tasteless. The disappearance of

sweetness may be a result of chain lengthening (Kirimura *et al*., 1969). The combination of an acidic and a basic amino acid such as L-Lys-L-Glu and L-Arg-L-Glu is also tasteless. This may be due to interactions between the amino groups and the carboxyl groups in the dipeptide molecule (Kirimura *et al*., 1969). Dipeptides composed of two aromatic amino acids, such as L-Phe-L-Phe, had essentially bitter taste. As for amino acids the possible significance of peptides in marama flavour has not been investigated.

Table 2: Taste of some amino acids in aqueous solution at PH (6-7) (Belistz *et al.*, 2009)

Amino acid	**Taste**			
	L-compound		**D-compound**	
	Quality	**Intensity** (mmol/l.)	**Quality**	**Intensity** (mmol/l.)
Arginine	bitter		neutral	
Histidine		45-50	sweet	2-4
	bitter			
Isoleucine		10-12	sweet	8-12
	bitter			
Leucine		11-13	sweet	2-5
	bitter			
Lysine		80-90		
	bitter			
Phenylalanine		5-7	sweet	1-3
	bitter			
Proline		25-27		
	bitter			
		25-40	neutral	
	sweet			

2.4 Human perception of bitter taste and different methods of bitterness evaluation.

In human beings, the tongue is the primary organ of gustation (Kim, Breslin, Reed & Drayna, 2004; Latha & Lakshmi, 2012). The upper surface of the tongue is covered in papillae, taste buds and pink tissue called mucosa are also present (Latha & Lakshmi, 2012). According to these authors, the function of mucosa is to keep the tongue moist.

Tiny bumps called papillae gives rough structure to the tongue. Several thousands of taste buds are present on the surfaces of the papillae. Taste buds are collection of nerve cells that connect to nerves running into the brain. Different lipid molecules present in the taste buds of tongue plays a key role in sensing taste of food materials. There are five basic tastes: sweet, sour, salty, bitter and umani (Kim *et al*., 2004; Latha & Lakshmi, 2012). The fifth taste, called umami, results from tasting glutamate (Latha & Lakshmi, 2012). Since the tongue has many nerves that help detect and transmit taste signals to the brain, because of this, all parts of the tongue can detect the basic tastes (Latha & Lakshmi, 2012). Research has shown that TAS2Rs (taste receptors, type 2, also known as T2Rs) such as TAS2R38 coupled to the G protein gustducin are responsible for the human ability to taste bitter substances (Bartoshuk, 1993). Bitter taste is perceived by many to be unpleasant, sharp, or disagreeable (Ikeda, 2002). According to Bartoshuk (1993), bitter taste perception has been linked to sensitivity to 6-n-propylthiouracil (PROP), in that some people can taste bitter (taster) whilst others cannot (non tasters).

Currently, various methods have been developed for bitterness evaluation, such as the human gustatory test, the animal gustatory test, electronic tongues (taste sensors), etc. Human gustatory test is the most direct method for bitter evaluation and can directly express taste perception exactly after taste tests (Chen, Wu, Zhao, Xu & Hu, 2012). According to these authors, normal subjects without genetic defects in bitter perception can qualify bitterness evaluation without taste training. However, results from gustatory tests are reliable only after statistical analysis and results from individuals sometimes contain errors (Chen *et al*., 2012). But after the training process, the accuracy of bitter evaluation can be significantly increased. Human gustatory test is hard to perform due to the high cost and long testing time. The potential safety and ethical problems also need to be considered (Chen *et al*., 2012). Animal gustatory test is an alternative to the human gustatory test where animals (e.g., rodents, dogs and some other mammals) are used for bitterness evaluation in view of their similar bitter taste receptors to those of human beings (Meyerhof, 2005; Stark, Bareuther & Hofmann, 2005). The electronic tongue is an instrument that measures and compares taste (Chen *et al*., 2012). The electronic tongue includes benefits like screening the taste attributes of formulations in a rapid time frame and reducing reliance on human panel (Liyama, Ezaki, Toko, Matsuno & Yamafuyi, 1995).

Sufficient aqueous solubility of test compounds is necessary for application of the electronic tongue. Normally, the electronic tongue has three principal components, sensory array, the equipment of emitting and receiving signals and patter recognition (Liyama *et al*., 1995). The electronic tongue consist in imitates what is happening when molecules with specific taste nature interact with taste buds on the human tongue (Latha & Lakshmi, 2012; Chen *et al*., 2012). The taste buds are represented by sensors which interact with these molecules at the surface initiating changes in potential. These signals are compared with physiological action potentials which are recorded by computer which correspond to the neural network at the physiological level (Latha & Lakshmi, 2012; Chen *et al*., 2012). The data obtained can further be evaluated on the basis of already existing matrix of sensors responses which can be compared with human memory or association to already existing taste patterns (Liyama *et al*., 1995).

2.5 Method of analysis and quantification of phenolic compounds

Since marama bean cotyledons contain phenolic compounds, a review on the method of analysis and quantification of phenolic compounds is important because potential bitter phenolics in marama cotyledons will have to be identified and quantified. Usually, before any analysis, phenolics must first be extracted from their source. Previously van Zyl, (2007) and Kayitesi (2009) identified phenolic compounds in marama bean using acidified methanol as the extraction solvent. Various solvents including ethanol, acetone, water, etc. have been used for extraction of phenolic compounds (Shahidi & Naczk, 2004). Different solvents with differing polarities will extract phenolics differently (Shahidi & Naczk, 2004). Yu, Ahmedna & Goktepe (2005) reported that ethanol and methanol were more effective in extracting phenolic compounds from peanut skin than water, with ethanol (80%) being the most efficient extraction solvent. Phenolic extracts of plant materials are always a mixture of different classes of phenolics that are soluble in the particular solvent system used (Shahidi & Naczk, 2004). Additionally, the recovery of phenolics can be affected by the extraction period. Extraction periods varying from 1min (Price & Butler, 1977) to 24h (Burns, 1971; Maxson & Rooney, 1972) have been reported. However, longer extraction times increase the possibility of oxidation of phenolics unless reducing agents are added to the solvent system (Shahidi & Naczk, 2004). For example the

optimum extraction time required for dry bean phenolics has been reported to be 50 to 60 min (Deshpande, 1985). Moreover, the recovery of polyphenols from food products is also influenced by the ratio of sample to solvent. Shahidi & Naczk (2004) reported that changing the solvent from 1: 5 to 1:10 increases the extraction of condensed tannins (257.3 to 321.3 mg/100g) and total phenolics (773.5 to 805.8mg/100g) from commercial canola meals when using 70% acetone. The sample particle size also plays an important role in recovery of phenolics. Previously, Deshpande & Cheryan (1985) showed that the yield of tannin recovery for dry beans is strongly influenced by variation in the sample particle size. They found that 0.5% vanilin-assayable tannins decrease by about 25 to 49% as the minimum size is reduced from 820 to 250 *u*m.

Methods used for analysis of phenolic compounds may be classified as those that determine total phenolics content and do not distinguish between types of phenolic compounds such as spectrophotometric assays like the Folin-Ciocalteu assay (Shahidi & Naczk, 2004) or those indentify and quantifying an individual phenolic e.g. High Performance Liquid Chromatography (HPLC).

2.5.1 Spectrophotometric assays

A number of assays for phenolic compounds are based on the use of UV-visible spectrophotometry (Shahidi & Naczk 2004). It involves measuring the amount of light of a particular wavelength (UV or visible 220 – 850 nm) that is absorbed by a liquid sample or phenolic extract using a spectrophotometer (Shahidi & Naczk 2004). The absorbance is used to determine the concentration of a particular group of phenolics or a range of phenolics. Spectrophotometry is governed by the Beer-Lambert law (Huber, 2007). This law states that the fraction of light absorbed by a solution is proportional firstly to the distance the light travels through the solution (the path length), secondly to the chemistry of the substance or part of the substance responsible for absorbing the light (the chromophore), and thirdly to the concentration of that chromophore. Intensity of incident light (Ib) and emergent light (Ia) that has passed through the solution is related to the concentration (c) and distance travelled (l) by log Ib/Ia = a.l.c (where a is a constant) (Lakowicz, 1999).

The Folin-Ciocalteu assay is often used to determine the total content of dry beans phenolics (Shahidi & Naczk, 2004). The Folin-Ciocalteu assay is not specific and detects all phenolic groups found in extracts including those found in extractable proteins (Shahidi & Naczk, 2004). A disadvantage of this assay is the interference of reducing substances such as ascorbic acid with the determinations (Shahidi & Naczk, 2004).

2.5.2 High Performance Liquid Chromatography (HPLC) analysis

In HPLC analysis, separation of compounds in a mixture occurs more efficiently and also more quickly than that of traditional column chromatography (Shahidi & Naczk, 2004). The separation of compounds is due to their relative differences in the rate at which they move through the column on application of pressure exerted through a mobile phase or carrier liquid (Huber, 2007). The compound of the mixture travel with different rates due to their relative affinities to the solvent and stationary phase. Compounds with higher affinity towards the stationary phase of the column travel slowly and vice versa. The separation is more effective due to greater surface area achieved due to very small particle size of the stationary phase in comparison to that used in column chromatography (Huber, 2007).

2.5.2.1 Types of HPLC

HPLC analyses can differentiated based on the stationary phase in the column used or based on the purpose of use (Huber, 2007). The stationary phase in the column can be either normal or reverse phase. In the normal phase chromatography, the column stationary phase is made of polar compounds like silica gel, alumina etc. The polar compounds or molecules in the sample being analysed have higher affinity for the stationary phase and so they are retained longer in the column than non polar ones. Hence non-polar compounds are eluted first due to their affinity for the non-polar mobile phase while polar ones are eluted later (Huber, 2007). In the reverse phase HPLC, exactly the opposite of normal phase happens. The stationary phase is made of non polar compounds like C_{18} and C_8 type of organic compounds. The mobile phase used is polar. So compounds of high polarity elute first while those of low polarity or no-polarity are eluted last (Huber, 2007). Based on the purpose of use, HPLC can used for either analytical or preparative purposes. In analysis mode: the procedure is done to estimate different types of molecules and their individual quantities in a

mixture with the help of a detector (Huber, 2007). In preparative mode, the intension of the process is to separate large amounts of a specific compound or substance from a mixture. The substance eluted is of highest purity. The column and sample size are comparatively larger than that of analytical mode (Huber, 2007).

An advantage of HPLC is the fact that it can be used for both qualitative and quantitative analysis. The HPLC method evaluates almost all the compounds within the same family (Shahidi & Naczk, 2004). Molecules with small differences in absorption wavelengths can be detected well due to their differences in separation time (Huber, 2007). Substances in very low concentration like nanogram quantities can be detected due to the sensitivity of HPLC detectors used such as electrochemical and fluorescence detectors. Due to its separation efficiency, the quality of substance obtained by preparative HPLC tends to be of high purity (Shahidi & Naczk, 2004). HPLC is an expensive technique as it requires costly HPLC instrumentation, columns and also use of highest grade purity solvents, buffer, chemicals etc (Huber, 2007).

2.6 Gaps in knowledge

One of the major forms in which the marama bean is utilized is as a roasted snack. Research has been done on chemical and nutritional composition, physicochemical, functional and sensory properties of the marama flour. However, little information is available on sensory properties of the roasted bean. No research has been published on the compounds that could be responsible for a bitter taste in heated in marama beans. The bitterness of marama has not been studied and /or quantified. This study investigates some of the compounds that may contribute to bitterness in marama bean. It envisaged to quantify them and to understand the relationship between the bitter compounds, the heat treatment and the sensory profiles of roasted marama beans. This would provide more information regarding utilization in the food systems, in terms of sensory properties of the roasted marama bean.

2.7 Hypotheses

The sensory profile of marama beans roasted at 150ºC for three different times periods (20, 25 and 30 min) will differ. Marama cotyledons served as pastes of beans roasted for more than 20 min will progressively increase in bitterness intensity as a function of time and will taste less nutty. This is because long roasting time is associated with bitter taste resulting from over-roasting (Tomlins, Rukuni, Mutungamiri, Mandeya, & Swetman, 2007). More extensive heating increases the amount of melanoidins formed via Maillard reaction (Baker, 2002). Melanoidins are responsible for the brown colour and may result in undesirable flavours such as bitterness. Consequently the nutty flavour will be masked by the bitter taste and burnt flavour.

Water extracts of marama bean flour prepared from roasted cotyledons will taste more bitter than water extracts prepared from flour of raw (unroasted) marama cotyledons. Heat treatment such as roasting can increase the permeability of cell walls (Ee, Agboola, Rehman & Zhao, 2011), cleave covalent bonds or break down insoluble phenolics and release bounds phenolic compounds from other compounds to which they are bound (Dvorak *et al*., 2005). Therefore the heating by roasting will increase the concentration of extractable phenolics. The higher concentration of free phenolics in roasted marama cotyledons and consequently in the water extracts prepared from its flours will be perceived as more bitter.

Heat treatment by roasting for different periods of time will affect the saponins content. Marama beans roasted for a longer period of time will have a lower concentration of saponins. This is because high temperature hydrolyses the glycosidic bond between the sapogenin and the glycosidic residue and thus causes a reduction in saponins (Drumm, Gray, Hosfield & Uebersax, 1990). The presence of saponins will also contribute to a more bitter taste in marama bean cotyledons and in its water extracts. Saponins are believed to impart a high level of bitter taste in edible legumes such as soybeans and peas (Heng *et al.*, 2006).

2.8 Objectives

- To determine the effect of roasting time at 150 ºC on the sensory properties of marama bean cotyledons in the form of pastes and the bitterness of water extracts of flours prepared from the cotyledons

- To determine the effect of roasting time at 150 ºC on the extractable quantities of total phenolic content and specific compounds (protocatechuic acid, sinapic acid, vanillic acid, ferulic acid, quercetin, narigin, as well as saponins) in the water extracts prepared from flours of marama bean cotyledons.

3. RESEARCH

Figure 3.1 show the experimental design of this study based on the specific objectives. The research chapter is presented in the format of a research paper submitted to the Journal of Agricultural and Food Chemistry.

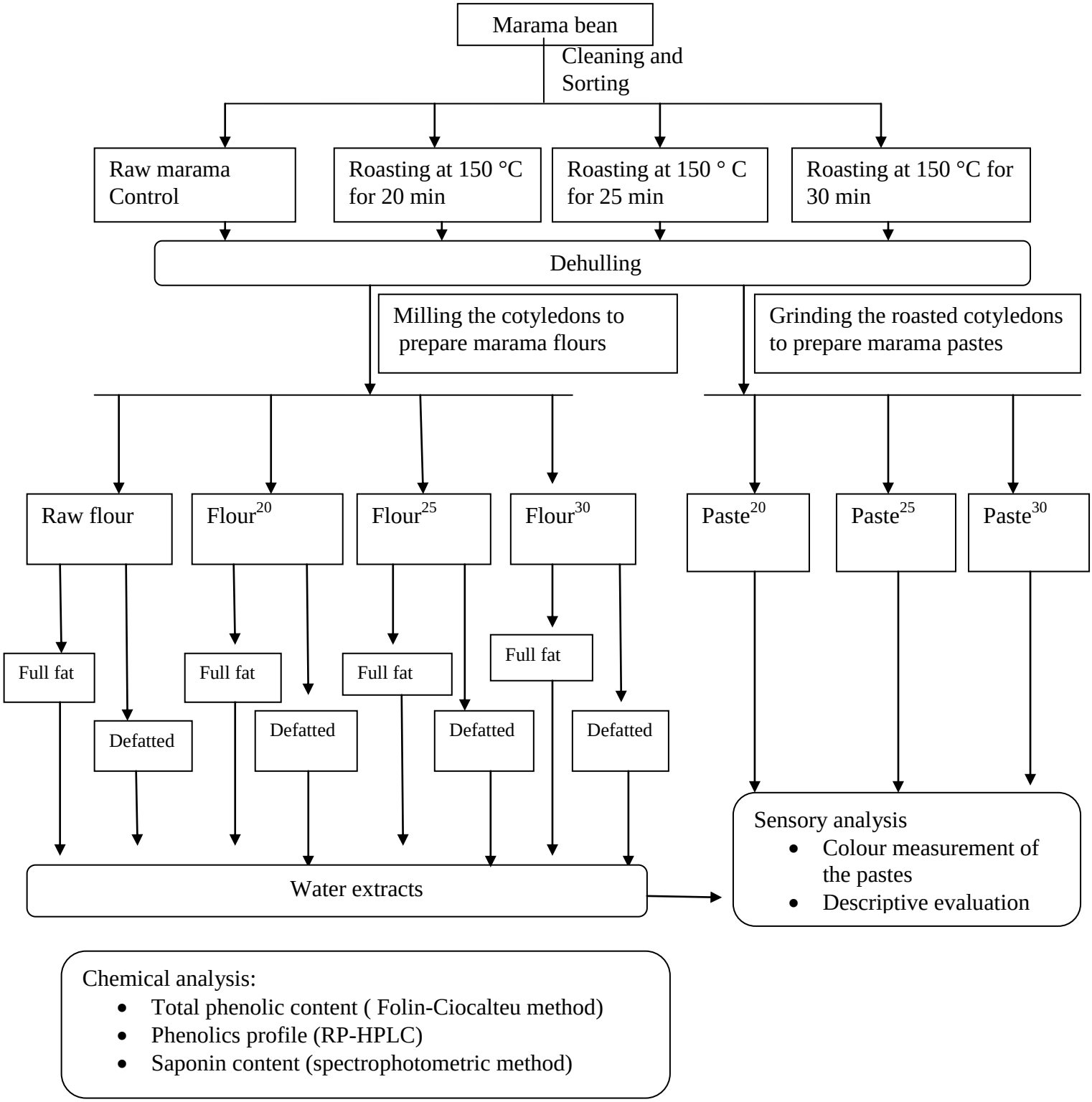

Figure 3.1: Experimental design of the study based on the specific objectives.

Abstract

Marama beans were roasted at 150 °C for different time periods 20, 25 or 30 min. Three pastes were prepared from the roasted cotyledons; paste^{20}, paste^{25} and paste ^{30}. Eight samples of water extracts were prepared from full fat and defatted flours from roasted and unroasted marama cotyledons. Descriptive testing was conducted to rate the intensity of flavour attributes of marama pastes and water extracts. Marama water extracts were analysed for total phenolic content, individual phenolic components as well as saponin content. Roasting marama for more than 20 min result in negative sensory properties such as bitterness. The major extractable phenolic acids present in marama water extracts were gallic and protocatechuic acids which increased as a function of roasting time and may be the cause of more intense bitterness. In addition the presence of high saponins content in marama beans might also contribute to the perceived bitterness.

Keywords: Marama beans, water extracts, roasting, descriptive sensory evaluation, bitter taste, phenolic compounds, saponins.

3.1. Introduction

Marama bean, (*Tylosema esculentum*) is an underutilized legume which grows wild in Southern Africa and forms part of the diet of some of the indigenous population (Jackson *et al*., 2010). Marama bean seeds are not eaten raw as they are tasteless with an unpleasant slimy texture, but after heating they have a delicious nutty flavour, resembling roasted peanuts (Mmonatau, 2005) although bitter types are known. The nutty flavour has been suggested to be caused by the formation and release of flavour compounds due to Maillard reaction as a result of heating (Kayitesi, 2009).

A further reason for heat treating marama beans is to inactivate antinutritional factors. Maruatona (2010) reported that roasting marama beans at 150 °C for 20min is required to inactivate trypsin inhibitors. This time/temperature combination is therefore regarded as a minimum requirement for consumption purposes. Depending on the extent of heating, bitter compounds may develop and contribute to perceived

bitterness in marama bean. Mmonatau (2005) reported an undesirable bitter taste in roasted marama bean when heated at 150 °C for 30 min. Furthermore, Kayitesi (2009) reported a slight bitter taste in sorghum porridges composited with defatted roasted and unroasted marama flours. The bitterness is more pronounced in roasted marama than in raw seed (personal observation). The perceived bitter taste can limit the utilisation and consumption of this pulse. However, no research has been published on the compounds that could be responsible for a bitter taste in marama bean. The present study aimed at determining the effect of roasting time on the perceived bitterness of marama bean in relation to content of compounds that could potentially contribute to bitterness.

3.2. Materials and methods

3.2.1. Preparation of marama paste and water extracts

Marama beans (*Tylosema esculentum*) were collected from Masokaphala and nearby the central district of Botswana. Marama beans were heated at 150 °C in a forced convection continuous tumble roaster (Roastech, Bloemfontein, South Africa). A speed set of 290, 240 and 180 rpm was selected and resulted in a heating time of 20, 25 and 30 min, respectively. A DF cracker (WMC Metal Sheet Works, Tzaneen, South Africa) was used to dehull marama beans and the roasted cotyledons were retained. In order to prepare homogenous samples for descriptive sensory analysis, three marama pastes namely paste^{20}, paste^{25} and paste^{30} were prepared by pulverizing 300 g batches of roasted cotyledons for 15 min using a pot blender.

Full fat and defatted marama flour were prepared from cotyledons of roasted and unroasted beans following the method as described by Maruatona (2008). Defatted marama flour was prepared by coarsely grinding cotyledons using a laboratory Waring blender (Lasec, Johannesburg, South Africa). Oil was extracted using hexane (flour/hexane 1:3w/v) for two hours and the process was repeated twice. The dry residue was then milled again using a laboratory mill (IKA Werke, Staufen, Germany) to pass through a 1000 µm mesh. The samples of marama flour were denoted as full fat and defatted raw marama flour, full fat and defatted roasted marama^{20} flour, full fat and defatted roasted marama^{25} flour, full fat and defatted roasted marama^{30} flour. All

the flours were vacuum packed and stored at 4 °C until used to prepare marama water extracts.

Water extracts were prepared by mixing 10 g of marama flour in 150 ml of deionised water and bringing it to boil for 10 min. The water was decanted and filtered using a tea strainer. Eight samples of water extracts were prepared fresh from full fat and defatted flours of roasted and unroasted marama beans and evaluated within 90 min of preparation. For chemical analysis the extracts were centrifuged at 7500 x g for 10 min (25 ºC) using a Rotanta 460 R centrifuge (Labotech T., Johannesburg, South Africa) prior to the assay.

3.2.2. Descriptive sensory evaluation

A ten person (five male, five female) trained sensory panel at University of Pretoria was used to evaluate the sensory profiles of marama products (pastes and water extracts). The ten panelists were selected from a pool of 16 people after undergoing screening tests. The screening tests included the basic tastes identification test (sweet, salt, bitter, sour and umami), the ability of panelist to correctly describe differences between two different commercial peanut butters (Black Cat and Thokozi peanut butter) and the propylthiouracil (PROP) test following the method of Tepper, Christensen & Cao (2001). The one solution PROP test was used to eliminate panellists who could not taste bitterness. Subjects received a set of two solutions; Salt NaCl (0.1 mol/l) and PROP (0.32 mM). They were asked to swirl the entire sample (10 ml) in their mouth, expectorate it and rate the intensity using a labeled magnitude scale. Subjects rinsed their mouths with filtered tap water before they began and between each sample. The NaCl was tasted first followed by the PROP solution. Subjects also then completed a questionnaire in which they were asked to list three other foods known as tasting bitter. The descriptive sensory panel was trained for 14 h, during hourly sessions twice a week for a period of seven weeks. The training sessions included familiarizing the panelists with the marama products (paste and water extract), assessment procedures, the computer system and sensory evaluation software (Compusense Five® version 5.2, Compusense Guelph, ON, Canada). Descriptive terms and scale anchors were developed, defined and agreed upon for evaluation. Before the actual evaluation the panellists' performance was check at least two times and the Compusense FCM® tool was used to facilitate the training.

3.2.3. Evaluation of marama pastes and water extracts

The sensory evaluation of the marama products was conducted in a sensory evaluation laboratory with individual booths. Panelists evaluated all samples in triplicate during three days with one session per day. Each panelist received three samples (20 g each) of marama bean paste in plastic cups with lids and a set of eight water extracts (10mL each) in glass size 8 polytop containers with lids (Figure 3.2). All samples were coded with 3-digit random numbers and served at ambient temperature (25 °C) on a white tray, with 3 plastic teaspoons for tasting pastes, a serviette and two plastic disposable cups. One cup contained pieces of carrot for mouth neutralizing, and the other cup was filled with filtered tap water for rinsing the mouth before and between tasting the samples. To avoid fatigue and bitter taste build up in the mouth, samples were tasted with a 2 min break in between. Red light in the tasting booths was used to mask the colour of the paste for the panelists in order to concentrate on aroma and flavour properties. The panel used sixteen descriptive terms, grouped under aroma, flavour, texture and aftertaste attributes as shown in Table 3.1. Aroma was evaluated immediately after removing the cover using short sniffs. Then a half spoon (5 mg) of the paste was chewed in the mouth to test for flavour and texture properties. After swallowing, the panelists analyzed the aftertaste properties. Nine-point structured line scales were used to measure the intensity of each attribute for a given sample. Product references to illustrate attribute intensities were provided. The minimum scale value was 1 denoting not intense, not sticky, not grainy or not creamy. The maximum point was 9 denoting very intense, very sticky, very grainy or very creamy. Panelists tasted and expectorated the entire water extract sample and then only rated the intensity of the bitter taste. Evaluation sequence was based on a randomized complete block design.

Figure 3.2: Descriptive sensory evaluation of marama bean paste and water extracts.

Table 3.1: Descriptive sensory attributes used by trained panel to evaluate marama bean paste and water extracts

Attributes	Definitions	References to clarify and anchor sensory attributes
Aroma		
Nutty aroma	The intensity of aroma resembling nuts	Smooth peanut butter (Black Cat) = 7
Roasted aroma	Intensity of aroma resembling roasted nuts	Raw marama bean = 1 Peanut butter (Black Cat) = 7
Burnt aroma	The intensity of aroma similar to burnt food (e.g. toast)	Over roasted marama beans at 150 ^{0} C for 35 min grounded to flour = 8
Flavour		
Nutty flavour	Intensity of flavour resembling nuts	Peanut butter (Black Cat) = 8
Bitter taste	Basic bitter taste associated with caffeine or quinine	10% Nescafe Ricoffee in water = 8
Burnt flavour	The intensity of flavour similar to burnt food	Over roasted marama beans at 150 ^{0} C for 35 min grounded to flour = 8
Roasted flavour	Intensity of flavour resembling roasted nuts	Peanut butter (Black Cat) = 6
Oily(fatty) flavour	Intensity of flavour associated with oils	
Rancid flavour	Intensity of flavour associated with oxidised fats and oils (e.g. rancid butter)	Oxidised stored peanut butter = 8
Texture		
Grainy	Amount of small particles perceived by the tongue when the mass is gently compressed between the tongue and palate.	Smooth paste with no particles= 1 Paste with a clearly detectable quantity of grainy particles= 9
Sticky	Having the property of adhering to a surface in the mouth	
Creamy	Intensity of mouth feel associated with fatty oily products such as dairy cream	Yum yum peanut butter = 7 Water =1
After taste		
Bitter after taste	Basic bitter aftertaste associated with caffeine or quinine	10% Nescafe Ricoffee in water = 8
Burnt after taste	The intensity of aftertaste similar to burnt food	Marama bean roasted at 150 ^{0} C for 35 min = 8

(1= not intense; 9 = very intense)

3.2.4. Colour measurements of marama bean pastes

The colour of the pastes was measured using a Chroma Meter CR-400 (Konica Minolta Sensing, Osaka, Japan). The instrument is calibrated against black and white and the resultant values are scaled on a measure of 0-100, where 100 is the white calibration standard and 0 is the black standard. The measurements included light reflectance expressed in terms of lightness (L*), red/green characteristics (a*) and blue/yellow characteristic (b*).

3.2.5. Determination of total phenolic content of marama bean water extracts

The total phenolic content of marama bean water extracts was determined as catechin equivalents (CE, mg catechin equivalents per g of dry marama flour) using the Folin-Ciocalteu method as described by Waterman & Mole (1994). Deionised distilled water was used as the extraction solvent. Marama water extract (0.5 mL) was added to a 25 mL volumetric flask containing 10 mL distilled water. Folin-Ciocalteu's phenol reagent (2.5 mL) was added and mixed. After 2 min, 7.5 mL sodium carbonate (Na_2CO_3) solution (20 g/ 100 mL) was added and the content was mixed and made up to volume with deionised water. The volumetric flask was then stoppered and mixed thoroughly by inverting several times and allowed to stand for 2 h from the addition of sodium carbonate. The absorbance was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA) and the estimation of total phenolics in the extracts was carried out in triplicate for all the samples. Calculations of total phenolic content were done with the aid of standard curves generated from the absorbances of catechin standard solution of concentration within the range 0 to 1.0 mg/mL.

3.2.6. Determination and quantification of phenolic compounds in water extracts of marama bean flours

Reverse phase HPLC (Kim, Hyun, Kim, Park, Kim, Lee, Chun & Chung, 2007) was used to characterise and quantify the phenolic compounds in the different marama water extracts. The HPLC system used for this analysis consisted on a Waters HPLC system which was equipped with a Waters 2487 dual wavelength absorbance detector and a Waters 1525 binary pump (Waters, Associates, Milford, MA, USA) (Figure

3.3). The separation of the phenolics was carried out on a C18 reversed phase YMC-Pack ODS AM-303 (250 x 4.6 mm i.d 5 µm particle size) column at room temperature (25 °C). A computer-controlled system with Breeze ™ software was employed for collection and analysis of chromatographic data. All the solvents and chemicals used during this assay were of HPLC grade. The two mobile phases used for gradient HPLC elution were (A) 0.1% (v/v) acetic acid in HPLC grade water and (B) 0.1% (v/v) acetic acid in acetonitrile (v/v). Gallic acid, protocatechuic acid, caffeic acid, vanillic acid ferulic acid, sinapic acid, naringin and quercertin were used as standards. The phenolic standards were prepared by dissolving in HPLC grade dimethylsulphoxide. Mixtures of standards were prepared at different concentrations (200 ppm, 100 ppm, 50 ppm, 25 ppm and 10 ppm) and filtered through a 0.45 *u*m PTFE syringe filters before injection into the chromatographic system. Marama water extracts samples in 2 mL eppendrof tubes were each hydrolyzed with a drop of concentrated hydrochloric acid (32 %) than filtered through 0.45 *u*m PTFE syringe filters before injection. The flow rate of mobile phase was set at 0.8 mL/min, and a 10 µL sample volume was injected for the running time of 60 min. The linear gradient of the solvents was as follows: solvent B was increased from 8 to 10 % in 2 min, then increased to 30 % in 25 min, followed by an increase to 90 % in 23 min, then increased to 100 % in 2 min, kept at 100% of B for 5 min and returned to the initial condition.

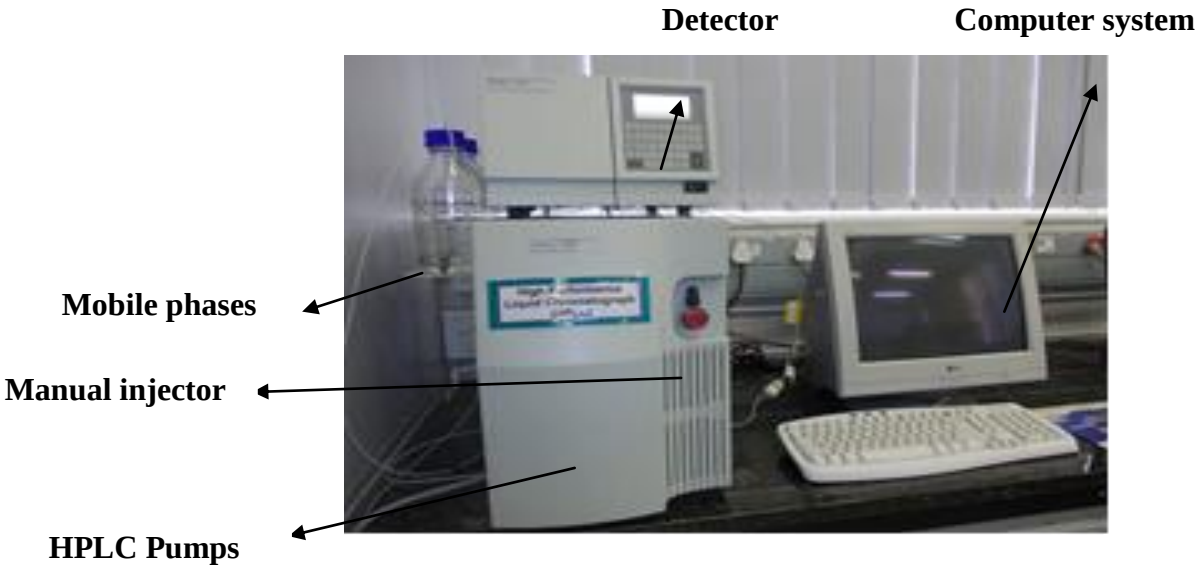

Figure 3.3: Picture of an Water HPLC system

The detection of phenolic compounds was monitored at 280 nm. Chromatographic peaks in the samples were identified by comparing their retention times with those of the reference standards. Quantification was performed from the peak area of each component and its corresponding calibration curve and the amounts were expressed as mg/100 g on dry basis.

3.2.7 Determination of total saponins from marama beans

The extraction of saponins was done following the method of Wang & Allister (2010). A sample (60 g) of coarsely ground cotyledon from unroasted and roasted beans was mixed with 200 mL of petroleum ether and continuously stirred for 2 h in a sealed container at room temperature (25 °C), then filtered through Whatman No. 1 filter paper. The residue was washed with 100 mL of petroleum ether and filtered one more time. After the oil extraction, the ether in the residue was allowed to volatize in a fume cupboard overnight. The sample was then finely milled again to pass through a 1000 µm mesh. The ether-extracted residue was dissolved in 200 mL of distilled water and continuously stirred for 60 min at room temperature, then filtered through Whatman No. 1 filter paper. The residue was washed with 100 mL of distilled water and filtered. The washing operation was repeated one more time and the filtrates were combined. Prior to centrifugation at 5000 x g (10 min, 4 °C) the filtrate was mixed with 5 g of polyvinyl-polypyrolidone to remove phenolic compounds. After freeze drying the supernatant, the dry material was mixed with 100 mL of distilled water and centrifuged at 10000 x g (20 min, 4 °C). The supernatant was mixed with 200 mL of n-butanol and 0.2 mL of concentrated HCl, then held at room temperature for 30 min in order to capture saponins. After centrifuging at 10 000 x g for 10 min at 4 °C, the butanol fraction was collected and the aqueous fraction was subjected to a second n-butanol extraction. Butanol fractions were combined and rotary evaporated at 50 °C to dryness. The residue was dissolved in 100 mL of distilled water, centrifuged (12 000 x g, 20 min, 4 °C), and freeze dried. The dried extracts were store in a sealed container covered with foil at 4 °C.

The dried extracts were analysed for total saponins content as describe by Baccou, Lambert & Sauvaire (1977). Soya saponin (Wako Chemicals, Inc. USA) was used as the standard. Two different mixtures of reagent A and B were prepared for the

analysis. Reagent A consisted of 0.5 mL of anisaldehyde plus 99.5 mL of ethyl acetate and Reagent B was 50 mL of concentrated sulphuric acid plus 50 mL of ethyl acetate. A volume of 1 mL of each reagent was added to test tubes containing soya saponin standard solutions prepared in ethyl acetate of concentration 0-40 *u*g/2 mL and the marama extract of concentration 40 *u*g/2 mL. The test tubes were placed in a water bath maintained at 60 °C for 20 min and then cooled for 10 min in water at room temperature. The absorbance was measured at 430 nm using a Lambda EZ150 spectrophotometer. The estimation of total saponins in the extracts was carried out in duplicate for all the samples by comparing the absorbance of the marama bean extracts with those of the soya saponin standard solutions and using regression equations obtained from standard curves.

3.2.8. Statistical analysis

All sensory and chemical data collected were analyzed using Statistica software Version 10 (Statsoft, Tulsa,OK,USA). Means and standard deviations of descriptive attribute ratings; colour of the pastes, total phenolic content, phenolic compounds profiles and saponins content were calculated separately for pastes and water extracts and analysed using one way analysis of variance (ANOVA). Fisher's least significant differences test at $p < 0.05$ was used to compare means.

3.3. Results

3.3.1. Marama pastes

Roasting marama bean at 150 °C for different time periods 20, 25 or 30 min, respectively, produced light, medium and dark-coloured marama bean pastes as shown in Table 3.2. and Figure 3.4. Paste^{20} was significantly lighter (as indicated by a higher L* value) and had higher b* value (yellowish), while paste^{30} had the lowest L* value and a relatively higher a* value (redness) compare to the others.

Table 3.2: L* a* b* colour values for marama bean paste manufactured from seed roasted at 150 °C for 20 (paste ^{20}), **25** (paste ^{25}) **and 30 min** (paste ^{30})**.**

Type of the paste	L*	a*	b*
Paste^{20}	55.7^{c}(0.17)	9.3 ^{a} (0.15)	29.6 ^{c} (0.12)
Paste^{25}	51.0^{b}(0.21)	11.1 ^{b} (0.06)	27.9 ^{b} (0.24)
Paste^{30}	43.9^{a}(0.30)	12.9 ^{c} (0.15)	23.2 ^{a} (0.15)

L* = Lightness (0=black, 100=white), +a* = red -a* = green, +b* = yellow, -b* = blue
^{abc}= Mean values with different letters within a row differ significantly (p <0.05); standard deviations are given in parentheses.

Paste^{20} **Paste^{25}** **Paste^{30}**

Figure 3.4: Colour of marama bean paste manufactured from seed roasted at 150°C for 20 (paste ^{20}), **25** (paste ^{25}) **and 30 min** (paste ^{30}).

Sensory testing showed that roasting marama beans at 150 °C for different time periods affected the intensity of the flavour and aroma. As shown in Table 3.3 marama pastes (paste^{20}, paste^{25} and paste^{30}) were judged to be significantly different (p < 0.05) from each other in terms of nutty aroma and flavour, roasted aroma and flavour, as well as burnt aroma and flavour. Paste^{20} was the nuttiest followed by paste^{25}, while paste^{30} was the least nutty. Paste^{30} was characterized by the most intense roasted aroma and flavour, burnt aroma and flavour among the pastes. In

addition, paste[30] tasted more bitter and had a more intense roasted, burnt and bitter aftertaste compared to paste[20] and paste[25].

Table 3.3: **Sensory flavour profiles (means ± standard deviations) of marama bean pastes manufactured from seeds roasted at 150 °C for 20** (paste [20]), **25** (paste [25]) **and 30 min** (paste [30])**.**

Attributes	**Paste[20]**	**Paste[25]**	**Paste[30]**
Overall nutty aroma	6.2[c] (1.1)	5.7[b] (0.7)	4.8[a] (0.7)
Roasted aroma	4.8[a] (1.4)	5.7[b] (0.8)	7.2[c] (0.7)
Burnt aroma	3.2[a] (1.4)	4.3[b] (1.7)	7.2[c] (1.8)
Nutty flavour	6.2[b] (1.0)	5.7[a] (0.9)	4.7[a] (0.9)
Roasted flavour	4.9[a] (1.4)	6.0[b] (0.7)	7.4[c] (0.7)
Burnt flavour	3.9[a] (1.6)	4.9[b] (1.8)	7.6[c] (1.0)
Bitter taste	4.8[a] (1.0)	5.5[b] (0.7)	6.7[c] (1.4)
Roasted aftertaste	3.5[a] (1.6)	4.6[b] (1.8)	5.6[b] (2.1)
Burnt aftertaste	3.0[a] (1.4)	3.9[b] (1.5)	6.0[c] (1.3)
Bitter aftertaste	4.1[a] (1.0)	4.7[a] (1.1)	5.6[b] (1.2)

[abc]= Mean values with different letters within a row differ significantly (p <0.05); standard deviations are given in parentheses.

Scale values:

The minimum point: 1 denoting not intense, not sticky, not grainy or not creamy.

The maximum point: 9 denoting very intense, very sticky, very grainy or very creamy

3.3.2. Marama water extracts

The sensory panel found significant differences in bitterness of marama water extracts. The effect of roasting marama beans seeds on perceived bitterness of water extracts prepared from its flours is shown in Figure 3.5. The water extracts from

flours (full fat and defatted) prepared from unroasted seeds were the least bitter compared to all the extracts prepared from flours of roasted seeds. The extract prepared from defatted roasted flour[30] was the most bitter among all the extracts.

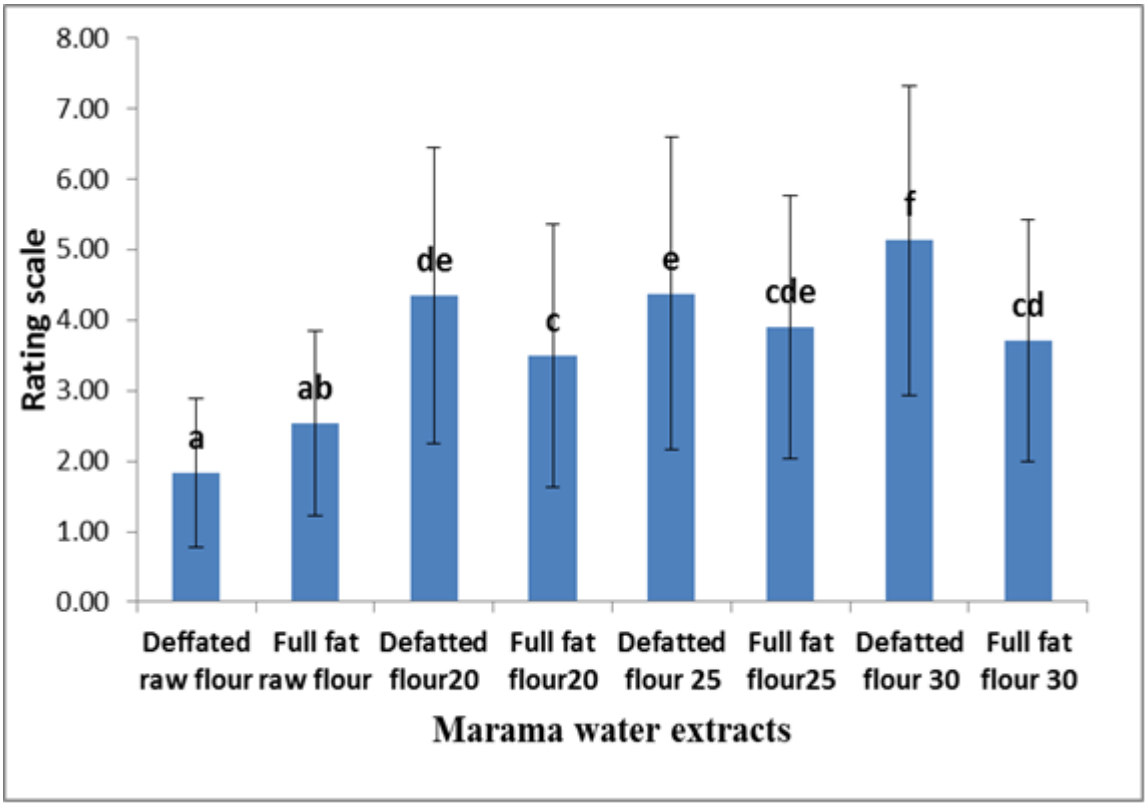

Figure 3.5: Measurement of bitterness intensity of water extract made form full fat and defatted flour of raw and roasted beans. Y- axis: Bitterness intensities, (1= not intense; 9 = very intense). X- axis: Marama beans water extracts prepared from flour of marama beans raw and roasted at 150 ºC for 20 (flour ^{20}), **25** (flour^{25}) **and 30 min** (flour ^{30})**.**

^{abcdef} = Mean values of graph bars with different letters differ significantly (P <0.05). Raw flour: flour prepared from unroasted marama beans. Errors bars represent the standard deviations.

3.3.3. Total phenolic content of marama water extracts

Figure 3.6 shows the effect of roasting of marama beans on total phenolic content of water extracts prepared from its flours. Results showed that the total phenolic content of water extracts prepared from flour of marama beans roasted for 25 min (31 mg CE/ g) and 30 min (32 mg CE/g) were similar and significantly higher than content of water extracts prepared from flour of marama beans roasted for 20 min (27 mg CE/g) and raw bean (24 mg CE/g) which had the lowest total phenolic content.

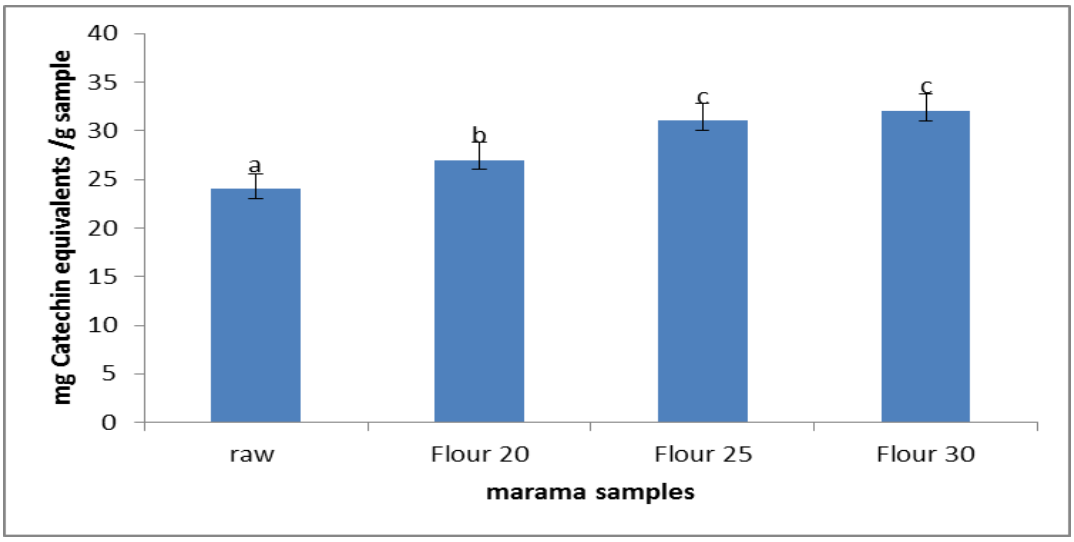

Figure 3.6: **Effect of roasting on total phenolic content of water extracts prepared from flour of marama beans roasted at 150 °C for 20** (flour ^{20}), **25** (flour ^{25}) **and 30 min** (flour ^{30})**.** ^{abc}= Mean values of graph bars with different letters differ significantly ($p < 0.05$). Errors bars represent the standard deviations.

3.3.4. Identification and quantification of individual phenolic compounds in marama water extracts from marama flours by HPLC

Figure 3.7 shows chromatograms of the phenolic compounds standards mixture and the water extracts prepared from raw and roasted marama beans. Two peaks were successfully identified as the phenolic acids gallic acid and protocatechuic acid based on retention time. The effect of roasting marama bean on phenolic acid content of the water extracts prepared from its flours is shown in Table 3.4

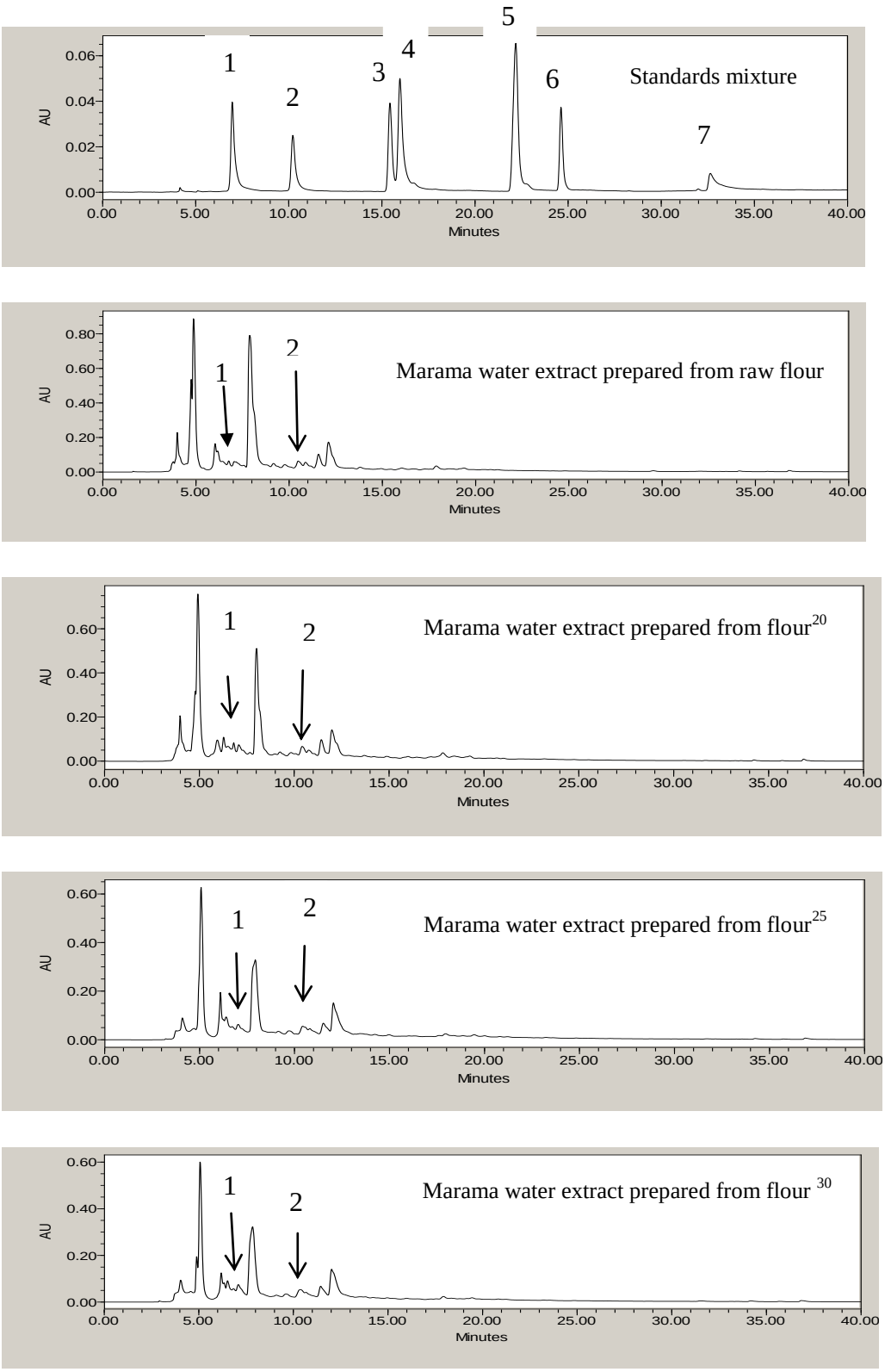

Figure 3.7: HPLC chromatograms of water extracts prepared from flours of unroasted and roasted marama beans at 150 °C 20 (flour ^{20})**, 25** (flour ^{25}) **and 30 min** (flour ^{30})**.**

1-Gallic acid; 2-Protocatechuic acid; 3- Vanillic acid; 4-Caffeic acid; 5-Sinapic acid; 6-Narigin; 7-Quercertin.

Table 3.4: **Phenolic acid content (means ± standard deviations) of water extracts prepared from flours of marama beans roasted at 150 °C for 20** (flour ^{20})**, 25** (flour ^{25}) **and 30 min** (flour ^{30}).

Flour	Gallic acid (mg/100 g) dry marama flour	Protocatechuic acid (mg/100 g) dry marama flour
Raw	34.8^{a}(2.4)	78.0^{a}(5.2)
Flour ^{20}	40.0^{b}(2.1)	85.0^{b}(2.3)
Flour ^{25}	70.0^{c}(5.5)	93.0^{c}(1.6)
Flour^{30}	81.0^{d}(4.3)	122.5^{d}(3.9)

^{abc}= Mean values in a column with different letters differ significantly ($p < 0.05$)

The results indicate that the extractable quantities of protocatechuic acid and gallic acid in marama water extracts significantly increased in concentration as the roasting time increased. The water extract prepared from flour of marama beans roasted for 30 min had the highest concentration of both gallic and protocatechuic acids. Water extract prepared from flour of marama beans roasted for 20 min had twofold less gallic acid content than the water extract prepared from flour of beans roasted for 30 min. The gallic acid content in the water extract prepared from flour of raw beans was twofold less than the content in water extracts prepared from beans roasted for 25 min and 30 min.

3.3.5. Saponin content of marama bean

Table 3.5 shows the percentage yield of freeze dried marama saponin extract made from flour of unroasted marama beans and beans roasted at 150 °C for different time periods.

The effect of roasting time on the resultant concentration of saponins in freeze dried marama extract, marama flour and water extracts tested by the sensory panel is presented in Table 3.6. Marama freeze dried saponins extracts prepared from flour of unroasted beans and flour of beans roasted for 30 min had similar concentrations of saponins but significantly less compared to the freeze dried saponins extracts prepared from flour of beans roasted for 20 min.

Table 3.5: Yield of freeze dried saponins extract prepared from flour of unroasted marama beans and beans roasted 150 °C for 20 (flour ^{20})**, 25** (flour ^{25}) **and 30 min** (flour ^{30})**, respectively**

Marama beans	Flour (g)	Marama freeze dried saponins extract(g)	Yield of freeze dried saponins extract prepared from marama flour (%)
Unroasted	60	7.96	13.25
Roasted ^{20}	60	7.78	12.95
Roasted ^{25}	60	7.90	13.16
Roasted ^{30}	60	7.67	12.24

Roasted^{20}: marama beans roasted at 150 ºC for 20 min

Roasted^{25}: marama beans roasted at 150 ºC for 25 min

Roasted^{30}: marama beans roasted at 150 ºC for 30 min

Table 3.6: Effect of roasting time on the resultant concentration of saponins in freeze dried marama extract, marama flour and water extracts tested by the sensory panel

Samples	Saponins content in freeze dried marama extract (mg/g)	Saponins content in marama flour (mg/100 g)	Saponins content in water extract tested by panelists (mg/l)
Raw	6.37^{a} (0.19)	84.44^{ab} (2.48)	56.33
Roasted ^{20}	7.23^{c} (0.23)	94.94^{c} (4.0)	62.53
Roasted ^{25}	6.91^{bc}(0.54)	91.09^{bc} (7.11)	60.73
Roasted ^{30}	6.46^{ab}(0.23)	82.53^{a} (2.90)	55.00

^{abc}= Mean values with different letters within a column differ significantly ($p < 0.05$)

Roasted^{20}: marama beans roasted at 150 ºC for 20 min

Roasted^{25}: marama beans roasted at 150 ºC for 25 min

Roasted^{30}: marama beans roasted at 150 ºC for 30 min

3.4. Discussion of results

Sensory panelists assigned higher bitter taste and burnt flavour ratings to pastes made from marama beans with longer roasting time. Consequently the paste made from beans roasted for 30 min had the highest intensities. The nutty flavour of paste[30] was probably masked by the bitterness and burnt flavour as a result of the sample being heat treated for a longer period of time. This finding is in agreement with Mmonatau (2005) who found that bitter taste increased in marama bean flour of seed roasted at different temperatures and for progressively longer time periods.

The low L* value and the dark roasted colour of the paste[30] are possibly due to higher level of melanoidin compounds formed from the Maillard reaction when increasing the duration of roasting. According to Baker (2002), colour development is dependent on the formation of brownish-coloured polymeric compounds known as melanoidins which are water-insoluble, high molecular weight compounds formed via Maillard reaction. The amount of melanoidins formed increases as the intensity of the thermal treatment e.g. time and temperature increases (Borrelli, Visconti, Mennella, Anese & Fogliano, 2002). Since melanoidins are also described as bitter tasting (Pedreschi, Kaak, Granby & Troncoso, 2007; Martins *et al*., 2001), these compounds probably contribute bitterness to paste[30]. In addition, furans and caramel compounds resulting from thermal decomposition reactions can significantly contribute to food flavour including bitterness or a burnt aroma (Bemiller & Whister 1996). These compounds probably contributed to the more intense burnt aroma and bitterness of the paste made from marama beans roasted for longer than 20 min. A study by Tomlins *et al.* (2007) on effect of roasting time on sensory attributes of peanut butter demonstrated that increased roasting time was associated with sensory attributes such as brown colour, roasted taste and bitter taste resulting from over-roasting. Longer roasting time are believe to lead to more thermal decomposition reaction as demonstrated in coffee beans by Sacchetti, Mattia, Pittia & Mastrocola (2009).

Yu *et al*., (2005) reported that during roasting, products formed due to the Maillard reaction might contribute to the increase of total phenolics or phenolic-like complexes that contribute to higher absorbance readings. Additionally, several studies (Lee, Jeong, Kim, Park, Nam, & Ahn, 2006; Jeong, Kim, Jo, Nam & Ahn, 2004; Kim,

Jeong, Park, Nam, Ahn, & Lee, 2006) have reported that heat treatment was effective at liberating phenolic compounds from residual sources, yielding higher total phenolic content in some food products (peanut hulls, citrus peels and grape seed extracts) and leading to an increase in extractable phenolics (Manzocco, Anese & Nicoli, 1998). Moreover heat treatments cleave covalent bonds and release phenolic compounds from other compounds to which they are bound and increase their concentration (Dvorak *et al*., 2005; Dewanto *et al*., 2002). Ee *et al*., (2011) suggested that roasting is likely to partially destroy the cell structure of the seeds, resulting in the release of some of the phenolics bound to the cell walls which otherwise would normally be insoluble in an aqueous media. These may be the reasons for the observed higher levels of total analyzable phenolic content and phenolic acids (gallic and protocatechuic acids) in water extracts from flour made from marama beans roasted for longer periods.

Robichaud & Noble (1990) found that the bitter threshold value for gallic acid is 10 to 1500 mg per litre in water and the perceived bitterness increases as a function of concentration. The gallic acids concentrations in water extracts prepared from marama beans flours were higher than this threshold value. According to Maga & Lorez (1973) the taste threshold value of protocatechuic acid is 3 mg per 100 g dry material. The observed levels of protocatechuic acid in all water extracts from marama beans flours were above the reported detection threshold for this compound. This individual compound may therefore have a sensory impact, as well as its combined effect with gallic acid. Maga & Lorez (1973) reported that a combination of phenolic acids resulted in much lower detection thresholds than those of their individual components. Thus the increase in concentrations of gallic acid and protocatechuic acid in the water extracts as a function of roasting time may lead to more bitterness.

Other phenolic compounds such as sinapic acid, caffeic acid, vanillic acid, ferulic acid, quercertin and naringin described as bitter tasting (Troszynska, 2004; Peleg & Noble, 1995) also present in marama bean cotyledons (van Zyl, 2007) were not detected by HPLC analysis in the marama water extracts in this study. This may possibly be due to relatively lower efficiency of extraction of phenolic compounds by water compared to an organic solvent such as methanol which was previously used by

van Zyl (2007). Yu *et al*. (2005) reported that ethanol and methanol were more effective in extracting phenolic compounds from peanut skin than water, with ethanol (80%) being the most efficient extraction solvent.

Several authors (Plug & Haring, 1993; Leland 1997) have reported that fat plays a dominant and multifunctional role in the flavour perception of food products. This includes the function of being a carrier or a sink for lipophilic flavour molecules or off-flavor molecules (Leland 1997). When fat is removed or reduced in a food, the flavour character contributed by fat is lessened or missing and the ingredient off-flavours may be more prominent as fat is not present to mediate in their vapour pressure or act as a sink (Leland 1997). Yackel & Cox (1992) also reported that fat may modify the intensity profile of flavours including nonvolatile tastants. Fat coating of the tongue tends to slow or reduce flavour perception (Yackel & Cox, 1992; De Roos, 1997). On replacing fat with water as evident in defatted marama samples, the increased moisture levels within a food system can create an unbalanced flavour and the perception of bitterness is often accentuated (Yackel & Cox 1992). In addition to the phenolic concentration that increased as emphasized earlier, removal of fat may explain why the water extract prepared from defatted flour was generally perceived as more bitter than the water extracts from full fat flours. This is in agreement with results of Kayitesi, Duodu, Minnaar & De Kock (2010) who reported that decreasing of fat content in marama bean flour unmasked the bitterness.

The mean bitterness values from water extracts made from defatted roasted flours had larger standard deviations compared to those of full fat flours. This relates to larger variation in rating the bitterness intensity of water extracts by the different panelits. Moreover, this probably indicated that panelists might have reached supra-threshold levels of bitterness and therefore had less agreement in ratings. It would be expected that the supra threshold bitterness levels of individual panelists would differ. Another possible explanation might simply be the differences in the number of taste buds in panellist individual's mouths, with more receptor cells potentially resulting in high perceived intensity of bitterness. Miller & Reedy (1990) demonstrated that taste sensitivity depends on multiple factors, including the nature and number of taste receptors on taste bud cells, and the number of taste buds a person has.

Marama flour made from bean roasted for 20 min had the highest concentration of saponins. This is probably due to heat treatment which could have increased permeability of cell membranes thus maximizing the extractable amount of saponins in water extract. Additionally, it might be possible that at this time and temperature combination (20 min at 150°C), saponins in the beans remain heat stable while longer heat treatment will cause saponins degradation. Previously, Cheng, Xie & Gong (2007) used microwave energy (800W) to assist extraction of saponins in *Ganoderma atrum*. The authors reported 20 min was the optimal time with the highest yield, and observed that longer extraction time at high temperature had negative effects resulting from degradation or conversion of saponins. It was suggested that the decrease in saponins content was due to more severe thermal treatment that may result in hydrolysis of the glycosidic bond between the sapogenin and glycosidic residue and therefore causing saponins reduction (Drumm *et al*., 1990). This may explain why the flour made from marama bean roasted for 30 min had the lowest saponins content. Moreover, Kavita, Tarade, Rekha, Singhal, Radha, Jayram, Aniruddha & Pandit (2006) also reported that the rate of saponins degradation increased with an increase in temperature. Since saponins degrades with heat, it would be excepted that roasting marama beans for a longer time duration at high temperature would degrade the saponins content in the beans.

Furthermore, in comparison to soya bean, the saponin content in marama bean flour seems higher than that of soya (0.58 mg per 100g) as reported by Gurfinkel and Rao (2002). However, the concentration of saponins in all marama extracts tested by panellist was higher than the reported bitter threshold value of saponins in water (2-12mg/l) (Heng *et al*., 2006).

3.5. Conclusion

Pastes and water extracts made from marama beans roasted for progressively longer time periods taste increasingly more bitter. Reasons include the presence of saponins and the increase in water extractable quantities of gallic and protocatechuic acid as a result of heating. Compounds resulting from Maillard reactions such as melanoidins, furans and caramel compounds possibly account for more bitterness in the pastes and

water extract made from beans roasted for longer period of time (30 min). Therefore roasting marama bean for more than 20 min should not be recommended to avoid negative sensory properties such as bitter taste, colour darkening and burnt notes. Different roasting temperature and time combinations should take development of bitterness into consideration.

4. GENERAL DISCUSSION

The first part of the general discussion is a critical review of methodology. It essentially discusses the different methods that were applied in this study. In this part of the research, suggestions for applying the methods better in future research are given. The second part proposes potential utilisation of marama beans and different methods of reducing bitterness. The third part critically discusses the value of the research on sensory and physico-chemical analyses of roasted marama beans with specific focus on compounds that may contribute to bitterness.

4.1. Critical review of methodology

There are four species of Tylosema that are used as food materials in Africa (Coetzer & Ross, 1977). In this study, only *Tylosema esculentum* were used because it is the unique specie of the genus Tylosema specific to the arid region of southern Africa (Jackson *et al*., 2010), where it grows in large population and therefore was available in enough quantity for this research. Very little information is known about the eating quality, antinutritional factors and potential bitterness of the other Tylosema species (e.g. *Tylosema fassoglense*).

The seed of marama beans were roasted at 150 °C in a forced convection continuous tumble roaster. Speed sets of 290, 240 and 180 rpm were selected and resulted in heating times of 20, 25 and 30 min, respectively. Roasting marama beans at 150 °C for 20 min is the minimum requirement for consumption purposes since this time and temperature combination inactivates antinutritional factors such as trypsin inhibitors (Maruatona, 2008). It is possible that roasting marama beans at other temperatures, either lower or higher will have different effects on the sensory properties including bitterness. Consideration of the specific heat capacity J/ (kg^{o}C) of marama beans is necessary for future experiments to optimise the roasting of marama beans while ensuring optimum sensory quality. Other methods of heating marama beans can also be considered. One such method is steam heating. Van Der Poel, Blonk, Van Zuilichem & Van Oort (1990) has used steam heating to inactivate trypsin in soya bean. Marama beans were roasted with the thick hulls intact requiring a lot of energy to heat the cotyledon inside. Heat treatment after removing the cotyledon from the

hull should possibly be investigated to more effectively control the heating of the cotyledon.

A mechanical cracker was used to dehull marama beans prior to flour or paste manufacturing. The process involved cracking the seed coat first, then manually separating the hulls from the cotyledons. Dehulling marama beans and removing the cotyledon pieces beans was a time consuming and labour intensive process because cracking had to be repeated more than once, particularly for small size marama beans. In addition it was difficult to remove the cotyledon piece from the small pieces of cracked marama beans thereby resulting in a low yield of roasted marama cotyledons. Developing equipments or technology that can more effectively crack marama hulls and separated them from cotyledons are strongly recommended.

The marama beans used in this study had different sizes and among them, with personal testing, some of the seed were slightly more bitter than others. This is possibly because marama beans grow wild and they were collected from different locations by local people who might have mixed different varieties together. No information is available on the variation in quantities of bitter compounds in marama beans harvested from different locations, climatic conditions. In addition, the difference in sizes and bitterness intensity are probably a function of influence of different horticultural and climatic conditions. Sorting marama by size before further experimentation will be of particular importance to allow an efficiently roasting and prevent over roasting of small size seed which may lead to more bitterness.

Full fat and defatted marama flour were prepared from cotyledons of roasted and unroasted beans following the method as described by Maruatona (2008). The process involved coarsely grind cotyledons using a blender. To produce the full fat flour, because marama beans contains high percentage of fat, coarsely grounded marama cotyledons were progressively milled and sieved to pass through a 1000 µm mesh in order to prevent the formation of a paste like. To produce defatted marama flour, coarsely grounded marama cotyledons were first defatted using hexane to obtain a defatted meal, which was then milled again to pass through a 1000 µm mesh and obtain defatted marama flour. Defatting marama beans was done manually and flour yield losses were the main problem encounter because some flour was decanted

with the hexane. Using equipment that can assist with oil extraction such an oil expeller to avoid flour losses would be strongly recommended for further study.

Distilled deionised water was used as the main solvent for extraction of bitter compounds in marama flours in this study. Only water soluble compounds can be detected as taste contributors in the mouth. This was for safety purposes because humans were used as subjects for sensory testing. Distilled water also contains no odour or flavour that could have affected the taste of the product. However water has a low efficiency of phenolic extraction compared to an organic solvent such as methanol or ethanol. Analysing directly the bitter components extracted by panelist's saliva will probably give a better indication of the types and quantities of compounds that contribute to bitter taste when humans are chewing and consuming roasted marama beans. The extraction of bitter taste components in the mouth due to saliva and mechanical breakdown is probably different to extraction by distilled water. The different components of saliva, especially proline rich proteins may potentially also bind with some of the compounds responsible for bitter taste affecting interaction with taste receptor cells.

Bitterness is perceived by a family of G protein-coupled receptors called hT2Rs (human bitter taste receptors), present on the surface of the tongue (Chen *et al*., 2012). "Descriptive sensory evaluation identifies, describe and quantify sensory attributes of a food material or a product using humans who have been specifically trained for this purpose" (Einstein, 1991). In this study descriptive sensory evaluation was conducted with human subjects using 9-point scales to rate the intensity of sensory attributes of marama pastes and bitterness of water extracts. Normally a human gustatory test is the most direct method used for bitterness evaluation because humans can directly express taste perception exactly after taste tests (Chen *et al*., 2012). This method is reliable; however, results from individuals sometimes contain large variations.

In this study, to avoid confusion in tastes and increase the accuracy of the evaluation, the panelists were screened using the basic tastes identification test following the one sample propylthiouracil (PROP) test as described by Tepper *et al*., (2001). The (PROP) test was of particular importance to this research because panellists who could not taste bitterness were eliminated. Tasters were therefore identified on the

basis of the perceived intensity of propylthiouracil (PROP) compared to a reference salt solution.

Before the actual evaluation, panelists were taken through training sessions to familiarize them with the marama products, to be able to identify and quantify correctly the differences between the products. Beside the between subjects varying sensitivity to bitter taste, and sensory training, other factors including sample order and rinsing of the mouth have been reported to affect the human perception (Chen *et al*., 2012). In this study, each panelist received a set of samples (pastes and water extracts) coded with 3-digit numbers and the order of sample presentation was randomized over the panel. Subjects ate carrot rings for neutralizing and rinsed their mouth with filtered tap water before they began and between each samples.

Taste cells receptors are unevenly distributed on the surface of the tongue (Chen *et al*., 2012). In this study, suitable procedures were taken in consideration to optimize taste perception. Each subject was asked to chew half a teaspoon of marama paste and swallow. For water extracts subjects were ask to swirl the entire sample (10ml) in their mouth, keep for few seconds, then expectorate it and rate the intensity. To avoid fatigue and bitter taste build up in the mouth, samples were tasted with a 2 min break in between.

Testing bitterness in marama products was quite unpleasant for panelists. Investigation of other methods of bitter taste evaluation such as electronic tongue (Chen *et al*., 2012) which is used in food industry to test and obtain data for bitterness intensity in foodstuffs such as beers, wines and olive oil using chemical taste sensor along with the pattern recognition techniques (Baldwin, Bai, Plotto & Dea, 2011) would be recommend for further research. However, humans have the advantage to receive signals from both olfactory and tongue receptors and integrate both sets of data in the brain to form judgments (Baldwin *et al*., 2011).

Moreover, although all panellists were PROP sensitive, their sensitivity in rating marama bitterness intensity was different. Taste sensitivity refers to the intensity at which tastes and flavours are perceived (Delwiche, Buletic & Breslin, 2001). Taste sensitivity depends on multiple factors, including the nature and number of taste

receptors on taste bud cells, and the number of taste buds a person has (Miller & Reedy, 1990). The numbers of taste buds is different among tasters and are highest in the supertasters (Delwiche *et al.*, 2001). The density of taste buds is directly correlated with the sensitivity of taste perception. It might be that subjects who tested marama products had different number of taste buds and therefore differ in bitterness sensitivity. In addition, five females and five males were selected as tasters in this study. Females are more likely to be supertasters than males because they have more taste buds and have more taste pores than males (Delwiche *et al.*, 2001). Considering a same group and/or sex of human subjects such as only supertasters for future research will help solve this problem.

A colour meter is a light sensitive instrument that measure surface colour of a product. In this study, colour analysis of the roasted marama beans was carried out using a colour meter. The instrument was standardized against a white tile before each measurement. Colour expressed in L*, a*, and b*scale parameters relate better how the colour are perceived and simplify understanding. Measuring colour using the colour meter gives objective results compare to the visual methods of specifying colour which are subjective. This is the reason why descriptive sensory evaluation was conducted under red light i.e. to mask the influence of varying colours of the pastes or water extracts.

In this study, water extracts were analysed for total phenolic content using the Folin-Ciocalteu assay as described by Waterman & Moles (1994). The Folin-Ciocalteu reagent reacts with phenolic hydroxyl groups in marama water extract and forms bleu chromogens that can be detected spectrophotometrically at 760 nm. This method is simple, sensitive and precise (Phipps, Sharaf & Butterweck, 2007) but is not specific as it detects all phenolic hydroxyl groups present in the extracts including those in extractable proteins e.g. tyrosine. Other reducing substances such as ascorbic acid may also interference with the assay.

The water extracts were analysed for their phenolic profile using reverse phase high performance liquid chromatography (RP-HPLC). This method identified and quantified specific phenolic compounds e.g. gallic and protocatechuic acids present in the water extracts. In RP- HPLC, the stationary phase is non-polar (made up of a non-

polar C_{18} and the mobile phase used is polar such as water and acetonitrile. So compounds of high polarity are first eluted while those of low polarity are eluted last. HPLC can be used for both qualitative and quantitative analysis. Molecule with small differences in absorption wavelengths can be detected well due to their differences in separation time. Substance with very low concentration can be detected well due to the sensitivity of HPLC detectors. However, this method requires preparation before use in processes such as mixing, homogenization, filtration, degassing etc. These techniques are also to be performed with proper care.

Saponins are known to form a soapy foaming substance when mixed with water (Rochfort & Panozzo, 2007; Heng *et al*., 2006; Khokhar & Apenten, 2003). Persistent foam tests are usually used as a rapid screening of saponins in a given plant before any quantification (Khokhar & Apenten, 2003). In this study, marama water extract was shaken in a glass (Figure 4.1C) and the formation of persistent foam lasting for about 10 min indicated the presence of saponins in the water extract.

Extraction of saponins from marama flour was done following the method of Wang & Mc Allister (2010). Defatted marama flour mixed with distilled water and continuously stirred at room temperature easily formed a gel/dough like substance. Marama proteins are extensible and can form sticky dough (Amonsou, 2010). The filtration of the mixture through filter paper (Whatman No.1) was therefore a major problem encountered and the vacuum pump was used to assist.

The measurement of saponins content was done by spectrophotometric assay as described by Baccou *et al*., (1997). The saponin extracts produced a chromophore (absorption at 430 nm) upon reaction with p-anisaldehyde and sulphuric acid in ethyl acetate. This method is easy to reproduce, accurate and very sensitive as the formation of the chromophore can be detected without difficulty for low concentrations of saponins. The use of other methods such as high performance liquid chromatography (HPLC) that is able to identify and quantify specific saponin compounds in marama would be strongly recommended for future studies.

4.2 Suggestions for potential utilisation of marama beans and methods to reduce bitterness.

The marama beans are somewhat bitter and might be a major problem for consumption and utilisation in the food industry. The use of techniques to effectively control bitter taste would be strongly recommended in further research and/or utilisation. Bitterness of marama can be masked with the use of bitter blockers. Sucrose (table sugar) or sweet tasting compounds, such as aspartame and sucralose have been widely used to mask the bitterness of pharmaceutical products such as quinine (Suzuki, Onishi, Hisamatsu, Masuda, Takashi, Iwata & Machida, 2004; Sohi, Sultana, & Khar, 2004) as well as food products such as juice, yogurt, soya (Szente, & Szeijtli, 2004; Szejt1i & Szente, 2005). Bitterness of marama products (paste, water extract) can be reduced upon addition of sugar. The whole roasted marama beans (Figure 4.1A) consumed as a snack may be coated with sugar or candy chocolate flavour. Roasted marama beans may also be made available in coarsely ground flour (Figure 4.1B), or sliced (flaked, slivered) and be sprinkled over breakfast products like muesli and desserts, particularly ice cream or other sweet desserts to mask the bitter taste. Further researches are called for to determine how much sugar and/or other sweeteners should be added to mask bitterness to levels acceptable by consumers.

Cyclodextrin a family of cyclic oligosaccharides, are also a common commercial product known for their ability to decrease bitterness due to their hydrophobic cavity and hydrophilic exterior shell (Szente & Szeijtli, 2004). Once bitter eliciting compounds interact with the interior of cyclodextrins, inclusion complexes are formed. As a result, bitterness is decreased because the bitter eliciting compounds are trapped within the cyclodextrin molecule, and therefore are unable to bind to taste receptors cells. For example, cyclodextrins are used to masks the bitterness of citrus juice mainly caused by flavonoids (limonin and naringin). Because flavonoids are still present in the juice, their bioactive potential is unchanged (Drewnowski & Gomez-Carneros, 2000). Other effective ways of masking bitterness in other plant foods involve the addition of fat, or salt. Fat is known as having a bitter masking effect through coating of the tongue and palate thus modifying the intensity profile of bitter taste (Yackel & Cox, 1992; De Roos, 1997). The suppression of bitterness by

salt is predominately dependant on the presence of sodium cation Na^+ (Breslin & Beauchamp, 1995). Na+ containing salts inhibit bitterness via oral peripheral interaction. The mechanism responsible for peripheral suppression of bitterness by sodium is not well known, however it might possibly due by blocking of taste receptors (TAS2Rs) (Breslin & Beauchamp, 1995). Bitter *Brassica* vegetables are thought to taste good only if sauces are added, that is, after the addition of fat, sugar, or salt (Reicks, Randall & Haynes, 1994).

The use of marama beans along with other carbohydrate starch foods like bread, or porridge to reduce the bitterness intensity should also be recommended in further application. Marama beans paste can also be used as butter/spread like peanut butter or almond butter on bread. Marama flour composited with sorghum flour in a porridge had a positive effect in lowering the bitterness intensity (Kayitesi *et al*., 2010). These techniques will improve palatability of marama bean products while preserving the bitter compounds e.g. saponins, phenolic compounds which are associated with health benefits (Rochfort & Panozzo, 2007).

Moreover, bitter compounds can also be removed during processing of marama beans as is usually done for soybeans, grape fruit, wine, tea, chocolate, brassica vegetables etc. (Drewnowski & Gomez-Carneros, 2000). Some of the common debittering processes are the use of solvents, micro-organisms, adsorption to resins, membrane ultrafiltration and precipitation (Drewnowski & Gomez-Carneros, 2000). For example, beany flavour and bitter taste, pose a major barrier to the inclusion of more soy products in the Western diet (Kay, 1998). Solvents (hexane), precipitation, filters, and microorganisms have all been used to produce non bitter and bland soy products. Addition of gelatin or maltodextrins to hydrolyzed soy proteins are used to minimized the bitterness (Maga, 1990). Bitter phenolic compounds are routinely adsorbed to resins, trapped on polymers, precipitated, extracted with solvents, or converted to non bitter compounds. For example, polyvinyl polypyrrolidone matrixes are used to adsorbed phenolic compound in wine (Noble, 1998). Protein "fining" of wine uses egg white, casein, gelatin to remove phenols and thereby lowering bitterness of the wine (Noble, 1998). Aging of wine reduces bitterness because phenols continue to polymerize and eventually precipitate (Noble, 1998). In many cases, these methods are patented and their details are guarded commercial secrets of a particular food

company (Puri, Marwaha, Kothari & Kennedy, 1996). Bitter saponins in legumes such as chickpeas and lentils are reduced by soaking the beans overnight in water, or cooking the beans (Rochfort & Panozzo, 2007). These are the possible techniques that may be considered to reduce or remove the bitter compounds when using marama beans.

Other potential approaches of bitter compound removal include developing new and less bitter varieties through domestication. An experiment on marama domestication was previously reported by Jackson *et al*., (2010). However this was not successful due to a lack of supervision of the cultivated plots and problems related to the cultivation of marama such as availability of water, land, soil quality and labour inputs.

Domestication is the process by which plants are genetically modified over time by humans for traits that are more advantageous or desirable for humans. (Zohary, Hopf, & Weiss, 2012). Normally, domestication tends to decrease genetic diversity, as only certain plants are selected and propagated (Zohary *et al*., 2012). As an example, almond is considered to be one of the earliest domesticated tree nuts (Delplancke, Alvarez, Espindola, Joly, Benoit, Brouck & Arrigo (2010). Wild almonds are bitter; the kernel produces deadly cyanide upon mechanical handling. Selection of the sweet type, from the many bitter types in the wild, marked the beginning of almond domestication. The wild almond species are bitter and toxic while domesticated almonds are not. Although marama beans do not contain cyanide glucoside (Holse *et al*., 2010) developing new marama varieties through plant breeding or domestication should also be taken into consideration for further research. Domesticated marama beans may then have shorter maturity, large seed with approximately the same size, and most important lower content of bitter substances which are not strongly perceived and thus not objectionable.

A

B

C

Figure 4.1: A) Picture of roasted marama beans cotyledons, B) Picture of coarsely grounded marama beans cotyledons, C) Picture of marama water extract showing a formation of a persistent foam, 10 min after agitation in a glass.

4.3 Value of the research on sensory and physico-chemical analysis of roasted marama beans with specifics focus on compounds that may contribute to bitterness

Unlike previous studies which focused on nutritional and chemical composition, as well as physicochemical, functional and sensory properties of marama flour, this is the first research investigating bitter eliciting substances that are perceived in marama beans. A selection of bitter compounds has been determined and quantify. This research provides a better understanding regarding the relationship between the bitter compounds, the heat treatment and the sensory profiles of dry roasted marama beans. The information is useful for planning options to utilize marama beans in food systems. Figure 4.3 illustrate the summary of the findings of the sensory and physico chemical analysis of marama beans pastes and water extracts.

The effect of thermally treated marama bean at 150°C on the sensory profile was studied as a function of time (20, 25, or 30 min). Changes in colour measured with colorimetric parameters (reflectance spectra, colour difference, *L**, *a** and *b** parameters), and the differences in sensory attribute ratings can be taken as evidence of the presence of Maillard reaction. As expected, marama paste samples made from beans roasted for longer periods of time became more reddish and suffered a loss of yellow colour. As studied by several authors, Martins *et al*. (2001), Smith, Taneda, Richey, Miyata, Du Yan, Stern, Sayre, Monnier, & Perry (1994), the Maillard reaction is initiated by the nonenzymatic condensation of a reducing sugar, such as glucose, with a protein amino group to form a Schiff base, which then undergoes an Amadori rearrangement to regenerate carbonyl reactivity (Figure 4.2). In the presence of heat, subsequent reactions involving dehydration, rearrangement, fragmentation, and further condensation reactions yield a variety of advanced Maillard reaction end products (Figure 4.2). Colour formation is attributed to the final stage of the reaction, where condensation between carbonyls (especially aldehydes) and amines occurs to give high molecular mass, coloured products known as melanoidins (Michalska, Amigo-Benaventb, Zielinski & Dolores Del Castillo, 2008). It is well known that the amount of melanoidins formed via Maillard reaction increase as the intensity of the thermal treatment e.g. time and temperature increases (Borrelli *et al*., 2002). The final stage of the reaction is known to be of great importance for flavour formation when

carbonyl compounds react with each other, as well as with amino compounds and amino acid degradation products, such as hydrogen sulphide and ammonia. It is these interactions that lead to the formation of flavour compounds, including important heterocyclics, such as pyrazines, pyrroles, furans, oxazoles, thiazoles and thiophenes (Mottram, 1991). These products are typical of those ingredients present in roasted, toasted, and cooked foods (Martins *et al*., 2001) and also possibly present in roasted marama bean. Maillard reaction is therefore responsible for the colour and the delicious nutty and roasted flavour perceived by the panelists in marama beans pastes. The differences in colour and flavour intensity of the paste are possibly due to the extent of the reaction.

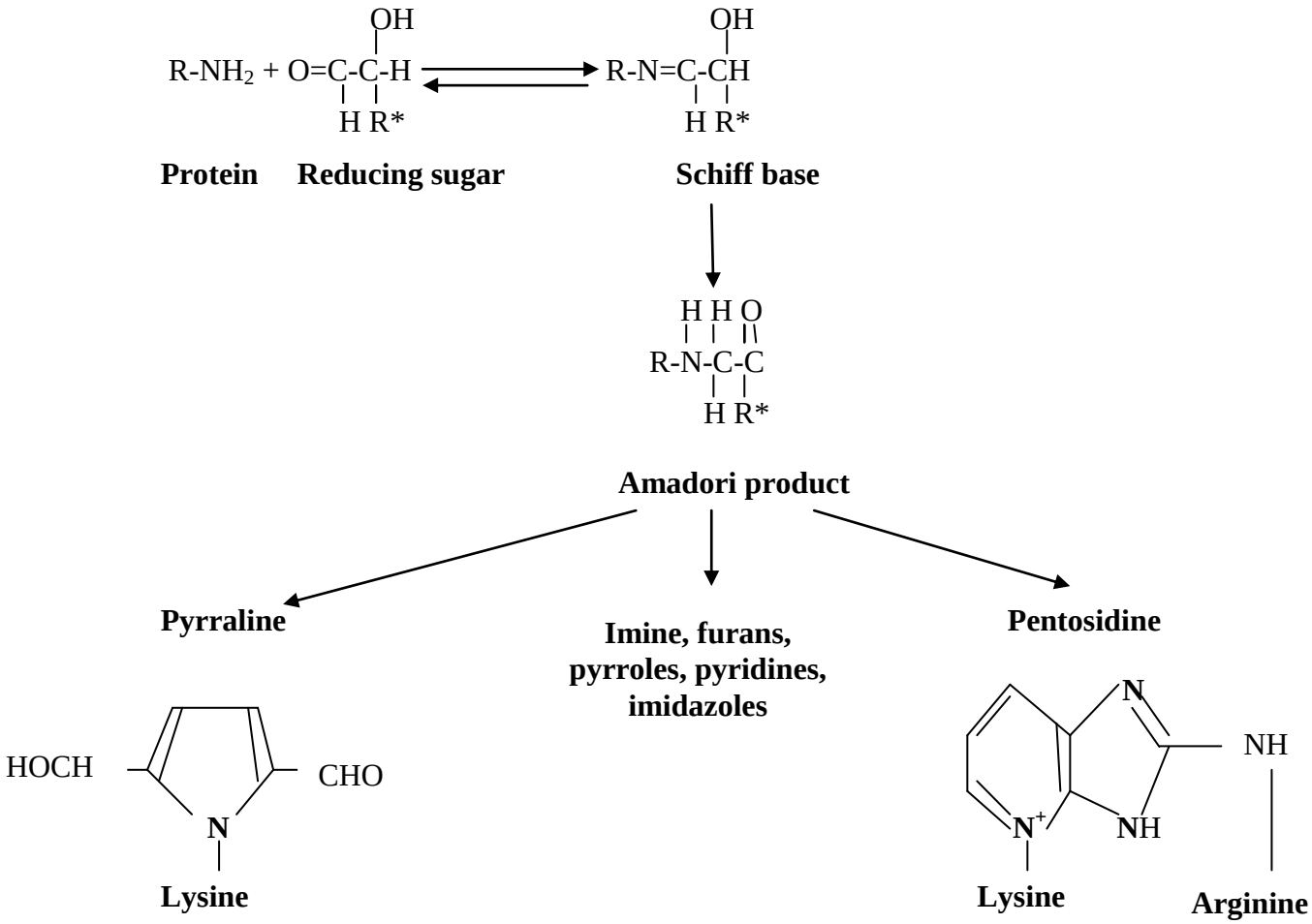

Figure 4.2: formation of advanced glycosylation end products (Smith *et al*., 1994).

R: Represents an intraproteinlysyl side chain (or N-terminal amino acid)

R*: Remainder of a reducing sugar or related carbonyl compound

Bitterness was more prominent in marama paste made with beans roasted for 30 min and in its water extracts. Bitterness is thought to be associated firstly with the amount of melanoidins formed via Maillard reaction or related to flavour compounds from Maillard reactions such as furan and caramel compounds (Be Miller & Whistler, 1996). Secondly to the presence of bitter phenolic compounds like gallic acid and protocateuic acid which were above the threshold value and therefore caused bitterness in marama bean pastes and water extracts. The extent of heating was positively correlated to the quantities of phenolic compounds detected by analysis. Thirdly to the high level of total saponins content that is also known to cause bitterness in legume (Heng *et al*., 2006).

An analysis of substances in marama beans can give a good indication of their negative effect e.g bitterness due to their concentration and possible synergistic effects. However some of these components like saponins and phenolic compounds are known to also contribute in food plants to health beneficial effects such as antioxidant activity, lowering blood cholesterol level, lowering the risk of cancer and cardiovascular disease as well as protecting against LDL oxidation through a reduction of free radicals (Rochfort & Panozzo, 2007; Heng *et al*., 2006; Khokhar & Apenten, 2003). These compounds, known as phytochemicals, hold major promise in the creation of designer foods for the dietary prevention of chronic diseases (Farnham, Simon & Stommel, 1999; Kochian & Garvin, 1999). However, this poses a dilemma in designing marama bean products as functional foods or nutraceutical ingredients because the content of bitter compounds which are good for health may be wholly incompatible with consumer acceptance. Therefore enhancing technology such the use of bitter blockers as emphasized earlier may be of great importance.

The effect of defatting marama flour was also noticeable by sensory testing. Marama water extract from defatted flour were generally perceived more bitter than the water extracts from full fat flour. This is possibly because the water extract from defatted flour probably contained more bitter compounds since the flour contained proportionally a higher amount of non fat substances compared to full fat flour. Fat in a product mask bitter perception due to coating the tongue and palate, thereby preventing water soluble bitter compounds to reach taste receptor cells (Yackel &

Cox, 1992; De Roos, 1997). Thus in addition to the phenolic concentration and saponins content that was above the threshold value as emphasized earlier, removal of fat possibly explain more bitterness in the water extract prepared from defatted flour.

Moreover, not all bitterness is rejected automatically. In selected foods and beverages such as coffee, beer, tonic water and wine, a certain degree of bitterness is expected. In such cases bitterness is usually paired with a desirable attribute: caffeine or alcohol (Mattes, 1994). Although liking for some degree of bitterness can be acquired in adult life as reported by Drewnowski & Gomez-Carneros (2000), excessive bitter taste e.g. in citrus juices, coffee, or beer is usually objectionable. The threshold for what is or is not acceptable may vary from one person to another because individual taste response to bitter varies enormously (Drewnowski & Gomez-Carneros, 2000). According to Rousseff (1990), inherited taste factours, compounded by sex and age, add an extra layer of complexity to the acceptance of bitter plant foods by the consumer. Food scientists acknowledge that it is difficult to blend concentrations so that bitterness levels are optimal for everyone (Rouseff, 1990). In that, some consumer may like marama beans considering the bitter taste as desirable attribute, while others will not. Bitter tasting nuts like kola nut (*Cola nitida)* are popular and consumed in some parts of West Africa (Kew Royal Botanical Gardens, 2013). The levels of bitter taste that consumers used to marama beans are willing to tolerate and that which consumers newly exposed to marama products will accept, remains to be determined.

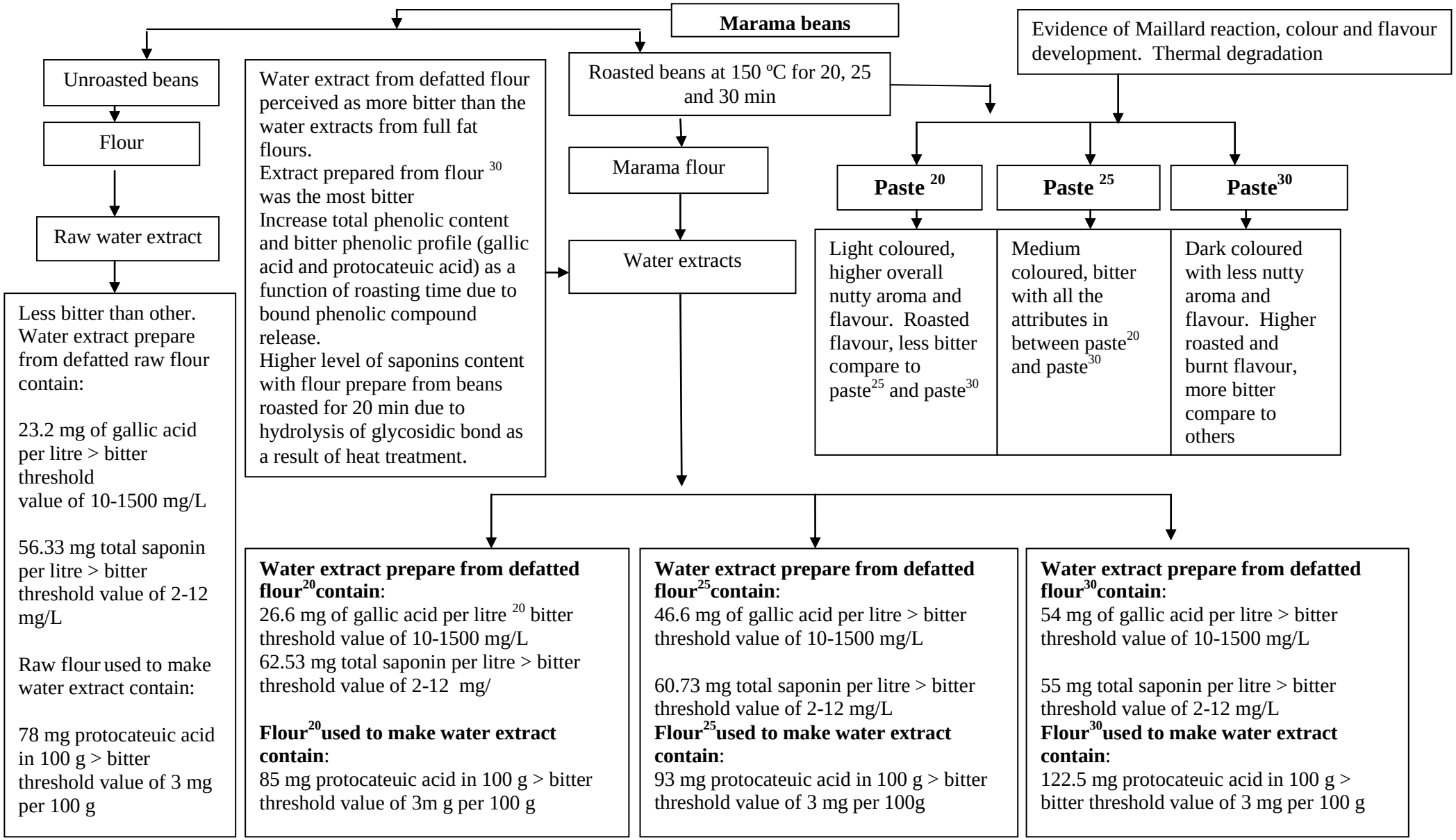

Figure 4.3: Summary of the findings of sensory and physico chemical analysis of marama beans pastes and water extracts.

5. CONCLUSIONS AND RECOMMENDATIONS

This study was the first research investigating potential bitter eliciting substances that are perceived in marama beans. Bitter phenolic content increase as a function of roasting time and the higher concentration of free gallic and protocateuic acid in roasted marama cotyledons and consequently in the water extracts prepared from its flours are perceived as more bitter. High saponins contents also contribute to a more bitter taste in marama bean cotyledons and in its water extracts. The knowledge of the bitter eliciting compounds in marama beans is therefore useful for planning options to utilize marama beans in food systems. At 150 ˚C roasting temperature, marama beans should not be roasted for more than 20 min because long time exposure to high temperature result in more bitter taste, burnt flavour as well as colour darkening.

It is important to acknowledge that a limited number of bitter eliciting components were selected to be determined in this study. Further researches are obviously required to explore other compounds e.g. alkaloids, amino acids, peptides, mineral salts with regard to bitterness of marama beans. It may also be useful to apply effective technologies as ways of reducing bitter substances to levels acceptable to consumers.

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