

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

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ABSTRACT

The role of phenolics and saponins in contributing to bitterness in marama beans, an underutilized legume, especially when roasted, was investigated. Marama beans were roasted at 150 °C for 20, 25 or 30 min, dehulled to separate cotyledons, and pastes were prepared from these. Water extracts were prepared from full fat and defatted flours from roasted and unroasted marama cotyledons. A sensory panel evaluated the sensory attributes of marama pastes and water extracts. Marama water extracts were analysed for total phenolic content, phenolic composition and saponin content. Roasting marama beans for more than 20 min resulted in negative 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. Saponin content of the water extracts was in the range of 55 to 63 mg/L. The identified phenolic acids, saponins and other as yet unidentified compounds may contribute to the perceived bitterness.

KEYWORDS: *Tylosema esculentum*; marama bean water extracts; roasting; descriptive sensory evaluation; bitterness; phenolic compounds; saponins.

1. INTRODUCTION

There is a search worldwide for food sources to alleviate malnutrition in developing countries due to a shortage of protein-rich foods. Utilisation of legume sources is one opportunity (Tresina & Mohan, 2013). Consumer acceptance of such food sources is highly dependent on their sensory properties. The marama bean [*Tylosema esculentum* (Burchell) A. Schreiber] is an underutilised 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 roasting they have a 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 the Maillard reaction as a result of heating (Kayitesi, Duodu, Minnaar & De Kock, 2010).

A further reason for heat treating marama beans is to inactivate anti-nutritional factors. Maruatona, Duodu & Minnaar (2010) reported that roasting marama beans at 150 °C for 20 min is required to inactivate trypsin inhibitors. This time/temperature combination is therefore regarded as a minimum requirement for marama beans for consumption. 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 *et al.* (2010) reported a slight bitter taste in porridges prepared from composite flours of sorghum and defatted roasted and unroasted marama flours. The perceived bitter taste can limit the utilisation and consumption of this pulse. No specific research has been published on the compounds that could be responsible for bitter taste in marama bean. 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 saponins are believed to impart a high level of bitterness in many edible legumes including lupins, lentils, chickpeas, soybean, various beans and peas (Rochfort & Panozzo, 2007). 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.

2. MATERIALS AND METHODS

2.1 Preparation of marama paste and water extracts

Marama beans were collected in the Masokaphala area, Central district of Botswana. Marama beans were heated at 150 °C in a forced convection continuous tumble roaster (Roastech, Bloemfontein, South Africa). Speed sets of 290, 240 and 180 rpm were selected resulting 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 the roasted marama beans and the cotyledons were retained. In order to prepare homogenous samples for descriptive sensory analysis, three marama pastes namely paste²⁰, paste²⁵ and paste³⁰ (referring to pastes from the cotyledons of marama beans roasted for 20, 25 and 30 min respectively) were prepared by pulverizing 300 g batches of roasted cotyledons for 15 min using a CT35 vertical cutter mixer (570 watt) with a 3.5 L bowl capacity.

Full fat and defatted marama flours were prepared from cotyledons of roasted and unroasted beans following the method described by Maruatona *et al.* (2010). Defatted marama flour was prepared by grinding cotyledons into a coarse flour using a laboratory Waring blender (Lasec, Johannesburg, South Africa) and oil was extracted twice, each time using hexane (flour-to-hexane ratio of 1:3 w/v) for 2 h. The dry residue obtained after removal of the hexane was milled using a IKA® A11 basic laboratory mill (IKA Werke, Germany) to pass

through a 1000 μm mesh. A total of eight samples of marama flour were prepared namely, full fat and defatted raw marama flour, full fat and defatted roasted marama (for 20 min) flour, full fat and defatted roasted marama (for 25 min) flour, full fat and defatted roasted marama (for 30 min) flour. All the flours were vacuum packed and stored at 4 °C until used to prepare marama water extracts.

Water extracts from the eight marama flour samples for descriptive sensory evaluation 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 and evaluated within 90 min of preparation. For chemical analysis the extracts were centrifuged at 7500 x g for 10 min (25 °C) using a Rotanta 460 R centrifuge (Labotech, Johannesburg, South Africa) prior to the assays.

2.2 Descriptive sensory evaluation

A ten person (five male, five female) trained sensory panel at the University of Pretoria was used to evaluate the sensory profiles of marama products (pastes and water extracts). The ten panelists were selected and screened for sensitivity for the basic tastes: sweet, salt, sour, umami and specifically bitter (using the one solution propylthiouracil test of Tepper, Christensen & Cao (2001), and also the ability to differentiate and describe commercial peanut butters. 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 familiarising the panelists with the marama products (paste and water extract), assessment procedures 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 panelists' performance was checked at least twice and the Compusense FCM® tool was used to facilitate the training.

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 (10 mL each) in size 8 glass polytop containers with lids. 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 neutralising, 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 twelve descriptive terms, grouped under aroma, flavour and aftertaste attributes (Table 1). Aroma was evaluated immediately after removing the cover using short sniffs. Then a half spoon (5 g) 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 and the maximum point was 9 denoting very intense. 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. Approval of the sensory evaluation protocol was granted by the Ethics Committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria (EC080825-035).

Table 1: Descriptive sensory attributes used by a trained panel to evaluate marama bean paste

Attributes	Definitions	References to explain 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. burnt peanut)	Overroasted marama beans (150 °C for 35 min) ground 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 Ricoffy in water =8
Burnt flavour	The intensity of flavour similar to burnt food	Overroasted marama beans (150 °C for 35 min) ground 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 (e.g. sunflower)	
Rancid flavour	Intensity of flavour associated with oxidised fats and oils (e.g. rancid butter)	Oxidised stored peanut butter =8
After taste		
Bitter aftertaste	Basic bitter aftertaste associated with caffeine or quinine	10% Nescafe Ricoffy in water =8
Burnt aftertaste	The intensity of aftertaste similar to burnt food	Overroasted marama beans (150 °C for 35 min) = 8
Roasted aftertaste	The intensity of aftertaste similar to roasted nuts	Peanut butter (Black Cat) =6

(1= not intense; 9 = very intense)

2.3 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 was calibrated against black and white and the resultant values were 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*).

2.4 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 marama flour) using the Folin-Ciocalteu method as described by Waterman and Mole (1994). 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 % m/v) was added, made up to volume with deionised water mixed thoroughly 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.

2.5 Characterisation and quantification of phenolic compounds in water extracts of marama bean flours

Reverse phase HPLC was used to characterise and quantify the phenolic compounds (Kim, Hyun, Kim, Park, Kim, Lee, Chun & Chung, 2007) in the marama water extracts. The HPLC system (Waters Associates, Milford, MA, USA) consisted of a model 1525 binary pump, model 2487 dual wavelength absorbance detector and a YMC-Pack ODS AM-303 (250 x 4.6 mm i.d., 5 μm particle size) column at room temperature (25°C) (Waters Associates, Milford, MA, USA). All solvents and chemicals used were of HPLC grade. Mobile phase A was 0.1% (v/v) acetic acid in HPLC grade water and mobile phase B was 0.1% (v/v) acetic acid in acetonitrile. Solvents were delivered in a linear gradient 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 before returning to the initial condition. Total run time was 60 min. Injection volume was 10 μ L, flow rate was 0.8 mL/min, phenolic compounds were detected at 280 nm and BreezeTM (Waters, Associates, Milford, MA, USA) software was employed for collection and analysis of chromatographic data. Different concentrations (200 ppm, 100 ppm, 50 ppm, 25 ppm and 10 ppm) of phenolic standards (gallic acid, protocatechuic acid, caffeic acid, vanillic acid, ferulic acid, sinapic acid, naringin and quercetin) were prepared in HPLC grade dimethylsulphoxide and filtered through 0.45 μ m PTFE syringe filters before injection into the chromatographic system. Marama water extracts in 2 mL Eppendorf tubes were acidified with a drop of concentrated hydrochloric acid (32 %) and filtered through 0.45 μ m PTFE syringe filters before injection. 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 flour on dry basis.

2.6 Determination of total saponins in marama beans

The extraction of saponins was done following the method of Wang and Allister (2010). A sample (60 g) of coarsely ground cotyledon from unroasted and roasted beans was defatted using 200 mL petroleum ether. 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 twice with 100 mL distilled water, filtered, the filtrates were combined, mixed with 5 g polyvinyl-polyrrolidone to remove phenolic compounds, centrifuged at 5000 x g (10 min, 4 °C) and the supernatant was freeze-dried. The dry material was then mixed with 100 mL distilled water and centrifuged at 10000 x g (20 min, 4 °C). The supernatant was mixed with 200 mL n-butanol and 0.2 mL concentrated HCl, and held at room temperature for 30 min to capture saponins. After

centrifuging at 10 000 x g (10 min, 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 distilled water, centrifuged (12 000 x g, 20 min, 4 °C), and freeze dried. The dried extracts were stored in a sealed container covered with foil at 4 °C.

The dried extracts were analysed for total saponins content as described by Baccou, Lambert & Sauvaire (1977). Soya saponin (Wako Chemicals USA, Inc.) 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 anisaldehyde plus 99.5 mL ethyl acetate and Reagent B was 50 mL concentrated sulphuric acid plus 50 mL ethyl acetate. Each reagent (1 mL) was added to test tubes containing soya saponin standard solutions prepared in ethyl acetate of concentration 0-40 $\mu\text{g}/2$ mL and the marama extract of concentration 40 $\mu\text{g}/2$ 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. 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.

2.7 Statistical analysis

All sensory, physical and chemical data collected were analysed 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 compound concentrations 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 $P \leq 0.05$ was used to compare means. Results reported in this study are averages of three replications.

3. RESULTS AND DISCUSSION

3.1 Colour and sensory properties of marama pastes

Roasting marama beans at 150 °C for different time periods 20, 25 or 30 min, respectively, produced light, medium and dark-coloured marama bean pastes. Paste²⁰ was significantly lighter as indicated by a higher L* value (55.7 ± 0.2) versus 51.0 ± 0.2 for paste²⁵ and 43.9 ± 0.3 for paste³⁰ and had higher b* value (yellowish) of 29.6 ± 0.1 versus 27.9 ± 0.2 for paste²⁵ and 23.2 ± 0.2 for paste³⁰. Paste³⁰ had a higher a* value (redness) of 12.9 ± 0.2 versus 11.1 ± 0.1 for paste²⁵ and 9.3 ± 0.2 for paste²⁰. Sensory panellists assigned higher bitter taste and burnt flavour ratings to pastes made from marama beans with longer roasting time (Table 2). The paste made from beans roasted for 30 min had the highest intensities. Paste²⁰ was the nuttiest followed by paste²⁵, while paste³⁰ was the least nutty in aroma and flavour. The nutty flavour of paste³⁰ was probably masked by the bitterness and burnt flavour as a result of the sample being heat treated for a longer period of time. Mmonatau (2005) also found that bitter taste increased in marama bean flour of beans roasted at higher temperatures and for progressively longer time periods. The low L* value and the dark roasted colour of the paste³⁰ were 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 in terms of time and temperature increases (Borrelli, Visconti, Mennella, Anese & Fogliano, 2002). Since melanoidins are also described as bitter tasting (Pedreschi, Kaack,

Table 2: Sensory profiles of pastes prepared from marama beans roasted at 150 °C for 20 min (Paste²⁰), 25 min (Paste²⁵) and 30 min (Paste³⁰)

Attributes	Paste ²⁰	Paste ²⁵	Paste ³⁰
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)

Mean values with different letters within a column differ significantly ($p \leq 0.05$); standard deviations are given in parentheses

Scale values: 1 denotes not intense, 9 denotes very intense

Granby & Troncoso, 2007; Martins, Jongen & Van Boekel, 2001), these compounds probably contributed to bitterness as a results of roasting. In addition, furans and caramel compounds resulting from thermal decomposition reactions can significantly contribute to food flavour including bitterness or a burnt aroma (BeMiller & Whistler, 1996). These compounds probably contributed to the more intense burnt aroma and bitterness of the pastes made from marama beans roasted for longer than 20 min. A study by Tomlins, Rukuni, Mutungamiri, Mandeya & Swetman (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 increase thermal decomposition reactions as demonstrated in coffee beans (Sacchetti, Mattia, Pittia & Mastrocola, 2009).

3.2 Bitterness properties of marama water extracts

The sensory panel found significant differences in bitterness of marama water extracts (Figure 1). The water extracts from flours (full fat and defatted) prepared from unroasted beans were the least bitter. The water extract prepared from defatted flours were generally perceived as more bitter than the water extracts from full fat flours. The extract prepared from defatted roasted flour³⁰ was the most bitter among all the extracts.

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 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 (Leland, 1997). Yackel & Cox (1992) also

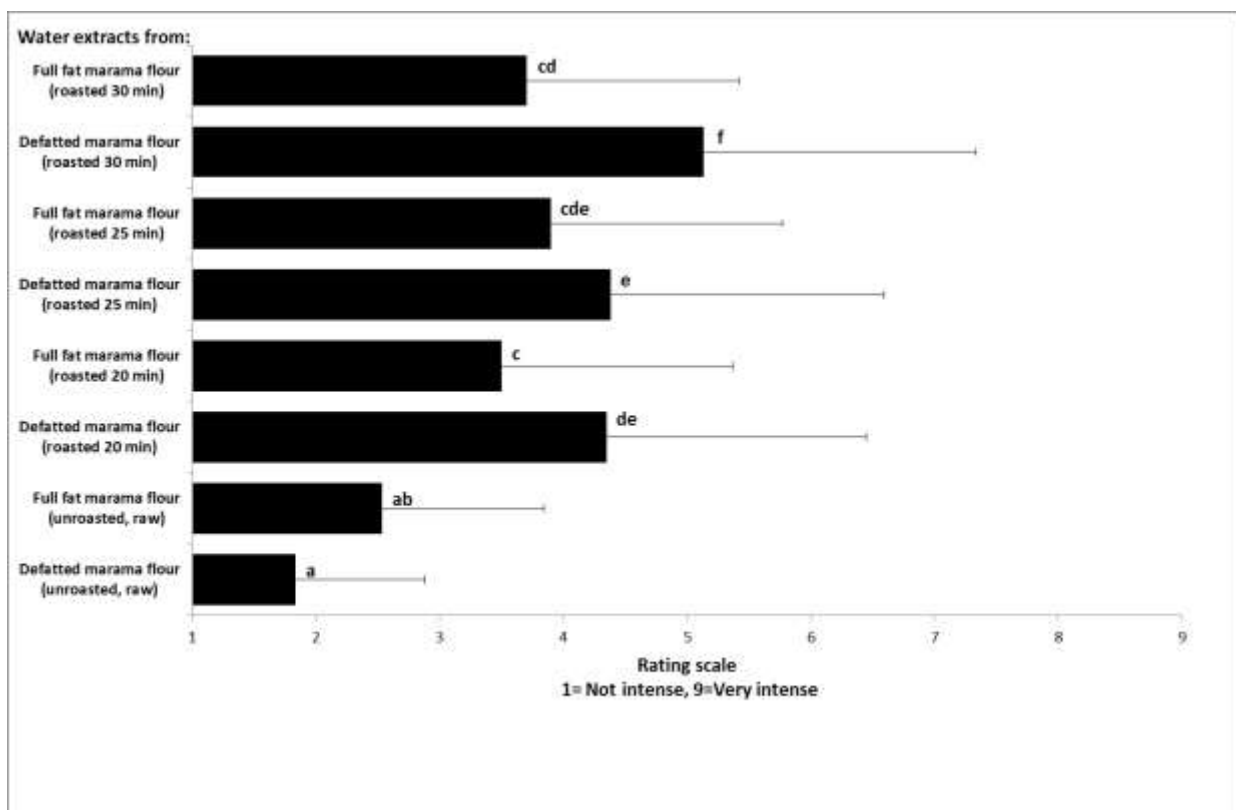


Figure 1: Bitterness intensity of water extracts from full fat and defatted flours of raw and roasted marama beans. Mean values of graph bars with different letters differ significantly ($p \leq 0.05$)

reported that fat may modify the intensity profile of flavours including non-volatile 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). Removal of fat may explain why the water extract prepared from defatted flours were generally perceived as more bitter than the water extracts from full fat flours. This is in agreement with results of Kayitesi *et al.* (2010) who reported that decreasing fat content in marama bean flour unmasked the bitterness in sorghum-marama porridges.

Large variation in individual panellists' ratings of the bitterness intensity of water extracts were observed as indicated by large standard deviations (Figure 1). This probably indicated that for some panellists the bitterness of the extracts may have been at supra-threshold levels limiting ability to distinguish intensity levels. It would be expected that the supra-threshold bitterness levels of individual panellists would differ. Another possible explanation might be the differences in the number of taste buds in individual panellists' mouths, with more receptor cells potentially resulting in higher 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.

3.3 Total phenolic content and phenolic profile of marama water extracts and their relation to bitterness

Table 3 shows 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 that of water extracts prepared from flour of marama beans roasted

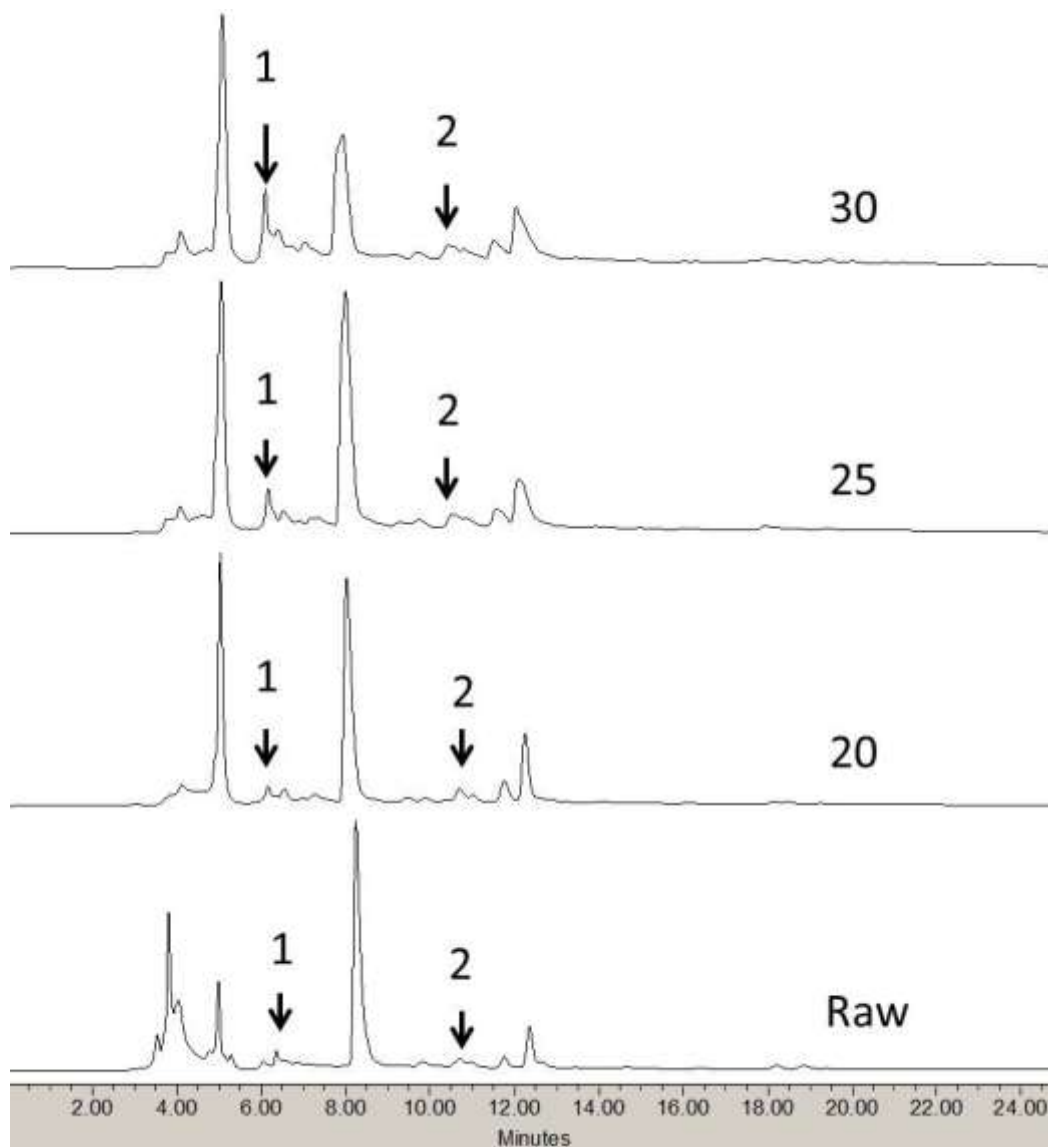


Figure 2: Chromatograms of water extracts prepared from raw marama flour (Raw) and flours from marama beans roasted at 150°C for 20 minutes (20), 25 minutes (25) and 30 minutes (30). 1 = gallic acid; 2 = protocatechuic acid

for 20 min (27 mg CE/g) and raw beans (24 mg CE/g). Gallic acid and protocatechuic acid were the only phenolic compounds identified in the water extracts from the HPLC analysis with the standards available (Figure 2). The levels of these phenolic acids in the marama water extracts significantly increased upon roasting and with increase in roasting time of the marama beans, with the water extract prepared from flour of marama beans roasted for 30 min having the highest concentrations (Table 3).

Table 3: Total phenolic content (TPC) and phenolic acid content of water extracts prepared from flours of marama beans roasted at 150 °C for 20 min (Flour²⁰), 25 min (Flour²⁵) and 30 min (Flour³⁰)

Flour	TPC (mg CE*/g flour) dry basis	Gallic acid (mg/100 g flour) dry basis	Protocatechuic acid (mg/100 g flour) dry basis
Raw	24 ^a (1.6)	34.8 ^a (2.4)	78.0 ^a (5.2)
Flour ²⁰	27 ^b (1.8)	40.0 ^b (2.1)	85.0 ^b (2.3)
Flour ²⁵	31 ^c (1.8)	70.0 ^c (5.5)	93.0 ^c (1.6)
Flour ³⁰	32 ^c (1.8)	81.0 ^d (4.3)	122.5 ^d (3.9)

Mean values in a column with different letters differ significantly (p <0.05); standard deviations are given in parentheses

*CE – catechin equivalents

Yu, Ahmedna & Goktepe (2005) reported that during roasting, colour complexes form due to the Maillard reaction which 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, Lee, 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 extractable concentration (Dvorak *et al.*, 2005; Dewanto, Wu & Liu, 2002). Ee, Agboola, Rehman & Zhao (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 medium. These may be the reasons for the observed higher levels of total assayable 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/L in water and the perceived bitterness increases as a function of concentration. Taking the flour-to-water ratio used in preparation of the water extracts into consideration (10 g flour to 150 ml water), the gallic acid concentrations in the water extracts work out to range from 24 mg/L in extract from raw flour to 54 mg/L in extract from flour prepared from marama beans roasted for 30 min. These concentrations are within the range of the bitterness threshold values for gallic acid indicated above. According to Maga & Lorenz (1973) the taste threshold value of protocatechuic acid is 3 mg per 100 g dry material (or 0.3 mg per 10 g dry material). The corresponding levels of protocatechuic acid in the 10 g flour used to prepare the water extracts range from 7.8 mg to 12.3 mg which are above the reported threshold for this compound. Protocatechuic acid may therefore have a sensory impact on bitterness, as well as its combined effect with gallic acid. Maga & Lorenz (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 perceived bitterness.

Other phenolic compounds such as sinapic acid, caffeic acid, vanillic acid, ferulic acid, quercetin and naringin described as bitter tasting (Troszynska, 2004; Peleg & Noble, 1995) and 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 be due to relatively lower efficiency of extraction of phenolic compounds by water compared to organic solvents 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. However, it is worth noting that there were other peaks in the chromatograms which could not be identified (Figure 2). These may be other phenolic compounds in forms such as glycosides or even thermal degradation products for which standards were not available. Liquid chromatography coupled with mass spectrometry (LCMS) would be useful in providing a more exhaustive analysis of the phenolic compounds in the extracts.

3.4 Saponin content of marama bean and relation to bitterness

The effect of roasting time on the concentration of saponins in marama flour and water extracts evaluated by the sensory panel is presented in Table 4. Marama flour from beans roasted for 20 min had higher concentration of saponins than flour from raw marama beans. This is probably due to heat treatment which could have increased permeability of cell membranes thus maximizing the extractable amount of saponins in water extracts. There

Table 4: Effect of roasting time on the concentration of saponins in freeze dried marama bean extract, marama bean flour and marama bean water extracts

Samples	Saponin content in freeze dried marama extract (mg/g)	Saponin content in marama flour (mg/100 g)	Saponin content in water extract evaluated 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

Mean values with different letters within a column differ significantly ($p \leq 0.05$); standard deviations are given in parentheses

Roasted²⁰: marama beans roasted at 150 °C for 20 min

Roasted²⁵: marama beans roasted at 150 °C for 25 min

Roasted³⁰: marama beans roasted at 150 °C for 30 min

appeared to be a progressive decrease in saponin content of marama flour with increase in roasting time of marama beans. It might be possible that at the time and temperature combination of 20 min at 150 °C, saponins in the beans remain heat stable while longer heat treatment may cause saponin degradation. Previously, Chen, Xie & Gong (2007) used microwave energy (800 W) to assist extraction of saponins in *Ganoderma atrum*. The authors reported 20 min microwave treatment as the optimal time with the highest yield, and observed that longer extraction time at higher temperature had negative effects resulting from degradation or conversion of saponins into other compounds. It was suggested that the decrease in saponin 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, Gray, Hosfield & Uebersax, 1990). This may explain why the flour made from marama beans roasted for 30 min had the lowest saponin content. Moreover, Tarade, Singhal, Jayram & Pandit (2006) also reported that the rate of saponin degradation increased with an increase in temperature. Since saponins degrade with heat, it would be expected that roasting marama beans for a longer time duration at high temperature would degrade the saponins in the beans. The concentration of saponins in all marama extracts evaluated by the panelists was higher than the reported bitter threshold value of saponins in water (2-12 mg/l) (Heng *et al.*, 2006). Therefore it may be concluded that based on this bitterness threshold, saponins may also contribute to bitterness in the marama water extracts.

Pastes and water extracts made from marama beans roasted for progressively longer time periods tasted increasingly bitter. This appears to be more in line with the increase in water extractable quantities of gallic acid and protocatechuic acid with increasing heating time (Table 3), but not with the saponin content which seemed to decrease (Table 4). This

notwithstanding, the possible contribution of saponins in the extracts to bitterness may not be ruled out because of its reported bitterness threshold as mentioned earlier. It is also possible that compounds resulting from Maillard reactions such as melanoidins, furans and caramel compounds which could not be identified in this research, could also contribute to bitterness in the pastes and water extracts made particularly from marama beans roasted for longer periods of time (30 min). It is also possible that various compounds including gallic acid, protocatechuic acid, saponins, their decomposition products and various Maillard reaction products may be acting together synergistically to elicit bitterness.

On the basis of these results, it may be suggested that roasting marama bean for more than 20 min is not recommended to avoid negative sensory properties such as bitter taste, colour darkening and burnt notes. The potential of marama beans as an alternate source of food protein should consider the development of bitter taste as a function of roasting temperature and roasting time combinations. This research makes an important contribution to the development of this underutilised legume as an acceptable source of protein for at risk communities.

It is important to acknowledge that a limited number of bitter eliciting components were selected to be determined in this study. Further research is obviously required to explore the contribution of other compounds to bitterness of marama beans. Such compounds may include decomposition products of saponins (sapogenins) and other phenolic compounds and their thermal decomposition products. It will also be useful to apply more powerful analytical techniques such as LCMS to enable the identification of phenolic compounds for which standards are not available and possibly their decomposition products.

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