



CHARACTERIZATION AND APPLICATION OF BAMBARA
GROUNDNUT STARCH-LIPID COMPLEXES

By

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B.Tech. (Hons) Food Science, M.Sc. Food Technology

Submitted in fulfilment of the academic requirement for the degree Doctor of
Philosophy (Ph.D) in Food Science and Technology

Department of Biotechnology and Food Technology
Faculty of Applied Sciences
Durban University of Technology, Durban, South Africa

Supervisor: Professor Eric Oscar Amonsou

Co-supervisor: Professor Suren Singh

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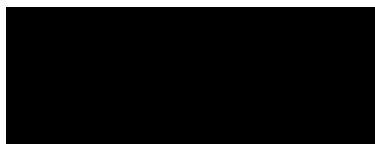
Co-supervisor: Professor Suren Singh

February, 2017

DECLARATION

I declare that the thesis herewith submitted to the Department of Biotechnology and Food Technology, Durban University of Technology for the award of PhD Degree in Food Science and Technology is my work and has not been previously submitted for a degree at any other University or Higher Institution of Education.

As the candidate's supervisors, we agree to the submission of this thesis.



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Date

Professor Eric Oscar Amonsou
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Professor Suren Singh
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Date

DEDICATION

This thesis is dedicated to the Almighty God for seeing me through the programme. I also like to dedicate this thesis to my lovely mother, late Pastor (Mrs.) Rhoda Olukemi Oyeyinka. Continue to rest in the bosom of our Lord Jesus Christ till we meet again.

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ABSTRACT

Bambara groundnut (*Vigna subterranea*) is an indigenous underutilised leguminous crop to Africa. It is a good source of protein and carbohydrate including starch. Bambara groundnut is a traditional crop grown mainly for subsistence in Southern Africa. Bambara groundnut has the advantage of being drought tolerant and can thrive in hot temperatures and poor soil conditions. Therefore, it has great potential as an alternative crop to soya bean and peanuts for cultivation and utilisation. Bambara groundnut starch can potentially be used for various industrial applications. However, native starches are not suitable for most industrial applications, hence the need for modification. Bambara groundnut starch has been previously modified using physical and chemical modification methods. Natural alternatives such as the use of lipids are being sought to modify starches due to the associated risk with chemically modified starch.

In this research, Bambara groundnut starch was modified with lipids to improve functional properties, utilisation and application. Specifically, the physicochemical properties of native Bambara groundnut starch obtained from five Bambara groundnut genotypes and three landraces (maroon, brown and cream) were determined. Bambara groundnut starch was modified with lipids (palmitic acid, stearic acid, oleic acid, linoleic acid and lysophosphatidylcholine) and the physicochemical properties of the modified starch were investigated. Further, the influence of high-pressure homogenization on complexation of Bambara groundnut starch with lipids was assessed in comparison with maize and potato starches. Lastly, an application of modified Bambara groundnut starch in biofilm production was also studied.

Bambara groundnut landraces generally showed higher amylose contents (approx. 33%) than the genotypes (approx. 28%). Differences were observed in the crystalline patterns of these starches. Bambara groundnut genotypes exhibited the C-type-crystallinity, while the landraces showed the unusual A-type pattern. In terms of functionality, landrace starches showed better swelling than the genotypes. Subsequent studies on modification used maroon Bambara groundnut starch since the amylose content was higher than other landraces and there was a consistent supply of the grains during the period of the study.

Generally, Bambara groundnut starch showed higher complexing ability with all the lipids than maize and potato reference samples. These differences in complexing ability among the starches could be due to the variation in amylose contents (Bambara groundnut starch: 31.5%, maize: 22.5% and potato: 24.6%). Fatty acids complexed better with Bambara groundnut starch than lysophosphatidylcholine, which could be due to the structural differences in comparison with the lysophosphatidylcholine molecule. The number of fatty acid in the glycerol backbone

and the additional steric hindrance of the polar phosphatidic acid group in the lysophosphatidylcholine may have reduced its complexing ability. Among the fatty acids, palmitic acid complexed better than stearic and the unsaturated fatty acids, possibly due to its short chain length compared to other fatty acids. Bambara groundnut starch showed reduced peak and setback viscosities in the presence of stearic acid, linoleic acid and lysophosphatidylcholine, suggesting the formation of V-amylose complex. Bambara groundnut starch pasted with lipids displayed reduced gelling ability compared to their unmodified counterparts. XRD studies of freeze-dried paste revealed peaks at $2\Theta = 7.4, 12.9$ and 19.9° confirming the formation V-amylose complexes in Bambara groundnut starch. Modification of Bambara groundnut starch with lipids resulted in reduced digestibility.

High-pressure homogenization significantly increased the complexing ability of Bambara groundnut starch with lipids. Homogenized Bambara groundnut starch-lipid complexes generally exhibited higher complex index than their unhomogenized counterparts. The higher complexing ability could be attributed to the effect of high-pressure which may have enhanced greater dispersion of lipids in the starch-water system. X-ray diffraction studies also revealed the formation of higher complexes as shown by high intensities at peaks ($2\Theta = 7.4, 12.9$ and 19.9°) corresponding to V-amylose complexes. Bambara groundnut starch-lipid complexes displayed significantly higher melting temperatures ($95.74\text{--}103.82^\circ\text{C}$) compared to native uncomplexed starch (77.32°C). Homogenized Bambara groundnut starch complexes were non-gelling while the unhomogenized types produced weak gels, with $G' > G''$ in the range of 0.1-10 Hz. Complexation of Bambara groundnut starch with lipids using high-pressure homogenization may be employed in the production of modified starch with non-gelling properties and higher thermal stability suitable for certain industrial application, such as fat replacers in mayonnaise, frozen foods and desserts for a better mouth feel.

The physicochemical and mechanical properties of biofilm prepared from Bambara groundnut starch modified with stearic acid at varying concentrations of 0, 2, 4, 6, 7 or 10% were further studied. By SEM, Bambara groundnut starch films containing stearic acid ($> 2\%$) showed a progressively rough surface compared to those with 2% stearic acid and the control. The addition of 2% stearic acid to Bambara groundnut starch film reduced water vapour permeability by approximately 17%. However, mechanical properties of starch films were generally negatively affected by stearic acid. Bambara groundnut starch film may be modified with 2% stearic acid for improved water vapour permeability and thermal stability with minimal effect on tensile strength.

PREFACE

This thesis is organised into nine chapters and presented as submitted for publication. Chapter one gives the general introduction to the thesis. Chapter two presents a critical review of legume seed microstructure, starch structure, crystallinity, yield, and composition. The methods of starch modification, a brief review of existing literature on Bambara groundnut starch modification and an extensive review on starch-lipid complexes including mechanism and functionality of modified starch were also discussed. The last part discussed application of starch in biofilm production with respect to their preparation methods, physicochemical and mechanical properties. Chapter three and four presents the characterization of native starch extracted from Bambara groundnut genotypes and landraces, respectively. Chapter five presents the effect of lipid types on complexation and some physicochemical properties of Bambara groundnut starch. Chapter six and seven investigated the effect of lipid types and high-pressure homogenization on complex formation and physicochemical properties of Bambara groundnut starch. Chapter eight presents the application of modified Bambara groundnut starch in bio-film production. Chapter nine is a general discussion of the entire findings. Possible future application of lipid modified Bambara groundnut starch were highlighted. Conclusions were drawn from the study and recommendations were proposed for future studies.

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ABBREVIATIONS

ABS = Absorbance

ANN: Annealing

ANOVA: Analysis of variance

B: Bambara groundnut starch

BDF: Biodegradable film

BV: Breakdown viscosity

CI: Complex index

CLSM: Confocal Laser Scanning Microscopy

CON: Control

CTAB: Cetyltrimethylammonium bromide

DP: Degree of polymerization

DSC: Differential scanning calorimetry

EB: Elongation at break

EM: Elastic modulus

ESF: Edible starch films

FTIR: Fourier transform infrared spectroscopy

FV: Final viscosity

G'' : Loss modulus

G' : Storage modulus

GI: Glycemic index

GMS: Glyceryl monostearate

HI: Hydrolysis index

HMT: Heat-moisture treatment

HPH: High-pressure homogenization

k: Consistency coefficient

KBR: Potassium bromide

KOH: Potassium hydroxide

Lau: Lauric acid

Lin: Linoleic acid

LPC: Lysophosphatidylcholine

MM: Monomyristin

MP: Monopalmitin

MS: Monostearin

Myr: Myristic acid
 n: Flow behaviour index.
 NaOH: Sodium hydroxide
 ND: Not detected
 Ole: Oleic acid
 P: Potato starch.
 Pam: Palmitic acid,
 PT: Pasting temperature
 PV: Peak viscosity
 RDS: Rapidly digestible starch
 RS: Resistant starch
 RVA: Rapid visco-analyzer
 RVU: Rapid visco-analyzer unit
 SDS: Slowly digestible starch
 SDS: Sodium dodecyl sulphate
 SEM: Scanning Electron Microscope
 SFA: Saturated fatty acids
 Ste: Stearic acid
 SV: Setback viscosity
 T_c : Conclusion Gelatinisation temperature
 T_m : Melting temperature
 T_o : Onset Gelatinisation temperature
 T_p : Peak Gelatinisation temperature
 TS: Tensile strength
 TV: Trough viscosity
 UFA: Unsaturated fatty acids
 WI: Whiteness index
 WVP: Water vapour permeability
 WVP: Water vapour permeability
 XRD: X-Ray Diffraction
 γ : Shear rate
 ΔH_{gel} : Gelatinisation enthalpy
 ΔH_m : Melting enthalpy
 τ : Shear stress

PUBLICATIONS AND CONFERENCES ATTENDED

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- **Oyeyinka, S.**, Singh, S. Adebola, P., Gerrano, A. & Amonsou, E.O. (2015). Physicochemical properties of starches with variable amylose contents extracted from Bambara groundnut genotypes. *Carbohydrate Polymers*, **133**: 171-178
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- **Oyeyinka, S.A.,** Ma, Y., Singh, S. & Amonsou, E.O. Enhancing the industrial potential of Bambara groundnut starch through complexation with fatty acids using high pressure homogenization. 18th World Congress of Food Science and Technology (IUFOST) Conference, Dublin, Ireland, 21st-25th August 2016.
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CHAPTER ONE

1.0 Introduction

Bambara groundnut (*Vigna subterranea*) is an indigenous leguminous crop to Africa. Nutritionally, the protein (16-27%) and carbohydrate (52-67%) contents of Bambara groundnut (Kaptso et al. 2016; Kaptso et al. 2014; Sirivongpaisal 2008) are similar to those reported for pulses such as cowpea (Campbell et al. 2016; Devi et al. 2015) and peas (Sharma et al. 2015). Bambara groundnut is resistant to drought and can grow in extreme conditions of poor soils and hot climates (Barimalaa and Anoghalu 1997). Hence, Bambara groundnut may serve as an alternative protein and energy source to other legumes such as soya bean and peanuts that cannot thrive under harsh growing conditions. Despite the nutritional and agronomic advantages of Bambara groundnut, the potential of this crop is not fully exploited. In many parts of Africa, including Southern Africa, Bambara groundnut is mainly cultivated for subsistence and traditionally consumed mainly after boiling or roasting (Swanevelder 1998).

Bambara groundnut is a good source of starch, which may have potential application in the food industry. The starch content of Bambara groundnut may vary between 35 and 45% depending on the source and grain variety (Adebowale et al. 2002; Afolabi 2012; Sirivongpaisal 2008). With the growing demand for starch, there is an opportunity for pulse starches such as those extracted from Bambara groundnut for various industrial applications. The application of starch in the industry is influenced by their physicochemical properties including pasting and thermal properties. These properties may vary with botanical origin, grain variety, and composition e.g. amylose content. Starch extracted from Bambara groundnut grown in Nigeria showed significantly higher Gelatinisation temperature (93°C) (Afolabi 2012), compared to those from Thailand (75°C) (Sirivongpaisal 2008) and Cameroon (78-82°C) (Kaptso et al. 2014). Bambara groundnut grown in South Africa are mainly the landrace types, but some genotypes have also been developed by the Agricultural Research Council of South Africa to improve agronomic traits. Breeding may significantly change starch composition, which may impact starch functionality. Knowledge of the physicochemical properties of Bambara groundnut starch is important to facilitate their utilisation in food applications.

Native starches are not suitable for most industrial applications, due to their poor resistance to high thermal and shear processing conditions. Hence, they are modified to improve their

functionality and application. Chemical modification of starch is most widely studied and its effect on starch functionality may vary with the type of chemical (Bemiller 1997). Most chemicals used in starch modification are synthetically derived (D'Silva et al. 2011) and their residues in foods present safety concerns (Li et al. 2009). Hence, naturally occurring compounds such as lipids are being considered in starch modification. The use of polar lipids in starch modification have long been known to influence starch functionality (Biliaderis and Tonogai 1991; Zobel 1988). Amylose in starch can form inclusion complexes with lipids known as V-amylose complexes. The degree of complexation of starch with lipids may vary with starch source (Exarhopoulos and Raphaelides 2012), amylose content (Eliasson et al. 1988; Exarhopoulos and Raphaelides 2012) and lipid types (Kawai et al. 2012; Tang and Copeland 2007). Shorter chain lipids are generally easily accommodated into the amylose helix compared to longer chain types (Cui and Oates 1999; Kawai et al. 2012). Kawai et al. (2012) demonstrated that lauric acid showed a higher complexing ability with potato starch compared to myristic, palmitic, stearic, oleic and linoleic acids. Furthermore, Cui and Oates (1999) reported that sago starch displayed a higher complexing ability with LPC than with monoglycerides. The LPC molecule presumably has less tendency to form micelles and may pass through the surface of starch granules more easily than the monoglycerides (Cui and Oates 1999).

V-amylose complexes are of interest in the food industry since they offer better functionality than their unmodified counterparts. These complexes have been used to retard bread staling (Riisom et al. 1984) and to produce slowly digestible starch (Kawai et al. 2012; Zhang et al. 2012). V-amylose complexes have also found application in improving the barrier properties of starch biodegradable films (Jiménez et al. 2012a; Liu et al. 2015b; Schmidt et al. 2013). Hence, researchers are exploring different methods for improving the degree of complexation of starch with lipids (Chang et al. 2014; Chang et al. 2013a; 2013b). Extending the pasting time of teff starch with stearic acid increased the degree of complexation by 83% (D'Silva et al. 2011). Other studies reported that the application of high-pressure homogenization (HPH) to gelatinized starch-lipid complex can improve the degree of complexation (Lesmes et al. 2008; Meng et al. 2014a; Meng et al. 2014b; Yamada et al. 1998). Meng et al. (2014a) found that the degree of complexation of maize starch with different fatty acids significantly increased by almost double after HPH.

Pulse starch may be explored in complexation with lipids to improve functionality, application, and utilisation. There are a few reports on the formation of inclusion complexes between some pulse starches and lipids (Biliaderis and Tonogai 1991; Exarhopoulos and Raphaelides 2012; Marinopoulou et al. 2016; Raphaelides and Georgiadis 2007; Sun et al. 2013). Biliaderis and Tonogai (1991), found that the rheological properties of lipid-modified pea and garbanzo bean starches depend on their amylose content and the lipid type. Furthermore, the modification of pea starch film using lipids such as beeswax, has been found to significantly reduce its tensile strength (Han et al. 2006). However, beeswax addition reportedly decreased water vapour permeability. Previous studies on Bambara groundnut starch modification primarily focused on chemical methods such as carboxymethylation (Afolabi 2012), acetylation, oxidation (Adebowale et al. 2002) and physical modification methods (annealing and heat-moisture treatment) (Adebowale and Lawal 2002). Therefore, it is important to investigate the influence of lipid type and high-pressure homogenization on the formation of V-amylose complexes in Bambara groundnut starch and how this could influence functionality and application.

CHAPTER TWO

2.0 Literature review

2.1 Legume seed microstructure

Food microstructure is an important concept in understanding the functionality of a food material (Amonsou et al. 2014). It may influence the extraction of food components such as protein and starch (Parada and Aguilera 2007). The microstructure of legumes such as soya bean and cowpea has been well researched (Avanza et al. 2012; Sefa-Dedeh et al. 1978; Wolf 1970). However, only a few studies exist on the microstructure of underutilized legumes such as Bambara groundnut (Enwere and Hung 1996). The microstructure of Bambara groundnut grain (Enwere and Hung 1996) is similar to those reported for cowpea (Avanza et al. 2012; Sefa-Dedeh and Stanley 1979; Sefa-Dedeh et al. 1978) and African yam bean (Enwere et al. 1990). Cross sections of cowpea revealed the presence of different shapes and sizes of starch granules in parenchymatous cells (Figure 2.1) embedded in a compact protein matrix (Avanza et al. 2012). A similar arrangement of starch granules has been reported for Bambara groundnut (Enwere and Hung, 1996). However, variations with respect to the size of the parenchymatous cells have been observed in some cowpea varieties (Sefa-Dedeh and Stanley 1979).

Variations in starch granule morphology have been reported for Bambara groundnut, which could be associated with the botanical origin and cultivar of the grains (Adebowale and Lawal 2002; Kaptso et al. 2014). Kaptso et al. (2014), observed polygonal shaped granules for starch extracted from white Bambara groundnut cultivar and spherical shaped granules for black cultivar (Figure 2.2). According to their report, starch from white Bambara groundnut showed a remarkably large granule size (10-35 μm) compared to the black cultivar (6-15 μm) (Kaptso et al. 2014). Differences in size and shape of starch granules from different pulses have also been reported (Hoover et al. 2010).

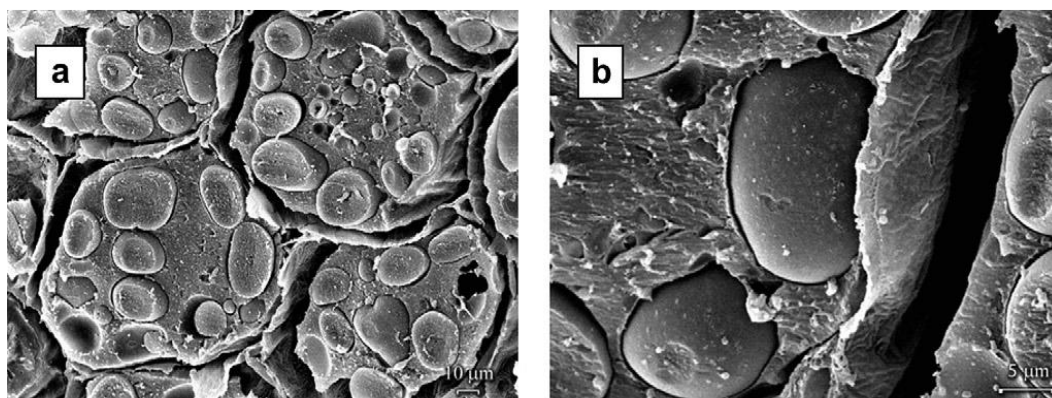


Figure 2.1 Micrograph of the cross section of cowpea grains

a: raw seed, b: starch granule (Avanza et al. 2012)

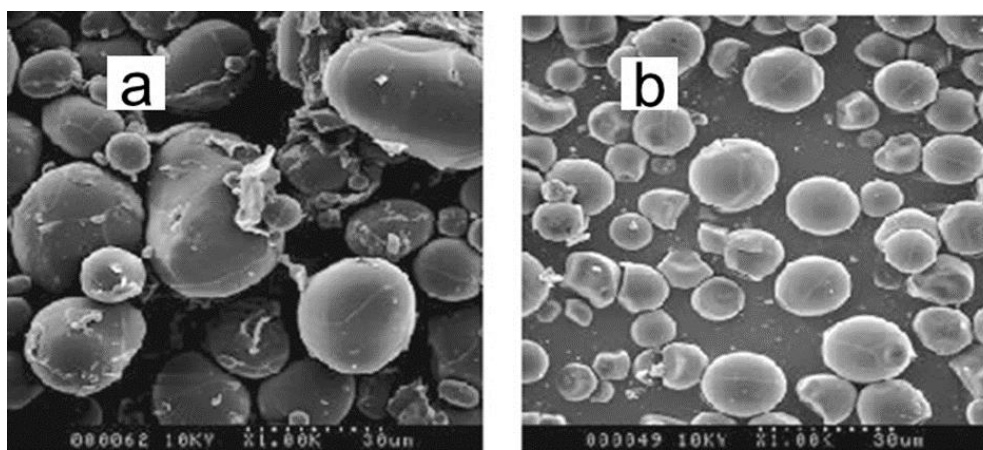


Figure 2.2 Micrograph of Bambara groundnut starch

a: white cultivar b: black cultivar (Kaptso et al. 2014)

2.2 Legume seed composition

Legumes belong to the Leguminosae family of the dicotyledonous seed of plants which can be classified into oil seeds and pulses. About 60 pulse species have been domesticated throughout the world (Hedley 2001). Among these pulses, pea (*Pisum sativum* L.) is the most widely grown and researched, especially in developed nations of the world. Underutilized pulses, such as Bambara groundnut, are traditional crops in developing countries including South Africa and are currently being explored as possible food security crops. Pulses contain significant quantities of protein and carbohydrate (Table 2.1), while fat, ash and fibre are present in relatively small quantities. The protein (24.6-25.3%) and carbohydrate (49.8-59.80%) contents of Bambara groundnut grain (Kaptso et al. 2016; Kaptso et al. 2014) are similar to those reported for other common pulses such as chickpea (Kaur and Singh 2005). In many parts of the world, pulses are consumed as whole grain because they are rich in fibre, B-complex vitamins and are an inexpensive source of protein in comparison to other crops (Singh et al. 2004). Other components such as fibers, resistant starch and polyphenols in pulses makes them suitable for various food applications (Avanza et al. 2013; Kaptso et al. 2016; Sridhar and Seena 2006). Thus, pulses represent significant food sources for developing nations, especially countries in the Africa region. However, some pulses such as Bambara groundnut are only consumed in small quantities and have limited utilisation and application. This could be associated with the hard-to-cook defect found in legumes such as cowpea (Akinyele et al. 1986) and Bambara groundnut (Ojimelukwe 1998). Furthermore, the limited research that has been done on Bambara groundnut may also have contributed to its underutilisation. Thus, focusing

on major components in Bambara groundnut, such as starch, may create an opportunity to improve the utilisation of this grain, for example, as a source of starch in the food industry.

Table 2. 1 Chemical composition of selected pulses

Legume types	Species	Moisture	Protein	Fat	Ash	Fibre	CHO
Bambara groundnut	<i>Vigna subterranea</i> ^a	11.60	24.60	6.00	3.75	3.50	59.80
Cowpea	<i>Vigna unguiculata</i> ^b	11.55	19.61	1.40	3.20	0.92	63.24
Lentil	<i>Lens culinaris</i> ^c	11.20	20.60	2.15	2.80	6.82	56.40
Broad bean	<i>Vicia faba</i> ^d	12.97	22.61	2.67	2.90	2.46	56.39
Kidney bean	<i>Phaseolus vulgaris</i> ^d	9.15	20.09	2.46	3.85	6.78	57.67
Chick pea	<i>Cicer arietinum</i> ^e	8.01	23.58	1.03	2.83	1.60	62.88

Values are reported in % wet basis. CHO: Carbohydrate (determined by difference)

Reference: ^a(Kaptso et al. 2014) ^b(Oyeyinka et al. 2013) ^c(de Almeida Costa et al. 2006) ^d(Qayyum et al. 2012) ^e(Kaur and Singh 2005)

2.2.1 Pulses as potential starch sources

Pulses are a good starch source, which may be used for various industrial applications. Starch (22-45%) is the main carbohydrate in the pulse grain (Hoover and Ratnayake 2002). However, unlike cereal starches, pulse starches have received less attention and their application is very limited. Currently, the majority of the starch used in the industry is obtained from cereals such as maize and tuber crops, for example, potato and cassava. Extensive research on starches from these crops has made them readily available for certain industrial applications (Hoover et al. 2010). Hence, more research is needed to unlock the potential in pulse starches such as those extracted from Bambara groundnut.

2.2.2 Starch extraction methods from pulses

Two extraction methods that are generally employed for isolating starch from pulses are dry and wet milling methods (Hoover et al. 2010). According to Hoover et al. (2010), dry milling is conventionally used in the industry, while wet milling method is widely used in the laboratory. The purity of starch extracted by wet milling method is reportedly higher than dry milling methods. Air classification employed during dry milling does not completely remove the protein fraction from starch granules (Hoover et al. 2010). Thus, the extraction method may influence the yield and purity of starch.

The wet extraction method may be further classified into two depending on whether the starch is extracted directly from soaked grains or from flour. In the first method, grains are soaked in NaOH solution (0.2%) for a predetermined time; sufficient to allow for the removal of the seed coat. Dehulled grains are wet-milled, re-suspended in NaOH solution with pH adjusted to

between 8 and 8.5 to allow for protein solubilization. The slurry obtained is shaken and sieved to remove non-starch components such as fibre. The starch filtrate is centrifuged several times, repeatedly washed with water and the resulting starch residue is dried. The second method, which is very similar to the above described method, uses flour as the starting material. The flour is usually obtained from the grains after dehulling. Besides extraction method, genetic differences among cultivar or species of pulses may also influence the starch yield (Singh et al. 2004). Singh et al. (2004) found significant variations in the starch yield (29.0-35.2%) of six chickpea cultivars grown in the same location. The starch yield (22-46%) from Bambara groundnut grain has been found to vary with the extraction method, cultivar differences and growth location (Adebowale et al. 2002; Afolabi 2012; Sirivongpaisal 2008).

2.2.3 Starch composition and purity

Starch granules are composed mainly of amylose and amylopectin, which represents about 98-99% of starch (dry weight) (Tester et al. 2004). The amounts of these two starch components may vary with the origin and variety of the grain. Previous studies found some differences in the amylose contents (21.67-27.80%) of Bambara groundnut starch (Kaptso et al. 2014; Sirivongpaisal 2008). These values are comparable to those reported for rice bean (Maaran et al. 2014), cowpea starch (Huang et al. 2007) (Table 2.1) and are in agreement with the literature (Hoover et al. 2010). Other factors such as the method of amylose determination and the physiological state of the seed have been reported to influence the amylose content of starch (Hoover et al. 2010). Starch may show the presence of lipids, proteins, and ash, which are present in minute quantities (Table 2.1). These minute components are frequently used as an index of starch purity (Maaran et al. 2014; Piecyk et al. 2013). Low ash contents of starches are associated with the absence of hydrated fine fibers found in the cell wall enclosing the starch granules, while low nitrogen content indicates the absence lipids associated with endosperm proteins (Zhou et al. 2004). Although endogenous lipids in starches such as in cereal starch may impact starch functionality, pulse starches generally contain little or no endogenous lipids. The absence of fissures on the surface of starch granules as observed under scanning electron microscopy is also frequently used to assess starch purity after extraction (Piecyk et al. 2013).

Table 2.2 Yield and composition of selected dry bean starches¹

Starch source	Scientific name	Yield	Amylose	Protein	Lipid	Ash
Bambara groundnut ^a	<i>Vigna subterranea</i>	45.57	21.67	0.61	0.44	0.47
Lablab bean ^b	<i>Lablab purpureus</i>	29.90	32.10	0.04	0.54	0.14
Navy bean ^b	<i>Phaseolus vulgaris</i>	24.30	26.50	0.06	0.56	0.35
Rice bean ^b	<i>Vigna umbellata</i>	20.60	22.10	0.13	0.55	0.58
Tepary bean ^b	<i>Phaseolus acutifolius</i>	27.90	30.00	0.02	0.62	0.11
Velvet bean ^b	<i>Mucuna deeringiana</i>	25.20	27.90	0.05	0.48	0.06
Wrinkled pea ^c	<i>Phaseolus sativum</i>	21.60	78.42	0.03	0.85	0.01
Chick pea ^d	<i>Cicer arietinum</i>	29.65	35.24	0.52	0.26	0.05
Cowpea ^e	<i>Vigna unguiculata</i>	37.00	25.80	0.49	0.07	-

¹Values are reported on % dry weight basis

References: ^aSirivongpaisal (2008); ^bMaaran et al. (2014); ^cZhou et al. (2004); ^dMiao et al. (2009); ^eHuang et al. (2007).

2.3 Starch organization

Amylose and amylopectin are biosynthesized and assembled in densely packed semi-crystalline aggregates, called granules (Jenkins and Donald 1995). These two polymers form clusters joined together forming lamellae and blocklets, which in turn build up to form growth rings. As observed by light microscopy (Figure 2.3a), the growth rings extend from the center to the surface and are approximately 120-400 nm in thickness (Copeland et al. 2009). These growth rings comprise of amorphous and crystalline regions alternating with higher and lower density respectively (Figure 2.3b). The first growth layer, usually less organised, is initiated at the center and is made up of a large proportion of the reducing ends. Newly synthesized rings are later deposited on the surface of the granules (Oates 1997). Amylose and amylopectin form the low and high- density growth layers respectively with amylose dispersed between the amylopectin lattice (Jane et al. 1994). The arrangement of amylose and amylopectin defines the starch source and determines to a large extent its functionality. Several models such as laminated structure comb-like (Staudinger 1937), bush structure (Meyer and Bernfeld 1940), a revision of the bush structure (Whelan 1971) and cluster models (French 1972; Hizukuri 1986) describing the arrangement of amylopectin molecule within the growth ring of starch granule (Figure 2.4 A-E) have been documented. The most widely accepted model is the cluster model proposed by French (1972). The model explained the high viscosity of amylopectin and the possibility of building high molecular weight amylopectin with short side chains. These authors found that it was possible to increase the molecular weight of amylopectin by simply increasing the number of clusters in the structure (Figure 2.4E). Further, the exterior chains of amylopectin molecules possess double helices which are interspersed with the amorphous regions (French 1972). The cluster model describes the three-dimensional structure of amylopectin, which

classifies amylopectin molecule into three broad classes of glucose chains, A, B and C chains. The shortest A-chains (DP 6-12) are connected by a single α -(1-6) linkage to the amylopectin molecule, while the B chains are classified into B1, B2, B3 and B4, depending on the number of clusters and the chain length (Hizukuri 1986).

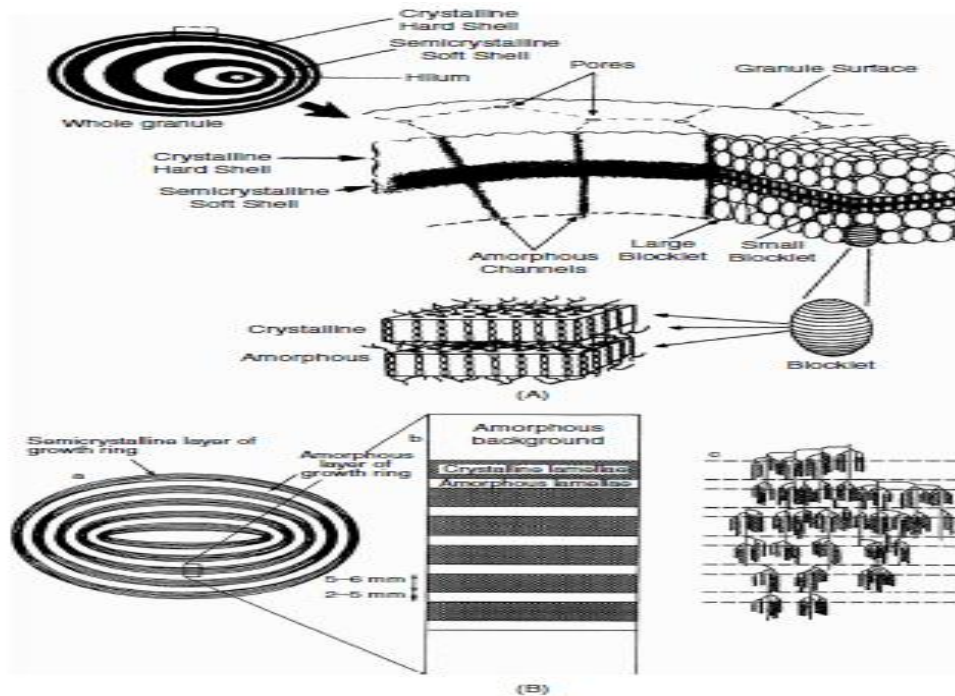


Figure 2.3 Schematic diagram of starch granule structure

(Bemiller 1997)

(a) Single granule comprising concentric rings of alternative amorphous and semi-crystalline composition. (b) Expanded view of the internal structure (c) Accepted cluster structure for amylopectin within the semi-crystalline growth ring. A-chain: sections of amylopectin with double helices, which are packed into crystalline lamellae. B-chain: provides inter-cluster connections. Branching points for both A and B chains are predominantly located within the amorphous lamellae.

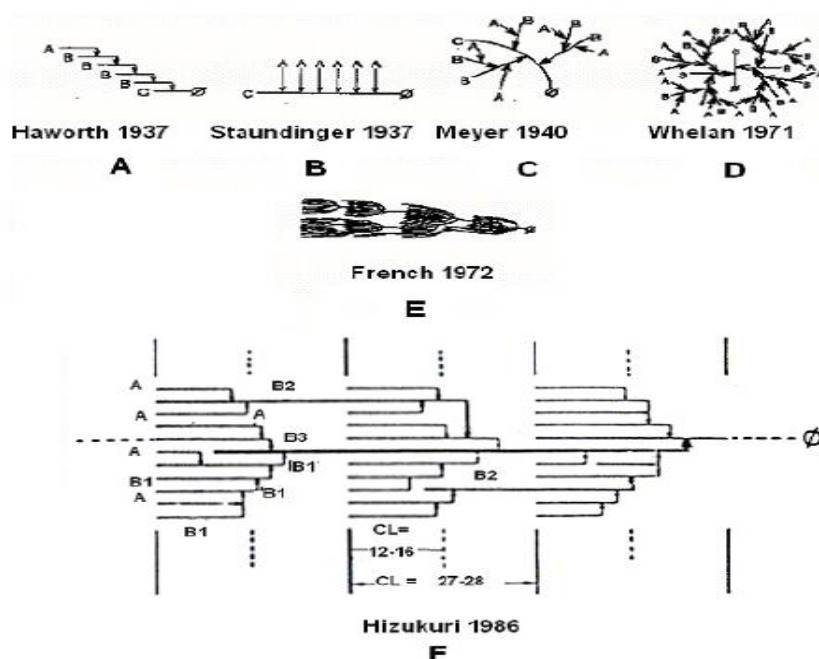


Figure 2.4 Various model of amylopectin structure

A: Laminated structure, B: Comb-like model, C: Bush structure (Staundinger 1937), D: Bush structure (Meyer and Bernfeld 1940), E: Revision of bush structure (Whelan 1971) F: Cluster model I (French 1972), Cluster model II (Hizukuri 1986)

2.3.1 Structure of amylose and amylopectin

Amylose is a straight chain polymer of starch linked with α -1, 4-glycosidic bonds, while amylopectin is a branched chain polymer linked with α -1, 4 and α -1, 6-glycosidic bonds (Figure 2.5). Depending on the starch source, the degree of polymerization (DP) of amylose may vary between 690 and 6340 (Hizukuri 1993) while the molecular weight of amylose may range from 1×10^5 to 1×10^6 (Hoover et al. 2010). Pulse starches, for example, cowpea starch, generally have higher DP than cereals and tuber starches (Chung et al. 1998; Won et al. 2000). Amylose in starch may exist in the amorphous growth ring, amorphous lamellae, or interspersed with amylopectin molecules (Atkin et al. 1999; Jane and Chen 1992; Jenkins and Donald 1995; Kasemsuwan and Jane 1994).

Amylopectin represents the major component of starch with an average molecular weight of 10^7 - 10^9 (Aberle et al. 1994). The structure of amylopectin is generally believed to consist of short amylopectin chains, which form double helices and are associated into clusters (Robin 1974).

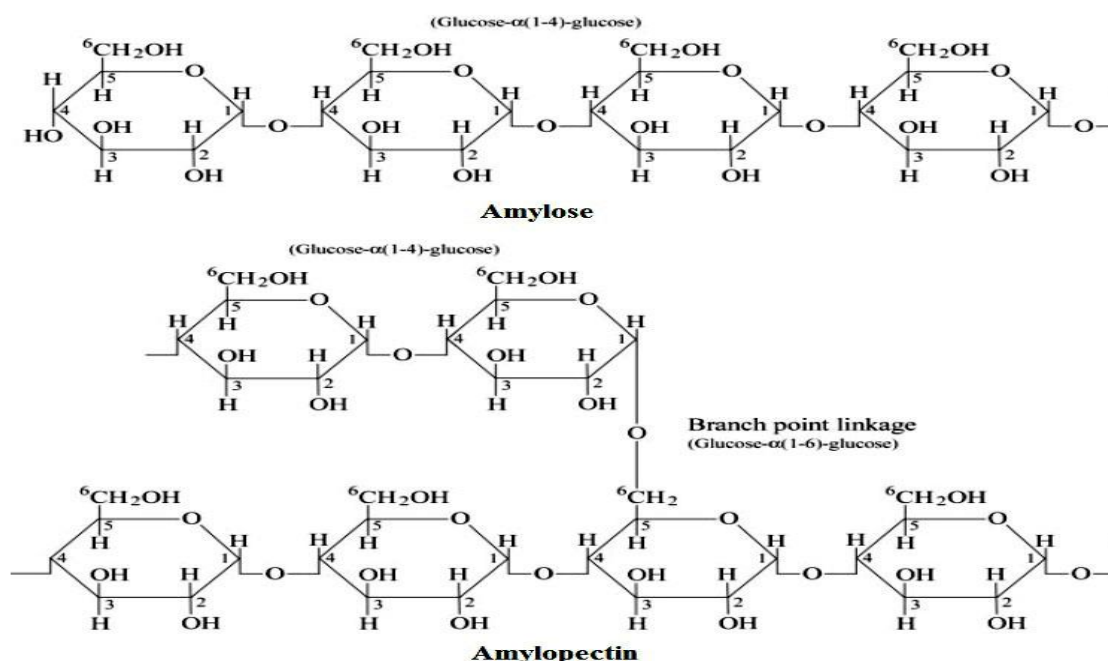


Figure 2.5 Structure of amylose and amylopectin

(Herrero-Martínez et al. 2004)

2.3.2 Starch crystallinity pattern

X-ray diffraction study revealed that the crystalline lamellae of amylopectin has three distinct crystalline patterns (Jenkins and Donald 1995). These patterns link the crystalline structure and the length of the amylopectin chains forming the clusters. Short A-chains are associated with A-type crystallinity, longer A-chains display B-type crystallinity, while intermediate-length A-chains show C-type crystallinity (Jenkins and Donald 1995). The A and B crystalline patterns are differentiated based on the packing arrangement of double helices within amylopectin and their level of hydration (Imberty and Perez 1988). The A-type is closely packed and are less hydrated, while the B-type has a more hydrated helical core (Cheetham and Tao 1998; Imberty and Perez 1988). Most cereal starches display A-type crystallinity pattern, tuber starches such as potato starch, mostly exhibit the B-type pattern, while pulse starches consist of mixtures of A and B polymorphic forms, and are categorized as C starches (Oates 1997). Starch may also exhibit the V-type diffraction pattern when complexed with lipids (Figure 2.6). X-ray diffraction studies generally reported the C-type pattern for pulse starches (Hoover et al. 2010; Kaur et al. 2010; Liu et al. 2015a). However, variations in starch crystallinity pattern have been observed for different or same pulse species (Afolabi 2012; Kaptso et al. 2014; Kawamura 1969; Sirivongpaisal 2008). For example, some authors found the A or C-type pattern for

mung bean starch (Hoover et al. 1997; Kawamura 1969; Ohwada et al. 2003) and Mexican yam bean starch (Forsyth et al. 2002). Variation in the crystalline pattern of Bambara groundnut starch have been reported for Bambara groundnut grown in different location (Afolabi 2012; Kaptso et al. 2014; Sirivongpaisal 2008). The C-type pattern (Figure 2.7) was reported for Bambara groundnut starch by Afolabi (2012), while other authors working with Bambara groundnut grown in different locations reported the A-type crystallinity (Kaptso et al. 2014; Sirivongpaisal 2008). Differences in the crystalline pattern of starches have been attributed to differences in growth conditions, growth locations, and inherent genetic differences among plant species (Agama-Acevedo et al. 2015; Bello-Perez et al. 1998; Kaptso et al. 2014; Waliszewski et al. 2003).

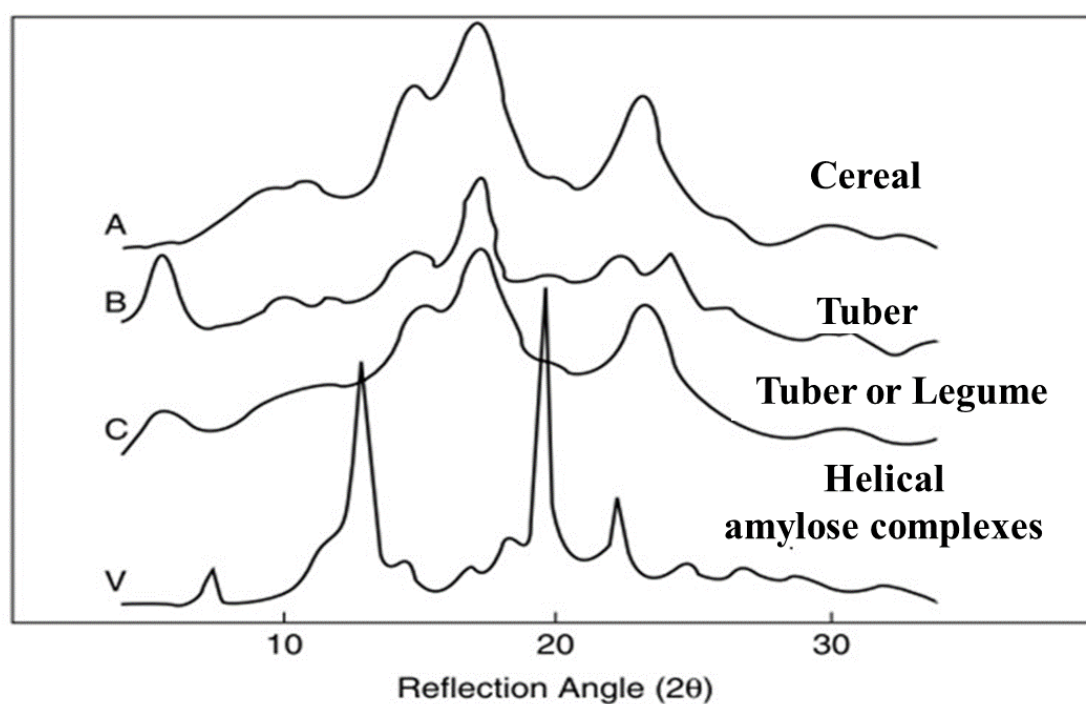


Figure 2.6 X-ray diffraction patterns for different starches A, B, C and V amylose (Cui 2005)

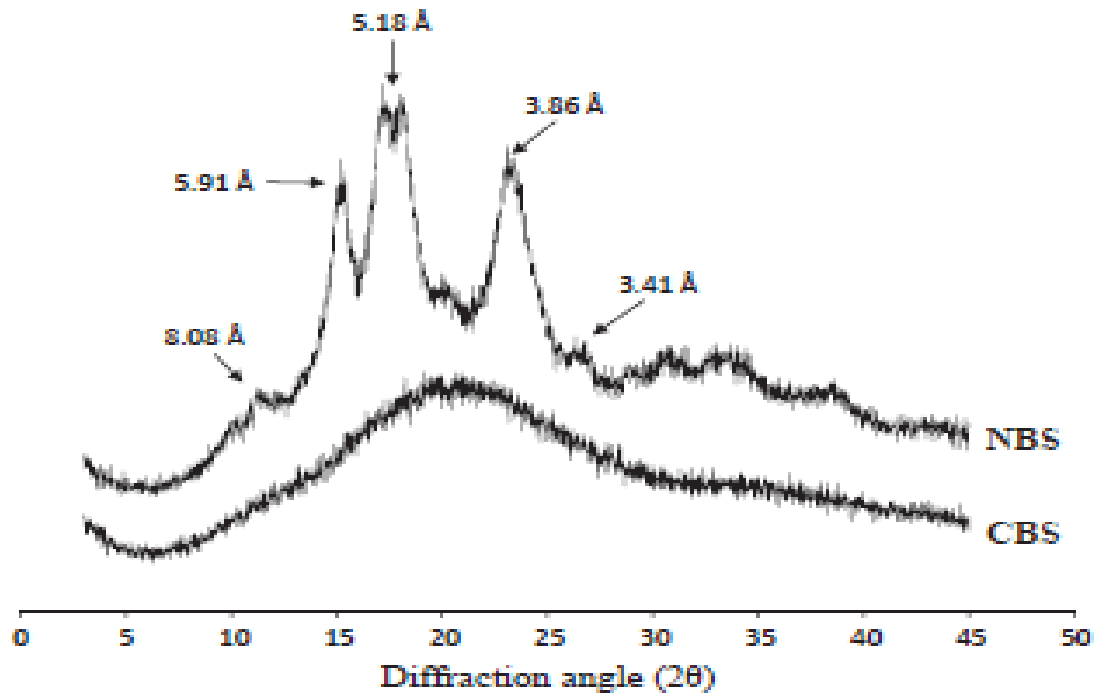


Figure 2.7 X-ray diffraction pattern of Bambara groundnut starch

(Afolabi 2012)

2.4 Starch functionality

Starch functionality is influenced by the ratio of amylose and amylopectin, the molecular structure of these two starch components and the minor components of starch (e.g. lipids). This section discusses the functional properties of pulse starches such as Gelatinisation, pasting and swelling in relation to starch composition and structure.

2.4.1 Gelatinisation

Starch Gelatinisation involves a phase change of starch granules from an ordered state to a disordered state (Hermansson and Svegmarm 1996; Hoover et al. 2010). The phase transition occurs in the presence of excess water and over a temperature range specific for starch from different origin. Starch Gelatinisation takes place in the amorphous region of starch and it is accompanied by subsequent swelling of starch granules. Other changes include loss of birefringence and crystalline order as well as dissociation of double helices and leaching of amylose into the surrounding medium (Hoover et al. 2010). Gelatinisation properties of starch have been studied using several methods, including the use of a differential scanning calorimeter. These starch properties (T_o : onset Gelatinisation temperature, T_p : peak Gelatinisation temperature T_c : conclusion Gelatinisation temperature and the enthalpy of Gelatinisation) are influenced by amylose content, botanical origin and the structure of

amylopectin. Low amylose starch is generally associated with high Gelatinisation temperature (Kaptso et al. 2016; Kaptso et al. 2014; Naidoo et al. 2015; Stevens and Elton 1971). Starch extracted from white Bambara groundnut cultivar with low amylose content (25%) reportedly showed high Gelatinisation temperature of 81.7°C compared with the black variety (77.5°C) with higher amylose content (27.8%) (Kaptso et al. 2016; Kaptso et al. 2014). However, some studies found that low amylose starch did not show high Gelatinisation temperature (Chung et al. 2008a; Joshi et al. 2013; Kaptso et al. 2016; Kaur et al. 2010; Li and Yeh 2001). Joshi et al. (2013) investigated the functional properties of starches with different amylose contents (lentil: 32.52%, maize: 24.78% and potato: 14.93%). Potato starch with the lowest amylose content displayed the lowest peak Gelatinisation temperature of 65.65°C, while maize starch showed the highest value (73.80°C). Lentil starch reportedly showed peak Gelatinisation temperature (68.32°C) which is intermediate to those of potato and maize starches. The high peak Gelatinisation temperature of maize starch was associated with the more compact granular structure and the presence of lipids which may form an inclusion complex with amylose (Singh et al. 2003). Starch extracted from cowpea varieties with significant differences in amylose contents (35.5-42.2%) was found to display similar Gelatinisation temperatures (approx. 82°C) (Kaptso et al. 2016). Nevertheless, Noda et al. (1996), working with sweet potato and wheat starches found that the T_o , T_p , T_c and the enthalpy of Gelatinisation (ΔH) are significantly influenced by the structure of amylopectin rather than amylose content. In general, 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). Huang et al. (2007) associated high Gelatinisation temperature in cowpea starch with the presence of higher amounts of long amylopectin chains.

2.4.2 Pasting

Pasting is a process that follows Gelatinisation (BeMiller 2011). During starch pasting, considerable granule swelling and leaching of amylose occurs, which contributes to the increased viscosity of starch after cooling. The pasting properties of starch is a reflection of its botanical origin as well as the composition of its major component (amylose and amylopectin). According to Hoover et al. (2010), most pulse starches display a high pasting temperature and a high setback viscosity, which could be associated with their relatively high amylose contents. Pasting temperature of Bambara groundnut starch may vary between 78 and 84°C depending on the source and variety of the grain (Adebawale et al. 2002; Afolabi 2012; Sirivongpaisal 2008). Other factors which could influence the pasting properties of pulse starches include the

presence of only trace amounts of lipid complexed amylose chains, the strong interaction between amylose–amylose and/or amylose–amylopectin chains and the molecular structure of amylose and amylopectin (Hoover et al. 2010). Huang et al. (2007), found that cowpea starch exhibited higher peak and final viscosities than did chickpea and yellow pea starches due to its higher amount of long amylopectin chains. Previous studies similarly reported that the amylose contents and the distributions of amylopectin chain length of starches from different botanical origin predominantly affected their pasting properties (Jane et al. 1999).

2.4.3 Swelling

Starch swelling involves interaction between the crystalline and amorphous regions of starch (Hoover 2001; Singh et al. 2003). Most pulse starches showed pronounced swelling only at temperatures above 70°C (Hoover et al. 2010). According to these authors, starch extracted from pulses may have amylose chains closely packed within the amorphous regions of the granule, resulting in stronger interactions through hydrogen bonding. The swelling properties of starch have been suggested to depend on the ratio of amylose to amylopectin contents of starch (Blazek and Copeland 2008; Tester and Morrison 1990a; Tester and Morrison 1990b). Differences in the swelling ability of Bambara groundnut starch have been reported by different authors, which could be due to variation in amylose contents (Adebowale et al. 2002; Afolabi 2012; Sirivongpaisal 2008). Tester and Morrison (1990b) reported that the swelling behaviour of starch is directly related to their amylopectin component. Low amylose starch would exhibit higher swelling. A study on the influence of starch structure on the swelling behaviour of wheat starches varieties, showed that amylose content and the structure of amylopectin accounted for approximately 89% of the total variation in swelling power (Sasaki and Matsuki 1998). Although higher amounts of long amylopectin chains ($DP \geq 35$) contributed to high swelling, these authors reported that amylose content had a greater influence than the chain length of amylopectin. The influence of amylose on starch swelling power may not be generalized to all starches as the study reported by these authors was on cereal starches (Sasaki and Matsuki 1998; Tester and Morrison 1990b). Furthermore, the dependency of swelling power on the amylose content of starches may vary with the botanical origin and cultivar. This seems plausible since previous researchers working with starches from different botanical origin found that swelling power may not be expressed as a function of amylose content alone (Li and Yeh 2001; Naidoo et al. 2015). Other authors found that starch from four field pea cultivars with significant differences in amylose contents showed similar swelling behaviour (Ratnayake et al. 2001). Thus, variation in the swelling power of different starches will depend on many

factors such as botanical origin, amylose content, starch granule size, the magnitude of interactions between amorphous and crystalline regions in the molecular structure of amylose and amylopectin.

2.5 Starch modification

Native starches are generally unsuitable for most industrial applications. Hence, they are modified to improve functionality and to enhance certain industrial applications. Modification of starch increases resistance towards extreme processing conditions such as high temperature and shear and may also slow down the extent and rate of starch retrogradation (D'Silva et al. 2011). Over the last decades, various starch modification processes such as physical, genetic, enzymatic, chemical have been studied (Kaur et al. 2012). Physical modification processes such as annealing and heat-moisture treatment can be safely used in starch modification, as it does not pose any major risk with regard to food safety (Kaur et al. 2012; Zavareze and Dias 2011). Other physical methods employed in modification of starch as reviewed by Kaur et al. (2012) include osmotic pressure treatment, microwave radiation, pulsed electric field treatment, multiple deep freezing and thawing cycles. Genetic modification of starch involves traditional plant breeding or the application of biotechnology to produce starch with desirable properties (Kaur et al. 2012). It involves manipulating the enzyme system of the starch biosynthetic pathway to alter the ratios of amylose and amylopectin (Hui 2006). The technology involved can produce novel starches, which may reduce the use of hazardous chemicals in starch modification (Kaur et al. 2012).

Chemical modification can be achieved by crosslinking, etherification, oxidation, esterification and grafting of starch molecules (Gao et al. 2014; Kaur et al. 2012; Kittipongpatana and Kittipongpatana 2013; Wongsagonsup et al. 2014). However, certain chemicals such as epichlorohydrin used in starch modification are reported to be unsafe in food applications (Li et al. 2009). More recently, the use of naturally occurring compounds such as amino acids (Cui et al. 2014), ionic gums (Pramodrao and Riar 2014), fatty acids (Kawai et al. 2012; Zhang et al. 2012) and lysophospholipids (Ahmadi-Abhari et al. 2013c; Cui and Oates 1999; Siswoyo and Morita 2003) are finding application in starch modification. The emergence of clean label starch technologies has further encouraged the use of naturally occurring organic compounds such as lipids in the starch modification.

2.5.1 Mechanism of starch-lipid complexation

The modification of starch with lipids results in the formation of inclusion complexes between added lipids and amylose in starch. These complexes are formed under controlled conditions of moisture, shear, and temperature. The ability of amylose in starch to form V-amylose complexes with lipids has been extensively researched (Carlson et al. 1979; Godet et al. 1993c). Amylose is believed to change its configuration from the coil to helix, which allows guest molecules to reside within the cavities of amylose helix. Starch-lipid complexes can be formed during Gelatinisation of starch in the presence of lipids or may be naturally present in starch (Putseys et al. 2010). The complex formed is known as V-amylose and has its inner surface lined with methylene groups and glycosidic linkages, resulting in a hydrophobic tube (Immel and Lichtenthaler 2000a). Methylene groups of lipids interact with the fifth hydrogen atom, of the glycosyl residues in a six-glycosyl-residue ring within the V-amylose helix through van der Waals interactions (Godet et al. 1993a). Lipids, including fatty acids, react with the hydrophobic core of the amylose helix (Raphaelides and Karkalas 1988). Starch modified with lipids has functional properties different from the unmodified counterparts. Understanding changes in the functional properties such as thermal, pasting and digestibility properties of modified starch have formed the basis of research reported in the literature.

The probable and most widely accepted mechanism of starch-lipid complexation involves hydrophobic forces that enable the transfer of a hydrophobic ligand component, such as aliphatic fatty acid chain into the hydrophobic environment within the V-amylose helix (Gelders et al. 2004). Fatty acids, such as stearic acid possess a polar head and non-polar tail (Figure 2.8). By molecular modelling (Godet et al. 1993a; Godet et al. 1993c), Raman spectroscopy (Carlson et al. 1979) and nuclear magnetic resonance (Kawada and Marchessault 2004), the polar head of the lipid i.e. the carboxylic acid group has been reported to be located outside the V-amylose due to electrostatic repulsions and steric hindrance (Figure 2.9). Different methods have been described for the formation of amylose-inclusion complexes. Putseys et al. (2010), summarized the methods into three categories: starting with starch and ligands or with amylose and ligands or synthesizing amylose in the presence of the ligands. The characteristics of the amylose-inclusion complexes formed depends on the method used (Putseys et al. 2010). Purer complexes are formed when starting with amylose and ligands or starch and ligands. The cavity (diameter) of V-amylose that forms complex with ligands are controlled by the size of the complexing agent, leading to helices with 6, 7 or 8 glycosyl residues per turn (Nuessli et al. 2003). Six-glycosyl-residues per turn are for lipids or linear

alcohols, 7 for branched chain alkyl compounds and 8 for more bulky compounds. The complexes formed are thus referred to as V6-, V7- and V8- amylose respectively (Le Bail et al. 2005). Among the V-amylose types, V-6 is the most widely studied and has greater potential in food applications.

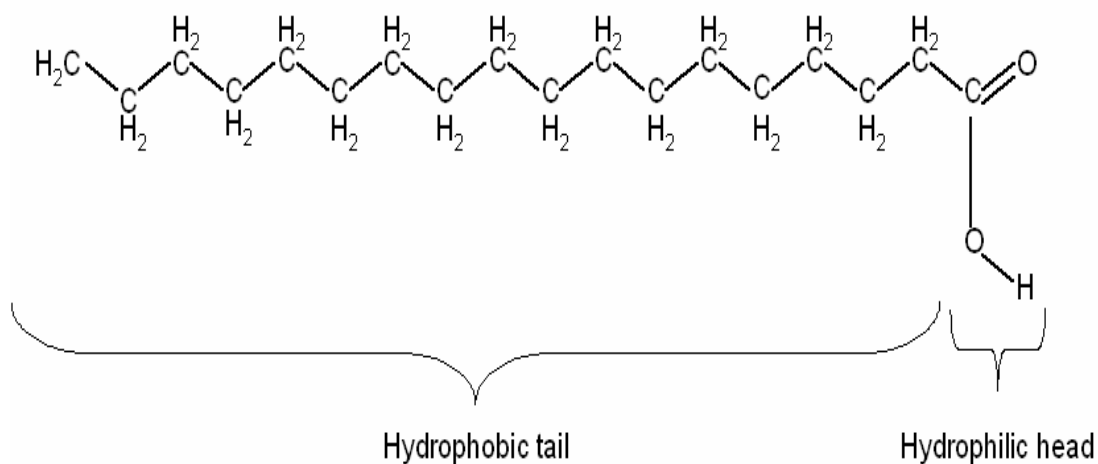


Figure 2.8 Stearic acid showing the hydrophilic carboxyl head and the hydrophobic tail.

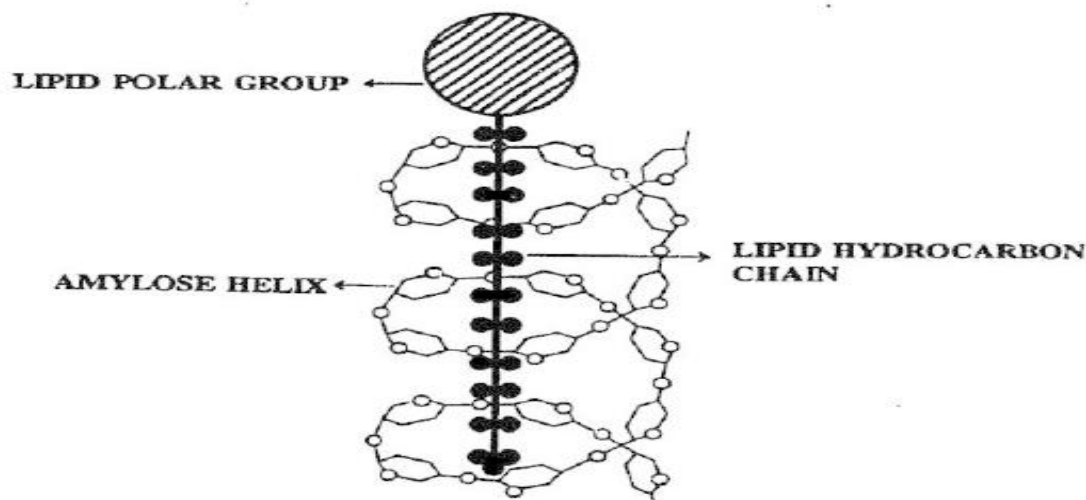


Figure 2.9 Schematic illustration of amylose inclusion complexes

(Carlson et al. 1979)

2.5.2 Methods of V-amylose preparation

Different methods of V-amylose preparation have been extensively described (Obiro et al. 2012b; Putseys et al. 2010). Obiro et al. (2012b) grouped these methods into classical, enzymatic and thermo-mechanical methods. The current study employed rapid visco-analyzer and homogenization for the preparation of V-amylose complexes. These methods are thermo-

mechanical methods and have been found to enhance the degree of complexation of starch with lipids. The increase in the degree of complexation is probably due to the simultaneous application of heat and shear (Obiro et al. 2012b). The following section discusses rapid visco-analyzer and homogenization as methods of V-amylose preparation.

2.5.2.1 Rapid Visco-analyzer

The use of rapid visco-analyzer (RVA) and amylograph instruments in the preparation of V-amylose complexes have been demonstrated in different studies (D'Silva et al. 2011; Obiro et al. 2012a; Tang and Copeland 2007). Tang and Copeland (2007) showed that V-amylose complexes can be formed between wheat starch and various lipids using a RVA. These authors pasted lipids with wheat starch directly in the RVA system without prior incubation of starch and lipid in a water bath system. X-ray diffraction of freeze-dried starch pastes revealed the formation of V-amylose complex (Figure 2.10) with peaks at $2\theta = 7.4^\circ$, 12.7° and 19.8° (Tang and Copeland 2007). Extending the pasting time of various starches with lipids using RVA has been shown to increase the degree of complex formation (D'Silva et al. 2011; Obiro et al. 2012a; Ocloo et al. 2016). D'Silva et al. (2011) found that the degree of complexation of teff starch with 0.25% stearic acid increased by approximately 83% when the holding time was increased from 5 to 120 minutes during starch pasting. According to these authors, the increase in complexation was due to prolonged interaction between the starch and the added stearic acid. Obiro et al. (2012a) also studied the occurrence of V-amylose complexes in maize or teff starch biphasic pastes i.e. peak viscosity paste as short (approx. 12 min) and prolonged (130 min) pasting times. Differential scanning calorimetry of freeze dried pastes showed that short pasting time produced type I V-amylose complexes, while maize or teff starch pasted with stearic acid for a prolonged time resulted in the formation of type II V-amylose complexes (Obiro et al. 2012a). Thus, increasing pasting time between starch and lipids can enhance the degree of complexation and may also increase the thermal stability of these complexes. Type II V-amylose complexes are reportedly more stable than type I V-amylose complexes since the former melt at higher temperatures ($> 105^\circ\text{C}$) (Raphaelides and Karkalas 1988).

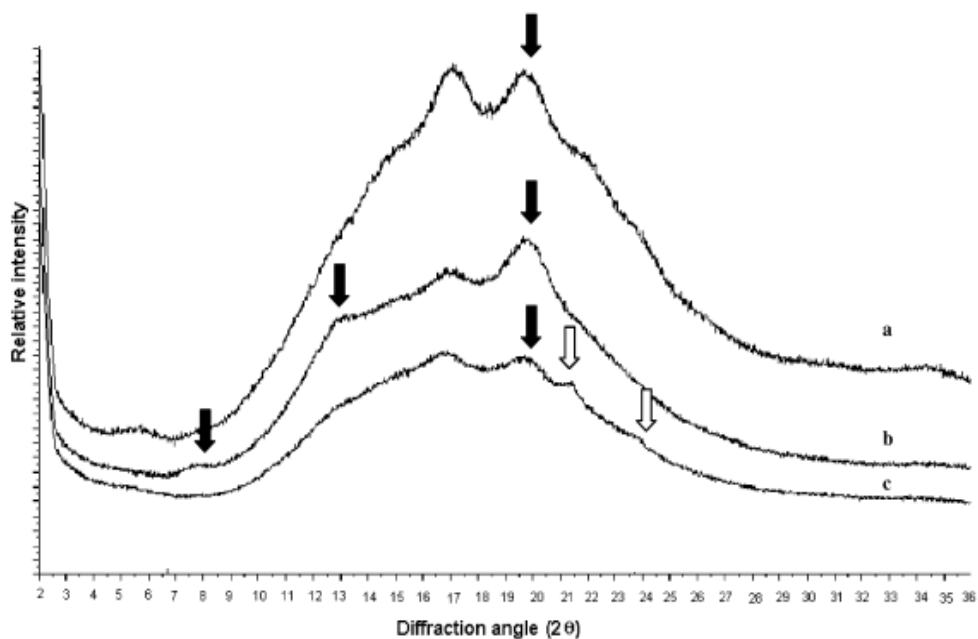


Figure 2.10 X-ray diffraction patterns of starch mixed with fatty acid

(Tang and Copeland 2007)

(a) Wheat starch mixed with 0.05 mmol stearic acid (b) Starch mixed with 0.14 mmol stearic acid (c) Peaks corresponding to amylose-lipid complexes and stearic acid micelles are designated by the filled and open arrows respectively.

2.5.2.2 High-pressure homogenization

Due to the limitations of some of the conventional methods used in the preparation of V-amylose complexes, for example, low complexation index, researchers are now focusing on methods of improving the degree of complexation. As noted above (section 2.5.2.2), extending the pasting time of starch with lipids has been found to increase the interaction between lipids and amylose in starch. The extended pasting time suggests high input of energy and more time to produce high amounts of V-amylose complex. A promising thermo-mechanical method which has been used to improve complexation of starch with lipids is the use of high-pressure homogenization (HPH). Homogenization is widely used in the industry to produce uniformity in fluids through the application of shear and turbulence. It has also been applied to enhance the degree of complexation of starch with lipids (Lesmes et al. 2008; Meng et al. 2014a; Meng et al. 2014b). Increased complexing ability of starch with lipids using HPH was attributed to two major factors. First, lipids are uniformly distributed in starch suspension as small droplets

under the influence of high-pressure, intense shear, turbulence and cavitation (Meng et al. 2014a). The second reason is that high pressure and shear conditions during HPH promotes the release of amylose from swollen starch granules (Meng et al. 2014a). Lesmes et al. (2008) developed a continuous, dual feed homogenization process for the formation of V-amylose complexes using starches differing in amylose contents. More recently, V-amylose complexes were prepared from maize starch and fatty acids with different chain length and degree of unsaturation using HPH (Meng et al. 2014a; Meng et al. 2014b). In these studies, maize starch and fatty acids were gelatinized prior to being fed into a high-pressure homogenizer. Maize starch-fatty acid complexes prepared by HPH showed higher complex index (almost double) than those prepared without HPH (Meng et al. 2014a). The complex index of the V-amylose complexes was reported to increase with an increase in homogenization pressure from 80 to 120 MPa (Meng et al. 2014b). However, the difference in complex index between 100 and 120 MPa was reportedly small (Meng et al. 2014b). This suggests that 100 MPa may be the optimum homogenization pressure for the release of amylose needed to form a complex with the dispersed lipids. The intensity of X-ray diffraction peaks, which corresponds to the formation of V-amylose complexes was found to be higher for homogenized complexes than the unhomogenized ones (Meng et al. 2014a; Meng et al. 2014b). Future studies may be required to further assess the influence of HPH on the formation of V-amylose using starches from different botanical origin. Furthermore, rheological properties of the complexed starch as well as retrogradation tendencies require further investigation.

2.5.3 Factors affecting starch-lipid complexation

The formation of V-amylose is affected by several factors such as starch type, amylose content and its molecular structure in terms of the degree of polymerization, the water content of the starch, lipid type in terms of the structure of ligand and ligand concentration (Obiro et al. 2012b).

2.5.3.1 Starch type and moisture content

Starch type and moisture content are also important factors that influence the formation of the starch-lipid complex. Derycke et al. (2005), observed that the formation of V-amylose in rice and maize starches with similar amylose contents (approx. 29%) enriched with oleic and linoleic acid was affected by the moisture content (25, 40 and 66%) of the starches. V-amylose was not formed at a higher moisture content (66%), but was formed at low (25%) and intermediate (40%) moisture contents in maize starch (Derycke et al. 2005). However, rice starch formed V-amylose at low, intermediate and high moisture contents. Chang et al. (2014),

studied the influence of heat treatment and varying moisture contents (10, 20, 30, 40 and 50%) on the formation of V-amylose between lauric acid and normal maize starch. Their findings indicated 40% moisture content as optimum for the formation of V-amylose complex. These results described above suggest that the formation of V-amylose is not only dependent on moisture content but may also depend on the starch type. It appears that relatively high moisture contents (> 40%) seem to inhibit complex formation. Derycke et al. (2005), suggested that under low moisture conditions (25 and 40%), crystalline V-amylose complexes are readily formed. The higher moisture content of starch probably hinders the starch-lipid system from attaining the activation energy required for complex formation (Jovanovich and Añón 1999). Other factors such as heating time, temperature, and shear during complexation may also influence the formation of V-amylose (Obiro et al. 2012b).

2.5.3.2 Amylose content and degree of polymerization

The amylose content of starch and its degree of polymerization have been shown to significantly influence the formation of V-amylose complexes. Starches with high amylose contents produce higher yield of V-amylose complex than those with low amylose (Eliasson et al. 1988; Ocloo et al. 2016). More importantly, the degree of polymerization (DP) seems to play a crucial role in complexation, since long amylose chains i.e. high DP has been found to produce high V-amylose yield. Godet et al. (1995) studied the formation of V-amylose complex from crystalline amylose with different degrees of polymerization (30, 40, 80 and 900) using caprylic, lauric and palmitic acids. The amount of complex formed was found to increase with an increase in the DP (Table 2.3). However, too long amylose chains may cause conformational disorder resulting in crystal defects (Gelders et al. 2004). Short chains e.g. DP of 20, on the other hand, may not form complex at all, as they are relatively too short to complex with lipid (Godet et al. 1995).

Table 2.3 Effect of degree of polymerization of amylose on complexed V-amylose yield

Fatty acid	DP 30	DP 40	DP 80	DP 900
Caprylic	56.10	62.30	85.60	95.90
Lauric	44.50	67.00	90.60	97.20
Palmitic	42.00	62.00	86.10	96.60

Adapted from (Godet et al. 1995) DP: Degree of polymerization.

2.5.3.3 Chain length and degree of lipid unsaturation

Lipid structure with regards to chain length and the degree of unsaturation may also affect the degree of complexation (Gelders et al. 2004; Gelders et al. 2006). The optimum chain length required for complex formation has been found to vary among authors. Some authors suggest that lipids with a chain length of 14 carbon atoms are ideal candidates for complex formation (Bhatnagar and Hanna 1994; Hoover and Hadziyev 1981; Krog 1971). Other studies reported 16 or 18 carbon atoms as the preferred lipid chain length (Krog 1971; Lagendijk and Pennings 1970). In general, shorter chain lipids complex better with starch than longer chains. However, some authors found that very short lipid chains, for instance, 10 or less carbon atoms appear too short to form complex with lipids (Godet et al. 1995; Lebail et al. 2000). It is suggested that shorter chain fatty acids (10 or less) are too soluble in the aqueous environment, and thus, are not properly retained in the hydrophobic helix of amylose. The relatively shorter chain glyceryl monostearate (GMS) was found to give higher complex yield than the longer chain docosanoic acid (Gelders et al. 2004). Kawai et al. (2012) also showed that lauric acid showed higher complexing ability than myristic, palmitic and stearic acids (Figure 2.11). The decrease in V-amylose yield with an increase in lipid chain length has been attributed to the activation energy needed for complex formation (Siswoyo and Morita 2003). The increased activation energy resulted from the extra energy needed to induce greater hydrophobic interactions between the ligand and the V-amylose helix (Obiro et al. 2012b; Siswoyo and Morita 2002; 2003). Beside the chain length of lipid, the degree of lipid unsaturation may also affect the degree of complexation. In general, saturated lipids show higher complexing ability with starch than unsaturated ones. Several early reports found that the complexing ability of cis-unsaturated lipids with amylose was low, resulting in low V-amylose yields (Bhatnagar and Hanna 1994; Eliasson and Krog 1985; Krog 1971; Lagendijk and Pennings 1970; Raphaelides and Karkalas 1988; Tang and Copeland 2007; Zhou et al. 2007). For example, stearic acid showed higher complexing ability with rice starch than did linoleic acid (Zhou et al. 2007). Yamada et al. (1998) reported that the less the number of double bonds in a lipid, the easier the lipid forms complex with starch. The low complexing ability of unsaturated lipids results from structural rigidity conferred by the presence of double bonds. Hence, saturated lipids are favoured over unsaturated ones in the formation of V-amylose complexes (Kaur and Singh 2000; Yamada et al. 1998). However, in some instances, the complexing ability of unsaturated fatty acids with amylose was higher than the saturated types (Annor et al. 2015; Kawai et al. 2012; Meng et al. 2014a). Karkalas et al. (1995) postulated that the double bonds in unsaturated lipids influence crystal structure more than yield. Amylose helix needs to be expanded in order

to accommodate the unsaturated portion of the lipid (Karkalas et al. 1995). Thus, differences in the complexing ability of lipids as observed in the literature suggest that the expansion of the V-amylose helix may depend on complexation conditions, amylose content and the molecular structure of amylose (degree of polymerization).

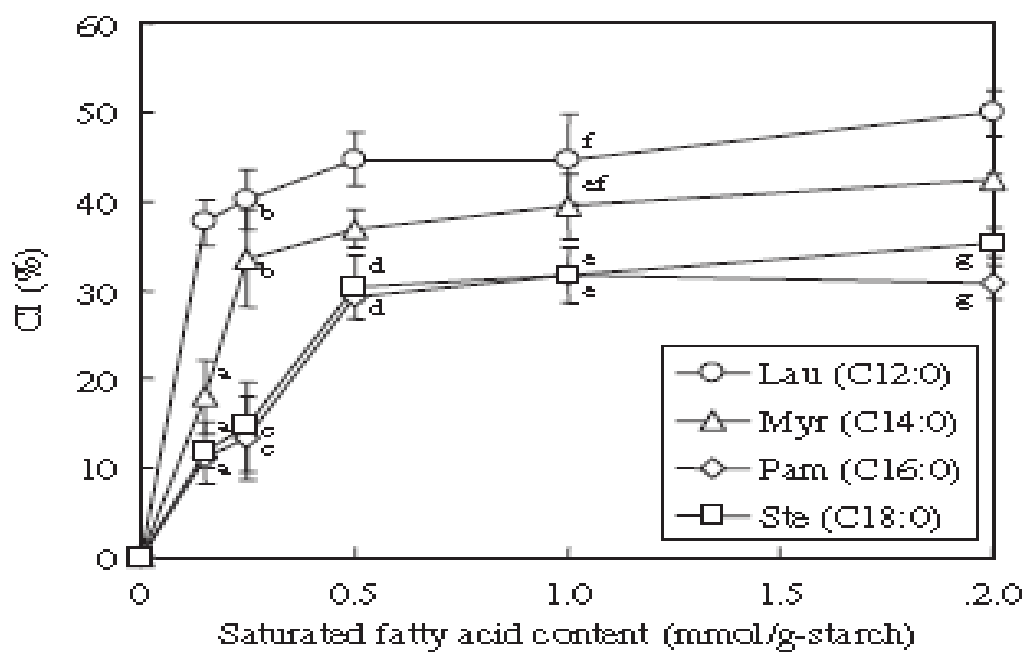


Figure 2.11 Effect of fatty acid addition on complex index of gelatinized potato starch

(Kawai et al. 2012)

Lau: Lauric acid, Myr: Myristic acid, Pam: palmitic acid, Ste: Stearic acid

2.5.4 Effect of modification on starch functionality

The modification of starch with lipids can significantly change the functional properties of starch. These changes possibly result from amylose inclusion complexes with lipids and has been found to make starches more suitable for many industrial applications. This section (2.5.4.1-2.5.4.6) discusses the influence of lipids on starch functionality. Specifically, Gelatinisation, pasting, syneresis, gel firmness and digestibility properties are reviewed.

2.5.4.1 Gelatinisation

The melting temperature (T_m) or peak Gelatinisation temperature (T_p) and melting enthalpy (ΔH_m) are important parameters that are also used to assess the degree of complexation of starch with lipids. The T_m allows for deductions to be made about the helical length of V-amylose complex, while ΔH_m provides information on the degree of order (crystallinity) in the complex and the amount of V-amylose complex formed (Kawai et al. 2012). One of the major

limitations of native starch is their poor resistance to high temperatures usually encountered during processing. The interaction of lipids with various starches is known to improve starch thermal stability. Native starches generally have been found to exhibit lower T_m values than complexed starches (Ahmadi-Abhari et al. 2013a; Biliaderis and Tonogai 1991; Obiro et al. 2012a; Ocloo et al. 2016; Wang et al. 2015b). The high T_m of V-amylose complexes is associated with the inclusion of the lipid in the amylose helix interior through hydrophobic interactions. According to Nimz et al. (2004), these interactions are dominated by hydrogen-to-hydrogen van der Waals forces between hydrogen bonded to the 3rd and 5th carbons of glucose molecules with the hydrogen from the aliphatic chain of the lipid molecule. Depending on the T_m value, V-amylose complexes may be categorized into two main forms; type I and type II V-amylose complexes (Figure 2.12). Type I V-amylose complexes may show T_m values between 80 and 104°C (Biliaderis and Galloway 1989; Raphaelides and Karkalas 1988). Type I V-amylose complexes have been reported to consist of partially ordered structure with no distinct crystalline regions (Biliaderis and Galloway 1989). Type II V-amylose complexes which appear to be more ordered and melt at higher temperatures (> 105°C) have been shown to display distinct crystalline and amorphous regions (Biliaderis and Seneviratne 1990; Karkalas et al. 1995). Type II complexes can be further classified into IIa and IIb. The formation of type II V-amylose complexes could probably result from slow nucleation followed by distinct crystal growth of type I V-amylose complexes (Biliaderis and Galloway 1989). The type II V-amylose complexes may also be formed due to annealing of type I V-amylose complexes during extended wet heat processing at elevated temperature (90°C) (Biliaderis and Galloway 1989; Karkalas et al. 1995; Tufvesson et al. 2003a; 2003b). Type I V-amylose complexes may be transformed into the more stable type II V-amylose complexes during extended heating at 100°C for 24 h (Tufvesson et al. 2003a). Type I or type II V-amylose complexes was reportedly formed when stearic acid was pasted with maize starch for short (approx. 12 min) and extended times (approx. 130 min) respectively (Obiro et al. 2012a).

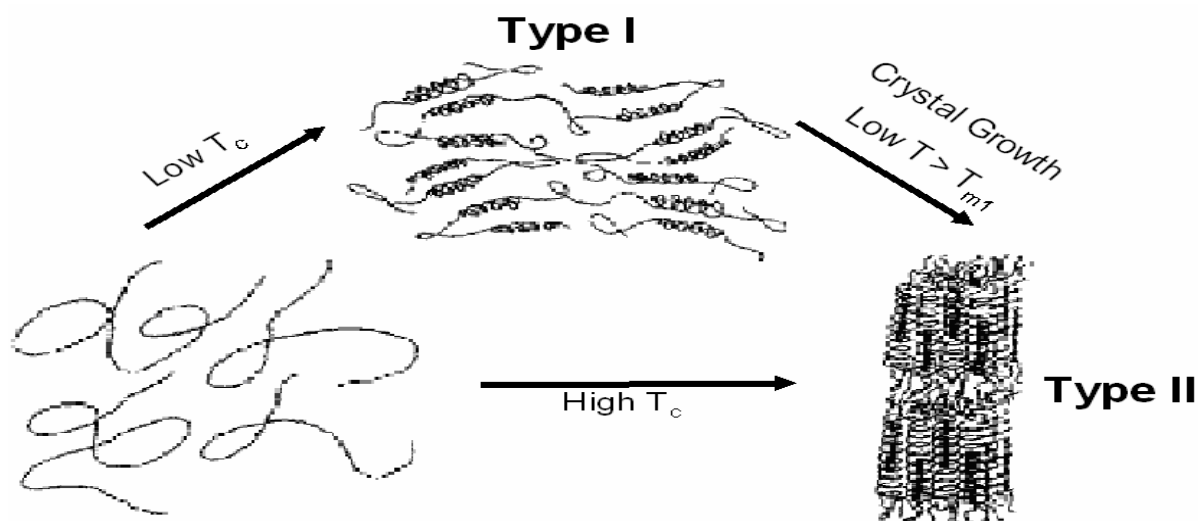


Figure 2.12 Mechanism of the formation of type I and II amylose-lipid complexes

(Biliaderis and Galloway 1989)

T_c : Temperature of crystallization T_{mI} : Temperature of melting of Type I complexes

Biliaderis and Tonogai (1991) studied the influence of different lipids; sodium dodecyl sulphate (SDS), Cetyltrimethylammonium bromide (CTAB) and lysophosphatidylcholine (LPC) at 2 or 4% on the Gelatinisation properties of starch (20-35%) gels. Native pea starch and defatted rice starch (35% w/w) complexed with lipids showed melting temperature varying between 89 and 106°C (Biliaderis and Tonogai 1991). Pea starch and defatted rice starch complexed with LPC formed type II V-amylose complexes with T_m values of approx. 106°C, while type I V-amylose complexes were formed in these starches complexed with CTAB or SDS (Biliaderis and Tonogai 1991).

The ΔH_m value of V-amylose complexes has been associated with the amount of V-amylose complex formed and the degree of order (crystallinity) in the complex. According to the report of Eliasson and Krog (1985), the contribution of the degree of order to the ΔH_m value is lower than that of the amount of V-amylose complex. Thus, high ΔH_m value may suggest the formation of more V-amylose complex (Kawai et al. 2012). The complex index which measures the extent of interaction between starch and lipids was found to correlate positively with the ΔH_m value of potato starch-fatty acid mixtures (Kawai et al. 2012). Cui and Oates (1999) similarly attributed higher ΔH_m value (3.2 J/g) of sago starch-LPC complex with high complexing ability compared with monomyristin (2.20 J/g), monopalmitin (1.38 J/g) and monostearin (0.31 J/g). Lipids with small chain length have also been found to form more

complexes than those with longer chain (Kawai et al. 2012; Meng et al. 2014a; Wang et al. 2015b). Potato starch complexed with lauric acid showed high ΔH_m value (Figure 2.13) which correlated positively with its high CI (Figure 2.11) (Kawai et al. 2012).

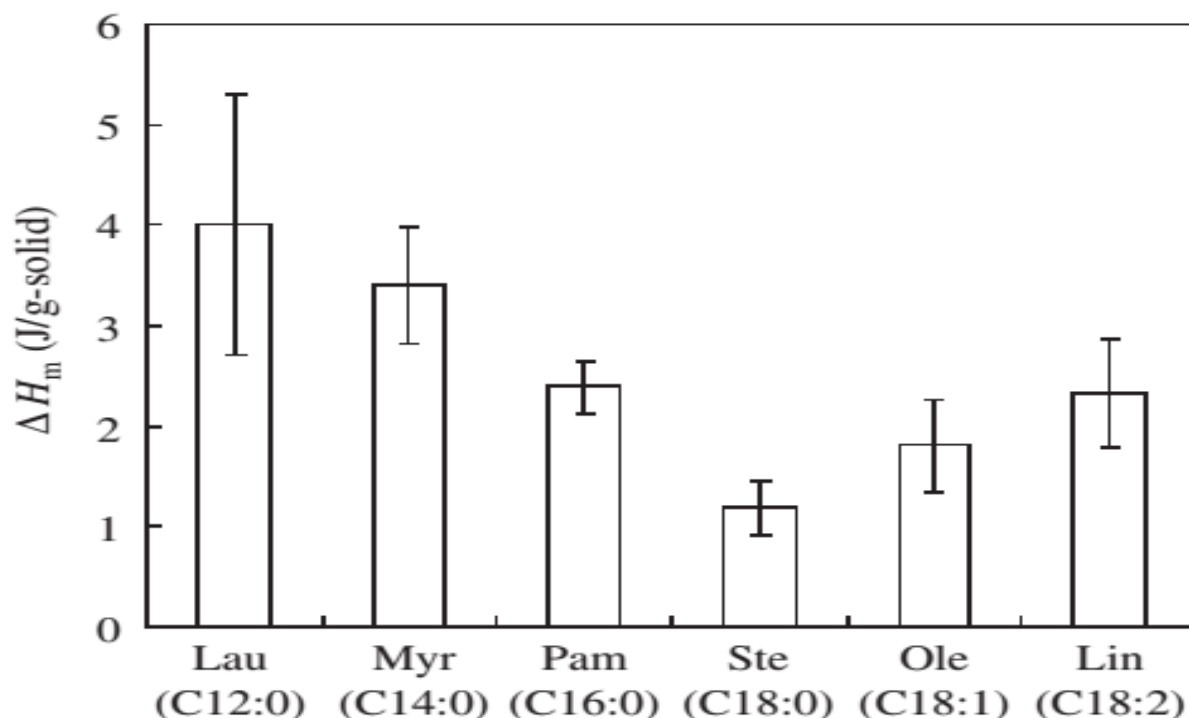


Figure 2.13 Melting enthalpy of potato starch-fatty acid mixtures at 0.50 mmol/g starch

(Kawai et al. 2012)

Lau: Lauric acid, Myr: Myristic acid, Pam: palmitic acid, Ste: Stearic acid, Ole: Oleic acid, Lin: Linoleic acid

2.5.4.2 Pasting

Starch pasting properties including peak, setback, and final viscosities are influenced by modification with lipids. During pasting, starch granules are hydrated with water followed by swelling and subsequent disintegration. These changes cause amylose to leach into the surrounding medium resulting in increased paste viscosity. However, in the presence of lipids, such as fatty acids, starch has been found to display reduced swelling with minimal amylose leaching (Krog 1971; Raphaelides and Georgiadis 2006; 2007). The RVA pasting profile for starch modified with lipids generally showed reduced peak and breakdown viscosities (D'Silva et al. 2011; Obiro et al. 2012a; Zhou et al. 2007). The reduction in viscosity of starch pasted with lipids may vary with lipid structure i.e. chain length and degree of unsaturation and heating conditions. Zhou et al. (2007) found that the modification of rice starch with 1% stearic acid resulted in a significantly higher reduction (8%) in peak viscosity compared to linoleic acid

(1%) which showed approximately 3% reduction. The reduction in peak viscosity of starch in the presence of added lipid presumably results from the formation of a V-amylose complex between starch and the added lipids. However, Kim and Walker (1992) suggested that the added lipid may also cover the starch surface with a film, increasing hydrophobicity. The film layer reduces the ability of the granule to absorb water, thus resulting in reduced peak viscosity. Nevertheless, some researchers found that fatty acid increased peak viscosity of starch, rather than reducing it (Liang et al. 2002; Wang et al. 2015b). Wang et al. (2015b), studied the complexing ability of normal and waxy wheat starches with different fatty acids, lauric, myristic and palmitic acids. Wheat starch pasted with palmitic acid showed the highest reduction in peak viscosity compared to lauric and myristic acids (Wang et al. 2015b). However, while waxy wheat starch pasted with these fatty acids displayed reduced peak viscosity, the peak viscosity of normal wheat starch pasted with lauric acid increased slightly by 2% (Wang et al. 2015b). Similar results were reported by Liang et al. (2002), who also found varied effects of different lipids on the peak viscosities of isolated or commercial rice starch. While the addition of some lipids increased the peak viscosities of rice starches, other lipids resulted in reduced peak viscosity (Liang et al. 2002). Differences in the peak viscosity results of starch in the presence of lipids, may thus depend also on starch type and the complexation conditions.

The final viscosity of starch generally increased with lipid modification (D'Silva et al. 2011; Maphalla and Emmambux 2016; Obiro et al. 2012a; Wang et al. 2015b). The addition of lauric, myristic and palmitic acids increased the final viscosity of normal and waxy wheat starches (Wang et al. 2015b). Lauric acid, a shorter chain fatty acid, reportedly showed significantly higher increase in final viscosity than myristic and palmitic acids (Wang et al. 2015b). Liang et al. (2002), studied the effect of three categories of lipids i.e. free fatty acids, glycerolipids and phospholipids on commercial and isolated rice starches. All the studied lipids increased the final viscosity of the rice starches. Among the lipids, monopalmitin brought about the highest increase in final viscosity, which could be associated with its short chain length (Liang et al. 2002). In the category of free fatty acids, palmitic acid showed the highest increase in final viscosity, while no particular trend was observed for the unsaturated fatty acids (Liang et al. 2002).

Setback viscosity of starch is also usually affected by lipid modification. Setback is associated with the extent of starch retrogradation and firming tendency of starch gels. A higher rate of

retrogradation is expected to occur when amylose is free to associate into crystallites (Liang et al. 2002). When starch is pasted with lipids, the amylose molecule which is responsible for short term starch retrogradation, forms V-amylose complex. The formation of this complex prevents or reduces the extent of amylose re-association during storage. The lipid prevents or slows down the formation of gel network during storage (D'Silva et al. 2011; Richardson et al. 2004). The setback viscosities of different starches have been found to generally reduce when pasted with lipids (Liang et al. 2002; Zhou et al. 2007). The reduction in setback viscosities, which is associated with retrogradation, was the basis for using emulsifiers in the production of foods such as bread, to delay retrogradation and maintain softness (Liang et al. 2002). Furthermore, the time to reach peak viscosity also increased in the presence of lipids. The reduced rate of swelling was also suggested to be responsible for the increased time taken to reach peak viscosity for maize starch (Raphaelides and Georgiadis 2006; Zhou et al. 2007). Differences in starch pasting properties such as peak, final and setback viscosities in the presence of lipids, could be attributed to a number of factors, such as the botanical source of the starch and its amylose content, lipid type in term of chain length, degree of unsaturation and on the complexation conditions such as pasting time and temperature.

2.5.4.3 Syneresis

Starch syneresis refers to the release of water from a firm gel due to retrogradation. Starch retrogradation has been widely studied by many researchers, mainly due to its detrimental effect on the sensory and storage qualities of many starchy foods (Wang et al. 2015a). Starch retrogradation may be desirable in certain food products such as in breakfast cereals due to structural and sensory changes (Karim et al. 2000). Further, starch retrogradation has also been found to be desirable in terms of nutrition due to the slower rate of starch digestion (Copeland et al. 2009; Wang and Copeland 2013; Wang et al. 2015a). Changes in the starch structure during storage have been found to depend on several factors such as starch source, the amylose content of starch, storage temperature and the presence of lipids. Lipids are generally known to slow down the rate and extent of starch retrogradation, by preventing crosslinking of starch molecules, formation of double helices and junction zones. These changes, according to Becker et al. (2001), limits the mobility of amylose due to the formation of inclusion complexes. Singh et al. (2002), investigated the extent of syneresis in maize and potato starches complexed with fatty acids of different chain length. The syneresis rate of maize and potato starches decreased with increasing fatty acid concentration. With or without added lipids, the syneresis rate of potato starch was generally higher than that of maize starch (Singh et al. 2002). Differences in

syneresis rate were attributed to the more fragile nature of potato starch granules and the variation in amylose contents of these starches (Singh et al. 2002). Takahashi and Seib (1988) similarly found that the addition of LPC can reduce the rate of firming and gelling of wheat starch gels when stored above freezing temperature.

2.5.4.4 Rheology

Dynamic rheological properties have been employed along with steady shear rheological properties to understand the structure of gelatinized starch (Clark and Ross-Murphy 1987). Depending on the frequency sweep data, dispersions including those obtained from starch can be classified into dilute, concentrated solution, a weak or strong gel (Clark and Ross-Murphy 1987). The dynamic rheological properties of starch may be influenced by starch concentration, lipid type and concentration. Biliaderis and Tonogai (1991), found variations in the rheological properties of concentrated starch (20-35%) complexed with different lipids. At 20% or 35% starch concentration, sodium dodecyl sulphate (SDS), Cetyltrimethylammonium bromide (CTAB) and lysophosphatidylcholine (LPC) at 2% decreased the elastic properties (G') of garbanzo bean starch gel (Biliaderis and Tonogai 1991). However, SDS, CTAB and LPC showed varied effects on the G' of pea starch gels. All the lipids increased the G' of pea starch gel (35%), but only SDS and CTAB at 2% were found to decrease the G' of pea starch gel at 20%. Singh et al. (2002) also found that myristic and stearic acids had a varied effect on the G' of maize and potato starches heated from 20°C to 85°C. While the addition of myristic acid decreased the G' of maize and potato starches, the addition of stearic acid decreased the G' of maize starch and increased the same parameter in potato starch (Singh et al. 2002). The variation in the rheological responses of these starches was attributed to differences in starch granules rigidity, amylose content and the phosphorus contents (Singh et al. 2002). Ahmadi-Abhari et al. (2015) who investigated the influence of LPC on wheat starch (6%) gel prepared at different gelation phases found that the G' of wheat starch gel significantly decreased during storage. The reduction in G' of wheat starch gel was attributed to the restriction of starch granules swelling by LPC (Ahmadi-Abhari et al. 2015). Thus, increase or decrease in the visco-elastic behaviour of starch gels prepared in the presence of lipids will vary with the starch source, amylose contents, lipid type, starch concentration and Gelatinisation temperature. It is also possible that the chain length of amylose in the starch may also influence the rheological properties of the resulting starch gel as reported in previous studies (Kweon et al. 1993; Won et al. 2000).

2.5.4.5 Gel firmness

The firmness of starch gel is highly dependent on the formation of junction zones. During storage of starch paste, amylose and amylopectin chains realign as the cooked starch paste cools to form a gel. The linear amylose chains, as well as the linear part of amylopectin presumably forms cross-links through hydrogen bridges (Wang et al. 2015a). Amylose chains may also form double helices and junction zones which enhance the formation of firm gel structure. These junction zones consist of segments of opposite starch chains that have interacted with each other and form a three-dimensional gel network structure (Whistler and BeMiller 1997). Studies by Raphaelides (1992) found that gels made from potato starch modified with myristic, palmitic and stearic acids in alkaline solution (0.01 M KOH) had reduced firmness. Whittam et al. (1986) similarly reported that addition of the fatty acids resulted in low gel firmness compared to unmodified starch gels. According to Raphaelides (1992), if the amylose helices are saturated, the repulsions between adjacent helices are strong enough to completely inhibit gel formation. Previous studies found that extended pasting of teff or maize starches with 1.5% stearic acid produced non-gelling paste (D'Silva et al. 2011). The similar non-gelling behaviour of potato starch paste with a high concentration of emulsifier has also been reported (Richardson et al. 2004). According to these authors, the added emulsifier resulted in the formation of thick opaque pastes, which had no distinct network. Hence, the gelling or non-gelling behaviour of gelatinized starch suspensions in the presence of lipids will depend on lipid type and concentration and possibly the starch concentration.

2.5.4.6 Starch digestibility

Lipid addition to starch has been found to significantly reduce starch digestibility (Cui and Oates 1999; Guraya et al. 1997; Zhang et al. 2012). In general, starch-lipid complex is more resistant to digestive enzymes than uncomplexed starch (Putseys et al. 2009). Reduction in starch digestibility presumably results from the formation V-amylose complexes, which has some resistance to enzymatic hydrolysis (Sievert and Wuesch 1993). In-vitro digestibility studies of potato starch complexed with different fatty acids at 0.05 mmol/g-starch found a slight reduction in the hydrolysis rate of complexed starches (Kawai et al. 2012). The decrease in susceptibility of complexed starch to enzymatic hydrolysis may vary with the lipid type in terms of concentration and structure. For example, non-waxy rice starch complexed with long chain (\geq C:18) saturated lipids exhibited reduced digestibility (in-vitro) than short-chain (<C:18) saturated and unsaturated lipids at the same concentration (Guraya et al. 1997). Approximately 33% reduction in digestibility was reported for complex formed between

saturated lipids and rice starch, while most of the unsaturated lipids showed 10% reduction in starch digestibility (Guraya et al. 1997). Cui and Oates (1999), also found that starch complexed with longer chain monoglycerides showed a higher reduction in starch hydrolysis than the shorter chain ones. According to Lesmes et al. (2009), starch complexed with saturated and longer lipid chain have higher resistance to enzyme hydrolysis. Lipid concentration has also been found to influence the extent of digestibility of complexed starch. Kawai et al. (2012) found that the extent of starch hydrolysis reduced further when the fatty acid concentration was increased from 0.15 mmol/g-starch to 0.50 mmol/g-starch. Wheat starch hydrolysis was also found to decrease with increasing concentrations of LPC from 0.5 to 5% (Ahmadi-Abhari et al. 2013b). It is however reported that, although the hydrolysis of complexed starch occurs at a much slower rate than uncomplexed starch, both forms are finally digested in-vivo (Holm et al. 1983). This suggests that V-amylose complexes and resistant starches are different entities, with the former being more susceptible to enzymatic hydrolysis than the latter (Czuchajowska et al. 1991).

2.6 Application of starch in bioplastics

There is a growing demand for environmentally friendly materials such as edible bioplastics. Bio-based materials are promising renewable feedstocks that are theoretically recyclable and have a production process which is more energy efficient than petroleum-based plastics (Álvarez-Chávez et al. 2012). The most promising natural polymer that has been widely used in biodegradable film (BDF) production is starch. It has an attractive combination of availability, price, performance and good film-forming ability (Wilhelm et al. 2003). The use of edible BDF in the food industry seems to be on the increase due to their wider application in extending the shelf life of foods by preventing changes in aroma, colour, texture, taste or handling characteristics (Tharanathan 2003). Edible BDF made from native starches generally have poor mechanical properties, provide a minimal barrier to moisture and are very brittle.

2.6.1 Preparation of edible starch-based films

Edible starch films (ESF) can be prepared from starch or its amylose and amylopectin components (Jiménez et al. 2012a). According to Bertuzzi et al. (2007), amylose is responsible for the film-forming capacity of starch-based films. Starch films are made by casting and drying gelatinized starch (wet method) or via thermoplastic processing also known as the dry method (Paes et al. 2008). However, the use of pre-gelatinized starch in the production of starch films has also been reported (Pagella et al. 2002). The wet method of preparing ESF is the most widely studied. In this method, starch granules are disrupted through Gelatinisation in excess

media usually water (>90% w/w), where they undergo an irreversible transition (Carvalho 2008). The entire process can be summarized into Gelatinisation, homogenization of the mixture, casting and drying. The processing conditions including Gelatinisation temperature, homogenization speed, and time, drying temperature and time reported for making starch films vary with authors (Jiménez et al. 2012a). For example in the preparation of ESF from maize starch, a temperature-time combination of 60°C for 8 h was reported (Garcia et al. 2000). Lower temperatures such as 25, 40 and 48°C for a relatively shorter time has also been used (Cano et al. 2014; Ortega-Toro et al. 2014). The homogenization step in film preparation is very important and can be avoided depending on the components in the film-forming dispersion (Jiménez et al. 2012a). However, when immiscible components such as lipids are added, the homogenization step is necessary. This will ensure the formation of a stable emulsion and adequate integration of all the components (Jiménez et al. 2012a). The drying step is the last stage in film preparation. According to Paes et al. (2008), there is no standard method for obtaining starch films with the required physicochemical properties. Hence, preliminary investigations may be necessary during film preparation.

2.6.2 Effect of lipids on physicochemical properties of edible starch based films

Starch-lipid interaction has an impact on the physicochemical and mechanical properties of edible starch films (ESF). The addition of lipid can reduce tensile strength, improve barrier properties to water vapour and oxygen (Jiménez et al. 2010a; Jiménez et al. 2012b; Ortega-Toro et al. 2014). The following sections (2.5.2.1-2.5.2.5) review studies on physicochemical properties of starch films modified with lipids.

2.6.2.1 Microstructural properties

Scanning electron microscope has been used to study the interaction between lipids and starch molecules in film matrices. Jiménez et al. (2012b) studied the effect of lipid structure on the microstructural properties of maize starch films. Fractured films containing fatty acids generally showed a rough surface, when compared with unmodified films, which showed a continuous and smooth surface (Figure 2.13). Saturated fatty acids (SFA), palmitic and stearic acids at 15% (starch weight) were reportedly more finely distributed in the starch matrix than oleic acid, which is unsaturated (Jiménez et al. 2012b). The films modified with oleic acid further showed several voids on the surface. The better distribution of SFA in the starch matrix suggest that the film-forming starch dispersion complexed better with SFA than the unsaturated fatty acids (Jiménez et al. 2012b). The presence of a double bond in oleic acid presumably

affected the formation of V-amylose complex. Films containing oleic acid showed voids on the surface which were attributed to molecular self-association in the initial dispersion phase (Jiménez et al. 2012b). Jiménez et al. (2013) similarly observed voids in maize starch films made with oleic acid. However, Schmidt et al. (2013) attributed the presence of voids in cassava starch film to bubbles formed during the homogenization process, rather than poor dispersion of fatty acids. The films were modified with 4% stearic acid (starch weight) and homogenized for 10 minutes at 12000 rpm prior to casting (Schmidt et al. 2013). The variation in homogenization time and possibly the absence or presence of vacuum during homogenization may have accounted for the voids in the stearic acid films. Further, high homogenization speed has also been found to result in films with irregular and rough surfaces (Garcia et al. 2000).

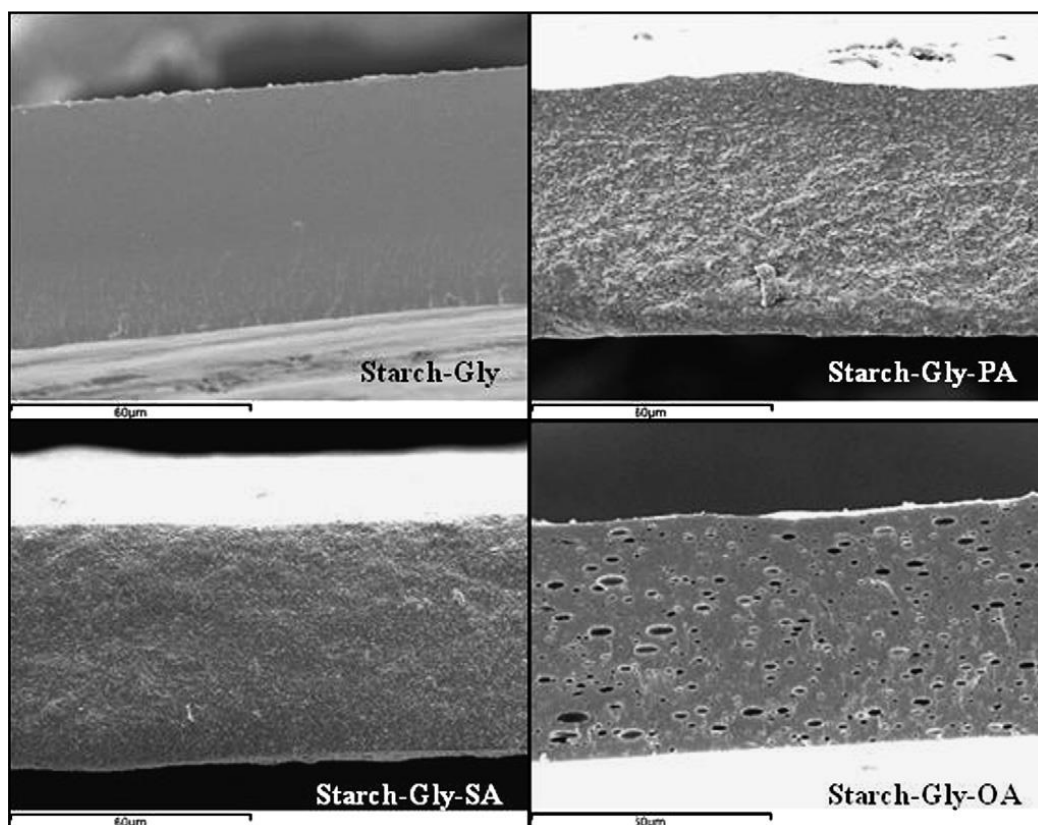


Figure 2.14 SEM micrographs of the cross-sections of maize starch-fatty acid films (Jiménez et al. 2012b)

2.6.2.2 Mechanical properties

The mechanical properties such as tensile strength (TS), elastic modulus (EM) and elongation at break (EB) of ESF have been found to be greatly influenced by lipid addition (Chen et al. 2009; Jiménez et al. 2012b; Ortega-Toro et al. 2014). In general, the TS, EM and EB of ESF

have been found to show significant reductions following lipid addition (Chen et al. 2009; Jiménez et al. 2012b; Ortega-Toro et al. 2014). The degree of unsaturation and chain length of lipid may influence the mechanical properties of ESF. For example, Jiménez et al. (2012b) found that oleic acid addition at 15% concentration substantially reduced the TS of maize starch film by approx. 64%, while palmitic acid and stearic acid resulted in 26% and 10% reduction respectively. Reduction in TS was associated with discontinuity introduced by lipids into starch matrix leading to a reduction in cohesive forces within starch molecules (Jiménez et al. 2012b). However, fatty acid addition was reported to increase the TS of sweet potato starch film (Liu et al. 2015b). Sweet potato starch-fatty acid films were prepared by heating 2% fatty acid (starch weight), 33% glycerol (starch weight) and potato starch (3% w/v) at 90°C for 90 min. The TS of potato starch-fatty acid films decreased with increasing fatty acid chain length (Liu et al. 2015b). According to these authors, the smaller size of short chain fatty acids allows for ease of incorporation into the polymer chains and enhances the strength of the starch films (Liu et al. 2015b). Unlike the study of Liu et al. (2015b), Jiménez et al. (2012b) prepared maize starch-fatty acid film by heating maize starch (2%) glycerol (25%) and fatty acids (15%) at 95°C for 30 minute. Thus, differences in the mechanical properties of starch-based films seem to depend on the starch botanical source, the composition of the film such as starch-to-plasticizer ratio and the heating conditions i.e. Gelatinisation temperature and time. Further, the pre-processing step such as adding starch to lipids which were previously dissolved in organic solvents and shaking the mixture under controlled conditions prior to heating as reported by Liu et al. (2015b), may also influence starch mechanical properties.

2.6.2.3 Water vapour permeability

Lipids are generally known to improve the barrier properties of starch films to water vapour. The reduction in water vapour permeability (WVP) of biopolymer films in the presence of different lipids has been documented for maize starch (Jiménez et al. 2010b; Jiménez et al. 2012b; Ortega-Toro et al. 2014), cassava starch (Schmidt et al. 2013) sweet potato starch (Liu et al. 2015b) and other hydrocolloids such as sodium and calcium caseinates (Fabra et al. 2010). Maize starch film with added fatty acids, displayed significantly lower water vapour permeability (WVP) as compared to the control film (Jiménez et al. 2012b). Saturated fatty acids (SFA) reportedly showed a higher reduction (25%) in WVP than unsaturated fatty acids (UFA) which showed approximately 12% reduction (Jiménez et al. 2012b). The reduction in WVP has been associated with increased hydrophobicity of the films due to the added lipids (Ayranci and Tunc 2001). Lipids presumably cover the starch surface with a film restricting

the flow of water through the starch film (Kim and Walker 1992; Liu et al. 2015b). The reduction in WVP of edible starch films may vary with lipid chain length and starch type. Some authors found that WVP of sweet potato starch film reduced as the chain length of fatty acids increased (Liu et al. 2015b). The addition of 2% arachidic acid resulted in greater reduction in WVP of sweet potato starch films compared to lauric, myristic, palmitic and stearic acids (Liu et al. 2015b). However, Jiménez et al. (2012b), observed no significant differences in WVP of films modified with palmitic acid or stearic acid. Thus, variation in WVP of starch films modified with lipids will depend on the starch source, the degree of lipid unsaturation, lipid chain length and structural rearrangement of lipid in the starch films. Further, the differences in WVP of starch films may also depend on the lipid concentration. Garcia et al. (2000) observed that by increasing the sunflower oil concentration above the optimum concentration of 2% (w/v starch), the WVP of commercial maize and high amylose maize starch films increased. The increase in WVP of the starch films was associated with migration of oil and decrease of the crystalline-amorphous ratio (Garcia et al. 2000). It is possible that excess lipid in the starch-lipid-water suspension self-associate into micellar structures as previously reported (Tang and Copeland 2007).

2.6.2.4 Thermal properties

The melting temperature (T_m) and enthalpy (ΔH_m) are two parameters used to describe the thermal properties of starch films (Garcia et al. 2000; Liu et al. 2015b). The T_m provides information on the dissociation temperature of the starch film and could be used to explain possible differences associated with starch-lipid complexation (Jiménez et al. 2013). Regardless of lipid type, the T_m of starch film containing lipid is generally higher than a film without lipid. A study on melting properties of films prepared from sweet potato starch and five saturated fatty acids revealed higher T_m (184.3-191.8°C) for lipid-modified films compared to the control film without lipid (182.7°C) (Liu et al. 2015b). This was associated with the possible formation of V-amylose complex between starch molecules and the fatty acids (Liu et al. 2015b). Storage conditions such as the water activity (a_w) of the films could also influence the thermal stability of the lipid-modified starch film. Jiménez et al. (2013), studied the influence of varying water activities (0, 0.53, 0.68 and 0.75) on melting properties of maize starch film modified with palmitic, stearic and oleic acids. According to their findings, neither the addition of fatty acid to starch matrix nor the water activities caused significant changes in the melting endotherm of the film. However, depending on the water activity of the film, the ΔH_m values differed significantly for a given fatty acid. Low a_w ($a_w < 0.68$) favoured

the formation of starch-lipid complex. High a_w ($a_w > 0.68$) provides greater molecular mobility which could result in the destruction of the complexes due to the predominance of starch chain hydration (Jiménez et al. 2013). Thus, variation in the formation and thermal stability of starch film containing lipids could be linked to differences in the degree of unsaturation of the fatty acid, which could result in different degrees of complexation. This seems plausible since previous studies reported that palmitic and stearic acids (saturated fatty acid) were more finely distributed in the starch matrix and gave a more homogeneous network than monounsaturated oleic acid (Jiménez et al. 2012b). This was associated with better complex formation between lipid and amylose.

2.6.2.5 Water Solubility

The water solubility of biofilm is primarily related to the hydrophilicity/hydrophobicity and the structure of the various components (Davanço et al. 2007). Depending on lipid concentration, the solubility of starch film in water may increase or decrease. Schmidt et al. (2013) studied the water solubility of cassava starch film containing varying concentrations of stearic acid. The solubility of cassava starch films containing 5% or 10% stearic acid was lower than the control film, while films containing higher concentrations (15% or 20%) of stearic acid, showed much higher water solubility than the control film (Schmidt et al. 2013). The lower value for film solubility in water, at low stearic acid concentrations, was associated with the greater interaction between components of the starch film. The solubility of starch film in water is very important especially when the films are intended for consumption together with products and may be an important factor that determines biodegradability when used as a packaging wrap (Bourtoom and Chinnan 2008).

2.7 Bambara groundnut starch modification

Previous studies on Bambara groundnut starch modification mainly involved annealing (ANN), heat-moisture treatment (HMT) (Adebowale and Lawal 2002), carboxymethylation (Afolabi 2012), oxidation and acetylation (Adebowale et al. 2002). Adebowale and Lawal (2002) found that ANN and HMT significantly reduced the swelling power and solubility of Bambara groundnut starch. However, reports on chemical modification of Bambara groundnut starch did show that changes in starch functionality may vary with the chemical used. For example, while hypochlorite oxidation significantly reduced the swelling ability of Bambara groundnut starch (Adebowale et al. 2002), acetylation (Adebowale et al. 2002) and carboxymethylation (Afolabi 2012) both resulted in increased swelling power. The increase in

swelling power after carboxymethylation was due to the incorporation of carboxyl group into Bambara groundnut starch. This resulted in a loss of crystallinity (confirmed by the X-ray diffraction) and the subsequent increase in the amorphous region that is hydrophilic (Afolabi 2012). Pasting properties such as setback viscosity of modified Bambara groundnut starch generally showed that physical and chemical modification can reduce retrogradation tendencies (Adebowale et al. 2002; Afolabi 2012). Due to the health and safety concerns arising from the use of chemicals such as hypochlorite, the modification of Bambara groundnut starch using lipids presents a more viable alternative. Furthermore, the reported studies on Bambara groundnut starch modification concluded that chemically modified Bambara groundnut starch may be suitable as a biodegradable polymer in the production of superabsorbent hydrogels and agricultural mulch, rather than in food applications (Afolabi 2012). Hence, alternative modification methods, such as the use of lipids, may be more promising for improving the functionality of Bambara groundnut starch.

2.8 Conclusions

The physicochemical characterizations of starch obtained from Bambara groundnut grown in Southern Africa has not been established. In addition, the influence of lipids on complexation and functionality of Bambara groundnut starch as well as the use of Bambara groundnut starch-lipid complex in the production of biodegradable films, is yet to be investigated. The review of literature showed that the effect of lipid modification on starch functionality varies with the starch source, amylose content, lipid type and complexation conditions. The relatively high amylose content of Bambara groundnut starch makes it suitable for complexation with lipids. Knowledge of the physicochemical properties of Bambara groundnut starch modified with lipids is, therefore, important to enhance the utilisation of Bambara groundnut starch in various food applications.

2.9 Aim, hypothesis and objectives

2.9.1 Aim

The aim of this research was to modify Bambara groundnut starch with lipids for improved functionality, utilisation and application.

2.9.2 Hypotheses

1. Bambara groundnut of different genotypes will show differences in starch composition e.g. amylose content, which will in turn influence starch functionality such as restricted swelling (Tester and Morrison 1990b). Starch extracted from Brazilian maize genotypes reportedly

showed higher amylose contents than starch from maize landraces (Uarrotta et al. 2013). Bambara groundnut genotypes and landraces will show the C-type diffraction pattern commonly found in legume starches (Afolabi 2012; Hoover et al. 2010).

2. Modification of Bambara groundnut starch with fatty acids and lysophosphatidylcholine (LPC) will reduce the peak viscosity and improve thermal stability due to a reduction in the rate of granule swelling and disruption (Richardson et al. 2004; Zhou et al. 2007). The digestibility of starch-lipid complexes may reduce because the amylose structure will change in the presence of lipids from a coil to a single helix which restricts access to digestive enzymes (Ahmadi-Abhari et al. 2013b; Kawai et al. 2012). Bambara groundnut starch will complex better with fatty acids than with LPC because fatty acids exist in a free form as compared to LPC, which has a complex structure with a glycerol backbone, which may introduce steric hindrance.

3. The use of high-pressure homogenization (HPH) will increase the complexation of Bambara groundnut starch with fatty acids or LPC. Maize starch complexed with different fatty acids using HPH showed a higher degree of complexation than unhomogenized samples (Meng et al. 2014a; Meng et al. 2014b). HPH promotes greater dispersion of lipids in starch suspension and enhances better complexation of starch with lipids (Meng et al. 2014a; Meng et al. 2014b).

4. Film produced using Bambara groundnut starch-lipid complex will have improved barrier properties to water vapour (Bertan et al. 2005; Jiménez et al. 2010b; Jiménez et al. 2012a). Incorporation of fatty acids to starch films have been reported to improve barrier properties to water vapour due to increased film hydrophobicity (Bertan et al. 2005; Jiménez et al. 2012b).

2.9.3 Objectives

1. To determine the physicochemical properties of starches from Bambara groundnut genotypes and landraces.
2. To determine the effect of lipid addition on physicochemical properties of Bambara groundnut starch and to investigate the in-vitro digestibility of the complexed starches.
3. To determine the influence of HPH and lipid type on complex formation and physicochemical properties of Bambara groundnut starch-lipid complexes and also to investigate changes in the dynamic rheological properties of the modified starch.
4. To investigate the application of the modified Bambara groundnut starch in biofilm production.

CHAPTER THREE

3.0 Physicochemical properties of starches with variable amylose contents extracted from Bambara groundnut genotypes

Abstract

The physicochemical properties of starches extracted from five Bambara groundnut genotypes were investigated. Bambara groundnut starch granules were predominantly oval shaped with a smooth surface and an average size of $26 \pm 0.2 \mu\text{m}$. The amylose contents (20-35%) varied significantly among genotypes. X-ray diffraction revealed the C-type pattern for all starches with relative crystallinity range: 29-35%. FTIR spectra of Bambara groundnut starches showed variable peak intensities at 2931, 1655 and 860 cm^{-1} , which corresponds to C-H stretching, H_2O bending vibrations and C-O stretching, respectively. Bambara groundnut genotype with the highest amylose content showed the lowest intensity at wavenumber 2931 cm^{-1} . With the exception of oil absorption which was similar, swelling power, water absorption and paste clarity of starches were significantly different among genotypes. Genotype with high amylose content showed restricted swelling, low paste clarity and great ability to absorb water. All Bambara groundnut starches displayed a shear thinning behaviour ($n < 1$).

3.1 Introduction

Bambara groundnut (*Vigna subterranea*) is a good source of protein (19-21%) and carbohydrate (57-67%) (Kaptso et al. 2014; Onimawo et al. 1998; Sirivongpaisal 2008), similar to legumes such as cowpea (Oyeyinka et al. 2013) and peas (Wang and Castonguay 2014). Bambara groundnut plant is highly drought tolerant and thus, well adapted to the changing climate. However, Bambara groundnut is neglected and under-utilized in Southern Africa. Traditionally, Bambara groundnut is consumed by boiling freshly harvested grains and eaten as a relish with maize-meal porridge (Hillocks et al. 2012). Matured grains are dried and ground into flour for making puddings. The under-utilisation of many crops, including Bambara groundnut, may be attributed to a lack of sufficient research to unlock their potential and value addition.

The major carbohydrate of Bambara groundnut is starch, which may have potential applications in the food industry. The starch contents of Bambara groundnut may vary between 35-46%

(Flour basis) (Adebowale et al. 2002; Afolabi 2012; Sirivongpaisal 2008). By microscopy, Bambara groundnut has been found to contain round and irregularly shaped or polygonal starch granules (Adebowale and Lawal 2002; Kaptso et al. 2014; Sirivongpaisal 2008) with an average size ranging from 6-61 μm depending on variety and source (Adebowale and Lawal 2002). According to Kaptso et al. (2014), starch extracted from white Bambara groundnut were larger (10-35 μm) than those extracted from black Bambara groundnut (6-15 μm). In terms of starch composition, Bambara groundnut starches have been found to contain varying amounts of amylose (21-28%) depending on source and variety (Kaptso et al. 2014; Sirivongpaisal 2008). The amylose content of starch can significantly influence its functional properties, including swelling and Gelatinisation (Stevens and Elton 1971; Xie et al. 2009). Native starch consists of semi-crystalline structure and Gelatinisation, which involves the heating of starch in water, disrupts the starch granular structure and causes changes from semi-crystalline to amorphous structure (Atwell et al. 1988). During the Gelatinisation process, starch granules absorb water and swell and the amylose leaches out of swollen granules, which causes an increase in viscosity of the medium (Hermansson and Svegmarm 1996). Previous studies showed that high amylose maize starch exhibited higher viscosity (Xie et al. 2009).

The molecular characterization of starch revealed differences in crystallinity patterns, which are associated with botanical origin. Based on X-ray diffraction patterns, starches have been classified as A-, B- or C-type polymorphs. The major difference between A and B is associated with the packing arrangement of double helices formed from short chains within the amylopectin molecule and their level of hydration (Imberty and Perez 1988). The A-form adopts a closely packed structure with water molecules between each double helix, while the B-type is less densely packed and more hydrated in the central cavity (Cheetham and Tao 1998; Imberty and Perez 1988). C-type starches contain mixtures of A- and B-forms. Generally, A-type crystallinity has been reported for cereal starches, B-type for tubers, high amylose cereal starches or retrograded starches and the C-type for pulse starches. Studies on the structure of Bambara groundnut starch revealed the C-type crystallinity (Afolabi 2012), which is similar to most legume starches (Hoover et al. 2010). However, some authors reported the A-type crystallinity for some varieties of Bambara groundnut (Kaptso et al., 2014; Sirivongpaisal, 2008). Variations in crystallinity patterns and amylopectin branch chain lengths have been found to influence starch functionality. For instance, with the same chain length, starches with B-polymorph displayed a lower Gelatinisation temperature compared to starches with A-polymorph (Jane et al. 1999; Whittam et al. 1990). Cai et al. (2014) found that Gelatinisation

temperature and water solubility significantly negatively correlated with amylopectin short branch chain. The crystalline structure of starch is primarily a property of amylopectin. However, the linear chain of amylose can form double helices structure and thus contribute to crystallinity. The transformation of amorphous amylose to crystalline form during heating and melting has been reported in the literature (Nordmark and Ziegler 2002; Qi et al. 2008; Singh et al. 2010).

So far, studies on Bambara groundnut varieties grown in Southern Africa have been limited to breeding to improve agronomic characteristics. Under marginal growth conditions, Bambara groundnut showed an improvement in yield from 0.3 to 4.2 t ha⁻¹ (Madamba 1995). Shegro et al. (2013) reported a wide genetic variability among 20 Bambara groundnut accessions in South Africa using morphological quantitative markers. These studies also revealed some high yielding accessions. Although the improvement in yield through conventional breeding is a positive outcome, breeding can significantly influence the composition of major components including starch of Bambara groundnut grains. For instance, starches from newly bred Brazilian maize showed higher amylose contents compared to starches from locally grown varieties (Uarrotta et al. 2013). Although breeding efforts have been largely successful in Southern Africa, the knowledge of the physicochemical properties of starch from newly bred genotypes may be important to facilitate their selection, introduction, and utilisation. Hence, this study investigated the composition, structure and functional properties of starch extracted from five Bambara groundnut genotypes.

3.2 Materials and methods

3.2.1 Plant materials

Five Bambara groundnut genotypes, SB7-1, SB8-1, BMB-29, BMB-16 and SB-1-1 of the same species were obtained from the Agricultural Research Council-Vegetable and Ornamental Plant Institute, Pretoria, South Africa. The accessions were collected in South Africa and evaluated for their agronomic characteristics such as days to 50% flowering, days to 50% maturity, plant height, terminal leaf length, terminal leaf width, leaf area, number of leaves, petiole length, number of pods, pod mass, fresh weight, dry weight, harvest index, yield per plant and hundred seed weight at Roodeplaat research farm in Pretoria, Gauteng province. The altitude of Roodeplaat is 1168 m above sea level. The location receives rainfall ranging from 350.00 mm to 431.30 mm and minimum and maximum recorded temperature ranged from 14.46°C to 30.31°C during the cropping season (2011-2012). The five genotypes used in this

study were selected based on some agronomic traits such as high yield, a high number of pod and maturity period for a future improvement programme (Shegro et al. 2013).

3.2.2 Flour preparation

Bambara groundnut was dehulled by soaking 250 g of grains in 1 litre of water for 12 h. The grains were manually dehulled and dried at 45°C in a hot air oven (D-37520, Thermo Fischer Scientific, South Africa). Dried dehulled grains were milled into flour using a Warring blender (HGBTWTS3, Torrington USA). The flours were sieved (355 µm), sealed and kept at 4 °C until analyzed (Sirivongpaisal 2008)

3.2.3 Starch extraction

Starch was extracted following the methods described by Sirivongpaisal (2008). Briefly, Bambara groundnut flour was dispersed in 0.3% (w/v) NaOH solution at a ratio 1:10 respectively. The slurry was stirred at room temperature on an orbital shaker for 4 h. After shaking, the supernatant was decanted, distilled water was added and the resulting slurry was sieved (sieve aperture size: 180 µm). The starch suspension was left for 12 h to settle and the starch obtained was washed repeatedly with water, centrifuged at 10,000×g and then neutralised with 0.1 N HCL. Extracted starch was dried at 45°C in a hot air oven (D-37520, Thermo Fischer Scientific, South Africa). Dried starch was kept at 4°C until analysis. The starch yield was calculated as a ratio of dried starch to Bambara groundnut flour. The purity of Bambara groundnut starch extracts was assessed by analysing their protein, fat and ash contents using AOAC (2000) methods.

3.2.4 Grain composition

Moisture, fat and ash contents of Bambara groundnut grains was determined using AOAC (2000) methods. Protein content was determined by Kjeldahl method ($6.25 \times N$) and total carbohydrate was calculated by difference.

3.2.5 Colour

Tristimulus L^* a^* b^* parameters of starch were determined after standardization using a colorFlex (A60-1014-593, USA). Snapshots in duplicates were taken and values were read directly from a digital print. Averages of the readings were computed and reported. Total colour difference (ΔE) and Hue angle (H) were calculated using equation 1 and 2, respectively (Falade and Okafor 2014). Potato starch (Sigma-Aldrich, South Africa) was used as a reference for comparison.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad 1$$

$$\text{Hue angle} = \tan^{-1}(b/a) \quad 2$$

3.2.6 Microscopy

Starch granule morphology was examined using Scanning Electron Microscope (EVO 15 HD SEM) with an accelerating potential of 4 kV. A thin layer of Bambara groundnut starch was mounted on the aluminium specimen holder by double-sided tape. Starch samples were coated with a thin film of gold, with a thickness of about 30 nm and the micrographs were obtained (Li et al. 2014b).

3.2.7 Amylose content

Amylose contents of the starches were determined by the iodine binding method (Williams et al. 1970).

3.2.8 X-ray diffraction

X-ray diffraction pattern of Bambara groundnut starch was done using the methods of (Li et al. 2014a) with a few modifications. X-ray diffractometer (Empyrean, PANalytical Netherlands) operating at 40 kV with a target current of 40 mA was used for the analysis. Starch samples were equilibrated at a temperature and relative humidity of 25 °C and 100% respectively in a low-temperature incubator (MTIE10, Labcon, South Africa) for 12 h. The equilibrated samples were tightly packed in a rectangular glass cell and scanned over a region of 4 to 40 (2θ)° at a scanning speed of 0.06°/ min. The relative crystallinity of the starch was calculated using equation 3.

$$\text{Relative crystallinity (\%)} = 100A_c/(A_c + A_a) \quad 3$$

A_c is the crystalline area and A_a is the amorphous area on the X-ray diffractogram.

3.2.9 Fourier transform infrared spectroscopy

Starch spectra were obtained using modified method of Afolabi (2012). Briefly, starch (3 mg) was mixed with 150 mg of FTIR-grade potassium bromide (KBR) and pressed using a 10 Mpa mechanical compressor system for 8 min to obtain a transparent pellet. The pellet formed was transferred into the FTIR system (Varian 800, Scimitor Series) and spectra obtained for the samples in percentage transmittance mode from 400 to 4000 cm⁻¹.

3.2.10 Oil and water absorption capacity

The oil absorption capacity of Bambara groundnut starch was determined as described by Falade and Okafor (2014) with a few modifications. Briefly, one gram of each sample was weighed into a dry, clean centrifuge tube. Sunflower oil (10 mL) with a density of 0.98 g/L was poured into the tube and properly mixed by vortexing. The suspension was centrifuged (Centrifuge Model: Eppendorf 5810R, Germany) at 3,500×g for 30 min. Supernatant was discarded and the tube, with its content, reweighed. The gain in weight expressed, as a percentage of oil bound, was calculated as the oil absorption capacity of the sample. The same procedure was repeated for water absorption capacity by replacing oil with water.

3.2.11 Swelling power

The swelling power of Bambara groundnut starch was determined by methods described by Madruga et al. (2014). Briefly, starch samples (0.1 g starch in 10 ml of distilled water) were stirred and placed in a water bath for 30 min at temperatures ranging from 50 to 90°C with constant stirring. The suspension was centrifuged (Centrifuge Model: Eppendorf 5810R, Germany) at 3400×g for 20 min and the supernatant discarded. Swelling power was obtained by weighing the swollen starch residue after centrifugation and dividing by the original weight of starch on dry weight basis.

3.2.12 Paste clarity

The paste clarity was determined using the method described by Liu et al. (2014) with a few modifications. Briefly, 1% starch (on dry weight basis) was prepared using distilled water. The suspensions were heated in a water bath at 90°C for 30 min (with occasional shaking). After cooling to room temperature, the percentage transmittance was determined at 650 nm against a water blank using a UV spectrophotometer (Jenway, 7305 Bibby Scientific UK). The paste clarity of the samples stored at 4°C for 24, 48 and 72 h was also examined.

3.2.13 Rheology

The rheological properties of Bambara groundnut starches were measured using the modified method of D'Silva et al. (2011). Briefly, Bambara groundnut starch (10% w/v) was gelatinized at 95°C for 30 min in centrifuge tubes and rapidly transferred into the Rheolab (80732808, Anton Paar Austria) cup. Starch pastes were allowed to equilibrate at 60°C for 10 min. The pastes were measured at shear rates ranging between 10 and 1000 s⁻¹. The data were fitted into a Power-Law Model Barnes et al. (1989) as follows:

$$\tau = k\dot{\gamma}^n$$

4

Where τ is the shear stress (Pa), k is the consistency coefficient, (Pa.s)ⁿ, γ is the shear rate (s⁻¹) and n is the flow behaviour index.

3.2.14 Statistical analysis

All experiments were conducted in duplicate. Data were analysed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference Test ($p < 0.05$).

3.3 Results and discussion

3.3.1 Proximate composition of Bambara groundnut

Composition data were similar across Bambara groundnut genotypes (Table 3.1). Carbohydrate (approx. 58%) and protein (approx. 24%) were the major components of Bambara groundnut grains. Fat and ash contents of Bambara groundnut genotypes were generally low. The protein contents of Bambara groundnut from this study were slightly higher than those reported for Bambara groundnut elsewhere (Adebowale et al. 2002; Onimawo et al. 1998; Sirivongpaisal 2008). Environmental growth conditions and genotypes may influence crop yield and grain composition. For instance, Jing et al. (2010) reported higher rice yield in subtropical environments than in tropical environments. However, the variation in protein contents of the rice genotypes was reportedly small. According to these authors, genotypic differences had more influence on grain composition than did the growth environments (Jing et al. 2010). Tahir et al. (2011) also found some variations in protein contents of lentil genotypes grown in the same environment.

Table 3.1 Proximate composition of Bambara groundnut (g/100 g)

Genotypes	Moisture	Protein	Fat	Ash	*Total Carbohydrate
SB7-1	7.5 ^a ±0.0	22.8 ^a ±0.8	8.2 ^b ±0.1	2.2 ^a ±0.0	59.5 ^a ±0.8
SB8-1	8.1 ^b ±0.2	24.9 ^a ±1.4	5.8 ^a ±0.6	2.9 ^b ±0.1	58.4 ^a ±2.2
BMB-29	8.7 ^b ±0.0	23.2 ^a ±0.1	8.5 ^b ±0.0	3.3 ^c ±0.0	56.5 ^a ±0.2
BMB-16	8.5 ^b ±0.0	23.5 ^a ±0.6	7.8 ^b ±0.8	2.9 ^b ±0.1	57.3 ^a ±1.5
SB-1-1	7.5 ^a ±0.1	25.4 ^a ±1.3	6.6 ^a ±0.1	2.2 ^a ±0.0	58.4 ^a ±1.1

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

*Total carbohydrate calculated by difference.

3.3.2 Yield, purity, amylose content and morphology

The starch yields varied significantly ($p < 0.05$) among Bambara groundnut genotypes with values ranging from 22-33%. Bambara groundnut genotype SB7-1 had the highest starch yield

(approx. 33%) followed by SB8-1 (approx. 26%). The starch yield of Bambara groundnut genotypes compares favourably with literature (Hoover et al. 2010). The lightness (L^*) value (approx. 94) of Bambara groundnut starches were very similar across genotypes (Table 3.2). These values compare favourably to that of the reference potato starch sample. The relatively high L^* values of starches is an index of purity. All the starch extracts had low contents of residual protein, ash and fat (Table 3.3).

Table 3.2 Colour, amylose content and relative crystallinity of Bambara groundnut starch

Genotypes	L^*	a^*	b^*	ΔE	Hue angle	Amylose	Relative crystallinity
*Potato	96.9 ^a ±0.1	0.8 ^a ±0.1	1.9 ^a ±0.0	-	-	24.6 ^{bc} ±0.8	30.3 ^b ±0.1
SB7-1	94.9 ^b ±0.1	-0.4 ^a ±0.1	2.9 ^a ±0.0	1.2 ^b ±0.0	-81.7 ^b ±0.0	26.7 ^c ±0.1	34.3 ^a ±0.1
SB8-1	94.3 ^b ±0.1	-0.2 ^b ±0.1	2.9 ^a ±0.0	0.9 ^a ±0.0	-86.7 ^a ±0.0	30.7 ^a ±0.6	33.8 ^a ±0.1
BMB-29	93.8 ^b ±0.1	0.1 ^c ±0.1	3.8 ^b ±0.0	1.8 ^b ±0.0	89.8 ^c ±0.0	35.1 ^a ±0.2	29.4 ^b ±0.1
BMB-16	91.8 ^c ±0.1	0.1 ^c ±0.0	7.3 ^c ±0.0	5.9 ^c ±0.0	89.3 ^c ±0.0	19.6 ^d ±0.3	35.3 ^a ±0.1
SB-1-1	94.1 ^b ±0.1	-0.1 ^c ±0.0	2.5 ^a ±0.0	0.7 ^a ±0.0	-87.4 ^a ±0.0	25.2 ^b ±0.1	35.0 ^a ±0.1

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

*Potato starch was added as reference for colour measurement

Table 3.3 Proximate composition of Bambara groundnut starch

Samples	Moisture	Protein	Fat	Ash
SB7-1	8.9 ^a ±0.3	0.3 ^c ±0.02	0.2 ^b ±0.01	0.3 ^a ±0.08
SB8-1	9.1 ^a ±0.2	0.3 ^c ±0.02	0.1 ^b ±0.01	0.1 ^b ±0.03
BMB-29	8.5 ^a ±0.1	0.8 ^a ±0.02	0.4 ^a ±0.07	0.2 ^b ±0.03
BMB-16	8.6 ^a ±0.7	0.6 ^b ±0.01	0.1 ^b ±0.01	0.2 ^b ±0.01
SB-1-1	9.3 ^a ±0.2	0.4 ^b ±0.01	0.1 ^b ±0.01	0.2 ^b ±0.06

*Mean ± SD. Values with different superscript along the column are significantly different ($p \leq 0.05$).

The amylose contents of Bambara groundnut starches varied significantly ($p < 0.05$) from 20-35% (Table 3.2). Among studied genotypes, BMB-29 starch had the highest amylose content (approx. 35%) while BMB-16 starch showed the lowest value (approx. 20%). Previous studies have similarly reported some variations in amylose contents of starches extracted from Bambara groundnut cultivars (Kaptso et al. 2014) and rice genotypes (Jing et al. 2010). Genotypic differences accounted for the main variation (68%) in amylose contents of rice starches compared to differences in environmental conditions (25% variation) (Jing et al. 2010). The variation in amylose contents among Bambara groundnut genotypes from this study could be attributed to inherent genetic differences rather than the prevailing environmental

conditions since these genotypes were grown in the same location. The amylose contents of starches from this study are within the range of values previously reported for pulse starches such as cowpea and pigeon pea (Hoover et al. 2010).

Bambara groundnut starch showed oval shaped granules with a few granules round and irregular in shape (Figure 3.1). All starch granules were smooth with no fissures or pin holes. The average length of the starch granules was approximately $26 \pm 0.2 \mu\text{m}$. The size and shape of Bambara groundnut starch genotypes compared favourably with previous reports on pulse starches (Adebowale and Lawal 2002; Hoover et al. 2010; Kaptso et al. 2014).

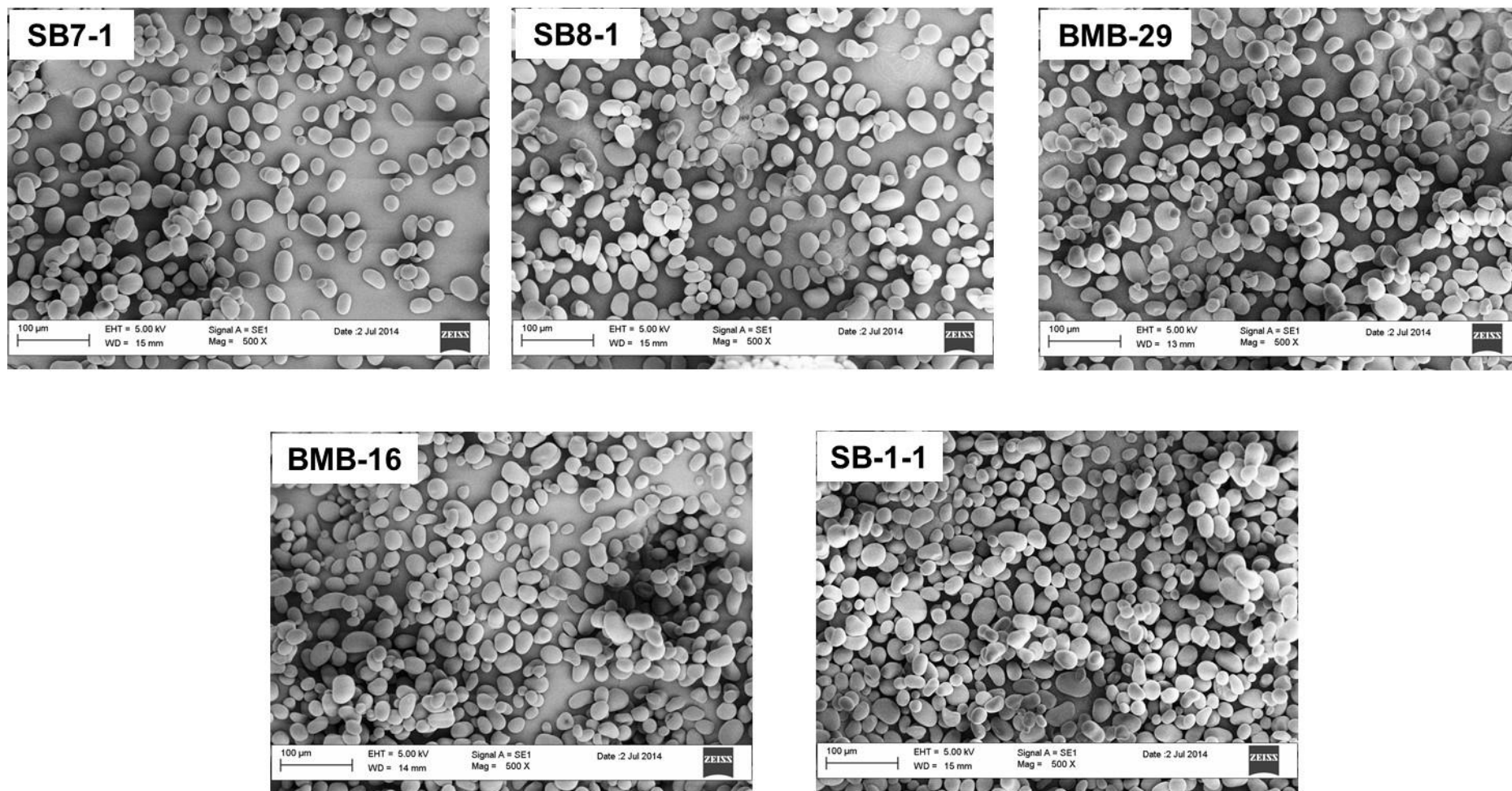


Figure 3.1 Representative micrograph of starch isolated from Bambara groundnut genotypes.

3.3.3 Crystallinity pattern

X-ray diffractogram of Bambara groundnut starch genotypes showed strong peaks at approximately 17, 20, 21 and 27 (2θ) and weak peaks at around 6, 23, 31 and 35 (2θ) (Figure 3.2) suggesting a C-type crystallinity. The C-type crystallinity is generally reported for pulse starches such as cowpea (Sandhu & Lim, 2008) and Bambara groundnut (Afolabi 2012). However, some authors have reported A-type pattern in Bambara groundnut starch (Kaptso et al. 2014; Sirivongpaisal 2008). This is very unusual for pulse starches. Kaptso et al. (2014) reported A-type crystallinity for two varieties of Bambara groundnut possibly due to the absence of a peak reflection around $2\theta = 5.6$. According to the literature, many authors have attributed a C-type crystallinity pattern for pulse starches where the peak at 5.6 (2θ) appeared very weak (Cai et al. 2014; Sun et al. 2015) or absent (Guo et al. 2015; Kaur et al. 2010; Sandhu and Lim 2008). C-type starches may contain varying proportions of pure 'A' and 'B' polymorphs (Gernat et al. 1990), which may influence the XRD reflection. A-polymorph reportedly showed strong reflection peaks at approximately 15, 23 and 27 (2θ), whilst B-polymorph showed a characteristic strong peak at approximately 5.6, 17 and few small peaks at around 15, 20, 22 and 24 (2θ) (Cai et al. 2014). In this study, a weak peak at approximately $2\theta = 6$ and other strong peaks observed from the XRD diffractogram of Bambara groundnut starches strongly suggest a mixture of A and B and hence these genotypes were classified as C-starch.

The relative crystallinity of Bambara groundnut starches varied from 29.4 to 35.3% (Table 3.2). Starch extracted from Bambara groundnut genotype BMB-29 had lower relative crystallinity (approx. 29%) compared to starches from other Bambara groundnut genotypes. According to previous studies, amylopectin molecules form the crystalline structure in starch granules, therefore, it is expected that relative crystallinity will be inversely related to amylose content (Hizukuri 1985; Hizukuri et al. 1983; Kaur et al. 2010; Sandhu and Lim 2008). Therefore, the relatively lower relative crystallinity of BMB-29 starch could be attributed to its relatively higher amylose content (Table 3.2). Comparable relative crystallinity values have been reported for pulse starches such as lentil starch (Kaur et al. 2010) and Bambara groundnut starch (Hoover et al. 2010).

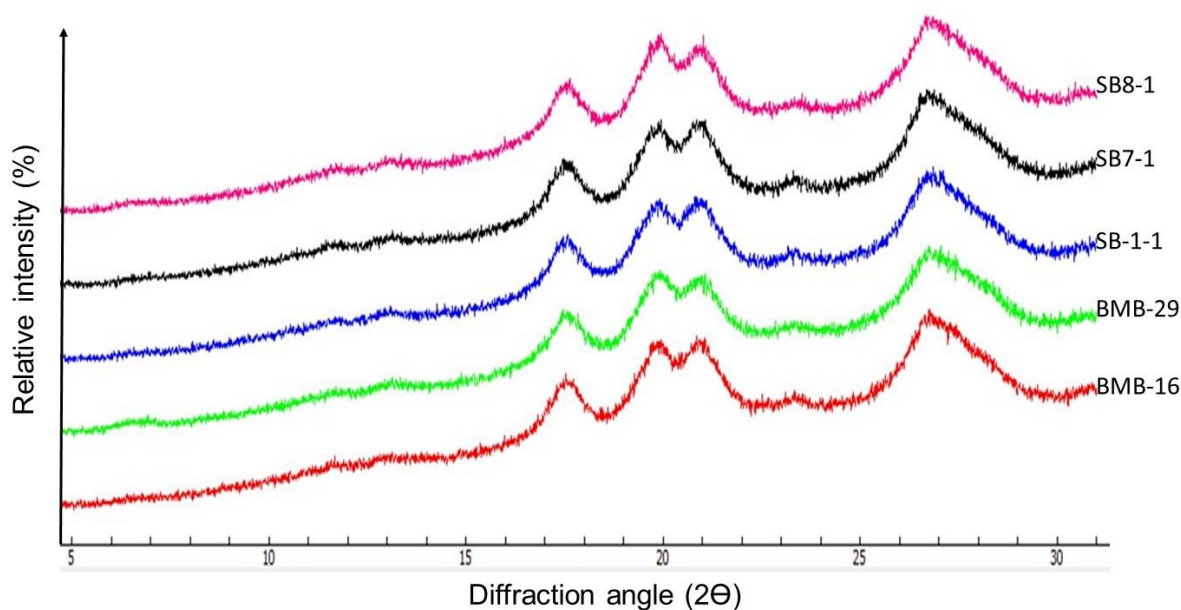


Figure 3.2 X-ray diffractograms of Bambara groundnut starches.

3.3.4 Fourier transform infrared spectroscopy

FTIR spectra of Bambara groundnut starches showed characteristic band with a peak at 2931 cm^{-1} in the region of $2800\text{--}3000\text{ cm}^{-1}$, that could be attributed to C-H bonds stretching (Figure 3.3). The amylose/amylopectin ratio may influence the absorbance of starch in this region. As discussed above, the amylose contents (Table 3.2) of starches were significantly ($p < 0.05$) different among genotypes and this seemed to have influenced the peak intensity at wavenumber 2931 cm^{-1} . Bambara groundnut genotype BMB- 29 with relatively high amylose contents (Table 3.2) showed weak peak intensity compared to other genotypes. Previous studies also attributed the variation in FTIR peak intensities at this wavenumber (2931 cm^{-1}) to differences in amylose and amylopectin contents of starches from potato, maize, wheat starches and waxy starch (Kizil et al. 2002). A broad band in the region of $3000\text{--}3600\text{ cm}^{-1}$ could be attributed to OH stretching (Ottenhof et al. 2003; Zhang and Han 2006). Below 800 cm^{-1} , that is, at low wavenumbers, FTIR of Bambara groundnut starches exhibited complex vibrations as a result of the skeletal mode vibration of glucose pyranose ring. This is in agreement with previous reports (Zeng et al. 2011). The sharp band at 1655 cm^{-1} could be attributed to H_2O bending vibrations (Zeng et al. 2011). Vibrations due to absorbed water in the amorphous regions of starch have been observed in a broader infrared range (Cael et al. 1974; Kizil et al. 2002). Kizil et al. (2002) attributed the vibration at 1642 cm^{-1} to water molecule in the non-crystalline region of starch. Further, these authors reported that the intensity of the observed peak may be related to the starch crystalline types as potato starch with B-type polymorph

displayed a sharper peak than other starches with A-type. All the Bambara groundnut genotypes from this study showed the C-type polymorph (Figure 3.2) consisting of the mixture of A and B (Gernat et al. 1990). The variation in peak intensity at 1655 cm^{-1} may be attributed to variation in proportion of A and B of the respective genotypes. The stretching of C–C and C–O bonds is normally observed in the region of $1300\text{--}800\text{ cm}^{-1}$ (Capron et al. 2007). The peaks occurring at 1164 cm^{-1} in Bambara groundnut starches may be attributed to coupling mode of C–O and C–C stretching, while peaks at 860 and 928 cm^{-1} could be attributed to C–O stretching. Comparative peaks have been reported in the literature (Capron et al. 2007; Kizil et al. 2002).

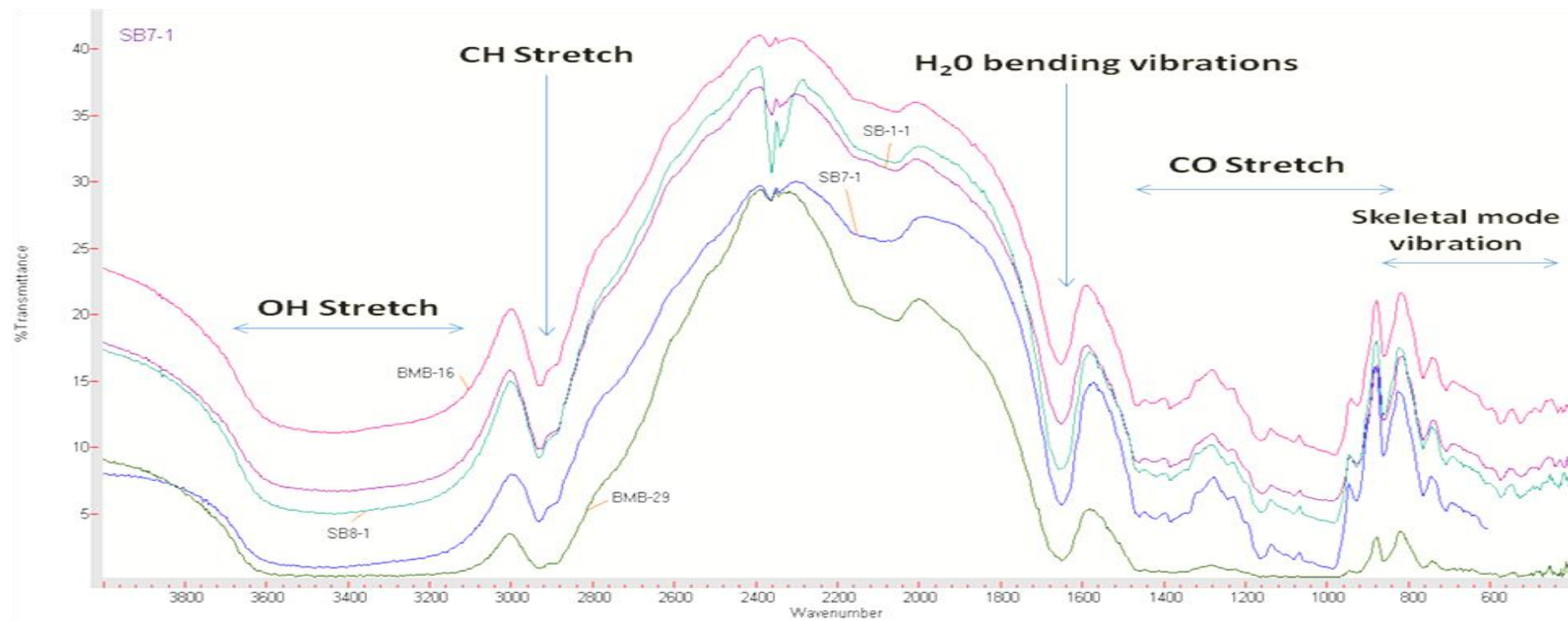


Figure 3.3 FTIR Spectra of a Bambara groundnut starches

3.3.5 Water and oil absorption capacities

The water absorption capacities of Bambara groundnut starches differed across genotypes (Figure 3.4). Among the studied genotypes, BMB-29 starch showed the greatest ability to absorb water (1.2 g water/ g starch). The significantly ($p<0.05$) higher water absorption capacity of BMB-29 starch compared to starches from other genotypes could possibly be attributed to its higher amylose content (approx. 35%) (Table 3.2). Similarly, high water absorption capacity has been reported for Chinese yam and chick pea starches containing higher amylose contents (Shujun et al. 2006; Singh et al. 2004). Unlike with the water absorption, the oil absorption capacities of Bambara groundnut starches were not very different among genotypes (Figure 3.4). Approximately 0.6 g of oil was absorbed per g of starch. The oil absorption capacities and water absorption capacities of the starches from the Bambara groundnut genotypes were similar to those previously reported for Bambara groundnut landrace varieties (Adebowale et al. 2002; Sirivongpaisal 2008).

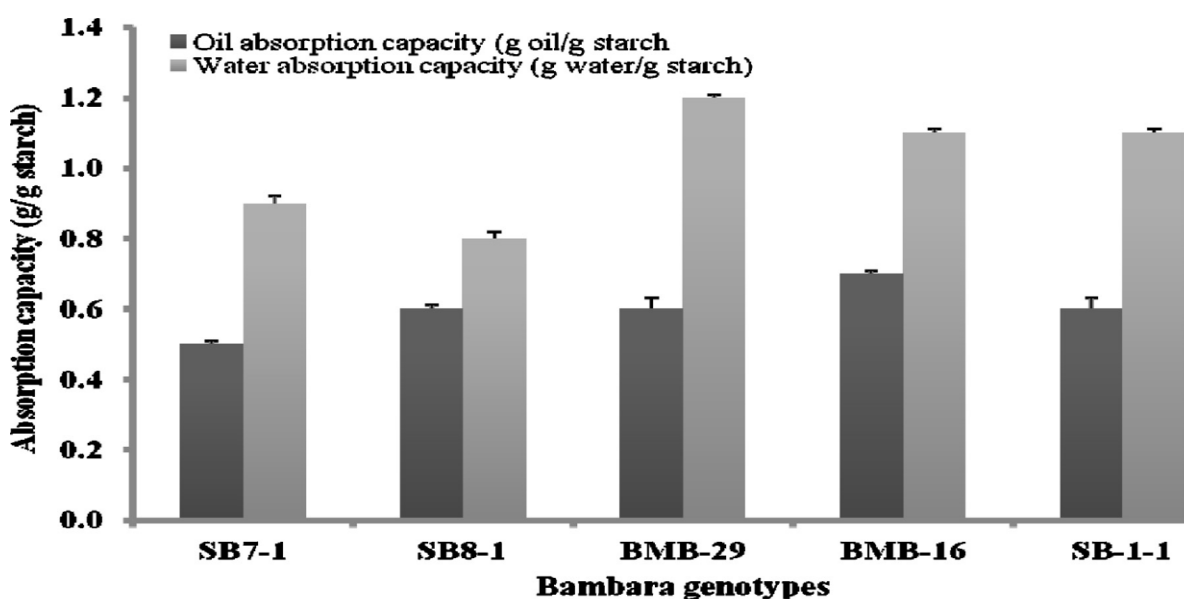


Figure 3.4 Oil and water absorption capacities of Bambara groundnut starches.

Error bars indicate standard deviation ($n=2$).

3.3.6 Swelling power

Bambara groundnut starches showed a progressive increase in swelling power as the temperature increased (Figure 3.5). However, a sharp increase in swelling power was observed between the temperature range of 80 and 90°C. This may be attributed to starch Gelatinisation. Previous studies on Bambara groundnut starch reported similar Gelatinisation temperature range (77-93°C) (Afolabi 2012; Kaptso et al. 2014; Sirivongpaisal 2008). Starch extracted from

Bambara groundnut genotype BMB-16 and SB-1-1 with lower amylose contents (Table 3.2) showed higher swelling power between 50 and 80°C. The swelling power of the starches above 80°C followed the order SB-1-1>BMB-29>SB7-1>BMB-16>SB8-1. Comparative studies on non-waxy and waxy maize starches indicated that amylose content of starch restricts its swelling behaviour (Ratnayake et al. 2002). Although the variation in the swelling power of Bambara groundnut genotypes may be associated with differences in amylose contents of starches, other factors such as molecular structure of amylopectin and the magnitude of interaction within the amorphous and crystalline regions may also play a role as indicated in previous studies (Singh et al. 2003). The swelling power in this study is similar to those reported for Bambara groundnut landrace (Adebowale et al. 2002; Sirivongpaisal 2008).

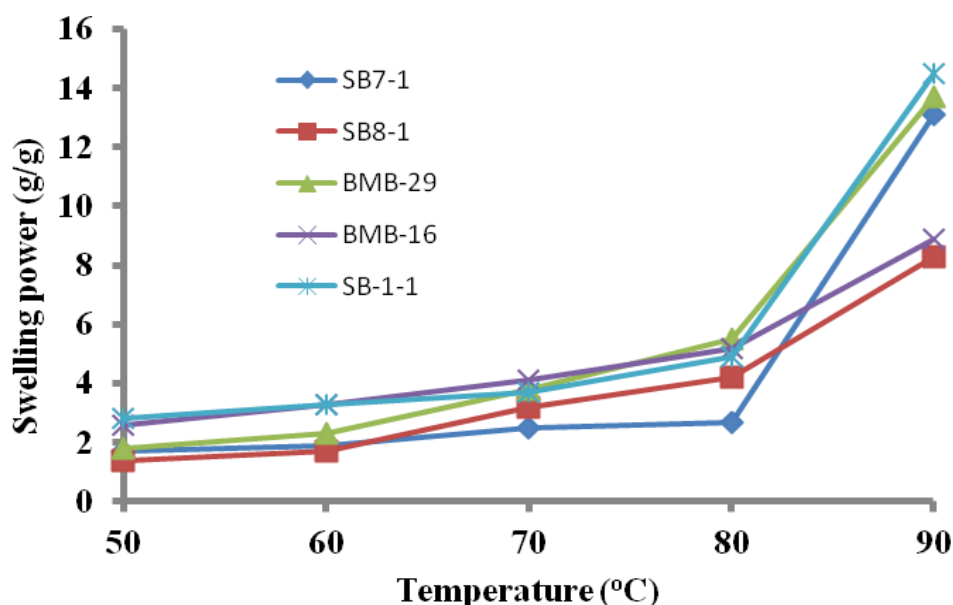


Figure 3.5 Swelling power of Bambara groundnut starch as a function of temperature

3.3.7 Paste clarity

Among studied genotypes, BMB-29 starch showed the lowest paste clarity (Figure 3.6), which could be attributed to its relatively high amylose content (Table 3.2). During paste formation, starches with relatively higher amylose contents have a higher tendency to leach into the surrounding medium and thus forming a cloudy paste. However, during storage at 4°C, all the starches showed a sharp decrease in paste clarity (75-87%) within 24 h. Beyond 24 h storage, the paste clarity remained almost constant throughout the storage period for all the starches. The reduction in paste clarity values may be attributed to aggregation and slow re-crystallization of amylopectin (Gani et al. 2010). A similar time-dependent decrease in paste

clarity has also been reported for canna starch cultivars (Thitipraphunkul et al. 2003), rice starch (Ashwar et al. 2014), and chestnut starch (Gani et al. 2010).

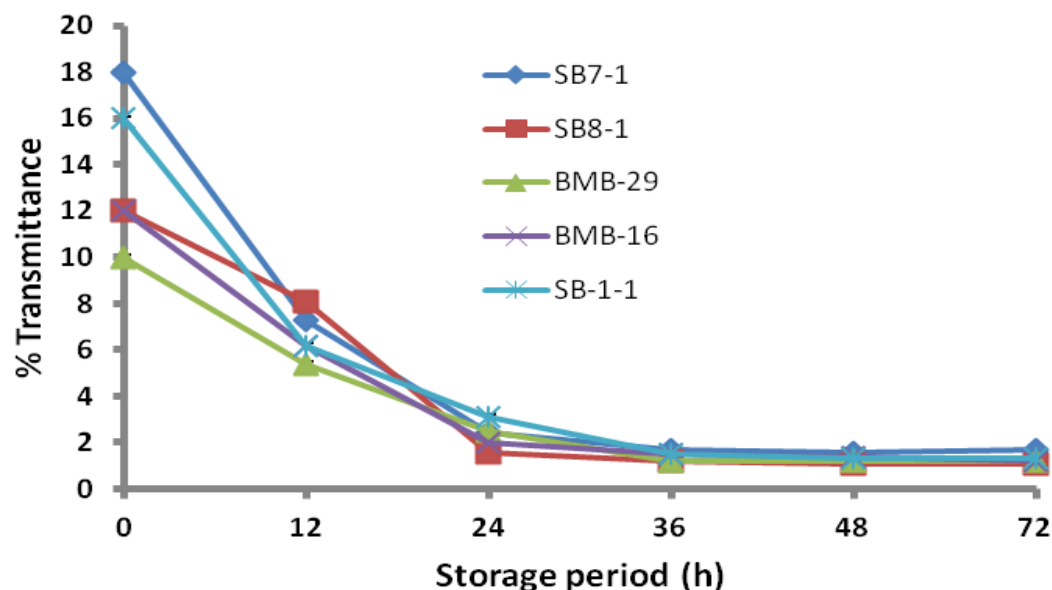


Figure 3.6 Paste clarity of Bambara groundnut starch genotypes as affected by storage (4°C)

3.3.8 Rheology

As expected, gelatinized Bambara groundnut starches showed a decrease in viscosity with increasing shear rates (Figure 3.7) suggesting a breakdown in the starch structure by the applied shear stress. This behaviour, typical of pseudoplastic macromolecules, varied across Bambara groundnut starch genotypes. Among the Bambara groundnut genotypes, BMB-29 starch showed the highest viscosity within a shear rate range of 10-100 s⁻¹, which may be associated with its relatively high amylose content (Table 3.2). Possibly, the shear induced during Gelatinisation process may have enhanced the solubilisation of amylose into the surrounding starch medium, which may have enhanced the viscosity of the starch pastes. Xie et al. (2009) reported a similarly high viscosity for maize starch with higher amylose content compared to maize starch with lower amylose.

The power-Law model was applied to describe the flow behaviour of Bambara groundnut starches. The flow properties of the starches followed the Power-Law model with the correlation coefficient ($r^2 \geq 0.9$) close to 1 (Table 3.3). Flow behaviour index ($n \sim 0.1$) was similar across starch genotypes (Table 3). However, the consistency index (K) of the starches varied among Bambara groundnut genotypes (1.5 to 1.8 Pa.sⁿ) (Table 3.3).

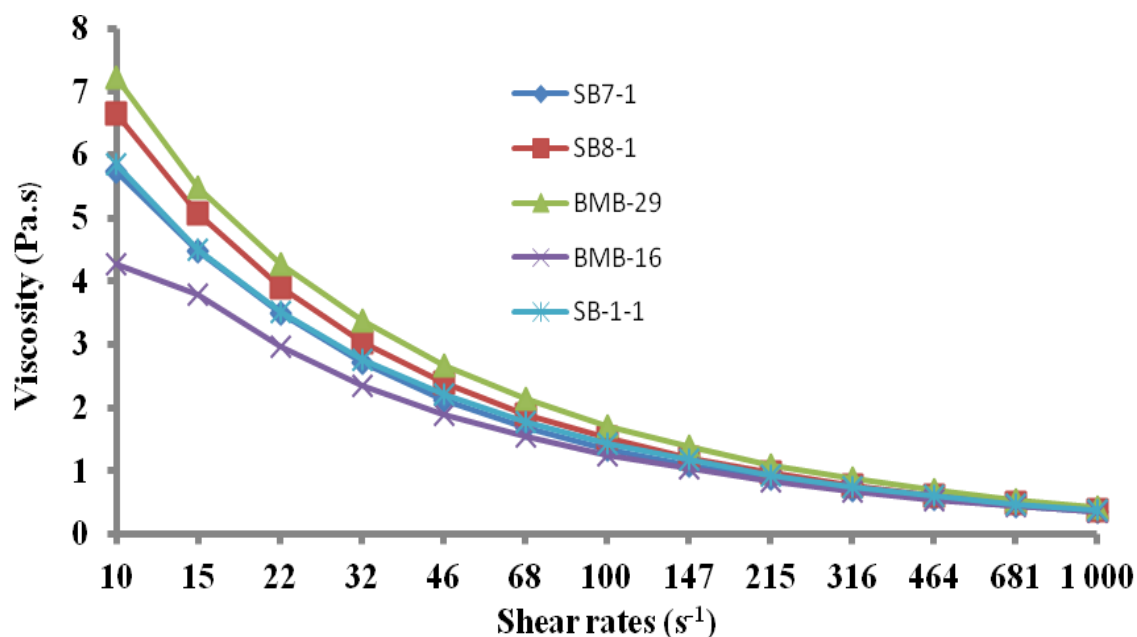


Figure 3.7 Rheogram of Bambara groundnut starch genotypes

Table 3.4 Power-Law model coefficients of gelatinised Bambara groundnut starch

Genotypes	n	K (Pa.s) ⁿ	r ²
SB7-1	0.1 ^a ±0.1	1.8 ^b ±0.0	0.9 ^a ±0.1
SB8-1	0.1 ^a ±0.1	1.7 ^b ±0.0	0.9 ^a ±0.0
BMB-29	0.1 ^a ±0.1	1.5 ^a ±0.1	0.9 ^a ±0.1
BMB-16	0.1 ^a ±0.1	1.7 ^b ±0.0	0.9 ^a ±0.0
SB-1-1	0.1 ^a ±0.1	1.7 ^b ±0.0	0.9 ^a ±0.1

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

K: Consistency index, n: flow behaviour index, r²: correlation coefficient.

3.4 Conclusions

Bambara groundnut genotypes are relatively good sources of carbohydrate including starch. Starches isolated from Bambara groundnut genotypes consists of predominantly oval shaped granules. Bambara groundnut starches contain variable amounts of amylose which significantly influence their functional properties such as water absorption, swelling, paste clarity, and viscosity. All Bambara groundnut starches display the C-type crystallinity pattern. FTIR of all Bambara groundnut starches present the same characteristic bands in the finger print regions (1300 - 800 cm⁻¹) with some variation in peak intensities at 2931 cm⁻¹ and 1655 cm⁻¹ which could be attributed to differences in amylose contents and degree of crystallinity of genotypes.

CHAPTER FOUR

4.0 Physicochemical properties of starches extracted from Bambara groundnut landraces

Abstract

The physicochemical properties of starches extracted from three Bambara groundnut landraces, maroon, brown and cream were studied. The amylose contents (31.5-34.6%) of Bambara groundnut starches were significantly different among landraces. All Bambara groundnut starches exhibited A-type crystalline pattern with an average relative crystallinity of 32%. The peak Gelatinisation temperature (approx. 73°C) of brown Bambara groundnut starch was slightly low compared to maroon (approx. 78°C) and cream (approx. 76°C) Bambara groundnut starch. Bambara groundnut starches showed a substantially high proportion of resistant starch (71%) and similar predicted glycemic index (40.1) among landraces. Bambara groundnut starch can potentially be used as thickening agents in food products and ingredient development.

4.1 Introduction

The starch industry relies mainly on cereals such as maize as major sources of starch. Pulses including pea (*Pisum sativum*), cowpea (*Vigna unguiculata*) and Bambara groundnut (*Vigna subterranea*) are relatively good sources of starch (18-49%) (Adebooye and Singh 2008; Hoover et al. 2010). These leguminous crops can play a role as alternative starch sources to the conventional cereal crops. Among pulses, pea starch has found some applications in the food and allied industries. However, traditional crops such as Bambara groundnut have not been extensively researched and their application remains limited in the food industry.

Botanical origin, composition (e.g. amylose content) and plant species may significantly influence the physicochemical properties of starch (Chung and Liu 2012; Kaur and Sandhu 2010; Kaur et al. 2010; Liu et al. 2015a). Potato starch granules appeared round or oval in shape with smooth surfaces compared to maize starch granules, which were irregular with many pores on the surface (Sujka and Jamroz 2009). Gelatinisation is a phase transition that occurs when heating starch in the presence of excess water. Gelatinisation changes the starch structure from semi-crystalline to amorphous phase (Joshi et al. 2013). This process is frequent in food processing and therefore, has been extensively studied. Many studies reported a

significant influence of amylose on starch melting temperature (Fredriksson et al. 1998; Joshi et al. 2013; Stevens and Elton 1971). Joshi et al. (2013), studied the physicochemical properties of lentil starch with higher amylose content than maize and potato starches. The melting temperature of lentil starch was found to be intermediate between potato and maize starches (Joshi et al. 2013). However, some studies did not find any relationship between amylose content and Gelatinisation temperature (Chung et al. 2008a; Kaur et al. 2010). For instance, Chung et al. (2008a), reported similar Gelatinisation temperatures for starches from three varieties of common bean differing in amylose contents. According to Jane et al. (1999), the fine structures of amylopectin play a significant role in Gelatinisation process and starch pasting. Pasting properties of starch such as peak viscosity, breakdown, and final viscosity, have been found to vary greatly among potato cultivars (dos Santos et al. 2016). Huang et al. (2007), also found some variations in the pasting properties of pulse starches. Cowpea starch with a higher amount of long amylopectin chains showed higher peak and final viscosities compared to chickpea and yellow pea starches (Huang et al. 2007).

There is a growing interest in pulse starches because of their high resistant starch contents, which are known to have positive physiological effects (Blazek and Copeland 2008; Skrabanja et al. 1999). Previous studies related the digestibility of starch to crystalline patterns. A-type is associated with cereals such as maize starch, B-type with tubers such as potato starch and high amylose maize starch, while C-type (a mixture of A and B) is often associated with legume starches. According to Srichuwong et al. (2005), A-type starch are more susceptible to digestive enzymes than B and C-starches. Hoover and Sosulski (1985), reported that approximately 74% of maize starch (A-type) was hydrolyzed compared to C-type dry bean starches (26-35%). The high proportion of resistant starch in pulse starches such as cowpea and peas have been attributed to their moderately high amylose content compared to cereal starches (Hoover et al. 2010). The United Nations General Assembly declared 2016 as the International Year of Pulses (A/RES/68/231). This is important in creating awareness on the importance of this category of crop in addressing food and nutrition challenges. By making this declaration, the United Nations hopes to position pulses as primary sources of protein and other essential nutrients such as dietary fiber and starch. More research is needed to tap into the potential of pulses grown in many parts of the world including Southern Africa.

Bambara groundnut (*V. subterranea*) is a starch-rich (22-45%) leguminous crop (Afolabi 2012; Kaptso et al. 2014), grown in many parts of Africa including Southern Africa. The Bambara

groundnut plant is highly drought tolerant and well adapted to the changing climate. Previous studies reported significant variations in amylose contents (21-35%) of Bambara groundnut starch depending on source and cultivar (Kaptso et al. 2014; Sirivongpaisal 2008). Other studies on the characterization of Bambara groundnut starch revealed different results on crystalline patterns (Afolabi 2012; Kaptso et al. 2014). Afolabi (2012) reported the C-type pattern for Bambara groundnut starch while others found the A-type crystallinity (Kaptso et al. 2014; Sirivongpaisal 2008). Differences in crystalline patterns of Bambara groundnut starches may be associated with the origin of the grains and variety. Many varieties of Bambara groundnut are grown in Southern Africa. Bambara groundnut landraces dominate the production areas and these varieties are grown by farmers mainly for subsistence. To facilitate the utilisation of Bambara groundnut landraces, knowledge of the physicochemical properties of their starch component may be important. Hence, this study investigated the physicochemical properties of starches extracted from three Bambara groundnut landraces.

4.2 Materials and methods

4.2.1 Materials

Three types of Bambara groundnut landraces harvested from Markathini farm station Jozini, South Africa, were used in this study. Bambara groundnuts were differentiated by their grain coat colours as maroon, brown and cream (Figure 4.1). All chemicals and solvents used were laboratory grade. Glucose oxidase and peroxidase assay kit (No. GAGO-20), amyloglucosidase (No. 7095), alpha-amylase (No. 7545), guar gum (No. 4129), potato and maize starch were purchased from Sigma-Aldrich (St. Louis, MO).

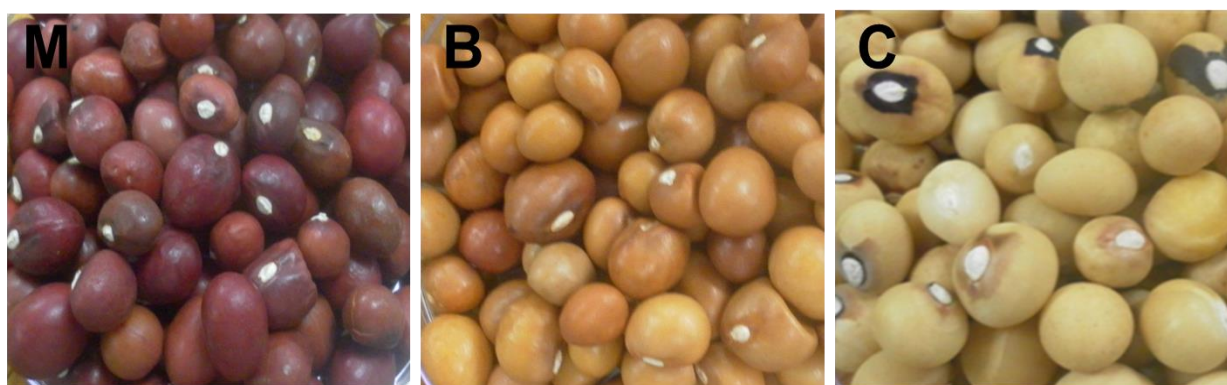


Figure 4.1 Grains of Bambara groundnut landraces

M: Maroon, B: Brown, C: Cream

4.2.2 Preparation of Bambara groundnut flour and starch extraction

Bambara groundnut flour was prepared according to the method of Sirivongpaisal (2008) with slight modifications. Briefly, Bambara groundnut were dehulled, dried, ground into flours and sieved (sieve aperture size: 355 µm). Starch was then extracted as reported by Sirivongpaisal (2008). The yield of starch was calculated as a ratio of dried starch to Bambara groundnut flour. Starch samples were stored at 4°C until analyzed.

4.2.3 Microscopy

Starch granule morphology was examined using a scanning electron microscope (EVO 15 HD) with an accelerating potential of 4 KV. Briefly, a thin layer of the starch granule was mounted on the aluminium specimen holder with double-sided tape. The starch sample was coated with a thin film of gold for 2 min with a thickness of about 30 nm (Naidoo et al. 2015). Average starch granule size was determined from the diameter of individual granules (N=40) on the basis of the scale bar provided on the captured scanning electron micrographs (Stevenson et al. 2006).

4.2.4 Amylose contents

Amylose contents of the starches were determined by iodine binding method (Williams et al. 1970).

4.2.5 X-ray diffraction

X-ray diffraction pattern of Bambara groundnut starch was done as described by Afolabi (2012). The relative crystallinity (RC) of the starch was calculated using equation 4.1

$$RC (\%) = \frac{100Ac}{(Ac+Aa)} \quad 4.1$$

where Ac is the crystalline area and Aa is the amorphous area on the X-ray diffractogram.

4.2.6 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra of the extracted starches were obtained using a spectrometer (Varian 800 Series) following the method reported by Afolabi (2012). Spectra were obtained in the transmittance mode with 64 scans from 400 to 4000 cm⁻¹.

4.2.7 Differential scanning calorimetry

Thermal properties of Bambara groundnut starches were determined using a differential scanning calorimeter (SDT Q600, USA) coupled with a thermal analysis data station and data

recording software. Starch samples were prepared in a ratio of 1:3 with distilled water in an aluminium DSC pan. The pans were allowed to equilibrate at 25°C for 12 h prior to DSC analysis. Samples were scanned from 10 to 120°C each with an interval heating rate of 10 °C/min. An empty pan was used as a reference for all measurements.

4.2.8 Swelling power

Swelling power of Bambara groundnut starch was determined as described by Madruga et al. (2014). Briefly, 1% starch suspension in water was heated for 30 min at temperatures ranging from 50 to 90°C with constant stirring. The suspension was centrifuged (Centrifuge model: Eppendorf 5810R, Germany) at 3400×g for 20 min and the supernatant discarded. Swelling power was obtained by weighing the residue after centrifugation and dividing by original weight of starch on dry weight basis.

4.2.9 Pasting properties

The pasting properties of Bambara groundnut starch were examined using a Rapid Visco-Analyzer (Newport Scientific Australia) according to the standard method provided by the instrument manufacturer. Briefly, starch (2.8 g) was weighed into the test canister containing 25 ml of distilled water. The mixture was agitated by mixing manually 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.

4.2.10 In-vitro digestibility

Digestibility of Bambara groundnut starch was done as previously reported (Kaur et al. 2010; Naidoo et al. 2015). Briefly, porcine pancreatic α -amylase (3.89 g) was dispersed in water (25.7 mL), centrifuged for 10 min at 2500xg, 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 assay kits. The glucose assay kit was used to analyze the glucose content of the starch fractions. Nutritional starch fractions based on digestibility were rapidly digestible starch (RDS), which represents the portion of starch that was hydrolyzed within 20 min of incubation, slowly

digestible starch (SDS), which represents the starch hydrolyzed between 20 and 120 min and resistant starch (RS), which represents the starch fraction not digested after 120 min of incubation. The predicted glycemic index was estimated using equation 2 as previously described (Goñi et al. 1997).

$$GI = 39.71 + 0.549HI \quad 4.2$$

GI: Glycemic index

HI: Hydrolysis index

4.2.11 Statistical analysis

Starch samples were prepared in duplicate and analyses were run at least in triplicate. Data were analyzed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference Test ($p < 0.05$).

4.3 Results and discussion

4.3.1 Starch yield, morphology and amylose contents

The starch yield was significantly different among Bambara groundnut landraces. Maroon Bambara groundnut gave a slightly higher starch yield (35%) compared to brown and cream, which were similar (approx. 29%) (Table 4.1). The starch yields observed in this study are within the range reported in the literature for pulse starches including Bambara groundnut (Afolabi 2012; Hoover et al. 2010; Sirivongpaisal 2008). Bambara groundnut starches showed low contents of residual ash, fat and protein (Table 4.1), suggesting that these starches were pure.

Table 4.1 Yield, proximate composition, granule size, amylose contents and relative crystallinity of Bambara groundnut starches

Parameters	Bambara groundnut landraces		
	Maroon	Brown	Cream
Yield (%)	35.0 ^a ±0.1	28.0 ^b ±0.1	29.0 ^b ±0.2
¹ Moisture	7.5 ^a ±0.2	7.0 ^a ±0.0	6.5 ^a ±0.2
¹ Protein	0.2 ^b ±0.1	0.5 ^a ±0.1	0.3 ^b ±0.1
¹ Fat	0.3 ^b ±0.1	0.5 ^a ±0.1	0.3 ^b ±0.1
¹ Ash	0.1 ^b ±0.0	0.1 ^b ±0.0	0.2 ^a ±0.1
Granule size (µm)	27 ^a ±4.7	24 ^a ±4.5	29 ^a ±5.5
Amylose content (%)	31.5 ^b ±0.4	34.6 ^a ±0.2	32.9 ^b ±0.2
Relative crystallinity (%)	33.0 ^a ±0.5	30.5 ^b ±1.0	32.5 ^a ±0.1

Mean ± SD. Mean with different superscript along a row are significantly different ($p < 0.05$).

¹Values expressed in percentage (%)

The majority of Bambara groundnut starch granules appeared oval with a few granules round and irregular in shape (Figure 4.2). All starch granules were smooth with no fissures. These granules were moderately large in size with a diameter ranging from 24 to 29 μm (Table 4.1). The shape and size of Bambara groundnut starch granules are in agreement with previous reports on pulse starches extracted from pea (Hoover 2001; Liu et al. 2015a), cowpea (Adebooye and Singh 2008) and Bambara groundnut varieties obtained elsewhere (Kaptso et al. 2014).

Starches extracted from Bambara groundnut landraces showed significantly different amylose contents (approx. 32-35%) (Table 4.1), which compared favourably with literature (Hoover et al. 2010; Kaptso et al. 2014; Sirivongpaisal 2008). A similarly high level of amylose contents (approx. 33-36%) has been reported for peas grown in China (Liu et al. 2015a). With the exception of high amylose maize starches, pulse starches have relatively high amylose contents compared to cereal and tuber starches. The amylose contents of Bambara groundnut starches from this study are higher than those reported for normal maize (24.8-25.1%) and potato (14.9-23.2%) starches (Joshi et al. 2013; Li and Yeh 2001). Among Bambara groundnut landraces, brown Bambara groundnut starch had a slightly higher amylose content (approx. 35%) than maroon and cream Bambara groundnut starches. Previous studies on Bambara groundnut similarly found some minor variations in amylose contents of starches extracted from Bambara groundnut varieties grown in Cameroon (Kaptso et al. 2014) and Thailand (Sirivongpaisal 2008). The amylose content of different starches may vary with the botanical source, growth location, and genotypic differences. Adebooye and Singh (2008), also reported variation in the amylose contents of starches extracted from two cowpea varieties grown in the same location.

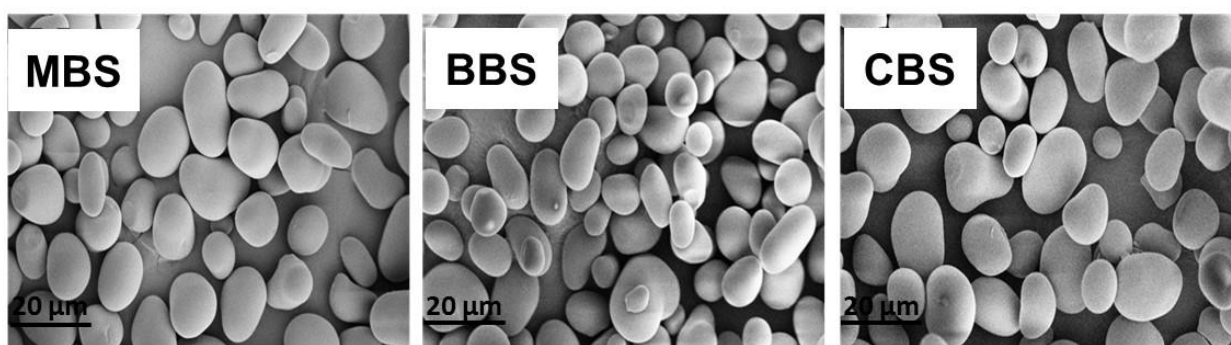


Figure 4.2 Micrographs of starches extracted from Bambara groundnut landraces
Magnification, $\times 1500$, MBS: Maroon Bambara groundnut starch, BBS: Brown Bambara groundnut starch, CBS: Cream Bambara groundnut starch

4.3.2 XRD

All Bambara groundnut starches exhibited strong peaks at 15° (2θ), a doublet at 17° and 18° (2θ) and a single peak at 23° (2θ) (Figure 4.3), suggesting the A-type crystallinity pattern. Similarly, Kaptso et al. (2014) and Sirivongpaisal (2008) reported the A-type pattern for starch extracted from Bambara groundnut grown in Cameroon and Thailand, respectively. However, other authors observed the C-type pattern typical of most pulse starches in Bambara groundnut starch (Afolabi 2012). It is possible to observe differences in crystallinity patterns of starch from the same species. These variations may depend on growing conditions and cultivar differences. For instance, both the A and C-type pattern were reported for some varieties of mung bean starch (Hoover et al. 1997; Ohwada et al. 2003) and Mexican yam bean starch (Forsyth et al. 2002).

The relative crystallinity of brown Bambara groundnut starch was slightly lower than those extracted from maroon and cream Bambara groundnut (Table 4.1). This may be explained by its slightly high amylose content (Table 4.1). Since the side chains of amylopectin form the crystalline structure in starches, the relative crystallinity should be inversely related to the amylose content (Sandhu and Lim 2008).

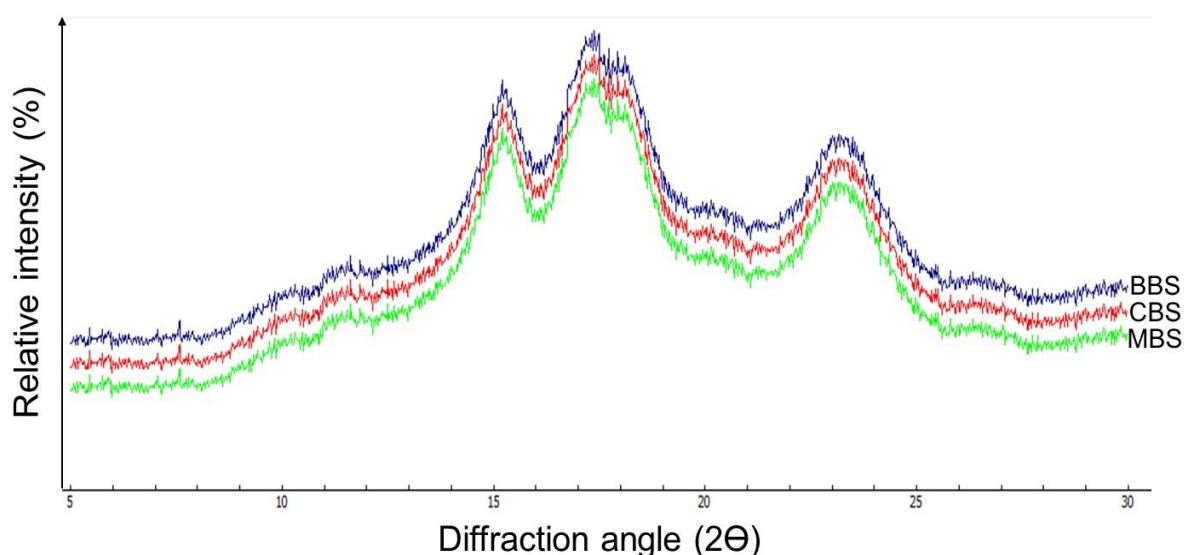


Figure 4.3 X-ray diffractograms of starches extracted from Bambara groundnut landraces

MBS: Maroon Bambara groundnut starch

BBS: Brown Bambara groundnut starch

CBS: Cream Bambara groundnut starch

4.3.3 FTIR

Bambara groundnut displayed characteristic FTIR bands associated with starch which was similar among landraces. All starches showed complex vibrations in the region below 800 cm^{-1} because of the skeletal vibration of the glucose pyranose ring. A broad band in the region of $3000\text{--}3600$ was observed with a peak at approximately 3431 cm^{-1} (Figure 4.4). This peak could be attributed to OH stretching (Ottenhof et al. 2003; Zhang and Han 2006). Similar FTIR band patterns were reported for Bambara groundnut starch (Afolabi 2012). Maroon Bambara groundnut starch showed lower peak intensities than did brown and cream Bambara groundnut in the C-H stretching region of $2800\text{--}3000\text{ cm}^{-1}$. These differences in peak intensities could be linked to variations in amylose contents (Kizil et al. 2002). Other peaks were observed around 1650 cm^{-1} , which could be attributed to bending vibrations of H_2O absorbed in the amorphous regions of starch (Cael et al. 1974; Kizil et al. 2002; Zeng et al. 2011). Kizil et al. (2002), also observed peaks in the same region (1650 cm^{-1}) for potato, maize and wheat starches.

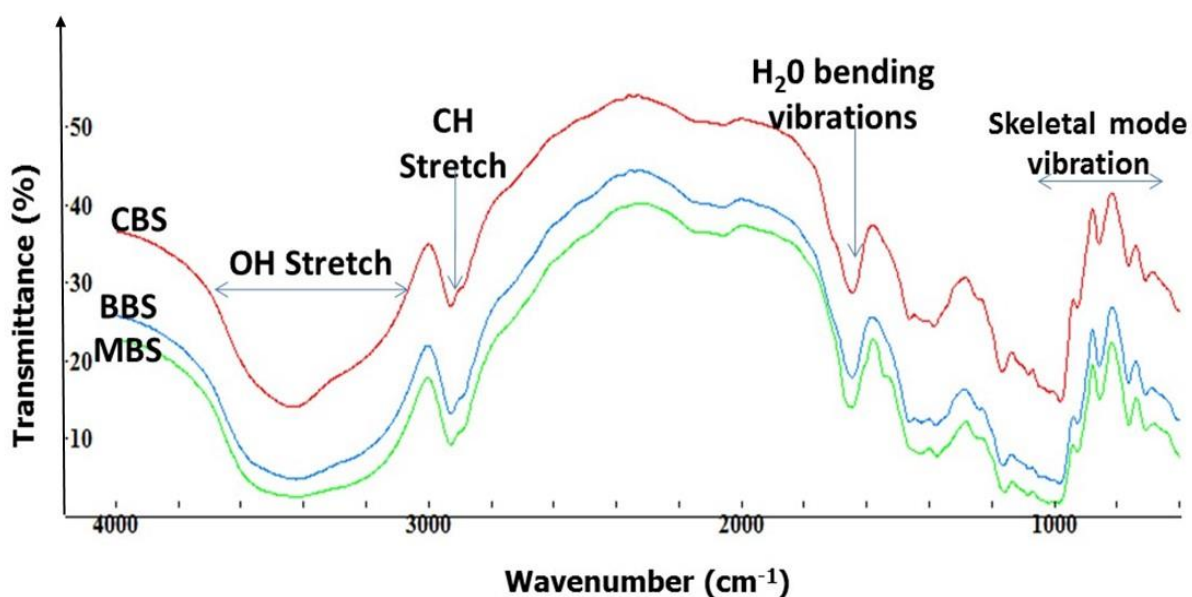


Figure 4.4 FTIR spectra of starches extracted from Bambara groundnut landraces

MBS: Maroon Bambara groundnut starch, BBS: Brown Bambara groundnut starch, CBS: Cream Bambara groundnut starch

4.3.4 DSC

Bambara groundnut starches showed slight differences in onset Gelatinisation temperature (T_o), peak Gelatinisation temperature (T_p), conclusion Gelatinisation temperature (T_c) and Gelatinisation enthalpy (ΔH_{gel}) (Figure 4.5 & Table 4.2). The T_p of Bambara groundnut starches

varied between 73 and 78 °C (Figure 4), which is in agreement with previous reports on Bambara groundnut (Afolabi 2012; Kaptso et al. 2014; Sirivongpaisal 2008). However, the peak Gelatinisation temperatures of Bambara groundnut starches were high in comparison with values reported for maize and potato starches, respectively (Joshi et al. 2013; Li and Yeh 2001). This could be attributed to differences in amylose contents of Bambara groundnut (Table 4.2), maize (approx. 25%) and potato (14.9-23.2%) starches (Joshi et al. 2013; Li and Yeh 2001). However, Liu et al. (2015a) found significantly reduced peak Gelatinisation temperatures for pea starches, which had comparable levels of amylose contents to Bambara groundnut starches (Table 4.2). These findings suggest that amylose content is not the only factor that could affect starch Gelatinisation. According to some reports, the melting temperature of starch could depend on the distribution of amylopectin short chains rather than the proportion of amylose to amylopectin (Noda et al. 1996).

The Gelatinisation enthalpy (ΔH_{gel}) of Bambara groundnut starches were not very different (approx. 13 J/g) (Figure 4.5). Kaptso et al. (2014), also observed slight differences in ΔH_{gel} for starch extracted from two Bambara groundnut varieties grown elsewhere. However, higher ΔH_{gel} values up to 25.2 J/g have been reported for Bambara groundnut starch by some authors (Afolabi 2012). Variation in ΔH_{gel} values may be attributed to differences in the extent of interactions between the double helices forming the crystalline region of the respective starches (Zhou et al. 2004).

Table 4.2 Thermal properties of Bambara groundnut starches

Parameters	Bambara groundnut landraces		
	Maroon	Brown	Cream
Onset temperature (°C)	72.0 ^a ±0.2	68.3 ^b ±0.3	70.9 ^a ±0.2
Peak temperature (°C)	77.5 ^a ±0.1	73.1 ^b ±0.4	76.4 ^a ±1.0
Conclusion temperature (°C)	84.4 ^a ±0.1	77.4 ^b ±0.1	82.5 ^a ±0.1
Enthalpy of Gelatinisation (J g ⁻¹)	12.9 ^b ±0.1	13.6 ^a ±0.2	12.7 ^b ±0.1

Mean ± SD. Mean with different superscript letters along a row are significantly different ($p < 0.05$).

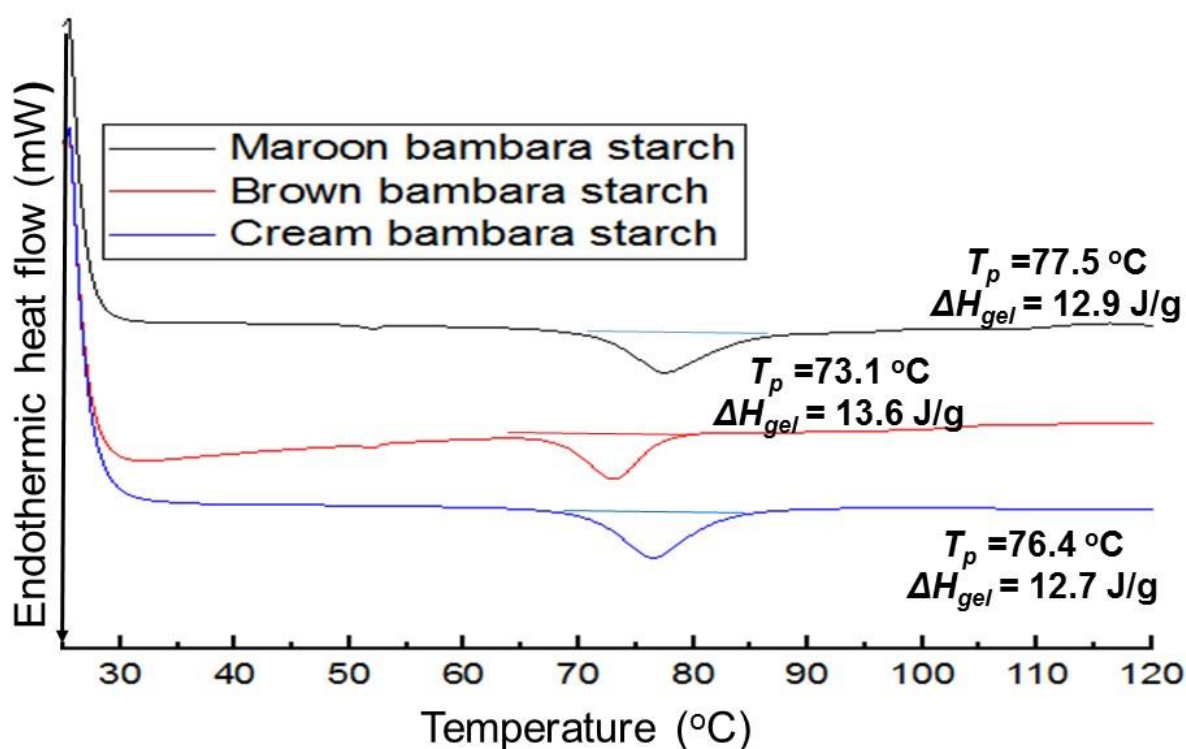


Figure 4.5 Typical thermograms of starches extracted from Bambara groundnut landraces

4.3.5 Swelling power

Bambara groundnut starches showed similar swelling behavior. The swelling power of Bambara groundnut starches progressively increased at a temperature range of 70 to 90°C (Figure 4.6). The rapid increase in swelling power at temperatures above 70°C have been attributed to melting of starch crystallites, which confirms Gelatinisation (Hoover and Sosulski 1985). Sirivongpaisal (2008), reported slightly high swelling power for Bambara groundnut starches, which could be attributed to their low amylose content (approx. 22%) compared to the studied landraces (Table 4.1). Amylose has been suggested to restrict starch swelling behavior (Naidoo et al. 2015). According to Tester and Morrison (1990b), swelling of starch is primarily a function of amylopectin while amylose and lipids act as a diluent. However, in some instances, starches with significantly high amylose content did not show restricted swelling (Kaur and Sandhu 2010; Kaur et al. 2010; Naidoo et al. 2015). Factors such as the molecular structure of amylopectin and the magnitude of interaction within the amorphous and crystalline regions have also been suggested to influence starch swelling (Singh et al. 2003).

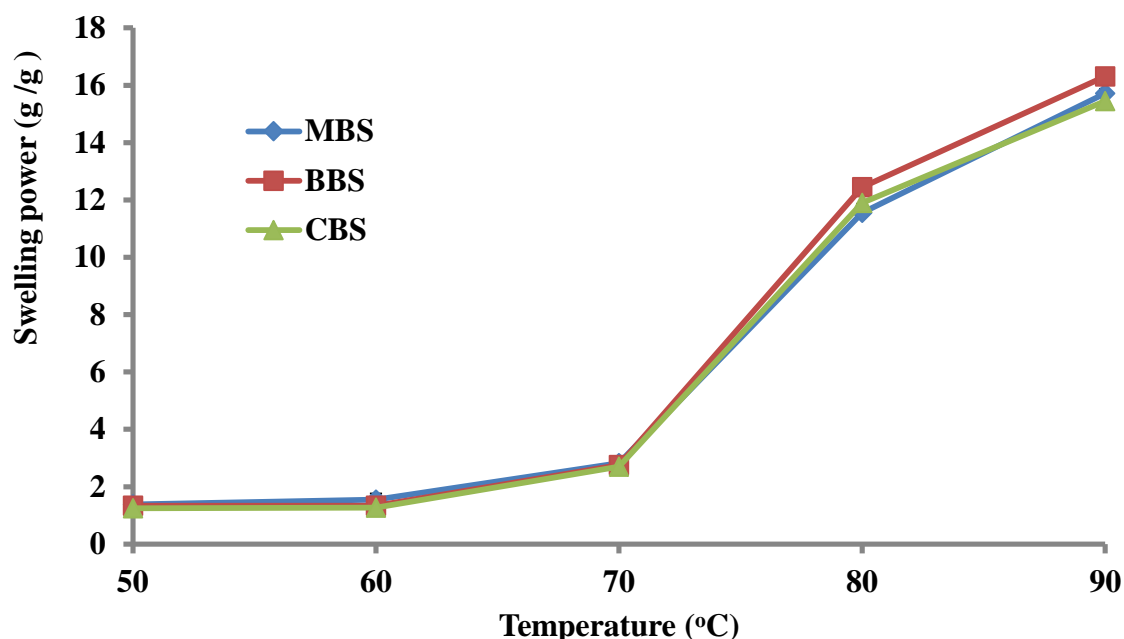


Figure 4.6 Swelling power of Bambara groundnut starches

MBS: Maroon Bambara groundnut starch, BBS: Brown Bambara groundnut starch, CBS: Cream Bambara groundnut starch.

4.3.6 Pasting

Bambara groundnut starches showed similar pasting profile curves for the three landraces (Figure 4.7). The pasting temperature of Bambara groundnut starches varied between 77.6-80.4°C (Table 4.3). These values are within the range reported in the literature for Bambara groundnut (Adebowale et al. 2002; Adebowale and Lawal 2002; Sirivongpaisal 2008) and cowpea starches (Adebooye and Singh 2008). In comparison with other crops, the pasting temperatures of Bambara groundnut starches are intermediate between the values reported for potato starch (66.2-68.6°C) (Gałkowska et al. 2014; Joshi et al. 2013) and normal maize starch (77-88°C) (Gałkowska et al. 2014; Joshi et al. 2013). This is in agreement with the previous report on lentil starch (Joshi et al. 2013). Starch extracted from cream Bambara groundnut showed the lowest peak viscosity compared to brown and maroon Bambara groundnut starches, which were similar (Table 4.3). The peak viscosity also referred to as “swelling peak” may be influenced by starch composition, structure and the presence of other minor components of starch such as lipids (Tester and Morrison 1990b). Starches with high amylose contents would show low peak viscosity due to restricted swelling of starch granules (Huang et al. 2015). However, in this study, the variation in peak viscosities did not seem to show any inverse correlation with amylose contents of starches. Previous studies similarly found that differences

in amylose contents were not sufficient to explain the variation in peak viscosity of starch (Adebooye and Singh 2008; Han and Hamaker 2001; Kaur and Sandhu 2010; Kaur et al. 2010). Huang et al. (2007), associated the presence of a high proportion of long amylopectin chains in cowpea starch with its high peak viscosity compared to those of chickpea and yellow pea starches. Thus, the variation in peak viscosity of Bambara groundnut starches could possibly be linked to differences in the chain length of amylose (Jane and Chen 1992; Ratnayake et al. 2001) and amylopectin components of these starches (Huang et al. 2007; Jane et al. 1999). Furthermore, among the studied landraces, brown Bambara groundnut starch showed the highest setback (127.1 RVU) and final (339.5 RVU) viscosities. This may be associated with its significantly higher amylose content compared to maroon and cream Bambara groundnut starches (Table 4.1). The re-association of starch as double helices upon cooling is responsible for the setback viscosity of starches and the extent of this re-association during the cooling phase is closely related to the amylose contents of Bambara groundnut starches. The final pasting viscosity results of Bambara groundnut landraces suggest that their starches can be potentially used as thickening agents in food application.

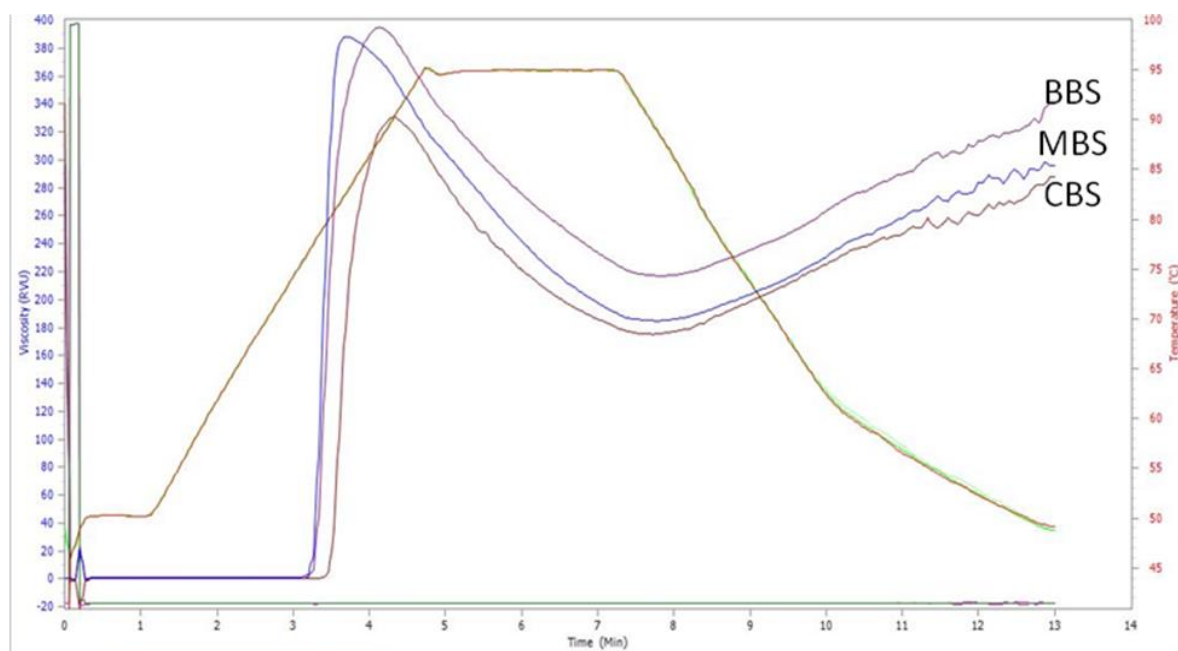


Figure 4.7 Typical pasting curves of Bambara groundnut landrace starches

Table 4.3 Pasting properties of starches extracted from Bambara groundnut landraces

Parameter	Bambara groundnut Landraces		
	Maroon	Brown	Cream
Peak viscosity (RVU)	383.1 ^a ±1.3	385.6 ^a ±1.5	329.3 ^b ±0.6
Trough viscosity (RVU)	184.5 ^b ±0.4	212.4 ^a ±1.0	174.3 ^b ±1.2
Breakdown viscosity (RVU)	198.7 ^a ±1.1	173.2 ^b ±0.9	155.0 ^c ±1.9
Final viscosity (RVU)	289.4 ^b ±0.4	339.5 ^a ±1.4	286.6 ^b ±1.2
Setback viscosity (RVU)	104.9 ^b ±0.4	127.1 ^a ±0.2	112.3 ^{ab} ±0.0
Pasting temperature (°C)	77.6 ^b ±0.1	77.6 ^b ±0.1	80.4 ^a ±0.5
Peak time (min)	3.7 ^c ±0.1	4.1 ^b ±0.0	4.4 ^a ±0.1

Mean ± SD. Mean with different superscript letters along a row are significantly different ($p < 0.05$).

4.3.7 In-vitro digestibility

The nutritional starch fractions were similar among Bambara groundnut landraces (Table 4.3). Rapidly digestible starch and slowly digestible starch fractions were about 12 and 16% of the total starch fractions, respectively. However, Bambara groundnut starches contained a substantial amount of resistant starch fractions (69.7-72.6%). This result is in agreement with previous reports on native legume starches (Du et al. 2014; Kaur and Sandhu 2010; Kaur et al. 2010; Liu et al. 2015a). Further, the resistant starch contents of Bambara groundnut starches were substantially higher than the values reported for native maize starches (5-20%) (Chung et al. 2009a; Chung et al. 2009b; Chung et al. 2008b). Cereal starches have relatively lower amylose contents compared to pulse starches including Bambara groundnut starch (Table 1). From previous reports, pulse starches with high amylose contents have been found to show high resistance to digestive enzymes (Hoover and Sosulski 1985). The double helical structure of amylose is presumably not accessible to the amylase enzyme (Naidoo et al. 2015). The proportion of resistant starch and predicted glycemic index (approx. 40) of starches from Bambara groundnut landraces compare favorably with reports on common bean and pigeon pea starches (Du et al. 2014; Kaur et al. 2010; Sandhu and Lim 2008).

Table 4.4 Nutritional starch fractions and predicted glycaemic index of starches extracted from Bambara groundnut landraces

Starch	RDS (%)	SDS (%)	RS (%)	GI
Maroon Bambara groundnut	12.3 ^b ±0.1	16.3 ^a ±0.2	71.4 ^a ±0.1	40.1 ^a ±0.1
Brown Bambara groundnut	13.3 ^a ±0.1	16.9 ^a ±0.1	69.7 ^b ±0.1	40.1 ^a ±0.2
Cream Bambara groundnut	11.5 ^b ±0.2	15.9 ^a ±0.2	72.6 ^a ±0.1	40.1 ^a ±0.1

Mean ± SD. Mean with different superscript letters along a column are significantly different ($p < 0.05$).

RDS: rapidly digestible starch, SDS: slowly digestible starch, RS: resistant starch, GI: glycemic index

4.4 Conclusions

Bambara groundnut starch consists mainly of oval shaped granules. The amylose contents of Bambara groundnut starches are significantly different among landraces. All starches exhibit the A-type crystalline pattern. Brown Bambara groundnut starch displays significantly low peak Gelatinisation temperature, which may be due to its high amylose content compared to cream and maroon Bambara groundnut landraces. However, Bambara groundnut starches show no inverse correlation of peak viscosities with their amylose contents. Bambara groundnut starches contain a considerable amount of resistant starch fractions. Starch extracted from Bambara groundnut landraces can potentially be used as thickening agents in food product and ingredient development.

CHAPTER FIVE

5.0 Effect of lipid types on complexation and some physicochemical properties of Bambara groundnut starch

Abstract

This study investigated the effect of stearic acid, linoleic acid and lysophosphatidylcholine on complex formation and physicochemical properties of Bambara groundnut starch in comparison with potato starch. Complexation index reached a maximum at 2% lipid concentration. Bambara groundnut 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 groundnut starch but increased the final viscosity. Pasting of Bambara groundnut and potato starches with lipids resulted in the formation of type I V-amylose complexes, with melting temperatures ranging from approx. 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 groundnut starch with lipids resulted in reduced digestibility suggesting their potential application in formulating foods for the management of diabetes.

5.1 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 (Kaur et al. 2012). Of these modification methods, chemical modification is the most widely studied (Bemiller 1997). However, chemicals such as epichlorohydrin, and hypochlorite solution used in starch modification have been found to raise food safety concerns (Li and Vasanthan 2003; Lui 2005). Consumers' awareness on food safety and the emergence of clean label starch technology has increased the search for natural alternatives in starch modification. Naturally occurring compounds like lipids have been reportedly used in starch modification for improved functionality (Kawai et al. 2012; Meng et al. 2014b; Zhang et al. 2012). Amylose can form single helical inclusion complexes known as V-amylose complex with ligands such as lipids. These complexes may be formed between amylose in native starch and endogenous lipids or formed upon Gelatinisation of starch in the presence of added lipids (Putseys et al. 2010). V-amylose complexes have been used to enhance starch pasting properties (D'Silva et al. 2011; Zhou et

al. 2007), prepare novel starches with slowly digestible property (Kawai et al. 2012; Zhang et al. 2012) and to protect volatile and sensitive ligands such as polyunsaturated fatty acids (Cohen et al. 2011; Zabar et al. 2010).

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 (Cui and Oates 1999; Kawai et al. 2012; Meng et al. 2014a; Siswoyo and Morita 2003). This is associated with the activation energy required for complex formation, which increases with increasing acyl chain length (Siswoyo and Morita 2003). Additional energy is required to enhance more hydrophobic interactions between the lipid and the amylose helix (Cui and Oates 1999; Siswoyo and Morita 2002). Kawai et al. (2012) observed higher complexing ability of lauric acid with potato starch than myristic, palmitic and stearic acids. Previous studies also found an increase in melting temperature of starch-lipid complexes compared to starch alone (Kawai et al. 2012). The transition temperature of these complexes may vary with the type of lipids. 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 (Kawai et al. 2012). Potato starch complexed with stearic acid similarly showed higher T_m (97°C) which was almost double that of potato starch-linoleic acid complex (Kawai et al. 2012). Two distinct types of V-amylose complex (type I and type II) based on thermal transitions may be observed for a given ligand (Biliaderis and Seneviratne 1990; Biliaderis and Galloway 1989; Karkalas et al. 1995). Type I complexes are predominantly amorphous and generally dissociate at low temperatures between 95 and 105°C (Karkalas et al. 1995). Type II complexes, which can be further subdivided into type IIa and IIb complexes are semi-crystalline and dissociate at higher temperatures up to about 121°C (Karkalas et al. 1995). So far, most studies on V-amylose complex mainly focused on conventional starch sources such as maize and potato. Only a few reports on the modification of pulse starch with lipids have been found in the literature (Biliaderis and Tonogai 1991; Exarhopoulos and Raphaelides 2012; Raphaelides and Georgiadis 2007; Sun et al. 2013). The effect of lipid on mung bean starch was studied by Sun et al. (2013). 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 Gelatinisation of maize,

high amylose maize, and pea starches retarded granules destruction (Exarhopoulos and Raphaelides 2012).

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 (Afolabi 2012; Kaptso et al. 2014). Previous studies on the modification of Bambara groundnut starch focused primarily on annealing, heat-moisture treatment (Adebowale and Lawal 2002), oxidation, acetylation (Adebowale et al. 2002) and carboxymethylation (Afolabi 2012). Bambara groundnut starch contains moderately high amylose content (21-35%) (Kaptso et al. 2014; Sirivongpaisal 2008), 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 groundnut remains an underutilized crop in Africa. The underutilisation of many traditional crops such as Bambara groundnut could be due to insufficient research. Due to the relatively low cost of traditional crops and their agronomic advantage of being drought tolerant, these crops are currently being considered as an alternative starch source for various industrial applications in Africa. To promote the utilisation of Bambara groundnut beyond traditional usage, it is important to modify its starch component with lipids. Thus, it was necessary to investigate the effects of lipid types, stearic acid, linoleic acid and lysophosphatidylcholine on complex formation and physicochemical properties of Bambara groundnut 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.

5.2. Materials and methods

5.2.1 Materials

Bambara groundnut was obtained from Markathini Research station, Jozini, KwaZulu-Natal province, South Africa. Starch (Amylose content: 31.5%) was prepared from Bambara groundnut flour as described by Sirivongpaisal (2008). Potato starch (Amylose contents: 24.6%), glucose oxidase assay kit (No. GAGO-20), amyloglucosidase (No. 7095), amylase (No. 7545), guar gum (No. 4129), stearic acid (STE), linoleic acid (LIN) and lysophosphatidylcholine (LPC) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents used were laboratory grade.

5.2.2 Preparation of starch–lipid complex

To determine the optimum lipid concentration needed to form a complex with Bambara groundnut starch, STE, LIN and LPC were added to the starch at varying concentrations of 0, 0.5, 1, 1.5, 2, 2.5, 3 or 4% as described by D'Silva et al. (2011). Commercial potato starch was used as a reference sample.

5.2.3 Complexation index

The extent of complex formation between starch and lipids were determined as previously reported (Meng et al. 2014b). The complexation index (CI), which is a measure of reduction in iodine binding capacity, was evaluated using equation 1. The optimum lipid concentration was established as 2% for the three lipid types. Hence, this concentration was used for complexation in subsequent experiments.

$$CI\% = 100 \times (\text{ABS of reference} - \text{ABS of sample}) / \text{ABS of reference} \quad 5.1$$

ABS = Absorbance, Reference = starch without lipids

5.2.4 Pasting properties of starch-lipid mixtures

Bambara groundnut and potato starches were pasted with lipids using a Rapid Visco Analyzer (Newport Scientific, Australia), according to the method of Tang and Copeland (2007). 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 it manually using the 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. Starch-lipid pastes were dried in a freeze dryer (CHRIST, Germany) and kept at 4°C until analyzed.

5.2.5 Gel strength

The texture of starch gels obtained after cooling the pastes at 4°C for 4 days was analyzed as described by Shaikh et al. (2015) except that gels were compressed to 50% of the original size at a speed of 2 mm/s. The firmness of gel formed in the canister was analyzed using a Shimadzu texture analyzer (EZ-SX, China).

5.2.6 Confocal Laser Scanning Microscopy

Starch-lipid complexes were prepared for microscopy analysis according to the modified method of D'Silva et al. (2011). 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 the dark cold room for 12 h.

5.2.7 Thermal properties of dried starch-lipid complex

Thermal properties of the starch-lipid complex were examined according to the methods of Chang et al. (2013a) with a few modifications. Briefly, pasted freeze-dried complexed starch (3 mg) was directly weighed into the aluminum DSC pan and distilled water (12 μ 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 a 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.

5.2.8 X-ray diffraction

X-ray diffraction of the starch-lipid complex was conducted using Empyrean PANalytical diffractometer (Netherlands) operating at 40 kV with a target current of 40 mA as previously reported by Afolabi (2012) with slight modifications. 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 to 40 (2θ)° at a scanning speed of 0.06°/min.

5.2.9 In-vitro digestibility

Digestibility of the freeze-dried starch-lipid complexes was done as previously reported (Naidoo et al. 2015; Sandhu and Lim 2008). Briefly, porcine pancreatic α -amylase (3.89 g) was dispersed in water (25.7 ml), centrifuged for 10 min at 2500xg, and 18.7 ml of supernatant was collected. Amyloglucosidase (1 ml) diluted with 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 assay kits. Nutritional 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.

5.2.10 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 ($p < 0.05$).

5.3 Results and discussion

5.3.1 Complex index

Regardless of lipid types, the complex index (CI) of Bambara groundnut and potato starch-lipid mixtures progressively increased with increasing lipid concentration, reaching a maximum at 2% (Figure 5.1). The fatty acid structure such as the degree of unsaturation and the presence of polar head (as is the case for complex lipid, LPC) significantly influenced the degree of complexation with starch. Bambara groundnut starch seemed to complex better with stearic acid (STE), than with linoleic acid (LIN) and lysophosphatidylcholine (LPC). The CI of Bambara groundnut starch complexed with STE (62.9%) was higher than Bambara groundnut starch complexed with LIN and LPC which showed approximately 54%. The low CI value of Bambara groundnut starch complexed with LIN can be linked to the presence of double bonds which gives a kink in the molecular structure of LIN (Yamada et al. 1998). According to Yamada et al. (1998) the kink in cis-unsaturated 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 (Bhatnagar and Hanna 1994; Eliasson and Krog 1985; Krog 1971; Lagendijk and Pennings 1970; Raphaelides and Karkalas 1988; Tang and Copeland 2007; Zhou et al. 2007). However, in some exceptional cases, unsaturated fatty acids were reported to show higher complexing ability than the saturated types (Annor et al. 2015; Kawai et al. 2012; Meng et al. 2014a). Kawai et al. (2012) 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 suggests that many factors other than the lipid structure may influence CI. It has been suggested that double bonds in unsaturated lipids influence the crystal structure of V-amylose more than the yield (Karkalas et al. 1995). 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. 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 groundnut starch complexed with LPC was lower compared to that of Bambara groundnut starch complexed with STE and LIN. The low CI may be attributed 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 (Hauser et al. 1980). Possibly, the angles of the polar groups on LPC reduced its ability to form a complex with Bambara groundnut starch. Cheng et al. (2015) reported that the inclusion rate of LPC into potato starch 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 (Cheng et al. 2015; Wang et al. 2014). Further, NMR studies showed that debranched-potato starch-LPC complexes were formed by hydrophobic interactions between the alkyl chains of LPC and the helix cavity of the debranched-starch, with the rest of the LPC molecule lying outside the helix (Cheng et al. 2015). This may further explain the low CI of Bambara groundnut starch complexed with LPC (Figure 5.1).

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 groundnut starch. The amylose content of Bambara groundnut (approx. 32%) was higher than that of potato starch (approx. 25%). High amylose starches form high amount of V-amylose complexes compared to starch with low amylose contents (Eliasson et al. 1988; Gelders et al. 2004; Obiro et al. 2012b). Further, the degree of polymerization of amylose in these starches as indicated by previous studies (Eliasson et al. 1988; Gelders et al. 2004; Obiro et al. 2012b) may also have influenced their CI values.

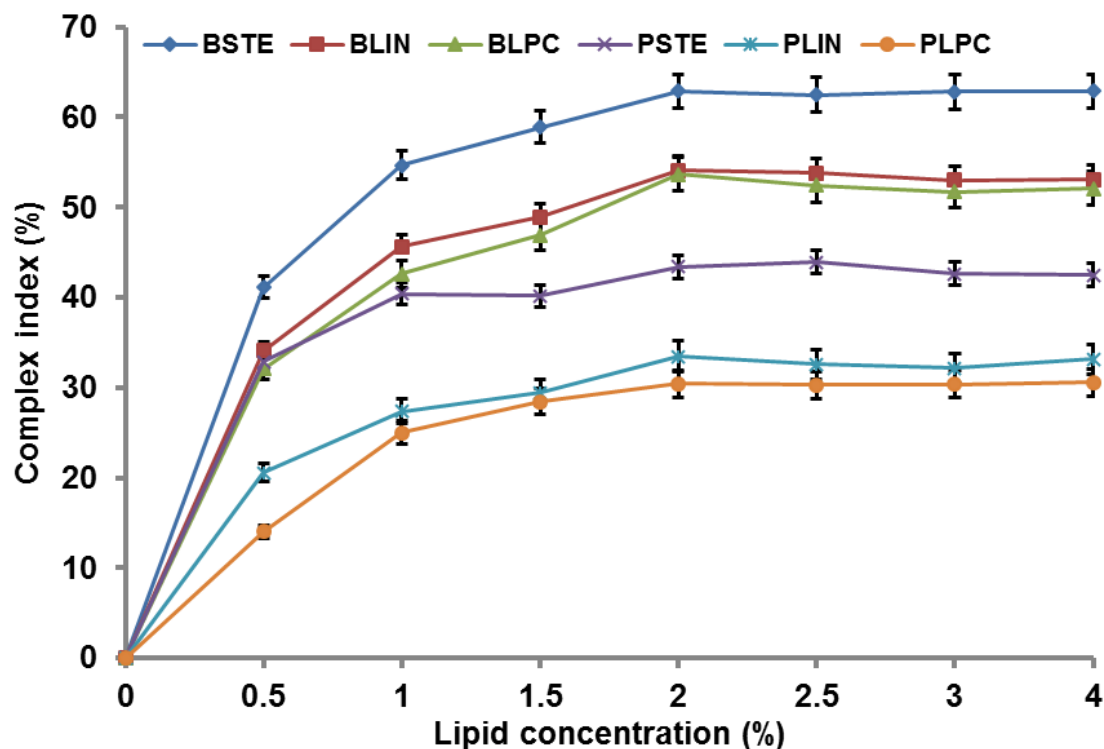


Figure 5.1 Effect of lipid on complex index of gelatinized Bambara groundnut and potato starches

B: Bambara groundnut starch, P: Potato starch, STE: Stearic acid, LIN: Linoleic acid, LPC: Lysophosphatidylcholine

5.3.2 Pasting

The pasting properties of Bambara groundnut and potato starch were significantly altered with lipid addition (Table 5.1). Peak viscosity (398.1 RVU) of Bambara groundnut starch reduced by approximately 9% when pasted with STE. Bambara groundnut starch complexed with LIN and LPC showed slightly lower reduction (approx. 7%) in peak viscosity. Variations in peak viscosity could be attributed to differences in the degree of complex formation of studied lipids with starch (Figure 5.1). Reductions in peak viscosity after pasting of rice starch with stearic or linoleic acid (Zhou et al. 2007), maize starch pasted with myristic, palmitic (Raphaelides and Georgiadis 2006) or stearic acid (D'Silva et al. 2011; Obiro et al. 2012a; Ocloo et al. 2016; Raphaelides and Georgiadis 2006) has been attributed to the formation of V-amylase complexes. Zhou et al. (2007) similarly reported higher reductions in peak viscosity of rice 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 (Kim and Walker 1992). Peak viscosity of potato starch with or without lipids was substantially higher compared to Bambara groundnut starch (Table 5.1).

The high phosphate monoester content of potato starch as reported by some authors (Jane et al. 1999; McPherson and Jane 1999), may have facilitated hydration and swelling of starch granules. This may have contributed to the higher peak viscosity of potato starch compared with Bambara groundnut starch. Some authors similarly observed an increase in peak viscosity of starches pasted with different lipids (Liang et al. 2002; Wang et al. 2015b). The setback viscosity (111.4 RVU) of Bambara groundnut starch reduced with lipid addition reaching approximately 104, 98 and 96 RVU for Bambara groundnut 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 towards 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 (D'Silva et al. 2011; Richardson et al. 2004).

Table 5.1 Pasting properties of Bambara groundnut 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 ^d ±0.1	189.9 ^d ±0.4	208.2 ^c ±0.3	280.7 ^{ab} ±0.2	111.4 ^{ab} ±0.1	77.6 ^a ±0.5	3.7 ^{ab} ±0.2
BSTE	361.1 ^e ±0.6	201.5 ^c ±0.9	164.9 ^c ±0.4	305.0 ^a ±1.0	103.6 ^b ±0.1	78.0 ^a ±0.5	4.0 ^{ab} ±0.1
BLIN	371.5 ^f ±0.1	199.1 ^c ±1.8	162.0 ^c ±1.2	297.2 ^{ab} ±1.2	98.1 ^c ±0.2	78.8 ^a ±0.4	4.1 ^{ab} ±0.1
BLPC	372.1 ^f ±0.2	198.1 ^c ±0.3	173.9 ^c ±0.4	294.0 ^{ab} ±0.4	95.9 ^c ±1.2	79.3 ^a ±0.7	4.3 ^a ±0.1
PCON	801.5 ^c ±0.7	138.3 ^e ±0.2	663.2 ^a ±0.1	259.8 ^b ±0.4	121.4 ^a ±0.4	68.2 ^b ±0.4	2.9 ^c ±0.2
PSTE	865.0 ^a ±0.2	246.8 ^a ±0.3	618.2 ^b ±0.1	277.5 ^{ab} ±0.8	30.7 ^e ±0.1	68.4 ^b ±0.4	3.1 ^c ±0.1
PLIN	834.0 ^b ±1.2	251.7 ^a ±0.3	582.3 ^b ±0.2	274.3 ^{ab} ±0.3	22.7 ^e ±0.3	68.7 ^b ±0.2	3.2 ^c ±0.1
PLPC	831.0 ^b ±0.5	233.5 ^b ±0.2	598.5 ^b ±0.4	272.5 ^{ab} ±0.1	41.3 ^d ±0.2	68.3 ^b ±0.1	3.2 ^c ±0.2

Mean ± SD. Mean with different superscript letters along the column are significantly different (p≤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 groundnut starch, P: potato starch.

5.3.3 Confocal Laser Scanning Microscopy

Confocal laser scanning micrographs of the Bambara groundnut starch pasted without lipids showed distorted granules which fluoresced green (Figure 5.2A). 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 (Figure 5.2B-D). The presence of lipids in starch suspension did not prevent starch granule disintegration. This could be attributed to 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 (Exarhopoulos and Raphaelides 2012).

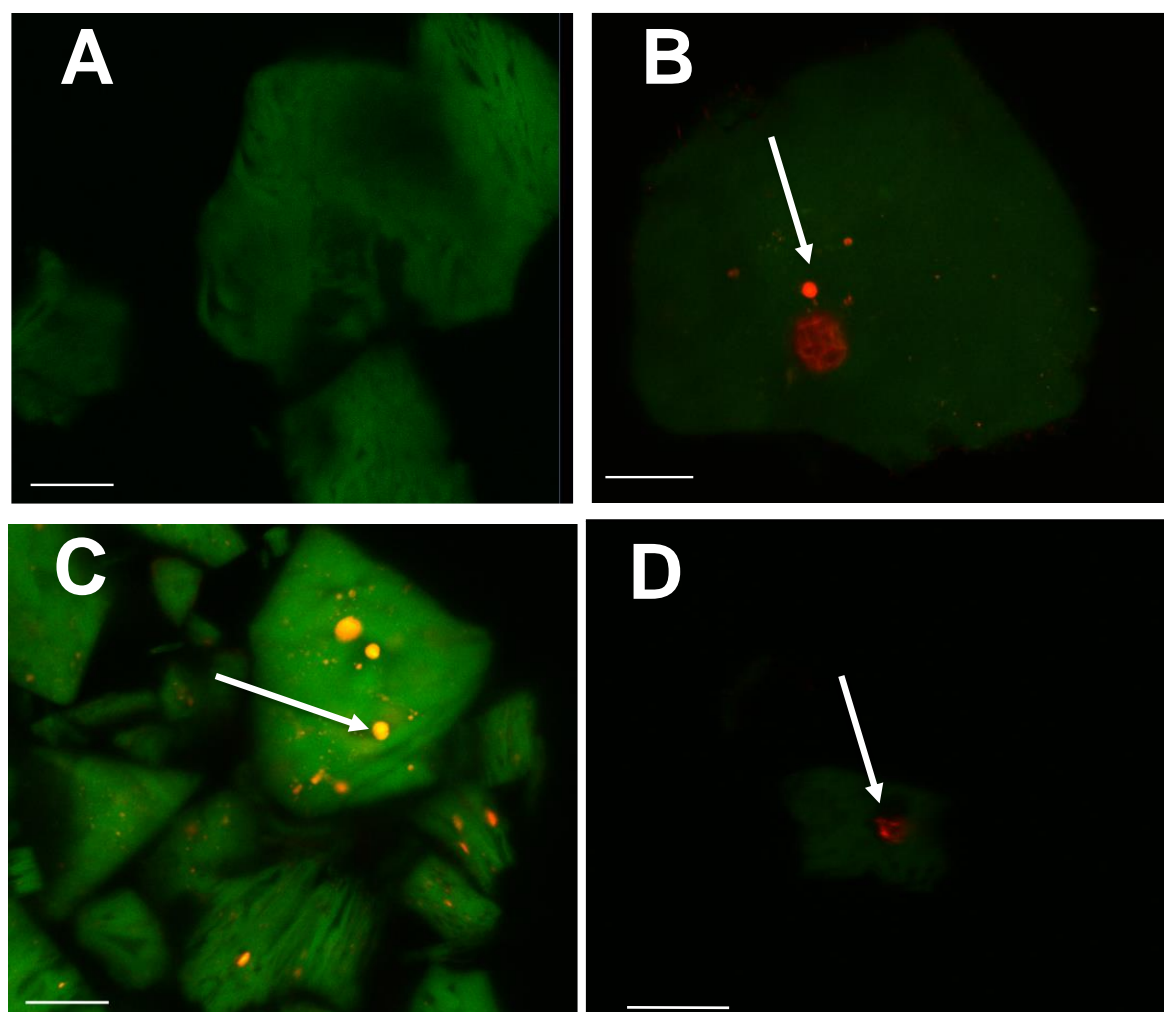


Figure 5.2 Confocal laser scanning micrographs of Bambara groundnut starch pasted with lipids

A: Control Bambara groundnut starch, B: Bambara groundnut starch pasted with stearic acid, C: Bambara groundnut starch pasted with lysophosphatidylcholine D: Bambara groundnut starch pasted with linoleic acid. Arrows indicate lipids within the starch granules. Scale bars for A, B, C and D are 20 μm

5.3.4 Texture of starch gels

Bambara groundnut starch gel with or without lipids showed higher gel firmness compared to potato starch (Figure 5.3). Bambara groundnut 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 maize starches Chung et al. (1998). However, with lipid addition, Bambara groundnut and potato starch gels showed a significant reduction in their firmness, suggesting that amylose in starch interacts with lipids forming 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 (D'Silva et al. 2011; Richardson et al. 2004).

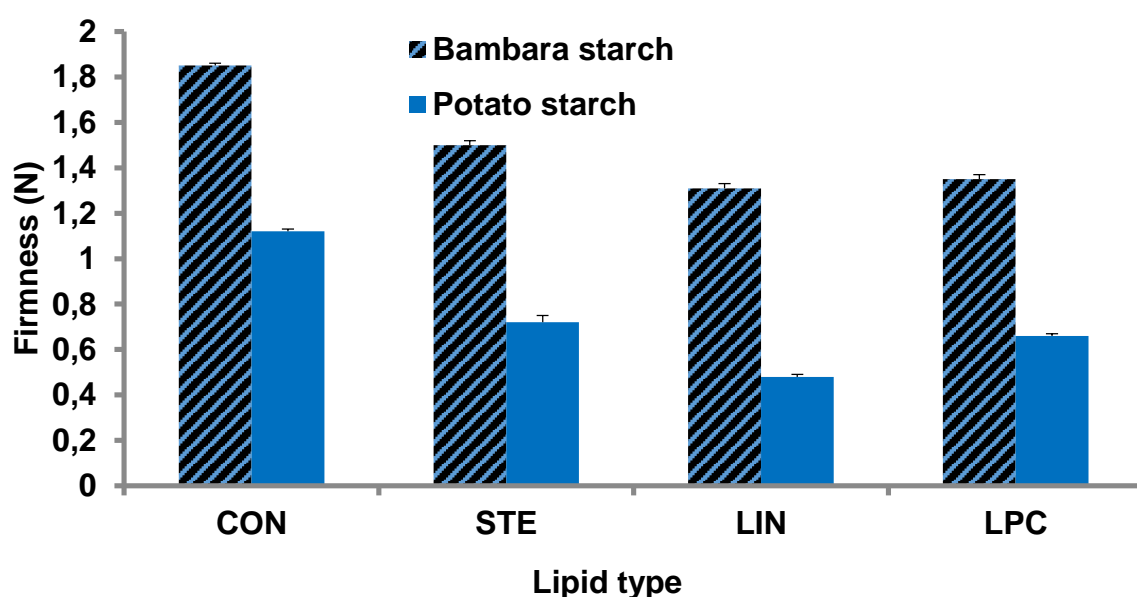


Figure 5.3 Gel strength of starch gels with or without lipid at 2%

CON: Control, STE: Stearic acid, LIN: Linoleic acid, LPC: Lysophosphatidylcholine

Error bars indicate standard deviation (N=3)

5.3.5 X-ray diffraction

X-ray diffraction (XRD) patterns of Bambara groundnut and potato starches pasted with lipids showed similar diffraction patterns (Figure 4 A&B) with a major peak at $2\theta = 19.9^\circ$ and minor peaks at $2\theta = 7.4^\circ$ and 12.9° suggesting the formation of the 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 (Liang et al. 2002; Obiro et al. 2012a; Zobel 1988). Among the starch-lipid complexes, Bambara groundnut starch complexed with STE showed higher intensity compared to those complexed with LIN and LPC, confirming better complex formation of Bambara groundnut with STE (Figure 1). The same trend was observed for potato starch-lipid complexes. Further, Bambara groundnut 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 (Chang et al. 2013a; 2013b; Obiro et al. 2012a; Ocloo et al. 2016).

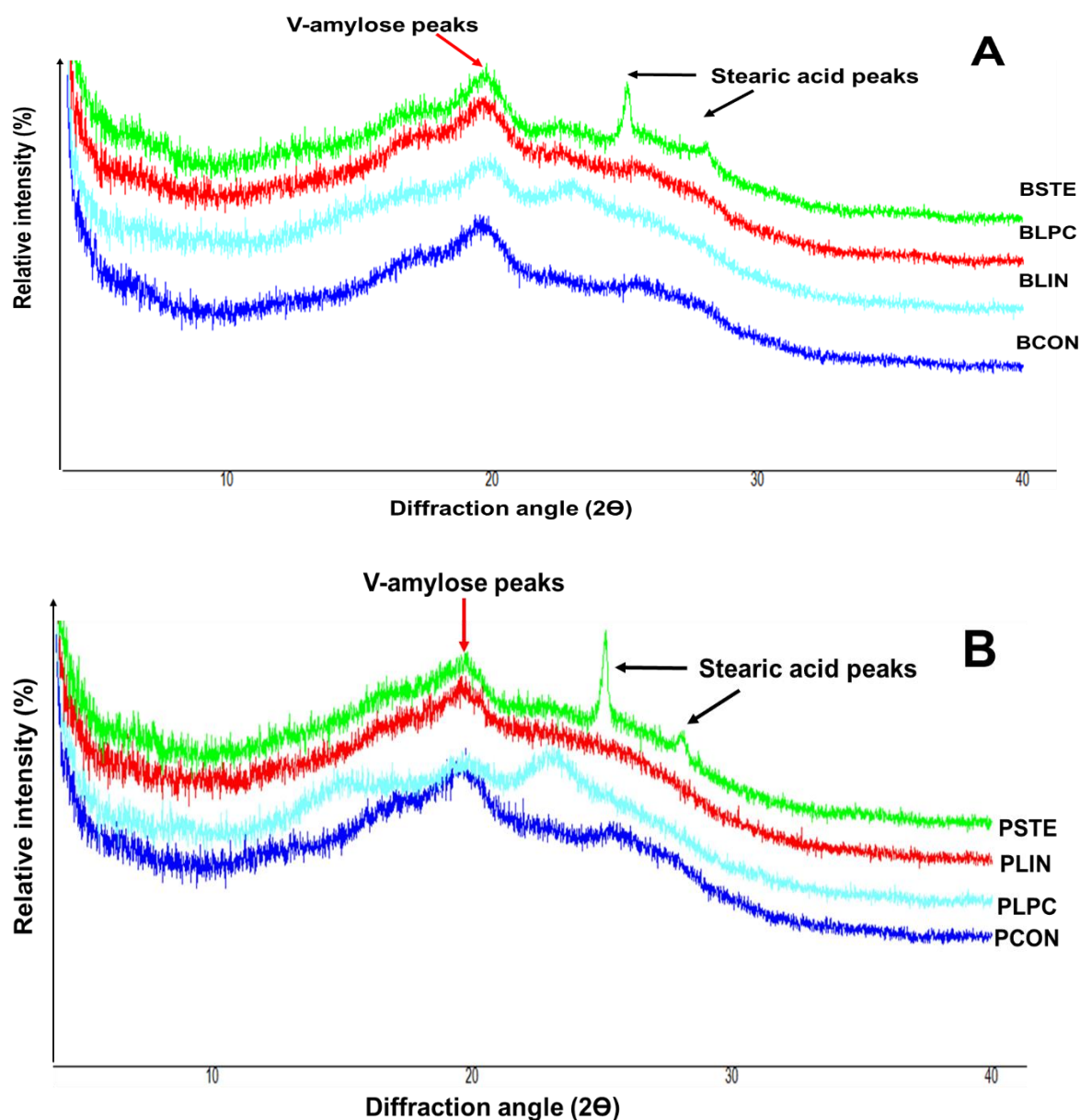


Figure 5.4 X-ray diffractograms of Bambara groundnut (A) and potato (B) starches pasted with lipids

B: Bambara groundnut starch, P: potato starch, STE: stearic acid, LIN: linoleic acid, LPC: lysophosphatidylcholine

5.3.6. Differential Scanning Calorimetry

Bambara groundnut and potato starches complexed with lipids generally showed higher melting temperatures (T_m) and enthalpies (ΔH_m) compared to starches pasted without lipids (Figure 5.5 & Table 5.2). Two endothermic transitions (Peak I & II) were observed in both starches complexed with stearic acid. The first transition (Peak I) which occurred at a temperature of approximately 68°C may be attributed to the melting of free uncomplexed stearic acid. This is in agreement with the XRD result (Figure 4 A&B) which also indicated the presence of free stearic acid.

Previous research on starch stearic acid complexes similarly observed a peak at about the same temperature (Obiro et al. 2012a; Ocloo et al. 2016; Raphaelides and Karkalas 1988). The second peak (Peak II) in Bambara groundnut and potato starches pasted with lipids showed T_m which varied from 97.5 to 100.9°C and suggests the melting of type I V-amylose complexes. According to previous research (Biliaderis and Seneviratne 1990; Raphaelides and Karkalas 1988), V-amylose complexes may show three endotherms with T_m values at <80°C, 80-104°C and at values > 104°C. These endotherms have been attributed to non-complex lipids, Type I V-amylose complexes, and Type II V-amylose complexes respectively (Biliaderis and Seneviratne 1990; Raphaelides and Karkalas 1988). Obiro et al. (2012a) similarly reported Type I V-amylose complex for teff and maize starches pasted with stearic acid for a short pasting time. High melting temperature of amylose-lipid complexes is reportedly associated with their stability (Kawai et al. 2012). Therefore, the stability of the Bambara groundnut and potato starch-lipid complexes were in the order BSTE > PLPC > PSTE > PLIN > BLPC > BLIN. Kawai et al. (2012) also observed a higher T_m (approx. 97°C) for potato starch complexed with STE than potato starch complexed with LIN (78°C). The reported T_m values of starch-fatty acid complexes increased with a decrease in the number of double bonds (Kawai et al. 2012).

The ΔH_m of Bambara groundnut 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 5.2). The ΔH_m of amylose-lipid complexes is suggested to reflect the amount of complex and the degree of order within the complex (Kawai et al. 2012). The higher ΔH_m of Bambara groundnut starch pasted with STE (Table 5.2) compared to other starch-lipid complexes may explain the higher complexing ability (Figure 5.1) and the higher intensity of the V-amylose peak (Figure 5.4).

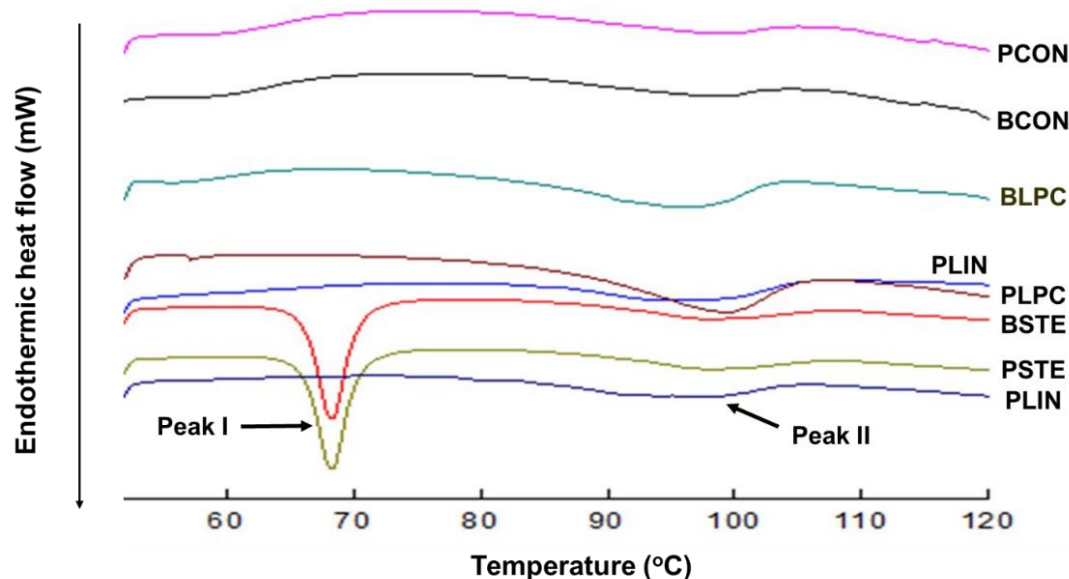


Figure 5.5 Typical thermograms of Bambara groundnut and potato starch pasted with lipids
 B: Bambara groundnut starch, P: Potato starch, CON: Control, STE: Stearic acid, LIN: Linoleic acid,
 LPC: Lysophosphatidylcholine, Peak I: Free stearic acid, Peak II: Melting of starch crystallites or V-amylose complexes

Table 5.2 Thermal properties of Bambara groundnut and potato starches pasted with different lipids

Sample	Peak I				Peak II			
	T_o (°C)	T_m (°C)	T_c (°C)	ΔH_m (J/g)	T_o (°C)	T_m (°C)	T_c (°C)	ΔH_m (J/g)
BCON	ND	ND	ND	ND	85.2 ^e ±1.0	93.1 ^d ±0.3	97.9 ^d ±0.1	0.4 ^f ±0.1
BSTE	66.0 ^a ±0.1	68.4 ^a ±0.1	70.9 ^a ±0.5	0.9 ^a ±0.1	90.4 ^d ±0.7	101.0 ^a ±0.6	103.7 ^c ±0.4	1.8 ^a ±0.1
BLIN	ND	ND	ND	ND	85.8 ^e ±0.1	97.5 ^c ±0.4	103.7 ^c ±1.3	1.1 ^b ±0.1
BLPC	ND	ND	ND	ND	97.5 ^a ±0.5	98.2 ^{bc} ±0.8	103.3 ^c ±0.3	1.0 ^b ±0.1
PCON	ND	ND	ND	ND	85.9 ^e ±0.9	90.0 ^e ±0.7	97.9 ^d ±0.3	0.2 ^g ±0.1
PSTE	67.1 ^a ±0.1	68.5 ^a ±0.1	70.7 ^a ±0.8	0.6 ^b ±0.1	97.2 ^a ±0.9	99.3 ^b ±0.8	105.3 ^b ±0.8	0.9 ^c ±0.1
PLIN	ND	ND	ND	ND	94.8 ^b ±0.2	98.3 ^{bc} ±0.5	106.9 ^a ±0.2	0.8 ^d ±0.1
PLPC	ND	ND	ND	ND	92.8 ^c ±0.8	101.9 ^a ±1.3	106.2 ^{ab} ±0.5	0.5 ^e ±0.2

Mean ± SD. Mean with different superscript letters along the column are significantly different ($p \leq 0.05$). CON: Control, STE: Stearic acid, LIN: Linoleic acid, LPC: Lysophosphatidylcholine, B: Bambara groundnut starch, P: potato starch. T_o , T_m , T_c and ΔH_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

5.3.7. In-vitro digestibility

The starch digestibility of Bambara groundnut was significantly influenced by complexation with lipid (Table 5.3). The addition of lipid caused about 7-14% reduction in RDS fractions depending on the lipid types. The same trend was observed for potato starch when complexed with lipid, but to a lesser extent. Among studied lipids, Bambara groundnut starch complexed with STE showed the highest level of SDS (approx. 12%) and RS (approx. 13%), similar to potato starch (SDS: approx. 5% and RS: approx. 10%). The reduction in digestibility of complexed starches may be attributed to the formation of amylose-lipid complexes as found in previous research (Meng et al. 2014b; Zhang et al. 2012). 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 restricts enzyme access into the starch granule interior. This may account for the reduction in the starch hydrolysis rate. The higher reduction in digestibility of Bambara groundnut starch complexed with STE may be linked to its high CI (Figure 5.1) and high ΔH_m (Table 5.2), which corresponds to complexing ability and the relative amount of formed complex respectively. Our results agree with previous findings on starch-lipid complexes (Guraya et al. 1997; Kawai et al. 2012; Lesmes et al. 2009). Kawai et al. (2012) similarly associated higher reduction in the amount of rapidly digested starch for potato starch-lauric acid complex, with high CI and high ΔH_m values. Guraya et al. (1997) also studied the complexing ability and digestibility of emulsifiers with different chain length and degree of unsaturation. Emulsifiers with saturated long-chain monoglycerides reportedly showed high complexing ability and reduced digestibility compared to unsaturated emulsifiers (Guraya et al. 1997).

Table 5.3 Nutritional starch fractions of Bambara groundnut starch pasted with different lipids

Sample	RDS (%)	SDS (%)	RS (%)
BCON	87.2 ^b ±0.1	3.7 ^d ±0.3	9.1 ^d ±0.4
BSTE	75.1 ^e ±0.1	12.0 ^a ±0.1	12.8 ^a ±0.1
BLIN	80.4 ^d ±0.3	7.6 ^c ±0.2	12.0 ^b ±0.1
BLPC	80.8 ^d ±0.1	8.5 ^b ±0.1	10.8 ^c ±0.1
PCON	90.6 ^a ±0.1	0.5 ^e ±0.2	8.9 ^e ±0.2
PSTE	85.2 ^c ±0.5	4.6 ^d ±0.6	10.1 ^c ±0.1
PLIN	86.1 ^c ±0.1	4.1 ^d ±0.2	9.8 ^d ±0.1
PLPC	86.5 ^c ±0.3	3.9 ^d ±0.4	9.3 ^d ±0.3

Mean ± 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 groundnut starch, P: potato starch.

5.4 Conclusions

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

CHAPTER SIX

6.0 Effect of high-pressure homogenization on structural, thermal and rheological properties of Bambara groundnut starch complexed with different fatty acids

Abstract

The effect of high-pressure homogenization (HPH) on the degree of complexation of different fatty acids with Bambara groundnut starch was studied. HPH significantly increased the complexation of Bambara groundnut starch with palmitic, stearic, oleic and linoleic acids. However, saturated fatty acids generally showed higher complexing ability than unsaturated types. For all fatty acids, Bambara groundnut starch showed high complex index than maize and potato starches, which could be associated with variation in amylose contents (22.5-31.5%). The formation of V-amylose crystalline types was confirmed by XRD with peaks at $2\theta = 7.4, 12.9$ and 19.9° . Bambara groundnut starch-fatty acid complexes displayed significantly higher melting temperatures (95.74 - 103.82°C) compared to native uncomplexed starch (77.32°C). Homogenized Bambara groundnut starch complexes were non-gelling while unhomogenized types produced weak gels, with $G' > G''$ in the range of 0.1 - 10 Hz. Complexation of Bambara groundnut starch with fatty acids using HPH may be employed in the production of modified starch with non-gelling properties and higher thermal stability suitable for certain industrial applications.

6.1 Introduction

Starches are modified to make them suitable for most industrial applications. Modification improves starch resistance to extreme processing conditions such as shear and heat that are usually encountered in the industry. Over the last few years, starch has been modified by physical, genetical, enzymatic and chemical methods (Bemiller 1997; Kaur et al. 2012). Chemical modification of starch seems to be the most widely used (Bemiller 1997). But, most of these chemicals (e.g. propylene oxide) are synthetically derived (D'Silva et al. 2011). Currently, natural alternatives, such as the use of lipids are being sought to produce clean label starches.

The structural and functional properties of starch-fatty acid complex may vary with fatty acid type and concentration (Exarhopoulos and Raphaelides 2012; Tang and Copeland 2007; Zhou et al. 2007), as well as processing conditions such as Gelatinisation time and temperature (Chang et al. 2014; Chang et al. 2013a; 2013b). The interaction between added lipids like fatty

acids and starch as revealed by X-ray diffraction studies, results in a distinct V-type crystalline structure known as V-amylose complex (Zobel 1988). Exarhopoulos and Raphaelides (2012) studied the morphological and structural studies of thermally treated starch-fatty acid systems. The presence of fatty acids effectively retarded the Gelatinisation process of maize starch, high amylose starch and pea starch (Exarhopoulos and Raphaelides 2012). Several starch modification processes using lipids such as fatty acids have been reported to improve starch functionality (Exarhopoulos and Raphaelides 2012; Obiro et al. 2012a; Ocloo et al. 2016; Zhou et al. 2007). For example, rice starch showed restricted granule swelling, reduced solubility and a lower rate of retrogradation with stearic acid addition (Zhou et al. 2007). Furthermore, starch-lipid complexes showed improved thermal stability compared to their native counterparts. (Kawai et al. 2012; Zhang et al. 2012). These changes in starch functionality are associated with the formation of amylose-inclusion complexes with added lipids. The rheological behaviour of starch-fatty acid complexes was investigated by Singh et al. (2002). Stearic acid addition reduced the storage modulus (G') in maize starch and increased the same parameter in potato starch. However, the addition of myristic acid decreased the G' in both starches (Singh et al. 2002). Thus, the rheological behaviour of various starches may depend on the starch source and fatty acid type.

Different methods have been explored in the preparation of starch-fatty acid complexes (Chang et al. 2014; Chang et al. 2013a; 2013b; D'Silva et al. 2011). The main purpose for using these methods is to increase the degree of complexation of starch with lipids. D'Silva et al. (2011), found that the degree of complexation of teff starch with 0.25% stearic acid increased by approximately 83% when the holding time was increased from 5 to 120 min during starch pasting. The increase in complexation was attributed to prolonged interaction between the starch and the added stearic acid (D'Silva et al. 2011). A promising method for increasing the degree of complexation of lipids with starch is the application of high-pressure homogenization (HPH) to gelatinized starch-fatty acid complexes. Previous research documented that the use of HPH can enhance the interaction between lipids and starch, resulting in better complexation (Lesmes et al. 2008; Meng et al. 2014a; Meng et al. 2014b; Yamada et al. 1998). Meng et al. (2014a) found that homogenization enhanced complexation of maize starch with fatty acids. Maize starch complexes prepared by HPH displayed higher complex index (almost double) compared to those prepared without homogenization (Meng et al. 2014a). However, the melting temperature of the complexes was not significantly affected by HPH (Meng et al. 2014a).

Most of the studies on V-amylose complex formation have focused on the use of conventional starch sources such as maize (Chang et al. 2013a; Meng et al. 2014a; Meng et al. 2014b; Obiro et al. 2012a) and potato (Kawai et al. 2012; Singh et al. 2002). However, pulse starches such as pea and Bambara groundnut (*Vigna subterranea*), which are generally high in amylose are also promising base material for the formation of V-amylose. Unlike pea starch, which has been reported to show some potential for industrial utilisation (Exarhopoulos and Raphaelides 2012; Hoover et al. 2010; Raphaelides and Georgiadis 2007), starch from Bambara groundnut has not been well researched. Bambara groundnut has potential for application in the industry, since it is a good source of starch (22-46%) (Adebowale et al. 2002; Afolabi 2012). Depending on source and variety, the amylose contents of Bambara groundnut starch have been found to vary between 20 and 35% (Kaptso et al. 2014; Sirivongpaisal 2008). A recent study showed that pasting Bambara groundnut starch with stearic acid, linoleic acid and lysophosphatidylcholine produced type I V-amylose complex which was confirmed by XRD. Complexing Bambara groundnut starch with fatty acids may be important to enhance its industrial potential and promote utilisation. Hence, this study investigated the effect of high-pressure homogenization on physicochemical properties of Bambara groundnut starch complexed with different fatty acids.

6.2 Materials and methods

6.2.1 Experimental materials

Bambara groundnut was obtained from Markathini Research station, Jozini, KwaZulu-Natal province, South Africa. Potato starch was obtained from Sigma-Aldrich (St. Louis, MO). Maize starch and fatty acids: palmitic, stearic, oleic and linoleic acids were purchased from Aladdin Chemistry Company (Shanghai, China).

6.2.2 Starch extraction and amylose contents determination

Starch was extracted from Bambara groundnut flour as described by Sirivongpaisal (2008). The amylose contents of extracted Bambara groundnut starch and those of maize and potato starches were 31.5%, 22.5% and 24.6% respectively, as determined by iodine binding method (Williams et al. 1970).

6.2.3 Preparation of starch fatty acid complexes by high-pressure homogenization

Starch-fatty acid complexes were prepared as previously reported by Meng et al. (2014b), except that fatty acids were added in concentration of 0, 1, 2, 3 and 4% to Bambara groundnut starch (dry weight basis). Starch-fatty acid mixtures were heated with constant stirring in a

water bath at 95°C for 20 min. The resulting starch-fatty acid pastes were homogenized using high-pressure homogenizer (APV2000 SPX, Germany) at 100 MPa for three passes. A portion of homogenized starch-fatty acid pastes was freeze-dried, while another portion was used for rheological measurement. Unhomogenized complexes were prepared in the same way as homogenized complexes but without homogenization. Maize and potato starches were included as reference samples.

6.2.4 Analyses

6.2.4.1 Complex index

The degree of complex formation between starch and fatty acids was determined using equation 1 as previously reported (Meng et al. 2014b). The complexation index (CI), is a measure of reduction in the iodine binding capacity of starch. The CI was determined at different fatty acid concentrations. After establishing the range of fatty acids for maximum complexation, structural; XRD, thermal and rheological properties of starch-fatty acid complexes were determined at 4% fatty acid concentration as described in sections below.

$$CI\% = 100 \times (\text{ABS of control} - \text{ABS of sample}) / \text{ABS of reference} \quad 6.1$$

ABS: Absorbance

6.2.4.2 X-ray diffraction

X-ray diffraction (XRD) of starch-fatty acid complexes was conducted using Empyrean PANalytical diffractometer (Netherlands). The diffractometer operated at 40 kV with a target current of 40 mA. Scanning was done from 5 ° to 30 ° (2θ) with an exposure time of 16 min 14 s, the step size of 0.026 ° and a time/step ratio of 229.5 s. (Obiro et al. 2012a).

6.2.4.3 Thermal properties of dried starch-lipid complex

Thermal properties of starch-fatty acid complex were determined as previously described by Meng et al. (2014a). Briefly, pasted freeze-dried complexed starch (3 mg) was directly weighed into the aluminum DSC pan and distilled water (9 µl) added. Pans were hermetically sealed and equilibrated for 24 h. Equilibrated pan containing the samples were directly heated from 20 to 140 °C at a rate of 10 °C/min. An empty pan was used as reference.

6.2.4.4 Rheology

The viscoelastic properties of starch gels were determined as previously reported by Wang et al. (2012) with slight modifications. Briefly, the viscoelastic properties of the gels were measured at 25°C and a strain of 1% which was in the range of the linear viscoelastic region

using a Rheometer (KNX2210 Malvern, UK). The spectra was obtained by recording storage modulus (G') and loss modulus (G'') as a function of angular frequency in the range 0.1 to 10 Hz.

6.2.4.5 Statistical analysis

All analyses were conducted in duplicate. Data were analyzed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference Test ($p < 0.05$).

6.3 Results and discussion

6.3.1 Complex index

Complex index (CI) of gelatinized Bambara groundnut starch-fatty acid complexes, generally increased with increased fatty acid concentration, reaching a plateau between 2-4% (Figure 6.1A&B). The degree of complexation of Bambara groundnut starch with fatty acids was affected by high-pressure homogenization (HPH) and fatty acids types. Homogenized Bambara groundnut starch-fatty acid complexes displayed significantly higher CI (Figure 6.1A) than unhomogenized ones (Figure 6.1B). This could be attributed to better dispersion of lipid in starch suspension during homogenization (Meng et al. 2014a; Meng et al. 2014b). Furthermore, saturated fatty acids, palmitic and stearic showed higher CI than unsaturated fatty acids (i.e. oleic and linoleic acids), even when unhomogenized. Molecular rigidity introduced by the presence of double bonds in unsaturated fatty acids have been reported to hinder access to the amylose helix (Yamada et al. 1998; Zhou et al. 2007) and thus reducing CI as observed for Bambara groundnut starch-lipid complexes. Our result is in agreement with previous research where cis-unsaturated fatty acids complexed poorly with amylose giving low V-amylose yields (Bhatnagar and Hanna 1994; Eliasson and Krog 1985; Krog 1971; Lagendijk and Pennings 1970; Raphaelides and Karkalas 1988; Tang and Copeland 2007; Zhou et al. 2007). Homogenized maize (Figure 6.1C) and potato (Figure 6.1E) starch-fatty acid complexes showed similar trend. However, the CI values of maize and potato starches were lower than those of Bambara groundnut starch. The amylose content of Bambara groundnut starch (31.5%) was higher than those of maize (22.5%) and potato (24.6%) starches respectively. The variation in CI among these starches could be due to differences in their amylose contents (Eliasson et al. 1988). Other factors such as the degree of polymerization of amylose (Gelders et al. 2004; Godet et al. 1993a) may also influence the degree of complexation.

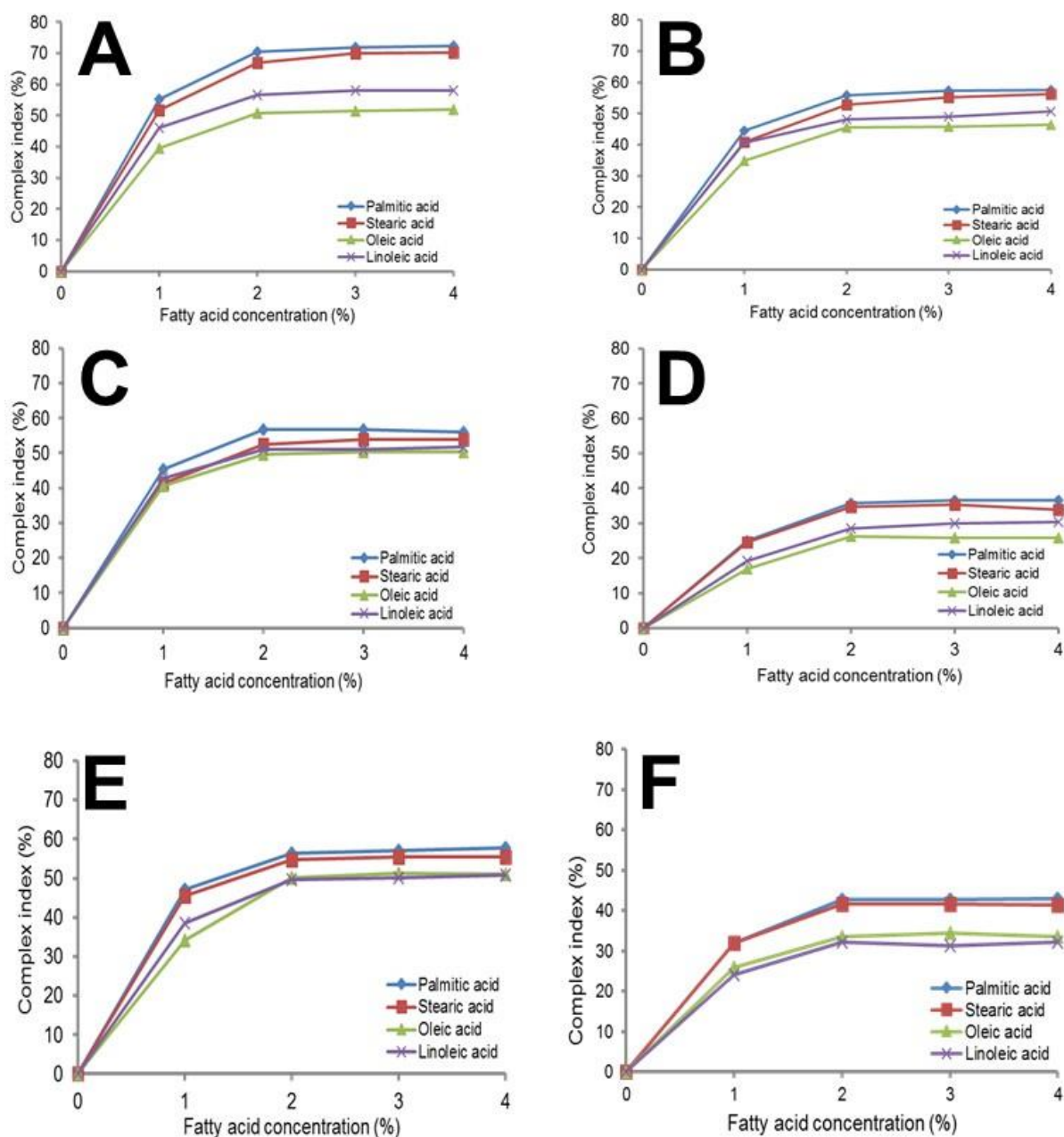


Figure 6.1 Complex index of gelatinized Bambara groundnut, maize and potato starches complexed with different fatty acids

- A: Homogenized Bambara groundnut starch-fatty acid complex
- B: Unhomogenized Bambara groundnut starch-fatty acid complex
- C: Homogenized maize starch-fatty acid complex
- D: Unhomogenized maize starch-fatty acid complex
- E: Homogenized potato starch-fatty acid complex
- F: Unhomogenized potato starch-fatty acid complex

6.3.2. XRD

The XRD pattern of native Bambara groundnut starch showed the A-type crystallinity pattern with strong peaks at 15° (2θ), a doublet at 17° and 18° (2θ) and a single peak at 23° (2θ) (Figure 6.2A). However, when Bambara groundnut starch was gelatinized with fatty acids, XRD analysis revealed the loss of its native crystallinity. Bambara groundnut starch complexed with fatty acids showed peaks at $2\theta = 7.4, 12.9$ and 19.9° (Fig. 2A-C), which is in agreement with the XRD peaks observed for V-amylose in previous studies (Exarhopoulos and Raphaelides 2012; Tang and Copeland 2007; Zobel 1988).

Similar V-amylose peaks were observed for maize (Figure 6.2B) and potato (Figure 6.2C) starch-fatty acid complexes. The peaks corresponding to V-amylose crystalline types were significantly higher for the homogenized starch-fatty acid complexes (Figure 6.2A-C), further suggesting better complexation with HPH. Peaks at $2\theta = 22^{\circ}$ and $2\theta = 24^{\circ}$ were observed in Bambara groundnut, maize and potato starches complexed with palmitic and stearic acids (Figure 6.2A-C). These peaks suggest the presence of free fatty acid aggregates (Chang et al. 2013a; 2013b; Obiro et al. 2012a; Ocloo et al. 2016).

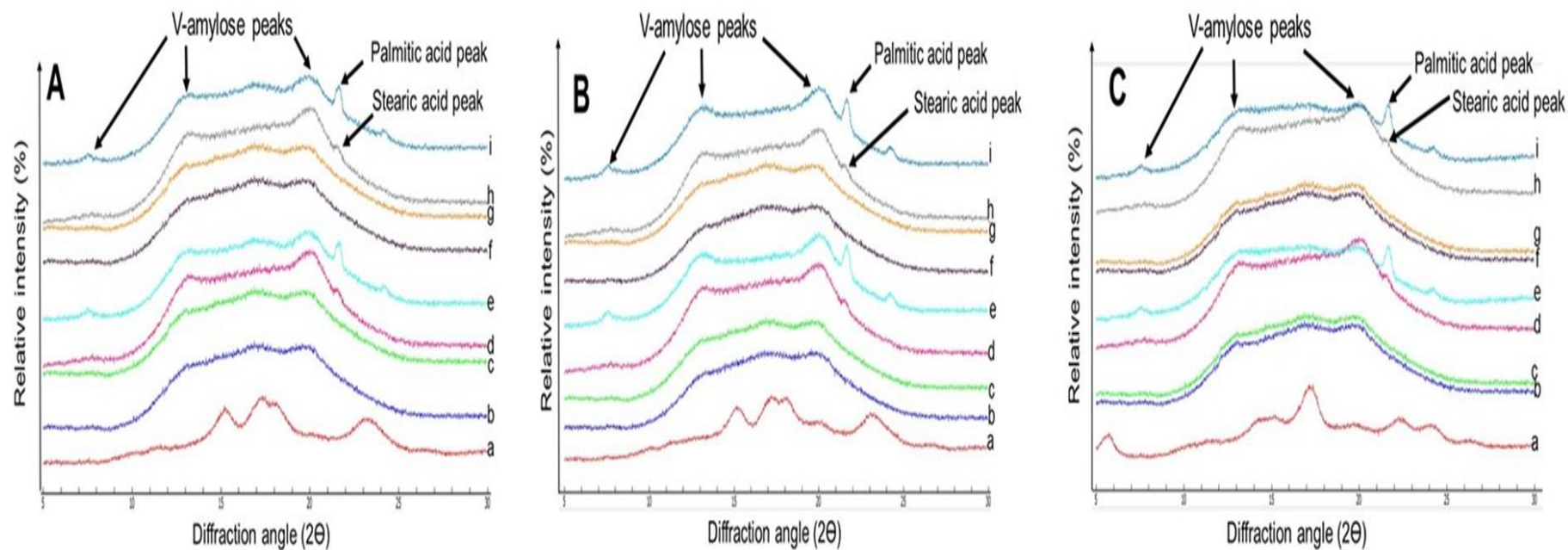


Figure 6.2 X-ray diffractograms of gelatinized Bambara groundnut, maize and potato starch complexed with different fatty acids

A: Bambara groundnut starch, B: Maize starch, C: Potato starch

a: native starch, b: oleic acid, c: linoleic acid, d: stearic acid, e: palmitic acid (unhomogenized complexes)

f: oleic acid, g: linoleic acid, h: stearic acid, i: palmitic acid (homogenized complexes)

6.3.3. DSC

The melting temperatures (T_m) of Bambara groundnut starch complexed with fatty acids were generally higher than their native counterparts (Figure 6.3a-b). Maize and potato starches displayed similar trend (Figure 6.3c-f). Homogenized starch-fatty acid complexes displayed broader transitions than unhomogenized samples (Figure 6.3). The T_m of Bambara groundnut starch-fatty acid complexes varied from 93.35-103.82°C (Table 6.1), which corresponds to the melting of type I V-amylose complex (Biliaderis and Seneviratne 1990; Raphaelides and Karkalas 1988). According to Kawai et al. (2012), T_m allows for the deductions of the helical length of V-amylose complexes and indicates their stability. For example, the T_m value of potato starch-fatty acid complexes was found to increase with increasing chain length of the fatty acid (Kawai et al. 2012). Cui and Oates (1999) reported similar trend for sago starch complexed with monoglycerides. Early studies also suggested that the length of a helical segment in the V-amylose structure is probably determined by the length of the lipid molecule within the helix, and by the number of molecules that lie end-to-end (Karkalas et al. 1995). Homogenized Bambara groundnut starch-fatty acid complexes, including those of maize and potato reference samples, displayed substantially higher ΔH_m values (4.68-6.77 J/g) than the unhomogenized ones (0.82-1.94 J/g) (Table 6.1). Previous studies reported that ΔH_m values of V-amylose complexes correspond to the amount of complex and the degree of order within the complex (Eliasson and Krog 1985; Kawai et al. 2012). The ΔH_m result is in agreement with the CI (Figure 6.1) and XRD (Figure 6.2). Fatty acid type significantly influenced the thermal stability of V-amylose complexes. Bambara groundnut starch complexed with saturated fatty acids (palmitic and stearic) displayed significantly ($p < 0.05$) higher thermal stability (i.e. T_m) than those complexed with unsaturated fatty acids (oleic and linoleic). Differences in thermal stability could be linked to the double bonds of unsaturated fatty acids, which give a kink and allow for partial inclusion into the amylose helix cavity. (Kaur and Singh 2000; Kawai et al. 2012). Bambara groundnut, maize and potato starches complexed with palmitic and stearic acid showed additional transitions at approximately 62°C (peak A) and 68°C (peak B) respectively (Fig. 3a-f). These transitions may be attributed to the melting of uncomplexed fatty acid. XRD results from this study (Figure 6.2) also revealed the presence of free fatty acids. Similar results have been reported in the literature (Chang et al. 2013a; Obiro et al. 2012a; Ocloo et al. 2016).

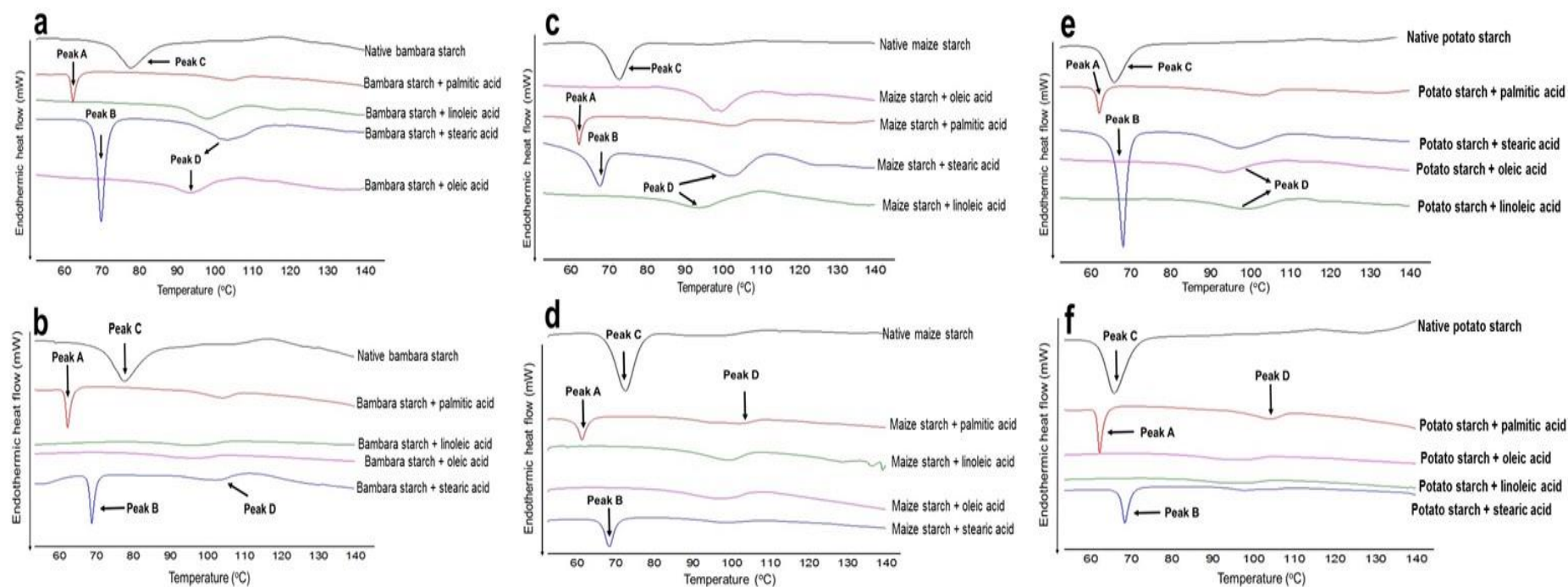


Figure 6.3 Thermograms of gelatinized Bambara groundnut, maize and potato starches complexed with different fatty acids

a: Homogenized Bambara groundnut starch, b: Unhomogenized Bambara groundnut starch, c: Homogenized maize starch

d: Unhomogenized maize starch, e: Unhomogenized potato starch, f: Homogenized potato starch

Table 6.1 Thermal properties of gelatinized starch-fatty acid complexes

Unhomogenized					Homogenized			
Bambara groundnut starch								
Fatty acid	T _o (°C)	T _m (°C)	T _c (°C)	ΔH _m (J/g)	T _o (°C)	T _m (°C)	T _c (°C)	ΔH _m (J/g)
Palmitic	89.55 ^{bc} ±0.85	103.82 ^a ±0.71	107.69 ^a ±1.30	1.94 ^a ±0.06	95.21 ^b ±0.04	103.62 ^a ±0.46	108.26 ^a ±0.19	6.77 ^a ±0.04
Stearic	92.13 ^b ±0.01	101.81 ^{ab} ±0.94	106.96 ^{ab} ±0.62	1.63 ^{ab} ±0.54	95.97 ^a ±0.11	103.67 ^a ±0.26	112.02 ^a ±0.18	6.52 ^a ±0.02
Oleic	89.14 ^{bc} ±0.18	95.74 ^c ±2.02	104.22 ^c ±0.79	1.34 ^{abcd} ±0.71	85.72 ^g ±0.70	93.35 ^d ±0.04	100.10 ⁱ ±0.06	5.11 ^b ±0.14
Linoleic	89.04 ^{bc} ±0.17	97.34 ^c ±0.23	103.09 ^d ±1.38	0.86 ^d ±0.03	90.55 ^d ±0.04	97.83 ^{bc} ±0.09	106.14 ^e ±0.30	5.69 ^b ±0.01
Maize Starch								
Palmitic	87.83 ^{cd} ±0.49	102.41 ^{ab} ±0.60	106.75 ^{abc} ±0.51	1.57 ^{abc} ±0.07	90.01 ^{de} ±0.16	102.39 ^a ±0.39	107.13 ^d ±0.17	6.76 ^a ±0.06
Stearic	90.04 ^{bc} ±0.11	97.99 ^{bc} ±0.47	105.22 ^{bcd} ±1.56	1.27 ^{bcd} ±0.02	92.35 ^c ±0.15	101.25 ^a ±0.19	109.06 ^b ±0.11	6.55 ^a ±0.06
Oleic	87.19 ^{cd} ±1.79	94.39 ^c ±1.42	104.24 ^d ±0.49	0.97 ^{cd} ±0.06	84.93 ^h ±0.02	92.56 ^d ±2.35	103.82 ^g ±0.74	5.41 ^a ±0.14
Linoleic	87.93 ^{cd} ±0.29	98.31 ^{bc} ±0.98	104.51 ^{cd} ±0.55	0.92 ^d ±0.08	91.69 ^c ±0.31	99.48 ^b ±0.27	105.03 ^f ±0.29	5.45 ^b ±0.05
Potato Starch								
Palmitic	95.60 ^a ±0.59	103.86 ^a ±0.45	108.43 ^a ±0.04	1.63 ^{ab} ±0.04	90.40 ^b ±0.72	102.24 ^a ±0.28	105.24 ^f ±0.02	5.87 ^b ±0.06
Stearic	92.28 ^b ±0.05	97.94 ^{bc} ±0.35	104.88 ^{bcd} ±0.28	1.43 ^{abcd} ±0.01	86.74 ^f ±0.04	96.27 ^c ±0.43	106.48 ^{de} ±0.03	5.64 ^b ±0.24
Oleic	85.64 ^d ±1.29	95.74 ^c ±1.02	103.22 ^d ±0.62	0.82 ^d ±0.02	84.75 ^h ±0.04	92.78 ^d ±0.16	101.14 ^h ±0.76	4.81 ^c ±0.01
Linoleic	89.22 ^{bc} ±0.75	95.49 ^c ±1.06	103.72 ^d ±0.33	0.83 ^d ±0.01	89.74 ^e ±0.21	98.29 ^b ±0.08	106.48 ^{de} ±0.06	4.68 ^c ±0.05

Mean ± SD. Mean with different superscript letters along a column are significantly different ($p < 0.05$).

T_o, T_m, T_c and ΔH_m are onset temperature, melting temperature, conclusion temperature and melting enthalpy respectively.

6.3.4. Rheology

The viscoelastic behaviour of Bambara groundnut starch gels was significantly affected by HPH and complexation with fatty acids (Figure 6.4A-B). The storage moduli (G') of Bambara groundnut starch gels were generally greater than their loss moduli (G'') (Figure 6.4A-B) in the frequency range of 0.1-10 Hz. Both G' and G'' showed a minor dependency on frequency. These results suggest that complexation produced weak gel structure, which is in agreement with previous studies (Martínez-Ruvalcaba et al. 2007). Bambara groundnut starch-fatty acid complexes produced gels with reduced G' and G'' compared to their native starch gel counterparts. The reduction in G' and G'' in the presence of lipids has been associated with the formation of V-amylose complex (Ahmadi-Abhari et al. 2015; Biliaderis and Tonogai 1991; Singh et al. 2002). Singh et al. (2002), also observed a reduction in G' and G'' of maize and potato starches complexed with myristic acid. In general, saturated fatty acids showed greater reduction in G' and G'' of Bambara groundnut starch gels when compared to the unsaturated types. This could be linked to better complexation of Bambara groundnut starch with saturated fatty acids as indicated by the CI (Figure 6.1). Furthermore, Bambara groundnut starch complexed with saturated or unsaturated fatty acids were non-gelling when prepared under homogenized condition. The non-gelling behaviour of starch paste with added lipids has also been observed in earlier studies (D'Silva et al. 2011; Richardson et al. 2004). Amylose-fatty acid complexes have been suggested to either prevent the formation or increase the spacing between junction zones. HPH may influence the rheological behaviour of starch-fatty acid gels in two ways. First, the applied pressure during homogenization enhances better interaction with fatty acid. Also, the higher level of starch disintegration during HPH may also slow down the re-association of amylose molecules during short term storage. Similar rheological behaviour was recorded for maize and potato starch gels. But the G' and G'' of Bambara groundnut starch gels were generally higher than the reference starches. Pulse starches are generally characterized by stronger gels compared to cereal and tuber starches. For instance, cowpea starch showed higher gel strength and higher G' and G'' compared to maize and potato starches, which was attributed to the presence of longer amylose chains in cowpea starch (Chung et al. 1998; Won et al. 2000). Differences in amylose contents (22.5-31.5%) may also have influenced the rheological behaviour of studied starches. Biliaderis and Tonogai (1991), attributed the substantially higher G' of legume starch (pea and garbanzo bean) gels (with or without lipids) to their high amylose contents compared to rice and wheat starch gels.

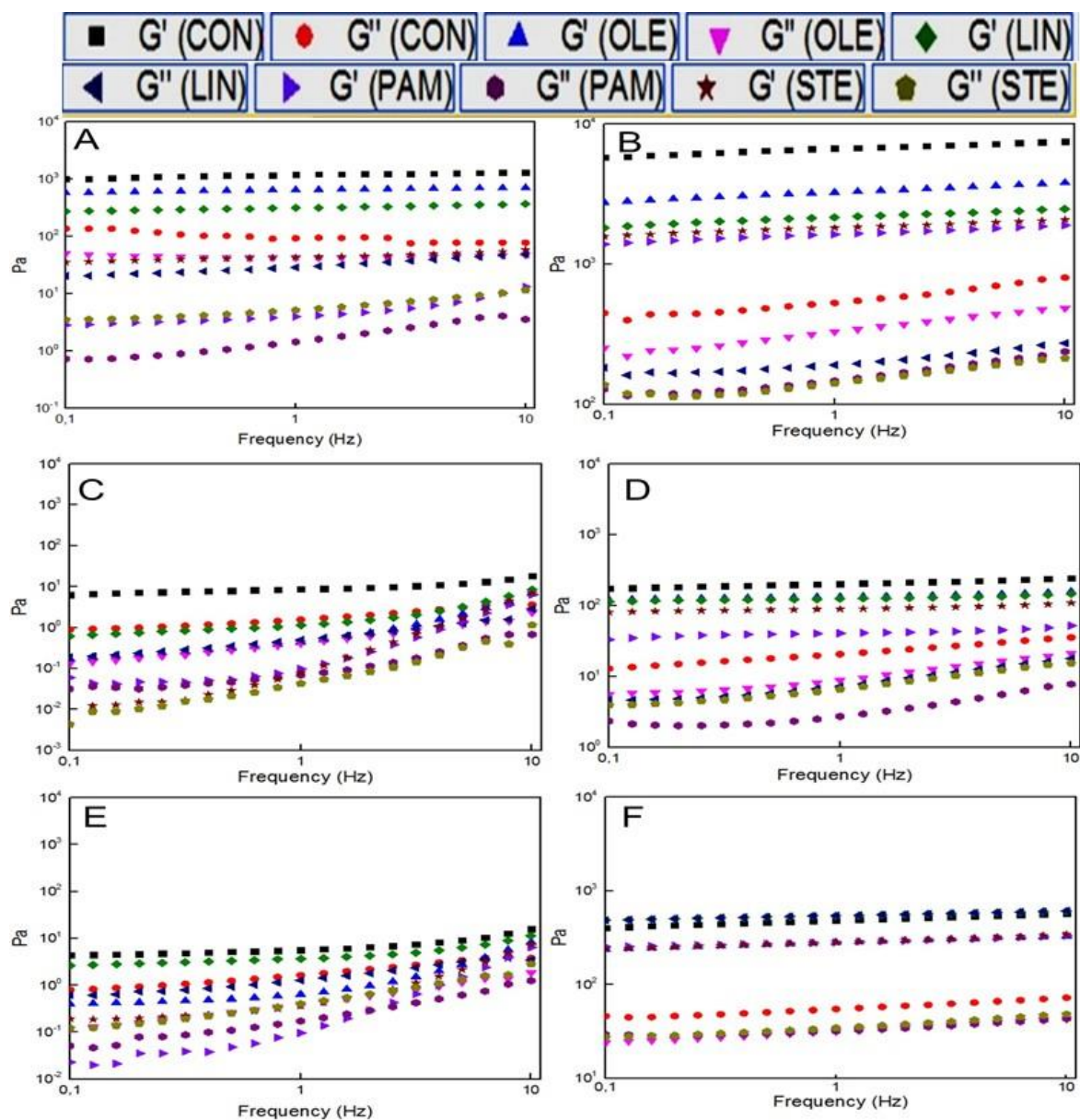


Figure 6.4 Effect of high-pressure homogenization on viscoelastic properties of gelatinized Bambara groundnut, maize and potato starch-fatty acid complexes

CON: Control starch, OLE: Starch + Oleic acid, LIN: Starch + Linoleic acid,
PAM: Starch + Palmitic acid, STE: Starch + Stearic acid

A: Homogenized Bambara groundnut starch B: Unhomogenized Bambara groundnut starch

C: Homogenized maize starch

D: Unhomogenized maize starch

E: Homogenized potato starch

F: Unhomogenized potato starch

6.4 Conclusions

High-pressure homogenization improved the degree of complexation of Bambara groundnut starch with fatty acids. Bambara groundnut starch seemed to complex better with fatty acids than did maize and potato starches. Saturated fatty acids showed higher complexing ability with Bambara groundnut, maize and potato starches than unsaturated fatty acids. Bambara groundnut starch formed type I V-amylose complexes with fatty acids. Starch-fatty acid complexes prepared under unhomogenized condition formed weak gels, while homogenized samples were non-gelling. Complexation of Bambara groundnut starch with fatty acids using HPH may be employed to produce modified starch with improved thermal stability and non-gelling behaviour that are more suitable for certain industrial applications such as in frozen foods and desserts for better mouth feel.

CHAPTER SEVEN

7.0 Influence of high-pressure homogenization on the physicochemical properties of Bambara groundnut starch complexed with lysophosphatidylcholine

Abstract

Amylose can form inclusion complexes with lipids for improved starch functionality. This study determined the influence of high-pressure homogenization on the physicochemical properties of Bambara groundnut starch complexed with lysophosphatidylcholine. Homogenization significantly increased the degree of complexation of Bambara groundnut starch with lysophosphatidylcholine. Bambara groundnut starch showed higher complex index than maize and potato starch reference samples. X-ray diffraction revealed the formation of V-amylase crystalline types with peaks at $2\theta = 7.4, 12.9$ and 19.9° . Complexing Bambara groundnut, maize and potato starches with lysophosphatidylcholine resulted in the formation of type I V-amylase complexes. These complexes had melting temperatures and enthalpies ranging from 91.5 to 98.6°C and 1.4 to 5.5 J/g respectively. Starch- lysophosphatidylcholine complexes displayed low syneresis rate. Homogenized Bambara groundnut starch complexes were non-gelling while unhomogenized types produced weak gels, with $G' > G''$ in the frequency range: 0.1 - 10 Hz. The non-gelling properties of homogenized Bambara groundnut starch-lysophosphatidylcholine complex suggest that the modified starch could be used in the food industry to provide a smooth texture in frozen foods and desserts.

7.1 Introduction

Native starches are unsuitable for most industrial applications because of their poor resistance to extreme processing conditions such as heat and shear. Therefore, starches are modified to improve their functional properties and application in foods. Starch modification mainly involves the interaction between the two major starch components (amylose and amylopectin) and additives such as ligands. The amylose component of starch can form inclusion complexes with lipids. During starch Gelatinisation, amylose undergoes a conformation change resulting in a single left-handed helix with a hydrophobic inner cavity (Putseys et al. 2010). Lipids can complex amylose in the hydrophobic tube (Immel and Lichtenthaler 2000b; Putseys et al. 2010) and are stabilized by van der Waals forces (Godet et al. 1993c).

Different lipids e.g. stearic acid and lysophosphatidylcholine (LPC) have been employed in starch modification. Lysophosphatidylcholine is the main endogenous phospholipid found in cereal starches (Hernández-Hernández et al. 2011; Morrison 1988). Starch modification using LPC has attracted the interest of many researchers, possibly due to its wider application in food, for example, as emulsifiers. Cui and Oates (1999) found that sago starch showed higher complexing ability with LPC than with monoglycerides. According to these authors, the LPC molecule presumably has less tendency to form micelles and may pass through the surface of starch granules more easily than monoglycerides. Siswoyo and Morita (2003), reported that the number of fatty acids bound to the glycerol backbone in a phospholipid can also influence the complexing ability of starch. Defatted wheat starch complexed with 1- or 2-glycerophosphatidylcholine (GPC) showed higher complex index (approx. 93%) than with 1, 2-GPC (approx. 63%) (Siswoyo and Morita 2003). Furthermore, the amylose contents and the degree of polymerization of amylose in different starches may influence the degree of complexation with lipids (Eliasson et al. 1988; Godet et al. 1995; Ocloo et al. 2016). Other studies reported on the influence of complexation on starch functionality. For instance, LPC at a concentration of 3% decreased the storage modulus (G') and loss modulus (G'') of freshly prepared and stored wheat starch gel (Ahmadi-Abhari et al. 2015). This was attributed to the restriction of starch granules swelling by LPC during heating. The addition of lipid to maize and potato starches has been reported to significantly slow down the rate of syneresis during storage (Singh et al. 2002).

The preparation of V-amylose complexes may be achieved using different methods. These methods include the classical method which involves heating starch with ligands under shear-less conditions, enzymatic or thermo-mechanical methods (Obiro et al. 2012b). The aim of these methods is to increase the degree of complexation of starch with lipids. Of these methods, the thermo-mechanical method such as pasting starch with lipids and the use of high-pressure homogenization (HPH) appears to be more effective due to the simultaneous use of heat and shear. The greater shear employed during HPH of V-amylose complexes makes it a more promising method for the dispersion of lipid in starch suspension. The improved dispersion of lipids in starch, as well as higher level of starch disintegration, has been found to promote better complexation (Meng et al. 2014a; Meng et al. 2014b; Yamada et al. 1998). V-amylose complex prepared from maize starch and fatty acids through HPH showed a higher degree of complexation than unhomogenized samples (Meng et al. 2014a; Meng et al. 2014b). Previous studies on complexation of lipid with starch have focused mainly on cereal and tuber crops,

possibly because these crops are good sources of starch. However, pulses such as Bambara groundnut (*Vigna subterranea*) are also relatively good sources of starch (22-45%) (Afolabi 2012; Kaptso et al. 2014). Chemical modification of Bambara groundnut starch using carboxymethylation (Afolabi 2012), acetylation, oxidation (Adebowale et al. 2002) and physical modification methods (Adebowale and Lawal 2002) have been reported in the literature. The aim of this study was to produce clean label starch through the following objectives: complex Bambara groundnut starch with LPC and homogenize the resulting V-amylose complex and to assess the influence of LPC and HPH on the physicochemical properties of Bambara groundnut starch in comparison to maize and potato starches.

7.2 Materials and methods

7.2.1 Experimental materials

Bambara groundnuts were obtained from Markathini Research station, Jozini, KwaZulu-Natal province, South Africa. Potato starch was obtained from Sigma-Aldrich (St. Louis, MO). Maize starch and LPC were purchased from Aladdin Chemistry Company (Shanghai, China).

7.2.2 Starch extraction and amylose contents determination

Starch was extracted from Bambara groundnut flour as described by Sirivongpaisal (2008). The amylose contents of extracted Bambara groundnut starch and those of maize and potato starches were 31.5%, 22.5%, and 24.6% respectively as determined by iodine binding method (Williams et al. 1970).

7.2.3 Preparation of starch-LPC complex by high-pressure homogenization

Starch-LPC complexes were prepared as previously reported by Meng et al. (2014b), except that LPC was added at 0, 1, 2, 3 or 4% starch weight (dry basis). Starch-LPC mixtures were heated with constant stirring in a water bath at 95°C for 20 min. The resulting pastes were homogenized using high-pressure homogenizer (APV2000 SPX, Germany) at 100 MPa for three passes. Homogenized starch-LPC pastes were freeze-dried and kept at 4°C until analyzed. Unhomogenized complexes were prepared in the same way as homogenized complexes but without homogenization. Maize and potato starches were included as reference samples and treated in the same way as Bambara groundnut starch.

7.2.4 Complex index

The extent of complex formation between starch and LPC was determined as reported by Meng et al. (2014b). Complex index (CI) is a measure of reduction in iodine binding capacity. The CI of the complexed starches was estimated using equation 1. The CI was determined at

different LPC concentration. After establishing the range of LPC for maximum complexation, structural; XRD, thermal and rheological properties of starch-LPC complexes were determined at 4% LPC concentration.

$$CI\% = 100 \times (\text{ABS of control} - \text{ABS of sample}) / \text{ABS of reference} \quad 7.1$$

ABS: Absorbance

7.2.5 X-ray diffraction

X-ray diffraction of starch-LPC complexes was conducted using Empyrean PANalytical diffractometer (Netherlands). The diffractometer operated at 40 kV with a target current of 40 mA. Scanning was done from 5° to 30° (2θ) with an exposure time of 16 min 14 s, the step size of 0.026° and a time/step ratio of 229.5 s. (Obiro et al. 2012a).

7.2.6 Thermal properties of dried starch-LPC complex

Thermal properties of starch-LPC complex were examined as described by Meng et al. (2014a) except that the ratio of starch-LPC complex to water was 1:3. Briefly, pasted freeze-dried complexed starch (3 mg) was directly weighed into the aluminium DSC pan and distilled water (9 µl) was added. Pans were then hermetically sealed and equilibrated for 24 h. The equilibrated pan containing the samples were directly heated from 20 to 140°C at a rate of 10°C/min. An empty pan was used as a reference.

7.2.7 Frequency sweep measurements

The modified method of Wang et al. (2012) was used to measure viscoelastic properties of the starch gel. The viscoelastic properties of the gels were obtained at the strain of 1% which was in the range of the linear viscoelastic region using a Rheometer (KNX2210 Malvern, UK). The spectra were obtained by recording storage modulus (G') and loss modulus (G'') as a function of angular frequency in the range 0.1 to 10 Hz.

7.2.8 Flow behaviour

The flow behaviour of Bambara groundnut starch-LPC complexes was determined as previously described with slight modifications (D'Silva et al. 2011). Briefly, gelatinized starch paste samples either homogenized or unhomogenized were cooled to 25°C. The flow behaviour

properties were measured at shear rates ranging between 10 and 1000 s⁻¹. The data were fitted into a Power-Law Model (Barnes et al. 1989) as follows:

$$\tau = k\gamma^n \quad 7.2$$

Where τ is the shear stress (Pa), k is the consistency coefficient, (Pa.s)ⁿ, γ is the shear rate (s⁻¹) and n is the flow behaviour index.

7.2.9. Syneresis

Syneresis of homogenized and unhomogenized starch pastes was determined as reported by Singh et al. (2002), except that starch pastes were heated at 95°C for 20 min. Starch pastes stored at 4°C for 24 and 48 h were centrifuged at 3000×g for 20 min. Syneresis was measured as the % of water released after centrifugation.

7.2.10 Statistical analysis

Samples were prepared in duplicate and analyses were done at least in triplicate. Data were analyzed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference Test ($p < 0.05$).

7.3 Results and discussion

7.3.1 Complex index

The CI of gelatinized Bambara groundnut starch-LPC complex increased with increasing concentration of LPC from 1 to 4% (at 1% increment) (Data not shown). The CI value (38%) increased by approximately 28%, when LPC was increased from 1 to 2%. Further increase in the concentration of LPC from 2 to 4% did not significantly ($p < 0.05$) change the CI values of Bambara groundnut starch. Maize and potato starch-LPC complexes similarly reached a plateau between 2-4%. For the subsequent experiment, LPC was added to Bambara groundnut starch at 4% concentration. The CI of homogenized Bambara groundnut starch-LPC complex was about 60%, which was higher than the unhomogenized complex (51%) (Figure 7.1). The high CI of the homogenized complex could be due to better dispersion of lipid in starch suspension during homogenization (Meng et al. 2014a; Meng et al. 2014b). Previous studies similarly reported better complexation of maize starch with fatty acids using HPH (Meng et al. 2014a; Meng et al. 2014b; Yamada et al. 1998). Homogenized maize and potato starch-LPC complexes showed a similar trend. But the CI value of Bambara groundnut starch-LPC

complex was about twice those of maize and potato starches. This could be due to differences in the amylose contents (maize: 22.5%, potato: 24.6%, Bambara groundnut: 31.5%) of these starches as previously reported by Eliasson et al. (1988) and possibly in the degree of polymerization (DP) of amylose. Godet et al. (1995) found that the amount of V-amylose complex formed with lipids increased with increasing DP of amylose from DP 30, DP 40, DP 80 and DP 900). The CI in this study is similar to values (approx. 63%) reported for wheat starch complexed with 1, 2-glycerophosphatidylcholine (GPC) (Siswoyo and Morita 2003). However, these authors reported higher CI (approx. 90%) for wheat starch complexed with 1- or 2- GPC. Variations in CI values could be attributed to differences in the molecular structure of amylose, structural difference between LPC and GPC, as well as the method V-amylose preparation. Siswoyo and Morita (2003) prepared V-amylose complexes from wheat starch and GPC at 60°C as compared to 90°C used in this study. According to previous research the inclusion rate of LPC into amylose decreased at temperatures greater than 60°C since LPC deteriorates at high temperatures (Cheng et al. 2015).

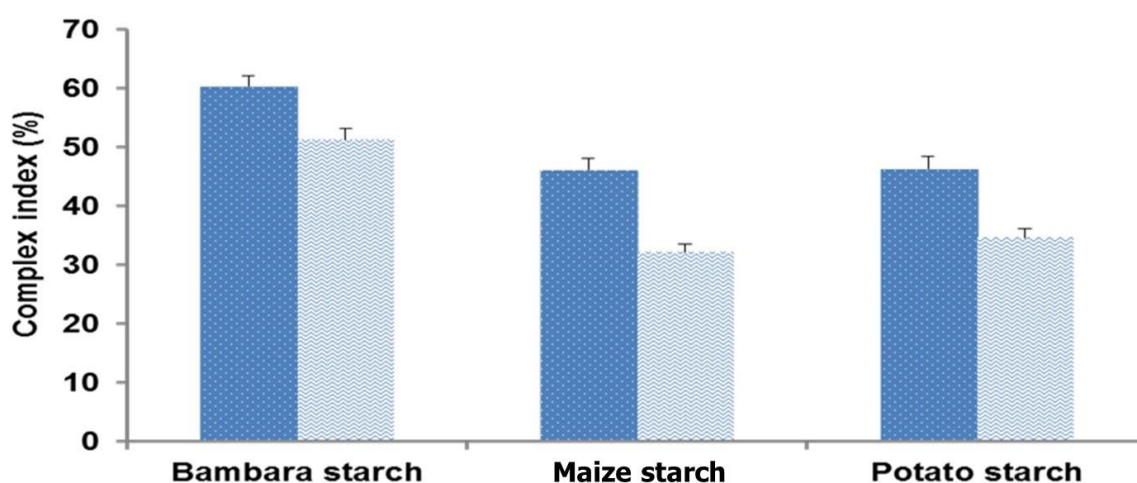


Figure 7.1 Complex index of gelatinized starch-Lysophosphatidylcholine complex

Homogenized (■) Unhomogenized (□)

7.3.2 XRD

The XRD pattern of native Bambara groundnut starch showed the A-type crystallinity pattern with strong peaks at 15 ° (2 θ), a doublet at 17 ° and 18 ° (2 θ) and a single peak at 23 ° (2 θ) (Figure 7.2A). However, when Bambara groundnut starch was gelatinized with LPC, XRD revealed the loss of its native crystallinity. Bambara groundnut starch complexed with LPC

showed peaks at $2\theta = 7.4, 12.9$ and 19.9° (Figure 7.2). These peaks are in agreement with the XRD peaks reported for V-amylose complex (Meng et al. 2014a; Ocloo et al. 2016; Zobel 1988). The V-amylose peaks were also observed in maize and potato starch-LPC complexes (Figure 7.2). The peaks corresponding to V-amylose crystalline types were sharper for the homogenized starch-LPC complexes (Figure 7.2A) than the unhomogenized samples (Figure 7.2B), further suggesting better complexation when HPH was utilized.

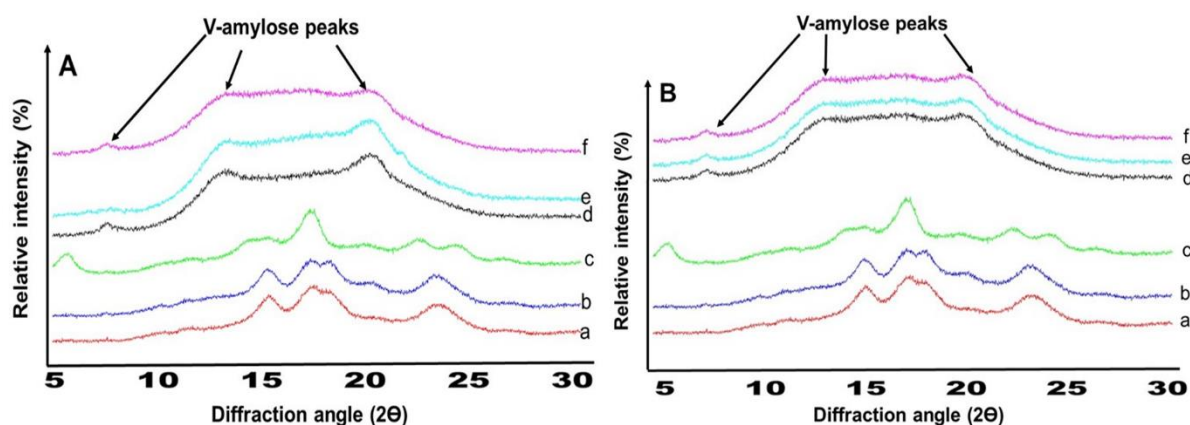


Figure 7.2 XRD of gelatinized starch-Lysophosphatidylcholine complexes

A: Homogenized complexes, B: Unhomogenized complexes

a: Bambara groundnut starch control, b: maize starch control, c: potato starch control, d: Bambara groundnut starch-LPC e: maize starch-LPC, f: potato starch-LPC, LPC: Lysophosphatidylcholine

7.3.3 DSC

Typical differential scanning calorimetric curves revealed that Bambara groundnut starch-LPC complexes generally had significantly higher melting temperature and broader transitions than the native Bambara groundnut starch (Figure 7.3 A&B). The higher melting temperature (T_m) of Bambara groundnut starch-LPC complexes suggest that LPC restricts swelling of starch granules during heating (Ahmadi-Abhari et al. 2015). The LPC molecule could also have increased the rigidity of the granules to water ingress, resulting in high T_m value (Hernández-Hernández et al. 2011). Similar results were observed for maize and potato starch-LPC complexes (Fig. 3A&B). The T_m values (94.39 - 98.55°C) of homogenized starch-LPC complexes were slightly higher than the values for unhomogenized samples (91.52 - 94.17°C) (Table 7.1). These transition temperatures correspond to the melting of type I V-amylose complexes (Biliaderis and Seneviratne 1990; Raphaelides and Karkalas 1988). Homogenized

starch-LPC complexes also showed higher melting enthalpy (ΔH_m) (approx. 5.40 J/g) than unhomogenized ones (approx. 1.50 J/g) (Table 7.1). Previous research correlated the T_m and ΔH_m with thermal stability and amount of V-amylose complexes respectively (Kawai et al. 2012). This result indicates greater stability of homogenized complexes and further confirms better complexation of starch with LPC. Cui and Oates (1999) studied the complexing abilities of lipids including LPC with 40% sago starch. Sago starch complexed with 2% LPC reportedly showed ΔH_m value of 3.56 J/g. However, Ahmadi-Abhari et al. (2013a) reported slightly higher ΔH_m value of 5.7 J/g for 20% wheat starch complexed with 2% LPC. Siswoyo and Morita (2003) also found that defatted wheat starch complexed with 1- or 2-glycerophosphatidylcholine (GPC) showed higher ΔH_m values (5.6-8.2 J/g) than wheat starch complexed with 1, 2-GPC (2.9-5.2 J/g). Variation in the complexing ability of starch with lipids could be attributed to differences in amylose contents, complexation conditions such as incubation temperature, time, starch moisture content and the molecular structure of the lipid.

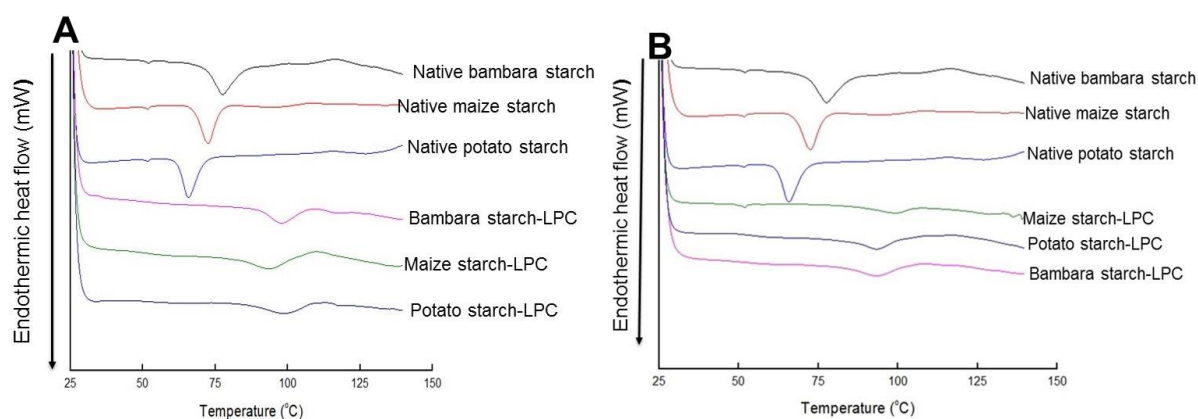


Figure 7.3 DSC Thermograms of gelatinized starch-lysophosphatidylcholine complexes

A: Homogenized complexes, B: Unhomogenized complexes, LPC: Lysophosphatidylcholine

Table 7.1 Thermal properties of gelatinized starch-lysophosphatidylcholine pastes

Starch type	Homogenized				Unhomogenized			
	T_o (°C)	T_m (°C)	T_c (°C)	ΔH_m (J/g)	T_o (°C)	T_m (°C)	T_c (°C)	ΔH_m (J/g)
Bambara groundnut	90.8 ^a ±0.1	97.8 ^a ±0.7	105.9 ^{ab} ±0.2	5.3 ^a ±0.1	86.3 ^c ±0.6	91.5 ^c ±0.1	100.2 ^b ±0.2	1.5 ^a ±0.1
Maize	84.5 ^b ±0.7	94.4 ^b ±0.2	104.6 ^c ±0.4	5.5 ^a ±0.1	91.2 ^a ±0.7	95.4 ^a ±0.2	98.2 ^c ±0.2	1.4 ^b ±0.1
Potato	89.6 ^a ±0.9	98.6 ^a ±0.3	106.4 ^a ±0.8	5.4 ^a ±0.7	88.6 ^b ±0.1	94.2 ^{ab} ±0.1	106.4 ^a ±0.8	1.6 ^a ±0.3

Mean ± SD. Mean with different superscript letters along a column are significantly different ($p < 0.05$).

T_o : onset temperature, T_m : melting temperature, T_c : conclusion temperature, ΔH_m : melting enthalpy respectively.

*Lysophosphatidylcholine was added to starch at 4% (dry weight basis)

7.3.4 Rheology

7.3.4.1 Apparent viscosity

The apparent viscosity of Bambara groundnut starch-LPC paste was generally low, about 3-4 times less than the starch without LPC (Data not shown). Theoretically, LPC can interact with amylose in starch during Gelatinisation to form a V-amylose complex. This change in the structure of amylose due to complexation with lipids, results in a reduction of the hydrodynamic volume of the amylose molecules (Raphaelides 1993; Raphaelides and Georgiadis 2006). Homogenization further brought about a significant ($p < 0.05$) reduction in the viscosity of Bambara groundnut starch. The low viscosity of homogenized samples could be attributed to extensive degradation of starch macromolecules through shear, cavitation, and collision during HPH (Wang et al. 2012).

The flow properties of the starches with or without LPC followed the Power-Law model (Table 7.2) with a fit of $r^2 \geq 0.96$. All starch-LPC complexes showed strong pseudoplastic behaviour with flow behaviour index (n) several times < 1 (Table 7.2). Homogenized samples generally showed higher n values, which was about double the value recorded for the unhomogenized ones (Table 7.2). The higher n value for homogenized pastes could be due to the extensive shear and greater reduction in particle size during HPH. The consistency coefficient (k) indicative of viscous properties of starch increased with the addition of LPC but decreased with homogenization. D'Silva et al. (2011), similarly observed increase in k values for maize and teff starches pasted with stearic acid. The reduction in k value after homogenization suggest that HPH may reduce the consistency of starch.

Table 7.2 Power Law parameters of homogenized starch-LPC pastes

Starch type	LPC (%)	Unhomogenized			Homogenized		
		n	k (Pa s ⁿ)	r^2	n	k (Pa s ⁿ)	r^2
Bambara groundnut	0	0.04 ^a ±0.01	10.47 ^a ±0.12	0.99	0.09 ^b ±0.01	0.09 ^a ±0.01	0.99
Bambara groundnut	4	0.03 ^c ±0.01	10.56 ^{bc} ±0.20	0.96	0.12 ^a ±0.01	0.03 ^c ±0.01	0.99
Maize	0	0.10 ^c ±0.01	0.09 ^{cd} ±0.01	0.99	0.12 ^a ±0.02	0.03 ^c ±0.01	0.99
Maize	4	0.06 ^d ±0.01	1.88 ^e ±0.02	0.99	0.12 ^a ±0.01	0.02 ^d ±0.01	0.99
Potato	0	0.05 ^c ±0.01	27.07 ^d ±0.14	0.99	0.11 ^a ±0.01	0.08 ^a ±0.01	0.99
Potato	4	0.04 ^b ±0.01	41.36 ^b ±0.21	0.99	0.11 ^a ±0.01	0.06 ^b ±0.01	0.99

Mean ± SD. Mean with different superscript letters along a column are significantly different ($p < 0.05$).

LPC: Lysophosphatidylcholine

k : consistency index, n : flow behaviour index

7.3.4.2 Viscoelasticity

The viscoelastic behaviour of Bambara groundnut starch gel was significantly affected by HPH and complexation with LPC (Figure 7.4 A&D). The storage modulus (G') and loss modulus (G'') of Bambara groundnut starch gels showed a minor dependency on frequency with $G' > G''$ in the range of 0.1-10 Hz. Gels prepared from Bambara groundnut starch-LPC complex exhibited lower G' and G'' compared to gels from the control Bambara groundnut starch. The reduction in G' and G'' in the presence of LPC could be attributed to the formation of V-amylose complex (Ahmadi-Abhari et al. 2015; Singh et al. 2002). The addition of LPC may have prevented or slowed down the interaction between starch molecules preventing the formation of double helices, junction zones and gel network during storage (D'Silva et al. 2011; Richardson et al. 2004). Ahmadi-Abhari et al. (2015) similarly observed a reduction in G' and G'' of wheat starch complexed with LPC. Food dispersions have been classified into dilute solutions, concentrated solutions, as well as weak and strong gels (Clark and Ross-Murphy 1987; Hesarinejad et al. 2014). Based on this classification, unhomogenized Bambara groundnut starch-LPC complex can be regarded as a weak gel. However, the homogenized Bambara groundnut starch-LPC complexes were non-gelling. They appeared to be highly concentrated solutions. Similar non-gelling behaviour was observed for homogenized maize and potato starch-LPC complexes. The non-gelling behaviour of starch paste with added lipids has previously been reported (D'Silva et al. 2011; Richardson et al. 2004). The non-gelling behaviour of homogenized starch-LPC complexes could be due to the dual effect of V-amylose complex formation and the applied pressure. Thus, HPH impacts a weak structure on the V-amylose complexes which may have increased further the spacing between junction zones during storage. Possibly, more complexes were formed between LPC and amylose, leaving fewer amylose chains available for junction zone formation during storage of starch-lipid complex paste. It has been suggested that the pressure applied during HPH improves the distribution of lipids within the starch suspension and enhances the release of more amylose from swollen starch granules due to intense shear and turbulence (Meng et al. 2014a; Meng et al. 2014b). This could explain why gels from homogenized starch-LPC complexes showed substantially lower G' and G'' (Figure 7.4 D-F) than the unhomogenized samples (Figure 7.4 A-C). Obviously, the low G' and G'' of homogenized starch-LPC complex may be linked to their lower k values (Table 7.2). In comparison with maize (Figure 7.4 B&E) and potato (Figure 7.4 C&F) starches, homogenized and unhomogenized Bambara groundnut starch-LPC gels (Figure 7.4 A&D) generally showed higher G' and G'' . The higher amylose content (31.5%) of Bambara groundnut starch compared to maize (22.5%) and potato (24.6%) starches may have

influenced their rheological behaviour. In a study conducted by Biliaderis and Tonogai (1991), pea and garbanzo bean gels with or without the addition of lipids, showed substantially higher G' than rice and wheat starch gels, which was associated with the high amylose contents of the legumes starches.

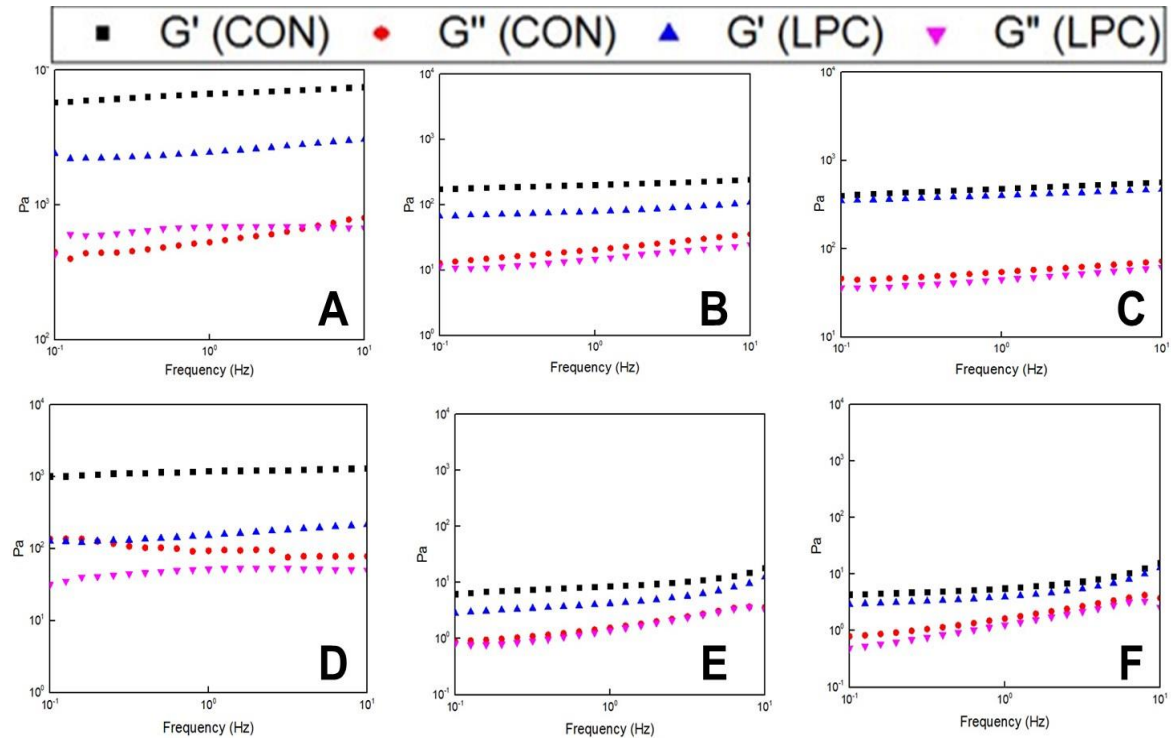


Figure 7.4 Viscoelastic properties of gelatinized starch- lysophosphatidylcholine complexes

CON: Starch without LPC

LPC: Starch complexed with lysophosphatidylcholine

A: Unhomogenized Bambara groundnut starch

D: Homogenized Bambara groundnut starch

B: Unhomogenized maize starch

E: Homogenized maize starch,

C: Unhomogenized potato starch

F: Homogenized potato starch

7.3.6 Syneresis

The amount of water released (syneresis) after storing starch pastes at 4°C for a period of 48 h increased with an increase in storage period (Table 7.3). However, starch complexed with LPC showed significantly lower syneresis than starch alone. Syneresis results from the re-association of the linear starch molecules, which are predominantly amylose. Thus, the reduction in syneresis rate of complexed starch could be due to the formation of V-amylose complexes. These complexes delay the release of water from within starch granules during storage (Singh et al. 2002). Homogenized starch pastes generally displayed significantly ($p < 0.05$) lower syneresis rates compared to unhomogenized samples. This is in agreement with the CI (Figure 7.1), XRD (Figure 7.2), and ΔH_m results (Table 7.1), further indicating better complexation by using HPH. No syneresis was observed in homogenized Bambara groundnut,

maize and potato starch-LPC complexes stored for a period of 24 h. Similarly, homogenized maize starch paste without LPC showed no syneresis after storage for 24 h. The formation of inclusion complexes between maize starch and endogenous lipids may have prevented loss of water from the control maize starch. The extent of syneresis was larger in the order of potato > Bambara groundnut > maize starch pastes. Differences in the degree of complexation, amylose contents and fragility of starch granules have been linked with variation in syneresis rate of starch from different botanical sources (Singh et al. 2002).

Table 7.3 Effect of high-pressure homogenization on syneresis (%) in starch-LPC complexes stored at 4°C

Starch type	LPC (%)	Unhomogenized paste		Homogenized paste	
		24 h storage	48 h storage	24 h storage	48 h storage
Bambara groundnut	0	40.82 ^b ±1.63	44.50 ^a ±1.29	26.38 ^b ±0.22	28.31 ^b ±0.71
Bambara groundnut	4	33.04 ^d ±0.71	38.02 ^c ±1.72	NWR	22.99 ^c ±0.02
Maize	0	38.92 ^c ±1.17	42.35 ^{ab} ±1.71	NWR	29.29 ^{ab} ±0.37
Maize	4	31.29 ^d ±0.28	33.94 ^d ±0.57	NWR	19.83 ^d ±0.06
Potato	0	43.32 ^a ±0.62	44.79 ^a ±0.45	27.73 ^a ±0.67	31.89 ^a ±0.16
Potato	4	37.88 ^c ±0.37	40.27 ^{bc} ±1.06	NWR	24.72 ^c ±0.61

Mean ± SD. Mean with different superscript letters along a column are significantly different ($p < 0.05$).

LPC: Lysophosphatidylcholine

NWR: No water was released.

7.4 Conclusions

High pressure homogenization improved the degree of complexation of Bambara groundnut starch with LPC. Bambara groundnut starch complexed better with LPC than maize and potato starches, which could be due to its high amylose content. Bambara groundnut starch-LPC complexes formed the type I V-amylose. Homogenized Bambara groundnut starch-LPC complexes were non-gelling and showed low syneresis rates. High pressure homogenization appears to be a promising method for enhancing the complexation of Bambara groundnut starch with LPC. Findings from this study suggest that HPH may be employed to produce modified starch suitable for certain industrial applications such as frozen foods and desserts.

CHAPTER EIGHT

8.0 Effect of stearic acid addition on physicochemical and mechanical properties of Bambara groundnut starch films

Abstract

The physicochemical and mechanical properties of biofilm prepared from Bambara groundnut starch modified with varying concentrations of stearic acid (0, 2.5, 3.5, 5, 7 and 10%) were studied. By scanning electron microscopy, Bambara groundnut starch films modified with stearic acid (≥ 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 groundnut starch film reduced water vapour permeability by approximately 17%. Bambara groundnut 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 groundnut starch film may be modified with 2.5% stearic acid for improved water vapour permeability and thermal stability with minimal effect on tensile strength.

8.1 Introduction

Bio-plastic packaging is receiving much attention in recent times mainly due to their biodegradability. This category of plastics also provides an alternative packaging option without contributing to environmental pollution (Cano et al. 2015; Jiménez et al. 2012a). 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 et al. 2014). The use of starch in bio-plastic packaging is limited due to its hydrophilic and poor mechanical property (Liu et al. 2015b; Ortega-Toro et al. 2014). The addition of plasticizers such as glycerol and sorbitol have been reported to improve film flexibility (Jiménez et al. 2012a). Plasticizers penetrate the amorphous regions of starch and interrupt hydrogen bonding along the polymeric network making the film more flexible.

The improvement in the barrier property of starch-based films following the addition of lipids to starch matrix has also been reported (Jiménez et al. 2012b; Liu et al. 2015b; Ortega-Toro et al. 2014; Schmidt et al. 2013). These improvements in barrier properties may vary with starch source and lipid type. For instance, approximately 27% reduction in water vapour permeability (WVP) was reported for maize starch film modified with 15% stearic acid compared to the control (Jiménez et al. 2012b). However, other authors working with cassava starch film reported higher reduction (39%) in WVP at the same stearic acid concentration (Schmidt et al. 2013). According to Jiménez et al. (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 et al. 2015b). Potato starch film modified with stearic acid was reported to exhibit higher melting temperature (191°C) compared to the control film, which melted at approximately 183°C (Liu et al. 2015b). 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 et al. 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 et al. 2012b; Liu et al. 2015). Most reported studies on improving physicochemical properties of starch films with lipids have focused mainly on conventional starch sources such as cereals and tubers. However, starches from pulses such as cowpea (*Vigna unguiculata*) and Bambara groundnut (*Vigna subterreneae*) with high amylose content (22-78%) (Hoover et al. 2010), may be promising matrix for making films. Bambara groundnut (*Vigna subterreneae*), is a pulse of African origin. The starch yield of Bambara groundnut may vary between 22 and 46% depending on source and cultivar (Sirivongpaisal 2008). In Southern Africa, this crop is neglected and grown mainly for subsistence. Recently, Bambara groundnut starch was modified with lipids for improved functionality.

The use of Bambara groundnut starch in complexation with lipids and in biofilm application is unique in many ways. Bambara groundnut starch has a moderate level of amylose content (20-35%) (Sirivongpaisal 2008), which is higher than those of maize and potato starches. High amylose content of starch improves strength and film flexibility (Peressini et al. 2003; Zhang and Han 2006; Zobel 1988) and also produces films with better gas barrier properties (Lourdin

et al. 1995; Palviainen et al. 2001; Wolff et al. 1951). Furthermore, in comparison with maize, Bambara groundnut is highly resistant to drought and therefore well adapted to the changing climate. Bambara groundnut starch as an alternative source of starch would be more sustainable for utilisation and application in the industry. To further increase the utilisation and application of Bambara groundnut 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 groundnut starch films modified with stearic acid.

8.2 Materials and Methods

8.2.1 Materials

Bambara groundnut with brown seed coat colour was obtained from Markathini Research station Jozini, South Africa. The brown type was used because there was a consistent supply from the farm during the experimental period. The seeds were cleaned and stored at 4°C prior to use. All chemicals and solvents used were laboratory grade.

8.2.2 Starch extraction

Bambara groundnut starch was extracted from Bambara groundnut flour as described by Sirivongpaisal (2008). The yield of starch, calculated as a ratio of dried starch to Bambara groundnut flour was 35%. Amylose content was 31.5% as determined by iodine-binding method (Williams et al. 1970). The extracted starch had low ash (0.1%), protein (0.2%) and fat (0.3%) contents, suggesting that it was relatively pure.

8.2.3 Preparation of films

Films were prepared by dispersing 3% (w/w) Bambara groundnut starch in distilled water. The dispersions were stirred at 95°C for 30 min to induce starch Gelatinisation. 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 (Ultraturax). Bambara groundnut 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, South Africa).

8.2.4 Colour

Tristimulus L^* a^* b^* parameters of Bambara groundnut starch films were determined after standardization using a colorFlex (A60-1014-593, USA). Five snapshots were taken for each film and the average of the readings were reported. Average of the readings were computed and reported. Whiteness index (WI) was calculated using equation 8.1 (Boun and Huxsoll 1991). Bambara groundnut starch films without stearic acid were used as a reference for comparison.

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad 8.1$$

8.2.5 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, UK) and absorbance was recorded at a wavelength of 550 nm. The opacity of the films was calculated according to the equation 8.2.

$$T = A_{550}/x \quad 8.2$$

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

8.2.6 SEM

The SEM images films surface was carried out using a 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 et al. 2015).

8.2.7 FTIR

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.

8.2.8 Water soluble matter

The water-soluble matter of the film was determined according to the method described by Bertan et al. (2005). Dried films (2 cm in diameter) 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.

8.2.9 Mechanical properties

Tensile strength (TS) and breaking force of the films were determined using a universal testing machine (EZ-SX, Shimadzu Japan). 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×1cm) and conditioned for 48 h at 53% RH. At least 6 films were tested for each treatment.

8.2.10 DSC

Thermal properties of films were measured using the modified method of Dai et al. (2015). Briefly, film (3 mg) was cut and placed into the aluminium pan and hermetically sealed using a DSC punch sealer. Samples were scanned from 25 to 250°C with an interval heating rate of 10°C/min in a differential scanning calorimeter (SDT Q600, USA) coupled with a thermal analysis data station and data recording software. An empty pan was used as a reference for all measurements.

8.2.11 Water vapour permeability (WVP)

The modified method of Taylor et al. (2005) based on the ASTM method E96- 97, (American Society for Testing & Materials, 1997) was used. Briefly, circles (30 mm diameter) were cut from cast films and the thickness measured in five places using a micrometer. Containers (50 ml) were modified by accurately drilling a hole in the centre 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 days.

8.2.12 Statistical analysis

All experiments were conducted in triplicate. Data were analysed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference Test ($p < 0.05$).

8.3 Results and discussion

8.3.1 Film morphology, colour and opacity

SEM image of Bambara groundnut starch film modified with 2% stearic acid appeared smooth and was comparable to the control (Figure 8.1). This could be attributed to better dispersion of stearic acid in the starch matrix. Films prepared with stearic acid concentration greater than 2% showed a progressively rougher surface. Lipids at relatively high concentrations may self-associate in preference to forming inclusion complexes with amylose in starch (Tang and Copeland 2007). Thus, the rougher surfaces of Bambara groundnut starch films at higher stearic acid concentrations may be attributed to molecular self-association of stearic acid.

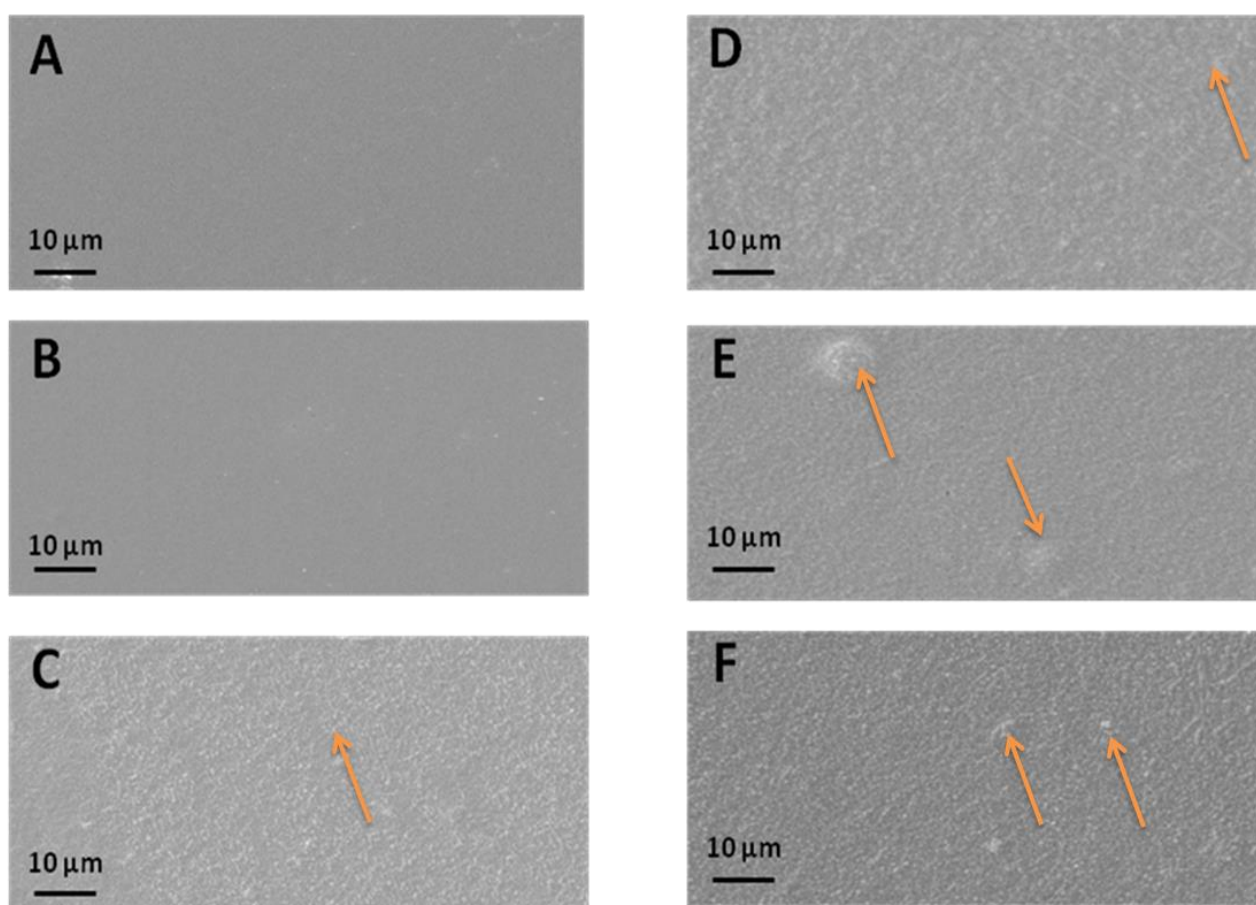


Figure 8.1 SEM images of Bambara groundnut starch films with or without stearic acid.

Arrows indicate stearic acid aggregates A: Bambara groundnut starch film without stearic acid, B: Bambara groundnut starch film with 2% stearic acid, C: Bambara groundnut starch film with 4% stearic acid, D: Bambara groundnut starch film with 6% stearic acid, E: Bambara groundnut starch film with 7% stearic acid, F: Bambara groundnut starch film with 10% stearic acid

The lightness (L^*) and whiteness index (WI) values of Bambara groundnut starch film modified with stearic acid were slightly lower compared to the control (Table 8.1). 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' colouration. Previous studies similarly reported a decrease in L^* value of high amylose maize starch film following the addition of lipids (Muscat et al. 2013) and tapioca starch film modified with potassium sorbate (Flores et al. 2007).

The opacity of Bambara groundnut films seemed to increase with increasing concentration of stearic acid in the starch matrix. Control Bambara groundnut starch film showed the lowest opacity value (1.14 AU nm), while starch film with 10% stearic acid had the highest value of 3.12 AU nm (Table 8.1). 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 8.1) and further suggests that stearic acid were embedded in the starch matrix (Figure 8.1). Thus, Bambara groundnut starch films modified with stearic acid have the potential for packaging light sensitive foods.

Table 8.1 Colour parameters, whiteness index and opacity of Bambara groundnut 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	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
4	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
6	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

8.3.2 FTIR of Bambara groundnut starch film

FTIR spectra of Bambara groundnut starch films with or without stearic acid were very similar (Figure 8.2). Nevertheless, some differences can be observed in the OH stretching region. The bands between 3200 and 3600 cm^{-1} which correspond to the stretching vibration mode of hydroxyl groups arose from absorbed water from the starch and stearic acid as previously reported (Cano et al. 2015; Jiménez et al. 2014). 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 groundnut 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 et al. (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 cm^{-1} 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 groundnut starch and stearic acid. Previous studies indicated that when two components are mixed, the physical blends may result in changes in the characteristic spectra peaks (Guan et al. 1998; Yin et al. 1999). The peak located at approximately 1650 cm^{-1} in all the starch films corresponds to absorbed water and carboxyl groups in their ionized state as previously reported (Karimi et al. 2014; Nacos et al. 2006).

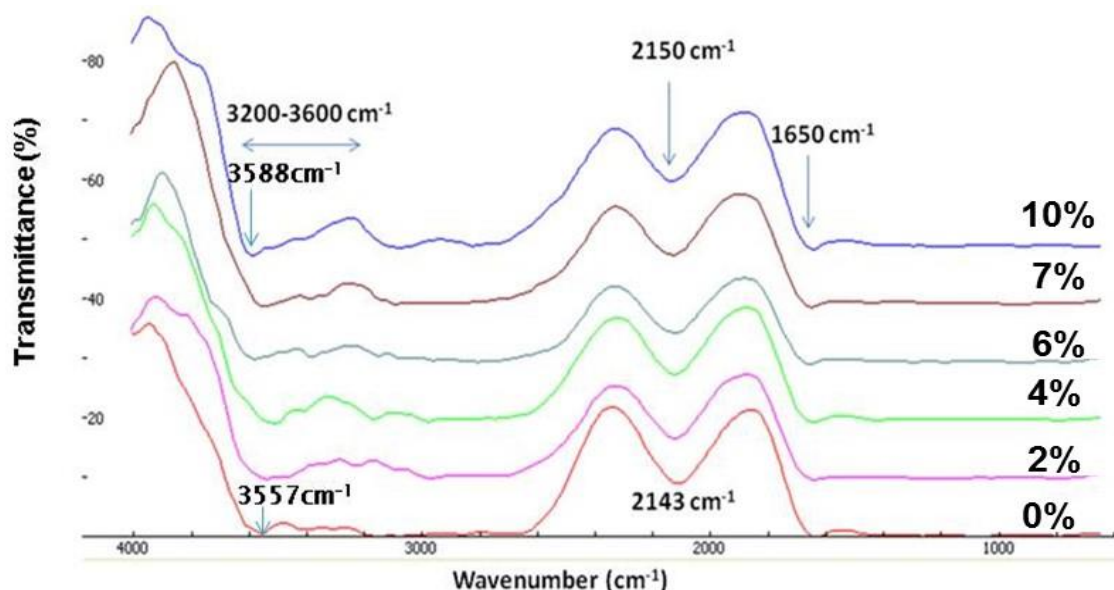


Figure 8.2 FTIR Spectra of Bambara groundnut starch films with or without stearic acid

Values in percentages represent stearic acid concentration per dry weight of starch

8.3.3 WVP

Expectedly, Bambara groundnut starch films modified with stearic acid were less permeable to water vapour compared to the control (Table 8.2). WVP was reduced by approximately 17% when Bambara groundnut starch film was prepared with 2% stearic acid. However, increasing the concentration of stearic acid above 2%, did not significantly change the WVP of modified

Bambara groundnut starch films. The reduction in WVP of the modified films could be linked to the hydrophobicity of stearic acid as indicated by previous authors (Jiménez et al. 2010a; Jiménez et al. 2012a; Jiménez et al. 2012b). Uncomplexed stearic acid at concentrations above 2%, which contributed to the surface roughness of the films (Figure 8.1), may have also restricted the passage of water vapour through the films. Jiménez et al. (2012b) reported approximately 27% reduction in water vapour permeability of maize starch films modified with 15% stearic acid. Other authors similarly reported a reduction in water vapour permeability following the addition of lipids to maize starch films (Jiménez et al. 2012b; Ortega-Toro et al. 2014), cassava starch films (Schmidt et al. 2013) and gelatin-based films (Bertan et al. 2005).

Table 8.2 Soluble matter, water vapour permeability, tensile strength, break force and moisture contents of Bambara groundnut starch films modified with stearic acid

Stearic acid (%)	Soluble matter (%)	WVP (10^{-3} g mm h ⁻¹ KPa ⁻¹ m ⁻²)	TS (MPa)	Break force (N)	Moisture content (%)
0	37.73 ^b ±0.12	2.15 ^a ±0.01	48.32 ^a ±0.25	6.67 ^a ±0.26	25.07 ^a ±0.21
2	38.89 ^b ±0.21	1.79 ^b ±0.01	33.88 ^b ±0.12	3.07 ^b ±0.27	22.11 ^b ±0.92
4	46.59 ^a ±0.14	1.76 ^b ±0.01	25.42 ^c ±0.06	3.01 ^{bc} ±0.14	21.51 ^b ±0.06
6	46.40 ^a ±0.10	1.48 ^c ±0.01	22.18 ^c ±0.24	2.57 ^c ±0.35	20.35 ^c ±0.25
7	45.73 ^a ±0.13	1.45 ^c ±0.01	18.62 ^d ±0.24	2.62 ^c ±0.59	20.49 ^c ±0.57
10	46.53 ^a ±0.86	1.44 ^c ±0.01	13.22 ^e ±0.28	1.93 ^d ±0.38	20.64 ^c ±0.19

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

8.3.4 Tensile Properties

The tensile strength (TS) of Bambara groundnut starch films substantially reduced with the addition of stearic acid (Table 8.2). The reduction in tensile strength of the films may have resulted from discontinuities introduced in the starch matrix by stearic acid (Jiménez et al. 2012b). Films prepared with 2% stearic showed a lower reduction (approx. 30%) in TS compared to films prepared at higher concentrations. Some authors reported higher reductions (approx. 40%) in TS for maize starch films modified with 15% stearic acid (Jiménez et al. 2012b) and approximately 51% reduction in TS for cassava starch films modified with 15% stearic acid (Schmidt et al. 2013). The differences in TS of the modified Bambara groundnut starch films compared to those reported by these authors could be attributed to starch type, lipid concentration, and differences in amylose content.

8.3.5 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 8.2). Bambara groundnut 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 the film modified with 10% stearic acid (Table 8.2). The control film and film prepared with 2% 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 et al. (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 tensile strength result (Table 8.2), facilitated leaching of soluble matter during mechanical stirring. Further, the separation of large fatty acid crystals causing disintegration of the starch network may also account for the increase in solubility of the films in water as previously reported (Sapru and Labuza 1994; Schmidt et al. 2013). The increased solubility of the films in water is advantageous especially when the films are intended for use in products to be consumed together with the film. Furthermore, film solubility may be an important factor that determines biodegradability when used as a packaging wrap (Bourtoom and Chinnan 2008). Previous studies also reported an increase in films solubility in water for composite films of soy protein isolate modified with lauric acid (Rhim et al. 1999), gelatin-based composite films modified with fatty acids (Bertan et al. 2005) and cassava starch film modified with stearic acid (Schmidt et al. 2013).

8.3.6 DSC

The addition of stearic acid to Bambara groundnut starch significantly ($p<0.05$) increased the melting temperatures and enthalpies of the films (Table 8.3). Both the control film and films modified with stearic acid exhibited two endothermic peaks (Figure 8.3). The first 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 of uncomplexed 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 (Chang et al. 2013a; 2013b; Obiro et al. 2012a). The second 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 V-amylose complexes. These complexes are known to melt at higher transition temperature and enthalpy (Chang et al. 2013a; 2013b; Obiro et al. 2012a). Films moisture content (Table 8.2), which decreased with increasing concentration of stearic acid, could also have influenced the melting peak position (Table 8.3). Furthermore, the peak shift in the hydroxyl group region as shown by the FTIR spectra (Figure 8.2), 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 maize starch nanocrystals (Li et al. 2015), and potato starch films modified with stearic acid (Liu et al. 2015b).

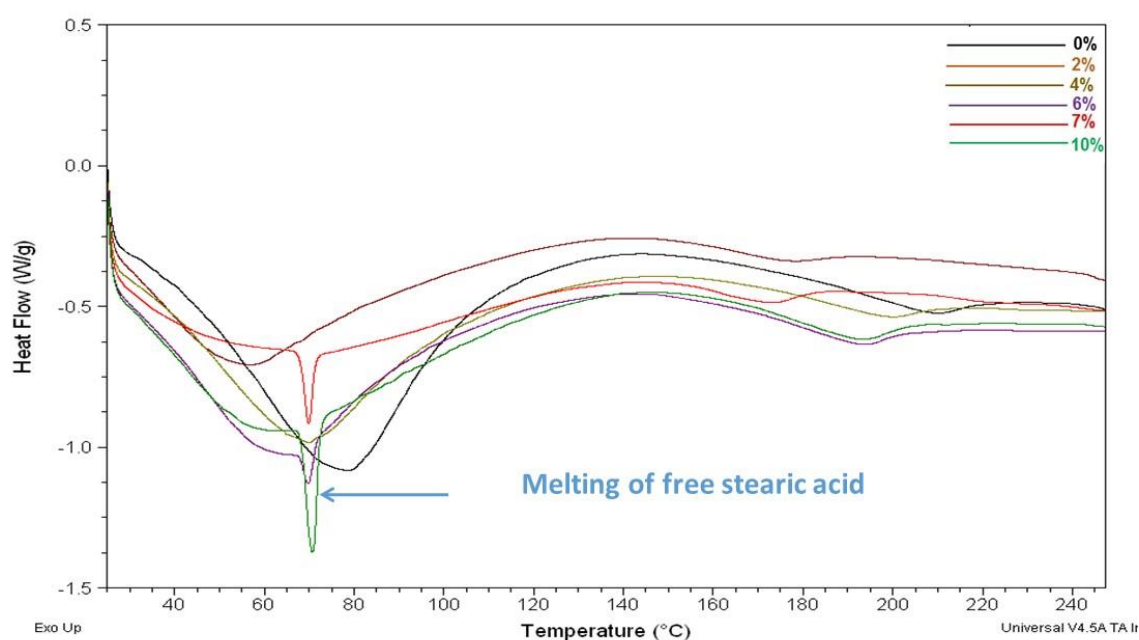


Figure 8.3 Thermograms of Bambara groundnut starch films with stearic acid at varying concentrations

Percentages represent stearic acid concentrations

Table 8.3 Thermal properties of Bambara groundnut 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	156.04 ^c ±1.18	174.25 ^{bc} ±1.81	189.42 ^d ±0.64	5.23 ^c ±0.08
4	162.05 ^b ±0.21	177.91 ^b ±0.15	193.00 ^c ±1.41	5.52 ^c ±0.08
6	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

8.4 Conclusions

Bambara groundnut starch film modified with stearic acid show increased films solubility and opacity. The surfaces of films prepared with stearic acid concentration greater than 2% appear rough, which may be associated with uncomplexed stearic acid. Bambara groundnut starch films modified with stearic acid have improved thermal stability compared to unmodified starch films. Stearic acid (up to 2%) may be added to Bambara groundnut starch matrix for the preparation of biofilms with improved water vapour permeability and acceptable tensile strength.

CHAPTER NINE

9.0 GENERAL DISCUSSIONS

In this thesis, Bambara groundnut starch was complexed with lipids for improved functionality, application and utilisation. The first part of this section discusses the composition and functionality of starches extracted from Bambara groundnut genotypes and landraces (Chapters 3 and 4). The second part focuses on the influence of lipid type and high-pressure homogenization on physiochemical properties of Bambara groundnut starch-lipid complexes (Chapters 5, 6, 7 and 8). The third part suggests potential applications of Bambara groundnut starch-lipid complexes in food and non-food systems.

9.1 Composition structure and functionality of starch from Bambara groundnut genotypes and landraces

Bambara groundnut landrace starches were characterized by slightly higher amylose content (approx. 33%) compared to the genotypes (approx. 28%). Bambara groundnut genotypes displayed the C-type pattern generally associated with pulse starch, while the landraces showed the A-type crystallinity. Differences in crystalline patterns may be observed among starch of the same species. C-type starch contain varying proportions of 'A' and 'B' polymorphs with the A-polymorph usually greater in amounts than the B-type. Wang et al. (2008), reported that the amorphous region of pea starch was mainly located in the core part of the starch granules and may be distributed alternately in the crystalline areas, which mainly exist in the peripheral region. Hence, 'A' and 'B' polymorphs may be arranged differently within starch granules and may influence the XRD reflection. For instance, Bogracheva et al. (1998), working with pea starches with the C-type pattern found that the 'A' and 'B' polymorphs are present within a granule, with the B-polymorph located in the center of the granule surrounded by the A polymorph. Thus, the differences in the crystallinity pattern between the genotype and landrace starches could be due to differences in the intra or inter granular arrangement of the 'A' and 'B' polymorphs and possibly the proportion of these polymorphs within the starch granules. Although the A-type pattern is generally reported for most cereal starches, some studies also found the A-type crystallinity for pulse starches including those isolated from mung bean (Kawamura 1969) and Bambara groundnut (Kaptso et al. 2016; Kaptso et al. 2014; Sirivongpaisal 2008).

The amylose content of starch, crystalline pattern and the degree of crystallinity may influence their functional properties. In general, starches with relatively high amylose contents would

show restricted swelling. However, some researchers found no inverse relationship between starch swelling power and its amylose content (Kaur and Sandhu 2010; Kaur et al. 2010; Naidoo et al. 2015). In this study, landrace starches with significantly higher amylose content exhibited higher swelling power than the genotypes. This result suggests that factors other than amylose content may have influenced the swelling power of these starches. Previous studies associated variation in swelling power to differences in the packing arrangement of amylose chains (Hoover et al. 2010; Joshi et al. 2013), the molecular structure of amylose and amylopectin components (Huang et al. 2007; Jane and Chen 1992; Jane et al. 1999; Ratnayake et al. 2001).

As previously noted, Bambara groundnut landraces were used in subsequent experiments due to raw material availability. Hence, the pasting, Gelatinisation and digestibility properties of the landrace starches were further investigated. Brown Bambara groundnut starch showed lower peak Gelatinisation temperature, but higher peak, setback and final viscosities compared to maroon and cream Bambara groundnut. Pasting and thermal properties of starches have been found to significantly correlate with amylose contents and chain length distribution of amylopectin, with the latter having more influence (Huang et al. 2007; Jane et al. 1999; Noda et al. 1996). In the current study, the peak viscosity of Bambara groundnut starches showed no correlation with their amylose contents. Hence, differences in peak viscosity of the landrace starches could be due to other factors such as distribution of the amylopectin components of these starches. Huang et al. (2007), attributed variation in peak viscosity among chickpea, yellow pea and cowpea starches to differences in their proportion of long amylopectin chains. Furthermore, landrace starches displayed high amounts of resistant starch (71%) and a similar glycemic index value of 40.1. The fairly high amylose contents of the landrace starches suggest that these starches could be better candidates for complex formation with lipids than the genotypes (Eliasson et al. 1988).

9.2 Complex index structure and functionality of Bambara groundnut starch as influenced by lipid addition and homogenization

Complex index (CI), is frequently used to determine the degree of complexation of starch with lipids. With or without homogenization, fatty acids (PAM, STE, OLE and LIN) generally showed higher complexing degree with Bambara groundnut starch than LPC. Among the studied fatty acid types, saturated fatty acids showed higher CI than the unsaturated types.

Differences in complexing ability of fatty acids with starch have been found to be influenced by structural differences in terms of lipid chain length and degree of unsaturation (Eliasson and Krog 1985; Kawai et al. 2012; Tang and Copeland 2007; Zhou et al. 2007). It is generally known that the double bonds in unsaturated fatty acids hinder the formation of V-amylose complex due to the formation of a kink within the amylose helix, resulting in low amount of V-amylose complex (Hahn and Hood 1987; Liu et al. 2016; Thachil et al. 2014). However, Karkalas et al. (1995) suggested that amylose helix may expand from six glucosyl residues per turn to seven in order to accommodate the unsaturated portion of acyl chain. Thus, it is hypothesized that the expansion of V-amylose helix may vary with the structure of the amylose contents in terms of chain length, starch moisture content, lipid concentration and the lipid type. This may explain the differences in the reported trend in the literature, in which some authors found that saturated lipids complexed better (Eliasson and Krog 1985; Liu et al. 2016; Raphaelides and Karkalas 1988; Tang and Copeland 2007; Zhou et al. 2007), while others found the opposite (Annor et al. 2015; Kawai et al. 2012; Meng et al. 2014a). As previously noted, LPC displayed lower complexing ability with Bambara groundnut starch compared to the fatty acids. The low complexing ability of LPC may be explained by its complex structure in terms of its molecular weight, number of fatty acids bound to the glycerol backbone and the presence of hydrophilic phosphate group. Previous studies found that the position of the fatty acid or the number of fatty acids present in the glycerol backbone of LPC influenced its complexing ability with starch (Siswoyo and Morita 2003). Another plausible reason for the low complexing ability of LPC with Bambara groundnut starch may be associated with the high Gelatinisation temperature (95°C) used in this study. LPC molecule could deteriorate at temperatures greater than 60°C, which could result in decrease in the inclusion rate of LPC into amylose (Cheng et al. 2015; Wang et al. 2014).

Pasting Bambara groundnut starch with lipids further confirmed better complexation with saturated fatty acid (STE) than the unsaturated type (LIN) and LPC. The addition of STE to Bambara groundnut starch gave the highest reduction in peak viscosity and highest increase in final viscosity. Reduction in peak viscosity of starch modified with lipids have been found to significantly correlate with the degree of complexation (D'Silva et al. 2011; Wang et al. 2015b; Zhou et al. 2007). Bambara groundnut starch gel complexed with lipids showed reduced firmness, suggesting that the lipid probably increased the spacing between junction zones formed by amylose or amylopectin chains during storage (D'Silva et al. 2011; Richardson et al. 2004). The complexed starches also showed a reduced hydrolysis rate compared to the

uncomplexed samples, which may further suggest complex formation between Bambara groundnut starch and the lipids.

High-pressure homogenization (HPH) improved the degree of complexation of Bambara groundnut starch irrespective of the lipid types. Homogenized complexes showed significantly higher CI than the unhomogenized samples. HPH is a promising technique for complexing starch with lipids to produce more stable and homogeneous products (Che et al. 2009). Homogenization may also enhance better starch functionality, for example, it can produce better film properties in starch and reduced syneresis rate due to improved distribution of macromolecules such as lipids within the starch structure (Nilsson et al. 2006). Bambara groundnut starch film modified with stearic acid showed a better barrier to water vapour and improvement in thermal properties. Modification of Bambara groundnut starch film with 2% stearic acid was found to be optimum for improving the physicochemical properties with minimal effect on the tensile strength.

A model is proposed to explain the differences in complexing ability of the lipids, the improvement in complexation of Bambara groundnut starch with lipids using high-pressure homogenization and the influence of these complexes on functionality (Figure 9.1). In the proposed model, STE and LPC are used to describe the possible interactions during complexation. The carboxylic polar head in STE and other fatty acids have been shown to be retained outside the amylose helix due to steric hindrance (Carlson et al. 1979; Godet et al. 1993a; Godet et al. 1993b; Kawada and Marchessault 2004). The V-amylose complexes formed between Bambara groundnut starch and fatty acids are considered to interact with one another (opposite or adjacent chains) through hydrogen bonding (Figure 9.1B). Polar head of the uncomplexed stearic acid may also interact with that of the V-amylose complexes. In contrast, electrostatic repulsions from the phosphate backbone in the LPC molecule between two V-amylose complexes, dominate the molecular interactions (Figure 9.1C). Hydrophobic interactions between the hydrophobic tube of amylose and lipids results in complex formation, and is well-known to reduce the firmness of starch gel. Starch gels are formed when the amylose chains form cross-links through hydrogen bridges during short storage (Wang et al. 2015a). These bridges create junction zones, due to the formation of a three-dimensional gel network structure (Whistler and BeMiller 1997). Thus, fatty acids can reduce the formation of junction zones in starch gel in two ways. Firstly, by forming complexes with amylose in starch and secondly, possibly by the close interactions between the V-amylose complexes due to

hydrogen bonding, further reducing the tendency of neighbouring amylose chains from forming junction zones. This may explain the higher CI of the fatty acids compared with the LPC molecule. Maize and potato starch reference samples showed lower CI values compared to Bambara groundnut starch, which could be due to differences in amylose contents (22.5-31.5%) (Eliasson et al. 1988; Obiro et al. 2012b).

Furthermore, as earlier stated, unhomogenized Bambara groundnut starch-lipid complexes produced weak or soft gels while the homogenized ones were non-gelling. The non-gelling behaviour of homogenized V-amylose complexes is considered to result from enhanced interactions between the polar head of V-amylose complexes and that of uncomplexed fatty acid as proposed above. The hydrogen bonds between the polar heads though, can easily separate during homogenization, they have been shown to interchange easily and reform during deformation (Belitz et al. 1986). It is also possible that the larger amounts of uncomplexed lipids (Figure 9B & C) and amylose chains may have reduced after homogenization, since there is the likelihood that more complexes are formed between the free lipid and the amylose (Figure 9.1D). It has been suggested that the applied pressure improves the distribution of lipids within the starch suspension and enhances the release of more amylose from swollen starch granules due to intense shear and turbulence (Meng et al. 2014a; Meng et al. 2014b). Non-gelling behaviour of starch-lipid complexes have been previously reported (D'Silva et al. 2011; Richardson et al. 2004). X-ray diffraction studies also revealed that more V-amylose complexes were formed by high-pressure homogenization due to the high intensities of the V-amylose peaks (Figure 6.2 & 7.2). Generally, Bambara groundnut starch complexed with lipids displayed higher T_m (92-104°C) than the native starch (78°C), which corresponds to the melting temperature of type I V-amylose complexes (Biliaderis and Seneviratne 1990; Raphaelides and Karkalas 1988). The melting enthalpy values (5.1-6.8 J/g) of the homogenized complexes, which were higher than the unhomogenized samples (0.86-1.94 J/g), also confirmed the formation of more V-amylose complex. High enthalpy values have been associated with the amount of V-amylose complex formed between starch and lipids (Kawai et al. 2012; Meng et al. 2014a; Wang et al. 2015b). Dynamic rheological data indicate low G' for Bambara groundnut starch-lipid complexes gels compared to gels from uncomplexed starch, which was due to V-amylose complex formation (Ahmadi-Abhari et al. 2015; Singh et al. 2002).

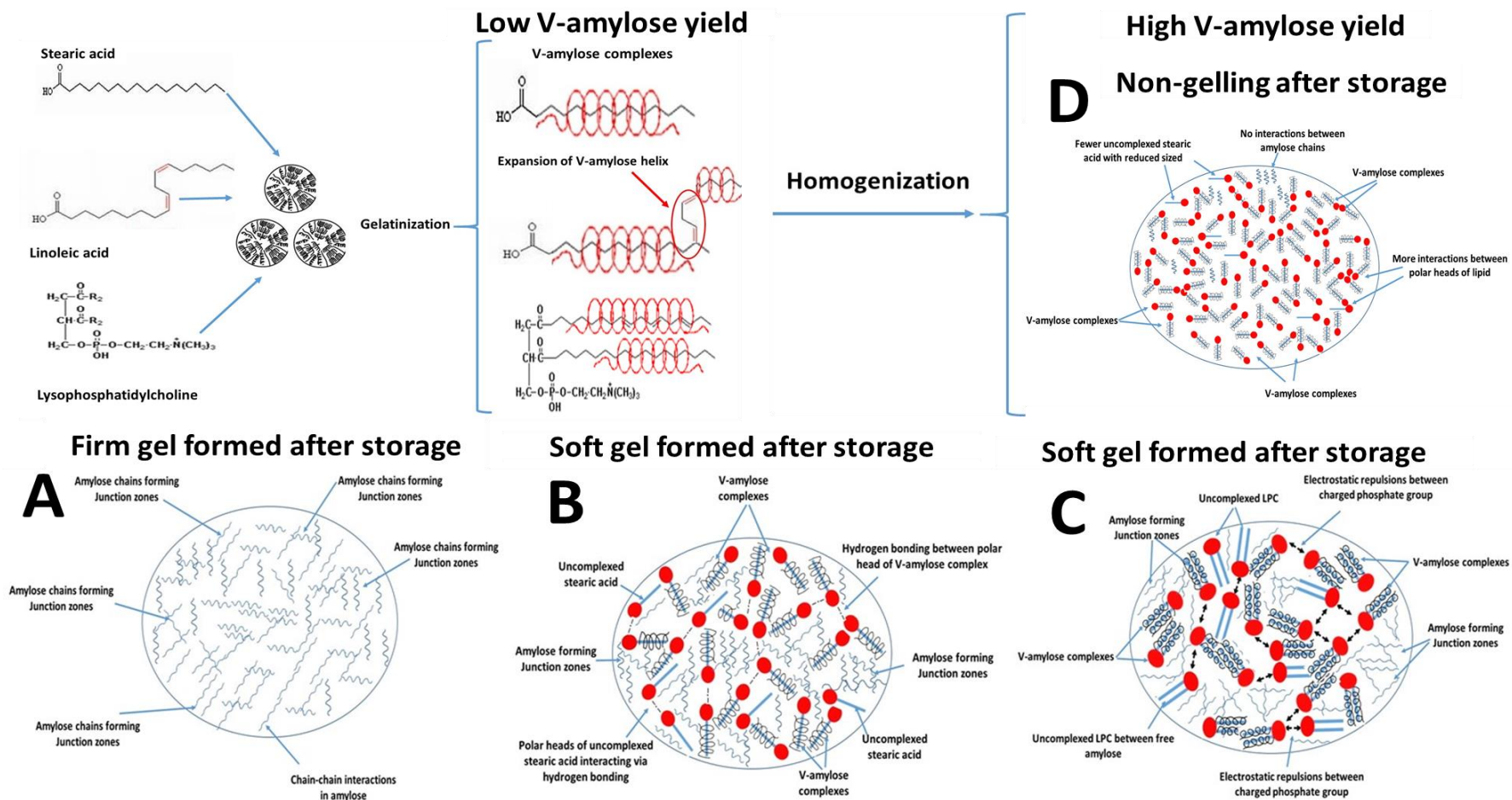


Figure 9.1 Proposed model showing the influence of homogenization on interactions between Bambara groundnut starch and lipids

A: Gelatinized Bambara groundnut starch without lipids, B: Unhomogenized gelatinized Bambara groundnut starch-stearic acid complex
 C: Unhomogenized gelatinized Bambara groundnut starch-lysophosphatidylcholine complex, D: Homogenised gelatinized Bambara groundnut starch-stearic acid complex

9.3 Potential application of Bambara groundnut starch-lipid complexes

Lipid modified Bambara groundnut starch displayed reduced digestibility (in-vitro), hence these modified starch can be potentially used in formulating food for the diabetics. The high final viscosities of Bambara groundnut starch, with the addition of lipids and consequent reduction in gelling ability, suggest the potential of the modified starch as fat replacers in foods. Lipid-modified Bambara groundnut starch may be useful in salad creams. The higher melting temperature of the modified Bambara groundnut starch suggests that these starches would be useful in food that requires high-temperature processing such as in extruded products. Homogenized Bambara groundnut starch-lipid complexes may also be employed in the production of modified starch with non-gelling properties suitable for certain industrial application such as in frozen foods and desserts for better mouth feel. Bambara groundnut starch film modified with lipids e.g. stearic acid may find application in coating of fruits and vegetables for extended shelf-life.

9.4 General conclusions and Recommendation

Bambara groundnut represents a good source of starch for the industry. Landrace starches generally showed fairly higher amylose contents and better functionality such as swelling power when compared with the genotypes. Bambara groundnut starch formed complex with the studied lipids as confirmed by V-amylose peaks and high melting temperatures corresponding to the type I V-amylose complex. Saturated lipids showed high complexing degree with Bambara groundnut starch due to ease of access into the amylose helix interior. Homogenization improves the complexing ability of Bambara groundnut starch with all the lipids. This was confirmed by high intensity of XRD peaks and high enthalpy values. Homogenized Bambara groundnut starch-lipid complexes are non-gelling and show a low syneresis rate. Bambara groundnut starch complexed better with the lipids than maize and potato starches, which could be due to its high amylose content. This is a major and interesting part of this research as the starch industry may find homogenized Bambara groundnut starch-lipid complexes suitable for use as an alternative to the conventional cereal starches. Bambara groundnut starch showed good film-forming properties, which could be modified with 2% stearic acid for improved water vapour permeability and thermal stability with minimal effect on tensile strength. Further research is required to understand the interaction between starch and lipids after homogenization. This may provide a better insight into the type and extent of fusion or mechanism that governs the interaction between starch and lipids after high-pressure

treatment. Further research may also be required to explore the unique non-gelling property of the homogenized Bambara groundnut starch-lipid complexes in food systems.

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Physicochemical properties of starches with variable amylose contents extracted from bambara groundnut genotypes



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ABSTRACT

The physicochemical properties of starches extracted from five bambara genotypes were investigated. Bambara starch granules were predominantly oval shaped with a smooth surface and an average size of $26 \pm 0.2 \mu\text{m}$. The amylose contents (20–35%) varied significantly among genotypes. X-ray diffraction revealed the C-type pattern for all starches with relative crystallinity range: 29–35%. FTIR spectra of bambara starches showed variable peak intensities at 2931, 1655 and 860 cm^{-1} , which corresponds to C–H stretching, H_2O bending vibrations and C–O stretching, respectively. Bambara genotype with the highest amylose content showed the lowest intensity at wavenumber 2931 cm^{-1} . With the exception of oil absorption which was similar, swelling power, water absorption and paste clarity of starches were significantly different among genotypes. Genotype with high amylose content showed restricted swelling, low paste clarity and great ability to absorb water. All bambara starches displayed a shear thinning behaviour ($n < 1$).

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1. Introduction

Bambara groundnut (*Vigna subterranea*) is a good source of protein (19–21%) and carbohydrate (57–67%) (Kaptso et al., 2014; Sirivongpaisal, 2008; Onimawo, Momoh, & Usman, 1998), similar to legumes such as cowpea (Oyeyinka et al., 2013) and peas (Wang & Castonguay, 2014). The bambara plant is highly drought tolerant and thus, well adapted to the changing climate. However, bambara groundnut is neglected and under-utilized in Southern Africa. Traditionally, bambara is consumed by boiling freshly harvested grains and eaten as a relish with maize-meal porridge (Swanevelde, 1998). Matured grains are dried and ground into flour for making puddings. The under-utilization of many crops including bambara may be attributed to lack of sufficient research to unlock their potential and value addition.

The major carbohydrate of bambara grain is starch, which may have potential applications in the food industry. The starch contents of bambara may vary between 35 and 46% (Flour basis) (Afolabi, 2012; Sirivongpaisal, 2008; Adebowale, Afolabi, & Lawal, 2002). By microscopy, bambara has been found to contain round and irregularly shaped or polygonal starch granules (Kaptso et al., 2014;

Sirivongpaisal, 2008; Adebowale & Lawal, 2002) with an average size ranging from 6 to $61 \mu\text{m}$ depending on variety and source (Kaptso et al., 2014; Sirivongpaisal, 2008; Adebowale & Lawal, 2002). According to Kaptso et al. (2014), starch extracted from white bambara were larger ($10\text{--}35 \mu\text{m}$) than those extracted from black bambara ($6\text{--}15 \mu\text{m}$). In terms of starch composition, bambara starches have been found to contain varying amounts of amylose (21–28%) depending on source and variety (Kaptso et al., 2014; Sirivongpaisal, 2008). The amylose content of starch can significantly influence its functional properties including swelling and gelatinization (Xie et al., 2009; Stevens & Elton, 1971). Native starch consists of semi-crystalline structure and gelatinization, which involves heating of starch in water, disrupts the starch granular structure and causes changes from semi-crystalline to amorphous structure (Atwell, Hood, Lineback, Varriano-Marston, & Zohel, 1988). During the gelatinization process, starch granules absorb water and swell and the amylose leaches out of swollen granules, which causes an increase in viscosity of the medium (Hermansson & Svegmarm, 1996). Previous studies showed that high amylose maize starch exhibited higher viscosity (Xie et al., 2009). Further, the high amylose contents of different starches have been found to inhibit swelling during gelatinization (Tester & Morrison, 1990).

The molecular characterization of starch revealed differences in crystallinity patterns, which are associated with botanical origin.

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RESEARCH ARTICLE

Physicochemical properties of starches extracted from bambara groundnut landraces

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The physicochemical properties of starches extracted from three bambara groundnut landraces, maroon, brown, and cream were studied. The amylose contents (31.5–34.6%) of the bambara starches were significantly different among the landraces. All the bambara starches exhibited an A-type crystalline pattern with an average relative crystallinity of 32%. The peak gelatinization temperature (approx. 73°C) of brown bambara starch was slightly low compared to maroon (approx. 78°C) and cream (approx. 76°C) bambara starch. The bambara starches showed substantially high proportion of resistant starch (71%) and similar predicted glycemic index (40.1) among landraces. Bambara starch can potentially be used as a thickening agent in food products and ingredient development.

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1 Introduction

The starch industry relies mainly on cereals such as corn as major sources of starch. Pulses including pea (*Pisum sativum*), cowpea (*Vigna unguiculata*), and bambara groundnuts (*Vigna subterranea*) are relatively good sources of starch (18–49%) [1–3]. These leguminous crops can play a role as alternative starch sources to the conventional cereal crops. Among pulses, pea starch has found some applications in the food and allied industries. However, traditional crops such as bambara have not been extensively researched and their application remains limited in the food industry.

Botanical origin, composition (e.g., amylose content) and plant species may significantly influence the physicochemical properties of starch [4–7]. Potato starch granules appeared round or oval in shape with smooth surfaces compared to corn starch granules, which were irregular with many pores on the surface [8]. Gelatinization is a phase transition that occurs when heating starch in the presence of moisture.

Gelatinization changes the starch structure from semi-crystalline to amorphous phase [10]. This process is frequent in food processing and therefore, has been extensively studied. Many studies reported a significant influence of amylose on starch melting temperature [9–11]. Joshi [10], studied the physicochemical properties of lentil starch with higher amylose content than corn and potato starches. The melting temperature of lentil starch was found to be intermediate between potato and corn starches [10]. However, some studies did not find any relationship between amylose content and gelatinization temperature [5, 12]. For instance, Chung [12], reported similar gelatinization temperatures for starches from three varieties of common bean differing in amylose contents. According to Jane [13], the fine structures of amylopectin play a significant role in gelatinization process and starch pasting. Pasting properties of starch such as peak viscosity, breakdown and final viscosity, have been found to vary greatly among potato cultivars [14]. Huang [15], also found some variations in the pasting properties of pulse starches. Cowpea starch with higher amount of long amylopectin chains showed higher peak and final viscosities compared to chickpea and yellow pea starches [15].

There is a growing interest in pulse starches because of their high resistant starch contents, which are known to have positive physiological effects [16, 17]. Previous studies

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Abbreviations: A_a , amorphous area; A_c , crystalline area; HI, hydrolysis index; RC, relative crystallinity

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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.

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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

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 properties [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 also found an increase in melting temperature of starch–

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

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Influence of high-pressure homogenization on the physicochemical properties of bambara starch complexed with lysophosphatidylcholine

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ABSTRACT

Amylose can form inclusion complexes with lipids for improved starch functionality. This study determined the influence of high-pressure homogenization on the physicochemical properties of bambara starch complexed with lysophosphatidylcholine. Homogenization significantly increased the degree of complexation of bambara starch with lysophosphatidylcholine. Bambara starch showed higher complex index than corn and potato starch reference samples. X-ray diffraction revealed the formation of V-amylose crystalline types with peaks at $2\theta = 7.4, 12.9$ and 19.9° . Complexing bambara, corn and potato starches with lysophosphatidylcholine resulted in the formation of type I V-amylose complexes. These complexes had melting temperatures and enthalpies ranging from 91.5 to 98.6°C and 1.4 – 5.5 J/g respectively. Starch-lysophosphatidylcholine complexes displayed low syneresis rate. Homogenized bambara starch complexes were non-gelling while unhomogenized types produced weak gels, with G' > G'' in the frequency range: 0.1 – 10 Hz. The non-gelling properties of homogenized bambara starch-lysophosphatidylcholine complex suggest that the modified starch could be used in the food industry to provide smooth texture in frozen foods and desserts.

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1. Introduction

Native starches are unsuitable for most industrial applications because of their poor resistance to extreme processing conditions such as heat and shear. Therefore, starches are modified to improve their functional properties and application in foods. Starch modification mainly involves the interaction between the two major starch components (amylose and amylopectin) and additives such as ligands. The amylose component of starch can form inclusion complexes with lipids. During starch gelatinization, amylose undergoes a conformation change resulting in a single left-handed helix with a hydrophobic inner cavity (Putseys, Lamberts, & Delcour, 2010). Lipids can complex amylose in the hydrophobic tube (Immel & Lichtenthaler, 2000; Putseys et al., 2010) and are stabilized by van der Waals forces (Godet, Tran, Delage, & Buleon, 1993).

Different lipids e.g. stearic acid and lysophosphatidylcholine

(LPC) have been employed in starch modification. LPC is the main endogenous phospholipid found in cereal starches (Hernández-Hernández et al., 2011; Morrison, 1988). Starch modification using LPC has attracted the interest of many researchers, possibly due to its wider application in food, for example, as emulsifiers. Cui and Oates (1999) found that sago starch showed higher complexing ability with LPC than with monoglycerides. According to these authors, the LPC molecule presumably has less tendency to form micelles and may pass through the surface of starch granules more easily than monoglycerides. Siswoyo and Morita (2003), reported that the number of fatty acid bound to the glycerol backbone in a phospholipid can also influence the complexing ability of starch. Defatted wheat starch complexed with 1- or 2- glycerophosphatidylcholine (GPC) showed higher complex index (approx. 93%) than with 1, 2-GPC (approx. 63%) (Siswoyo & Morita, 2003). Furthermore, the amylose contents and the degree of polymerization of amylose in different starches may influence the degree of complexation with lipids (Eliasson, Finstad, & Ljunger, 1988; Godet, Tran, Colonna, Buleon, & Pezolet, 1995; Ocloo, Minnaar, & Emmambux, 2016). Other studies reported on the influence of complexation on starch functionality. For instance, LPC at a

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Effect of high-pressure homogenization on structural, thermal and rheological properties of bambara starch complexed with different fatty acids

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The effect of high pressure homogenization (HPH) on the degree of complexation of different fatty acids with bambara starch was studied. HPH significantly increased the complexation of bambara starch with palmitic, stearic, oleic and linoleic acids. However, saturated fatty acids generally showed higher complexing ability than unsaturated ones. For all fatty acids, bambara starch showed a higher complex index than corn and potato starches, which could be associated with the variation in amylose contents (22.5–31.5%). The formation of V amylose crystalline materials was confirmed by XRD with peaks at 2θ 7.4, 12.9 and 19.9°. Bambara starch fatty acid complexes displayed significantly higher melting temperatures (95.74–103.82 °C) compared to native uncomplexed starch (77.32 °C). Homogenized bambara starch complexes were non gelling while unhomogenized complexes produced weak gels, with $G' > G''$ in the range of 0.1–10 Hz. Complexation of bambara starch with fatty acids using HPH may be employed in the production of modified starch with non gelling properties and higher thermal stability suitable for certain industrial applications.

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Introduction

Starches are modified to make them suitable for most industrial applications. Modification improves starch resistance to extreme processing conditions such as shear and heat that are usually encountered in industry. Over the last few decades, starch has been modified by physical, genetical, enzymatic and chemical methods.^{1,2} Chemical modification of starch seems to be the most widely used.² But most of these chemicals, for example, propylene oxide, are synthetically derived.³ Currently, natural alternatives, such as the use of lipids, are being sought to produce clean label starches.

The structural and functional properties of a starch–fatty acid complex may vary with fatty acid type and concentration,^{4–6} as well as processing conditions such as gelatinization time and temperature.^{7–9} The interaction between added lipids such as fatty acids and starch as revealed by X-ray diffraction studies results in distinct V-type crystalline structure known as V-amylose complex.¹⁰ Exarhopoulos and Raphaelides⁶ studied the morphological and structural studies of thermally treated starch–fatty acid systems. The presence of fatty acids effectively retarded gelatinization process of maize starch, high amylose

starch and pea starch.⁶ Several starch modification processes using lipids such as fatty acids have been reported to improve starch functionality.^{4,6,11,12} For example, rice starch showed restricted granule swelling, reduced solubility and lower rate of retrogradation with stearic acid addition.⁴ Furthermore, starch–lipid complexes showed improved thermal stability compared to their native counterparts.^{13,14} These changes in starch functionality are associated with the formation of amylose–inclusion complexes with added lipids. The rheological behaviour of starch complexed with fatty acid was investigated by Singh *et al.*¹⁵ Stearic acid addition reduced the storage modulus (G') in corn starch and increased the same parameter in potato starch. However, the addition of myristic acid decreased the G' in both starches.¹⁵ Thus, the rheological behaviour of various starches may depend on the starch source and fatty acid type.

Different methods have been explored in the preparation of starch–fatty acid complexes.^{3,7–9} The main purpose for using these methods is to increase the degree of complexation of starch with fatty acids. D'Silva *et al.*,³ found that the degree of complexation of teff starch with 0.25% stearic acid increased by approximately 83% when the holding time was increased from 5 to 120 min during starch pasting. The increase in complexation was attributed to prolonged interaction between the starch and stearic acid.³ A promising method for increasing the degree of complexation of lipids with starch is the application of high-pressure homogenization (HPH) to gelatinized starch–fatty acid complexes. Previous research documented that the use of HPH

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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).

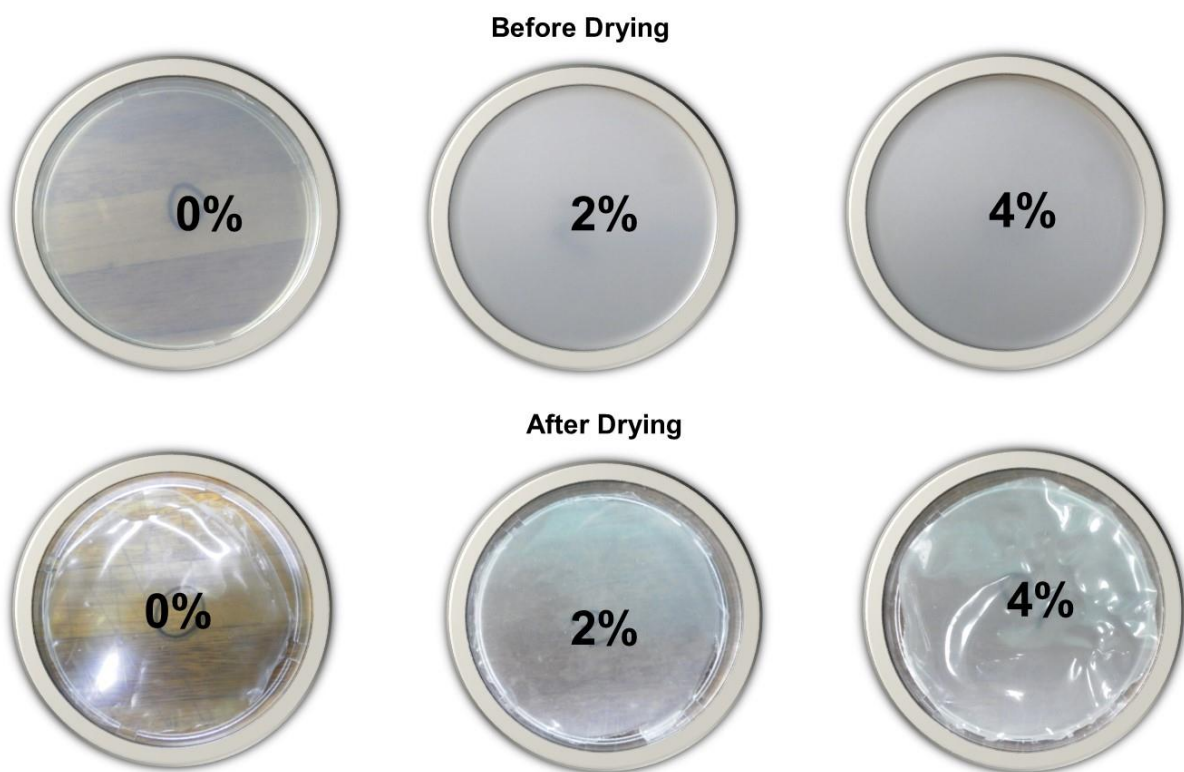
The modification of starch with lipid results in the formation of amylose inclusion complexes. These complexes may be explored in making films with improved barrier properties (Jiménez and others 2012b; Liu and others 2015). Most reported studies on improving physicochemical properties of starch films 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 subteranea*) 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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Values in percentages represents concentration of stearic acid per dry weight of starch