

Physicochemical and Mechanical Properties of Bambara Groundnut Starch Films Modified with Stearic Acid

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Abstract: The physicochemical and mechanical properties of biofilm prepared from bambara starch modified with varying concentrations of stearic acid (0%, 2.5%, 3.5%, 5%, 7%, and 10%) were studied. By scanning electron microscopy, bambara starch films modified with stearic acid ($\geq 3.5\%$) showed a progressively rough surface compared to those with 2.5% stearic acid and the control. Fourier transform infrared spectroscopy spectra revealed a peak shift of approximately 31 cm^{-1} , suggesting the promotion of hydrogen bond formation between hydroxyl groups of starch and stearic acid. The addition of 2.5% stearic acid to bambara starch film reduced water vapor permeability by approximately 17%. Bambara starch films modified with higher concentration of stearic acid were more opaque and showed significantly high melting temperatures. However, mechanical properties of starch films were generally negatively affected by stearic acid. Bambara starch film may be modified with 2.5% stearic acid for improved water vapor permeability and thermal stability with minimal effect on tensile strength.

Keywords: bambara starch, edible films, mechanical, physicochemical, stearic acid

Practical Application: Bambara starch film may be modified with 2.5% stearic acid for improved water vapor permeability and thermal stability, which could find application in the food industry for coating surfaces of highly perishable food commodities such as fruits and vegetables, thereby extending shelf life.

Introduction

Bio-plastic packaging is receiving much attention in recent time mainly due to their biodegradability. These category of plastics also provide alternative packaging option without contributing to environmental pollution (Jiménez and others 2012a; Cano and others 2015). Edible starch films and coatings, presents an alternative to petrol-based plastics because they are cheap, readily available, and are obtained from renewable sources. However, starch-based plastics are inferior to the petrol-based types (Ortega-Toro and others 2014). The use of starch in bio-plastic packaging is limited due to its hydrophilic and poor mechanical property (Ortega-Toro and others 2014; Liu and others 2015). Addition of plasticizers such as glycerol and sorbitol have been found to improve films flexibility (Jiménez and others 2012a). Plasticizers penetrate the amorphous regions of starch and interrupts hydrogen bonding along the polymeric network making the film more flexible.

The improvement in barrier property of starch-based films following the addition of lipids to starch matrix has also been reported (Jiménez and others 2012b; Schmidt and others 2013; Ortega-Toro and others 2014; Liu and others 2015). These improvements in barrier properties may vary with starch source and lipid type. For instance, approximately 27% reduction in water vapor permeability (WVP) was reported for corn starch film modified with 15% stearic acid compared to the control (Jiménez and others 2012b). However, other authors working with cassava starch film reported higher reduction (39%) in WVP at the same stearic acid concentra-

tion (Schmidt and others 2013). According to Jiménez and others (2012b), the improved barrier property of starch films modified with lipids is due to the overall increase in films hydrophobicity. Further, stearic acid addition has been found to improve the thermal stability of starch films (Liu and others 2015). Potato starch film modified with stearic was reported to exhibit higher melting temperature ($191\text{ }^{\circ}\text{C}$) compared to the control film, which showed approximately $183\text{ }^{\circ}\text{C}$ (Liu and others 2015). The formation of inclusion complexes between lipids and amylose in starch is well known. Beyond certain concentrations, which may vary with lipid type, the lipid molecules associate rather than form complex with amylose (Tang and Copeland 2007). In general, starches with high amylose contents have been reported to form more amylose-lipid complexes (Eliasson and others 1988).

The modification of starch with lipid results in the formation of amylose inclusion complexes. These complexes may be explored in making films with improved barrier properties (Jiménez and others 2012b; Liu and others 2015). Most reported studies on improving physicochemical properties of starch films with lipids have focused mainly on conventional starch sources like cereals and tubers. However, starches from pulses such as cowpea (*Vigna unguiculata*) and bambara (*Vigna subterrenea*) with high amylose content (22% to 78%; Hoover and others 2010; Oyeyinka and others 2015; Oyeyinka and others 2016b), may be a promising matrix for making films. Bambara groundnut is a pulse of African origin. The starch yield of bambara may vary between 22% and 46% depending on source and cultivar (Sirivongpaisal 2008; Afolabi 2012; Oyeyinka and others 2015; Oyeyinka and others 2016a). In Southern Africa, this crop is neglected and grown mainly for subsistence. Recently, bambara starch was modified with lipids for improved functionality (Oyeyinka and others 2016b,c). The use of bambara starch in complexation with lipids and in biofilm

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application is unique in many ways. Bambara starch has a moderate level of amylose content (20% to 35%; Sirivongpaisal 2008; Oyeyinka and others 2015, 2016a), which is higher than those of corn and potato starches. High amylose content of starch improves strength and film flexibility (Zobel 1988; Peressini and others 2003; Zhang and Han 2006) and also produces films with better gas barrier properties (Wolff and others 1951; Lourdin and others 1995; Palviainen and others 2001). Furthermore, in comparison with corn, the bambara plant is highly resistant to drought and therefore well adapted to the changing climate. Bambara starch as an alternative source of starch would be more sustainable for utilization and application in the industry. To further increase the utilization and application of bambara starch, we considered it necessary to explore its application in the production of edible starch films. Hence, this study investigated the physicochemical and mechanical properties of bambara starch films modified with stearic acid.

Materials & Methods

Materials

Bambara groundnut with brown seed coat color was obtained from Markathini Research station Jozini, KwaZulu-Natal Province, South Africa. The seeds were cleaned and stored at 4 °C prior to use. All chemicals and solvents used were laboratory grade.

Starch extraction

Bambara starch was extracted from bambara flour as described by Oyeyinka and others (2015). The yield of starch, calculated as a ratio of dried starch to bambara flour was 35%. Amylose content was 31.5% as determined by iodine-binding method (Williams and others 1970). The extracted starch had low ash (0.1%), protein (0.2%), and fat (0.3%) contents, suggesting that it was relatively pure.

Preparation of films

Films were prepared by dispersing 3% (w/w) bambara starch in distilled water. The dispersions were stirred at 95 °C for 30 min to induce starch gelatinization. Glycerol (0.3% starch basis) was added to the starch dispersion followed by the addition of stearic acid (0%, 2.5%, 3.5%, 5%, 7%, and 10%) on starch dry weight basis. The dispersions were homogenized at 95 °C for 5 min using a rotor-stator homogenizer (Ultra turax). Bambara starch films prepared in the same way as described above, but without stearic acid served as the control. Starch dispersions (25 mL) were spread evenly on petri-dishes. Films were formed by drying for approximately 48 h at 30 °C. These conditions were established from previous experimental trials to ensure that homogenous flawless films were obtained. Film thickness (average of 0.14 mm) was measured with an analogue micrometer to the nearest 0.001 mm at 6 random positions. Moisture content was determined by drying the films at 105 °C until constant weight in a hot air oven (D-37520, Thermo Fischer Scientific, Johannesburg, South Africa).

Color

Tristimulus L^* a^* b^* parameters of bambara starch films were determined after standardization using a colorFlex (A60-1014-593, Reston, Virginia, U.S.A.). Five snap shots were taken for each film and values were read directly from a digital print. Average of the readings were computed and reported. Whiteness index (WI)

was calculated using Eq. 1. Bambara starch films without stearic acid was used as a reference for comparison.

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad (1)$$

Opacity

The light barrier properties of the films were measured as described by Al-Hassan and Norziah (2012). Briefly films were exposed to light absorption at wavelength 550 nm. Rectangular films (1 × 4 cm) were placed in the cuvette of a UV-visible spectrophotometer (Jenway 7305, Loughborough, U.K.) and absorbance was recorded at a wavelength of 550 nm. The opacity of the films was calculated according to the Eq. 2.

$$T = A_{550}/x \quad (2)$$

where A_{550} is the absorbance at a wavelength of 550 nm and x is the film thickness (mm).

Scanning electron microscopy

The scanning electron microscopy (SEM) images films surface was carried out using scanning electron microscope (EVO 15 HD) with an accelerating potential of 4 kV. Films were equilibrated at 53% RH in desiccators for 48 h. equilibrated films were fixed on copper stubs, gold coated, and observed using an accelerating voltage of 2 and 5 kV (Cano and others 2015).

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra of the films were obtained using a FTIR spectrometer (Varian 800, Scimitor Series). The films were placed directly into the cell. For each spectrum, 64 consecutive scans at a percentage transmittance mode from 400 to 4000 cm^{-1} were recorded.

Water soluble matter

Water-soluble matter was determined according to the method described by Bertan and others (2005). Dried films (2 cm in dia) were immersed in centrifuge tubes containing 50 mL distilled water. The tubes were stirred mechanically at 25 °C for 24 h. After this period, the films were removed from the solution and dried at 105 °C for 24 h in a forced air oven (D-37520, Thermo Fischer Scientific, South Africa). The initial solid content was determined from the sample moisture content. The difference in weight was used to calculate the water soluble matter as a percentage of the initial weight.

Mechanical properties

Tensile strength (TS) and breaking force of the films were determined using a universal testing machine (EZ-SX, Shimadzu Scientific Instruments, Columbia, Maryland U.S.A.). The initial distance of separation and velocity were adjusted to 40 mm and 1 mm/s, respectively. Before analysis, the films were cut into a rectangle (6 cm × 1 cm) and conditioned for 48 h at 53% RH. At least 6 films were tested for each sample.

Differential scanning calorimetry

Thermal properties of films were measured using the modified method of Dai and others (2015). Briefly, film (3 mg) was cut and placed into the aluminum pan and hermetically sealed using a differential scanning calorimetry (DSC) punch sealer. Samples were scanned from 25 to 250 °C with an interval heating rate of

Table 1—Color parameters, whiteness index, and opacity of bambara starch films modified with stearic acid.

Stearic acid (%)	L^*	a^*	b^*	WI	Opacity(AU nm)
Control	92.21 ^a ± 0.01	-0.87 ^a ± 0.01	3.17 ^b ± 0.01	91.55 ^a ± 0.01	1.23 ^d ± 0.00
0	92.78 ^a ± 0.01	-0.95 ^a ± 0.01	2.64 ^c ± 0.02	92.25 ^a ± 0.01	1.14 ^d ± 0.00
2.5	90.97 ^b ± 0.01	-0.91 ^a ± 0.01	3.21 ^b ± 0.02	90.38 ^b ± 0.01	2.46 ^c ± 0.00
3.5	90.96 ^b ± 0.02	-1.15 ^b ± 0.01	2.68 ^c ± 0.01	90.50 ^b ± 0.02	2.49 ^c ± 0.00
5	90.14 ^b ± 0.01	-1.03 ^b ± 0.01	2.69 ^c ± 0.03	89.72 ^b ± 0.02	2.60 ^b ± 0.01
7	90.83 ^b ± 0.01	-1.29 ^c ± 0.01	2.88 ^c ± 0.02	90.30 ^b ± 0.01	2.69 ^b ± 0.05
10	88.35 ^c ± 0.03	-1.72 ^c ± 0.01	4.22 ^a ± 0.02	87.49 ^c ± 0.03	3.12 ^a ± 0.00

Mean ± SD. Mean with different superscript along the column are significantly different ($P < 0.05$). WI, Whiteness index.

10 °C/min in a differential scanning calorimeter (SDT Q600, TA Instruments, New Castle, U.S.A.) coupled with a thermal analysis data station and data recording software. An empty pan was used as reference for all measurements.

Water vapor permeability

The modified method of Taylor and others (2005) based on the ASTM method E96- 97 (American Society for Testing & Materials, 1997) was used. Briefly, circles (30 mm dia) were cut from cast films and the thickness measured in 5 places using a micrometer. Centrifuge tube (50 mL) were modified by accurately drilling a hole in the center of the plastic screw top. Films were mounted on top of the modified containers filled with distilled water (45 mL). The films were placed between the lid and the containers to ensure a watertight seal was maintained throughout the experiment. Containers with the films were placed in a fume cupboard at 25 °C and relative humidity of 40%, with the fan switched on. Weight loss was recorded daily for up to 14 d.

Statistical analysis

Except otherwise stated, all experiments were conducted in triplicate. Data were analyzed using analysis of variance and means were compared using Fischer's least significant difference test ($P < 0.05$).

Results and Discussions

Film morphology, color, and opacity

SEM image of bambara starch film modified with 2.5% stearic acid appeared smooth and was comparable to the control (Figure 1). This could be attributed to better dispersion of stearic acid in the starch matrix. Films prepared with stearic acid concentration greater than 2.5% showed progressively rougher surface. Lipids at relatively high concentrations may self-associate in preference to form inclusion complexes with amylose in starch (Tang and Copeland 2007). Thus, the rougher surfaces of bambara starch films at higher stearic acid concentrations may be attributed to molecular self-association of stearic acid.

The lightness (L^*) and whiteness index (WI) values of bambara starch film modified with stearic acid were slightly lower compared to the control (Table 1). These values showed a decrease, which was not significant with increasing stearic acid concentration up to 7%. The decrease in L^* and WI values suggest that stearic acid caused films' coloration. Previous studies similarly reported decrease in L^* value of high amylose corn starch film following the addition of lipids (Muscat and others 2013) and tapioca starch film modified with potassium sorbate (Flores and others 2007).

The opacity of bambara the films seemed to increase with increasing concentration of stearic acid in the starch matrix. Control bambara starch film showed the lowest opacity value (1.14 AU nm), whereas starch film with 10% stearic acid had the highest

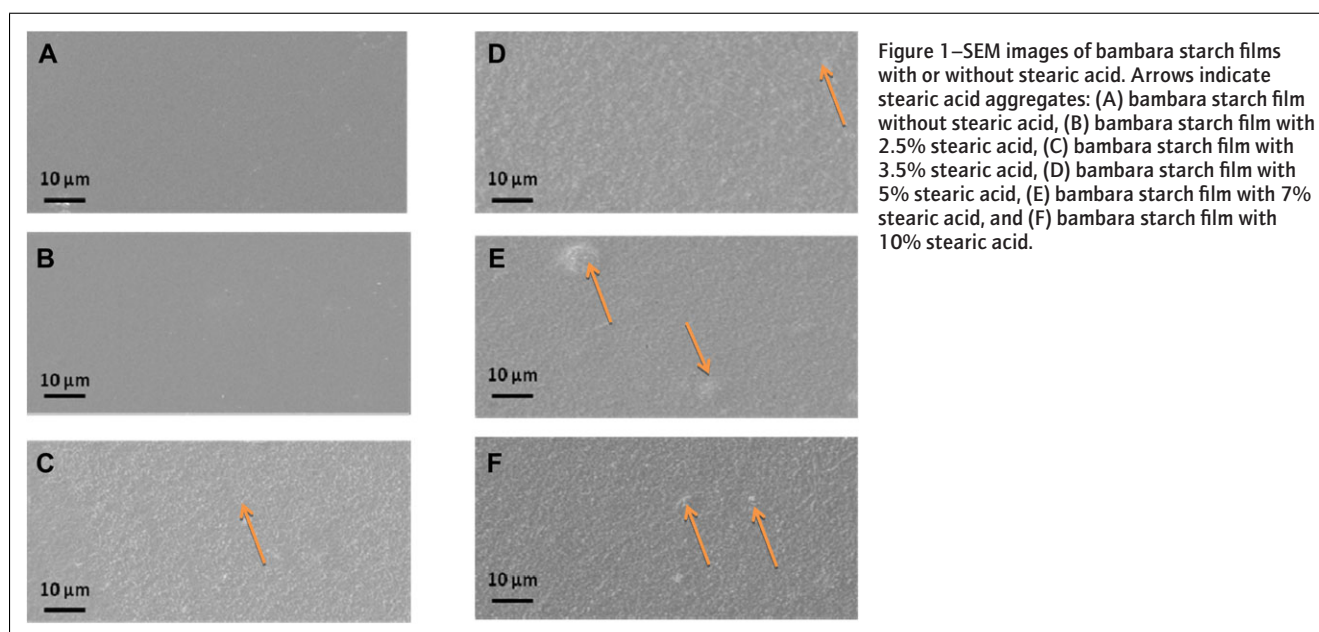


Figure 1—SEM images of bambara starch films with or without stearic acid. Arrows indicate stearic acid aggregates: (A) bambara starch film without stearic acid, (B) bambara starch film with 2.5% stearic acid, (C) bambara starch film with 3.5% stearic acid, (D) bambara starch film with 5% stearic acid, (E) bambara starch film with 7% stearic acid, and (F) bambara starch film with 10% stearic acid.

Table 2–Soluble matter, water vapor permeability, tensile strength, break force, and moisture content of bambara starch films modified with stearic acid.

Stearic acid (%)	Moisture content (%)	Soluble matter (%)	WVP (10^{-3} g mm h $^{-1}$ KPa $^{-1}$ m $^{-2}$)	TS (MPa)	Break force (N)
0	25.07 ^a ± 0.21	37.73 ^b ± 0.12	2.15 ^a ± 0.01	48.32 ^a ± 0.25	6.67 ^a ± 0.26
2.5	22.11 ^b ± 0.92	38.89 ^b ± 0.21	1.79 ^b ± 0.01	33.88 ^b ± 0.12	3.07 ^b ± 0.27
3.5	21.51 ^b ± 0.06	46.59 ^a ± 0.14	1.76 ^b ± 0.01	25.42 ^c ± 0.06	3.01 ^{bc} ± 0.14
5	20.35 ^c ± 0.25	46.40 ^a ± 0.10	1.48 ^c ± 0.01	22.18 ^c ± 0.24	2.57 ^c ± 0.35
7	20.49 ^c ± 0.57	45.73 ^a ± 0.13	1.45 ^c ± 0.01	18.62 ^d ± 0.24	2.62 ^c ± 0.59
10	20.64 ^c ± 0.19	46.53 ^a ± 0.86	1.44 ^c ± 0.01	13.22 ^e ± 0.28	1.93 ^d ± 0.38

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

value of 3.12 AU nm (Table 1). The reduction in light transmitting property of films confirms the lower values of L^* and WI observed for starch films with added stearic acid (Table 1) and further suggests that stearic acid were embedded in the starch matrix (Figure 1). Thus, bambara starch films modified with stearic acid has the potential for packaging light sensitive foods.

FTIR

FTIR spectra of bambara starch films with or without stearic acid were very similar (Figure 2). Nevertheless, some differences can be observed in the OH stretching region. The bands between 3200 and 3600 cm^{-1} , which corresponds to the stretching vibration mode of hydroxyl groups arose from absorbed water from the starch and stearic acid as previously reported (Jiménez and others 2014; Cano and others 2015). The peak wavenumber in the OH stretching region increased from around 3557 cm^{-1} for the control film up to approximately 3588 cm^{-1} in bambara starch film modified with 10% stearic acid. This corresponds to a peak shift of approximately 31 cm^{-1} , which could be attributed to the promotion of hydrogen bond formation between hydroxyl groups of starch and stearic acid (Cano and others 2015). Cano and others (2015) working with films made

from pea starch and blend of polyvinyl alcohol similarly observed a peak shift of approximately 40 cm^{-1} in the same region. Notable peaks at around 2143 and 2150 cm^{-1} were found in the control film and films modified with stearic acid, respectively. The slight shift in peak position, which was accompanied with a slight peak broadening, further confirms the interactions between bambara starch and stearic acid. Previous studies indicated that when 2 components are mixed, the physical blends may result in changes in the characteristic spectra peaks (Guan and others 1998; Yin and others 1999). The peak located at approximately 1650 cm^{-1} in all the starch films corresponds to adsorbed water and carboxyl groups in their ionized state as previously reported (Nacos and others 2006; Karimi and others 2014).

WVP

Expectedly, bambara starch films modified with stearic acid were less permeable to water vapor compared to the control (Table 2). WVP was reduced by approximately 17% when bambara starch film was prepared with 2.5% stearic acid. However, increasing the concentration of stearic acid above 2.5% stearic acid did not significantly change the WVP of modified bambara starch films. The reduction in WVP of the modified films could

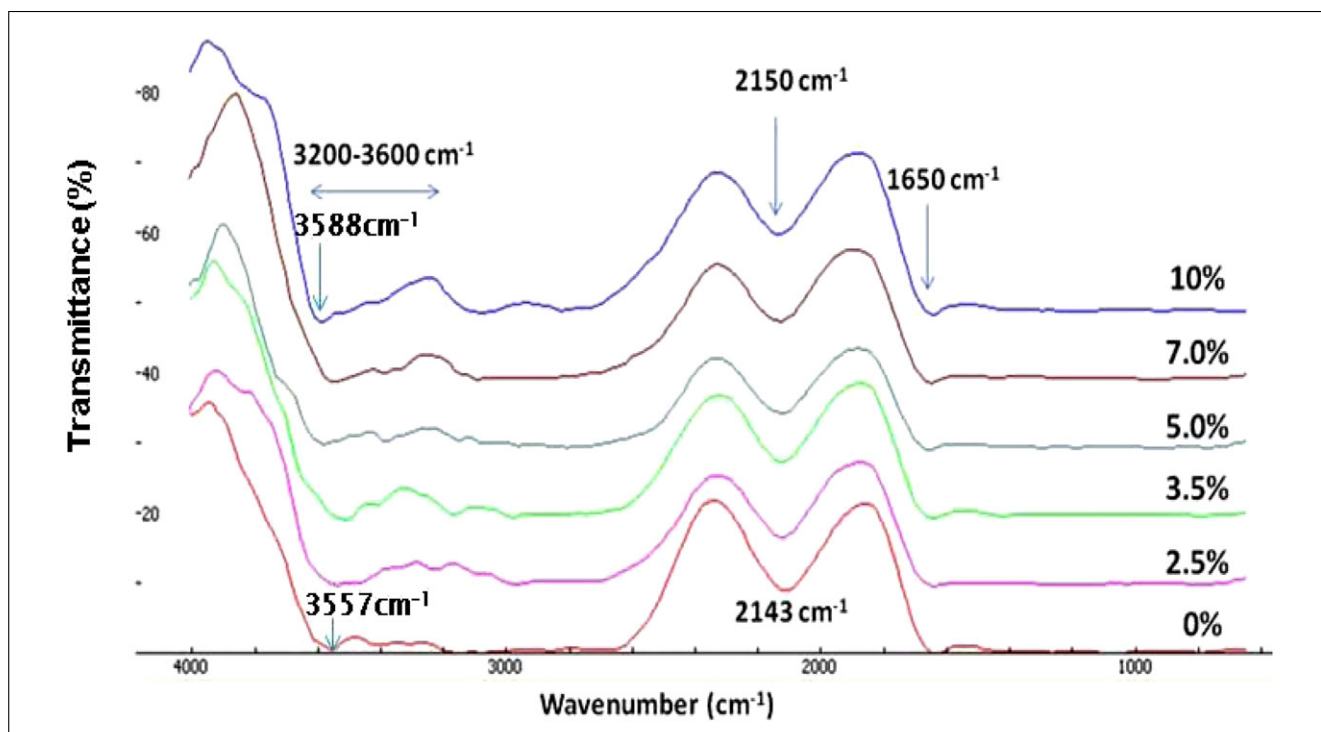


Figure 2–FTIR Spectra of bambara starch films with or without stearic acid. Values in percentages represent stearic acid concentration.

be linked to the hydrophobicity of stearic acid as indicated by previous authors (Jiménez and others 2010, 2012a,b). Uncomplexed stearic acid at concentrations above 2.5%, which contributed to the surface roughness of the films (Figure 1), may have also restricted water vapor through the films. Jiménez and others (2012b) reported approximately 27% reduction in WVP of corn starch films modified with 15% stearic acid. Other authors similarly reported reduction in WVP following the addition of lipids to corn starch films (Jiménez and others 2012b; Ortega-Toro and others 2014), cassava starch films (Schmidt and others 2013), and gelatin based films (Bertan and others 2005).

Tensile properties

The TS of bambara starch films substantially reduced with the addition of stearic acid (Table 2). The reduction in TS of the films may have resulted from discontinuities introduced in the starch matrix by stearic acid (Jiménez and others 2012b). Films prepared with 2.5% stearic showed lower reduction (approximately 30%) in TS compared to films prepared at higher concentrations. Some authors reported higher reductions (approximately 40%) in TS for corn starch films modified with 15% stearic acid (Jiménez and others 2012b) and approximately 51% reduction in TS for cassava starch films modified with 15% stearic acid (Schmidt and others 2013). The differences in TS of the modified bambara starch films compared to those reported by these authors could be attributed to starch type, lipid concentration, and differences in amylose content.

Moisture content and soluble matter

The moisture content of the control film was significantly ($P < 0.05$) higher than films modified with stearic acid (Table 2). Bambara starch film modified with stearic acid had reduced moisture content, which could be attributed to increase in film hydrophobicity. However, increasing the concentration of stearic acid beyond 3.5% did not significantly ($P < 0.05$) change the moisture content of the films.

Films solubility in water significantly ($P < 0.05$) increased with increasing concentration of stearic acid from approximately 38% for the control film up to 47% for film modified with 10% stearic acid (Table 2). The control film and film prepared with 2.5% stearic acid showed similar and low solubility values of approximately 38%. This may be attributed to better dispersion of stearic acid in the starch matrix (Schmidt and others 2013). The increase in film solubility with increasing concentrations of stearic remains unclear. It is possible that the weak structure of the films with high lipid content as reflected in the TS result (Table 2), facilitated leaching of soluble matter during mechanical stirring. Fatty acids may also contribute to water solubility due to the presence of the polar carboxylic head. Further, the separation of large fatty acid crystals causing disintegration of starch network may also account for the increase in solubility of the films in water (Sapru and Labuza 1994; Schmidt and others 2013). Previous studies also reported increase in films solubility in water for composite films of soy protein isolate modified with lauric acid (Rhim and others 1999), gelatin-based composite films modified with fatty acids (Bertan and others 2005) and cassava starch film modified with stearic acid (Schmidt and others 2013). The increased films solubility in water is advantageous especially when the films are intended for consumption together with products. Also, film solubility may be an important factor that determines biodegradability when used as a packaging wrap (Bourtoom and Chinnan 2008).

DSC

The addition of stearic acid to bambara starch significantly ($P < 0.05$) increased the melting temperatures and enthalpies of the films (Table 3). Both the control film and films modified with stearic acid exhibited 2 endothermic peaks (Figure 3). The 1st peak of the control film was broader and occurred at approximately 80 °C. This peak may be due to the melting of starch crystallites. However, films modified with stearic acid showed relatively lower transition temperatures with sharper peaks at approximately 70 °C. This peak may be attributed to the melting

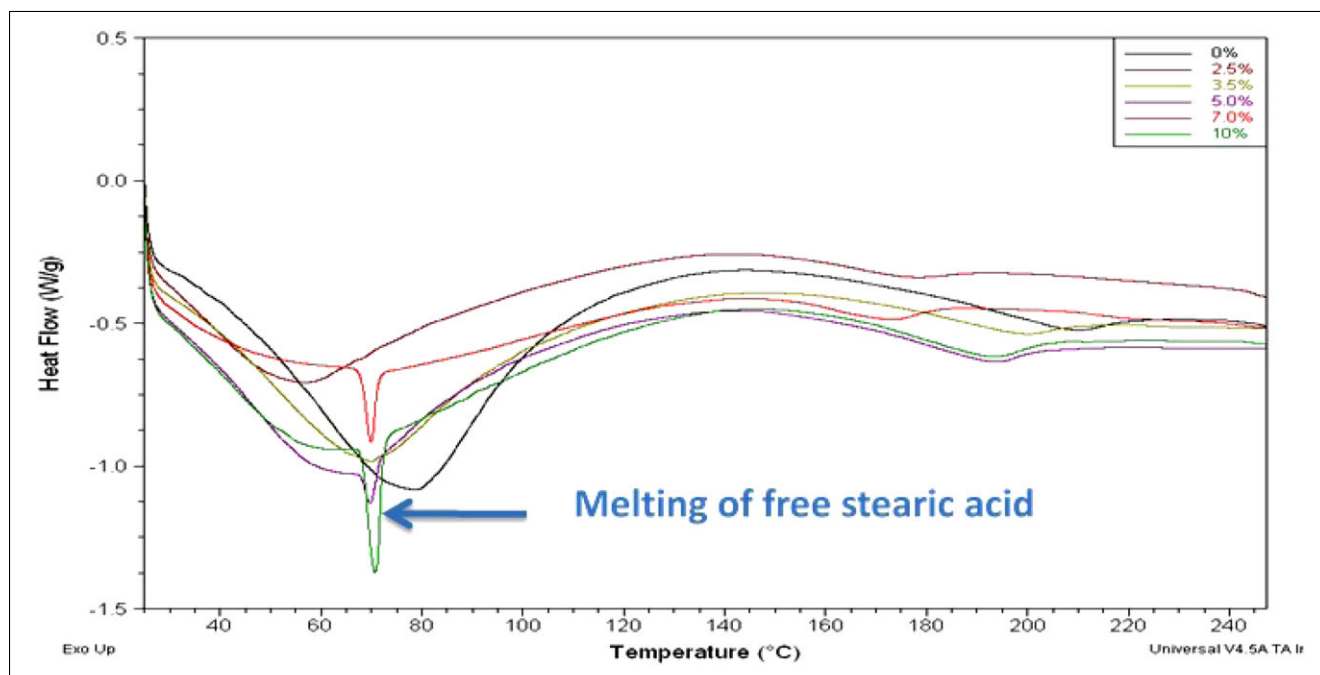


Figure 3—Thermograms of bambara starch films with stearic acid at varying concentrations. Percentages represent stearic acid concentrations.

Table 3—Thermal properties of bambara starch films modified with stearic acid.

Stearic acid (%)	T_o (°C)	T_m (°C)	T_c (°C)	ΔH_m (J/g)
0	156.13 ^c ± 0.18	172.38 ^c ± 0.03	186.44 ^d ± 2.31	4.39 ^d ± 0.33
2.5	156.04 ^c ± 1.18	174.25 ^{bc} ± 1.81	189.42 ^d ± 0.64	5.23 ^c ± 0.08
3.5	162.05 ^b ± 0.21	177.91 ^b ± 0.15	193.00 ^c ± 1.41	5.52 ^c ± 0.08
5	169.97 ^a ± 2.12	192.32 ^a ± 0.66	212.27 ^a ± 2.13	8.61 ^b ± 0.59
7	171.65 ^a ± 2.14	191.80 ^a ± 0.18	206.47 ^b ± 1.30	9.87 ^a ± 0.61
10	173.73 ^a ± 0.71	198.87 ^a ± 0.37	216.86 ^a ± 1.54	8.63 ^b ± 0.09

Mean ± SD. Mean with different superscript along the column are significantly different ($P < 0.05$). T_o , onset temperature; T_m , melting temperature; T_c , conclusion temperature; ΔH_m , melting enthalpy.

of uncomplexed or free stearic acid aggregates within the starch film matrix. Peak melting temperature of about 69 °C has previously been reported for uncomplexed stearic acid in starch matrix (Obiro and others 2012; Chang and others 2013a, 2013b). The 2nd peak observed in the films modified with stearic acid displayed higher melting temperatures than that of the control film and could be attributed to the formation of amylose–lipid complexes. These complexes are known to melt at higher transition temperature and enthalpy (Obiro and others 2012; Chang and others 2013b, 2013a). Films moisture content (Table 2), which decreased with increasing concentration of stearic acid, could also have influenced the melting peak position (Table 3) Furthermore, the peak shift in the hydroxyl group region as shown by the FTIR spectra (Figure 2), which suggest possible promotion of hydrogen bond formation, may further explain the higher melting temperature of these films. The supposedly formed hydrogen bond will require more energy for melting. The melting temperatures of the films modified with stearic acid are within values previously reported for pea starch films modified with corn starch nanocrystals (Li and others 2015), and potato starch films modified with stearic acid (Liu and others 2015).

Conclusions

Bambara starch film modified with stearic acid show increased films solubility and opacity. The surfaces of films prepared with stearic acid concentration greater than 2.5% appear rough, which may be associated with uncomplexed stearic acid. Bambara starch films with added stearic acid have improved thermal stability compared to unmodified starch films. Stearic acid (up to 2.5%) may be added to bambara starch matrix for the preparation of biofilms with improved WVP and acceptable TS.

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