# Research Article

# Effect of lipid types on complexation and some physicochemical properties of bambara groundnut starch

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This study investigated the effect of stearic acid, linoleic acid, and lysophosphatidylcholine on complex formation and physicochemical properties of bambara starch in comparison with potato starch. The complexation index reached maximum at 2% lipid concentration. Bambara starch complexed better with stearic acid than with linoleic acid and lysophosphatidylcholine. A similar trend was observed for potato starch but to a lesser extent. All lipids significantly reduced the peak and setback viscosities of bambara starch, but increased the final viscosity. Pasting of bambara and potato starches with lipids resulted in the formation of type-I V-amylose complexes, with melting temperatures ranging from ~98 to 102°C. X-ray diffraction of these complexes showed the crystalline V-amylose pattern with a major peak at  $2\Theta = 19.9^{\circ}$  and minor peaks at  $2\Theta = 7.4^{\circ}$  and 12.9°. Modification of bambara starch with lipids resulted in reduced digestibility, suggesting their potential application in formulating foods for the management of diabetes.

#### Keywords:

Amylose-lipid complex / Bambara starch / Linoleic acid / Lysophosphatidylcholine / Stearic acid

## 1 Introduction

Starches have limited industrial application due to their poor resistance to extreme processing conditions of pH, heat, and shear. To overcome these shortcomings, starches are often modified by physical, enzymatic, genetic, and chemical methods [1]. Of these modification methods, chemical modification is the most widely studied [2]. However, chemicals such as epichlorohydrin, and hypochlorite solution used in starch modification have been found to present food safety concerns [3, 4]. Consumers' awareness on food safety and the emergence of clean label starch technology have increased the search for natural alternatives in starch modification. Naturally occurring compounds such as lipids have been reportedly used in starch modification for improved functionality [5–7]. Amylose can form single Received: May 10, 2016 Revised: June 3, 2016 Accepted: June 4, 2016

helical inclusion complexes known as V-amylose complex with lipids. These complexes may be formed between amylose in native starch and endogenous lipids or formed upon gelatinization of starch in the presence of added lipids [8]. V-amylose complexes have been used to enhance starch pasting pro34perties [9, 10], prepare novel starches with slowly digestible property [5, 6] and to protect volatile and sensitive ligands such as polyunsaturated fatty acids [11, 12].

Differences in lipid structures including chain length of fatty acids and degree of unsaturation may influence the formation and stability of V-amylose complex. In general, the amount of V-amylose formed during complexation of starch with lipid has been found to decrease with increased lipid chain length [5, 13–15]. This is associated with the activation energy required for complex formation, which increases with increasing acyl chain length [14]. Additional energy is required to enhance more hydrophobic interactions between the lipid and the amylose helix [13, 16]. Kawai [5] observed higher complexing ability of lauric acid with potato starch than myristic, palmitic, and stearic acids. Previous studies

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Abbreviations: CI, complex index; LIN, linoleic acid; LPC, lysophosphatidylcholine; STE, stearic acid

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also found an increase in melting temperature of starchlipid complexes compared to starch alone [5]. The transition temperature of these complexes may vary with the type of lipid. Higher melting temperature  $(T_m)$  of 97°C was reported for potato starch complexed with stearic acid compared to those complexed with lauric, myristic and palmitic acids which showed  $T_m$  of approximately 90°C [5]. Potato starch complexed with stearic acid similarly showed higher Tm (97°C) which was almost double that of potato starch-linoleic acid complex [5]. Two distinct type of V-amylose complex (type I and type II) based on thermal transitions may be observed for a given ligand [17-19]. Type-I complexes are predominantly amorphous and generally dissociates at low temperatures between 95 and 105°C [19]. Type-II complexes, which can be further subdivided into types IIa and IIb complexes are semi-crystalline and dissociates at higher temperatures up to about 121°C [19]. So far, most studies on V-amylose complex mainly focused on conventional starch sources such as corn and potato. Only a few reports on the modification of pulse starch with lipids have been found in literature [20-23]. The effect of lipid on mung bean starch was studied by Sun [20]. These authors noted approximately 45% reduction in the firmness of mung bean starch gel pasted with 6% lipid. Other reports on starch-lipid complexes found that the interaction between amylose and fatty acids that took place during gelatinization of maize, high amylose maize, and pea starches retarded granule destruction [23].

There is growing demand for starch by the food industry and hence alternative starch sources have been considered. Pulses such as bambara groundnut (Vigna subterranea) can play a role as alternative starch sources to these conventional crops [24-26]. Previous studies on the modification of bambara starch focused primarily on annealing, heat moisture treatment [27], oxidation, acetylation [28], and carboxymethylation [26]. Bambara starch contain moderately high amylose content (21-35%) [24, 25, 29], thus making it suitable for complex formation with lipids. Unlike pea which is currently being used as a starch source in the Canadian industry, bambara remains an underutilized crop in Africa. The underutilization of many traditional crops such as bambara groundnut has been attributed to insufficient research [24]. Due to the relatively low cost of traditional crops and their agronomic advantage of being drought tolerant, these crops are currently being considered as alternative starch source for various industrial application in Africa. To promote the utilization of bambara beyond traditional usage, it is important to modify its starch component with lipids. To our knowledge, there is no report on the modification of bambara starch with lipids. Thus, we considered it necessary to investigate the effects of lipid types, stearic acid, linoleic acid, and lysophosphatidylcholine on complex formation and physicochemical properties of bambara starch. The fatty acids were chosen because

they are frequently used in starch modification, while lysophosphatidylcholine was included due to its use as an emulsifier in various food applications.

# 2 Materials and methods

# 2.1 Materials

Bambara groundnut were obtained from Markathini Research station, Jozini Kwazulu-Natal province, South Africa. Starch (amylose content: 31.5%) was prepared from bambara flour as described by Oyeyinka [24]. Potato starch (amylose contents: 24.6%), glucose oxidase assay kit (no. GAGO-20), amyloglucosidase (no. 7095),  $\alpha$ -amylase (no. 7545), guar gum (no. 4129), stearic acid (STE), linoleic acid (LIN), and lysophosphatidylcholine (LPC) were purchased from Sigma–Adrich (St. Louis, MO, USA). All other chemicals and solvents used were laboratory grade.

#### 2.2 Preparation of starch-lipid complex

To determine the optimum lipid concentration needed to form complex with bambara starch, STE, LIN, and LPC were added to the starch at varying concentrations of 0, 0.5, 1, 1.5, 2, 2.5, 3, and 4% as described by D'Silva [10]. Commercial potato starch was used as a reference sample.

#### 2.2.1 Complex index

The extent of complex formation between starch and lipids was determined as previously reported Meng [7]. The complex index (CI), which is a measure of the reduction in iodine binding capacity, was evaluated using Equation (1). The optimum lipid concentration was established as 2% for the three lipids. Hence, this concentration was used for complexation in subsequent experiments.

$$CI\% = \frac{ABS \text{ of reference} - ABS \text{ of sample}}{ABS \text{ of reference}} \times 100$$
(1)

where ABS = absorbance and reference = starch without lipids.

#### 2.2.2 Pasting properties of starch-lipid mixtures

Bambara and potato starches were pasted with lipids using a Rapid Visco Analyzer (Newport Scientific, Australia), according to the method of Tang and Copeland [30]. Briefly, lipids (2% starch weight basis) were weighed accurately into a test canister. Distilled water (25 mL) and starch (2.5 g) were added and the mixture was agitated by mixing manually using the plastic paddle before inserting the canister into the instrument. Starch was stirred at 960 rpm for 10 s before the

(25.7 mL), centrifuged for 10 min at 2500×g, and 18.7 mL

of supernatant was collected. Amyloglucosidase (1 mL) diluted in deionized water (2 mL) was added to the

supernatant. The solution was freshly prepared for the digestion analysis. Aliquots of guar gum (10 mL, 5 g/L) and

sodium acetate buffer (5 mL, 0.5 M) were added to the starch samples (0.5 g, dry basis) in plastic centrifuge tubes. Seven

glass balls (10 mm diameter) and 5 mL of enzyme solution

were then added to each tube, following the incubation in a

water bath (37°C) with agitation (170 rpm). Aliquots (0.5 mL)

were taken at intervals and mixed with 4 mL of 80% ethanol,

and the glucose contents in the mixture were measured

using glucose oxidase and peroxidase assav kits. Nutritional

shear input was decreased and held constant at 160 rpm during the subsequent heating and cooling cycles. Starch-lipid pastes were dried in a freeze dryer (CHRIST, Germany) and kept at 4°C until analyzed.

#### 2.2.3 Gel strength

The texture of starch gels obtained after cooling the pastes at 4°C for 4 days were analyzed as described by Shaikh [31] except that gels were compressed to 50% of the original size at speed of 2 mm/s. The firmness of gel formed in the canister was analyzed using a Shimadzu texture analyzer (EZ-SX, China).

#### 2.2.4 Confocal laser scanning microscopy

Starch–lipid complexes were prepared for microscopy analysis according to the modified method of D'Silva [10]. Nile Red and fluorescein-isothiocyanate (FITC) were used to stain lipids and starch, respectively. Briefly, approximately 5 mg of freeze-dried samples obtained after pasting were stained in the dyes and left overnight in dark cold room for 12 h.

# 2.2.5 Thermal properties of dried starch-lipid complex

Thermal properties of starch–lipid complex were examined according to the methods of Chang [32] with few modifications. Briefly, pasted freeze-dried-complexed starch (3 mg) was directly weighed into an aluminum DSC pan and distilled water (12  $\mu$ L) added. Pans were hermetically sealed and equilibrated for 12 h. 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 ( $T_{\rm o}$ ), melting temperature ( $T_{\rm m}$ ), conclusion temperature ( $T_{\rm c}$ ), and melting enthalpy ( $\Delta H_{\rm m}$ ) were obtained for the starch–lipid complexes.

#### 2.2.6 X-ray diffraction

X-ray diffraction of starch–lipid complex was conducted using Empyrean PANalytical diffractometer (Netherlands) operating at 40 kV with a target current of 40 mA as previously reported [24]. Starch samples were equilibrated for 12 h at 25°C and 100% in a low temperature incubator (MTIE10, Labcon, South Africa). The equilibrated samples were scanned over a region of 4–40 (20)° at a scanning speed of 0.06°/min.

#### 2.2.7 In vitro digestibility

Digestibility of the freeze-dried starch–lipid complexes was done as previously reported [33, 34]. Briefly, porcine pancreatic  $\alpha$ -amylase (3.89 g) was dispersed in water

starch fractions based on digestibility was rapidly digestible starch (RDS) represents portion of starch that was hydrolyzed within 20 min of incubation, slowly digestible starch (SDS) represents the starch hydrolyzed between 20 and 120 min while resistant starch (RS) was estimated as the starch not digested after 120 min of incubation. imately g were **2.3 Statistical analysis** All experiments were repeated three times. Data were analyzed using analysis of variance (ANOVA) and means were compared using Fischer's least significant difference test (p < 0.05). amined th few **3 Results and discussion** 

#### 3.1 Complex index

Regardless of lipid type, the complex index (CI) of bambaraand potato-starch–lipid mixtures progressively increased with increasing lipid concentration, reaching a maximum at 2% (Fig. 1). The fatty acid structure such as the degree of



**Figure 1.** Effect of lipid type on complex index of gelatinized bambara and potato starches. B, bambara starch; P, potato starch; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine. Error bars indicate standard deviation (N=3).

unsaturation and the presence of polar head (as is the case for complex lipid, LPC) significantly influenced the degree of complexation with starch. Bambara starch seemed to complex better with STE, than with LIN and LPC. The CI of bambara starch complexed with STE (62.9%) was higher than bambara starch complexed with LIN and LPC which showed approximately 54%. The low CI value of bambara starch complexed with LIN can be linked to the presence of double bonds which gives a kink in the molecular structure of LIN [35]. According to Yamada [35], the kink in cisunsaturated lipids such as linoleic acid only allows for partial inclusion into the amylose helix cavity. Our results agree with several other early reports where cis-unsaturated fatty acids complex poorly with amylose giving low V-amylose yields [9, 30, 36-40]. However, in some exceptional cases, some researchers reported that unsaturated fatty acids showed higher complexing ability than the saturated types [5, 15, 41]. Kawai [5] reported a CI value of 31.3% for potato starch complexed with stearic acid and a higher value (47.6%) for linoleic acid. These variations in reported CI values for saturated and unsaturated fatty acids suggest that many factors other than the lipid structure may influence CI. It has been suggested that double bonds in unsaturated lipids influences the crystal structure of V-amylose more than the yield [19]. These authors postulated that the amylose helix needed to be expanded from six glucosyl residues per turn to seven in order to accommodate the unsaturated portion of acyl chain [19]. Although the mechanism for the expansion of the amylose helix in the presence of unsaturated ligands remains unclear, it is possible that the expansion of the amylose helix may vary with complexation conditions such as moisture content, starch type, amylose contents, and the degree of polymerization of amylose used in various studies.

As noted above the CI values of bambara starch complexed with LPC was lower compared to that of bambara starch complexed with STE. The low CI is probably attributable to the structural conformation of the LPC molecule. LPC is packed head-to-tail forming a common hydrocarbon layer which is bordered on each side by a region of polar groups [42]. Possibly, the angles of the polar groups on LPC reduced its ability to form complex with bambara starch. Cheng [43] reported that the inclusion rate of LPC into potato starch amylose decreased at temperatures higher than 60°C. The decreased inclusion rate was attributed to the fact that LPC is prone to deterioration at higher temperatures [43, 44]. Further, NMR studies showed that debranched-potato starch–LPC complexes were formed by hydrophobic interactions between the akyl chains of LPC and the helix cavity of the debranched-starch, with the rest of the LPC molecule lying outside the helix [43]. This may further explain the low CI of bambara starch complexed with LPC (Fig. 1).

Potato starch complexed with the lipids showed similar CI trend but with lower values (STE = 43.4%, LIN = 33.5%, LPC = 30.4%) when compared to bambara starch. The amylose content of bambara ( $\sim$ 32%) was higher than that of potato starch ( $\sim$ 25%). Starches with high amylose contents have been reported to form more starch–lipid complex [45–47]. Further, the degree of polymerization of amylose in these starches as indicated by previous studies [45–47] may also have influenced their CI values.

## 3.2 Pasting

The pasting properties of bambara and potato starch were significantly altered with lipid addition (Table 1). Peak viscosity (398.1 RVU) of bambara starch reduced by approximately 9% when pasted with STE. Bambara starch complexed with LIN and LPC showed slightly lower reduction (~7%) in peak viscosity. Variations in peak viscosity could be attributed to differences in the degree of complex formation of studied lipids with starch (Fig. 1). Reductions in peak viscosity after pasting of rice starch with stearic or linoleic acids [9], maize starch pasted with myristic, palmitic acids [48], or stearic acid [10, 48–50] has been attributed to the formation of V-amylose complexes. Zhou [9] similarly reported higher reductions in peak viscosity of rice

 Table 1. Pasting properties of bambara and potato starches as affected by lipid type

Sample	PV (RVU)	TV (RVU)	BV (RVU)	FV (RVU)	SV (RVU)	PT (°C)	Peak time (min)
BCON	$398.1^{\text{d}}\pm0.1$	$189.9^{\rm d}\pm0.4$	$208.2^{\rm c}\pm0.3$	$280.7^{ab}\pm0.2$	$111.4^{ab}\pm0.1$	$77.6^{a}\pm0.5$	$3.7^{ab}\pm0.2$
BSTE	$\mathbf{361.1^{e}\pm0.6}$	$201.5^{\circ}\pm0.9$	$164.9^{\circ}\pm0.4$	$305.0^{\text{a}}\pm1.0$	$103.6^{b}\pm0.1$	$78.0^{\rm a}\pm0.5$	$4.0^{\text{ab}}\pm0.1$
BLIN	$371.5^{\text{f}} \pm 0.1$	$199.1^{\circ}\pm1.8$	$162.0^{\circ}\pm1.2$	$\mathbf{297.2^{ab}\pm 1.2}$	$98.1^{\circ}\pm0.2$	$\mathbf{78.8^a} \pm 0.4$	$4.1^{ab}\pm0.1$
BLPC	$372.1^{\text{f}}\pm0.2$	$198.1^{\circ}\pm0.3$	$173.9^{\circ}\pm0.4$	$\mathbf{294.0^{ab}\pm0.4}$	$95.9^{\circ}\pm1.2$	$\mathbf{79.3^{a}\pm0.7}$	$4.3^{\text{a}}\pm0.1$
PCON	$801.5^{\circ}\pm0.7$	$138.3^{ extsf{e}} \pm 0.2$	$663.2^{a}\pm0.1$	$\mathbf{259.8^b} \pm 0.4$	$121.4^{\text{a}}\pm0.4$	$68.2^{b}\pm0.4$	$2.9^{\circ}\pm0.2$
PSTE	$865.0^{\text{a}}\pm0.2$	$246.8^{\text{a}}\pm0.3$	$618.2^b\pm0.1$	$\mathbf{277.5^{ab}\pm 0.8}$	$30.7^{\text{e}}\pm0.1$	$68.4^{b}\pm0.4$	$3.1^{\circ}\pm0.1$
PLIN	$834.0^{b}\pm1.2$	$251.7^{\text{a}}\pm0.3$	$582.3^{b}\pm0.2$	$\textbf{274.3}^{ab} \pm \textbf{0.3}$	$22.7^{e}\pm0.3$	$68.7^{b}\pm0.2$	$3.2^{\circ}\pm0.1$
PLPC	$831.0^{\text{b}}\pm0.5$	$233.5^b\pm0.2$	$\mathbf{598.5^b} \pm 0.4$	$\mathbf{272.5^{ab}\pm 0.1}$	$41.3^{\text{d}}\pm0.2$	$\mathbf{68.3^b} \pm 0.1$	$3.2^{c}\pm0.2$

Mean  $\pm$  SD. Mean with different superscript letters along the column are significantly different ( $p \le 0.05$ ).

PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SV, setback viscosity; PT, pasting temperature; CON, control; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine; B, bambara starch; P, potato starch.

starch pasted with STE compared to rice starch pasted with LIN. Lipids presumably restrict starch granule hydration and swelling. It is also hypothesized that lipids may cover starch granule surface with a film [51]. Peak viscosity of potato starch with or without lipids were substantially higher compared to bambara starch (Table 1). The high phosphate monoester content of potato starch as reported by some authors [52, 53], may have facilitated hydration and swelling of starch granules. This may have contributed to the higher peak viscosity of potato starch compared with bambara starch. Some authors similarly observed an increase in peak viscosity of starches pasted with different lipids [54, 55]. The setback viscosity (111.4 RVU) of bambara starch reduced with lipid addition reaching approximately 104, 98, and 96 RVU for bambara starch pasted with STE, LIN, and LPC, respectively. Potato starch pasted with these lipids similarly showed significant reduction in setback viscosity. Low setback viscosity of starches is indicative of low tendencies toward retrogradation. This further suggests that the presence of lipids in starch during pasting prevents the re-association of amylose chains during cooling and storage. Amylose-lipid complexes reportedly prevent the formation of junction zones which takes place during short-term storage [10, 56].

#### 3.3 Confocal laser scanning microscopy

Confocal laser scanning micrographs of the bambara starch pasted without lipids showed distorted granules which fluoresced green (Fig. 2A). In contrast, the starch–lipid complexes revealed the presence of lipids with orange to red spots which were localized within the starch granules confirming complex formation (Fig. 2B–D). The presence of lipids in starch suspension did not prevent starch granule disintegration. This could be attributed high temperature treatment and shear during pasting. According to previous reports, the interaction of amylose–fatty acids could retard granule disintegration, but this highly depends on the heating temperature [23].

#### 3.4 Texture of starch gels

Bambara starch gel with or without lipids showed higher gel firmness compared to potato starch (Fig. 3). Bambara starch has a higher amylose content than potato, which could be responsible for the observed differences in gel firmness. Higher gel strength was similarly reported for cowpea starch with high amylose content compared to potato and corn starches [57]. However, with lipid addition, bambara and potato starch gels showed significant reduction in their firmness, suggesting that amylose in starch interacts with lipids to form inclusion complexes. These complexes may have prevented or slowed down the interaction between starch molecules preventing the formation of double helices, junction zones, and gel network during storage [10, 56].

# 3.5 X-ray diffraction

X-ray diffraction (XRD) patterns of bambara and potato starches pasted with lipids showed similar diffraction patterns (Fig. 4A and B) with a major peak at  $2\Theta = 19.9^{\circ}$ 



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Figure 2. Confocal laser scanning micrographs of bambara starch pasted with lipids. (A) Control bambara starch, (B) bambara starch pasted with stearic acid, (C) bambara starch pasted with lysophosphatidylcholine, (D) bambara starch pasted with linoleic acid. Arrows indicate lipids within the starch granules. Scale bars for A–D are 20  $\mu$ m.



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**Figure 3.** Gel strength of starch gels with or without lipid at 2%. CON, control; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine. Error bars indicate standard deviation (N=3).

and minor peaks at  $2\Theta = 7.4^{\circ}$  and  $12.9^{\circ}$  suggesting the formation of V-amylose complex. These peaks were absent in the unpasted starch without lipids. Similar V-amylose diffraction peaks have previously been reported for starch–lipid complexes [50, 54, 58]. Among the starch–lipid complexes, bambara starch complexed with STE showed higher intensity compared to those complexed with LIN and LPC, confirming better complex formation of bambara with STE (Fig. 1). The same trend was observed for potato starch–lipid complexes. Further, bambara and potato starches complexed with STE showed additional diffraction peak at  $2\Theta = 25.1^{\circ}$  and  $2\Theta = 27.1^{\circ}$ . These peaks which

were absent in starches pasted with LIN and LPC could be associated with free stearic acid aggregates. V-amylose patterns with additional peak indicating the presence of fatty acid aggregates have previously been reported [32, 49, 50].

#### 3.6 Differential scanning calorimetry

Bambara and potato starches complexed with lipids generally showed higher melting temperatures  $(T_m)$  and enthalpies  $(\Delta H_{\rm m})$  compared to starches pasted without lipids (Fig. 5 and Table 2). Two endothermic transitions (Peaks I and II) were observed in both starches complexed with stearic acid. The first transition (Peak I) which occurred at temperature of approximately 68°C may be attributed to the melting of free uncomplexed stearic acid. This is in agreement with the XRD result (Fig. 4A and B) which also indicated the presence of free stearic acid. Previous research on starchstearic acid complexes similarly observed peak at about the same temperature [39, 49, 50]. The second peak (Peak II) in bambara and potato starches pasted with lipids showed T<sub>m</sub> which varied from 97.5 to 100.9°C and suggest the melting of type-I V-amylose complexes. According to previous research [18, 39], V-amylose complexes may show three endotherms with  $\mathit{T}_{\rm m}$  values at <80°C, 95–105°C, and at values >105°C. These endotherms have been attributed to non-complex lipids, type-I V-amylose complexes and type-II



**Figure 4.** Effect of added lipids on X-ray diffractograms of bambara (A) and potato (B) starches. B, bambara starch; P, potato starch; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine.



**Figure 5.** Typical thermograms of bambara and potato starches pasted with lipids. B, bambara starch; P, potato starch; CON, control; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine, Peak I, free stearic acid; Peak II, melting of starch crystallites or V-amylose complexes.

V-amylose complexes, respectively [18, 39]. Obiro [50] similarly reported type-I V-amylose complex for teff and maize starches pasted with stearic acid for short pasting time. High melting temperature of amylose–lipid complexes is reportedly associated with their stability [5]. Therefore, the stability of the bambara and potato starch lipid complexes were in the order BSTE > PLPC > PSTE > PLIN > BLPC > BLIN. Kawai [5] also observed higher  $T_{\rm m}$  (~97°C) for potato starch complexed with STE than potato starch complexed with LIN (~78°C). The reported  $T_{\rm m}$  values of starch–fatty acid complexes increased with decrease in the number of double bonds [5].

The  $\Delta H_{\rm m}$  of bambara-starch–lipid complex varied from 1.0 to 1.8 J/g, while those of potato-starch–lipid complex ranged from 0.5 to 0.9 J/g (Table 2). The  $\Delta H_{\rm m}$  of amylose– lipid complexes is suggested to reflect the amount of complex and the degree of order within the complex [5]. The higher  $\Delta H_{\rm m}$  of bambara starch pasted with STE (Table 2) compared to other starch–lipid complexes may explain the higher complexing ability (Fig. 1) and the higher intensity of the V-amylose peak (Fig. 4).

#### 3.7 In vitro digestibility

The starch digestibility of bambara was significantly influenced by complexation with lipid (Table 3). The addition of lipid caused about 7–14% reduction in RDS fractions depending on the lipid type. The same trend was observed for potato starch when complexed with lipid, but to a lesser extent. Among studied lipids, bambara starch complexed with STE showed the highest level of SDS (~12%) and RS (~13%), similar to potato starch (SDS: ~5% and RS: ~10%). The reduction in digestibility of complexed starches may be attributed to the formation of amylose–lipid complexes as found in previous research [6, 7]. Lipid molecule interacts with amylose in the hydrophobic tube and prevents starch granule hydration and swelling. The amylose–lipid interaction results in the formation of V-amylose single helical structure with a conformational hindrance that possibly

Tabl	e 2.	Thermal	properties of	bambara	and	l potato starc	hes pasted	d with	different	lipids
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	Peak I				Peak II			
Sample	<i>T</i> ₀ (°C)	<i>T</i> <sub>m</sub> (°C)	<i>T</i> <sub>c</sub> (°C)	$\Delta H_{\rm m}$ (J/g)	<i>T</i> ° (°C)	<i>T</i> <sub>m</sub> (°C)	<i>T</i> c (°C)	$\Delta H_{\rm m}~({\rm J/g})$
BCON	ND	ND	ND	ND	$85.2^{ extsf{e}}\pm1.0$	$93.1^d\pm0.3$	$97.9^{d}\pm0.1$	$0.4^{f} \pm 0.1$
BSTE	$66.0^{a}\pm0.1$	$68.4^{a} \pm 0.1$	$\textbf{70.9}^{a} \pm \textbf{0.5}$	$0.9^{a}\pm0.1$	$90.4^{d}\pm0.7$	$101.0^{\texttt{a}}\pm0.6$	$103.7^{\circ}\pm0.4$	$1.8^{a}\pm0.1$
BLIN	ND	ND	ND	ND	$85.8^{\text{e}}\pm0.1$	$97.5^{\circ}\pm0.4$	$103.7^{\circ}\pm1.3$	$1.1^{ extsf{b}}\pm0.1$
BLPC	ND	ND	ND	ND	$97.5^{a}\pm0.5$	$98.2^{\text{bc}}\pm0.8$	$103.3^{\circ}\pm0.3$	$1.0^{\text{b}}\pm0.1$
PCON	ND	ND	ND	ND	$85.9^{\text{e}}\pm0.9$	$90.0^{\text{e}}\pm0.7$	$97.9^{d}\pm0.3$	$0.2^{g}\pm0.1$
PSTE	$67.1^{a}\pm0.1$	$68.5^{\text{a}}\pm0.1$	$70.7^{a}\pm0.8$	$0.6^{b}\pm0.1$	$97.2^{a}\pm0.9$	$99.3^{b}\pm0.8$	$105.3^{\text{b}}\pm0.8$	$0.9^{\circ}\pm0.1$
PLIN	ND	ND	ND	ND	$94.8^{b}\pm0.2$	$98.3^{\text{bc}}\pm0.5$	$106.9^{a}\pm0.2$	$0.8^{\text{d}}\pm0.1$
PLPC	ND	ND	ND	ND	$92.8^{c}\pm0.8$	$101.9^{a}\pm1.3$	$106.2^{\text{ab}}\pm0.5$	$0.5^{e}\pm0.2$

Mean  $\pm$  SD. Mean with different superscript letters along the column are significantly different ( $p \le 0.05$ ).

CON, control; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine; B, bambara starch; P, potato starch.

 $T_{o}$ ,  $T_{m}$ ,  $T_{c}$ , and  $\Delta H_{m}$  are onset temperature, melting temperature, conclusion temperature, and melting enthalpy, respectively. ND, no transition detected; Peak I, stearic acid endotherm; Peak II, melting of starch crystallites and V-amylose complexes.

Table 3. Nutritional starch fractions of bambara starch pasted with different lipids

Sample	RDS (%)	SDS (%)	RS (%)
BCON	87.2 <sup>b</sup> ± 0.1	$3.7^{d}\pm0.3$	$9.1^{d}\pm0.4$
BSTE	$75.1^{ extsf{e}}\pm0.1$	$12.0^{\text{a}}\pm0.1$	$12.8^{a} \pm 0.1$
BLIN	$\mathbf{80.4^d} \pm 0.3$	$7.6^{c}\pm0.2$	$12.0^{b}\pm0.1$
BLPC	$\mathbf{80.8^d} \pm 0.1$	$8.5^{b}\pm0.1$	$10.8^{\circ}\pm0.1$
PCON	$90.6^{\text{a}}\pm0.1$	$0.5^{\text{e}}\pm0.2$	$8.9^{e}\pm0.2$
PSTE	$85.2^{c}\pm0.5$	$4.6^{d}\pm0.6$	$10.1^{\circ}\pm0.1$
PLIN	$86.1^{\circ}\pm0.1$	$4.1^{d}\pm0.2$	$9.8^{d}\pm0.1$
PLPC	$86.5^{\rm c}\pm0.3$	$3.9^{d}\pm0.4$	$9.3^{\text{d}}\pm0.3$

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

RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; CON, control; STE, stearic acid; LIN, linoleic acid; LPC, lysophosphatidylcholine; B, bambara starch; P, potato starch.

restricts enzyme access into the starch granule interior. This may account for the reduction in starch hydrolysis rate. The higher reduction in digestibility of bambara starch complexed with STE may be linked to its high CI (Fig. 1) and high  $\Delta H_{\rm m}$  (Table 2), which corresponds to complexing ability and relative amount of formed complex, respectively. Our results agrees with previous findings on starch-lipid complexes [5, 59]. Kawai [5] similarly associated higher reduction in the amount of rapidly digested starch for potato starch-lauric acid complex, with high CI and high  $\Delta H_{\rm m}$ values. Guraya [59] also studied the complexing ability and digestibility of emulsifiers with different chain length and degree of unsaturation. Emulsifiers with saturated longchain monoglycerides reportedly showed high complexing ability and reduced digestibility compared to unsaturated emulsifiers [59].

#### 4 Concluding remarks

Pasting bambara and potato starches with STE, LIN, and LPC produce type-I V-amylose complex which was confirmed by XRD. Bambara starch complexed better with lipids than potato starch due to its higher amylose content. Bambara starch pasted with lipids show reduction in peak and set back viscosities. Stearic acid form more complex with bambara starch as shown by its higher complexing index, higher reduction in peak viscosity and higher melting enthalpy compared to LIN and LPC. Complexed starches generally display a reduction in digestibility suggesting their potential in formulating foods for the management of diabetes.

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