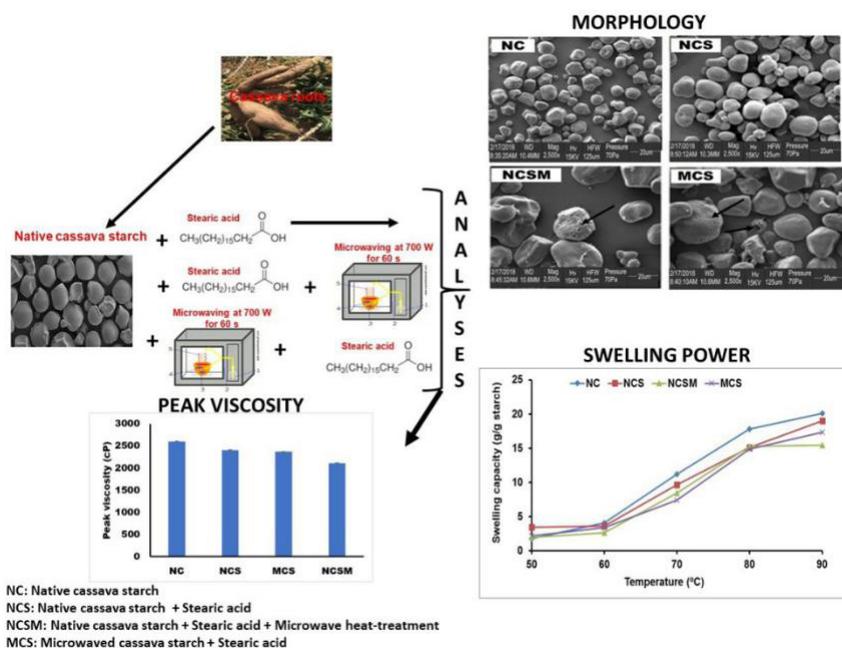


Morphology, pasting and thermal properties of microwave-assisted cassava starch- stearic acid complex

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Highlights

- Microwaving and stearic acid addition reduced the swelling power of cassava starch.
- Microwaving in combination with stearic acid showed a greater reduction in peak viscosity.
- Pasting of native or microwaved cassava starch with stearic acid produced type I amylose-lipid complexes.

RESEARCH ARTICLE

Morphology, pasting and thermal properties of microwave-assisted cassava starch- stearic acid complex

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Abstract: In this study, the physicochemical properties of microwave-assisted cassava starch-stearic acid complex was investigated. The effects of the modification methods were assessed using a scanning electron microscope, differential scanning calorimeter and rapid visco analyser. Native cassava starch showed mostly polygonal shape with some granules irregular and round in shape. Native cassava starch with added stearic acid had slight changes in shape while microwaved cassava starch showed irregular and dented granules with pin-holes or fissures. Native cassava starch modified with stearic acid alone or microwaved cassava starch with added stearic acid showed decreased swelling power as compared to the native starch. Pasting properties of cassava starch were significantly affected by stearic acid addition or microwaving. Stearic acid addition alone reduced the peak viscosity slightly (approx. 7%), adding stearic acid to microwaved cassava starch reduced the peak viscosity by approximately 9%, while microwaving cassava starch-stearic acid mixture showed greater reduction in peak viscosity (approx. 19%). Pasting of native or microwaved cassava starch with stearic acid resulted in the formation of type I amylose-lipid complexes, with melting temperatures ranging from approx. 92 to 102°C. Microwaving can improve complexation of stearic acid with cassava starch to produce modified starch with higher thermal stability and increased final viscosity.

Keywords: Amylose-lipid complexes, Cassava starch, Stearic acid, Microwaving.

INTRODUCTION

Cassava (*Manihot esculanta* Crantz) is a root widely consumed in many parts of the world including Africa. It is a good source of starch, which may be explored in various industrial applications due to its remarkable characteristics such as high freeze-thaw stability, high paste viscosity and clarity which are advantageous for applications industrially (Nwokocha *et al.*, 2009). Although cassava starch represents a valuable ingredient in food and pharmaceutical industries, in the native form, the starch is unsuitable for most industrial applications. This is because native starches generally they have poor resistance to processing conditions such as shear, pH and heat frequently encountered in the industry.

Hence, they must be modified to eliminate these shortcomings and to improve their physicochemical and functional properties (Kaur *et al.*, 2012). According

to Oyeyinka and Oyeyinka (2018), starch may be modified by chemical, physical, enzymatic genetic and a combination of these methods. Due to increased awareness of the relationship between diet and health, consumers are demanding foods with natural chemicals rather than synthetic ones. Hence, researchers are now focusing on the use of environmentally friendly method such as the use of natural chemicals e.g. lipids (Oyeyinka *et al.*, 2018; Oyeyinka *et al.*, 2017a) and physical methods such as microwaving (Lewandowicz *et al.*, 2000; Oyeyinka *et al.*, 2019).

Cassava starch has been chemically modified using lipids such as stearic acid (Maphalla and Emmambux, 2016; Schmidt *et al.*, 2013; Varavinit *et al.*, 2001) and physically by microwaving (Colman *et al.*, 2014; Lewandowicz *et al.*, 1997; Oyeyinka *et al.*, 2019). Starch-lipid complexes also known as V-amylose complexes are important in food applications because they have been reported to reduce tendency towards retrogradation (Oyeyinka *et al.*, 2016b), produce starch with slowly digestible property (Oyeyinka *et al.*, 2017c) and enhanced final viscosity (D'silva *et al.*, 2011; Oyeyinka *et al.*, 2017c).

The interaction between starch and lipids is influenced by several factors including amylose content, lipid type, starch moisture content and complexation conditions. Due to the benefit of starch-lipid complexes, efforts are on-going to improve the degree of complexation of starch with lipids using different method such as extended pasting of starch with lipids (D'silva *et al.*, 2011), increased incubation time of starch with lipids (Chang *et al.*, 2014), use of high pressure homogenization (Meng *et al.*, 2014a; Meng *et al.*, 2014b; Oyeyinka *et al.*, 2016a, 2016b) as well as a combination of physical and chemical methods (Oyeyinka *et al.*, 2018).

Microwave in combination with chemical methods have been reportedly used in starch modification for improved functionality. For instance, Zhao *et al.* (2018) reported that microwave pretreated esterification improved the substitution degree, structural and physicochemical properties of potato starch esters. Furthermore, other authors reported that microwave-assisted rapid heating of

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starch is an effective method for increasing the production of starch acetate (Lin *et al.*, 2017). Other studies found that microwaving alone altered the structure of starch granules (Lewandowicz *et al.*, 2000) and created pin holes on granule surface (Luo *et al.*, 2006; Oyeyinka *et al.*, 2019). Hence, it is hypothesized that the pores created during microwaving may facilitate the movement of lipids into the starch granule interior during heating. Hence, this study investigated morphology, pasting and thermal properties of microwave-assisted cassava starch- stearic acid complex.

MATERIALS AND METHODS

Cassava roots (TMS 326) were harvested from University of Ilorin research farm, Ilorin, Kwara state, Nigeria. Stearic acid (STE) was purchased from Ninoflex chemical stores, Ikeja, Lagos state. All chemicals and solvents used were laboratory grade.

Starch extraction

Starch was extracted as previously reported (Osunsami *et al.*, 1989). Cassava roots (2 kg) were peeled, washed and grated using a grating machine powered by Lister Diesel engine (5-1 6HP 650RPM, UK). The mash was submerged in water and sieving was done using a muslin cloth into a fresh bowl of potable water. The extract was left to settle for 24 hrs. After settling, the supernatant was disposed and fresh water (10 litres) was added to the sediment. The washing procedure was repeated five times until a clean white starch was obtained. Starch slurry was pressed in a muslin cloth to remove excess water and the starch sample was oven dried at 60 °C for 24 hrs. Dried samples were milled using a warring blender (Model: 8010S Torrington USA), sieved (sieve size: (180 µm) and packaged in Ziplock bags prior to analyses.

Microwaving of starch

Starch was microwaved as previously reported except that a maximum time of 60 s was used (Oyeyinka *et al.*, 2019). Briefly, the moisture content of the starch was adjusted to 30% with distilled water. The moistened starch was allowed to equilibrate for 2 h, prior to heating. After equilibration, starch samples were sealed with perforated polyethylene foil (Macro Packaging, Ivychurch, Kent, UK) designed for microwave ovens. Starch was heated using a microwave (Model MEJ11K, LG, Kuala Lumpur, Malaysia) at 700 W output and a frequency of 2450 MHz for 60 s. After heating, the sample was allowed to cool at room temperature and stored at 4°C until analysed.

Incorporation of stearic acid into starch

The complexing of stearic acid to the starch granules was done according to previous methods (Oyeyinka *et al.*, 2017c). Cassava starch was suspended in minimum amount of ethanol containing 2% stearic acid. The suspension was stirred with a magnetic stirrer at 50°C for 1 hr and then at room temperature for an additional 12 hr. Thereafter, the starch sample was oven dried and used immediately for subsequent analyses. Native cassava starch was treated the same way as the stearic acid treated starch.

Swelling power

Swelling power was determined as previously reported (Oyeyinka *et al.*, 2015). Briefly, a 1% starch suspension in water was heated for 30 min from 50 to 90°C with constant stirring. The suspension was centrifuged (model 5810R, Eppendorf International, Frankfurt, Germany) at 3400×g for 20 min at 25°C and the supernatant discarded. Swelling power was obtained by weighing the residue after centrifugation and dividing by the original weight of starch on a dry weight basis.

Microscopy

Starch granule morphology was studied using a scanning electron microscope (EVO 15 HD, Carl Zeiss, Jena, Germany) (Oyeyinka *et al.*, 2019). Average starch granule size was determined from diameter of individual granules (N=50) on the basis of the scale bar provided on the captured scanning electron micrographs (Stevenson *et al.*, 2006).

Pasting properties

The pasting properties of the starch samples were measured using a Rapid Visco-Analyzer (RVA 4500, Perten Instruments, Sydney, NSW, Australia). Briefly, 2.5 g of each sample was weighed and distilled water was added after accounting for the variation in moisture content of the microwaved samples. The mixture was agitated by mixing manually using a plastic paddle before inserting the canister into the instrument. Starch was stirred at 960 rpm for 10 s before the shear input was decreased and held constant at 160 rpm during the subsequent heating and cooling cycles. The peak viscosity, hot paste viscosity, cold paste viscosity, breakdown viscosity, peak time and pasting temperature of the samples were obtained from the instrument.

Thermal properties

Thermal property was examined according to a previously reported method (Oyeyinka *et al.*, 2015). Briefly, pasted freeze-dried complexes starch (3 mg) was directly weighed into an aluminum DSC pan and distilled water (12 µl) added. Pans were hermetically sealed and equilibrated for 12 hrs. Samples were heated from 20 to 120°C at a rate of 10°C/min; an empty pan was used as reference. The onset temperature (T_o), melting temperature (T_m), conclusion temperature (T_c) and melting enthalpy (ΔH_m) were obtained for the starch-lipid complexes.

Statistical analysis

Duplicate samples were prepared and analyses done in triplicate. Data was analysed using one-way analysis of variance (ANOVA) and means were compared using the Fisher Least Significant Difference (LSD) test ($p \leq 0.05$) using the Statistical Package for the Social Sciences (SPSS) Version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Morphology of native and microwaved starches

Native cassava starch granules had a different morphological appearance compared to starch granules from microwaved cassava starch (Figure 1). The starch granules were mostly polygonal with some granules irregular and round in shape. The average size of the native cassava starch granules ranged between 4 and 10 μm , which is in the range of values (2–20 μm) reported for cassava starch (Rolland-Sabaté *et al.*, 2013; Rolland-Sabaté *et al.*, 2012; Zhu, 2015).

Microwave heat treatment modified the surface properties of the cassava starch granules with obvious significant changes in the granule shapes and sizes. However, the addition of stearic acid to native cassava starch showed only a slight change in granule surface properties. Native cassava starch with added stearic acid had slight changes in shape, while the microwaved starch samples showed evidence of pores on the starch granules (Figure 1). The addition of stearic acid prior to microwaving or after microwaving had similar effect on starch granule morphology. Microwaving of starch has been found to alter starch structure (Lewandowicz *et al.*, 2000) and created pin holes on granule surface (Luo *et al.*, 2006; Oyeyinka *et al.*, 2019).

Swelling power of native and microwaved starches

Native cassava starch showed an increase in swelling power with increase in temperature, especially (Figure

2), which may be attributed with starch gelatinization (Oyeyinka *et al.*, 2015). Cassava starch complexed with stearic acid showed reduction in swelling at all the test temperatures compared with the native starch sample. Microwaving of starch prior to complexation with stearic acid or microwaving complexed starch resulted in further reduction in swelling of starch, indicating that microwaving further enhanced the interaction between starch and the lipids. It has been reported that reduction in swelling of starch following lipid addition results from interaction of lipids with amylose in the hydrophobic tube, which prevents starch granule hydration and swelling (Oyeyinka *et al.*, 2017c). Furthermore, the lipid molecule may also cover starch granule surface with a film (Kim and Walker, 1992). Thus, the greater reduction in swelling after microwaving suggest that microwaving enhanced the interaction of the lipids with amylose.

Pasting properties

Native and microwave-assisted starch-stearic acid complexes showed similar pasting curves, but significantly ($p < 0.05$) different pasting properties (Table 1). Native and microwaved cassava starch showed reduction in the peak viscosities after stearic addition. Cassava starch containing stearic acid with subsequent microwaving showed higher reduction (approx. 19%) in peak viscosity compared with native starch with added stearic acid (approx. 7%) and microwaved starch with added stearic acid (approx. 9%). The reduction in peak viscosity of starch in the presence of added lipid such as stearic acid presumably results from

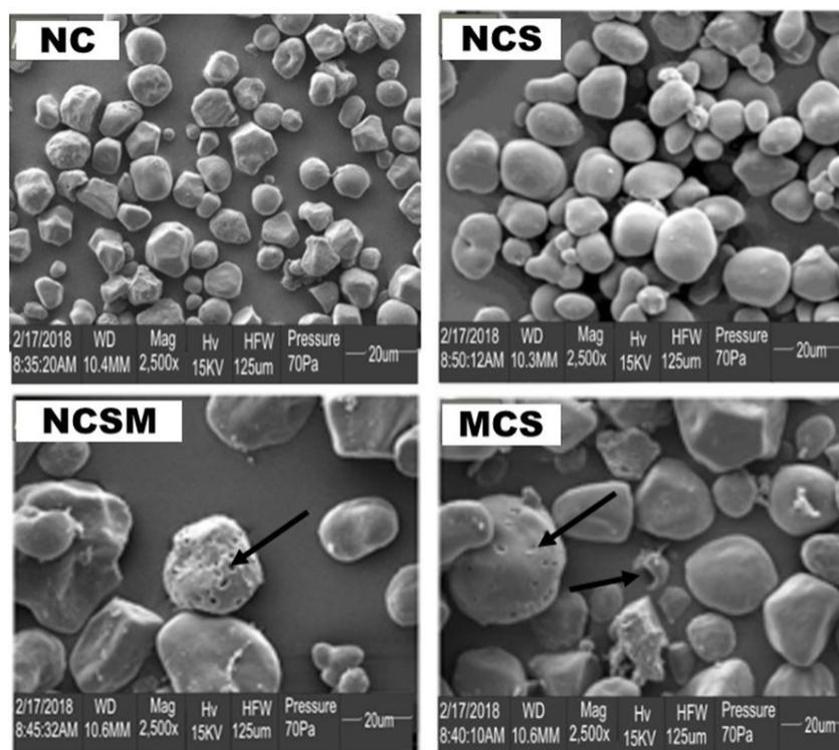


Figure 1: SEM images of native and microwave-heated starches, Scale bar represents 20 μm ;

NC: Native cassava starch, NCS: Native cassava starch + Stearic acid, NCSM: Native cassava starch + Stearic acid + Microwave heat-treatment, MCS: Microwaved cassava starch + Stearic acid.

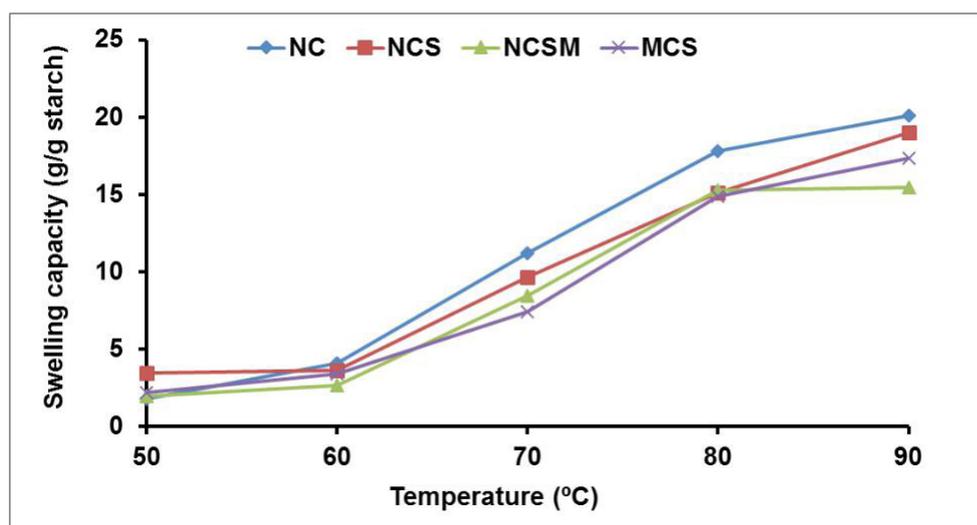


Figure 2: Swelling power of native and microwave-assisted cassava starch-stearic acid complexes.

NC: Native cassava starch, NCS: Native cassava starch + Stearic acid, NCSM: Native cassava starch + Stearic acid + Microwave heat-treatment, MCS: Microwaved cassava starch + Stearic acid.

Table 1: Pasting properties of native and cassava starches modified with microwave and stearic acid.

Parameters	NC	NCS	MCS	NCSM
PV (cP)	2596.67 _a ±7.05	2404.01 _a ±6.23	2370.13 _b ±2.94	2111.67 _a ±1.41
HPV (cP)	1672.67 _c ±1.45	1707.33 _b ±1.04	1654.64 _c ±4.32	2049.33 _a ±2.28
CPV (cP)	2585.06 _c ±5.00	2762.00 _b ±2.00	2734.00 _b ±1.00	3261.67 _a ±3.25
BDV (cP)	697.33 _b ±7.68	899.33 _a ±5.20	457.00 _c ±4.04	354.67 _c ±4.67
Setback ratio	1.54 _a ±0.03	1.62 _a ±0.17	1.38 _c ±0.04	1.59 _b ±0.02
Stability ratio	0.71 _c ±0.01	0.66 _d ±0.01	0.97 _a ±0.03	0.85 _b ±0.01
PT (°C)	74.90 _a ±0.20	73.57 _a ±0.08	74.17 _a ±0.15	74.20 _a ±0.40
Peak time (min)	4.14 _b ±0.01	4.14 _b ±0.04	5.04 _a ±0.04	5.04 _a ±0.01

Mean±SD. Mean with the same superscript along a row are not significantly different ($p < 0.05$)

PV: Peak viscosity; HPV: Hot paste viscosity; CPV: Cold paste viscosity; BDV: Breakdown viscosity; PT: Pasting temperature.

NC: Native cassava starch, NCS: Native cassava starch + stearic acid, NCSM: Native cassava starch + stearic acid + Microwave heat-treatment, MCS: Microwaved cassava starch + stearic acid.

the formation of amylose-lipid complex between starch and the added lipid. Previous studies similarly attributed reductions in peak viscosity to the formation of amylose-lipid complexes after pasting rice starch with stearic or linoleic acids (Zhou *et al.*, 2007) or potato starch with different fatty acids (Kawai *et al.*, 2012).

The higher reduction in peak viscosity of cassava starch containing stearic acid with subsequent microwaving suggest that more complexes were formed between the starch and the stearic acid. This may explain why cassava starch containing stearic acid with subsequent microwaving had lower swelling power compared to other starch samples (Figure 2). With or without added stearic acid, cold paste viscosities of cassava starch were generally higher than their hot paste viscosities (Table 1). This is expected since the viscosities of biological materials are temperature dependent. However, the cold paste viscosities

significantly increased with the addition of stearic acid or microwaving. Earlier studies also found increase in the final viscosity of starch after lipid addition (Maphalla and Emmambux, 2016; Obiro *et al.*, 2012; Wang *et al.*, 2015).

The pasting temperature (74.9°C) of native cassava starch was higher than values reported for potato starch (66.2-68.6°C) (Gałkowska *et al.*, 2014; Joshi *et al.*, 2013). Microwaving and stearic addition did not significantly ($p < 0.05$) influence the pasting temperature of cassava starch. Native cassava starch had similar pasting temperature (74.9°C) with those of the modified starch samples (average of 72.98°C). Pasting temperature represents the temperature at which the starch samples will cook. Hence, it seems that microwaving cassava starch with added stearic acid or the addition of stearic acid to native starch did not significantly ($p < 0.05$) affect the pasting temperature. Setback ratio significantly reduced from 1.62 to 1.38. The re-association

of starch as double helices upon cooling is responsible for the setback viscosity of starches (Oyeyinka *et al.*, 2017b) and indicates the retrogradation tendencies of starch (Liang *et al.*, 2002). Stearic acid addition alone to native cassava showed approximately 2% reduction in setback ratio compared with stearic acid addition to microwaved cassava starch (approx. 5%) and cassava starch containing stearic acid with subsequent microwaving (approx. 15%). Low setback viscosity of starches is indicative of low tendencies towards retrogradation and further suggests that the presence of lipids in starch during pasting prevents the re-association of amylose chains during cooling and storage (Oyeyinka *et al.*, 2017c).

Thermal properties

The thermal properties of microwaved cassava starch stearic-acid complexes are shown in Table 2. Native cassava starch showed peak gelatinization temperature (T_p) of 65.10°C which is within the range (56.2-74.7°C) reported for cassava starch in the literature (Zhu, 2015). Modification of cassava starch with stearic acid alone or in combination with microwaving shifted the T_p to a higher endotherm (92.04-101.85°C) compared with the native starch. The shift to a higher endotherm suggests that the added stearic acid possibly formed complexes with amylose in cassava starch. Lipids such as stearic acid are hydrophobic and may form inclusion complexes with amylose in starch. The amylose-lipid complexes are known to melt at varying temperature: <80°C, 80-104°C and at values >104°C (Biliaderis and Seneviratne, 1990; Raphaelides and Karkalas, 1988). These endotherms have been attributed to non-complex lipids, type I amylose-lipid complexes, and type II amylose-lipid complexes respectively (Biliaderis and Seneviratne, 1990; Raphaelides and Karkalas, 1988). In this study, the addition of stearic acid to cassava starch alone or to microwaved cassava starch or to cassava starch with subsequent microwaving all formed type I amylose-lipid complexes. Previous studies reported type II amylose-lipid complex for cassava starch pasted with stearic acid and xanthan gum (Maphalla and Emmambux, 2016). The variation in the thermal stability may be due to the extended pasting time used by these authors which possibly allowed greater interaction between the starch and the stearic acid (D'silva *et al.*, 2011).

The melting enthalpies (ΔH_m) of the modified starches varied between 0.57 and 1.07 J/g (Table 2). Native cassava

starch with added stearic acid had the lowest ΔH_m , while cassava starch microwaved prior to the addition of stearic acid had the highest value. The ΔH_m provides information on the degree of order (crystallinity) in the complex and the amount of amylose-lipid complex formed (Kawai *et al.*, 2012). Thus, the higher ΔH_m value of cassava starch microwaved prior to the addition of stearic acid suggests that more complexes were formed. The reason for this remains unclear. However, it seems that microwaving disrupts starch granular structure creating pores which may facilitate better interaction of lipids with amylose during incubation. Some authors working with annealed starch complexed with lipids similarly found that the annealed starch had pores on its surface which presumably enhanced the complexing ability of the starch (Oyeyinka *et al.*, 2018).

CONCLUSIONS

Microwaving enhanced better complexation of amylose in cassava starch with stearic acid and improved thermal stability of the starch. The modified starch showed better functionality such as low setback viscosities and higher gelatinisation temperatures. Native or microwaved cassava starch complexed with stearic acid both produced type I amylose-lipid complexes. Cassava starch modified with microwave and stearic acid complex can potentially be used in food formulations that require high temperature for processing such as in extrusion. Further research is required to understand the interaction between starch and lipids after microwave heat-treatment. This may provide a better insight into the extent and type of fusion that governs the interaction between starch and lipids after complexation and heat treatment. In addition, the application of the modified starch in food systems should also be considered in future studies.

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STATEMENT OF CONFLICT OF INTEREST

Authors declare no conflict of interest.

Table 2: Thermal properties of cassava starch-stearic acid complexes;

Samples	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH_m (J/g)
NC	59.85c±0.02	65.10c±0.82	71.13b±0.20	12.06a±0.24
NCS	87.66b±1.34	92.04b±0.77	100.22a±0.14	0.57d±0.04
NCSM	92.12a±0.01	101.85a±0.94	106.95a±0.61	0.86c±0.02
MCS	87.82b±0.48	101.40a±0.60	106.75a±0.50	1.07b±0.07

Mean with different superscript along the column are significantly different ($p < 0.05$).

To: Onset temperature; T_p : Peak temperature; T_c : Conclusion temperature; H: Gelatinisation enthalpy

NC: Native cassava starch, NCS: Native cassava starch + Stearic acid, NCSM: Native cassava starch + Stearic acid + Microwave heat-treatment, MCS: Microwaved cassava starch + Stearic acid

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