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Extraction and characterisation of cassava starch cultivated in different locations in Sabah, Malaysia

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Abstract

This work aimed to determine the physicochemical and functional properties of starch cassava grown in two different locations in Sabah, namely Tawau and Semporna. In this study, the starch of the cassava was extracted using a wet method and analysed for its physicochemical and functional properties. The total starch content of cassava grown in Semporna showed a higher value (61.21 g/100 g) compared to the sample from Tawau (51.77 g/100 g). No significant difference ($p>0.05$) observed for starch yield extracted from these two locations. However, there was a significant difference ($p<0.05$) showed for total starch, resistant starch and amylose content. The amylose content of starch isolated from Semporna (23.16%) was higher than from Tawau (13.87%). Scanning electron microscope revealed that starch isolated from both locations had smooth surfaces with some granules spherical, elongated and irregular in shape. Cassava starch from Semporna exhibited a high value of swelling power (6.85%) compared to Tawau (4.07%), and they were significantly different ($p<0.05$). The solubility values of the starch samples from Tawau and Semporna were 28.48% and 24.34%, respectively. The pH was observed to be lower for cassava starch isolated from Tawau (4.80) than for starch obtained from Semporna (5.49). The water absorption capacity of starch from Semporna absorbed slightly more water than starch from Tawau, with values of 76.51% and 63.64%, respectively. Pasting properties results showed no significance for all profiles measured except for setback viscosity. No significant differences ($p>0.05$) were observed for all gelatinisation and retrogradation properties. This study suggests that location influenced the physical, chemical and functional properties of cassava's starch.

1. Introduction

Cassava (*Manihot esculenta,* Crantz) is one of the most important root crops in tropical countries that providing energy to human due to a significant amount of carbohydrates and high nutritional value in its roots. Typical mature roots have an average composition of 60–70% moisture, 30–35% carbohydrate, 1–2% fat, 1–2% fibre and 1–2% protein, with trace quantities of vitamins and minerals. Mature roots can range in starch content from as low as 15% to as high as 33%, depending on the climate and harvest time (Breuninger *et al.,* 2009).

Starch is one of the most important functional food biopolymers (Mamat and Hill, 2018). It is one of the main components in cassava that have been utilised in food industries to facilitate the processing method as well as improved final food products. The starch content of the fresh cassava root is about 30% and gives the highest yield of starch per unit area of any crop known (Tanukari, 2004). It's a complex carbohydrate and among the most abundant of plant products, a major food reserve providing nutrient and energy source.

Starch is the most consumed carbohydrates in the human diet. It is deposited in the fruit in the form of granules, partially crystalline, whose morphology, chemical composition, and supermolecular structure are characteristic of each particular plant species. Starch owes much of its functionality to two major high molecular weight carbohydrate components, amylose and amylopectin, as well as to the physical organisation of these macromolecules into the granular structure

(French, 1984). Amylose is a linear polymer consisting of α-D-glucopyranose residues are linked through α-(1→4) glycosidic bonds whereas amylopectin is a branched polymer with α-(1→4) glycosidic bonds, having periodic branches at the O-6 position (Buléon *et al.*, 1998)

Asoaka *et al.* (1991) studied the effect of cultivar and seasonal towards physicochemical properties of cassava starch. They found that even though there are differences in organoleptic properties of cooked cassava root, yet, there is only a minor difference in physicochemical properties of starch granules. Tester *et al.* (1997) reported that high temperature would give an effect on the biosynthesis process in cassava starch causes depressed starch deposition to lead to low yield of starch because the synthesis of starch granules is small and little.

Functional and pasting properties of cassava flour and starch are important in the food industry since they will directly affect food products (Niba *et al.*, 2002; Mishra and Rai, 2006; Hasmadi *et al.*, 2020). However, the study on the characterisation of cassava flour in Malaysia, especially in Sabah, is scarce. Therefore, the objective of this study is to determine the physicochemical and functional properties of cassava starch extracted from two different districts in Sabah, namely Tawau and Semporna.

2. Materials and methods

2.1 Raw materials

The matured white variety of cassava (9 months of age) was harvested from Tawau and Semporna, Sabah, Malaysia.

2.2 Starch extraction and yield

Extraction of cassava starch was conducted using the wet method as described by Noorfarahzilah *et al.* (2020) with slight modifications. Cassava was mixed with water in a ratio of 1:10 and blended at high speed until smooth slurry formed. After blending, filtration of the slurry was done using a double layer of cheesecloth. The filtered mixture then was allowed to rest for two hrs to help the sedimentation process of starch and liquid at the top was decanted and discarded. The sediment then undergoes a drying process at 45°C for 24 hrs and stored in an airtight container for further analysis. Percentage of extraction yield was calculated using the formula as follow:

$$\text{Extraction Yield (\%)} = \frac{Starch\ weight\ (g)}{Cassava\ weight\ (g)} \times 100$$

2.3 Determination of total starch content

Total starch content was determined using a kit assay (Megazyme International, Wicklow, Ireland) based on the AOAC method 996.1 (2000) by enzymatic hydrolysis of starch using amylase/amyloglucosidases and quantification of glucose using glucose oxidase/peroxidase reagent.

2.4 Determination of amylose/amylopectin content

The amylose content was determined using an amylose/amylopectin assay kit (Megazyme International, Wicklow, Ireland) by selective quantitative precipitation of amylopectin with concanavalin A (Con A) and removing it by centrifugation.

2.5 Determination of resistance starch

Resistant starch was determined using a kit assay (Megazyme International, Wicklow, Ireland). This kit follows the protocols of the AOAC method 2002.02 (2000) procedures described by Niba and Hoffman (2003).

2.6 Determination of water absorption capacity

The water absorption capacity of samples was determined according to the method described by Mustapha *et al.* (2015). The water absorption capacity of samples was then calculated by using a formula as follow:

$$\text{Water absorption capacity} = \frac{W^2 - W^1}{W^0}$$

Where, W^O = Sample weight (g), W^1 = Tube weight (g) + sample weight (g), W^2 = Tube weight (g) + sediment (g).

2.7 Determination of swelling power and solubility

Swelling power and solubility was determined by the method described by Ikegwu *et al.* (2010) with a slight modification. The supernatant after the centrifugation process was poured into an aluminium plate and dried using an oven at 100±5°C for 4 hrs. The weight of the aluminium plate was taken every four hrs until there are no weight changes recorded. Properties of swelling power and solubility are then calculated based on the equation as follow:

$$\text{Solubility (\%)} = \frac{weight\ of\ dried\ supernatant}{Sample\ weight}$$

$$\text{Swelling Power (\%)} = \frac{W^2 - W^1}{W^0}$$

Where, W^0 = Sample weight (g), W^1 = Sample weight (g) + test tube weight (g), W^2 = Weight of swelled sediment (g) + test tube weight(g)

2.8 Determination of pH

The pH of the cassava starch was determined by using pH-meter based on the method described by Eriksson (2013).

2.9 Determination of pasting properties

The pasting properties were studied by using Rapid Visco Analyzer (RVA) as described by Chandanasree *et al.* (2016). Pasting profile recorded as peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, pasting temperature and peak time.

2.10 Determination of gelatinisation properties

Gelatinisation properties were determined by using Differential Scanning Calorimeter (DSC) based on the method described by Mamat *et al.* (2010). Several parameters, such as onset temperature (T_o), peak temperature (T_p), end temperature (T_c) and gelatinisation enthalpy (ΔH) were calculated and recorded.

2.11 Determination of retrogradation properties

Retrogradation properties were determined by using Differential Scanning Calorimeter (DSC) based on the method described by Raphael *et al.* (2011) with several modifications. The time used to allow retrogradation to occur completely was extended until 21 days. Several parameters, such as onset temperature (T_o), peak temperature (T_p), end temperature (T_c) and retrogradation enthalpy (ΔH) was calculated and recorded.

2.12 Determination of starch granule morphology

The starch granule morphology was observed by using a Scanning Electron Microscope (SEM) based on the method as described by Chandanasree *et al.* (2016).

2.13 Statistical analysis

The values reported are the mean of three replicates. The significance of differences ($p<0.05$) was assessed using an independent t-test performed by SPSS software.

3. Results and discussion

3.1 Starch yield

Starch yield is the amount of starch physically recoverable from cassava root. Starch yield for cassava starch from two different locations is given in Table 1. It was observed that cassava grown in Semporna exhibited a high extraction yield compared to cassava starch grown in Tawau with values of 22.58% and 21.71% respectively. However, statistical analysis showed that there was no significant difference (*p>0.05*) in the percentage of extraction yield obtained from both

locations. Zhu (2015) reported extraction yield of starch from cassava is varies depending on the method of extraction used and the area of cassava planted in which extraction can be as high as 80% and as low as 11%. Various artificial factors affecting the starch yield include the drying conditions of raw materials, harvesting time and root storage, the status of the roots used for extraction and the environmental conditions for growing the crop (Zhu, 2015). The results recorded in agreement with the findings reported by Abera and Rakashit (2003) and Joseph *et al.* (2004). However, Olomo and Ajibola (2003) reported a higher percentage of starch extraction, ranged from 48.8% to 79.9%.

Table 1. Extraction yield, total starch content, amylose/amylopectin content of cassava starch

Analysis	Tawau	Semporna
Extraction yield (%)	21.71±0.50[a]	22.58±1.48[a]
Total starch (g/100 g)	51.77±0.07[b]	61.21±0.17[a]
Amylose (%, w/w)	13.87±0.05[b]	23.16±0.52[a]
Amylopectin (%, w/w)	85.44±1.10[a]	74.28±0.12[b]

Values are expressed as mean±SD. Values with different superscript within the row are significantly different (p<0.05).

3.2 Total starch

The total starch content of cassava grown in Semporna showed a high value (61.21 g/100 g) compared to the sample obtained from Tawau (51.77 g/100 g), as shown in Table 2. Results obtained are higher than values reported by Charles *et al.* (2004) (34.2 to 35.1%) but lower than the finding reported by Aprianita *et al.* (2014) (77.4%). Cassava is one of the four most important sources of starch worldwide, together with rice, maise and wheat. Niba *et al.* (2002) recorded starch content of flour from 62.8±1.13 to 75.7±0.01 g/100 g dry weight for eleven cassava root genotypes in Nigeria. Aniedu and Omodamiro (2012) analysed six newly bred β-carotene cassava and found that starch contents of the flours produced from the roots ranged from 23.18 to 61.05%, which in agreement with our findings.

Table 2. Swelling power, solubility, pH, water absorption capacity, resistance starch of cassava starch

Analysis	Tawau	Semporna
Swelling power (%)	4.07±1.65[b]	6.85±0.34[a]
Solubility (%)	28.48±1.13[a]	24.34±4.91[b]
pH	4.71±0.21[b]	5.49±0.03[a]
Water absorption capacity (mL/g)	63.64±3.58[b]	76.51±0.11[a]
Resistant starch (g/100 g, dwb)	6.99±0.28[a]	4.65±1.04[b]

Values are expressed as mean±SD. Values with different superscript within the row are significantly different (p<0.05).

3.3 Amylose/amylopectin content

Amylose and amylopectin composition play essential roles in cassava starch, influencing thermal properties such as gelatinisation, retrogradation, gelling, pasting as well as crystallinity (Blazek and Copeland, 2008). In this study, a significant difference (p<0.05) was observed for amylose content recorded for Semporna and Tawau with values of 23.16% and 13.87% (Table 1), respectively. Similar observations have been reported earlier (Mbougueng *et al.,* 2009). Also, Li *et al.* (2001) and Charles *et al.* (2004) reported that the amylose content of cassava growth in Thailand falls ranged between 18% until 25%. Amylopectin content of cassava flour from Semporna was higher (85%) compared to Tawau (74%) (Table 1).

Amylose and amylopectin content is important as it determines the functional properties of cassava starch. Cassava with low amylose content (as exhibit by cassava grown in Tawau), will decrease the amorphous region between starch granules thus lead to the increase in crystallinity properties (Tukomane *et al.,* 2007). Furthermore, amylose content is one of the factors that influence the retrogradation properties of cassava starch in which high amylose content causes the ability of starch to retrograde increase due to the aggregation process of amylose that acts as the nucleus (Rodriguez-Sandoval *et al.,* 2008). Amylose content also influenced the pasting properties of starch. A high amount of amylose content will increase the pasting temperature because the releasing of amylose from the amylopectin network will be longer during gelatinisation, thus higher temperature required to form a starch paste (Moorthy, 2002).

3.4 Resistant starch

Resistant starch is defined as the fraction of starch that is resistant to digestion by α-amylase and pullulanase enzymes in vitro and may be fermented in the colon (Ashwar *et al.,* 2015). The results show that there were significant differences (p<0.05) in the resistant starch content of the starch extracted from both locations (Table 2). The values recorded in this study are lower than reported in past research (Martín Bernabé *et al.,* 2011). Resistant starch is indigestible by body enzymes and divided into 4 fractions namely RS1, RS2, RS3, and RS4 or also called type I, II, III, and IV starches (Sajilata *et al.,* 2006). Most of the cassava starch is categorised as resistant starch type II (RS2) where starch is in a specific granular form and resistant to enzyme digestion. It is measured chemically as the difference between the glucose released by the enzyme digestion of a boiled homogenised food sample and that from an unboiled, non-homogenized food sample. In raw starch granules, starch is tightly packed in a radial pattern and is relatively dehydrated (Sajilata *et al.,* 2006). Resistant starch gives several benefits to human health (Ashwar *et al.,* 2015). Apart from providing benefits to human, resistance starch as well contributes to the quality of the final product in food such as crunchiness, mouth-feel and palatability (Buttriss and Stokes, 2008).

3.5 Water absorption capacity

From the results (Table 2), it was observed that starch from Semporna held slightly more water than starch from Tawau, with values of 76.51% and 63.64%, respectively. Significant differences in the water absorption capacity were observed in this study. The protein content is one of the factors that influenced water absorption capacity in starch (Abdalla *et al.,* 2009). Protein molecules trap and form a bond with water molecule through hydrogen bonding and electrostatic attraction. Thus, the high content of protein leads to increased water absorption capacity. Apart from that, Nuwamanya *et al.* (2010) reported that protein and carbohydrate content in cassava would influence water absorption capacity because both components have hydrophilic regions such as polar network and amino acid network. Hoover and Sosulski (1986) reported that the variation in water absorption capacity of starches could be due to the difference in the degree of the engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains.

3.6 Swelling power

Swelling power provides evidence of non-covalent bonding between starch molecules. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation determine the degree of swelling and solubility (Rickard *et al.,* 1991). Results for the swelling power of cassava starch are presented in Table 2. Cassava starch from Semporna exhibits a high value of swelling power (6.85%) compared to Tawau (4.07%), and they are significantly different (p<0.05). The results obtained in this study were slightly lower compared to previous findings. A study conducted by Charles *et al.* (2005) shows a swelling power value can be as high as 27.2% until 42.3%, while another study conducted by Onitilo *et al.* (2007) shows a reading between 9.0% until 16.9% for swelling power at temperature 80°C. Delpeuch and Favier (1980) described that swelling volume also depend on the presence of various chemicals and treatments carried out on starch.

3.7 Solubility

The solubility values of the starch samples from

Tawau and Semporna were 28.48% and 24.34%, respectively. The solubility values of the samples were significantly different (p<0.05). Past studies found that solubility properties of starch have an inversely proportional relationship with amylose content; meanwhile, swelling power also have an inversely proportional relationship with amylopectin content in cassava starch (Kruger *et al.,* 2003). The data obtained in this study is in agreement with the findings by Kruger *et al.* (2003) in which an inversely proportional relationship occurs between amylose content and solubility of cassava starch from Tawau and Semporna. The same occurrence can be observed between amylopectin content with swelling power.

According to Aryee *et al.* (2006), low swelling power followed by high solubility properties of starch indicates weak associative forces in the starch granules. However, a weak associative force between starch granules is desired attribute in the processing of high glucose syrup since it favours the process of hydrolysis compared to starch that has high associative forces (Aryee *et al.,* 2006). One of the factors that lead to weak associative power between starch granules is due to damage starch that occurs during its extraction. Amongst the factors that determine starch solubility are a source of starch, inter-associate forces, swelling power and presence of other components (Buckman *et al.,* 2017).

3.8 pH

The pH was observed to be lower for cassava starch isolated from Tawau (4.80) than for starch from Semporna (5.49) (Table 2). The pH values of the samples were significantly different (p<0.05). The lower pH of cassava starch might be due to the presence of comparatively more impurities that incorporated into the starch during processing (Mishra and Rai, 2006). Sangeetha and Rai (2006) reported that the pH value of starch is influenced by two main factors that are the soil condition in the planted area as well as the compositional content of starch itself. Besides, the presence of foreign matter such as peel of cassava will as well influenced pH value in cassava starch (Mbougueng *et al.,* 2008).

3.9 Pasting properties

The pasting properties of cassava starch are one of the factors to be considered to maximise the application of cassava starch in the food industry, mainly as a food thickener. The viscosity of paste formed after the heating process is a vital aspect to be studied to determine the type of instrument that suitable to be used during food processing (Zhu, 2015; Noorfarahzilah *et al.,* 2017). Table 3 shows the pasting profiles of starch from two different locations. The results show that there were

significant differences (p<0.05) for peak, trough, breakdown, and final viscosity, but there is no significant difference observed for setback viscosity. The peak viscosity obtained in this study is higher than reported by Colman *et al.* (2012). Peak viscosity indicates the water-binding capacity of the starch or mixture. It is often correlated with final product quality. The peak viscosity occurs at the equilibrium point between swelling, causing an increase in viscosity and rupture and alignment, causing its decrease (Onitilo *et al.,* 2007).

Table 3. Pasting properties of cassava starch

Parameter	Tawau	Semporna
Peak viscosity (cP)	5575.33±209.00[a]	5550.67±231.31[a]
Trough viscosity (cP)	1948.00±83.54[a]	2039.33±55.19[a]
Breakdown viscosity (cP)	3627.33±127.45[a]	3511.33±285.28[a]
Final viscosity (cP)	2868.00±119.75[a]	2799.33±102.79[a]
Setback viscosity (cP)	920.00±37.47[a]	870.00±205.85[b]
Pasting temperature (°C)	72.98±0.53[a]	72.67±0.06[a]
Peak time (min)	4.98±0.12[a]	3.07±0.04[a]

Values are expressed as mean±SD. Values with different superscript within the row are significantly different (p<0.05).

The breakdown viscosity was 3627.33 cP and 3511.33 cP for starch from Tawau and Semporna, respectively. Adebowale *et al.* (2005) described that the higher the breakdown in viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking. According to Sandhya Rani and Bhattacharya, (1995), the breakdown is caused by the disintegration of gelatinised starch granule structure during continued stirring and heating. The difference in breakdown viscosities is related to the difference in rigidity of swollen granules.

Results of the setback viscosity were 920.00 cP and 870.00 cP for starch from Tawau and Semporna, respectively. There is a significant difference (p<0.05) in the setback viscosity of the starch samples. The values recorded are lower than the results obtained by Colman *et al.* (2002). The authors reported that the setback viscosity for native cassava starch was 948±6.93 cP. The pasting temperature of the starches obtained from Tawau (72.98°C) and Semporna (72.67°C) are within the range of the values previously reported for tuber (Charles *et al.,* 2004; Abera and Rakshit, 2004; Charles *et al.,* 2005). Pasting temperature represents the minimum temperature at which the sample will cook, which can have implications on the stability of other components in a formula and also indicate energy costs. In general, higher pasting temperature showed a higher degree of crystallinity of the starch (Mishra and Rai, 2006).

3.10 Gelatinisation properties

The gelatinisation properties of cassava starch are

given in Table 4. There are no significant differences observed for onset, peak and final temperature, as well as enthalpy. A study conducted by Sandoval and Fernandez, (2013) show a higher result compared to the data obtained from this study in which the range for value T_o, T_p and T_c is falls between 60.9 – 66.51°C, 65.44 – 72.00°C and 69.91- 71.36°C respectively. Hashimoto and Grossmann (2003) reported that gelatinisation temperature is influenced by the surrounding temperature during the growing of cassava. Besides, Gerard *et al.* (2001) reported that a low value of T_o, T_p and T_c is due to the low amylopectin network in starch. Data obtained in this study supporting the idea of Gerard *et al.* (2001), where gelatinisation temperature of Tawau starch T_o, T_p, T_c show high reading compared to Semporna in which both with 68°C, 72°C, 77°C and 62°C, 71°C and 72°C respectively.

Table 4. Gelatinisation properties of cassava starch

Parameter	Tawau	Semporna
Onset (°C)	68.93±0.21[a]	62.59±1.08[a]
Peak (°C)	72.25±0.27[a]	71.15±0.13[a]
End (°C)	77.30±0.44[a]	73.53±0.69[a]
Entalphy (J/g)	3.56±0.19[a]	10.70±0.75[a]

Values are expressed as mean±SD. Values with different superscript within the row are significantly different (p<0.05).

3.11 Retrogradation properties

Starch retrogradation is a process in which disaggregated amylose and amylopectin chains in a gelatinised starch paste re-associate to form more ordered structures (Wang *et al.,* 2015). The retrogradation properties of cassava starch are presented in Table 5. Statistical analysis shows there is no significant difference (p>0.05) observed for all parameters, for both samples of starch. However, the Tp value obtained from this study (55.54°C and 55.62°C) was higher compared to study conducted by Raphael *et al.* (2011) that is ranged from 51.01°C to 53.35°C whereas the enthalpy value in this study is lower (0.40 and 0.79 J/g) compared to study conducted by Raphael *et al.* (2011) that is ranged from 1.60 to 5.25 J/g. Moorthy (2002) reported that retrogradation for starch extracted from tubers is slower compared to starch extracted from cereals. A similar finding was reported by Yoo and Jane (2002). Lin *et al.* (2006) described that cassava starch contains more short-chain in amylopectin causes the retrogradation process to occur slower. Wang *et al.* (2015) reported that starch retrogradation is affected by several factors including water content, storage temperature, storage time, and additives (carbohydrates, salts, proteins, lipids and other food additives) in the system.

Table 5. Retrogradation properties of cassava starch

Parameter	Tawau	Semporna
Onset (°C)	45.01±0.16[a]	43.87±0.16[a]
Peak (°C)	55.54±0.59[a]	55.62±0.41[a]
End (°C)	61.24±0.45[a]	62.74±0.48[a]
Entalphy (J/g)	0.40±0.02[a]	0.79±0.03[a]

Values are expressed as mean±SD. Values with different superscript within the row are significantly different (p<0.05).

3.12 Starch granule morphology

The morphology of cassava starch plays a vital role in its functional properties. By Scanning electron microscope (SEM), the starches isolated from Tawau and Semporna had smooth surfaces with some granules spherical, elongated and irregular in shape (Figure 1), with a diameter ranging between 2.81 and 21.8 μm, while those of Semporna ranged between 2.89 and 20.7 μm. All the starch granules shape revealed by the SEM micrograph are as expected based on the previous study reported by Chandanasree *et al.* (2016) in which native starch granules are sphere, round and oval. However, several starch granules are concave in shape. Nuwamanya *et al.* (2010) reported that the starch granules that exist in concave shape are caused by excessive handling during its process of extraction from cassava. Differences in starch granules size may be attributed to the botanical origin as well as the variety of the crop.
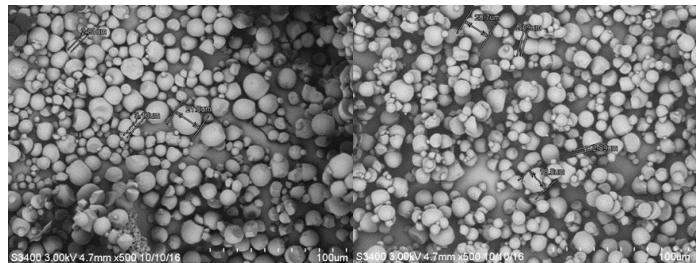

Figure 1. Granule morphology of cassava starch planted in Tawau (left) and Semporna (right) observed under scanning electron microscopy (SEM).

4. Conclusion

This study found that cassava starch extracted from different location varies in its properties. Starch isolated from Semporna showed a high amount of yield, total starch and amylose content compared to starch obtained from Tawau. A similar trend was also observed for swelling power, solubility and water absorption capacity. No significant difference observed for thermal profiles such as pasting, gelatinisation and retrogradation. Data obtained from this study could be used in food formulations as well as improving the functionality of cassava starch when used as a supplement ingredient in food production or to be used as biopolymer components in edible film preparations.

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Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

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