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Original article

Effect of annealing on the functionality of Bambara groundnut (*Vigna subterranea*) starch–palmitic acid complex

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Summary Bambara groundnut is an underutilised African leguminous crop. This study investigated the effect of annealing on the complexing ability and functionality of Bambara groundnut starch with palmitic acid. Corn starch was included as the reference. Annealing created cracks and pores on the surface of Bambara groundnut and corn starches, respectively. Bambara groundnut starch had significantly higher amylose content, higher peak and final viscosities than corn starch. The peak viscosities of native Bambara groundnut and corn starches significantly reduced with palmitic acid addition. Greater reduction in peak viscosities was observed when the annealed starches were complexed with palmitic acid, suggesting that more palmitic acid was complexed after annealing. This was confirmed by XRD peaks and melting enthalpies. Pasting of native Bambara groundnut and corn starches with palmitic acid resulted in the formation of type I V-amylose complexes, while type II complexes were formed from annealed starches pasted with palmitic acid.

Keywords Annealing, Bambara groundnut, corn, functionality, palmitic acid, starch.

Introduction

Starches in the native form are generally unsuitable for industrial applications. They are usually modified to improve functionality and application. Currently, starches from corn, potato and tapioca dominate the category of starch used in the industry. The physicochemical properties of these starches have been widely researched. However, due to the pressure on some starch sources, for example corn for uses other than starch, there is a growing demand for alternative starch sources. Furthermore, many underutilised leguminous crops such as pea (*Pea sativum*) and Bambara groundnut (*Vigna subterranea*) are now being proposed as possible starch source for the industry. Bambara groundnut is reportedly rich in starch (18–45%) (Afolabi, 2012; Oyeyinka *et al*., 2015, 2016a). Hence, this crop has potential as an alternative starch source to commercial starch sources.

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Previous research modified Bambara groundnut starch (BGS) using annealing (Adebowale & Lawal, 2002), oxidation, acetylation (Adebowale *et al*., 2002), carboxymethylation (Afolabi, 2012) or lipids (Oyeyinka *et al*., 2016b,c,d). Adebowale & Lawal (2002) found that ANN caused significant reduction in swelling power and peak viscosity of BGS. Other reports on BGS modification found that lipids can improve its thermal stability (Oyeyinka *et al*., 2016b,c,d) and reduce its tendencies towards retrogradation (Oyeyinka *et al*., 2016c). BGS pasted with stearic acid caused significant reduction in peak viscosity, which was associated with the formation of amylose–lipid complex (Oyeyinka *et al*., 2016c).

Several researchers are currently focusing on improving the degree of complexation of starch with lipids, either by increasing the incubation time of starch with lipids (Chang *et al*., 2014), using high pressure homogenisation (Oyeyinka *et al*., 2016b,d) or extending the pasting time of starch with lipids (D'Silva *et al*., 2011). Nakazawa & Wang (2004) studied the effect of annealing on starch–palmitic acid interaction. According to these authors, more palmitic

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acid (PAM) was complexed by annealed starches than by native starches. Saturated fatty acids such as PAM have been widely used in starch modification for improving functional properties of starch (Nakazawa & Wang, 2004; Kawai *et al.*, 2012; Oyeyinka *et al.*, 2016b). PAM seems to be the predominant fatty acid in starch lipids (Nakazawa & Wang, 2004) and has been found to show higher complexing ability with amylose than longer chain fatty acids such as stearic acid or the unsaturated types such as oleic and linoleic acids (Oyeyinka *et al.*, 2016b). Hence, this study investigated the effect of annealing and palmitic acid alone and in combination on pasting and thermal properties of BGS. Corn starch was included as the reference sample.

Materials and methods

Materials

Bambara groundnut was obtained from Markathini Research Farm station, Jozini KwaZulu-Natal province, South Africa. Corn starch and palmitic acid were purchased from Aladdin Chemistry Company (Shanghai, China).

Preparation of BGS

Starch was extracted from BGS following the methods reported by Oyeyinka *et al.* (2015). Extracted starch was dried at 45 °C in hot air oven. Dried starch was kept at 4 °C until analysis. The extracted starch had low contents of ash (0.14%), fat (0.08%) and protein (0.12%) contents suggesting that the starch is pure.

Amylose contents

Amylose contents of starches were determined by the iodine-binding method previously reported (Williams *et al.*, 1970).

Annealing of starch

Annealing of starch samples was done according to the modified method of Jacobs *et al.* (1995). Starch suspension in distilled water (1:2 w/v) was heated for 24 h in a sealed container in a water bath at 50 °C. After 24 h incubation, the suspension was filtered through a Whatman No. 1 filter paper and air-dried for 12 h. It was sealed polyethylene bags and stored at 4 °C prior use.

Incorporation of PAM to starch

PAM (2% w/w) was added to BGS following the procedure previously reported except that the starch-PAM suspension was stirred with a magnetic stirrer at 50 °C for 3 h and then at room temperature for an additional 24 h (Oyeyinka *et al.*, 2016c). Native BGS was treated in the same way as the PAM-treated starch except that PAM was not added.

Microscopy of native and annealed starch

Starch granule morphology was examined using a scanning electron microscope (EVO 15 HD, Jena Germany) with an accelerating potential of 4 kV. Average starch granule size was determined from the diameter of individual granules ($N = 50$) on the basis of the scale bar provided on the captured scanning electron micrographs (Stevenson *et al.*, 2006).

Swelling power of native and annealed starch

Swelling power of starch was determined as previously reported (Afolabi, 2012).

Pasting properties

The pasting properties of the starch samples were examined using a Rapid Visco-Analyzer (Newport Scientific, Australia). Briefly, native starch and starch–palmitic acid mixtures (2.8 g) were weighed into the test canister containing 25 mL of distilled water and the analysis done according to the manufacturer's instruction.

Thermal properties of starch

The thermal properties such as onset gelatinisation (T_o), peak gelatinisation (T_p), conclusion (T_c), gelatinisation temperatures and gelatinisation enthalpy (ΔH) of the native and modified starch samples were determined using a differential scanning calorimeter (SDT Q600, USA) as previously reported by (Oyeyinka *et al.*, 2016b).

Statistical analysis

Duplicate samples were prepared and analyses performed in triplicate. Data were analysed using one-way analysis of variance (ANOVA), and means were compared using the Fisher least significant difference (LSD) test ($P < 0.05$).

Results and discussion

Amylose contents

BGS showed relatively higher amylose content (32 ± 2.4%) than corn starch which showed 21.5 ± 1.2%. The amylose content of BGS is in agreement with the literature on pulse starches (Hoover *et al*., 2010) including BGS (Kaptso *et al*., 2014; Oyeyinka *et al*., 2015, 2016a,d). Generally, pulses are characterised by high levels of amylose content when compared to most cereal and tuber starches (Oyeyinka *et al*., 2016a).

Morphology

Native BGS showed predominantly oval shaped granules with few granules round and irregular in shape (Fig. 1a). All starches granules were smooth with no fissures or pin hole, suggesting that the extracted starches are relatively pure. On the other hand, native corn starch was mostly irregularly shaped with a few of the granules polygonal while some were round in shape (Fig. 1d). BGS appeared bigger (average diameter: to 27 ± 3.1 μm) than corn starch (14 ± 2.3 μm). The size and shape of the two starches in this study are similar to previous reports on Bambara groundnut (Oyeyinka *et al*., 2015, 2016a) and corn starches (Joshi *et al*., 2013). BGS had a cracked surface (Fig. 1b & c), while the corn starch showed obvious pores on its surface after annealing (Fig. 1d & f). Our result on starch granule morphology is in agreement with the literature where annealing was reported to slightly deform wheat starch granules (Kiseleva *et al*., 2005) and created pores on the surface of high-amylose rice starch (Dias *et al*., 2010). However, no observable changes were reported in the granule morphology of BGS after ANN (Adebowale & Lawal, 2002). Thus, the impact of annealing on starch granule morphology may depend on annealing temperature, incubation time and the starch source used in various studies.

Swelling power of native and annealed starch

Native Bambara groundnut and corn starches showed a progressive increase in swelling power with increase in temperature (Fig. S1). The increase in swelling ability of starches has been linked with the melting of starch crystallites, which confirms gelatinisation (Oyeyinka *et al*., 2015). BGS displayed a lower swelling power compared to corn starch, which may be associated with differences in their amylose contents. The amylose content of starches has been suggested to restrict their swelling behaviour (Tester & Morrison, 1990).

Annealed starches displayed reduced swelling power compared to their native counterparts (Fig. S1). Adebowale & Lawal (2002) also found significant reduction in the swelling power of BGS after annealing. The reduction in swelling power of Bambara groundnut and corn starches after annealing may be due to increase in molecular organisation of starch components (Gomes *et al*., 2005), resulting from perfection of starch crystallites (Waduge *et al*., 2006). According to these authors, crystallites perfection reduces the degree of hydration of the amorphous regions and hence, a reduction in swelling power.

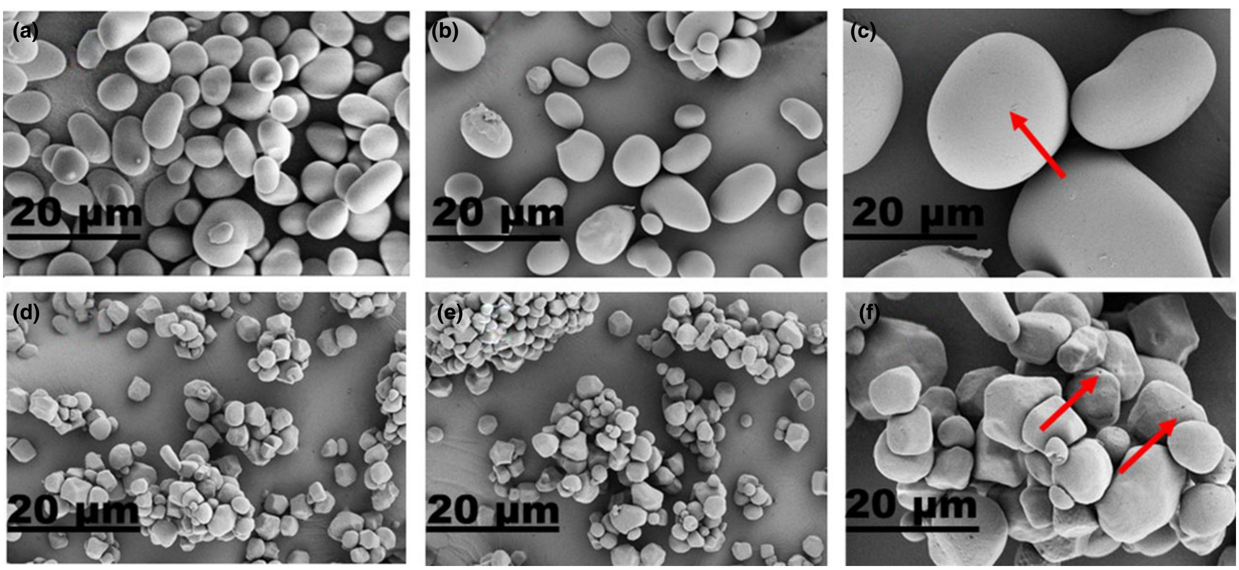

Figure 1 SEM images of Bambara groundnut and corn starches. Arrows indicate cracks and pores on Bambara groundnut starch and corn starch, respectively. (a) Native Bambara groundnut starch (Magnification 500×). (b) Annealed Bambara groundnut starch (Magnification 500×). (c) Annealed Bambara groundnut starch (Magnification 1500×). (d) Native corn starch (Magnification 500×). (e) Annealed corn starch (Magnification 500×). (f) Annealed corn starch (Magnification 1500×). [Colour figure can be viewed at wileyonlinelibrary.com]

Pasting properties

Native and annealed BGS complexed with palmitic acid showed similar pasting profile curves (Fig. S2). The same trend was observed for the control corn starch (Fig. S2). BGS displayed significantly ($P \leq 0.05$) higher peak, trough, breakdown, setback and final viscosities than corn starch (Table 1). Peak viscosity of starch may be influenced by the ratio of amylose to amylopectin, the chain length distribution of amylopectin as well as the presence of other minor components of starch such as lipids (Tester & Morrison, 1990). Starches with low amylose contents would normally display high peak viscosity due to restricted swelling of starch granules (Huang *et al*., 2015). However, in this study, the variation in peak viscosities did not show any inverse relationship with amylose contents. Previous studies similarly found that differences in amylose contents were not sufficient to explain the variation in starch peak viscosity (Kaur & Sandhu, 2010; Kaur *et al*., 2010; Oyeyinka *et al*., 2016a). Residual or endogenous lipids in corn starches have been reported to restrict starch granular swelling during pasting (Tester & Morrison, 1990). Thus, it is possible that the presence of these lipids may have accounted for the difference in peak viscosity of these starches. Other factors that may explain the variation in peak viscosity of the native Bambara groundnut and corn starches may be linked with differences in the chain length of amylose and amylopectin components of these starches (Jane *et al*., 1999; Huang *et al*., 2007).

Annealing significantly ($P \leq 0.05$) reduced the peak, breakdown and setback viscosities of BGS, but increased the trough and final viscosities (Table 1). This is in agreement with the literature on BGS (Adebowale & Lawal, 2002). However, annealing seems not to have any significant changes ($P \leq 0.05$) on the peak, trough, breakdown, setback and final viscosities of corn starch (Table 1). According to da Rosa Zavareze & Dias (2011), the impact of annealing on starch pasting properties is very controversial. Different results have been reported on the influence of annealing on starch pasting properties. For example, while some authors found a reduction in the peak viscosity of annealed potato starch (Jacobs *et al*., 1995), other authors working with peak, rice and wheat starches found the opposite (Jacobs *et al*., 1996). ANN also increased the pasting temperature of BGS, which could be due to bond strengthening (Gomes *et al*., 2004).

Native BGS pasted with palmitic acid showed further reduction in peak and breakdown viscosities, while annealed BGS pasted with palmitic acid further showed a greater reduction in these parameters (Table 1). Previous studies similarly found significant reductions in peak viscosity after pasting of BGS with stearic acid, linoleic acid and lysophosphatidylcholine (Oyeyinka *et al*., 2016c). The reductions in peak viscosity have been attributed to the formation of amylose–lipid complexes (Oyeyinka *et al*., 2016c). Worthy of note in the pasting properties of BGS is the final viscosity which increased after annealing and with palmitic acid addition (Table 1). Several studies similarly reported increase in the final viscosity of starch after lipid modification (Liang *et al*., 2002; D'Silva *et al*., 2011; Wang *et al*., 2015; Oyeyinka *et al*., 2016c). Wang *et al*. (2015) found that the addition of lauric, myristic

Table 1 Pasting and thermal properties of Bambara groundnut and corn starches

	NBS	NCS	ABS	ACS	NBS + P	NCS + P	ABS + P	ACS + P
Pasting								
PV (cP)	$6480.5^{a} \pm 0.7$	$3833.5^{de} \pm 1.4$	$6286.0^{b} \pm 1.8$	$3895.5^{d} \pm 0.7$	$6011.0^{c} \pm 1.1$	$3763.5^{e} \pm 1.9$	$5984.0^{c} \pm 1.0$	$3479.0^{f} \pm 1.7$
TV (cP)	$3269.0^{c} \pm 1.5$	$2278.0^{e} \pm 1.6$	$3766.0^{a} \pm 1.3$	$2361.5^{e} \pm 1.1$	$3406.0^{b} \pm 1.5$	$2582.0^{d} \pm 1.3$	$3822.5^{a} \pm 1.7$	$2529.0^{d} \pm 1.6$
BV (cP)	$3211.5^{a} \pm 1.1$	$1555.5^{d} \pm 1.3$	$2520.0^{b} \pm 1.5$	$1534.0^{d} \pm 0.8$	$2605.0^{b} \pm 1.5$	$1181.5^{e} \pm 1.2$	$2161.5^{c} \pm 1.7$	$950.0^{f} \pm 1.2$
SV (cP)	$1704.0^{bc} \pm 1.2$	$1620.0^{bc} \pm 1.8$	$1691.5^{bc} \pm 1.3$	$1668.0^{bc} \pm 1.4$	$1788.5^{b} \pm 1.3$	$1321.0^{cd} \pm 1.6$	$2752.5^{a} \pm 1.4$	$1090.5^{c} \pm 1.5$
FV (cP)	$4973.0^{c} \pm 1.6$	$3898.0^{d} \pm 1.4$	$5457.5^{b} \pm 1.8$	$4029.5^{d} \pm 0.7$	$5194.5^{bc} \pm 1.8$	$3894.0^{d} \pm 1.3$	$6575.0^{a} \pm 1.2$	$3619.5^{d} \pm 1.1$
PT (°C)	$78.2^{b} \pm 0.1$	$75.8^{d} \pm 0.3$	$79.9^{a} \pm 0.1$	$76.0^{d} \pm 0.2$	$77.9^{b} \pm 0.4$	$76.7^{c} \pm 0.3$	$80.0^{a} \pm 0.1$	$77.1^{c} \pm 0.4$
Thermal								
T_o (°C)	$70.41^{d} \pm 0.21$	$68.15^{e} \pm 0.11$	$74.01^{c} \pm 0.12$	$72.12^{c} \pm 0.04$	$86.52^{b} \pm 0.41$	$87.75^{b} \pm 0.64$	$94.84^{a} \pm 0.14$	$99.05^{a} \pm 0.40$
T_p (°C)	$75.66^{d} \pm 0.14$	$73.52^{de} \pm 0.02$	$78.14^{c} \pm 0.13$	$77.50^{c} \pm 0.12$	$102.42^{ab} \pm 1.72$	$99.25^{b} \pm 0.81$	$106.09^{a} \pm 0.12$	$105.85^{a} \pm 0.51$
T_c (°C)	$84.12^{c} \pm 0.04$	$77.51^{d} \pm 0.21$	$86.20^{c} \pm 0.11$	$80.25^{c} \pm 0.04$	$108.44^{a} \pm 0.22$	$105.05^{ab} \pm 0.56$	$112.63^{a} \pm 0.14$	$110.51^{a} \pm 0.12$
ΔH (J g^{-1})	$13.07^{b} \pm 0.12$	$12.56^{b} \pm 0.10$	$15.98^{a} \pm 0.02$	$15.25^{a} \pm 0.01$	$4.40^{e} \pm 0.12$	$3.75^{e} \pm 0.76$	$6.84^{c} \pm 0.18$	$5.95^{d} \pm 0.12$

Mean ± SD. Mean with different superscript along the row is significantly different ($P < 0.05$).

PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; SV, setback viscosity; FV, final viscosity; PT, pasting temperature; T_o, onset gelatinisation temperature; T_p, peak gelatinisation temperature; T_c, conclusion gelatinisation temperature; ΔH, enthalpy of gelatinisation; NBS, Native Bambara groundnut starch; NCS, Native corn starch; ABS, Annealed Bambara groundnut starch; ACS, Annealed corn starch; NBS + P, Native Bambara groundnut starch pasted with palmitic acid; NCS + P, Native corn starch pasted with palmitic acid; ABS + P, Annealed Bambara groundnut starch pasted with palmitic acid; ACS + P, Annealed corn starch pasted with palmitic acid.

and palmitic acids increased the final viscosity of normal and waxy wheat starches.

Thermal properties

The native BGS showed a significantly ($P < 0.05$) higher onset gelatinisation (T_o), peak gelatinisation (T_p), conclusion (T_c), gelatinisation temperatures and gelatinisation enthalpy (ΔH) than corn starch (Table 1). The T_p of BGS (approx. 76 °C) is in agreement with the literature (Afolabi, 2012; Kaptso *et al.*, 2014; Oyeyinka *et al.*, 2016a). In this study, corn starch with lower amylose content displayed lower T_p than BGS. The thermal properties of the native starches suggest that the ratio of amylose to amylopectin is not the only factor that can influence the T_p of starches. Previous studies also found that low amylose starch did not show high T_p (Chung *et al.*, 2008; Joshi *et al.*, 2013). According to Noda *et al.* (1996), starches with higher proportion of long amylopectin chains would display high T_o, T_p, T_c and ΔH, while those with abundant short amylopectin chains would exhibit low T_o, T_p, T_c and ΔH (Noda *et al.*, 1996). Thus, the difference in gelatinisation temperatures could be associated with the presence of higher amounts of long amylopectin chains in BGS than in corn starch.

Annealing significantly ($P < 0.05$) increased the T_o, T_p, T_c and ΔH of Bambara groundnut and corn starches (Table 1). The increase in T_o, T_p and T_c could be attributed to the reduction in swelling power (Fig. S1), which is reflected in the higher onset gelatinisation temperature (Table 1). According to Adebowale *et al.* (2005), the increase in T_o, T_p and T_c reflects the melting of crystallites that are formed as a result of amylose–amylose and amylose–amylopectin interactions along the starch chains. These interactions reduce the swelling power of the granule resulting in delayed gelatinisation and higher values T_o, T_p and T_c (da Rosa Zavareze & Dias, 2011).

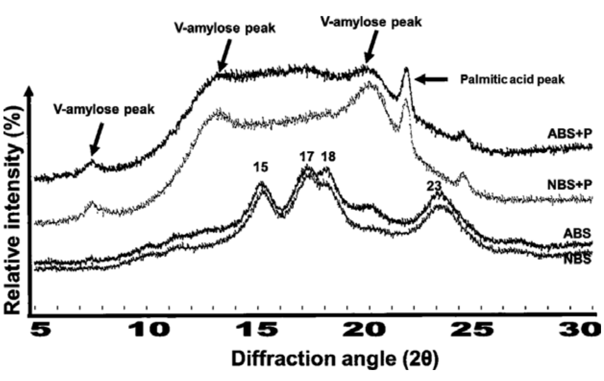

Figure 2 XRD of native, annealed Bambara groundnut starch complexed with palmitic acid. NBS, Native Bambara groundnut starch; ABS, Annealed Bambara groundnut starch; NBS + P, Native Bambara groundnut starch pasted with palmitic acid; ABS + P, Annealed Bambara groundnut starch pasted with palmitic acid.

Native or annealed BGS without palmitic acid melted at lower temperatures (75.66–78.14 °C) than these starches complexed with palmitic acid (102.42–106.09 °C) (Table 1). The same trend was observed for the corn starch reference sample (Table 1). The higher melting temperatures of the starches complexed with lipids may be attributed the formation of amylose–lipid complexes (Oyeyinka *et al.*, 2016b,c,d) Amylose–lipid complexes may show three endothermic peaks with values at <80 °C, 95–105 °C and at values >105 °C (Raphaelides & Karkalas, 1988; Biliaderis & Seneviratne, 1990). These endotherms have been attributed to noncomplex lipids, type I amylose–lipid complexes and type II amylose–lipid complexes, respectively (Raphaelides & Karkalas, 1988; Biliaderis & Seneviratne, 1990). The melting temperatures of native BGS (102.42 °C) and corn starch (99.25 °C) complexed with palmitic acid corresponds to the melting of type I amylose–lipid complexes, while the annealed starches with melting temperatures range of approximately 106 °C correspond to the melting of type II amylose–lipid complexes. The higher melting temperatures of the annealed starches complexed with palmitic acid suggest that annealing can improve the complexing ability of starch with lipids. This observation is similar to previous reports on corn starch complexed with palmitic (Nakazawa & Wang, 2004). These authors found type II amylose–lipid complexes with melting temperature of about 110 °C in common corn starch complexed with palmitic acid. type II amylose–lipid complexes are reportedly more ordered than the type I and show distinct crystalline and amorphous regions (Biliaderis & Seneviratne, 1990; Karkalas *et al.*, 1995). type II complexes can be further classified into IIa and IIb. The formation of type II amylose–lipid complexes probably results from slow nucleation followed by distinct crystal growth of type I (Biliaderis & Galloway, 1989). Furthermore, type II amylose–lipid complexes may also be formed due to annealing of type I during extended wet heat processing at elevated temperature (90 °C) (Biliaderis & Galloway, 1989; Karkalas *et al.*, 1995; Tufvesson *et al.*, 2003a,b) or during extended heating at 100 °C for 24 h (Tufvesson *et al.*, 2003a).

The ΔH (6.84 J g^{-1}) of annealed BGS-palmitic acid complex was significantly ($P < 0.05$) higher than the native starch complexed with palmitic acid (4.4 J g^{-1}) (Table 1). The same trend was observed for the reference corn starch (Table 1). However, ΔH values of native or annealed BGS complexed with palmitic acid were higher than the values observed for the corn starch samples. Kawai *et al.* (2012) suggested that ΔH

reflect the amount of complex and the degree of order within the complex. Thus, it appears that BGS complexed better with PAM compared with the control corn starch, which could be associated with the higher amylose content of BGS. Starches with high-amylose contents produce higher yield of V-amylose complex than those with low amylose (Eliasson *et al.*, 1988). Similar results where BGS complexed better with palmitic, stearic oleic and linoleic acids compared with corn and potato starches have been reported (Oyeyinka *et al.*, 2016b).

X-ray diffraction

Native BGS showed strong peaks at 15° (2θ), a doublet at 17° and 18° (2θ) and a single peak at 23° (2θ) (Fig. 2). Similar peaks were observed in the corn starch reference sample (Figure not shown). These peaks correspond to the A-type crystallinity pattern. Previous research on native BGS found the A-type (Kaptso *et al.*, 2014; Oyeyinka *et al.*, 2016a) or C-type crystallinity pattern (Afolabi, 2012; Oyeyinka *et al.*, 2015). Annealing seems not to change the crystallinity pattern of the starch samples (Fig. 2). The XRD result on annealed starch is in agreement with previous findings where annealing did not change the crystalline type of potato (Jacobs *et al.*, 1998; Vermeylen *et al.*, 2006) and wheat starches (Jacobs *et al.*, 1998).

Pasting of native or annealed BGS with palmitic acid revealed the loss of its native crystallinity (Fig. 2). BGS pasted with palmitic acid showed peaks at 2θ = 7.4, 12.9 and 19.9° (Fig. 2), confirming the formation of amylose–lipid complexes (Zobel, 1988; Oyeyinka *et al.*, 2016b,d). The peaks corresponding to amylose–palmitic acid complex displayed higher intensity, although similar to the native starch complexed with palmitic acid. Thus, it appears that annealing improves the complexation of palmitic acid with BGS. This observation may explain the higher reduction in peak viscosity (Table 1) and higher ΔH values (Table 1) of annealed starch complexed with palmitic acid.

Conclusion

BGS granules were bigger than the corn starch control. Annealing created cracks and pores on the surface of Bambara groundnut and corn starches, respectively. Swelling power of annealed starches reduced compared with the native starches. Pasting of native Bambara groundnut and native corn starches with palmitic acid resulted in the formation of type I amylose–lipid complexes, while type II complexes were formed from annealed starches pasted with palmitic acid. This was confirmed by XRD reflection peaks corresponding to amylose–lipid complex. Annealing in combination with complexation of BGS with palmitic acid may be employed to produce modified starch with improved thermal stability and increased final viscosity.

Conflict of interest

Authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Swelling power of Bambara groundnut and corn starches.

Figure S2. Typical pasting curves of Bambara groundnut and corn starch-palmitic acid complexes.