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Isolation and physicochemical characterization of starch from Marama Beans (*Tylosema Esculentum*)

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Abstract
One of the most popular types of beans grown in South Africa is Marama bean seeds (*Tylosema esculentum*) which are still widely underused although it is being consumed by the local population. To determine its qualities and assess its appropriateness for application in various sectors, starch was isolated from the plant's seeds and subjected to physicochemical characterization. Starch was isolated from the seeds using the crushing method along with sodium metabisulphite to prevent fermentation. Physicochemical parameters were determined using standard procedures and amylose / amylopectin ratio evaluated using the UV method by comparison with a standard. The starch was physically and chemically characterized, and it was found to have physicochemical values as follows – gelatinization temperature 64 °C; foam capacity 6%; bulk density 0.769 gcm^{-3}; emulsion capacity 38%; pH 7.77; water holding capacity 21.67%; tapped density 0.857 gcm^{-3}; browning temperature range 235.2 – 256.0 °C; charring temperature range 271.9 – 287.3 °C. At 95 °C, the starch percentage solubility was 3.5%, and the swelling power was 11.059. *Tylosema esculentum* starch was found to be made up of 73.523 % amylopectin and 26.477 % amylose. Given that its qualities favorably compare with those of starch used in industries especially the pharmaceutical sector, the varied physicochemical parameters results of starch isolated from *Tylosema esculentum* demonstrated potentials as a promising starch source for use in industries like the pharmaceutical, food, paper and textile as a biomaterial.

Keywords: Marama, Pharmaceutical, Physicochemical, Starch, *Tylosema esculentum*

1. Introduction
Tylosema esculentum, a [Fabacea] leguminous plant specie, is a legume that grows in Southern Africa's arid regions like Botswana and Namibia. The Marama Bean [*T. species*] is a potentially useful source of protein from leguminous oilseeds in Southern Africa. In Kaduna North, Nigeria, where they are cultivated, Marama beans are also referred to as 'Wawa-mata'. Marama beans seeds are a great source of protein and have a dry matter percentage of 30 – 39%. In developing nations, the Marama bean has enormous potential as a wholesome and nourishing crop, but none of its carbs have been properly investigated. The average starch content of marama seeds is 0.2% dry mass, according to Mosele et

al. (2011), although there is a dearth of knowledge regarding the physicochemical properties of Marama starch to establish its potential uses. For rural people that gather Marama beans, the possible use of the carbohydrate fractions in food applications has the potential to be very profitable. In the food industry, polysaccharides in particular, which can serve as thickeners, stabilizers, texturizers, and gelling agents, are important.

Based on the importance of starch and the need to investigate more sources from which starch with excellent characteristics for use in various studies can be found and the dearth of knowledge on physicochemical characteristics of starch from Marama beans; this research work is therefore intended to isolate and evaluate the characteristics (physicochemical) of starch gotten from *Tylosema esculentum* commonly known as Marama beans.

2. Literature review

Tylosema esculentum, the marama bean, is an underused legume that grows wild in portions of southern Africa and is consumed by the local population there. The Marama bean is found in various provinces throughout South Africa, as well as sparingly in Mozambique and Zambia. South Africa's Northern Cape, Limpopo, Gauteng, and North West provinces are home to the Marama bean, according to the National Department of Agriculture, Forestry, and Fisheries. Marama bean, a species of *Tylosema*, is a Southern African native oilseed legume that is underutilized. Like soy beans and peanuts, it is an excellent source of protein, it also has starch content. Marama's exceptionally high nutritional value as well as the amount of starch stored in the tuber makes it a potential new crop in Africa.[6] Although Marama beans have the potential to be a nutritious food and cash crop, very limited research has been done on them, including domestication efforts. Marama beans seeds are not usually eaten raw possibly because of the slimy texture and the unpleasant taste. Trypsin is an enzyme found in raw seeds that can have a negative effect on nutrition. The underutilization of Marama bean tuber starch can be attributed to inadequate understanding of its physicochemical characteristics in this particular plant species. This research work will lay the groundwork for the potential application of native Marama starch for both food and non-food uses, with the goal of encouraging people to preferentially use underutilised food sources like Marama bean tubers.

Starch is a large carbohydrate, a major source of energy for plants, and a crucial industrial resource on a worldwide scale because of its availability and quantity. Demand for starch is always high, especially in other businesses apart from food. For both humans and animals, the most significant dietary source of energy comes from starch found in storage organs (kernels, roots, tubers, or stems) derived from diverse botanical sources. Four - fifths of energy needed by humans are from major cereals (wheat, rice, and maize) and essential tuber crops (potato, cassava, and yam). Plants have both non-storage organs like leaves and roots and storage organs like seeds and tubers which contain the complex carbohydrate polymer known as starch. It exists as glucan polymers that are insoluble in water and take the shape of the plastid's semi-crystalline granular structure. Two distinct glucosyl polymers make up a starch granule (the amylose portion is straight essentially, while the amylopectin is massively branched). The amylopectin portion and amylose portion combine together to produce semi-crystalline granules having an interior structure which is lamellar and insoluble. Amylopectin is typically the main component, typically comprising up to 75% or more of the starch granule, and most of these polymers' ratios within starch granules are genetically defined. According to Seung

(2020), the amylose concentration of starch normally ranges from 20 to 30%. However, some mutant plant genotypes, particularly those found in maize, can have up to 70% amylose content in their starch. Conversely, other genotypes, referred to as waxy genotypes, are found in a range of plant species and include starch with less than 1% amylose (maize, barley, rice, and amaranth).

3. Research methodology

3.1. Materials

Marama beans were purchased at Gwagwalada Market in Abuja, Nigeria. All reagents used were analytical grade reagents and gotten from the CARC, Sheda Science and Technology Complex. The standard used was Corn starch (BP) and equally gotten from CARC.

3.2. Methods

Isolation of starch from Marama beans Seeds

Marama bean seeds were selected to remove dirt and then cleaned under running water. About 2 L of 1% w/v Sodium metabisulphite solution was used to soak the separated seeds (0.95 Kg) for an overnight period. The moistened seeds were then taken out and ground into a slurry with a lab blender. A considerable volume of 1% sodium metabisulphite solution was used to disperse the paste, which was then repeatedly filtered through muslin cloth. To help remove debris, the suspension gotten was spun in a centrifuge machine at a high speed of 3500 rotations per minute for 10 minutes. After spinning, the top liquid was gently poured off, and the soft, sticky part on top was removed. This step was repeated three times to make sure only the pure starch was left. Each time, any remaining sticky substance was taken off the starch. Once separated, it was first left out in the sun and then placed in a warm oven at 60 °C to dry. The starch finally gotten was crushed into powder, weighed, and then put into sample jars for later study.

Physicochemical Properties Determination

Emulsion Capacity

Using a vortex mixer, 30 seconds were spent dispersing 1 g of the material in 5 ml of distilled water. Once everything had been thoroughly mixed, 5 millilitres of vegetable oil was gradually added and mixed for a further 30 seconds. The suspension was centrifuged at 1600 rpm for five minutes. The amount of oil extracted from the sample was measured straight out of the tube. The emulsion capacity is the amount of oil emulsified and retained per gramme of sample.

Foam Capacity

Modifications were made to Omojola et al. (2012) methodology and used to determine the foam capacity. One gram of starch was homogenised over a period of five minutes using a vortex mixer (vortex 2 Genie set at shake 8) and fifty millilitres of distilled water. The homogenate was then poured into a one hundred millilitre measuring cylinder, and the volume was recorded 30 seconds later. The percentage increase in volume was used to express the foam capacity.

Water Holding Capacity

To ascertain this, the Fagbohun et al. (2013) approach was applied. In a centrifuge tube that had already been pre-weighed, the 5% w/v starch sample was diluted. For two minutes, the tube was stirred in a vortex mixer. Thereafter, the supernatant gotten was removed, and then the tube containing hydrated starch sample together was weighed. The calculation was done as the amount of water (in grams) bound by 100 g dry starch sample.

Gelatinization Temperature

Using Adebiyi et al. (2011) methodology, about ten millilitres of distilled water were added to a 20 millilitre beaker containing the 1 gram starch sample. The solution was heated and subsequently, a thermometer submerged in the solution was used to measure the temperature of gelatinization.

pH

A pH meter was used to measure this in a solution of 2 gram starch in 10 milliliter solution of the sample that had been agitated in water for five minutes.[17]

Browning / Charring Temperature

With slight modifications, the procedure of Omojola et al. (2012) was employed in determining this. A melting point device (model Electrothermal 9100) was used to measure the browning / charring temperatures of a portion of the sample placed in a capillary tube. This was read as temperature at which the sampled first turned brown and then blackish on heating.

Bulk / Tapped Density

With a few minor adjustments, the technique outlined by Adebiyi et al. (2011) was used to determine the bulk density of the sample. Sample (50 g) poured using a short glass funnel with a stem into a measuring cylinder calibrated to hold 250 cm^3. Bulk density was calculated by taking note of the volume that the starch occupied in the cylinder and dividing by the weight of sample used To find the tapped density, the cylinder was repeatedly tapped using a ruler, until a constant volume was obtained and then calculated by dividing the volume gotten with the weight of sample used.

Solubility Index

Procedure outlined by Michael et al. (2020) was utilized with small adjustments. Exactly 0.5 g of starch sample and 10 ml of distilled water were mixed together and then heated for thirty minutes at fifty °C. After that, it was centrifuged for a further half hour at 1500 rpm. A 5 milliliter decant of the top liquid was gotten then dried. The solubility was expressed as the percentage of sample that dissolved.[18] This was done within a temperature range of 50 °C – 95 °C.

Swelling Power

Again, the process outlined in Michael et al. (2020) was employed to calculate this while making a few little adjustments. Ten milliliters of distilled water and 0.1 gram of the sample were added together and the mixture was heated for 30 mins while being constantly shook in a water bath at 50 °C. Ultimately, the tube and contents was centrifuged for 20 minutes at a speed of 1500 rotations per minute to make it easier to remove the top liquid, which was then removed and the weight of the remaining paste was recorded. The result was computed by dividing weight of the resultant sample paste with the starting weight. The experiment was done within 50 °C – 95 °C.

Determination of Amylose / Amylopectin Constituents

Test tubes containing 0.1 g of the starch sample and corn starch standard were weighed separately. Carefully, 1 cm^3 ethanol together with 9 cm^3 of 1 mol / dm^3 sodium hydroxide solution were introduced to the samples. After completely mixing, the samples were covered. To gelatinize starch, the test tubes were set in a water bath and heated for ten minutes and then thoroughly cooled. Ten times the suspensions were diluted. For analysis, a volume of 0.5 cm^3 was set aside, and acetic acid (0.1 cm^3) together with iodine solution (0.2 cm^3) were added in that order. It was filled to a capacity of 10 cm^3 using 9.2 cm^3 of distilled water. After about 20 minutes during which the colour of the solution would have developed, it was vortexed and the absorbance was read at 620 nm.

Amylose content was expressed as:

Percent amylose content = {% Amylose of standard X Absorbance of sample} / Absorbance of standard

The % amylopectin content was then determined by subtracting the amylose content from 100.

4. Data analysis

The data are presented as the average standard deviation after each experiment was run in triplicate.

5. Results and discussions

The starch that was extracted had a yield of approximately 27 percent and was discovered as a bright whitish, crystalline powder that doesn't absorb water and without any odour. The yield is thought to be highly noteworthy, particularly in comparison to starches derived from samples like maize, cassava and potatoes. Physicochemical parameters results of Marama beans starch are outlined in Table 1. Figures 1 and 2, respectively, display the solubility profile and the swelling profile of the starch extracted from Marama beans.

Table 1: Physicochemical Characteristics of Marama Beans Starch

Parameter	Value
pH	7.77
Amylose Content	26.477%
Tapped Density	0.857 gcm^{-3}
Water Absorption Capacity	21.67 mg/100g
Gelatinizing Temperature	64 °C
Foam Capacity	6%
Browning Temperature	235.2 – 256.0 °C
Emulsion capacity	38%
Charring Temperature	271.9 – 287.3 °C
Bulk Density	0.769 gcm^{-3}
Amylopectin Content	73.523%

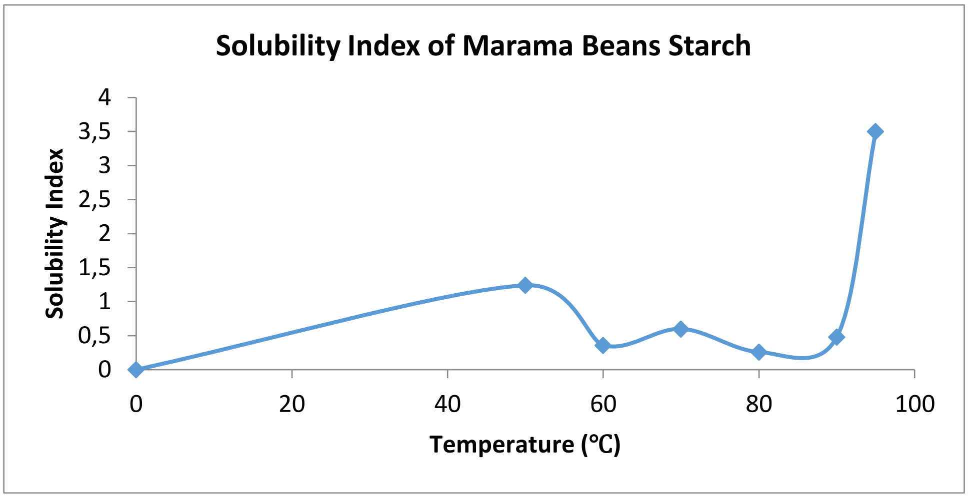

Figure 1: Solubility Index of Marama Beans Starch

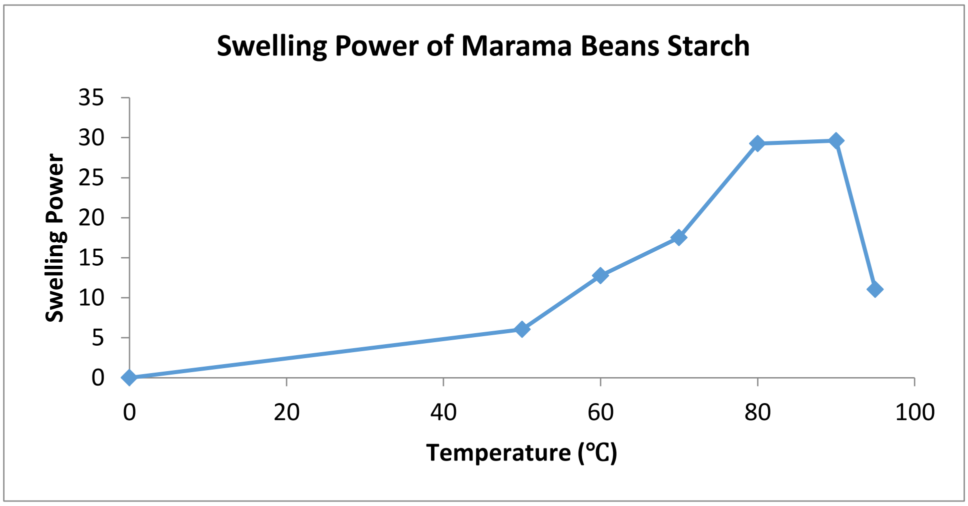

Figure 2: Swelling Power of Marama Beans Starch).

As is typical of pure starch, the starch produced from *Tylosena esculentum* seeds was a powder which had a yield of about 27% and was non-hygroscopic in nature. The bright white and crystalline powder after all preparations was also odourless. As compared to other starch sources such as cassava (33%), and maize (61.4%), the yield is believed to be quite significant. Its gelatinization temperature of 64 °C, is within the range of starch gelatinization temperatures that are typically observed. According to the study, the starch also had a 38% emulsion capacity and a 6% foam capacity which are a bit higher than what have been generally reported for starches from other sources. This is not unexpected and may result from the source material's high protein and fat content.

The temperature that starches may be cooked to without browning or charring is indicated by their browning and charring temperatures. Marama starch can still be used in some industries which utilize starch at quite elevated temperatures even though it has a browning temperature which is considerably lower than what has been reported for starches extracted from tiger nut, maize and many more. But compared to other starches, the charring temperature is about the same. This is in tandem with previous findings for starches from corn, *Anchomanes difformis*, tiger nut, ginger and *Icacina trichantha*.

The water holding capacity was recorded as 21.67 ml water in 100 g of starch sample which is quite lower in comparison with results from other starch samples. The variation in the granule's crystalline and amorphous area proportions may be the cause of the variance in water absorption capacity. The bulk and tapped densities fall within the range suggested for maize starch and are consistent with the results as reported. This demonstrates the starch's excellent compressibility. However, it was found that the starch has a quite high amylose content of 26.477%, which may mean that it is not a healthy food option for those with diabetes or other health concerns.

Figures 1 and 2 show the Marama beans starch patterns of swelling and solubility through temperature range from 50 – 95 °C. While the solubility index shows a slightly two-stage swelling pattern, the solubility profile of the starch shows a general trend of increasing with increasing temperature. This demonstrates the ability of the granules to absorb water while being heated. A minor fall occurs between 50 and 60 °C, followed by an increase up to 70 °C, a slight leveling off period, and then another swift increase starting at 90 °C. At different temperatures, there are two compositions of forces that bond internally- and then relax which have been suggested as the cause of this pattern and are quite similar to what has been observed for other starches like corn, potato and yam.

The swelling power is extremely noticeable; it is greater than that of maize starch but less than that of tiger nut, ginger, and anchovy starches. The starch's swelling curve indicates a rapid and progressive increase of swelling power with increase in temperature up to 90 °C before taking a dip at 95 °C. This signifies that Marama beans starch may be utilized successfully as a disintegrant in tablet formulation in pharmaceutical industries because an increase in swelling power indicates that a particular starch may be suitable for use as a possible disintegrant in drug formulation.

6. Conclusion

Starch from *Tylosema esculentum* has been isolated, and some of its physicochemical characteristics have been studied. According to the study, these characteristics are quite favorable when compared to those of other starches. *Tylosema esculentum* is a good source of starch, and the study has consequently demonstrated that it is a promising biomaterial for industrial usage (especially for pharmaceutical use because of the excellent swelling power), and particularly if the physicochemical qualities of the starch are enhanced to fulfill basic industrial biomaterial grade requirements. The raw starch and modified starch from marama beans can be further subjected to use in the industries in order to ascertain if the properties are suitable or further need to be modified.

The study on starch isolated from marama beans has indicated that it is a potential source of starch for both local and industrial use which can be exploited. This ultimately will make starch more affordable and there will be less burden on sourcing starch from some other popular and already over burdened sources for instance cassava, potatoes, and corn.

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