Physicochemical and functional characteristics of starch extracted from Marama bean tuber (*Tylosema esculentum* Burchell A. Schreiber)

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Abstract

Marama bean (*Tylosema esculentum* Burchell A. Schreiber) is a highly nutritious plant and is currently regarded as a prospective crop for the future in arid zone agri-ecologies of the world. Starch is a major storage component in higher plants and it is used in both food and non-food industries. The marama bean plant is a creeper with stems that lie prostrate on the ground in several directions up to six metres long in length which spread from a tuberous root below the ground. The tuber has a reddish brown bark and it can weigh about 1 kg and up to more than 10 kg. The plant produce attractive bright yellow flowers along the stems, each with erect petals and stamens, and are followed by marama fruits or bean. Mature marama bean cotyledons are white to cream in colour, encapsulated in hard, woody seed coats, reddish to brownish black in colour. Until recently, the basic knowledge of the physicochemical and functional properties of marama tuber starch is has not been yet reported. The present study reports for the first time the physicochemical and functional properties of marama tuber starch and makes a possible provision for a new starch source. Native marama

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starch content was 87.38 mg starch/gram fresh weight and the total amylose content was 35%. Phosphate at the C-6 position determined as Glucose-6-Phosphate was 0.788 nmol G6P/mg. The starch granules were round to elliptical with smooth surfaces and their sizes ranged from 8 -20 μm. The pasting properties of pasting temperature, host paste, peak, final viscosity, breakdown and set back showed higher values for marama starch in contrast to commercial potato starch. This study has clearly contributed to starch biology by making known for the first time the physicochemical and functional properties of marama tuber starch. It is hoped that by further exploring the potential of marama starch as a raw material, it can be applied in various applications in both industry and food processing that will produce high valued products.

Keywords: *Tylosema esculentum*; starch; pasting properties; amylose/amylopectin/starch granule.

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1 Introduction

Starch is the primary energy reserve in most plants and is the second most abundant carbohydrate in the biosphere after cellulose. Plants accumulate and mobilize starch in both non-photosynthetic storage tissues and photosynthetic tissue. In the plant, starch is deposited as granules in chloroplasts of photosynthetic tissues (leaves) as transient starch or in the amyloplasts of storage tissues (such as tuber, stem and seed endosperm) as storage starch. Physicochemical and functional properties of native tuber starches from arid agro ecological zones has been reported in species of major economic and nutritional importance such as Irish potatoes (*Solanum tuberosum*, family Solanaceae), cassava or manioc (*Manihot esculenta*, family Euphorbiaceae), sweet potatoes (*Ipomoea batatas*, family Convolvulaceae), yams (*Dioscorea* spp., family Dioscoreaceae) and cocoyams (*Colocacia* and *Xanthosoma* spp., family Araceae) Hoover, 2001). Starch is a major biopolymer in the food and non-food industries where it is utilized in diverse applications, such as a food thickener, , stabilisers, text modifier and gelling agents (Viñarta, MMolina, Figueroa and Farina, 2006; Khurana and Kanawjia, 2007), but also acts as a feedstock for bioethanol production as well as in the brewing industry (Wang et al., 2011). Starch varies greatly in form and functionality between and within botanical species, and even from the same plant cultivar grown under different conditions. Its different properties are utilized for making diverse food products. An increasing demand for starches from the food and non-food industries has created the need for new sources of this polysaccharide. A potential new starch source is the legume *Tylosema esculentum* (marama bean), a prospective new crop to semi-arid regions of southern Africa because of the outstanding high nutritional value of its seeds (Holse et al., 2010 Mosele, Hansen, Schulz and Martens, 2011; Hartley, Tshamekeng and Thomas, 2002) as

well as the starch (Coetzer, Robbertse and Grobbelaar, 1983) stored in the tuber. *T. esculentum* belongs to the Family *Fabacae* is a long lived perennial tuberous legume and a desiccation-tolerant species which thrives in semi-arid environments of Namibia, Botswana and the northern part of South Africa, with high temperatures, low rainfall and long periods of drought (van der Maesen, 2006). A number of studies have evaluated the domestication of the plant and it is potential as a food crop (Monaghan and Halloran, 1996; Holse et al., 2010; Mosele et al. 2011). In spite of its significant potential, the marama bean has not yet been studied very comprehensively in terms of starch. Carbohydrates or simple sugars such as fructose, glucose and arabinose have been reported for marama immature and mature seeds (Mosele et al. 2011), however the overall amount was less than 1%. The amount of starch in both immature and mature seeds was approximately $< 1\%$ (Mosele et al., 2011). There are no available studies on the analysis of physico-chemical and functional properties of marama tuber starch. Therefore, the aim of this study was to evaluate the functional and physico-chemical properties of native marama tuber starch. In an attempt to increase preference towards using underutilized food sources such as marama bean tubers, this study provides a beginning stage for possible application of native marama starch for both food and non-food uses. Establishing the commercial application of marama tuber starch will promote its demand, and local communities can plant and harvest marama for starch purpose with minimal inputs. This in turn would provide food security and generate income for the local communities for betterment of lives.

2 Materials and methods

2.1 Samples

Marama bean tuber samples were grown in marama experimental field of Omipanda (S20 27.3931 E16 39.443) and Otjovanatje (S21 19.355 E20 04.553) in Omaheke region, Namibia (Nepolo, Takundwa, Chimwamurombe, Cullis and Kunert, 2009). These sites are characterized by low rainfall ranging from 50 to 500 millimeters annually and long periods without precipitation (Powell, 1987). The soil at these sites is neutral to acidic, leached infertile and has low phosphorus content (Mmanatau, 2005). These sites were selected because they host marama experimental plots, making it possible to assess marama plants planted at the same time. Tuber samples were collected from the field after 12 months after planting and used directly in experimental analysis. These samples were collected by digging out the entire tuber and placed in Ziploc bags until the starch extraction process. Samples were immersed in distilled water upon arrival in the laboratory (Department of Biological Sciences, Faculty of Sciences, UNAM) prior to starch extraction which was performed within 48 hours.

2.2 Extraction of starch from marama tuber

Marama tuber starch was isolated by the method as described by Edwards, Marshall and Sidebottom, (1995) with some modifications. Two hundred grams of peeled and sliced tuber was homogenized with 500 mL of extraction buffer (50mM Tris/HCl, pH 7.5, containing 1mM diaminoethanetetraacetic (EDTA), 1mM dithiothreitol (DTT) and 0.1% (w/v) sodium metabisulfite) using a Waring commercial blender. The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged for 5 minutes at 1000 g. The supernatant was discarded and the starch was re-suspended in buffer (50mM Tris/HCl, pH 7.5, containing 1mM EDTA and 1mM DTT), then centrifuged for 5 min at 1000 g. This was repeated a further three times. After the last wash with buffer, the starch was re-suspended in cold acetone and left to settle at -20°C and the supernatant was discarded. This was repeated twice, until the starch started to appear white. The starch was allowed to air dry and stored in air-tight containers until analysis.

2.3 Determination of total starch

Concentration of starch in marama tuber was determined using amyloglucosidase/α-amylase method as described by McCleary, Solah and Gibson, (1994) (Total Starch, Megazyme International Ireland Inc). Fresh marama tubers were finely milled using a mortar and a pestle. A 100-mg milled sample was wet with 0.2 mL of 80% (v/v) ethanol and treated by boiling for 6 min in 300 units thermostable α-amylase to partially hydrolyse the starch. The pH of the samples was adjusted by adding 4 mL of 200 mM sodium acetate buffer pH 4.5. Dextrins were quantitatively hydrolysed to glucose by incubation at 50°C for 30 min with 20 units amyloglucosidase at pH 4.5. The samples were adjusted to 100 mL total volume and centrifuged at 2,000 × g for 10 minutes. The amount of glucose in the supernatant was determined using glucose oxidase/peroxidase (GOPOD) reagent (Megazyme International Ireland Inc). One litre of GOPOD reagent contains $\geq$ 12,000 units glucose oxidase, $\geq$ 650 units peroxidase, and 0.4 mmol 4-aminoantipyrine in glucose reagent buffer (1 M potassium dihydrogen orthophosphate, 200 mM para-hydroxybenzoic acid). A 0.1-ml aliquot of the supernatant as described above was incubated with 3 mL GOPOD reagent at 50°C for 20 min. The absorbance at 510 nm of each sample was read against the reagent blank. The concentration of starch in a sample was calculated by comparing the amounts of glucose produced from the sample and from glucose standard (1 mg/mL). Concentration of starch was expressed as a percentage of fresh flour weight.

$$\begin{aligned}\textbf{Starch } \% &= \Delta A \times F \times 1000 \times (1/1000) \times (1001W) \times (162/180) \\ &= \Delta A \times (FIW) \times 0.9\end{aligned}$$

where

ΔA = absorbance read against the reagent blank
F = conversion from absorbance of glucose standard to μg
= 100 μg of glucose/ absorbance for 100 μg of glucose
1000 = volume correction (0.1 mL taken from 100 mL)
1/1000 = conversion from μg to ml
$100/W$ = factor to express starch as a percentage of flour weight
W = the weight in mg of the flour analysed
162/180 = adjustment from free glucose to anhydro-glucose which occurs in starch

2.4 Determination of Amylose/Amylopectin

Amylose was determined using the Concanavalin A precipitation method as described by Gibson, Solah and McCleary, (1996) (Amylose/Amylopectin Assay Kit, Megazyme International Ireland Inc). A 25-mg sample was solubilized by boiling for 15 min in 1 mL dimethyl sulfoxide (DMSO). Lipids were removed by precipitating the starch in 6 mL of 95% (v/v) ethanol. The starch pellet was recovered by centrifugation at 2,000 × g for 5 min, then, redissolved by boiling for 15 min in 2 mL DMSO. The starch solution was diluted to 25 mL total volume with Concanavalin A solvent (180 mM sodium acetate, 900 mM $NaCl_2$, 0.9 mM CaCb, 0.9 mM $MgCl_2$ and 0.9 mM MnCl−2 pH 6.4). Starch from a 0.5-mL aliquot was hydrolysed with 16.5 units amyloglucosidase and 2.5 units α-amylase. The sample was incubated with 4 mL GOPOD reagent at 40°C for 20 min, and the absorbance of the sample was read at 510 nm against the reagent blank. Amylopectin in another aliquot (1.0 mL) was precipitated at room temperature for 1 h in the presence of 1.3 mg/mL Concanavalin A. After centrifugation at 14,000 × g for 10 minutes, amylose content in the supernatant was determined by treating the sample with amyloglucosidase/α-amylase solution. The percentage of amylose was expressed as a proportion of total starch. The amount of amylopectin was indirectly determined by subtracting the amounts of amylose from total starch concentration.

$$\textbf{Amylose},\%\ (\textbf{w/w}) = \frac{\text{Absorbance (Con A Supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

where: 6.15 = dilution factor for Con A-treated sample, and 9.2 = dilution factor for total starch extracts.

2.5 Determination of phosphate at the C-6 -position of the glucose residues

Phosphate at the C-6 position was determined as Glc-6-P after acid hydrolysis of the starch as described by Nielsen, Wischmann, Enevoldsen and Møller, (1994) with modifications. Starch (125 mg) was suspended in 0.5 mL of 0.7 M hydrochloric acid and kept at 100°C for 4 h. An aliquot of 30 μL was mixed with 230 μL of assay buffer containing 100 mM MOPS-KOH (pH 7.5), 10 mM $MgCl_2$, 2 mM EDTA in a microtitre well and neutralized with 100 μL of 0.7 N KOH. NAD (final concentration 0.4 mM) and 2 units of Glc-6-P dehydrogenase from Leuconostoc mesenteroides (Sigma-Aldrich, USA) were then successively added. Glc-6-P was determined by the absorption change at 340 nm caused by the Glc-6-P dehydrogenase-mediated reduction of NAD^+. Glucose residue was determined as the Glc-6-P formed by acid hydrolysis of starch. Potato starch was concurrently analysed for phosphate as a control.

2.6 Pasting properties

The pasting properties of marama starch were determined using a rapid visco-analyzer (Perten RVA 4500, Australia). An amount of 4.0 g of starch was directly weighed into aluminum RVA canisters and 25 mg of distilled water was added to form a starch suspension. The starch suspension was stirred in the RVA container initially at 11,000 × g for 10 sec and finally at 3000 x g for the remainder of the test. The temperature profile was started from 50°C for 1 min followed by raising the temperature linearly to 95°C in 3 min and 42 sec, holding for 2 min and 30 sec, cooling the system to 50°C in 3 min and 48 sec and holding at 50°C for 2 min. Peak viscosity (PV), temperature at PV ($P_{temp.}$), hot paste viscosity (HPV) or holding strength, cool paste viscosity (CPV) or final viscosity, breakdown (BD) or (PV-HPV), Set back (SB) or (CPV-HPV) and stability ratio HPV/PV were determined from the viscosity proïňĄle curve and expressed in centipoise (cP). The Thermocline windows software was used to process the data (Perten RVA 4500, Australia).

2.7 Starch granular characterization

The starch granules were observed using a Scanning Electron Microscope (Leo-Zeiss, Germany). Starch granules were sprinkled onto a double-sided tape attached to a stub and coated with gold using a sputter coater (s150A, Edwards) and placed in the SEM chamber. Photomicrographs were taken using a SEM apparatus at an accelerating voltage of 25 kV.

Figure 1: Marama tuber slices (A) White starch extracted from marama tuber (B).

3 Results and Discussion

3.1 Marama tuber starch extraction

Starch was successfully extracted from marama tuber and starch isolated was white in colour to naked eyes with a smooth texture (Figure 1, plate A and B). Colour is an important criterion in evaluating starch usage as any form of pigmentation on starch will adversely affect its acceptability and that of its products (Morell and Myers, 2003; Galvez and Resurrection, 1992).

3.2 Determination of marama tuber total starch

The total starch content, determined by enzymatic digestion was 87.38 mg starch/gFW as amyloglucosidase/α-amylase method. This compares unfavourably with industrially important starch sources such as potato tuber (15.44% of the fresh weight; Hoover, 2001), cassava roots (18.1-23.4% of the fresh weight, Defloor, Dehing and Delcour, 1998) and maize (65% of the dry weight, Morell and Myers, 2003). It should be considered, however, that the higher starch contents in those species are the result of many years of breeding for increased starch, so it is likely that increased starch content could also be attained in marama bean.

Table 1: Starch content, G6P content and amylose content of the marama tuber‡

Starch content (mg starch/gFW)	G6P Content (nmol G6P/mg starch)	Amylose (% w/w)
87.38 ±18.24	0.788 ± 0.159	35.74 ± 5.18

‡The data presented are the mean of samples taken from 4 independent tubers.
From each tuber five samples were taken and analysed individually.
Final data represents the mean and computed standard deviation.

3.3 Determination of phosphate at the C-6 -position of the glucose residues

The phosphate monoester content on the 6-position of the glucose monomer within marama tuber starch determined as Glucose-6-Phosphate after acid hydrolysis was 0.788 nmol G6P/mg starch (Table 1). The level amounts of Glc-6-bound phosphate was lower than that of potato (7.8- 33.5 nM Glu-6-P/ mg starch), cassava (2.5 nM Glu-6-P/ mg starch), and Maranata arundinacea (Arrow root) (4.5 nM Glu-6-P/ mg starch) observed by Blennow, Bay-Smidt, Olsen and Møller, (2000). However, the figure obtained in this study is higher than those reported for cereal starches, such as barley and maize (0.0 nM Glu-6-P/ mg starch) (Blennow et al., 2000). The starch phosphate content has been reported to be influenced by growing conditions and temperature and varies with the botanical origin of the starch (Nielsen et al., 1994). Low starch phosphate content of marama tuber could be influenced by the arid growing condition, higher temperature and low amount of phosphate in the soil. The presence of phosphate groups in starch is known to confer increased hydration capacity of starch pastes after gelatinization and the starch-phosphate content is correlated to starch-paste peak viscosity and gel-forming capacity (Blennow et al., 2002).

3.4 Determination of amylose/amylopection content of marama tuber starch

Determination of the amylose/amylopectin content by enzymatic digestion showed that in marama tuber amylopectin is the major constituent comprising about 65% (w/w) with amylose constituting the remaining 35% (w/w) (Table 1). The amylose content for marama tuber starch determined in this study was 35%, higher than that reported for potato (18.7%) (Lloyd et al., 1999) and 19.1% for sweet potato (Collado, Mabesa and Corke, 1999), but within the range (10-38%) of amylose content of native tuber starches (Hoover, 2001). This is also in line with amylose contents for legume starches (32.5-35.6%) reported by Singh, Nakuara, Inouchi and Katsuyoshi (2008). Amylose and amylopectin content plays an important role in influencing the functional properties of starches. High amylose starches are

characterized by their high gelling strength which suggests their usefulness in the production of pasta, sweets, bread and in the coating fried products (Vignaux et al., 2005).

3.5 Pasting properties

The pasting properties of the native marama starch dispersion and commercial potato at the same concentrations are presented in Table 2. Marama tuber starch showed higher pasting temperature (91.15°C) than potato starch. The peak viscosities (PV) observed for marama and potato starches were 2087 and 1075 cP respectively. The hot paste viscosity of two starch samples was comparable with marama starch showing a higher value of 829.3 cP. The breakdown viscosity (BD) of potato was 1025.5 cP, lower than 1257.7 cP of marama starch. The marama starch showed higher value of cold paste viscosity/final viscosity 1276.3 cP than potato starch. The setback viscosity (SB) was much higher for marama starch (447 cP), than that observed for potato starch (69 cP).

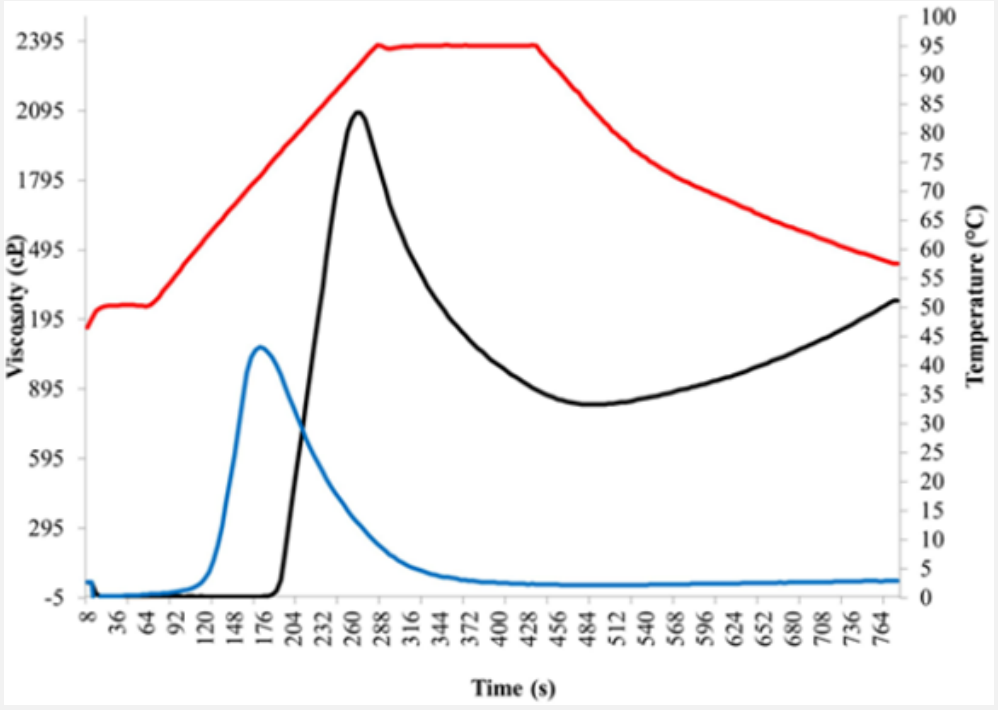

Figure 2: Pasting properties of native marama tuber and potato starch suspensions studied by Rapid visco-analyser. Red = temperature; Black = marama tuber starch; Blue= potato starch.

To compare the pasting properties of the different starches, gelatinization temperature

Table 2: Pasting properties of starches from *T. esculentum* and commercial potato‡

Starch Sample	P_{Temp} (°C)	PV(cP)	HPV(cP)	CPV(cP)	BD(cP)	SB(cP)
Marama	91.15	2087	829.3	1276.3	1257.7	447
Potato	72.5	1075	49.5	69	1025.5	19.5

‡P_{Temp} = Pasting temperature; PV= Peak viscosity; HPV =Hot paste viscosity; CPV= Cool paste viscosity; BD=Breakdown;SB= Setback.

(GT), peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown (PV-HPV) and setback (CPV-HPV) were calculated from the viscosity-temperature versus time curves obtained (Figure 2).

The heating of a starch-water dispersion under shear above its gelatinization temperature yields starch pastes. The pasting profiles of a starch is an effective method for relating starch functionality with its structural features and access the potential industrial application in products dependent on the viscosity and thickening behaviour of starch. The marama tuber starch pasting temperature was higher than that observed for commercial potato starch.

Pasting properties may be influenced by factors such as degree of branching of amylopectin and starch granule structure (Kim, Wiesenborn, Lorenzen and Berglund, 1996). The peak viscosities observed for marama tuber starch was higher than that observed for potato. The peak viscosity is an indicator of water binding capacity and ease with which the starch granules are disintegrated and often correlated with the final quality of the end product (Ragaee and Abdel-Aal, 2006). Higher peak viscosity is an indication that more starch has been gelatinized during processing (Suhendro, Kunetz, Mcoonough, Rooney and Waniska, 2000).

The hot paste viscosity of potato starch samples was comparable, with marama tuber starch showing a higher value. A higher hot paste viscosity represents low cooking loss and superior eating quality in food products (Cruz, Abraão, Lemos and Nunes, 2013). Marama tuber starch showed higher value of cold paste viscosity. A higher paste viscosity relates to the stability of the cooled, cooked starch paste to shear and it is due to the formation of an amylose network resulting in gel structure (Miles, Morris, Orford and Ring, 1985). Setback viscosity is a measure of retrogration of starch after cooling of the cooked starch paste. The higher value of setback in marama may be the result of higher amylose content as the linear amylose molecule favours more intermolecular hydrogen bonding than amylopectin, which has a branched structure. Marama tuber starches possible application could, therefore, exist in the food industry as its gelling property is double that of potato starch.

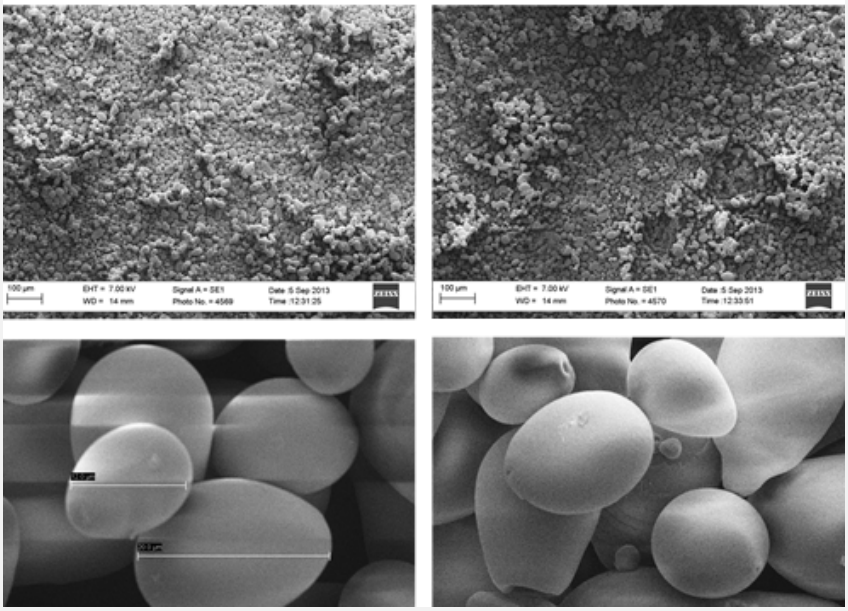

Figure 3: Scanning electron micrographs of native marama tuber starch granules (Nepolo, unpublished).

3.6 Granular morphology

The micrographs of the starch granules of marama tuber exhibited a variety of shapes, varying from small round (8 μm) to the larger elliptical and oval (20 μm). The surface of starch granules appeared to be smooth and showed no evidence of fissures or ruptures. Some of the starch granules had deep indentations (Figure 3). This observation was in accordance with previous results reported for starch granules isolated from legume species such *C. sativa* (Cruz et al., 2013) and *P. sativum* (Wang, Sharp and Copeland, 2011). Starch granule size plays a significant role in influencing the pasting parameters of starches and may have influence on the physicochemical and digestibility properties of starch (Kaur, Sandhu and Lim, 2010). Fine starch granules could be used as fat substitutes in high fat foods (Wang et al., 2011).

4 Conclusions

Native marama tuber starch reported in this study presented different physicochemical and functional properties to those previously reported for other legumes. This study makes

known for the first time the physicochemical and functional properties of native tuber starch and makes a possible provision for a new starch source. The relatively high proportion of amylose present in the starch and it is functional properties make it a possible industrial source of starch. Native marama tuber starch presented a pasting profile similar to potato starch, but with higher pasting gelatinization and peak viscosity temperature. This makes native marama tuber starch a potential technological alternative to potato starch especially in food application requiring higher pasting gelatinization. Future studies should therefore focus on transforming marama tuber starch into value added products such as thickener in tomato sauce and custard food type. Further information on marama starch physical and chemical properties need to made available to establish the specific usefulness of marama tuber starch.

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Conflicts of Interest

The authors declare no conflict of interest

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