FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF BAMBARA GROUNDNUT STARCH-CATECHIN COMPLEX OBTAINED BY CHEMICAL GRAFTING.

by

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DECLARATION

I, **Nontobeko B. Gulu**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

The aim of this study was to produce Bambara groundnut (BGN) starch-catechin complex using chemical initiators (ascorbic acid and hydrogen peroxide) and cyclodextrin (alpha and beta) with the view to obtain a functional ingredient for the food industry. BGN starch was successfully extracted from BGN flour through dry milling method, yielding 32% of BGN starch. Native BGN starch was chemically modified using ascorbic acid (1% w/w) and hydrogen peroxide (165% w/w) as redox, biocompatible initiator for grafting catechin to the BGN starch. In addition, cyclodextrin (alpha and beta) were also used as initiators for modifying BGN starch through complexation methods. Complexation methods used included the microwave, co-evaporation and kneading. The characterization of native and modified BGN starches was carried out by performing scanning electron microscopy, powder X-ray diffraction, Fourier Transform Infrared (FTIR) and fluorescence spectroscopy analysis. Functional, thermal and rheological properties of native and modified BGN starches were evaluated. The pasting properties of BGN starches were determined using the Rapid Visco Analyser (RVA). According to the SEM profile, native BGN starch had round, oval and elliptical shapes typical for legume starches. Native BGN starch displayed a typical type-C crystallinity which is common among legumes with strong peaks at 2θ of 15^{o}, 17^{o} and 23^{o}. BGN starches modified through complexation methods had sharper peaks indicating an increase in starch crystallinity; however, following chemical modification there was loss in starch crystallinity which was evidenced by the amorphous region in the chemically modified BGN starches. Structure of native and modified BGN starches was confirmed by FTIR. The FTIR spectra of native BGN starch showed variable peaks at 3285.34 cm^{-1}, 2931.69 cm^{-1}, 1634.36 cm^{-1}, 1336.77 cm^{-1} which are attributed to OH stretching, C-H stretching, water bending vibrations and C-O stretching, respectively. Furthermore, the FTIR results confirmed that native BGN starch is made up of glucose molecules just like all other starches. All modified BGN starches displayed a new absorption peak at 1020 cm^{-1} wavelength, thus indicating that starch modification was successful. On the other hand, all BGN starch-catechin complexes displayed a new absorption peak in the range of 1520 -1560 cm^{-1}, attributed to the C-C stretching within the aromatic ring of the catechin. The successful grafting of catechin to BGN starch was also confirmed by the fluorescence spectroscopy results, where all the BGN starch-catechin complexes had an emission peak at 320 nm while native BGN starch had an emission peak at 270 nm. Antioxidant capacity of BGN starch was determined through DPPH and ORAC antioxidant assays. Within the DPPH assay, the antioxidant activity ranged from 2.26 to 38.31 µmol TE/g. The antioxidant activity of modified BGN starch-catechin complexes was significantly ($p \leq 0.05$) higher than the ones modified without catechin. On the other hand, within the ORAC assay, the antioxidant activity ranged from 0.07 to 126.71 µmol TE/g. As opposed to the results obtained in DPPH assay, the

antioxidant activity of chemically modified BGN starch-catechin complexes was significantly ($p \leq 0.05$) higher than that of complexed BGN starch-catechin complexes. Chemical modification significantly increased the swelling capacity of native BGN starch while complexation methods significantly reduced it. The water absorption of native and modified BGN starches ranged from 0.23 to 3.10 g/g. Water absorption capacity of chemically modified BGN starches (2.86 and 3.10 g/g) was significantly higher than the water absorption capacity of native BGN starch (1.17 g/g). There was no significant difference ($p > 0.05$) in the oil absorption capacities of BGN starches and they ranged from 1.02 to 1.07 g/g. Breakdown viscosity, set back viscosity and final viscosity all reduced following starch modification. Lastly, microwave and chemically modified starches displayed a shear thinning behaviour while those modified through co-evaporation and kneading methods displayed a shear thickening behaviour.

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DEDICATION

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APPENDIX

APPENDIX: Approved Ethic clearance

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
AA	Ascorbic acid
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
AGU	Anyhydroglucose units
BGN	Bambara groundnut
BGNF	Bambara groundnut flour
BGNS	Bambara groundnut starch
BSE	Backscatter Electron detection
CGTase	Cyclodextrin glucanotransferase enzyme
cP	Centipoise units
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimetry
EC	Epicatechin
ECG	Epicatechin gallate
EDS	Energy Dispersive X-Ray Spectrometry
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
GDP	Gross domestic product
GI	Gylcemic index
HDL	High-density lipoproteins
HPV	Hot paste viscosity
H_2O_2	Hydrogen peroxide
LDL	Low-density lipoproteins
MANOVA	Multivariate analysis of variance
NADPH	Nicotinamide adenine dinucleotide phosphate
ORAC	Oxygen Radical Absorbance Capacity
OSA	Octenylsuccinic anhydride
PCA	Principal Component Analysis
ROS	Reactive oxygen species
RVA	Rapid visco analyser
SB	Setback viscosity
SE	Secondary Electron
SEM	Scanning electron microscopy
SOD	Superoxide dismutase

CHAPTER ONE
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Most plants store their carbohydrate in the form of starch and its main sources are tubers (potato), cereal grains (rice, wheat, maize), and root crops (sweet potato, cassava) (Adebowale & Lawal, 2002; Shamekh, 2002; Singh *et al.*, 2003; Singh *et al.*, 2007; Mensah, 2011). However, new product development as well as improving product and process quality, prompts the need to source new starches with various properties. As a result, expanding starch requirements together with its extending industrial usage call for the use of starch from underutilised legumes such as Bambara groundnut (BGN). BGN is a crop native to Africa, which produces an almost balanced food, as it has significant amounts of protein, carbohydrate and fat. It is a good source of starch because of its high carbohydrate content of about 65%. Adebowale & Lawal (2002) reported that BGN has the potential of being a good source of starch.

Mensah (2011) reported that BGN starch has high amylose content with granules being oval shaped. The starch shows a two stage swelling pattern with a similar viscosity profile to cereal starches. It also exhibits good resistance to acid at a pH range of 4.6 to 7.0. BGN starch has good thickening, gelling and film forming characteristics (Adebowale & Lawal, 2002). However, it being a native starch limits the industrial applications, hence, there is need to chemically modify it to extend its industrial usage.

Chemical modification is a method used to modify native starch molecules. This method introduces functional groups onto the starch molecule. Starch chemical modification increases the wide range of starch applications in various industries (Kaur *et al.*, 2012). With the aim of making starch acceptable in various food and industrial applications, chemical modification therefore, stabilises starch granules during processing (Xie *et al.*, 2005). Furthermore, it improves the good characteristics and eliminates native starch shortcomings.

Natural or synthetic polymers are modified to improve their characteristics through a method known as grafting (Contreras *et al*., 2008; Spizzirri *et al.*, 2010). This is a method whereby monomers are joined into other polymer chains through covalent bonding (Bhattacharya & Misra, 2004). Grafting involves the incorporation of chemical groups to the modified polymers with the view to bestow new beneficial characteristics to widen the use of polymers in the industry (Dergunov *et al*., 2008; Joung *et al*., 2008; Shin *et al*., 2008; Spizzirri *et al.*, 2010). In this study, grafting initiated by chemical means will be used and the initiator system will be the ascorbic acid and hydrogen peroxide pair. Initiators generate free radicals, which in turn react with monomers resulting in graft copolymers being formed. Formation of ascorbate and hydroxyl radical intermediates occur at room temperature when hydrogen peroxide oxidises ascorbic acid (Kitagawa & Tokiwa, 2006; Puoci *et al.,* 2008;

Spizzirri *et al.*, 2010), which then initiates the reaction. The hydrogen peroxide and ascorbic acid mechanism, firstly allows the activation of the polysaccharide chains towards radical reaction then, secondly, it covalently binds the antioxidant molecule to preformed macro radical. The initiator system will produce hydroxyl radicals that will abstract the H-atoms from the polysaccharide's hydroxyl groups. This will result in free radical sites being formed and the new functional group (catechin) being inserted (Toti & Aminabhavi, 2004; Spizzirri *et al.*, 2010). Catechins will be grafted onto the BGN starch molecule leading to the formation of a new BGN starch-catechin complex. Catechins are a group of flavonoids belonging to the flavan-3-ols (Kesteloot, 1992; Jeong & Kong, 2004; Wan *et al.*, 2008) and they are mainly found in green tea. Catechins are phytochemicals mostly found in different food and beverages in appreciable amounts. Black grapes, red wine, strawberries, broad beans and apricots are food products known to have high quantities of catechin. They are known to have protective effects against inflammatory and cardiovascular diseases as well as cancer (Osada *et al*., 2001; Arakawa *et al*., 2002; Yaping *et al*., 2003; Lee *et al.*, 2005).

In addition to grafting initiated by chemical means; it is hoped that cyclodextrin will allow the grafting of catechin on the BGN starch molecule. Cyclodextrins are cyclic oligomers of α-D-glucopyranose made up of glucose molecules joined by α- (1, 4) glycosidic bonds (Singh *et al.*, 2002; Astray *et al.*, 2009; Yavuz *et al.*, 2010; Celebioglu *et al.*, 2013; Mura, 2014). Three categories of cyclodextrins are (1) α-cyclodextrin made of six glucose units, (2) β-cyclodextrin made of seven glucose units and (3) γ-cyclodextrin made of eight glucose units; these glucose units are all joined by α-1, 4 gylcosidic bonds (Singh *et al.*, 2002; Del Valle, 2004; Astray *et al.*, 2009; Mura, 2014). Cylodextrins have the inclusion complex forming ability, which results in the characteristics of complexed materials being modified. Inclusion complex formation of cyclodextrins results in beneficial modifications of the guest molecules which cannot be achieved in any other way (Singh *et al.*, 2002). Cyclodextrins are known to have wide application in various industries such as the food industry, textile industry, pharmaceuticals, packing, cosmetics, bioconversion and environment protection (Singh *et al.*, 2002; Del Valle, 2004).

1.2 Statement of the research problem

Bambara groundnut (*Vigna subterranea*) is high in carbohydrates (65%) thus making it a good source of starch. However, being native starch it has limitations like high susceptibility to retrogradation, thermal and low shear resistance, thermal decomposition, hydrophilicity, poor mechanical properties, insolubility in cold water and dimensional instability (Xie *et al.*, 2005; Singh *et al.*, 2007; Mensah *et al.*, 2011; Neelam *et al.*, 2012). These shortcomings of starch limit its industrial usage, thus the need to improve starch functionality. It is hoped that grafting catechin onto the starch molecule will improve its functionality and nutritional properties. Hence, it is thought that cyclodextrin and initiators (hydrogen peroxide and

ascorbic acid) will graft catechin functional group onto the BGN starch molecule, resulting in the new BGN starch-catechin complex being formed. Therefore, this prompts the need to investigate the functional and rheological properties of BGN starch-catechin complex obtained by chemical grafting and cyclodextrin inclusion complex formation.

1.3 Broad objective

The aim of this study was to produce BGN starch-catechin complex using chemical initiators and cyclodextrin with a view to obtain a functional ingredient for the food industry.

1.4 Specific objectives

Specific objectives of the research include:

1. Production of BGN starch-catechin complex as a food ingredient using chemical initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin.
2. Establishing the structure of the new BGN starch-catechin complex.
3. Establishing functional, rheological and thermal properties of the new BGN starch-catechin complex, compared to the native starch.

1.5 Hypotheses

It is hypothesised that:

1. A new BGN starch-catechin complex will be produced as a food ingredient using chemical initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin.
2. A new catechin functional group will be grafted onto the BGN starch molecule.
3. The new BGN starch-catechin complex will have improved functional, rheological and thermal properties compared to the native starch.

1.6 Delineations of the research

Delineations of the research include:

1 Grafting using chemical initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin will be used in this research study.

1 Catechin is the only antioxidant that will be grafted into BGN starch.

1.7 Significance of the research study

The high carbohydrate content (65%) of BGN qualifies it to be a good source of starch. Consequently, BGN starch can form part of the starch source to be used in the food industry. The significance of this study is that new knowledge about BGN starch will be generated. BGN is a low cost crop, and cultivated mainly by small-holder African farmers for their own subsistence and for upkeep. Therefore, this study will contribute towards reaching the national development plan of realising a food trade surplus, with one-third being produced by

small-scale farmers, thereby ensuring household food and nutrition security. The new BGN starch-catechin complex will result in the formation of new markets for farmers. As a result, farmers will be sensitised to produce more BGN, thus leading to them having improved lives as their income will be improved. This will also improve the economy and contribute to food security. This study will result in BGN starch being applicable in the industry thereby, leading to job creation and growth which then leads to economic growth and international competitiveness. Furthermore, the expanding use of BGN crop will result in an increased production (harvesting). This will therefore result in the exportation of the crop thereby improving the economy of the country by creating new markets as well as improving and creating new investment opportunities.

BGN is mostly cultivated by women. This study will therefore, offer a convenient way for economically empowering women, leading to them improving their lives as well as the lives of their families. This study will therefore contribute towards promoting gender equality. Lastly, this research study will generate and apply knowledge thereby contributing to the national system of innovation by increasing the number of Masters students by supporting partnerships for research.

1.8 Expected outcomes

New knowledge about BGN being a carrier of an antioxidant will be generated. New knowledge with regards to BGN starch will also be generated. Production of new BGN starch-catechin complex using chemical initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin as a food ingredient is expected.

At least one article will be published in accredited journal, and the research output will be presented in at least one national or international conference. A Master's degree is also expected from this research study.

1.9 Thesis Overview

This thesis consists of five chapters and was structured in article format where each chapter is an individual manuscript. The structure of the thesis is shown in Figure 1.1. Chapter one titled motivation and design of the study, introduces the research overview which includes, the research problem, objectives, hypothesis, delineations of the research, significance of the study as well as expected outcomes.

Chapter two is the literature review which elaborates further on the background of the research study. An overview of starch, its composition and structure was reviewed, including its importance in the food industry, limitations and shortcomings of native starch, reasons for modifying starch as well as the different methods used for starch modification mostly focusing on the chemical modification methods, particularly graft polymerisation as a means of chemically modifying BGN starch. Furthermore, background on Bambara groundnut was

outlined, including its utilisation in different parts of Africa and its nutritional composition which lead to the review of BGN starch. In addition, grafting of antioxidant particularly catechin to BGN starch was also reviewed, including the beneficial effects associated with consumption of catechins and their mechanisms. Lastly, the use of cyclodextrins as complexing agents was reviewed, since they (cyclodextrins) were used as initiators for BGN starch modification through inclusion complex formation. Formation of inclusion complexes as well as different complexation techniques used in this research study was outlined.

Chapter three is the first research chapter focusing on the effect of some chemicals and cyclodextrin as initiators for grafting catechin onto the Bambara groundnut starch. Bambara groundnut starch was modified through chemical modification as well as through complexation methods using alpha and beta cyclodextrin. Complexation methods used included the microwave, co-evaporation and kneading method. The characterization of the modified BGN starch and BGN starch-catechin complexes was carried out by performing FTIR, fluorescence spectroscopy analysis, powder X-ray diffraction as well as the scanning electron microscopy. Lastly, the amount of catechin grafted on each modified BGN starch-catechin complex was determined using DPPH and ORAC assay.

Chapter four is the second research chapter focusing on the effect of initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin on the functional and rheological properties of Bambara groundnut starch-catechin complex. The functional and thermal properties of native and modified BGN starches were assessed. Rheological properties of modified BGN starches were also evaluated and the data was modelled using Power law. Chapter five is a summary of the research, detailing the general findings conclusions and recommendations.

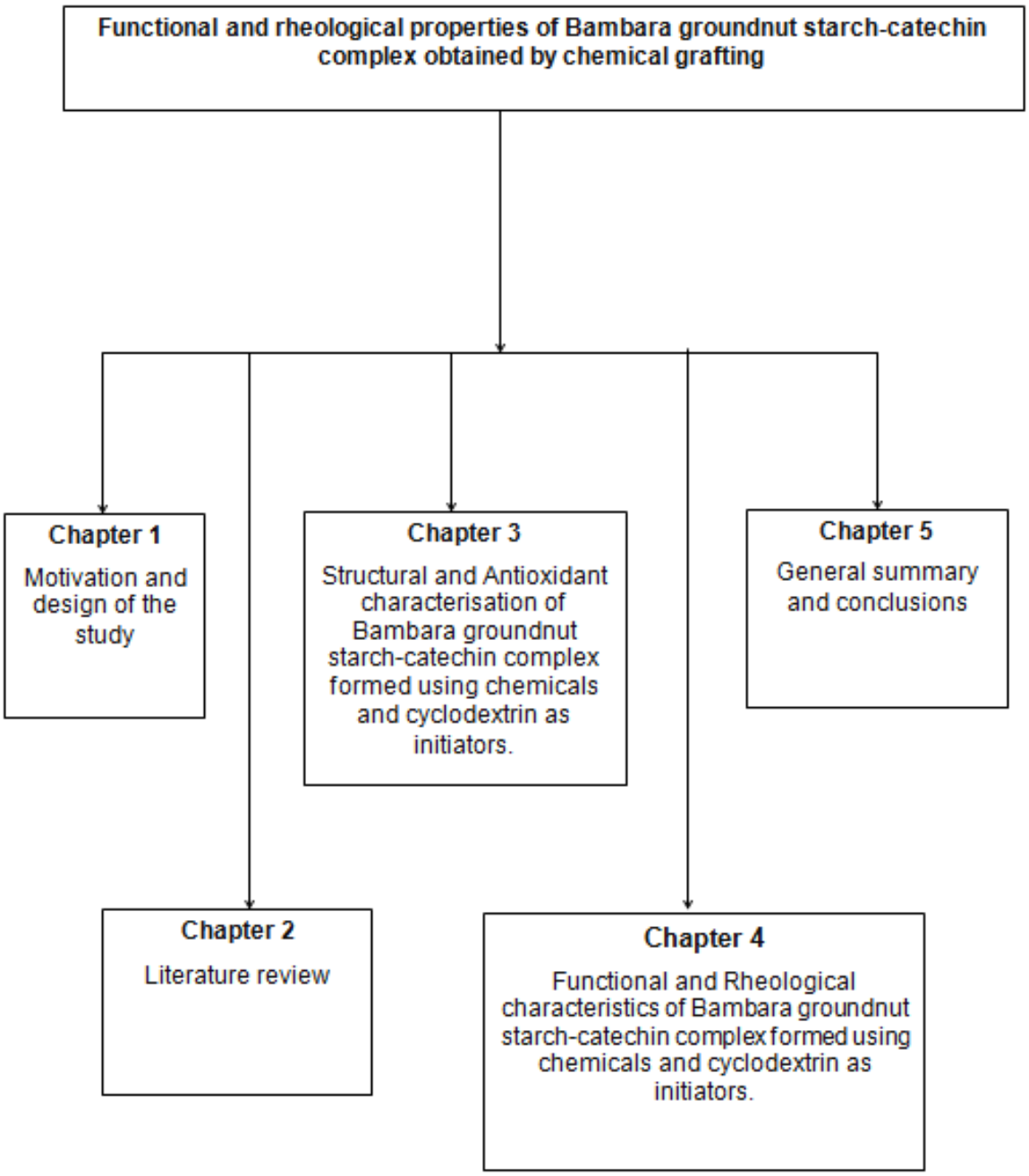

Figure 1.1 Thesis overview

1.10 References

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CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and description of Bambara groundnut

Legumes have formed part of the affordable meals across the world because they play an important role in combating malnutrition. They serve as source of non-processed protein, good source of fibre, resistant starch and other nutrients; also they are one of the low glycemic index sources of carbohydrates (Bamshaiye *et al.*, 2011). In the developing countries, legumes play a vital role in the diet of many populations, as they are a source of macronutrients like protein, carbohydrate and dietary fibre. Expanding starch requirements together with its extending industrial usage call for the use of starch from underutilised legumes such as Bambara groundnut (BGN). This is because of the legumes' affordability and availability. BGN is a native African crop mainly grown for its seeds by female subsistence farmers. It has significant amounts of protein, carbohydrate and fat; therefore it is usually referred to and used as a complete food (Baryeh, 2001; Bamshaiye *et al.*, 2011). It is grown in many parts of Africa (Muhammad, 2014; Yao *et al.*, 2015), where it is known by various names according to different local languages. Table 2.1 shows different common names of BGN from different countries (Bamshaiye *et al.*, 2011; Murevanhema & Jideani, 2013). Among the underutilised crops, BGN has the potential to justify investments for its improvement.

Bambara groundnut (*Vigna subterranea*), is a leguminous plant belonging to the *fabaceae* family, sub-family *Faboidea* and genus *Vigna* (Mpotokwane *et al.*, 2008; Bamshaiye *et al.*, 2011; Gabriel *et al.*, 2013; Yao *et al.*, 2015). According to Yao *et al.* (2015) there are two botanical varieties namely *Vigna subterranea* var *spontanea*, with wild varieties, and *Vigna subterranea* var *subterranea* with cultivated varieties. The common name used in literature, is derived from the tribe called "Bambara", who are from Mali (Murevanhema & Jideani, 2013; Yao *et al.*, 2015). Hillocks *et al.* (2012) reported that BGN originated from West Africa but it has become widely distributed in the semi-arid zone of sub-Saharan Africa. It is not only grown in many parts of Africa but it is now grown in parts of Asia, especially Indonesia, India, Malaysia, Philippines, Thailand and Sri Lanka. Furthermore, it is also found in south and central America as well as in northern Australia (Linnemann & Azam-Ali, 1993; Basu *et al.*, 2007; Muhammad, 2014). In South Africa, local people mostly grow BGN for home consumption. It was only recently that it is now being sold in local markets as boiled groundnut. BGN is mostly grown in three provinces, which are Kwazulu Natal, Limpopo, and Mpumalanga provinces. In Mpumalanga, small holder

farmers grow it in the low and middle level veld areas, both as a food and cash crop (Swanevelder, 1998).

Table 2.1 Common names of BGN used in different countries[1]

Country	Language	Name
Central Africa		Njogo bean
DRC		Congo groundnut
		Congo goober
Ghana		Akwei
		Aboboi akyii
Indonesia		Kacangbogor (bogor peanut)
Kenya		Njugu mawe
Madagascar		Pistache
		Voanjobory
Malawi		Nzama
		Njama
		Voandzounzama
Nigeria	Hausa	Guijiya
		Gujuya
		Kwaruru
	Ibo	Okpa
	Yoruba	Epa roro
Sierra Leone	Creole	Agbaroro
South Africa	Tsonga	Kokane
		Nyume
		Ndlowu
	Venda	Ndhu nwa tzidzimba
	Afrikaans	Jugoboon
	Xhosa	Jugo
	Zulu	Indlubu
Swaziland	SiSwati	Tindlubu
Zambia		Juga bean
		Ntoyo ciBemba
Zimbabwe	Ndebele	Indlubu
	Shona	Nyimo

[1]Bamshaiye *et al.*, 2011; Murevanhema & Jideani, 2013

It is the third significant crop grown in the low veld regions after maize and groundnuts in Mpumalanga (Swanevelder, 1998; Bamshaiye *et al.*, 2011). It is grown individually as well as an intercrop with maize, cowpeas and melons and it is always the dominant component in any intercrop.

BGN compares to the common peanut as it is a low, flat annual legume with compound leaves of three leaflets (Bamshaiye *et al.*, 2011). It is a difficult crop to harvest mechanically as it grows close to the ground and produces nuts underground. This then has a negative impact when it comes to large-scale commercialization; however, method of harvesting peanuts may be applicable. BGN is grown predominantly on the flat but sometimes, on moulds or ridges, as they may be beneficial in wetter areas since the crop does not tolerate water logging. Generally, BGN is a short-day plant, as a result flowering and nut development may be delayed by long day conditions (Mkandawire, 2007; Hillocks *et al.*, 2012).

BGN is an herbaceous, intermediate, annual legume with a compact well-developed taproot with many short (up to 20 cm long) lateral stems on which the leaves are borne (Swanevelder, 1998; Bamshaiye *et al.*, 2011). The leaves are trifoliate (± 5 cm long), the petiole (up to 15 cm) long, stiff, grooved, and the base is green or purple in colour (Swanevelder, 1998; Basu *et al*., 2007). Flowers are typically *papilionaceuos* and are borne in a raceme on long, hairy peduncles, which arise from the nodes on the stem (Massawe *et al*., 2002). The spreading types are usually cross-pollinated by ants while the branching types are usually self-pollinated. The pod is small (1.5 cm), round or slightly oval in shape and wrinkled with mostly one or two seeds (Swanevelder, 1998; Bamshaiye *et al.*, 2011). The unripe pod is yellowish green while the mature pods range from yellowish to a reddish dark brown colour or purple. After fertilisation the flower stem elongates, the sepal enlarges and the fruit develops above or just below the soil surface. The colour of the testa varies according to ripeness from light yellow to black, purple as well as other shades.

The plant has potential to improve malnutrition and boost food availability. BGN has prominent attributes such as drought tolerance, nitrogen fixation, high nutritional value and the ability to produce yields in marginal soils, among others, despite the fact that it still exists as land races (Azam-Ali *et al.*, 2001; Bamshaiye *et al.*, 2011; Muhammad, 2014; Yao *et al.*, 2015). It is ranked the third most important legume after groundnut (*Arachis hypogaea L*.) and cowpea (*Vigna unguiculata*); however, it is seen as a snack or food supplement rather than a lucrative cash crop and thus owing to its low status (Baryeh, 2001; Bamshaiye *et al.*, 2011; Mensah, 2011). It is cultivated both as an intercrop with maize, cowpeas and melons and as a sole crop; and it is always the dominant component in any intercrop.

BGN is a drought tolerant and easy to cultivate crop that makes very little demand on the soil. According to Baryeh (2001), it grows on any well-drained soil, but light sandy loams

with a pH of 5.0 ± 6.5 and 600 ± 1200 mm annual rainfall per year. An average day temperature of 20 to 28^{o}C is ideal for the crop. Furthermore, BGN is exceptionally versatile to hot temperatures; however, it additionally tolerates rainfall. For the crop to develop, it has an average growth period of 110 to 150 days (Basu *et al*., 2007). Consequently, it is not inclined to the risk of total crop failure, particularly in low and uncertain rainfall regions (Baryeh, 2001). BGN is resistant to high temperatures and it can grow on poor marginal soil not suitable for other leguminous crops. Although pests and diseases do not attack it in any of its production areas, it however becomes susceptible to different fungal diseases in damp conditions (Baudoin & Mergeai, 2001). It is grown without fertilisers and chemicals that can be costly and not easily accessible in remote areas. Like any other agricultural produce, it also has soil requirements, hence, it grows well on poor soil that is low in nutrients and on acidic laterite soils commonly found in Africa, however, it grows poorly in calcareous soils (Mkandawire, 2007). Disease and pests in any of its production regions do not attack it; however, in damp conditions it may be susceptible to various fungal diseases (Baudoin & Mergeai, 2001). It has a very low insect pest and disease susceptibility.

2.1.1 Cultivars and varieties of Bambara groundnut

The seeds are round, smooth and varying in size up to about 1.5 cm in diameter and very hard when they are dried. BGN seeds differ in terms of size, shape and seed coat colour. They may be round or elliptical in shape with cream, brown, red, mottled and black eyed, with or without hilum colouration, with seed weight ranging between 280 to 320 g (Bamshaiye *et al.*, 2011). According to the Department of Agriculture, Forestry and Fisheries (2011), there are several types of BGN varieties as illustrated in Table 2.2. Figure 2.1 gives a pictorial representation of the BGN different varieties (Diedericks, 2014).

Table 2.2 Bambara groundnut varieties

BGN variety	Description
Black	Matures early and its kernels are small to medium in size.
Red	Matures late and has larger kernels. It is susceptible to rooting.
Cream/black eye	Has large kernels and usually has high produce.
Cream/brown eye	Have an average kernel usually with high percentage produce.
Cream/no eye	Its percentage produce is usually low and it has small kernels and pods.
Speckled/flecked/spotted	It has small kernels one-seeded pods. Majority of this variety is purple in colour.
Brown	This is a light and dark brown variety, having medium to large kernels.

Source: Department of Agriculture, Forestry and Fisheries, 2011.

Figure 2.1 Different BGN varieties [Source: (Diedericks, 2014)]

2.1.2 Utilization of Bambara groundnut in different parts of Africa

In the light of BGN's drought tolerant characteristics, it has the potential to alleviate malnutrition and poverty in Africa. In addition, it has the potential to contribute towards food security and help to solve the problem of global warming in Africa. Traditionally BGN is used for various food uses and recipes throughout Africa. Essentially, it is mainly grown for human consumption and known to be a good protein source as well as a good supplement to maize based diets because of its high lysine content. The seeds can be eaten either while still immature or when they are fully mature. The immature seeds are usually boiled and eaten as an early source of food. On the other hand, the fully matured seeds are either cooked or pounded into flour. The seeds are often roasted and ground into nutritious flour. The mature seeds are sweet and pleasant tasting either roasted or boiled.

BGN seeds are eaten in different ways, fresh seeds may be eaten raw, boiled, grilled while flour from dry seeds can be used to make cakes (Adebowale & Lawal, 2002). In Mpumalanga, seeds are pounded into flour, which is then used to make porridge with maize or groundnuts. Seeds are also nutritious feed for chickens and the leaves, which are rich in nitrogen and phosphorus, are most suitable for animal grazing. Seed coats of legumes, BGN included are usually removed to reduce the anti-physiological factors and fiber content. This enhances the cooking quality, appearance, palatability, digestibility and texture of the

products (Akinjayeyu & Enude, 2002; Bamshaiye *et al.*, 2011). The type and quantity of legume determines the method to be used for dehulling the seeds, which can be done either manually or mechanically. Despite the seed's low oil content, some tribes in Congo roast the seeds and pound them for oil extraction. In comparison to other common pulses like cowpea, lentil and pigeon pea, BGN's gross energy value is higher (Bamshaiye *et al.*, 2011).

In Botswana, BGN is mostly grown for human consumption. Consumers prefer immature seeds, which are boiled in the pod, salted and consumed either on their own or mixed with maize seeds. Ripe and dry seeds are pounded into flour, which then is used to make a variety of cakes. Alternatively, they can be mixed with cereals and used to prepare several types of porridge that has relatively long shelf life. Roasted seeds can be boiled, crushed and eaten as a relish. After the pods have been harvested, livestock, in particular goats, are allowed to graze on the stem or stalk which they are very fond of, at the end of the season. The seeds of the mature black landrace are used in traditional medicine (Bamshaiye *et al.*, 2011).

In Burkina Faso, BGN is primarily consumed by its producers during the period before the cereals are harvested. BGN along with cowpea (*Vigna unguiculata (L) Walp*), constitutes the main source of vegetable protein for the rural populace (Bamshaiye *et al.*, 2011). Its leaves, which are rich in protein and phosphorus are used as fodder for livestock (Bamshaiye *et al.*, 2011).

In South Africa, BGN ('Njugo' beans), are often eaten when still immature. They are boiled until soft and then shelled. When they are quite dry, they are generally shelled and then boiled to make a stiff porridge.

In northern Ghana, the fresh immature beans are boiled and consumed after adding a little salt. The dry beans are also boiled, crushed and made into cakes or balls, which are then fried and used to prepare stews (Bamshaiye *et al.*, 2011). In southern Ghana, they soak the beans overnight, then boil them till soft to produce a kind of porridge/blancmange. Capsicum pepper and salt may be added during the boiling process. This preparation called 'abobo', is served with 'gari' (roasted, grated cassava) or with mashed, fried, ripe, plantain (Bamshaiye *et al.*, 2011). In 1960s, BGN was canned in Ghana, in tomato sauce with pieces of meat, in brine or as 'aboboi'.

In Nigeria, the freshly harvested pods are cooked, shelled and eaten as a vegetable snack, while dry seeds are either roasted and eaten as a snack in a manner similar to boiled peanuts or milled into flour and used in preparation of 'moi-moi' (Olapade *et al*., 2005) or 'okpa' among the Igbo tribe of Nigeria. Paste from BGN flour is used in the preparation of steamed product such as okpa in Nigeria. Okpa is a cooked dough-like gel that is made from BGN paste. It is wrapped in banana leaves before it is boiled.

In Cote d'voire, the seed is used to make flour, thus making it more digestible. In Zambia, bread is made from BGN flour. In Zimbabwe, the nuts are eaten fresh. In addition,

they can also be dried and stored for later consumption. The fresh nuts may also be roasted and eaten as a snack. BGN can be pounded and made into a relish mixed with onions, tomatoes and oil. The seeds may be milled into flour and used to make small flat cakes or biscuits. They can also be mixed with cereals and used to make porridge. The seeds are sometimes boiled and eaten together with plantains. The seed's nutritional content makes them suitable as chicken feed and after harvesting animals are allowed to graze on the dried leaves. Table 2.3 is a summary of the culinary uses of BGN in Africa as reported by Bamshaiye *et al.* (2011), Mensah (2011), Hillocks *et al.* (2012) and Murevanhema (2012). The different food uses of BGN throughout Africa are an indication that the legume can potentially improve food availability and malnutrition. Therefore, this knowledge prompts the need to expand on the nutritional composition of BGN.

2.1.3 Nutritional composition of Bambara groundnut

BGN has high nutritive value containing 53-65% carbohydrate, about 20% protein, 6.5% fat, 6.1% fibre. 3.4% ash, as well as low amounts of calcium, iron, sodium and potassium (Baryeh, 2001; Bamshaiye *et al.*, 2011; Mensah, 2011; Afolabi, 2012; Gabriel *et al.*, 2013). As a result it compares favourably in nutritional status, with other well-known and highly commercialised legumes such as cow pea and soya (Baryeh, 2001; Bamshaiye *et al.*, 2011; Afolabi, 2012). Table 2.4 gives the nutritional status of Bambara as compared to other common legumes as reported by Bamshaiye *et al.* (2011) and Hillocks *et al.* (2012). The gross energy value of BGN is higher than that of common pulses like cowpeas, lentil and pigeon pea. The red seeds play a significant role in combating iron deficiency especially in areas where it is a problem, this is because of their high iron content which is approximately double the amount of iron contained in cream seeds (Bamshaiye *et al.*, 2011; Hillocks *et al.*, 2012). Muhammad (2014) reported that when BGN is compared to other food legumes, it could complement other cereals because of its rich iron content and its protein which consists of high lysine and methionine. BGN can be used as an alternative to other legumes in low-rainfall areas and it provides a balanced diet for a demographic with low animal protein intake.

The higher lysine content in the seed makes it a high protein source and a supplement to maize based diets. Furthermore, BGN is reported to be richer than other groundnuts in essential amino acids such as valine, phenylalanine, threonine, methionine, leucine and isoleucine (Bamshaiye *et al.*, 2011). It has been reported that there is no difference in BGN varieties in terms of its composition; therefore, all the embedded nutrients will be available when consumed. Table 2.5 gives the approximate composition of BGN varieties in terms of the seed, seed coat and flour as reported by Bamshaiye *et al.* (2011). The black variant has the highest protein content while the cream variant has the lowest.

Table 2.3 Culinary uses of Bambara groundnut in Africa[1]

Country	Name	Culinary uses
Botswana		Immature seeds, which are boiled in the pod, salted and consumed, either on their own or mixed with maize seeds.
Northern Ghana		Immature seeds are boiled and consumed after adding a salt. Dry beans are also boiled, crushed and made into cakes or balls which are then fried and used to prepare stews.
Southern Ghana		Beans are usually soaked overnight, after which they are boiled till soft, to produce a kind of porridge/blancmange. Capsicum pepper and salt may be added during the boiling process. This preparation is called 'aboboi'.
South Africa	Sekome (Sesutho) Tihove (Shangaan) Shidzimba (Venda)	Adding beans and peanuts, or just one of the two to, maize or millet-meal and boiling the mixture until it forms a stiff dough. This is salted and pounded into a ball, and will keep fresh for several days.
	Tshipupu (Venda)	Boiled and then stirred, to make a thin porridge, like maize, they may also be added to a porridge made from peanuts.
	Dithaku (Sesutho)	Cooked with maize and pounded into thick, sticky dough.
East Africa		Roasted, pulverized and used to make a soup with or without condiments.
West African countries		Fresh pods are boiled with salt and pepper and eaten as a snack. They are also used in the preparation of stews. Roasted BGN seeds are eaten as a snack together with palm kernel.
Zimbabwe	Mutakura (Shona) Nkobe (Ndebele)	Boiled with maize, peanuts, cowpea salted served with mahewu

[1](Bamshaiye *et al.*, 2011; Mensah *et al.*, 2011; Hillocks *et al.*, 2012; Murevanhema, 2012)

Table 2.4 Nutritional comparison of some legume crops[1]

Nutrient g/100 g	Bambara groundnut	Soya	Cowpea	Kidney	Broad bean	Chickpea
Calories (kCal)	390	416	343	333	341	364
Protein	20.8	36.5	23.8	23.6	26.1	19.3
Carbohydrates	61.9	30.2	59.6	60	58.3	60.6
Fat	6.55	19.9	2.1	0.8	5.7	6.0

[1](Bamshaiye *et al.*, 2011; Hillocks *et al.*, 2012)

Table 2.5 Proximate composition of different varieties of BGN's seed, seed coat and flour[1]

Cultivars Seeds	Crude Protein (%)	Fat (%)	Moisture (%)	CHO* soluble (%)	CHO* (%)	Ash (%)
Red	19.5	6.5	8.0	7.6	54.4	3.0
Black	21.7	8.5	9.0	4.0	52.8	3.5
Cream	19.5	6.0	9.7	6.5	56.0	2.5
Brown	19.0	6.5	10.3	12.0	54.4	3.0
FLOUR						
Red	20.9	3.0	9.3	2.2	48.0	2.0
Black	22.6	4.0	9.0	1.4	32.0	2.0
Cream	22.3	3.0	9.0	1.6	49.6	1.5
Brown	19.4	3.5	10.0	2.9	48.0	2.0
SEED COAT						
Red	5.7	0.5	3.0	2.6	8.4	1.0
Black	6.1	2.0	3.5	3.0	6.0	1.5
Cream	6.8	1.0	3.0	1.8	9.2	1.0
Brown	6.3	2.0	3.0	0.5	9.1	1.0

[1](Bamshaiye *et al.*, 2011)
*CHO means carbohydrate

High protein content of BGN is an indication of its prospective use as a functional ingredient. BGN contains a considerable quantity of lysine and least quantities of trypsin and chymotrypsin. Furthermore, it contains carotene and vitamins such as niacin, thiamine and riboflavin however, its ascorbic acid is relatively low. Bamshaiye *et al.* (2011) reported that BGN's essential amino acid content such as lysine (6.82 g/16 gN), methionine (1.85 g/16 gN)

and cysteine (1.24 g/16 gN) is comparable to that of soya bean (6.24 g/16gN) lysine, (1.14 g/16gN) methionine and (1.80 g/16gN) cysteine. The fatty acid content of BGN oil is mainly linoleic, palmitic and linolenic acid.

BGN has substantial macro elements like nitrogen, calcium and iron, however, it is poor in phosphorus and magnesium as shown in Table 2.6 (Bamshaiye *et al.*, 2011). BGN is reported to have relatively high soluble fibre content among other beans and legumes. Soluble fibre is a non-nutrient common in oat bran and is believed to reduce the incidence of heart disease and to help prevent colon cancer. The carbohydrate content of BGN is predominantly comprised of starch. A smaller non-starch polysaccharide fraction occurs. This consists of reducing and non-reducing sugars. The high carbohydrate content of BGN makes it a good source of starch.

Table 2.6 Macro-elements of Bambara groundnuts (% dry matter)[1]

	K	Mg	Ca	P	N
Roots	1.5	0.6	0.9	0.2	2.7
Leaves	1.1	0.5	2.6	0.2	1.8
Seed	1.6	0.2	0.9	0.6	3.9

[1](Mkandawire, 2007; Bamshaiye *et al.*, 2011)

2.1.4 Bambara groundnut starch

BGN's high carbohydrate content of about 65% makes it a good source of starch. Adebowale & Lawal (2002) reported BGN to be a promising source of starch because of its outstanding caloric value (367cal/ 100 g) and nutrient source. Its carbohydrate content principally consists of starch that has the potential for industrial application. Afolabi (2012) reported that the starch yield of BGN gives an indication that the legume has promising potential as an industrial crop. The viscosity profile of BGN starch is comparable to that of cereal starches and it shows a two stage-swelling pattern. It indicates a reasonably restricted swelling starch and this is a typical swelling pattern of legumes. BGN starch is valued for some of its properties such as thickening, gelling and film forming (Adebowale & Lawal, 2002).

BGN starches contain varying amounts of amylose in the range 21 to 35% depending on source and variety (Sirivongpaisal, 2008; Kaptso *et al.*, 2015; Oyeyinka *et al.*, 2015). Functional properties such as swelling and gelatinisation are significantly influenced by the amylose content of starch (Xie *et al*., 2009; Oyeyinka *et al.*, 2015). A few studies have been reported on the functional and rheological properties of BGN starch. Adebowale *et al.* (2002) reported that the swelling capacity of BGN starch increased with an increase in temperature.

When starch is heated in water, its granules absorb water, swell and lose their molecular order thus resulting in a process known as gelatinisation. Gelatinisation causes a very big change in the rheological properties of starch suspensions (Eliasson & Gudmundsson, 2006). In addition, the process of gelatinisation increases the viscosity of the medium due to the leaching out of amylose from the starch granules.

Based on X-ray diffraction patterns, starches can be classified as A-, B- or C- type polymorphs. The A- and B- types can be differentiated by the packing arrangement of double helices formed from short chains within the amylopectin molecule as well as their level of hydration. The A- type is more compact with water molecules between each double helix while the B-type is loosely packed with a hydrated central cavity (Oyeyinka *et al.*, 2015). The C-type starches comprise of a combination of A- and B-type starches. It has been reported that A-type crystallinity is for cereal starches; B-type is for retrograded starches or tuber, cereal starches with high amylose content, and the C-type is for pulse starches (Oyeyinka *et al.*, 2015). Previous studies on the structure of BGN starch indicate a typical C-type crystallinity (Afolabi, 2012; Hoover *et al.*, 2010) characterised by strong intensity peaks corresponding approximately to 15^{o}, 17^{o} and 23^{o} 2θ. However, some researchers reported the A-type crystallinity for some varieties of BGN (Sirivongpaisal, 2008; Mensah, 2011; Kaptso *et al.*, 2015). These crystallinity patterns can influence the functionality of starch.

Microscopically, BGN starch has been reported to have round, oval and irregularly shaped starch granules with high amylose content, with an average size in the range of 6 to 61 µm, which is determined by seed variety as well as its source (Adebowale & Lawal, 2002; Sirivongpaisal, 2008; Mensah, 2011; Kaptso *et al.*, 2015; Oyeyinka *et al.*, 2015). The high amylose content of BGN starch is an indication that BGN starch can form stronger starch gels with elasticity. Sirivongpaisal (2008) assessed the thermal transition temperature of BGN starch by DSC was 71.69^{o}C at onset (T_o) while gelatinisation enthalphy (ΔH) was 11.73 J/g. Furthermore, water and oil absorption capacity of BGN starch were 1.67 and 1.01 ml/g, respectively. Oyeyinka *et al.* (2015) reported that the FTIR spectra of BGN starches showed variable peak intensities at 2931, 1655 and 860 cm¯1, which corresponds to C-H stretching, water bending vibrations and C-O stretching, respectively. Sirivongpaisal (2008) also reported that the pasting properties of the starch showed pasting temperature, breakdown and setback of 77.7^{o}C, 170 BU, 220 BU, respectively. The starch exhibits a good resistance to acid at a pH range of 4.6 to 7.0, also at this pH the starch showed similar pasting characteristics. In comparison to BGN flour, BGN starch exhibits higher swelling power, breakdown and setback, however, it has lower gelatinisation temperature, pasting temperature, water and oil absorption capacity (Sirivongpaisal, 2008).

2.2 Overview of starch

The expanding use of starch in the food industry is attributable to its convenience in various food products. Starches are the principal food reserve polysaccharides in plants and are therefore the major source of carbohydrates in the human diet. The major botanical sources for most commercial starches include cereal grains (rice, wheat, maize), tubers (potato) and root crops (sweet potato, cassava) (Adebowale & Lawal, 2002; Shamekh, 2002; Singh *et al.*, 2003, Singh *et al.*, 2007; Mensah, 2011). Table 2.7 gives the global major crop sources of starch as reported by Imam *et al.* (2012).

Starch being a significant functional biopolymer it greatly influences properties of various food products (Singh & Kaur, 2004). It modifies the texture and consistency of food products. Furthermore, it plays a vital role in the textural characteristics of various food products as it has many functions. These functions include being a gelling agent, thickener, water retention agent, colloidal stabilizer, bulking agent, an absorber of water, adhesive, as a source of energy in fermentation and as both an anti-sticky or sticky agent (Adebowale & Lawal, 2002; Singh *et al.*, 2003; Eliasson & Gudmundsson, 2006). There are varieties of attributes that starch greatly influences in the food products and these include gel formation, texture, adhesion, viscosity, binding, film formation, moisture retention, and product homogeneity (Kaur *et al*., 2012). However, successful development of value-added products is very dependent on having an in-depth understanding of starch structure as well as its functional properties. Starch can be used either as a raw material or as a food additive. It is used in a wide range of products such as in sauces, gravies, soups, batters, bakery products, coatings, dairy confectionery, snacks, and meat products. Apart from food applications, starch can also be used in alcohol-based fuels, pharmaceuticals, adhesives and textile (Kaur *et al.*, 2012).

Biodegradable packaging materials, low calorie substitutes, enhanced thermal and mechanical properties of thermoplastic materials, are all novel uses of starch (Kaur *et al.*, 2012). Moreover, starch is used in foods of different water contents, as an example it can be used in high water content products like dressings and in low water content products like liquorice. This therefore, shows the various aspects covered by starch functional properties. The physicochemical properties and functional characteristics of starch systems and their uniqueness in various food products vary with starch biological origin (Eliasson & Gudmundsson, 2006). This therefore, shows the many aspects portrayed by starch functional properties. Starch botanical origin determines the physiochemical and functional properties of starch systems

Table 2.7 Main crop sources of starch[1]

Source	Where it is produced
Corn/maize	North America, South America, Europe, Asia
Potato	United States, Europe
Sweet potato	Asia
Rice	Europe, United States, Asia
Wheat	Europe, United States, South America, Asia
Cassava	Asia, Africa, South America, United States, Europe
Tapioca	Asia, Africa, Europe
Sorghum	Asia, Africa, North America, South America, Europe
Banana	Africa, South America, Asia, Caribbean
Sago palm	Mostly Southeast Asia

[1]Adapted from Imam *et al.* (2012)

2.3 Starch composition and structure

Starch is a glucose polymer which is one of the most abundant natural polysaccharides synthesized by plants (Imam *et al.*, 2012). Morphologically, starch consists of spherical granules with diameters ranging between 1 to 150 µm depending on its botanical origin, while chemically it is composed of two distinct molecules namely amylose and amylopectin (Imam *et al.*, 2012). Normal starches are made up of 75% amylopectin and 25% amylose; waxy starches consists mainly of amylopectin and 0-8% amylose, while high–amylose starches consist of 40-70% amylose (Jane, 2004). In addition, most cereal starches also consist of lipids and phospholipids that greatly influence starch-pasting property. Amylose and amylopectin interaction with lipids and phospholipids greatly influences the paste viscosity, pasting temperature, clarity, and stability as well as retrogradation rate of starch pastes. Further discussion on amylose and amylopectin fractions is discussed in the next sections.

2.3.1 Amylose

Amylose is predominantly a linear polysaccharide molecule made of α-D-glucose units joined together by α-D-1-4 gylocosidic bonds resulting in helical conformations (Figure 2.2) (Jane, 2004; Mensah, 2011; Imam *et al.*, 2012). These helical conformations encourage starch interaction with lipids, iodine and other polar substances. Amylose makes up about 25% of starch, molecular weight ranging from 500 anyhdroglucose units (AGU) for high amylose maize starch to over 6000 AGU for potato starch (Jane, 2004). Amylose is hydrophilic in nature because of its many hydroxyl groups, and the uniform linear-chain nature permits

crystallisation from solution as well as in semisolid state in films and coatings (Jane, 2004). On cooling, it crystalizes and precipitates. On slow cooling; the molecules align resulting in intermolecular hydrogen bonding. However, gel formation through hydrogen bonding results when a concentrated amylose solution is cooled fast. Amylose greatly contributes to the swelling, gelation and water absorption properties of starch in food processing (Niba, 2005). As a result, high amylose starches are mostly used in food products requiring quick-setting gels like confectionery. Furthermore, amylose is mostly used in the formation of resistant starch because it is prone to gelatinization and retrogradation. Amylose has a high tendency to retrograde thus resulting in the formation of strong gels.

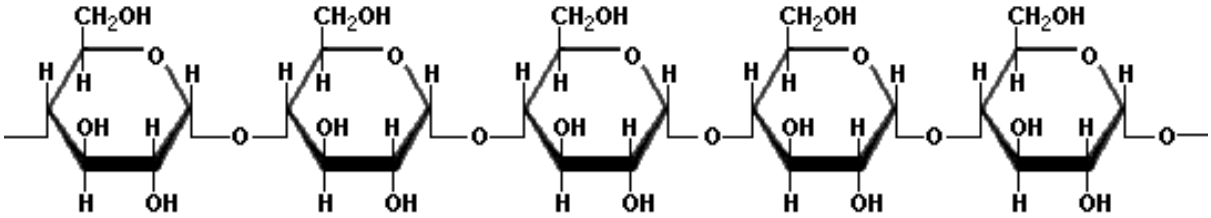

Figure 2.2 Basic structure of amylose (Mensah, 2011)

2.3.2 Amylopectin

Amylopectin is a highly branched molecule made up of α-D glucose units joined together by α-D-1-4 gylcosidic bonds at linear points and at branch points its joined by α-D-1-6 glycosidic bonds (Jane, 2004; Mensah, 2011). Amylopectin makes up about 75% of starch and its molecular weights ranges from hundreds of thousands to tens of millions anhydroglucose units (Jane, 2004; Mensah, 2011; Imam *et al.*, 2012). The amylopectin branching chains (Figure 2.3) differ with starch botanical source and they are the ones responsible for the starch crystalline structure, retrogradation, pasting as well as gelatinization properties of starch (Jane, 2004). Unlike amylose, amylopectin is insoluble in cold water and that is because of the hydrogen bonding of amylopectin's polymer chains. When amylopectin granules are heated, they absorb water and swell thereby breaking the hydrogen bonds (Mensah, 2011). In comparison to amylose, amylopectin is much more resistant to retrogradation and as such, it produces gels, which are more flexible and resistant. In aqueous solutions, amylopectin is stable and it produces weak films and soft gels. High amylopectin starches are used to improve freeze-thaw stability of food products because of amylopectin's resistance towards retrogradation. In addition, high amylopectin starches (waxy starches) are mostly used in the noodle processing industry as well as in some baked products with the aim of extending shelf life.

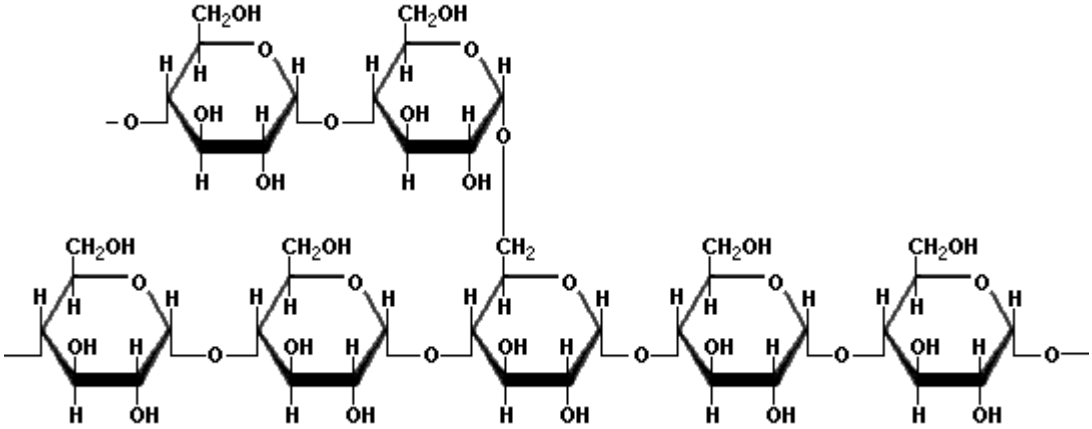

Figure 2.3 Basic structure of amylopectin (Mensah, 2011)

2.4 Importance of starch

In the food industry starch plays many important roles, it can be used either as a food additive or as a raw material. It is utilized in an extensive variety of products because it has great functional properties (Eliasson & Gudmundsson, 2006). Due to its relatively low cost, universal abundance and ability to impart a broad range of functional properties to food and non-food products, starch is therefore, used in many food and industrial applications (Bertolini, 2009). Starch is used in the food industry as a food ingredient, especially for its functional properties and as new food products are developed. Specific starch properties bestow noteworthy functional attributes to pharmaceutical and food products (Mensah, 2011).

The unique character of starch granules make them to be used for various industrial applications in different forms. These different forms include the use of starch granules in their swollen granular state, as intact granules where they are used directly, as film dried from dispersion, in the dispersed form, as an extrudate powder or after being subjected to different methods of starch modification (BeMiller, 2007). The main trend in starch application within the food industry, is in the formulation of different sauces and ready to eat meals as well as in syrup production (Bertolini, 2009). Starches may function as protective edible coatings and films because of their aroma and oxygen barrier characteristics.

2.5 Modification of starch

Depending on the properties exhibited, native starch has limited use in food products. Their poor functional characteristics restrict their wide industrial application. Native starches are insoluble in water, lose their thickening power after cooking and they also lose viscosity, hence they have limitations in industrial applications (Xie *et al.*, 2005; Singh *et al.*, 2007;

Mensah, 2011; Neelam *et al.*, 2012). They are good stabilizers and regulators in food systems, however, limitations such as thermal decomposition, low shear resistance, high tendency towards retrogradation and thermal resistance, limit its use in some industrial food applications (Xie *et al.*, 2005; Singh *et al.*, 2007; Mensah, 2011; Neelam *et al.*, 2012). Other disadvantages include hydrophilicity, dimensional instability and poor mechanical properties, which limit its industrial usage. Therefore, these shortcomings prompt the need to improve starch functionality through modification.

Various modification methods are used to enhance the properties of starch to meet the consumer need. This modification is directed at correcting one or some of the native starch shortcomings, consequently upgrading its versatility, stabilizing its granules during processing and satisfying consumer demand for a variety of industrial application (Xie *et al.*, 2005; Neelam *et al.*, 2012). Chemically modifying food grade starches increases clarity, smoothness and paste consistency, bestows cold storage and free-thaw stabilities to the food product. Starch modification has brought about advanced new processing techniques and creation of new market trends. Modified starch is used in food products because (1) of the functional features they provide in food applications, (2) of their availability and (3) their affordability (Teresa & Silva, 2012).

Starch modification is therefore essential to widen a range of desired functionalities, as it improves starch properties such as shelf life stability, solution viscosity and association behavior in the final product (Xie *et al.*, 2005). Furthermore, starches are modified to make their positive characteristics prominent, to diminish their undesirable qualities as well as to add new attributes. Modified starch can therefore function as emulsifiers (e.g. in French dressing), thickening agents (e.g. for pizza toppings), gelling agents, and binders in paper industries (Gabriel *et al.*, 2013).

2.5.1 Methods of starch modification

Different methods are used to modify starch with the aim of attaining suitable starch functionalities for different industrial applications. Starch modification techniques are broadly classified into four categories namely, physical, enzymatic, genetic and chemical modification (Xie *et al.*, 2005; Kaur *et al.*, 2012; Neelam *et al.*, 2012). Illustration of different kinds of methods used to modify starch is shown inFigure 2.4. These methods of starch modification aim at producing different innovative derivatives with enhanced physicochemical properties coupled with useful structural attributes. Two different types of modification can be combined to create a new type of modification; this is done with the aim of attaining modified starch with specific functional properties (Kaur *et al.*, 2012). In addition, these types of modification are advantageous as they result in increased production within a short space of time.

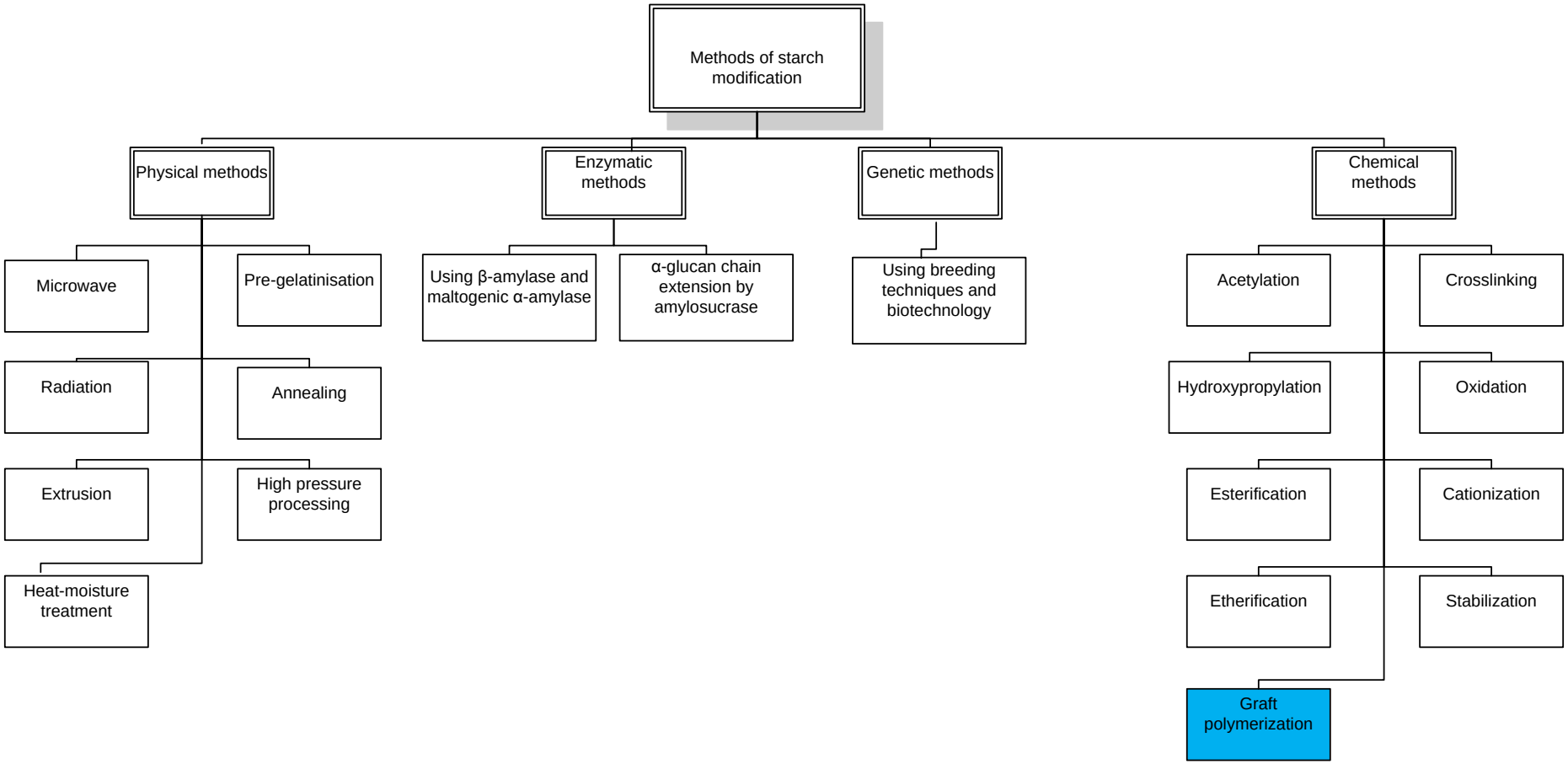

Figure 2.4 Different methods of starch modification Adapted from (Neelam *et al.*, 2012)

For example, chemical modification methods can be used in conjunction with physical modification methods like microwave. Physical modification is the type of modification preferred used in food products, owing to its safety as it does not involve chemicals which might have negative effects on humans (Kaur *et al.*, 2012). During the process of physical modification, native starch is subjected to various treatment conditions which include pressure, different temperature/moisture combinations, irradiation and shear (Xie *et al.*, 2005). In addition, physical modification results in size reduction of the starch granules leading to the formation of small-crystalline starch, and consequently soluble in cold water (Xie *et al.*, 2005; Neelam *et al.*, 2012).

Enzymatic modification is achieved when starch is treated with hydrolyzing enzymes, with the aim of attaining improved starch functionalities (Neelam *et al.*, 2012). Glucose and fructose syrup are products that can be obtained through enzymatic modification. Biotechnology and plant breeding methods are generally the methods for genetic modification. The following section covers chemical modification in detail.

2.6 Chemical modification

Chemical modification results in new functional groups being incorporated onto the starch molecule thereby resulting in starch with improved physiochemical properties (Singh *et al.*, 2007; López *et al.*, 2008; Afolabi, 2012). This can be done by decomposition (oxidation or enzymatic or acid hydrolysis) or derivatization reactions (esterification, etherification, grafting or cross-linking) (Tomasik, 2004; López *et al.*, 2008). Chemically modified starch has positive attributes such as having an increased ion interaction, desirable viscosity, reduced rate of retrogradation and enhanced molecular stability (Xie *et al.*, 2005). With the aim of making starch acceptable in different food and industrial applications, chemical modification therefore, stabilizes starch granules during processing. Furthermore, it results in starch with improved characteristics and it eliminates the native starch shortcomings. Chemical modification is known to result in molecular scission; and it favors molecular rearrangements and oxidation. Chemical modification of starch can be done when starch is in the following states (1) in suspension, (2) in a paste and (3) while in solid state (Tomasik & Schilling, 2003; Xie *et al.*, 2005). The most commonly used methods of starch chemical modification are acetylation, hydroxypropylation, esterification, etherification, crosslinking, oxidation, cationization, stabilization and graft polymerization.(Imam *et al.*, 2012; Kaur *et al.*, 2012) and they are extensively discussed in the following sections.

2.6.1 Acetylation

Acetylation is chemical modification of esterification, in which the hydrophilic hydroxyl groups are replaced with hydrophobic acetyl groups (Adebowale & Lawal, 2003; Lawal *et al.*, 2004; García *et al.*, 2012; Neelam *et al.*, 2012). These acetyl groups provide solution stability and

enhanced functional properties like hydrophilicity, cationic or anionic character at relatively low cost; they also change the physiochemical and functional properties of starch (Adebowale & Lawal, 2003; García *et al.*, 2012). According to Adebowale & Lawal (2003), Lawal *et al.* (2004) and García *et al.* (2012) starches modified through acetylation showed increased resistance to retrogradation, better paste clarity, stability and increased freeze-thaw stability. Acetylation results in hydrophobic starch because there is no hydrogen bond formed between water molecules and the hydroxyl groups. Acetylation determines interactions between starch chains by steric hindrance. This alters starch hydrophilicity and hydrogen bonding. Acetylation is of great importance in various food and industrial applications because they prevent syneresis and cloudiness in starch dispersions, by inhibiting interaction between amylopectin outer branches (Neelam *et al.*, 2012).

Starch modified using octenylsuccinic anhydride (OSA) as a reagent has been reported to be a beneficial emulsifier in the food, cosmetic and pharmaceutical industries. According to Chen *et al.* (2014) OSA results in the addition of hydrophobic chains to the hydrophilic starch structure. In comparison to native starch, Berski *et al*. (2011) reported acetylated starch to have enhanced solubility and swelling capacity. Furthermore, Bello-Pérez *et al*. (2000) results concur with what was reported by Berski *et al*. (2011). The results show that acetylation significantly increased starch's swelling capacity and solubility when compared to native starch. In addition, acetylated starch has reduced tendency towards retrogradation. Bello-Pérez *et al*. (2000) also reported the acetylated modified banana starch to have increased the paste viscosity in comparison to its native starch. In comparison to native starch, Mirmoghtadaie *et al.* (2009) reported acetylated oat starch to have increased swelling power, reduced gelatinization temperature and syneresis. Figure 2.5 gives an example of acetylation reaction mechanism where starch is acetylated with acetic anhydride in the presence of an alkali catalyst.

2.6.2 Hydroxypropylation

Hydroxylpropylated starches are usually prepared by etherification of native starch with propylene oxide in the presence of an alkaline catalyst (Lawal, 2009; Neelam *et al.*, 2012). During hydroxypropylation, the hydroxyl groups are primarily introduced into the starch chains in the amorphous regions mainly composed of amylose, and they are capable of disrupting intra and inter molecular hydrogen bonds, thereby weakening the granular structure of starch (Singh *et al.*, 2007; Lawal, 2009; Neelam *et al.*, 2012). This then results in an increase in motional freedom of starch chain in the amorphous regions. Interaction between starch molecules can be influenced by hydroxypropylation either by altering starch hydrophilicity or by steric hindrance (Singh *et al.*, 2007). During the formation of hydroxypropylated starches, some of the hydroxyl groups of the anhydroglucose unit are converted to –o-(-2-hydroxypropyl) groups (Figure 2.6). Hydroxypropylated starches have a

reduced tendency towards retrogradation, improved clarity, cold storage and viscosity stability.

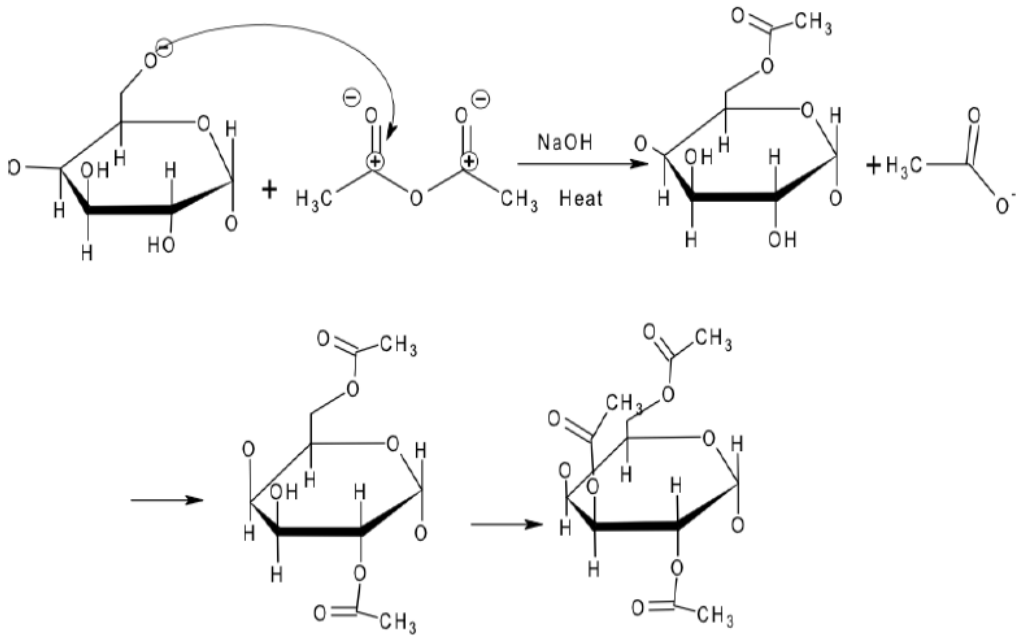

Figure 2.5 Chemical reaction during acetylation (Della, 2007; García et al., 2012)

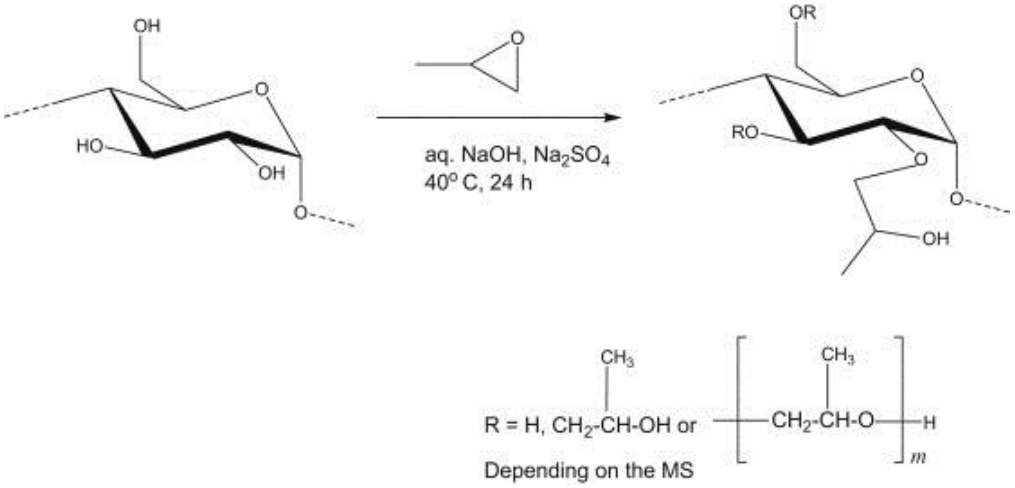

Figure 2.6 A typical reaction for preparation of hydroxylpropyl starch derivative (Lawal,2009)

Combining cross-linking and hydroxypropylation methods produces modified starches with desirable texture, also the resulting starch will have enhanced viscosity stability especially at low pH, high temperatures and when subjected to mechanical shear (Xie *et al.*, 2005). Hydroxypropyl starches impart viscosity and freeze-thaw stability in food products. In addition, due to their greater compatibility with proteins, hydroxypropyl starches are effective stabilizing and thickening agents in low fat dairy products. Furthermore, they are used as thickeners in different food products such as in puddings, salad dressing, pie fillings, gravies and sauces (Xie *et al.*, 2005). Hydroxypropylation is more effective than acetylation in improving the solubility of amylose complexes and in imparting low temperature stability. According to Senanayake *et al*. (2014), hydroxypropylated sweet potato starch had enhanced water solubility, digestibility as well as storage and gel stability in comparison to its native form in the cold storage.

2.6.3 Esterification

Esterification is achieved through the incorporation of acetate groups to the starch molecule (Eliasson & Gudmundsson, 2006). Starch can be esterified with inorganic and organic acids, however, phosphates are the only inorganic acid applicable in food processing (Tomasik, 2004). Esterification of starch with organic acids, mainly carboxylic acids, proceeds readily with acid anhydrides and chlorides. Esters of acetic and adipic acids with a low degree of esterification are used in the food industry because carboxylic acids readily esterify starch upon heating (Tomasik, 2004). During esterification process, starch hydroxyl groups are substituted, resulting in modified starch with noteworthy properties like enhanced freeze-thaw stability, increased viscosity, improved clarity, reduced syneresis, and hydrophobic, cationic or anionic character (Eliasson & Gudmundsson, 2006). In addition, esterification minimizes the association of amylopectin's outer branches. To achieve starch with typical emulsifying properties, starch hydroxyl groups must be substituted with hydrophobic reagents. Starches modified through esterification find application in baked, frozen, dry foods and canned products.

2.6.4 Etherification

Etherification offers a wide range of starch derivatives. Etherification with compounds carrying amino alkyl amino and dialkylamino as well as quaternised dialkylamino groups leads to cationic starches, which are interesting additives to cellulose pulp and sizes (Tomasik, 2004). Etherification can be performed with ethylene oxide and aziridine and their derivatives. Etherification gives starch excellent viscosity stability, better paste clarity, increased viscosity, reduced freeze-thaw stability and syneresis (Singh *et al.*, 2007). Starches modified through this method are widely used in different food products like fruit pie fillings, dips, puddings, sauces and gravies.

2.6.5 Cross linking

Cross linking reaction is whereby multifunctional reagents are added to the granular starch to react with its hydroxyl groups thereby leading to the formation of intermolecular linkages between starch hydroxyl groups (Eliasson & Gudmundsson, 2006; Singh *et al.*, 2007; Mensah *et al.*, 2011). Cross linking reinforces the hydrogen bonds in the starch granule with chemical bonds, which link the starch molecules. Furthermore, cross linking is intended to add intra- and inter- molecular bonds at random locations in the starch granule that stabilize and strengthen the granule (Singh *et al.*, 2007). Cross linking reactions are implemented so as to strengthen the structure of swollen granules upon gelatinization, enhancing the resistance to viscosity breakdown due to mechanical shear, acid conditions or high temperature (Xie *et al.*, 2005; Eliasson & Gudmundsson, 2006; Huber & BeMiller, 2010; Mensah *et al.*, 2011). To minimise granule rupture, loss of viscosity and the formation of stringy paste during cooking, starches are cross linked, resulting in starch that is suitable for canned products and other food applications (Singh *et al.*, 2007). Cross-linked starches have found application in the food industry as viscosifiers and texturizers in gravies, dairy products, soups, bakery and sauces. Nutritional benefits of cross linked starch as a new source of dietary fiber have also been reported. Starch botanical origin and cross linking reagents determine the effect cross linking reaction mechanism. However, apart from altering starch physical properties, cross linking reactions also alters the thermal characteristics of starch (Neelam *et al.*, 2012). In comparison to native starches, cross-linked starches have more distinct syneresis due to their ordered starch paste. This results in cross-linked starches with high degree of retrogradation.

Cross linking is used in conjunction with other starch modification methods like oxidation or etherification with the aim of imparting desirable textural, viscosity and gelatinization properties to different food products (Xie *et al.*, 2005). According to Singh *et al.* (2007), cross linked starch had increased gelatinisation temperature and reduced retrogradation rate. However, Jyothi *et al*. (2006) and Neelam *et al.* (2012) reported cross-linked starch to having ordered structure in the starch paste and consequently it has more distinct syneresis than has native starch, thereby resulting in a higher degree of retrogradation. Cross linked starches find application in retorted and high acidity foods because of their high resistance towards breakdown when subjected to severe agitation, extended cooking times and acidic concentrations in comparison to native starches (Kau *et al.,* 2006*;* Mirmoghtadaie *et al.*, 2009a). Starch cross linking enhances the starch granule rigidity by the formation of a three dimensional network. Cross linking starch modification greatly influences starch swelling capacity and paste clarity. However, the properties of the final cross linked starch are dependent on the botanical source of starch, parameters and methods used to cross-link the starch (Alcázar-Alay & Meireles, 2015a). The pastes of cross

linked starches are suitable for use in frozen food product formulations because of the good characteristics they possess such as good clarity, resistance towards retrogradation, thickening and stabilizing abilities. Figure 2.7 gives an example of starch cross linking reaction process with carboxylic acid as reported by Osorio *et al.* (2014). This reaction mechanism, firstly, involves molecular dehydration of the carboxylic acid, which then is followed by esterification reaction. Osorio *et al.* (2014) reported this reaction mechanism to be a proposed cross linking reaction mechanism for cellulose, however, results from previous reports indicates its applicability to starch as well.

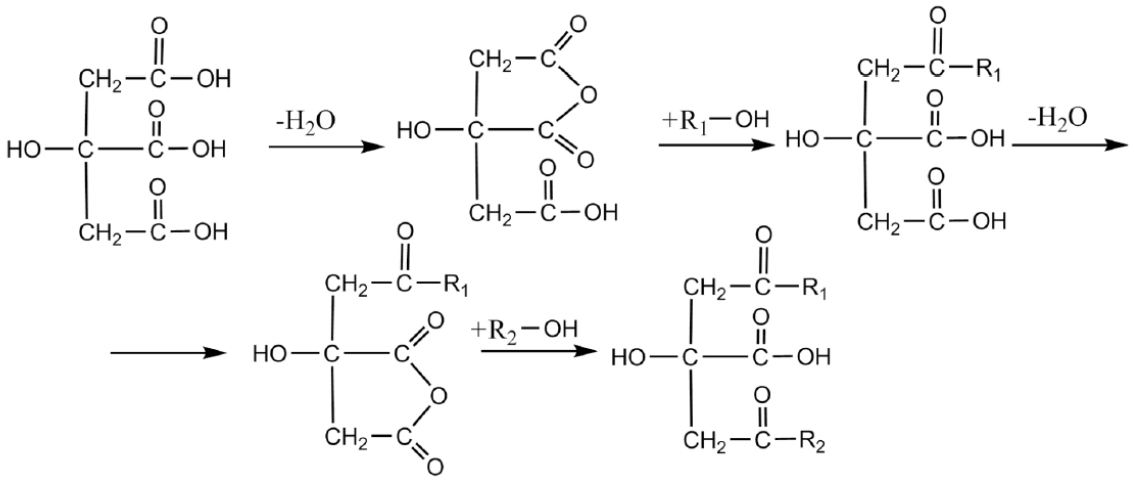

Figure 2.7 Cross-linking reaction mechanism using carboxylic acids, where R1 and R2 can either be cellulose or starch (Osorio et al., 2014)

2.6.6 Oxidation

Starch oxidation is achieved through the process of depolymerization, which results in the starch hydroxyl groups being oxidized to carbonyl and carboxyl groups (Lawal *et al.*, 2004; Neelam *et al.*, 2012). From previous studies, oxidation brings about improvement in the whiteness of the starch and restricted retrogradation (Adebowale *et al.*, 2002; Lawal *et al.*, 2004). In the food industry, the use of oxidized starches is increasing owing to their low viscosity, high stability, and clarity, binding properties, high solid dispersions and resistance to viscosity increases upon gelling in aqueous dispersions (Knill & Kennedy, 2005; Xie *et al.*, 2005; Eliasson & Gudmundsson, 2006; Neelam *et al.*, 2012). Sodium hypochlorite is the reagent that is mainly used to oxidize starch to be used in food. Oxidized starches are heat sensitive because when they are subjected to heat or stored for long periods they turn yellow. When compared to native starches they gelatinise at lower temperatures producing

aqueous dispersions of greater clarity and lower viscosity with fewer tendencies to retrograde, thus the pastes are more fluid (Knill & Kennedy, 2005). Moad (2011), proposed starch oxidation reaction mechanism by periodate, during which starch is oxidized to dialdehyde starch then finally to carboxyl functional starch as shown in Figure 2.8.

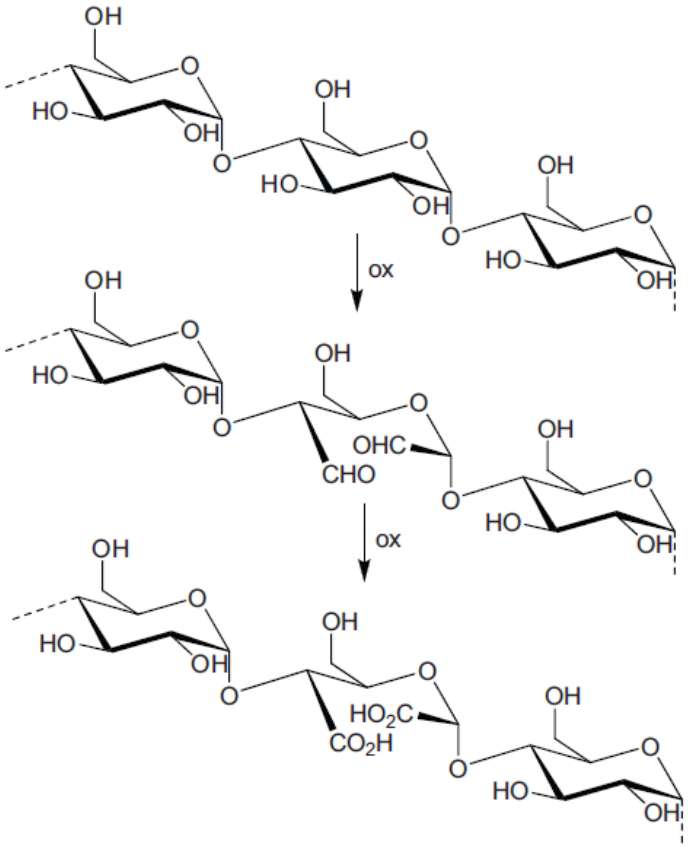

Figure 2.8 An example of starch oxidation reaction mechanism (Moad, 2011)

2.6.7 Cationization

Cationization is a process whereby positive ionic charges are introduced to the starch molecules through derivatization. This is achieved by incorporating amino, phosphonium, ammonium, sulfonium or imino groups onto the starch molecule (Xie *et al.*, 2005; Huber & BeMiller, 2010). In the paper industry, cationic starch derivatives are used as wet end additives, coating binders as well as surface sizing additives with the aim of improving sheet strength and to retain fines (Huber & BeMiller, 2010). In addition, they can function as industrial flocculants in mines and in sludge dewatering operations. Cationic starches can be used to produce paperboard used for packaging food products, however, the reagents used must be permitted by FDA. Cationic groups have the ability to improve starch's emulsion

stability as well as its fat-binding capacity. Figure 2.9 gives an example of cationization reaction process where the hydroxyl groups of the glycosyl starch units are substituted by functional groups.

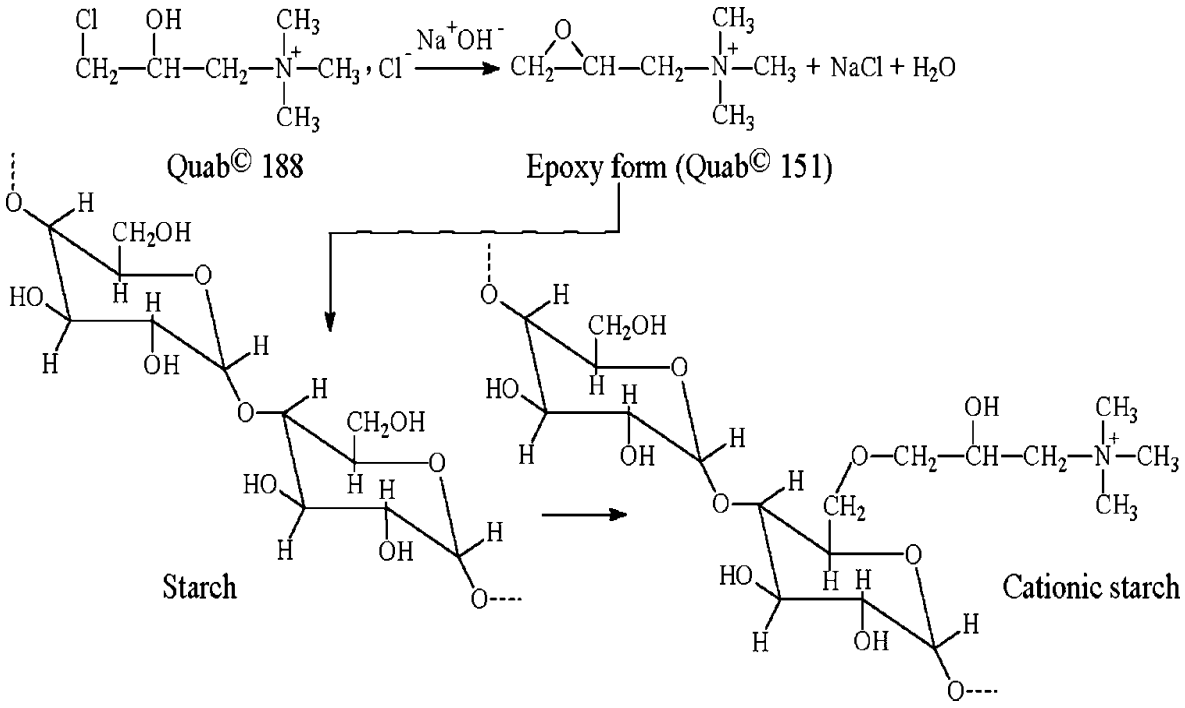

Figure 2.9 Cationization reaction mechanism (Sengupta et al., 2007)

2.6.8 Stabilization

Starch molecules react with blocking groups to retard retrogradation through a process known as stabilization (Mensah *et al.*, 2011). This results in the starch molecules crystallizing thereby altering structure of the food products. Stabilization is therefore, beneficial in the food industry because it extends the shelf life of food products by imparting freeze-thaw and textural stability. Stabilization is mostly used in frozen foods since starch retrogradation increases at low temperatures.

2.6.9 Graft polymerization

Grafting is a method that modifies natural and/or synthetic polymers thereby improving their characteristics (Contreras *et al.*, 2008; Spizzirri *et al.*, 2010). Graft polymerization does not alter the basic properties of modified polymers (Pathania & Sharma, 2012). During grafting process, covalent bonds link monomers to other polymer chains (Bhattacharya & Misra, 2004). Graft copolymers are formed when the substrates to be grafted bind to the active

sites in the polymer molecule; these active sites are formed in a polymer molecule during the process of graft polymerization. Grafting involves the incorporation of chemical groups to the modified polymers with the view to bestow new beneficial characteristics to widen the use of polymers in the industry (Dergunov *et al*., 2008; Joung *et al*., 2008; Shin *et al*., 2008; Spizzirri *et al.*, 2010). Polysaccharide molecules grafted with antioxidants become functionalized molecules possessing both properties of the natural and grafted polymer molecule (Curcio *et al*., 2009; Spizzirri *et al.*, 2010).

Starch graft polymers can be formed when starch molecules react with vinly monomers (Tomasik, 2004). The general structure of these vinyl monomers is CH_2=CH-X; where X can be H, Cl, COOH, $CONH_2$, $COOCH_3$, $OCOCH_3$. Chemical initiated grafting can follow either ionic or free radical pathway. The type of initiator determines the grafting pathway. The initiator impacts final product's structure and properties (Tomasik, 2004). Furthermore, chemical initiated grafting results in the formation of graft polymers due to the reaction that occurs between the monomer and free radicals generated by initiators. Free radicals generated from the starch hydroxyl groups are allowed to serve as micro initiators for the acrylic or vinyl monomers thereby resulting in the formation of graft polymers (Bertolini, 2009; Huber & BeMiller, 2010). Free radicals are produced by chemical methods or by high-energy radiation. The redox system in redox-initiated grafting provides optimum conditions for grafting to be performed under milder conditions thereby minimizing side reactions (Liu *et al.*, 2005).hence, redox-initiated grafting is the most preferred chemical initiation grafting method. Graft polymerisation method is used in the development of materials with specific physical and chemical structures. The grafting of the molecule onto a natural polymer like starch is of significant value in the development of new materials because it combines the properties of the natural polymer and the grafted molecule. Figure 2.10 shows possible grafting reaction process between starch and acrylic acid.

Previous work on BGN starch modification has been reported, however no work has been reported using the grafting method on starch. The starch modification methods that have been used on modifying BGN starch include, carboxymethylation (Afolabi, 2012), phosphorylation (Mensah, 2011), acetylation and oxidation (Adebowale *et al.*, 2002) as well as annealing and heat moisture treatment (Adebowale & Lawal, 2002). Spizzirri *et al.* (2010) grafted catechin to alginate and inulin using ascorbic and hydrogen peroxide as a redox initiator. The synthesised catechin-alginate/inulin complexes were characterised by FTIR, DSC and fluorescence analysis. The complexes showed good antioxidant properties. The authors then concluded that using ascorbic acid and hydrogen peroxide as a redox initiator system is an efficient method that imparts new characteristics to the macromolecules of natural origin. The new functionalised materials find application in the industry for different applications; they also play an important role in the optimisation of food preservation as well as in helping the manufacturers in packaging and new food product development. In

addition, Liu *et al.* (2014) and Arizmendi-Cotero *et al.* (2016) reported that grafting catechin to inulin using ascorbic acid and hydrogen peroxide as a redox initiator resulted in a new inulin-catechin polymer. This polymer displayed anti-diabetic properties which were confirmed via in vitro analysis of α-glucosidase activity inhibition. Furthermore, inulin-catechin polymer was reported to have improved crystallinity and thermal stability in comparison to inulin. This prompted the need to investigate grafting as a chemical modification method by grafting an antioxidant molecule (catechin) onto BGN starch.

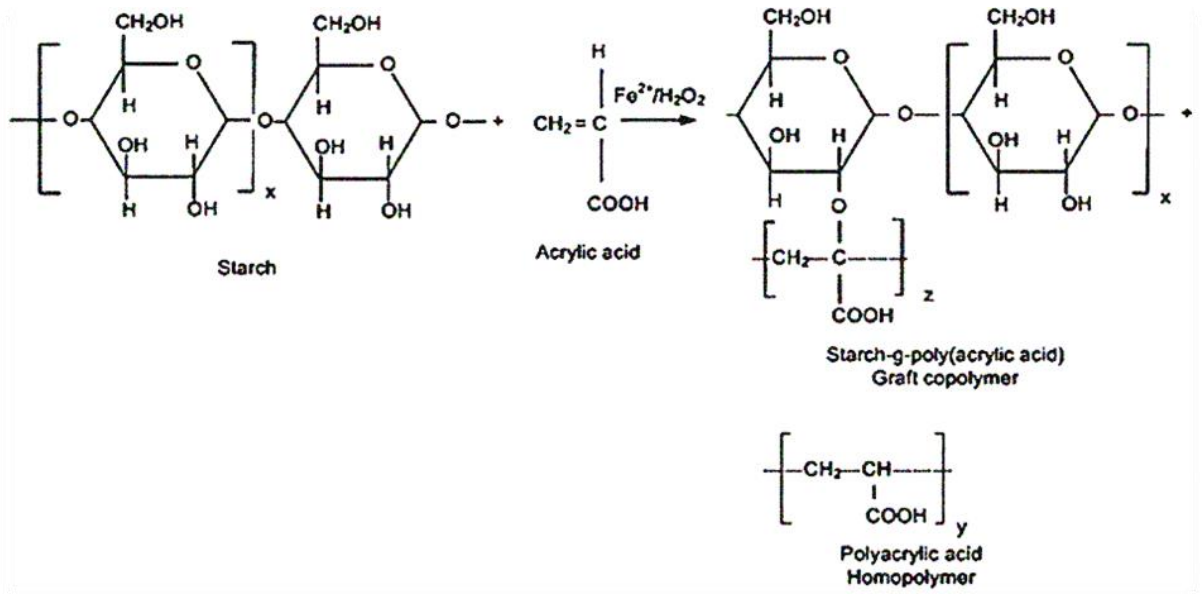

Figure 2.10 Possible grafting reaction mechanism (Witono et al.,2012)

2.7 Classification of antioxidants

Antioxidants are classified into three different categories namely: (1) primary, (2) secondary and (3) tertiary antioxidants. Primary antioxidants are responsible for the prevention of oxidant formation. Their mode of action is to supress the formation of free radicals. Secondary antioxidants are known to be scavengers of reactive oxygen species (ROS). These act by supressing chain initiation and breaking chain propagation reactions. Lastly, tertiary antioxidants play a vital role in repairing the oxidised molecules by using dietary antioxidants. In addition, antioxidants can also be classified as enzymatic or non-enzymatic antioxidants as illustrated in the diagram in Figure 2.11 (Iannitti & Palmieri, 2009).

Flavonoids are a large group of low molecular weight, secondary plant phenolics with significant antioxidant and chelating properties (Heim *et al.*, 2002). The basic flavonoid structure is the flavan nucleus. Structurally flavonoids are made up of diphenyl propane (C6 C3 C6) skeleton (Mehta *et al.*, 2015; Pietta, 2000; Gharras, 2009), which is labelled A, B and

C (Figure 2.12). Classes of flavonoids differ in their level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings. In plants, they occur as glycosylated derivatives. Flavonoids are found mainly in fruits, vegetables, wines, tea, cocoa, seeds, nuts, grains and spices (Pietta, 2000; Heim *et al.*, 2002).

The phenolic hydroxyl groups attached to the flavonoids ring structures give them their antioxidant activity. Flavonoids play many roles such as being singlet oxygen quenchers, reducing agents, hydrogen donators, superoxide radical scavengers, well as metal chelators. In addition, they activate antioxidant enzymes; reduce α-tocopherol radicals, mitigate nitrosative stress, inhibit oxidases as well as increase the levels of uric acid and low molecular weight molecules (Mehta *et al.*, 2015). Furthermore, flavonoids exhibit cardioprotective effects due to their ability to inhibit lipid peroxidation.

2.7.1 Catechin

Catechins are a group of flavonoids belonging to the flavan-3-ols (Kesteloot, 1992; Jeong & Kong, 2004; Wan *et al.*, 2008) and they are mainly found in green tea.. They account for 60% to 80% of the total flavonoids in green tea. Tea catechins are a class of flavonols with C-15 compounds and their derivatives are composed of two phenolic nuclei (A ring and B ring) connected by three carbon units (C-2, C-3 and C-4) (Juneja *et al.*, 2000). The flavonol structure of catechin contains two asymmetric carbon atoms at C-2 and C-3. Catechins and their derivatives have nucleophilic centers at C-6 and C-8, which are reactive with electrophilic specimens (Juneja *et al.*, 2000). Hence, it is thought that BGN starch will react with these sites. Chemically, catechins are highly reactive, with properties of metal chelation, oxidative radical scavenging, nitrosation inhibition (Juneja *et al.*, 2000). Figure 2.12 gives a basic flavonoid structure.

The most abundant catechins in tea products include catechin, epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin gallate (EGCG) (Kesteloot, 1992; Jeong & Kong, 2004; Lee et al., 2005; Wan et al., 2008). The structures of representative catechins are shown in Figure 2.13. Among these catechins, EGCG is the most abundant as it accounts approximately 50% to 80% of total catechins in green tea, followed by EGC, ECG and EC (Jeong & Kong, 2004; Wan *et al.*, 2008).

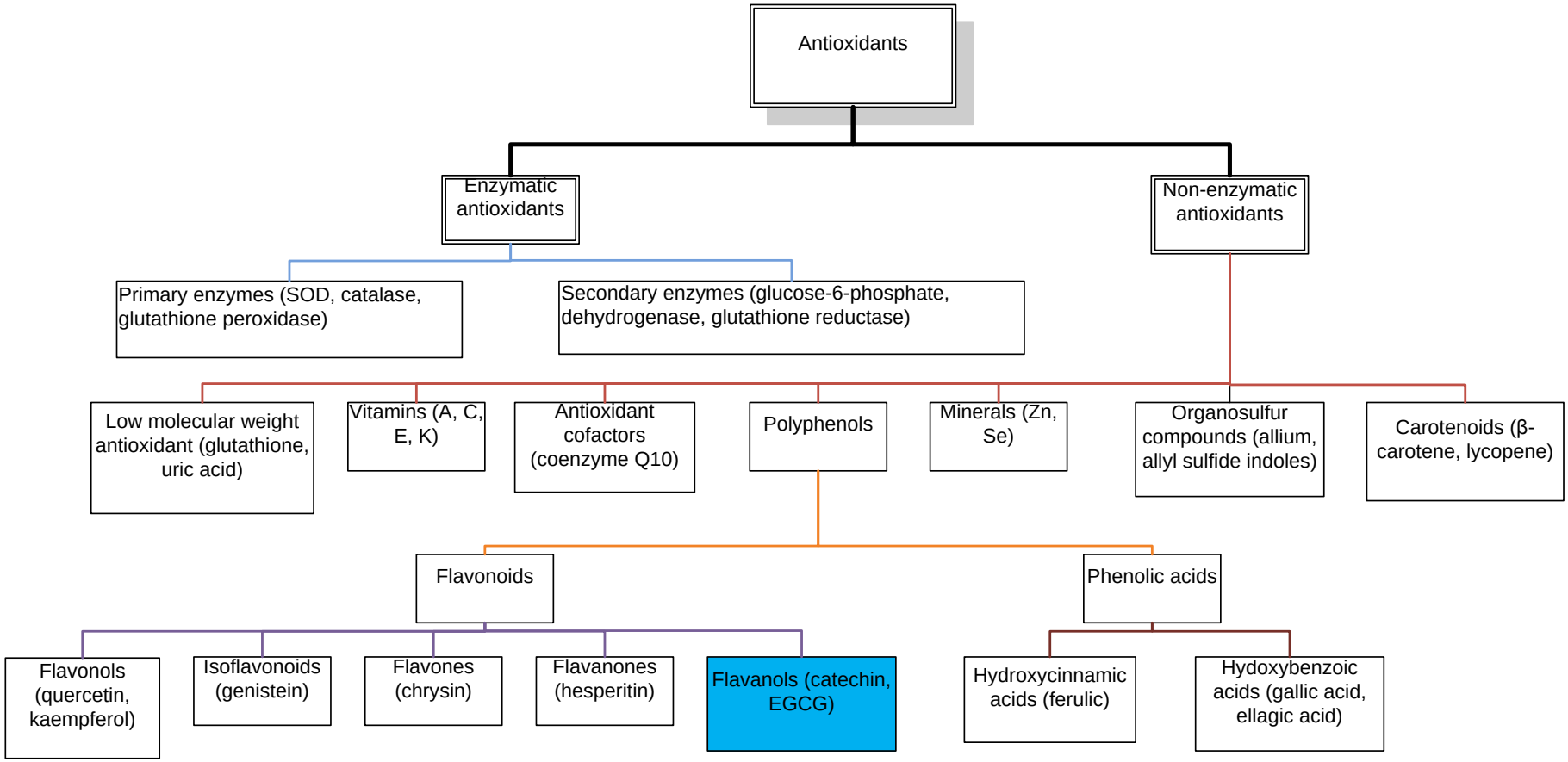

Figure 2.11 Classification of antioxidants. Adapted from: (Iannitti & Palmieri, 2009)

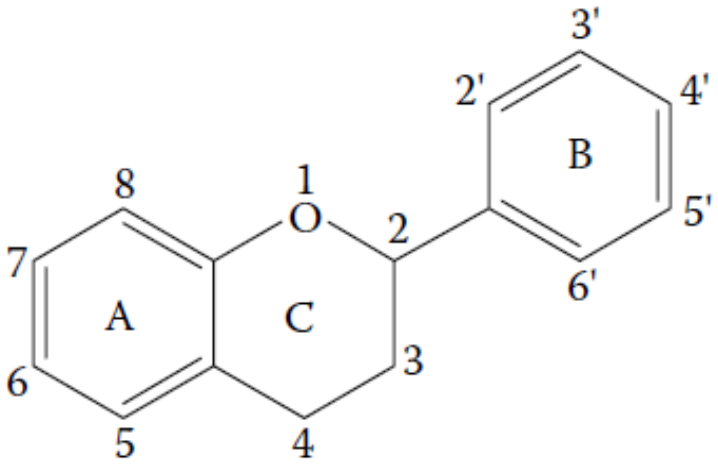

Figure 2.12 Basic flavonoid structure (Wan et al., 2008)

Catechins are phytochemicals mostly found in appreciable concentrations in different foods and beverages. Other than green tea, catechins are found in various food sources such as apples, peaches, buckwheat, red wine, broad beans, black grapes, apricots, strawberries and cocoa beans (Kesteloot, 1992; Wei burger, 2001; Van Der Sluis et al., 2002; Jeong & Kong, 2004). High concentrations of epicatechin are found in apples, pears, cherries, blackberries, raspberries, broad beans, black grapes and chocolate. Epigallocatechin, epicatechin gallate and epigallocatechin gallate are found in high concentrations in both black and green tea (Kesteloot, 1992; Willamson & Manach, 2005). Table 2.8 shows the catechin content in some common foods as reported by Willamson & Manach (2005).

2.7.2 Beneficial effects associated with consumption of catechins

Various beneficial effects have been associated with the consumption of catechins. According to Kesteloot (1992) and Willamson & Manach (2005), the consumption of catechins increases the resistance of low-density lipoproteins (LDL) to oxidation, fat oxidation, brachial artery dilation and plasma antioxidant activity. Catechins are considered to exert protective effects against cancer and inflammatory and cardiovascular diseases (Osada *et al.,* 2001; Arakawa *et al*., 2002; Yaping *et al*., 2003; Lee *et al.*, 2005). This then suggests that polyphenolic compounds like catechins may assume an imperative role in scavenging free radicals such as hydroxyl radicals, peroxyl radicals, superoxide anion radicals 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical, singlet oxygen and nitric oxide in living systems (Santos-Buelga & Scalbert, 2000; Sang *et* al., 2003; Lou *et al*., 2004; Lee *et al.*,

2005). Apart from the nutritional benefits, catechins contribute to the organoleptic properties of foods, especially by their astringency, bitter taste and colour and by their participation in haze formation.

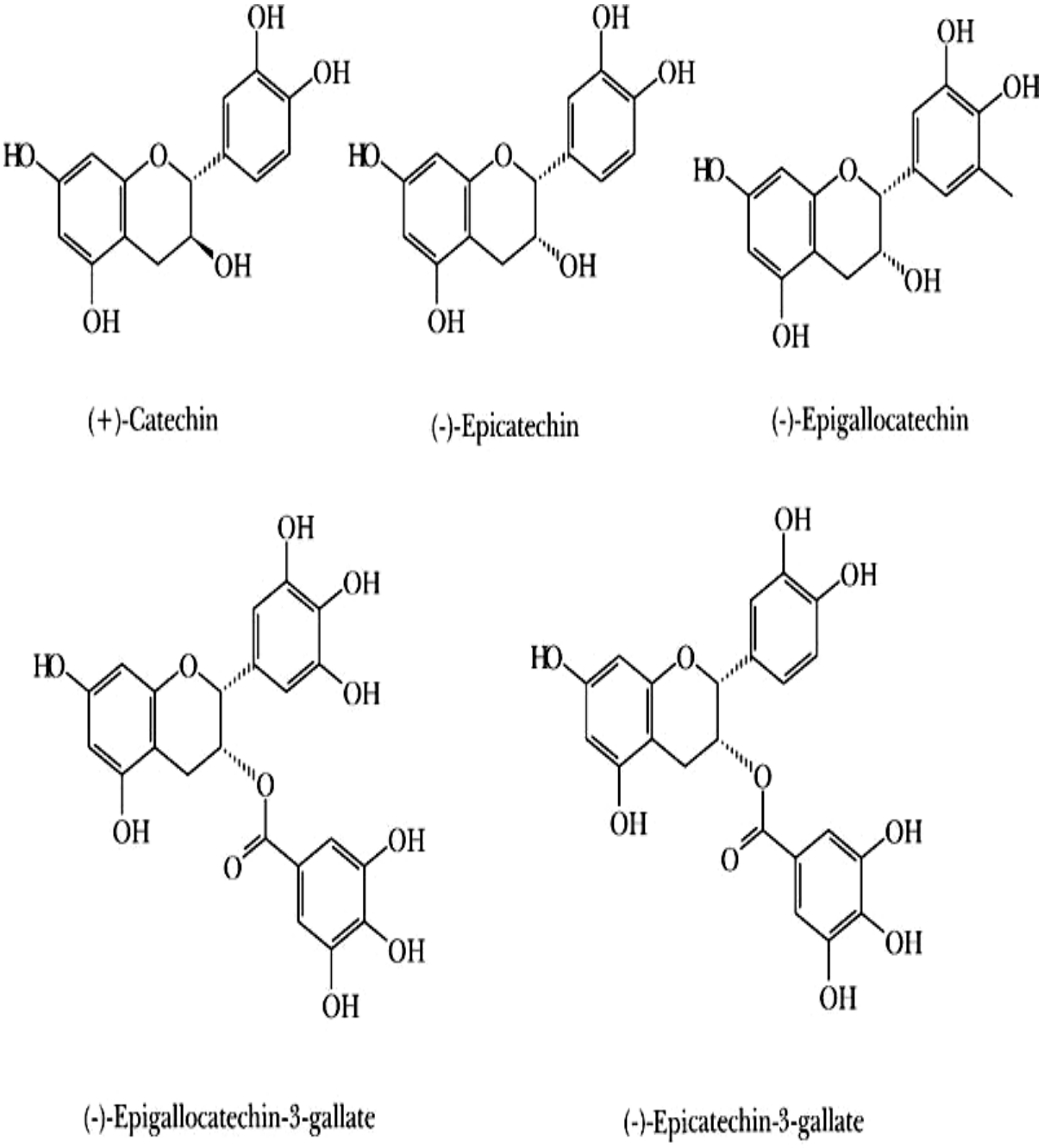

Figure 2.13 Structures of catechins (Jeong & Kong, 2004)

Damaged free radicals results in chronic health problems like cancer, cardiovascular diseases and inflammatory, furthermore they also play a role in contributing towards the etiology of aging (Kesteloot, 1992; Spiteller, 2001; Jeong & Kong, 2004). Major classes of biomolecules such as nucleic acids, lipids and proteins can be chemically altered by reactive oxygen species (ROS) and other free radicals. This results in a change in their (biomolecules) structures and function because of the free radicals mechanism, leading to the development of chronic diseases and aging.

Catechins do not have the ability of preventing platelet aggregation, lowering levels of plasma cholesterol and inhibiting LDL oxidation, and because of that, they are therefore thought to prevent cardiovascular diseases this way. Oxidation of LDLs plays a crucial role in the development of atherosclerosis. However, green tea catechins protect LDLs from oxidation by regenerating α-tocopherol in LDL particles and thus making catechins to be beneficial in humans (Jeong & Kong, 2004). To achieve the enhancement of lipid removal from the peripheral tissues and arterial wall, LDL cholesterol have to be lowered while the high-density lipoprotein (HDL) cholesterol is increased in plasma.

Table 2.8 Catechin content of some common foods[1]

Food	Catechin (mg/100 g)	Epicatechin (mg/100 g)	Epigallocatechin, Epicatechin gallate & Epigallocatechin gallate (mg/100 g)
Apples	0.9	6.1	0.6
Blackberries	37.1	4.7	0.8
Black grapes	10.1	8.7	2.8
Brewed black tea	1.5	2.1	23.1
Brewed green tea	2.6	8.3	114.3
Cherries	1.3	7.0	0.4
Cocoa	0.0	26.2	0.0
Dark chocolate	12.0	41.5	0.0
Fava beans	8.2	7.8	4.7
Milk chocolate	2.1	6.3	0.0
Pears	0.3	3.8	0.8
Raspberries	1.6	4.1	1.0
Red table wine	7.0	3.3	0.1

[1] Adapted from Willamson & Manach, 2005

This also acts as means of protection against heart diseases. Catechins are also accounted for having cancer chemopreventive properties. According to Kang *et al*. (2001) and Jeong & Kong (2004), green tea catechins significantly influence antithrombotic activity by inhibiting cytoplasmic calcium increase.

2.7.3 Antioxidant mechanism of catechins

Catechins can function as antioxidants through either one of the following ways, (1) by chelation metal ions, (2) by scavenging free radicals and lastly (3) by modulating oxidant/antioxidant enzymes or genes. Catechins have an important role in scavenging reactive oxygen species, such as nitrogen dioxide, superoxide radical, nitric oxide, singlet oxygen, peroxynitrite and hydroxyl radical, which may play important roles in carcinogenesis (Santos-Buelga & Scalbert, 2000; Sang *et* al., 2003; Lou *et al*., 2004; Lee *et al.*, 2005; Wan *et al.*, 2008). Catechins trap peroxyl radicals thereby inhibiting radical chain reactions and lipid peroxidation. In addition, they are capable of inhibiting the consumption of α-tocopherol and the metmyoglobin-initiated peroxidation of LDLs (Wan *et al.*, 2008). EGCG among tea catechins, is the most effective in reacting with most reactive oxygen species (Yang *et al*., 2002; Wan *et al.*, 2008).

Catechins prevent oxidation by chelating metal ions. They chelate mostly free radical reaction catalyst such as Fe and Cu due to their vicinal dihydroxy or thihydroxy structures (Wan *et al.*, 2008). This prevents the generation of free radicals. Catechins also achieve their antioxidant activity by regulating enzymes or genes related to oxidant/antioxidant. In addition, they enhance the expression of intracellular endogenous antioxidants such as glutathione, glutathione reductase, glutathione peroxidase, glutathione-S-reductase, quinine reductase and catalase (Valerio *et al*., 2001; Wan *et al.*, 2008).

Another approach of grafting an antioxidant (catechin) functional group to BGN starch is by using cyclodextrins. They do this through the formation of inclusion complexes.

2.8 Cyclodextrin

Cyclodextrins are cyclic oligomers of α-D-glucopyranose composed of glucose units linked by α-(1,4) glycosidic bonds (Singh *et al.*, 2002; Astray *et al.*, 2009; Yavuz *et al.*, 2010; Celebioglu *et al.*, 2013; Mura, 2014). Intramolecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase enzyme (CGTase) results in the production of cyclodextrins (Singh *et al.*, 2002; Del Valle, 2004; Astray *et al.*, 2009). They are naturally occurring water-soluble glucans. They are also known as Schardinger dextrins, cyclomaltoses and cycloamyloses (Singh *et al.*, 2002; Del Valle, 2004).

Cyclodextrins are of three types: α-cyclodextrin consisting of six glucopyranose units, β-cyclodextrin consisting of seven such units and γ-cyclodextrin consisting of eight such units joined together by α- (1, 4)glycosyl bonds (Dass & Jessup, .2000; Singh *et al.*, 2002; Del Valle, 2004; Astray *et al.*, 2009; Mura, 2014). Cyclodextrins are crystalline, homogeneous and non-hygroscopic substances, which are torus-like, built up from glucopyranose units. Each cyclodextrin is a torus (doughnut-shaped) molecule. The internal cavity of the doughnut is hydrophobic, whereas the external surface is hydrophilic. Consequently, these act as a host for entrapping either wholly or partially other chemicals without the formation of covalent bonds (Singh *et al.*, 2002). The hydrogen atoms and the glycosidic oxygen bridges line the cavity. The non-bonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the cavity producing a high electron density here. The C-2-OH group of one of the glucopyranose unit can form a hydrogen bond with the C-3-OH group of the adjacent glucopyranose unit. In the cyclodextrin molecule, these bonds form a complete secondary belt and that is why β-cyclodextrin has a rather rigid structure. Of all the cyclodextrins, β-cyclodextrin is the one with the lowest water solubility owing to this intramolecular hydrogen bond formation. Figure 2.14 gives the chemical structures of cyclodextrins [α, β and γ] (Astray *et al.*, 2009).

Depending on the type of cyclodextrin and guest compound, cyclodextrins crystallise in two main types of crystal packing, channel structures and cage structures. These crystal structures give an indication that cyclodextrins in complexes adopt the expected 'round' structure with all glucopyranose units in the $^4C_1$ chair conformation (Del Valle, 2004). Among all the main cyclodextrin types, β-cyclodextrin is the one that is most accessible, lowest-priced and generally the most useful (Singh *et al.*, 2002; Del Valle, 2004). Furthermore, β-cyclodextrin is widely used because of its cavity size, which is suitable for common guests with molecular weights ranging from 200 to 800 g/mol.

Cyclodextrins are applied in food processing and as food additives with a variety of aims. These aims include: (1) to protect lipophilic food components that are sensitive to oxygen and light or heat-induced degradation, (2) to solubilise food colourings and vitamins, (3) to stabilize fragrances, flavours, vitamins and essential oils against unwanted changes, (4) to suppress unpleasant odours or tastes, and (5) to achieve a controlled release of certain food constituents. Table 2.9 gives the characteristics of cyclodextrins or their derivatives that make them suitable for applications in analytical chemistry, agriculture, pharmaceutical field as well as food industry as reported by Del Valle (2004).

2.8.1 Formation of inclusion complexes

The most notable feature of cyclodextrin is its ability to form solid inclusion complexes (host-guest complexes) with a very wide range of solid, liquid and gaseous compounds during molecular complexation (Singh *et al.*, 2002; Del Valle, 2004; Astray *et al.*, 2009).

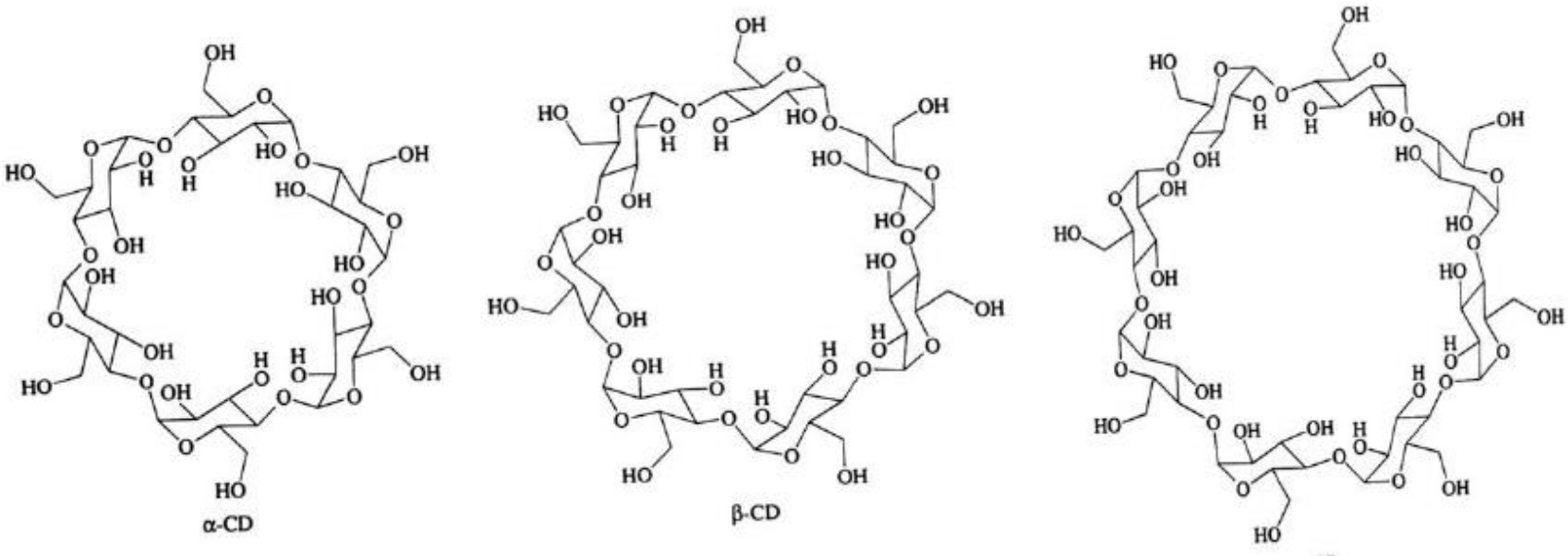

Figure 2.14 Chemical structures of α, β and γ cyclodextrins (Astray et al., 2009)

Table 2.9 Characteristics of cyclodextrin that make them suitable for different applications[1]

Characteristics of cyclodextrin that make them to be suitable for different applications
Stabilisation of light-or oxygen-sensitive substances
Modification of the chemical reactivity of guest molecules
Fixation of very volatile substances
Improvement of solubility substances
Modification of liquid substances to powders
Protection against degradation of substances by microorganisms
Masking of off odours and taste
Masking pigments or the colour of substances
Catalytic activity of cyclodextrins with guest molecules

[1]Adapted from Del Valle (2004)

In these complexes, a guest molecule is held within the cavity of the cyclodextrin host molecule as shown in Figure 2.15. Inclusion complexes are a combination of two or more molecules. One of the molecules known as the "host", includes totally or partly the "guest" molecules by physical forces. As a result, cyclodextrins are considered to be typical host molecules (Astray *et al.*, 2009). The dimensional fit that occurs between host cavity and the guest molecule is known as complex formation (Singh *et al.*, 2002; Del Valle, 2004). To form inclusion complexes, cyclodextrin's lipophilic cavity allows a suitable environment for appropriately sized non-polar moieties to form such complexes. (Singh *et al.*, 2002; Del Valle, 2004). During the formation of the inclusion complex, there are no covalent bonds either formed or broken (Schneiderman & Stalcup, 2000). The release of enthalpy-rich water molecules from the cavity is the main driving force of complex formation. To attain an apolar-apolar association as well as the decrease of cyclodextrin ring strain, hydrophobic guest molecules present in solution displaces water molecules. This leads to a more stable lower energy state (Singh *et al.*, 2002; Del Valle, 2004). The binding of guest molecules within the host cyclodextrin is a dynamic equilibrium. Binding strength is the measure of the "host-guest" complex bond and the local interactions between surface atoms (Singh *et al.*, 2002; Del Valle, 2004). Complexes can be formed in solution or crystalline state, water being the typical solvent used. Inclusion complexation can be achieved in a co-solvent system and in the presence of any non-aqueous solvent. Inclusion in cyclodextrins exerts a significant effect on the physiochemical properties of guest molecules as they are temporarily locked or confined within the host cavity. This then gives rise to beneficial modifications of guest molecules, which cannot be achieved in any other way.

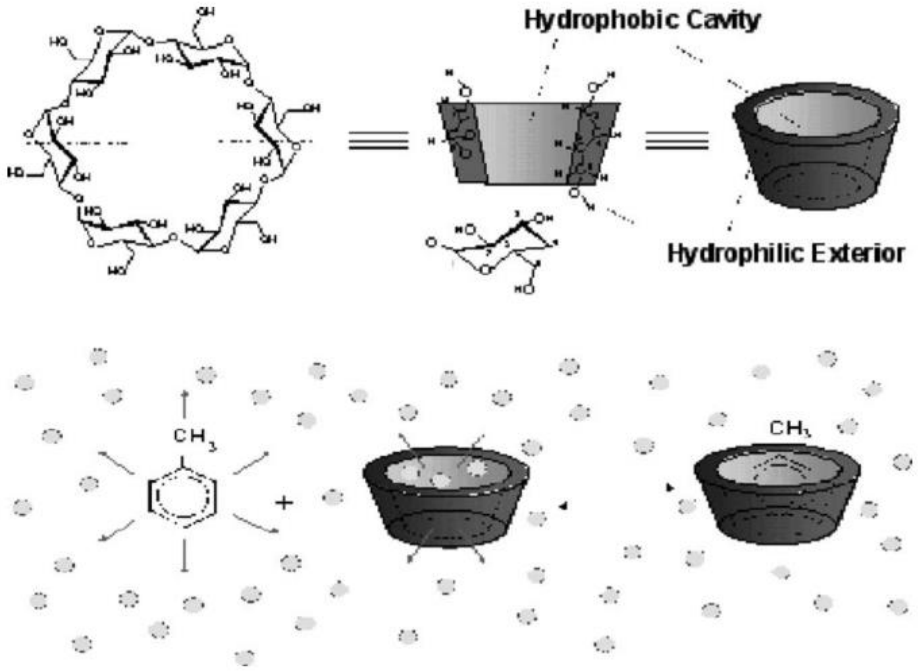

Figure 2.15 Illustration of cyclodextrin inclusion complex formation. Source: (Del Valle, 2004)

These properties include, solubility enhancement of highly insoluble guests, stabilization of labile guests against the degradative effects of oxidation, visible or UV light and heat control of volatility and sublimation, physical isolation of incompatible compounds, chromatographic separations, taste modification by masking off flavors, unpleasant odors and controlled release of drugs and flavors. Therefore, cyclodextrins are used in food, pharmaceuticals, cosmetics, environment protection, bioconversion, packing and textile industry (Bhardwaj *et al*., 2000; Fujishima *et al*., 2001; Singh *et al.*, 2002; Del Valle, 2004).

2.8.2 Complexation techniques

There are various techniques used to form inclusion complexes between cyclodextrins and the guest molecule. These techniques include but not limited to kneading, microwave and co-evaporation and they are discussed next.

Kneading

This method involves adding small amount of water to cyclodextrin to form a paste. Thereafter, the guest molecule is slowly added to the cyclodextrin paste while grinding. To

facilitate the dissolution of the guest molecule a hydro-alcoholic solution is added. The mixture is kneaded for a specified time which is dependent on the guest molecule. The kneaded mixture is then dried in a vacuum oven. The obtained dry powder is then ground to fine powder and screened through a sieve. The kneading method can be done using a mortar and pestle in a laboratory scale while extruders can be used when scaling up (Del Valle, 2004; Patil *et al.*, 2010; Savjani *et al.*, 2012; Saravana *et al.*, 2013; Sapana & Shashikant, 2014). This is considered a simple method for inclusion complex formation because of its low production costs.

Co-evaporation

The co-evaporation method involves dissolving cyclodextrin and the guest molecule in ethanol and hydro-alcoholic solution (1:4 ethanol: water) respectively. Thereafter, the two solutions are mixed to obtain molecular dispersion of the guest molecule and cyclodextrin [complexing agent] (Patil *et al.*, 2010). The resulting mixture is then stirred for 24 hours, then air dried in a vacuum oven at 45^{o}C to a constant weight. The dried complexes are then ground to fine powder and screened through a sieve. Patil *et al.* (2010); Savjani *et al.* (2012) and Saravana *et al.* (2013) reported co-evaporation method to be a simple and economic method on large scale production and laboratory scale. In addition, this method can be used as an alternative to spray drying method.

Microwave irradiation

This method uses the microwave oven to form inclusion complexes between cyclodextrin and the guest molecule. Definite molar ratio of the guest molecule and cyclodextrin are dissolved in ethanol: water (1:1) solution in a flask. The mixture is then allowed to react in the microwave oven at 600 watt for one to two minutes. After the reaction time, sufficient amount of solvent mixture is used to remove residual, un-complexed guest molecule and cyclodextrin from the reaction by thoroughly rinsing the flask with the solvent mixture (Patil *et al.*, 2010; Mishra *et al.*, 2014). The obtained precipitate is then separated using Whatman No. 1 filter paper, thereafter; dried for 48 hours in a vacuum oven at 40^{o}C. Microwave irradiation method is considered the best method for industrial scale production because of its high product yield and the short reaction time (Patil *et al.*, 2010; Savjani *et al.*, 2012; Saravana *et al.*, 2013; Sapana & Shashikant, 2014).

2.9 Analytical techniques used to characterize native and modified BGN starches

There are different techniques used to characterize starch that have been reported by different authors. These techniques include but not limited to powder X-ray diffraction, scanning electron microscopy, fluorescence as well as Fourier transform spectroscopy (FTIR) analysis. Powder X-ray diffraction is used as a characterization technique to

determine starch crystallinity and to determine its implication thereof. Based on the X-ray diffraction patterns, starches can be classified as A-, B- or C- type crystallinity. The A- and B- types can be differentiated by the packing arrangement of double helices formed from short chains within the amylopectin molecule as well as their level of hydration. The A-type is closely packed with water molecules between each double helix, while the B-type is less densely packed and more hydrated in the central cavity (Oyeyinka *et al.*, 2015). Type C starch crystallinity has been reported to be a mixture of A- and B- types. In addition type C is characteristic for legume starches (Hoover *et al.*, 2010). Afolabi (2012) and Oyeyinka *et al.* (2015) reported native BGN starch to exhibit type C crystallinity, which is typical of legume starches. However, some researchers reported the A-type crystallinity for some varieties of BGN (Sirivongpaisal, 2008; Kaptso *et al.*, 2015). According to literature, type A has been reported to be typical for cereal starches, with that in mind, it can therefore be concluded that BGN starch is more likely to be type C than A because is a legume.

Scanning electron microscopy is used to determine the starch morphology. It serves as a control for the process of starch isolation, showing the integrity of the granule. According to reports made by Adebowale & Lawal (2002); Sirivongpaisal (2008); Kaptso *et al.* (2015); Oyeyinka *et al.* (2015) and Ma *et al.* (2017), native BGN starch displayed round, oval and elliptical shapes, which is typical of legume starches. In addition, the granule surfaces all appeared to have smooth surfaces with no evidence of ruptures. Zhang *et al.* (2009) reported yellow ginger starch to be oval to ellipsoid in shape and only a few spherical ones. Jack bean starch was reported to be oval and round in shape with heterogeneous sizes (Lawal & Adebowale, 2005). Lastly, yellow sorghum starch micrographs displayed round, polygonal shapes with heterogeneous sizes (Olayinka *et al.*, 2015).

Fourier transform spectroscopy (FTIR) analysis works on the basis of functional groups and provides information in the form of peaks. It is sensitive to changes in molecular structure. Furthermore, it provides information on the basis of chemical composition and physical state of the whole sample. FTIR therefore confirms if starch is made up of carbon, hydrogen and oxygen which make up the gross starch structure. In addition, it is done to confirm the successful grafting of catechin (in this research study) to the starch molecule which will be evidenced by shifting of the spectral peaks as well as the appearance of new absorption peaks attributed to the aromatic ring of catechin.

Fluorescence spectroscopy is a reliable method that can be used to accurately measure the catechin content in starch. The method is precise and rapid and does not use special reagents or incubations (Ramirez-Sanchez *et al.*, 2010) thus making it adequate to improve measurements in starch-catechin complexes. Fluorescence spectroscopy can yield low detection limits, high sensitivity and high specificity (Skoog *et al*., 2006). The high specificity is largely due to the fact that fluorophores exhibit excitation (absorption) and emission (fluorescence) wavelengths. These wavelengths can be determined via the

collection of two spectra, an excitation spectrum and an emission spectrum. It can be used as a non-destructive analytical technique to provide information on the presence of fluorescent molecules. All these four analytical techniques were done so as to confirm the successful grafting of catechin to the BGN starch, successful starch modification having native starch as a control. Most researchers usually use only one or two methods to characterize the starch; however, in this research study four methods were used, because we wanted to see if the methods changed results.

2.10 Conclusion

The high carbohydrate content of BGN makes it a potential source of starch which can find application in the food industry. This will therefore, reduce the load on the existing starches being used in the food industry. Using grafting as a chemical modification method rather than the existing chemical starch modification methods is advantageous. It involves the incorporation of a new functional group to the modified starch molecule resulting in a modified starch with a new functional group, antioxidant as an example. The ability of cyclodextrins to form inclusion complexes is another approach to be used to graft a functional group to compounds.

2.11 References

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CHAPTER THREE

STRUCTURAL AND ANTIOXIDANT CHARACTERISATION OF BAMBARA GROUNDNUT STARCH-CATECHIN COMPLEX FORMED USING CHEMICALS AND CYCLODEXTRINS AS INITIATORS

Abstract

Bambara groundnut (BGN) starch (extracted from BGN flour) was modified through chemical modification as well as through complexation methods using alpha and beta cyclodextrin. Complexation methods used included the microwave, co-evaporation and kneading methods. Chemical modification was achieved by using ascorbic acid (1% w/w) and hydrogen peroxide (165% w/w) as a redox, biocompatible initiators for grafting catechin to the BGN starch. Cyclodextrin (alpha and beta) were also used as initiators for grafting catechin to the BGN starch molecule. The starch yield obtained from BGN flour was 32%. Starch crystallinity was determined using powder X-ray diffraction with native BGN starch being a typical C-type which is common among legumes, displaying strong diffraction peaks at 2θ of 15^{o}, 17^{o} and 23^{o}. Chemically modified BGN starch lost its crystallinity which was evidenced by the amorphous region, while the starches modified through complexation methods increased in crystallinity. The FTIR spectra of native BGN starch showed variable peaks at 3285.34 cm^{-1}, 2931.69 cm^{-1}, 1634.36 cm^{-1}, 1336.77 cm^{-1} attributed to OH stretching, C-H stretching, water bending vibrations and C-O stretching, respectively. All modified BGN starches displayed a new absorption peak at 1020 cm^{-1} wavelength, thus indicating that starch modification was successful. All BGN starch-catechin complexes displayed a new absorption peak in the range of 1520 -1560 cm^{-1}, attributed to the C-C stretching within the aromatic ring of the catechin. The fluorescence spectrum of native BGN starch gave an emission peak at 270 nm, while that of modified BGN starch-catechin complexes was 320 nm. This confirms the successful graft polymerization of catechin to BGN starch. Lastly, the antioxidant activity for BGN starch-catechin complex was determined using DPPH and ORAC assay. Within the DPPH assay, the antioxidant activity ranged from 2.26 to 38.31 µmol TE/g, antioxidant activity of BGN starch-catechin complexes was significantly ($p \leq 0.05$) higher than the modified starch without catechin. Complexation methods had significantly ($p \leq 0.05$) higher antioxidant scavenging activity in comparison to the chemically modified BGN starch-catechin complex. Within the ORAC assay, the antioxidant activity ranged from 0.07 to 126.71 µmol TE/g. Unlike in DPPH assay, the chemically modified BGN starch-catechin complex had the highest antioxidant activity (126.71 µmol TE/g) in comparison to the BGN starch-catechin complexes modified through complexation methods.

3.1 Introduction

Despite the main sources for commercial starch that are already available in the market, there is a need to look for alternative sources of starch. This is to reduce the load on the existing starches. The constant search for new starches with different properties is due to the continuous need to improve quality of products and processes as well as to develop new products. Consequently, the expanding demand for starch combined with its expanding industrial usage necessitated the need to source starch from underutilised and lesser known cereals and legumes. One such crop is Bambara groundnut (BGN). BGN is an indigenous African crop which produces an almost balanced food, as it contains sufficient quantities of protein, carbohydrate and fat (Baryeh, 2001; Bamshaiye et al., 2011). It is ranked the third most important grain legume after groundnut (*Arachis hypogaea L*) and cowpea (*Vigna unguiculata*); however, it is seen as a snack rather than a lucrative cash crop owing to its low status (Baryeh, 2001; Bamshaiye *et al*., 2011; Mensah, 2011). BGN has high nutritive value containing 53-65% carbohydrate, about 20% protein, 6.5% fat, 6.1% fibre, 3.4% ash, as well as appreciable amounts of calcium, iron, sodium and potassium (Baryeh, 2001; Bamshaiye *et al*., 2011; Mensah, 2011; Afolabi, 2012; Gabriel *et al*., 2013). It therefore, compares favourably with other well-known and highly commercialised legumes such as cowpea and soya (Baryeh, 2001; Bamshaiye *et al*., 2011; Afolabi, 2012). Its carbohydrate fraction is predominantly composed of starch and non-starch polysaccharides with lesser amount of reducing and non-reducing sugar. The high carbohydrate content of BGN makes it a good source of starch. Adebowale & Lawal (2002) reported that BGN is a promising source of starch, because of its high yielding potential, excellent caloric and nutrient content. However, being native starch there is need to chemically modify it to extend its industrial usage.

Chemical modification involves the incorporation of functional groups into the starch molecule. It also enhances the positive attributes and eliminates the shortcomings of the native starches. Chemical modification is done to stabilize starch granules and make it suitable for many food and industrial applications (Xie *et al.*, 2005). Xie *et al.* (2005) stabilised starch granules by reacting starch with sodium hypochlorite through oxidation as a starch modification method. Grafting is a method wherein monomers are covalently bonded (modified) onto other polymer chains (Bhattacharya & Misra, 2004) and requires the introduction of chemical groups to impart new characteristics to the modified polymers for different applications (Dergunov *et al*., 2008; Joung *et al.*, 2008; Shin *et al*., 2008; Spizzirri *et al*., 2010).

To improve the antioxidant properties of starch, the challenge will be to covalently bind catechin to starch. Catechins are a group of flavonoids belonging to the flavan-3-ols (Kesteloot, 1992; Jeong & Kong, 2004; Wan *et al*., 2008). They are a group of polyphenolic compounds abundantly contained in green tea as well as in a variety of food and beverages. They are potentially beneficial to human health as they are strong antioxidants; have

protective effects against cancer, inflammatory and cardiovascular diseases (Osada *et al.*, 2001; Arakawa *et al.*, 2002; Yaping *et al.*, 2003; Lee *et al*., 2005). Spizzirri *et al.* (2010) grafted catechin to alginate and inulin using ascorbic acid and hydrogen peroxide as a redox initiator. The obtained catechin-alginate/inulin complexes were characterised by FTIR, DSC and fluorescence analysis. It is reported that the new complexes showed good antioxidant properties. Furthermore, the authors concluded that using ascorbic acid and hydrogen peroxide as an initiator system significantly imparted new properties to the alginate and inulin polymers. The new functionalised material plays an important role in the optimisation of food preservation as well as in helping the manufacturers in packaging and new food product development.

Apart from grafting initiated by chemical means, cyclodextrin is another way of grafting catechin on to starch molecule. Cyclodextrins (α, β and γ) are cyclic oligomers of α-D-glucopyranose composed of glucose units linked by α- (1, 4) glycosidic bonds (Singh *et al*., 2002; Astray *et al*., 2009; Yavuz *et al*., 2010; Celebioglu *et al*., 2013; Mura, 2014). Cyclodextrins have the ability to form inclusion complexes and hence, the properties of the materials with which they complex can be modified significantly. Inclusion complex formation of cyclodextrins gives rise to beneficial modifications of the guest molecules which cannot be achieved otherwise (Singh *et al.*, 2002). These properties include solubility enhancement of highly insoluble guests, stabilization of labile guests against degradation effects of oxidation, taste modifications by masking of flavours, unpleasant odours and controlled release of drugs and flavours, among others. Therefore, cyclodextrins are used in food, pharmaceuticals, cosmetics, environment protection, bioconversion, packing and textile industries (Bhardwaj *et al*., 2000; Fujishima *et al*., 2001; Singh *et al*., 2002; Del Valle, 2004). Tian *et al.* (2010) reported the use of β-cyclodextrin in the food industry to extend the shelf life of food products, improve rice starch gelatinisation properties and reduce the undesirable taste in foods by flavour encapsulation.

The objective of this study was to (1) produce BGN starch-catechin complex as a food ingredient using chemical initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin (α and β), (2) establish the structure of the new BGN starch-catechin complex and (3) evaluate the antioxidant capacity of the BGN starch–catechin complex.

3.2 Materials and Methods

3.2.1 Source of materials and equipment

Bambara groundnuts were purchased from Triotrade, Johannesburg, Gauteng province, South Africa. Catechin and cyclodextrin (alpha and beta) were purchased from Sigma Aldrich. All other materials and equipment were obtained from the Oxidative Stress Laboratory and the Department of Food Science and Technology laboratories of the Cape Peninsula University of Technology, Bellville. Analytical grade chemicals were used.

The major equipment used in this study include; a water bath, cabinet dryer (Model 1069616), Hammer mill (Trapp TRF 40, Animal ration shredder/Hammer mill foliage, Jaraqua do sul-sc, Brasil), FTIR spectrophotometer (Spectrum two, Perkin Elmer UATR Two), powder X-ray diffractometer (D2 Phaser, Bruker AXS, D2 Phaser A26-X1-A2BOB2A, D 76181 Karisruhe, Germany), vacuum oven, specadie, fluorescence spectrophotometer (Perkin, Ltd), centrifuge (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc, USA), orbital shaker and magnetic stirrer. Figure 3.1 outlines the analyses that were carried out in this chapter.

3.2.2 Production of Bambara groundnut flour

Whole BGN seeds were screened and sorted to eliminate the defective ones and any physical hazards like stones and twigs. The seeds were then washed and dried at 50^{o}C for 24 h in the cabinet dryer (Cabinet dryer, Model 1069616). They were then milled using the hammer mill (Trapp TRF 40, Animal ration shredder/Hammer mill foliage, Jaraqua do sul-sc, Brasil) with sieve of 250 µm. The flour was then stored in plastic bags (zip lock bags) and kept in the refrigerator at 4^{o}C before use.

3.2.3 Extraction of Bambara groundnut starch

The method reported by Gabriel *et al.* (2013) was used to extract starch from Bambara groundnut flour (BGNF). Figure 3.2 gives a schematic diagram of the BGN starch extraction procedure. A typical extraction procedure involved the mixing of weighed BGNF with water (1:10 w/v) at room temperature for 1 h continuously using the heavy duty food mixer (Kenwood 7-qt major stand mixer). After mixing, the mixture was allowed to stand for 5 h, thereafter; it was centrifuged (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc., USA) for 30 minutes at a speed of 3 500 x *g*. The supernatant was discarded and the residue was subjected to a similar procedure, first with water containing 2% w/v NaCl (10 min mixing and 12 h standing); and then 0.03 M NaOH (10 min mixing and 12 h standing). The resultant residue was then re-constituted in water and passed through first a 75 µm then 45 µm sieve to remove the fibre. The sieved mixture which contains the pure starch was left for 2 h to sediment after which the supernatant was decanted. The residue was air-dried (at room temperature) to yield the BGN starch (BGNS).

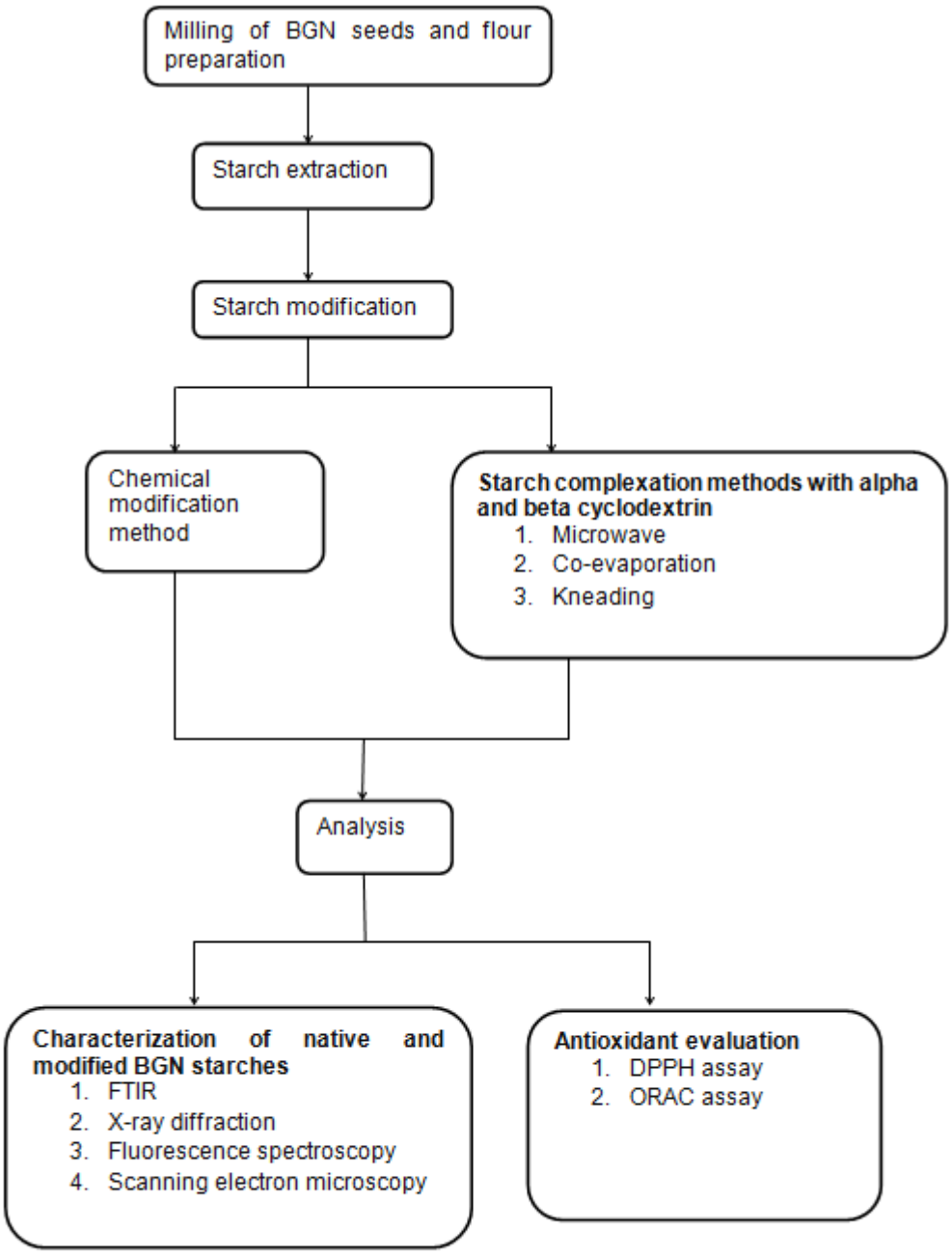

Figure 3.1 Chapter 3 outline

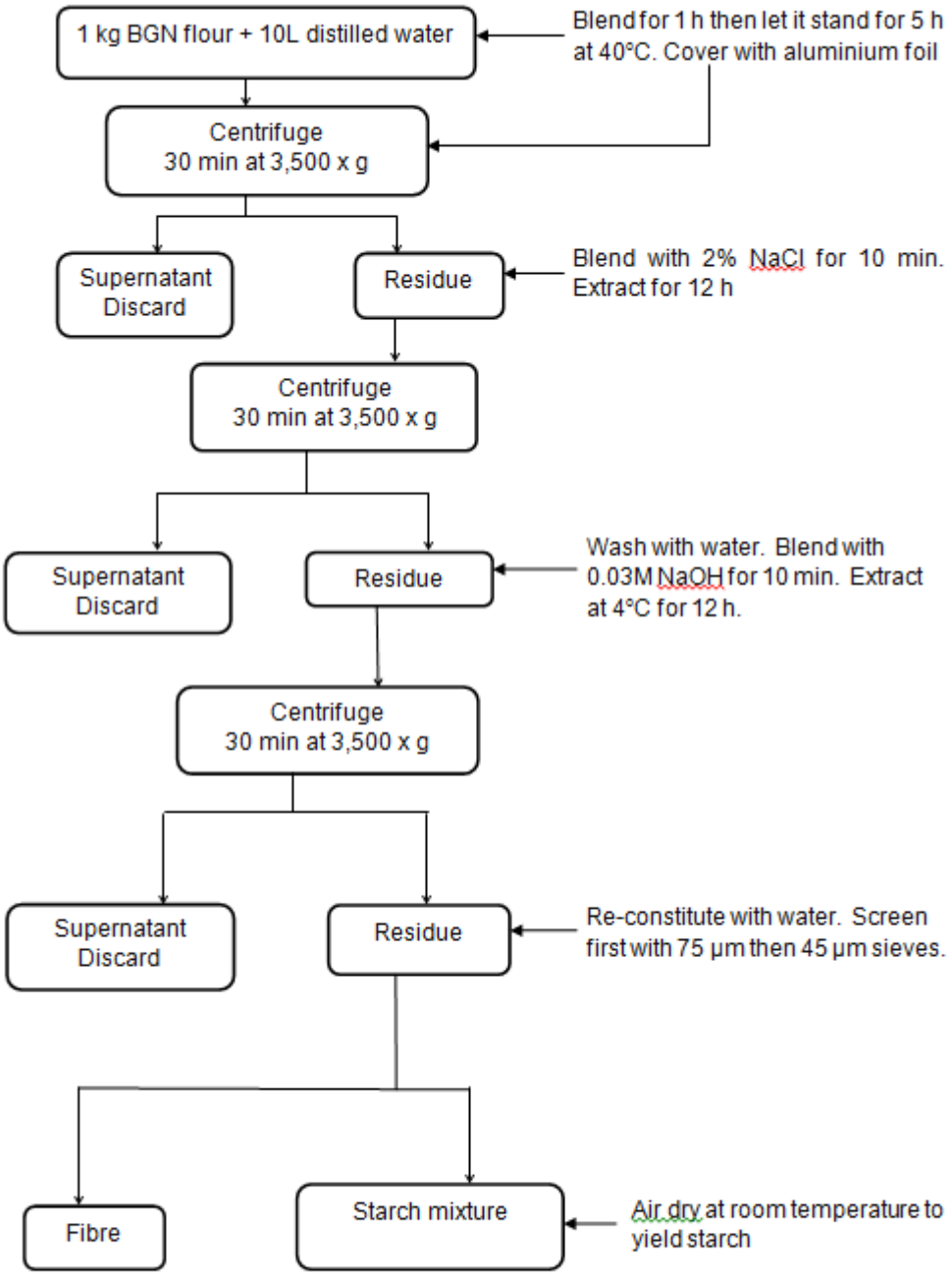

Figure 3.2 Schematic diagram of the BGN starch extraction procedure

3.2.4 Modification of BGN starch by grafting using chemical initiators

The method of Spizzirri *et al*. (2010) was used to modify BGN starch by grafting with slight modifications. The initiators used were ascorbic acid and hydrogen peroxide redox pair. The process was carried out in a 50 ml glass flask, 37.5 ml of distilled water was used to dissolve 10 g of BGN starch, and then 165% (w/w) hydrogen peroxide (120 v) and 1% (w/w) ascorbic acid were added as determined from preliminary studies by Gulu (2014). The mixture was incubated at 90^{o}C for 45 minutes. Thereafter, it was allowed to cool to room temperature. The method reported by Dong *et al.* (2015) was used with slight modifications to precipitate the pre-gelatinised starch from solution. Absolute ethanol was added drop wise while the BGN starch solution was continuously agitated in a sonicator bath. The mixture was sonicated for 10 min at high speed. Thereafter, the resulting mixture was then centrifuged at 3 783 x g for 5 minutes. The supernatant was discarded and the obtained residue presented the regenerated BGN starch particles which were then rinsed three times with absolute ethanol to remove hydrogen peroxide and ascorbic acid. For the BGN starch-catechin complex, 0.1 g of catechin was then added to the obtained BGN starch particles and this was left to react for 24 h. Thereafter the BGN starch particles were dried in a vacuum oven at 50^{o}C overnight.

3.3 Formation of BGN starch-catechin complex using cyclodextrin (alpha and beta) as an initiator

Three different methods were employed to complex cyclodextrin, BGN starch and catechin. These methods were kneading, co-evaporation and microwave irradiation and are discussed in sections 3.3.1 to 3.3.3.

3.3.1 Kneading method

The kneading method adapted from Liu & Zhu (2006); Yavuz *et al*. (2010) and Sharma & Sharma (2011) with slight modifications was used. Equal amounts 1:1 molar ratio of BGN starch and cyclodextrin were accurately weighed. Thereafter cyclodextrin (alpha and beta) was mixed with small amounts (3%) of water in a mortar to form a homogeneous paste. BGN starch was then added slowly while grinding, a small amount (5%) of hydro-alcoholic solution (ethyl alcohol) (1:1 ratio) was added to facilitate dissolution of BGN starch. The obtained mass was then dried at 40-50^{o}C in a vacuum oven for 24 hours. The dried complex was ground to a fine powder. This was repeated in the same manner; however, 1% catechin was now added to the cyclodextrin and BGN starch.

3.3.2 Co-evaporation method

The co-evaporation method adapted from Ribeiro *et al.* (2008); Patil *et al.* (2010) and Sharma & Sharma (2011) with slight modifications, was used. Equimolar amounts 1:1 molar

ratio of cyclodextrin (alpha and beta) and BGN starch were dissolved in 15 ml ethanol and hydro-alcoholic solution (1:4 ethanol-water), respectively. These two solutions were then mixed to obtain a molecular dispersion of BGN starch and cyclodextrin (alpha and beta). The mixture was stirred at 300 rpm using magnetic stirrer at 37^{o}C for 24 h, until a clear solution was obtained. The solvents were then air-dried in a vacuum oven at 45-50^{o}C (Laborota 40001, Heidolph) to constant weight. The resultant solids were then ground. This was repeated in the same manner; however, 1% catechin was now added to the cyclodextrin and BGN starch.

3.3.3 Microwave irradiation method

The microwave irradiation method adapted from Patil *et al.* (2010); Savjani *et al.* (2012); Kumar *et al.* (2013); Bhopate & Dhole (2014) and Mishra *et al.* (2014) with some modifications, was used. Ethanol and water solution (1:1 water: ethanol v/v) were used to dissolve BGN starch and cyclodextrin (alpha and beta) in a 250 ml glass beaker. The mixture was reacted in a microwave oven at a temperature of 60^{o}C for 90 min. When the reaction was complete, a sufficient amount of solvent mixture was used to remove residual, un-complexed BGN starch and cyclodextrin from the reaction by thoroughly rinsing the beaker with the solvent mixture. Obtained precipitate was then separated using Whatman No. 1 filter paper; thereafter it was dried for 48 h in a vacuum oven at 40^{o}C. This was repeated in the same manner; however, 1% catechin was now added to cyclodextrin and BGN starch.

3.4 Characterization of the modified BGN starch and BGN starch-catechin complex

3.4.1 Scanning electron microscopy

Starch granule morphology was examined using the scanning electron microscopy. The samples were transferred onto double-sided conductive carbon tape and mounted onto an aluminum SEM stubs. To enhance conductivity, the stubs were coated with a thin layer of gold using an Edwards S105A sputter-coater. Thereafter, the sample was loaded into a Zeiss MERLIN Field Emission Scanning Electron Microscope (Carl Zeiss Microscopy, Munchen, Germany) at the Electron Microbeam Unit of Stellenbosch University's Central Analytical Facility (CAF). A Zeiss Inlens Secondary Electron (SE) Detector, Zeiss Backscatter Electron (BSE) Detectors and Zeiss Smart SEM software were used to generate images, while the samples were chemically quantified by quantitative Energy Dispersive X-Ray Spectrometry (EDS) using an Oxford Instrument® X-Max 20 mm^2 detector and Oxford Aztec software (Oxford Instruments, Oxfordshire OX13 5QX, United Kingdom).

For InLens SE detection operating conditions of 3kV accelerating voltage and 80pA bean current with a working distance of 4.4 mm were applied. For Backscatter Electron detection (BSE), operating conditions of 20kV accelerating voltage and 11nA beam current

with a working distance of 9.5 mm, were applied. Images were captured in random areas and at a range of magnifications, to characterize grain morphology. Beam conditions during the quantitative analysis on the Zeiss MERLIN were 20kV accelerating voltage, with a working distance of 9.5 mm and a beam current of 11nA. Regions of interest were randomly selected, for EDS analysis a counting time of 10 seconds live-time was applied. Gold was automatically excluded from the analysis since the sample was coated with gold. The physical limitations of EDS do not allow for the analysis of elements lighter than Boron (B), therefore the elements Hydrogen (H), Helium (He), Lithium (Li) and Beryllium (Be) were not included in the analysis. Carbon and aluminum were also excluded.

3.4.2 Fluorescence spectroscopy analysis of grafted BGN starch

Grafted and blank BGN starch (10 mg) individually were weighed and dissolved in Millipore water in a 50 ml volumetric flask. This was then mixed until it was completely dissolved. Thereafter, the sample was poured into a cuvette (1 cm path length) and then placed inside the fluorescence spectrophotometer for analysis. This was repeated in the same manner for the blank without adding the sample. The method adapted from Insińska-rak *et al.* (2007) and Singh *et al.* (2010) was used. The settings used were 2.5 nm excitation and emission slit, acquisition interval of 1 nm and integration time kept at 0.1 s for the total luminescence spectra and 0.05 s in the synchronous scan method. The excitation emission matrices spectra were recorded for excitation spectra from 250 to 500 nm at 5 nm intervals, while the emission spectra ranged between 280 to 600 nm at 5 nm intervals and 0.1 sec integration time. Fluorescence measurements were made using synchronous three-dimensional fluorescence spectroscopy and single synchronous scan. Excitation and emission monochromator in the range of 250-500 nm were run concurrent to obtain a synchronous fluorescence spectrum. To obtain a 3-D spectrum, a single synchronous fluorescence spectrum was folded.

3.4.3 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy analysis of the modified BGN starch and BGN starch-catechin complex samples was carried out using the method reported by Afolabi (2012). The samples were milled using a coffee mill. Finely powdered sample and dry KBr (1:100, sample to KBr ratio) were accurately weighed and mixed. The mixing was performed in a vibratory ball mill capsule for 5 min. The ground mixture was then transferred to a specadie, which then resulted in an 8.5 mm diameter film being produced, which was then analyzed in the beam of the FTIR spectrophotometer. The spectrum was analyzed in the resolution interval of 400-4000 cm^{-1}.

3.4.4 Powder X-Ray diffraction

The method adapted from Ribeiro *et al.* (2008) and Afolabi (2012) was used to determine the structure of modified BGN starch and BGN starch-catechin complex using powder X-ray diffraction. X-ray diffractograms of the powdered BGN starch (1 g) were acquired with an X-ray diffractometer with Bragg-Brentano geometry. The settings used were the diffraction angle-scanning region from 3 to 40º, target voltage 40 kV, target current 100 mA, ageing time 5 min and radiation wavelength of 0.1542 nm.

3.4.5 Evaluation of antioxidant activity

Determination of scavenging activity on DPPH radicals

The method adapted from Spizzirri *et al.* (2010) was used. DPPH stock solutions (5 ml) at concentrations of 2.5 mM/ml were made before every analysis. A concentration of 1 mM/ml methanol was used to prepare the trolox stock solution, which was kept at -20^{o}C. A polymer of 25 mg was dissolved in 1 ml distilled water in a 25 ml volumetric flask. Thereafter, there was the addition of 4 ml of ethanol and 5 ml of ethanol solution of DPPH (200 µm), this resulted in a final concentration of 100 µm DPPH solution. The DPPH absorbance was determined using a UV-visible spectrophotometer (Perkin Elmer Lambda 25) at 517 nm. The blank polymers were subjected under the same process to act as a control sample. All measurements were done in triplicate and the DPPH values were expressed as µmol TE/g.

Total antioxidant activity determination using ORAC assay

Fluorescein stock solution was prepared by weighing 44 mg of fluorescein then dissolving it in 100 ml of phosphate buffer (75 mM, pH 7). It was then stored in a dark place under refrigeration temperatures. The AAPH radical (221 mM) was prepared by making 600 mg of AAPH up to 10 ml with the phosphate buffer. A 20 µM trolox solution was used as a reference standard and it was freshly prepared in phosphate buffer from 1 mM stock standard solution kept in the freezer.

Samples were diluted to avoid interference prior to analysis. A 5 times dilution was done on the modified BGN starches without catechin while a 10 times dilution was done on those with catechin. The method reported by Ou *et al.* (2002) and Zulueta *et al.* (2009) was used to determine the total antioxidant activity. The automated ORAC assay was carried out with fluorescence filters for an excitation wavelength of 485 nm and emission wavelength of 535 nm. The samples were placed in plates with 96 white flat-bottom wells and the reaction was carried out at 37^{o}C. In each well, 50 µl of fluorescein and 50 µl of sample, blank or standard (trolox) were placed, and the 25 µl of AAPH (221 mM) was added. Immediately after the addition, fluorescence was measured and measurements were taken in 5 min intervals. All measurements were done in triplicate and the ORAC values were expressed as µmol TE/g.

3.5 Statistical Analysis

All results were reported as means ± standard deviation of three independent trials. Multivariate analysis of variance (MANOVA) was used to establish difference between treatments. Duncan's multiple range test was used to separate means where significant difference existed (IBM SPSS version 22, 2013).

Principal Component Analysis (PCA) was used to extract the components that explained the variability in the fluorescence of native and modified BGN starches (Unscrambler ver 10.4). Smoothing Savitsky-Golay method was used.

3.6 Results and Discussion

3.6.1 BGN starch yield

Bambara groundnut starch yield obtained from the flour (BGN flour) was 32%. This was lower than the yield reported by Adebowale & Lawal (2002) [38.2%], Adebowale *et al.* (2002) [37.5%] and Afolabi (2012) [40.4%]. The lower yield could be attributed to the differences in the methods used for starch extraction. The aforementioned authors used wet milling whilst dry milling was used in this study. Using the wet milling method involves soaking the seeds overnight before milling. It is speculated that the enzymes break down the starch molecules while the seeds were soaked thereby releasing the starch molecules from the seed which in turn increases the amount of starch extracted. On the other hand, dry milling involves flour hydration and it is speculated that during milling there was loss of flour which in turn reduces the amount of starch to be extracted from the flour. In addition, dry milling methods have been reported to have a significant influence on starch damage, that can also contribute to the lower yield obtained in this study. However, using the dry milling method is more sustainable as it uses less water and energy in comparison to the wet milling method. Another difference is attributed to the seed cultivar difference; the aforementioned authors used seeds from Nigeria which is the cream variety while in this study mixed seeds from South Africa were used. The cream seeds (dehulled) have no tanning but the mixed variety (whole) used had tanning which might have caused the binding of starch thereby resulting in a lower starch yield. The starch obtained was whitish, powdery, tasteless and odourless.

3.6.2 Morphology of native and modified BGN starches

Scanning electron microscopy (SEM) was used to determine the morphology of native and modified BGN starches. There was a significant difference between granular morphology of native and modified BGN starches. Native BGN starches displayed round, oval and elliptical shapes (Figure 3.3) which is typical of legume starches. This results concurs with reports made by Adebowale & Lawal (2002); Sirivongpaisal (2008); Kaptso *et al.* (2015); Oyeyinka *et al.* (2015) and Ma *et al.* (2017) for BGN starch granular morphology. The granule surfaces all appeared to have smooth surfaces with no evidence of ruptures.

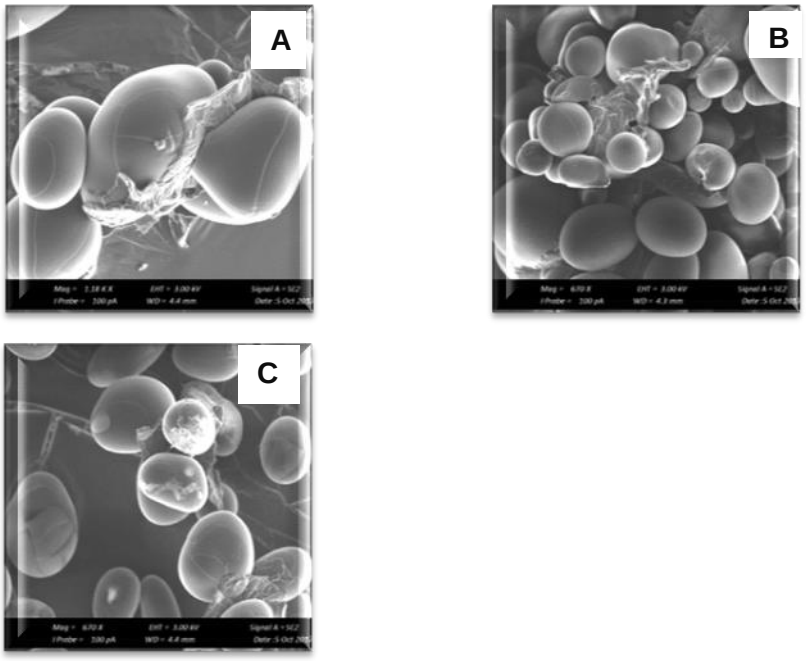

Figure 3.3 Scanning micrographs of native Bambara groundnut (BGN) starch. **A**: 1.18 K X, **B**: 670 X, **C**: 670 X

Following chemical modification, the round, oval granules lost their smooth surface texture, i.e. they were deformed; this may be due to the introduction of hydroxyl groups to the native starch. According to the SEM profile of chemically modified starches (Figure 3.4 and Figure 3.5), the starch granule crystalline structure was converted to an amorphous state. This result is in agreement with the X-ray diffraction patterns (starch crystallinity) of chemically modified BGN starches as discussed and shown in section 3.6.5 and Figure 3.29b, respectively. Furthermore, the loss of starch granule morphology of these starches suggests the successful chemical modification of BGN starch as well as grafting of the antioxidant functional group. Lastly, the SEM images of complexed BGN starches (Figure 3.6 to Figure 3.17) show clearly the characteristic BGN starch granules with cyclodextrin particles adhered to their surface, thus indicating the inclusion complex formation. The X-ray diffraction results for complexed BGN starches concurred with the SEM image findings, showing that all the complexed BGN starches retained their crystallinity while the chemically modified BGN starches had destroyed surface showing loss of starch crystallinity.

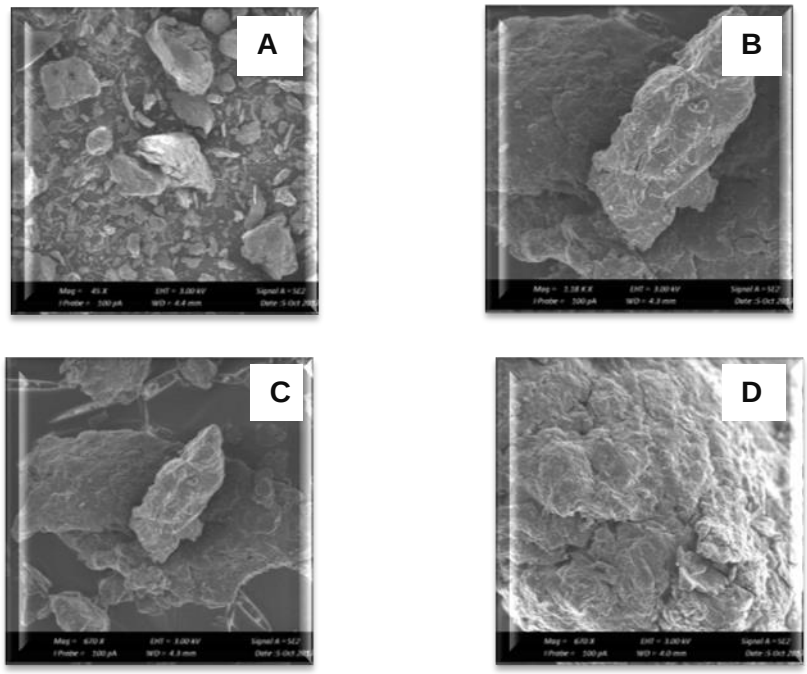

Figure 3.4 Scanning micrographs of chemically modified BGN starches. **A**: 45 X, **B**: 1.17 K X, **C**: 670 X, **D**: 670 X

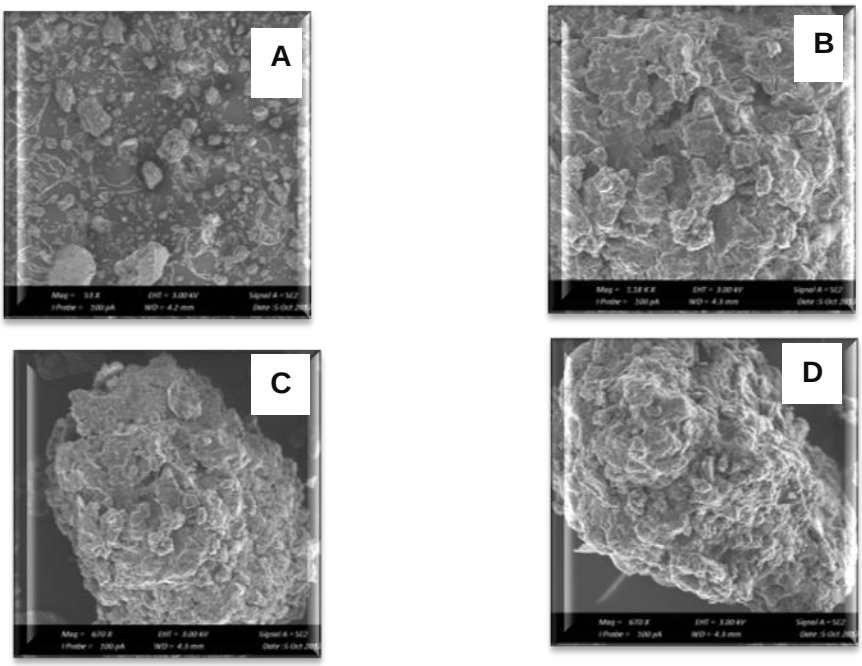

Figure 3.5 Scanning micrographs of chemically modified BGN starch-catechin complexes. **A**: 53 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

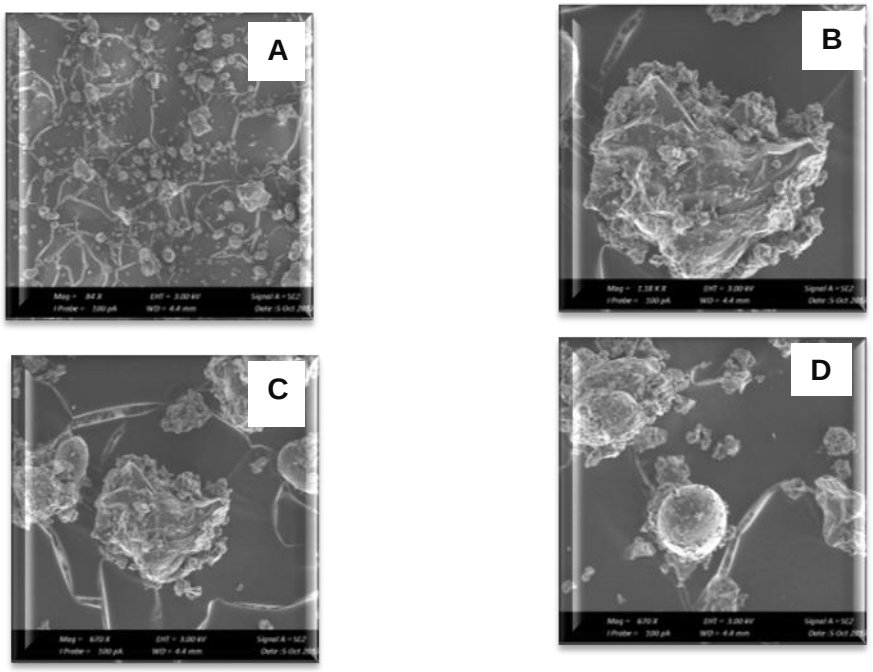

Figure 3.6 Scanning micrographs of complexed BGN starches modified through microwave method using alpha cyclodextrin. **A**: 84 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

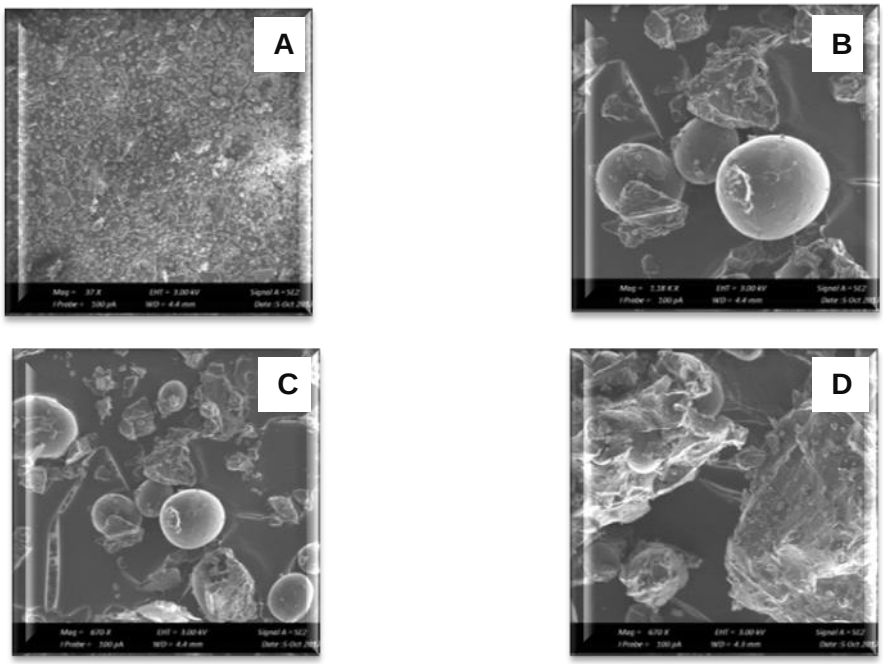

Figure 3.7 Scanning micrographs of BGN starch-catechin complexes modified through microwave method using alpha cyclodextrin. **A**: 37 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

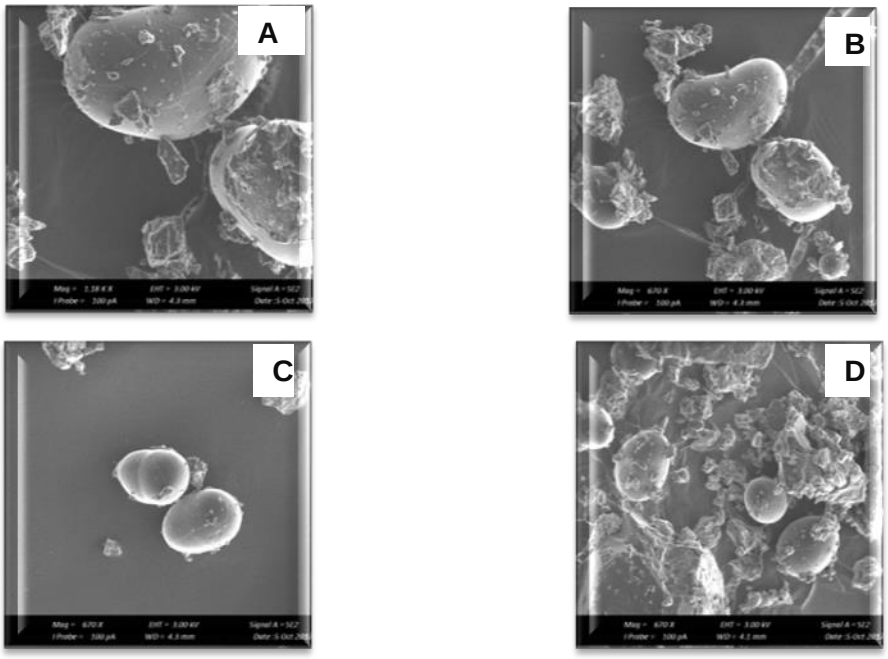

Figure 3.8 Scanning micrographs of complexed BGN starches modified through microwave method using beta cyclodextrin. **A**: 1.18 K X, **B**: 670 X, **C**: 670 X, **D**: 670 X

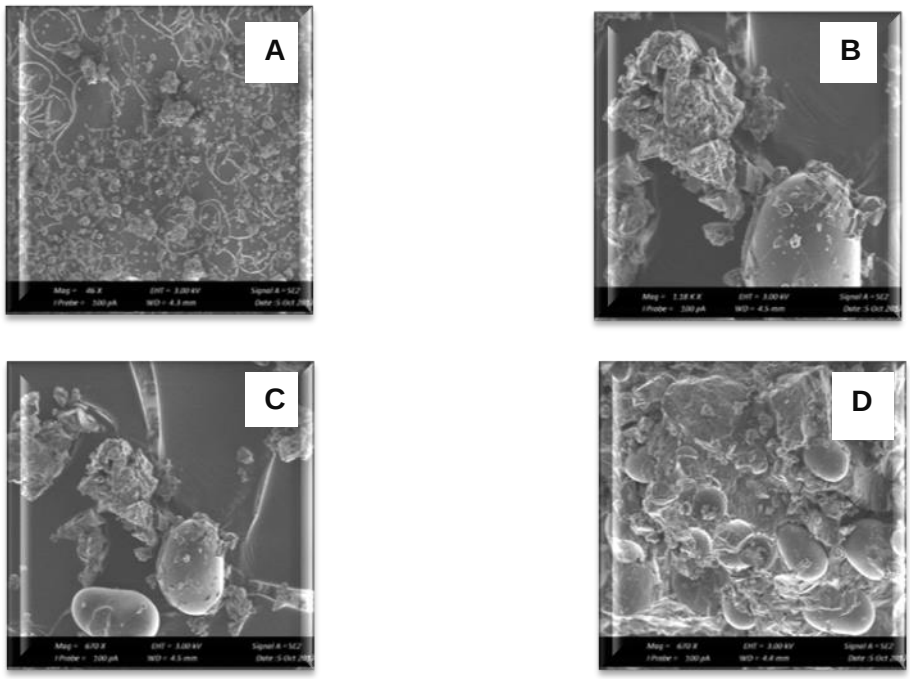

Figure 3.9 Scanning micrographs of BGN starch-catechin complexes modified through microwave method using beta cyclodextrin. **A**: 46 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

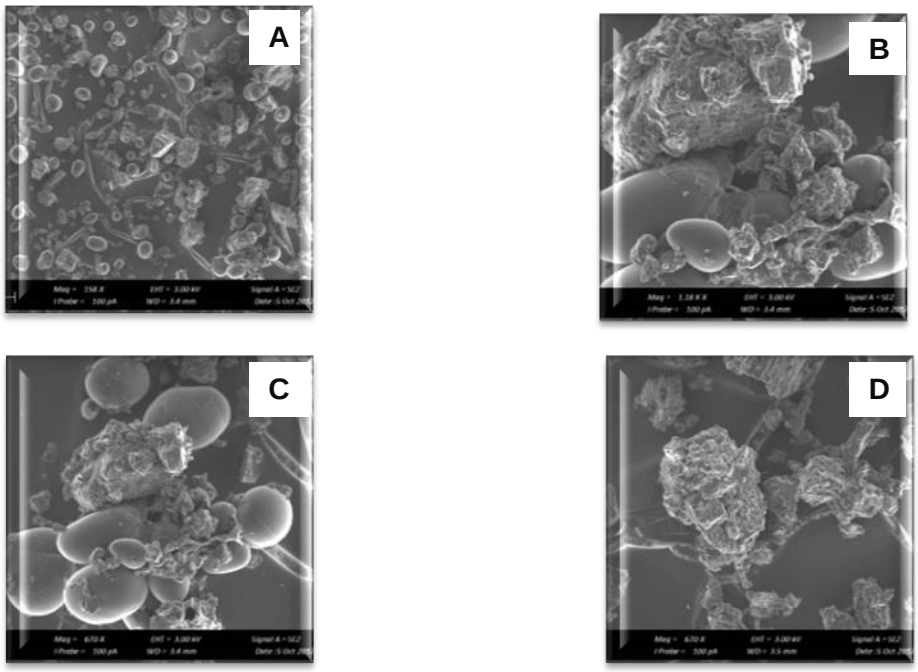

Figure 3.10 Scanning micrographs of complexed BGN starches modified through co-evaporation method using alpha cyclodextrin. **A**: 158 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

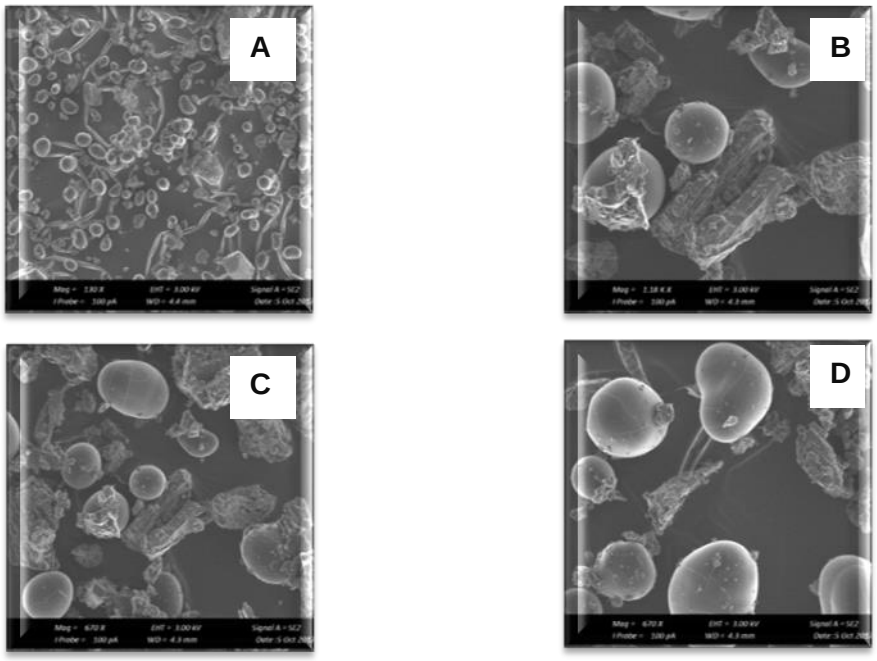

Figure 3.11 Scanning micrographs of BGN starch-catechin complexes modified through co-evaporation method using alpha cyclodextrin. **A**: 130 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

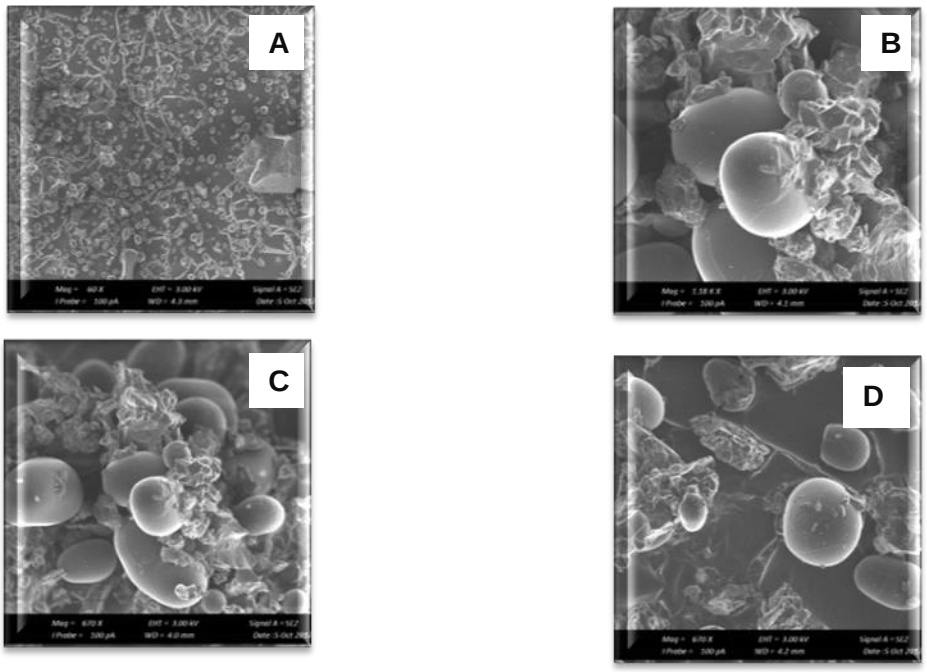

Figure 3.12 Scanning micrographs of complexed BGN starches modified through co-evaporation method using beta cyclodextrin. **A**: 60 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

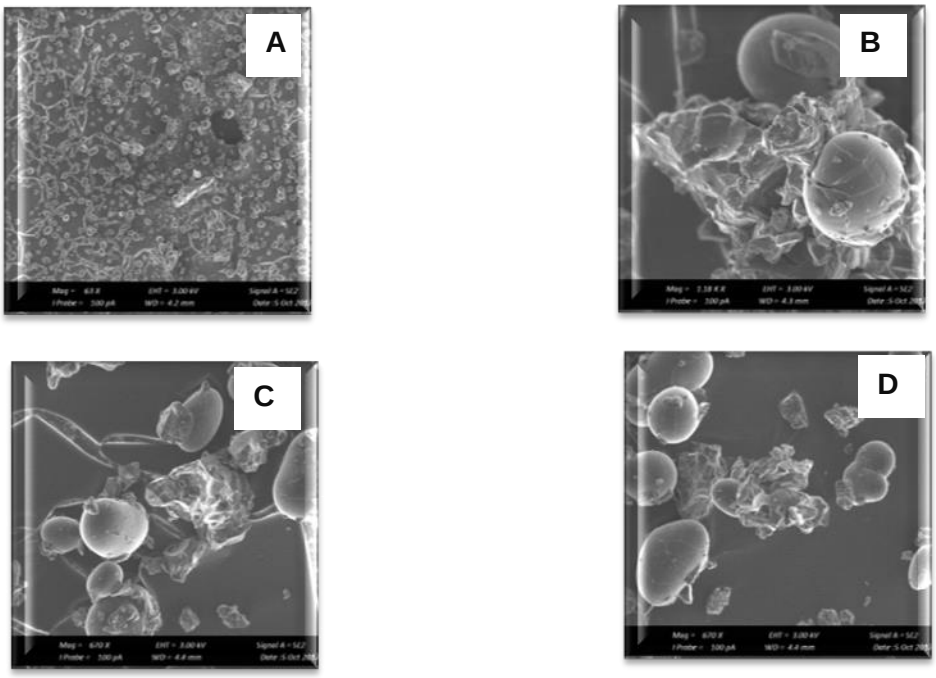

Figure 3.13 Scanning micrographs of BGN starch-catechin complexes modified through co-evaporation method using beta cyclodextrin. **A**: 63 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

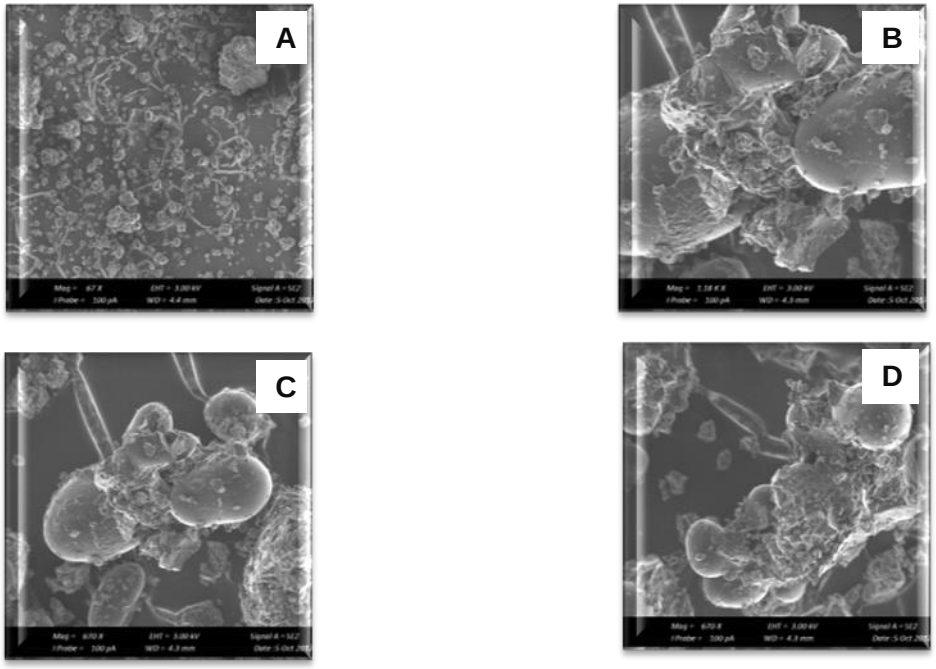

Figure 3.14 Scanning micrographs of complexed BGN starches modified through kneading method using alpha cyclodextrin. **A**: 60 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

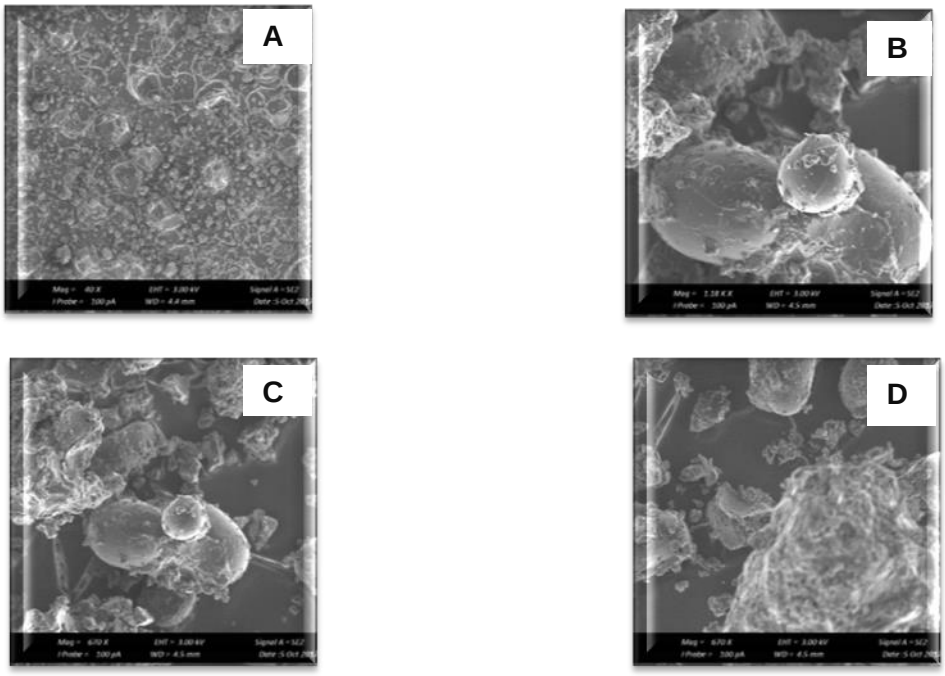

Figure 3.15 Scanning micrographs of BGN starch-catechin complexes modified through kneading method using alpha cyclodextrin. **A**: 40 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

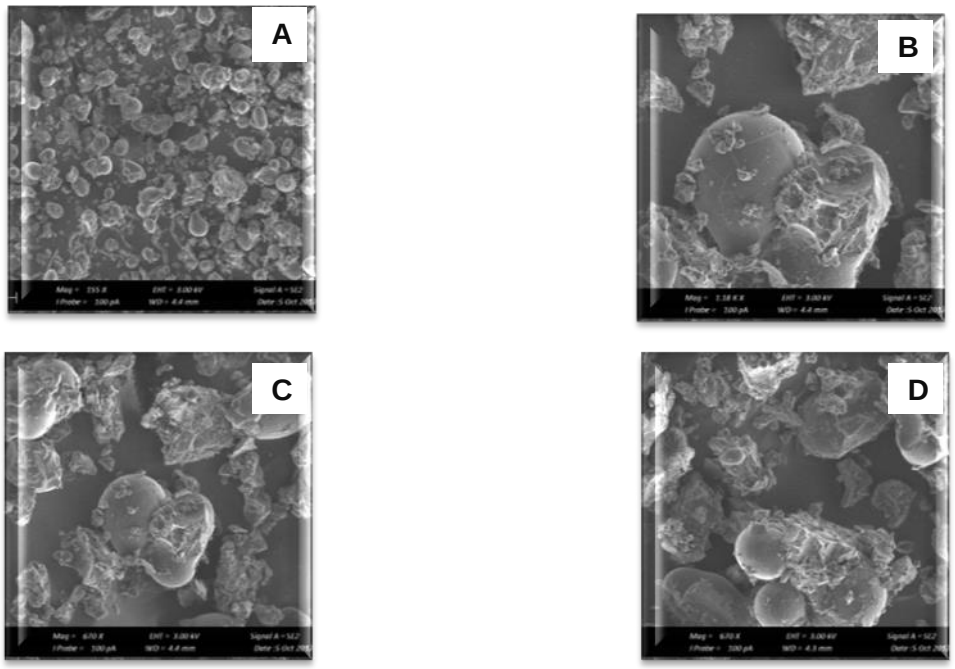

Figure 3.16 Scanning micrographs of complexed BGN starches modified through kneading method using beta cyclodextrin. **A**: 155 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

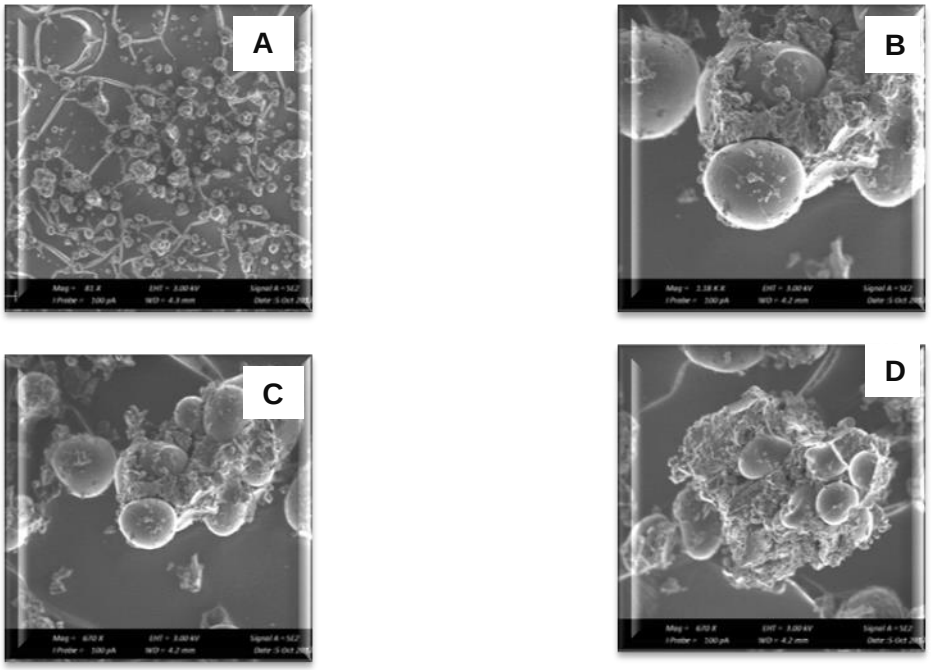

Figure 3.17 Scanning micrographs of BGN starch-catechin complexes modified through kneading method using beta cyclodextrin. **A**: 81 X, **B**: 1.18 K X, **C**: 670 X, **D**: 670 X

3.6.3 Fluorescence spectroscopy of grafted BGN starch

The fluorescence spectra of native and modified BGN starches are shown in Figure 3.18. Native BGN starch had an emission spectrum at 270 nm. Following modification, there was an observable shift in the emission spectra of the starches. Modified BGN starch–catechin complexes all showed a peak at 320 nm, while those modified without catechin showed a peak at 350 nm. Starch modification processes changes the intra- and intermolecular bonds within the starch molecules, thereby causing the functional reactive groups of native starch to be exposed which then can lead to the covalent binding of catechin. This explains the shift in the emission wavelength of native starch from 270 nm increasing to 320 nm (modified BGN starch–catechin complexes) and 350 nm in the modified starches. This gives an indication that catechin was successfully grafted onto the BGN starch hence the peak at 320 nm. This result is in agreement with reports made by Ramirez-Sanchez *et al.* (2010) and Liu *et al.* (2014) where catechin spectra was observed to be in the 280 nm to 320 nm range. The peak at 320 nm was attributed to the B ring of the catechin moiety, suggesting that a new functional group (catechin hydroxyl group) was successfully grafted onto the BGN starch molecule.

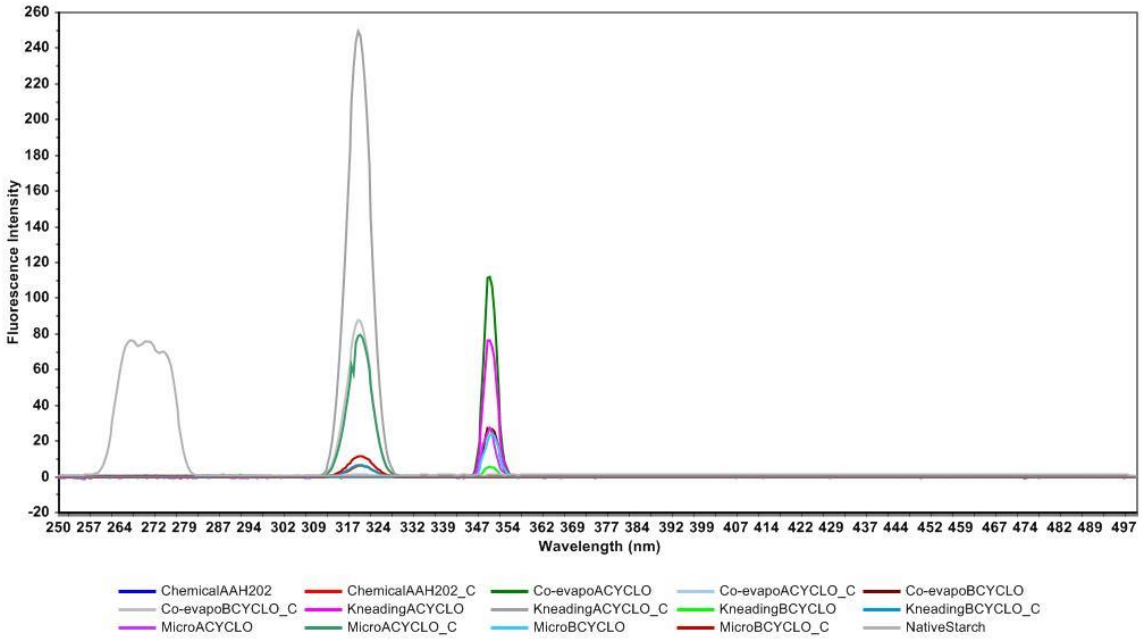

Figure 3.18 Fluorescence spectra of native and modified BGN starches. ChemicalAAH2O2: Chemical modification (ascorbic acid + peroxide and catechin); Co-evapoACYCLO: Co-evaporation (α-cyclodextrin); Co-evapoACYCLO_C: Co-evaporation (α-cyclodextrin + catechin); Co-evapBCYCLO: Co-evaporation (β-cyclodextrin); Co-evapBCYCLO_C: Co-evaporation (β-cyclodextrin + catechin); KneadingACYCLO: Kneading (α-cyclodextrin); KneadingACYCLO_C: Kneading (α-cyclodextrin + catechin); KneadingBCYCLO: Kneading (β-cyclodextrin); KneadingBCYCLO_C: Kneading (β-cyclodextrin + catechin); MicroACYCLO: Microwave (α-cyclodextrin); MicroACYCLO_C: Microwave (α-cyclodextrin + catechin); MicroBCYCLO: Microwave (β-cyclodextrin); MicroBCYCLO_C: Microwave (β-cyclodextrin + catechin)

Principal components explaining the variability in fluorescence emission spectra of BGN starches

Variation in the emission spectra of the BGN starches can be described by two principal components with eigenvalues exceeding one. Principal component 1 (PC1) described the greatest source of variability and it accounted for 83% of variability, while principal component 2 (PC2) accounted for 17% of variability. The cumulative variability of the two components added up to 100%. PC1 correlates with chemically modified starches, microwave (alpha cyclodextrin, beta cyclodextrin and beta cyclodextrin + catechin), co-evaporation (alpha cyclodextrin, alpha cyclodextrin + catechin and beta cyclodextrin) and kneading (alpha cyclodextrin, beta cyclodextrin and beta cyclodextrin + catechin). Figure 3.19 shows the score plot differentiating the emission spectra of BGN starches with respect to PC1 and PC2. The clustered variables are closely correlated. Starch modified through the kneading method (alpha cyclodextrin + catechin) is far off from all others and it can therefore be concluded that it is different from others. Furthermore, native BGN starch emission spectrum was significantly different from the spectra of modified starches; this then gives an indication that it was successfully modified. Hence, PC2 separates modified BGN starches from native BGN starch, though the modified starches are closely correlated as they were all clustered. From Figure 3.20, PC1 is highly loaded on the complexed BGN starches with catechin having emission spectra at wavelength 320 nm, while native BGN starch with wavelength of 270 nm is highly loaded in PC2. The emission spectrum of native BGN starch is different from the emission spectra of modified starches thus suggesting that starch was successfully modified.

3.6.4 Structure of native and modified BGN starches determined by Fourier Transform Infrared spectroscopy

The FTIR spectrum of native BGN starch is shown in Figure 3.21 with a broad peak at 3285.34 cm^{-1} that is in the range of 3200–3600 cm^{-1}. This is characteristic of OH stretching due to the hydrogen bonded hydroxyl groups contributing to the vibrational stretches associated with intra and intermolecular bound hydroxyl groups which make up the gross starch structure (Zhang & Han, 2006; Afolabi, 2012; Oyeyinka *et al.*, 2015). Similar results were reported for BGN starches by Afolabi (2012) and Oyeyinka *et al.* (2015). The sharp peak at 2931.69 cm^{-1} is attributed to the C-H stretching. The sharp bend at 1634.36 cm^{-1} was attributed to the water bending vibrations. The tightly bound water absorbed by the amorphous regions in the starch molecules gives rise to these vibrations. Comparable results have been reported for pea and BGN starch by Zhang & Han (2006) and Oyeyinka *et al.* (2015), respectively. The wavelength of 1336.77 cm^{-1} is attributed to the stretching vibrations of C-O and C-C bonds which are found in the wavelength region of 800–1300 cm^{-1} (Oyeyinka *et al.*, 2015; Wang *et al.*, 2015).

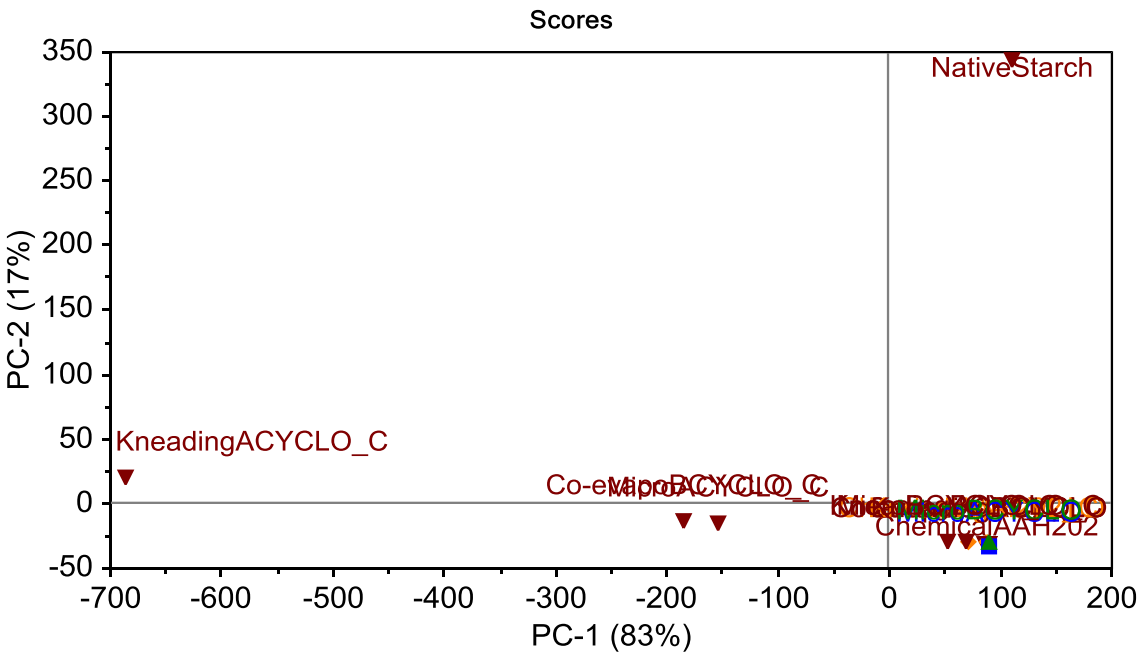

Figure 3.19 Score plot showing the emission spectra of native and modified BGN starches

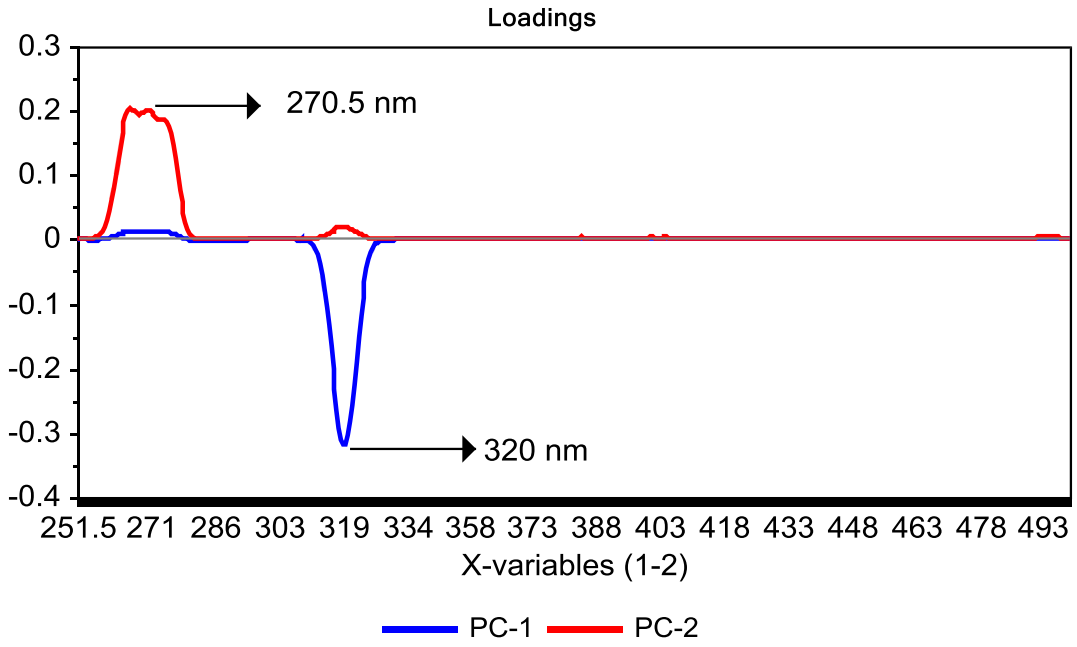

Figure 3.20 Loading plot displaying the emission spectra of native and modified BGN starches

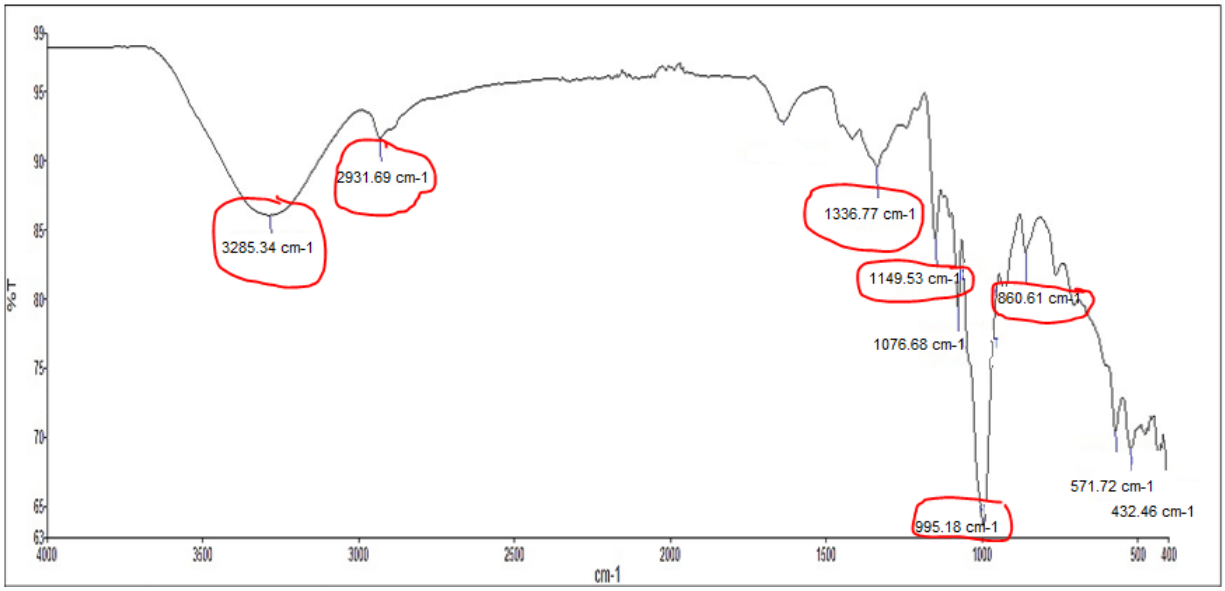

Figure 3.21 FTIR spectrum of native BGN starch

The peak at 1149.53 cm^{-1} in the BGN native starch is due to the C-O, C-C and C-O-H stretching. Comparable results have been reported by Oyeyinka *et al.* (2015) and Warren *et al.* (2016). Zhang & Han (2006) reported the peaks at 1076.68cm^{-1} and 995.18 cm^{-1} to be characteristic of the anhydroglucose ring O-C stretch. Furthermore, the peak at 995 cm^{-1} is known to be sensitive to water. The peak at 860.61 cm^{-1} is attributed to the C-O stretching in the native BGN starch as well as to the deformation of C-H vibrations. The native BGN starch exhibited complex vibrations at wavelengths below 800 cm^{-1}, that is, 571.72 and 432.46 cm^{-1} thus owing to the glucose pyranose ring skeletal mode vibration. This result is in agreement with reports made by Oyeyinka *et al.* (2015). Native BGN starch is therefore made up of glucose molecules just like all the other starches. In addition, it confirms that it is made up of carbon, hydrogen and oxygen atoms which make up the gross starch structure just like other starches.

The FTIR spectra of modified BGN starches are shown in Table 3.1. After modification, the peak at 2931.61 cm^{-1} (in native BGN starch) attributed to the C-H stretching disappeared in all modified BGN starches. It is speculated that starch modification disrupted the C-H bonds as new bonds were formed during the starch modification process.

Table 3.1 The FTIR spectra of native and modified BGN starches[1]

Modification method	Initiator	Wavelength cm^{-1}
Native starch	Native starch	3285.34, 2931.69, 1634.36, 1336.77, 1149.53, 1076.68, 995.18, 860.61, 571.72, 432.46
Chemical	AA + H_2O_2	3282.16, 1362.31, 1148.45, 1077.44, 1002.03, 426.87
	AA + H_2O_2 + catechin	3294.56, 1362.84, 1545.59, 1148.67, 1077.50, 1017.33, 571.81, 479.58, 406.83
Co-evaporation	Alpha cyclodextrin	3280.80, 1333.89, 1151.41, 1075.55, 1024.53, 860.30, 571.46, 521.03, 406.70
	Alpha cyclodextrin + catechin	3279.44, 1334.02, 1521.25, 1151.72, 1075.21, 1025.27, 862.72, 571.99, 523.06, 431.90
	Beta cyclodextrin	3294.53, 1336.14, 1151.79, 1077.82, 1021.10, 573.60, 526.06, 421.26
	Beta cyclodextrin + catechin	3298.73, 1335.44, 1526.36, 1151.75, 1077.17, 1020.84, 573.38, 525.74, 403.07
Microwave	Alpha cyclodextrin	3283.30, 1337.82, 1151.47, 1075.82, 1023.53, 572.58, 520.55
	Alpha cyclodextrin + catechin	3276.35, 1333.87, 1528.51, 1150.41, 1075.54, 1023.27, 572.17, 521.39, 436.82, 404.59
	Beta cyclodextrin	3288.74, 1334.49, 1151.60, 1077.58, 1020.80, 574.37, 526.99
	Beta cyclodextrin + catechin	3298.74, 1333.96, 1530.08, 1151.61, 1077.52, 1021.59, 572.59, 525.47, 427.32
Kneading	Alpha cyclodextrin	3269.02, 1332.05, 1151.35, 1075.48, 1024.97, 572.52, 519.99
	Alpha cyclodextrin + catechin	3275.19, 1332.42, 1520.25, 1151.27, 1075.53, 1024.89, 859.64, 572.21, 521.85, 405.78
	Beta cyclodextrin	3292.29, 1335.25, 1152.22, 1077.70, 1022.73, 574.87, 526.20, 427.53, 406.36
	Beta cyclodextrin + catechin	3295.33, 1334.71, 1522.25, 1152.28, 1077.84, 1022.62, 574.48, 528.21, 434.59

[1]AA: Ascorbic acid; H_2O_2: Hydrogen peroxide

The starch modification process involves the incorporation of new hydroxyl groups onto the starch molecule, which then form bonds with the carbon atoms in the starch resulting in C-OH bonds instead of C-H. Furthermore, the peaks at 1634.36 cm^{-1} and 860 cm^{-1} wavelength were not found in modified BGN starches. This gave an indication that native starch was successfully modified as new bonds (C-OH) were formed leading to the disappearance of the aforementioned bonds.

New peaks around 1020 cm^{-1} were observed in all the modified BGN starches. These new absorption peaks indicate that native BGN starch was successfully modified. Furthermore, the modified starches with catechin all showed a new absorption peak in the range 1520 - 1560 cm^{-1}. The new absorption peaks were attributed to the C-C stretching within the catechin aromatic ring. This result is in agreement with reports made by Spizzirri *et al.* (2010) in which they awarded the 1557 and 1525 cm^{-1} peaks to the C-C stretching found within the catechin aromatic ring. In addition, similar results were reported by Curcio *et al.* (2009) where a new absorption peak at 1558 cm^{-1} was awarded to the aromatic C-C stretch. This then suggests that catechin was grafted onto the BGN starch molecules. Following modification of native BGN starch some spectral bands disappeared as well as the appearance of new peaks. However, since BGN starch, beta and alpha cyclodextrin are all carbohydrates this therefore means that they have similar groups. This resulted in the spectral peaks of BGN starch being covered by similar groups of cyclodextrins (alpha and beta). This explains the similarity of modified BGN starch spectral peaks with those of modified BGN starch complexed with cyclodextrin (alpha and beta).

FTIR spectra within the chemical modification method

Chemical modification using hydrogen peroxide and ascorbic acid as redox pair initiators resulted in the introduction of the hydroxyl groups (new functional groups) onto the BGN starch molecule. The interaction mechanism of redox agents involves the oxidation of ascorbic acid by hydrogen peroxide at room temperature, which leads to the formation of hydroxyl radical and ascorbate radical intermediates that initiate the reaction (Spizzirri *et al.*, 2010). It is therefore speculated that the newly formed hydroxyl groups reacted with carbon molecules in the starch thereby resulting in the formation of C-OH bonds. This explains the increase in the 1336 cm^{-1} spectral band in the native starch to 1362 cm^{-1} in the chemically modified starch. The 995 cm^{-1} wavelength was not found in all modified BGN starches. Starch modification resulted in increased water absorption capacity of native starch, since this peak is sensitive to water it is speculated that the increased water absorption capacity of modified BGN starches resulted in the disappearance of this spectral band (995 cm^{-1}).

Addition of catechin caused a shift in the broad peak at 3285 cm^{-1} in native BGN starch to 3294 cm^{-1} in modified BGN starch-catechin complex. This is because catechin has hydroxyl groups which are speculated to have contributed to the vibrational stretches

associated with intra and intermolecular bound hydroxyl groups making up starch gross structure. Within the chemical modification method, new peaks were observed in the modified BGN starch-catechin complex indicating that a new complex was formed. These new peaks were displayed at 1017.33, 571.81, 479.58 and 406.83 cm^{-1}. Lastly the peak at 1545.59 cm^{-1} in the modified BGN starch-catechin complex (as shown in Figure 3.22) is attributed to the C-C stretching of the catechin aromatic ring. This peak gave an indication that catechin was grafted to the BGN starch.

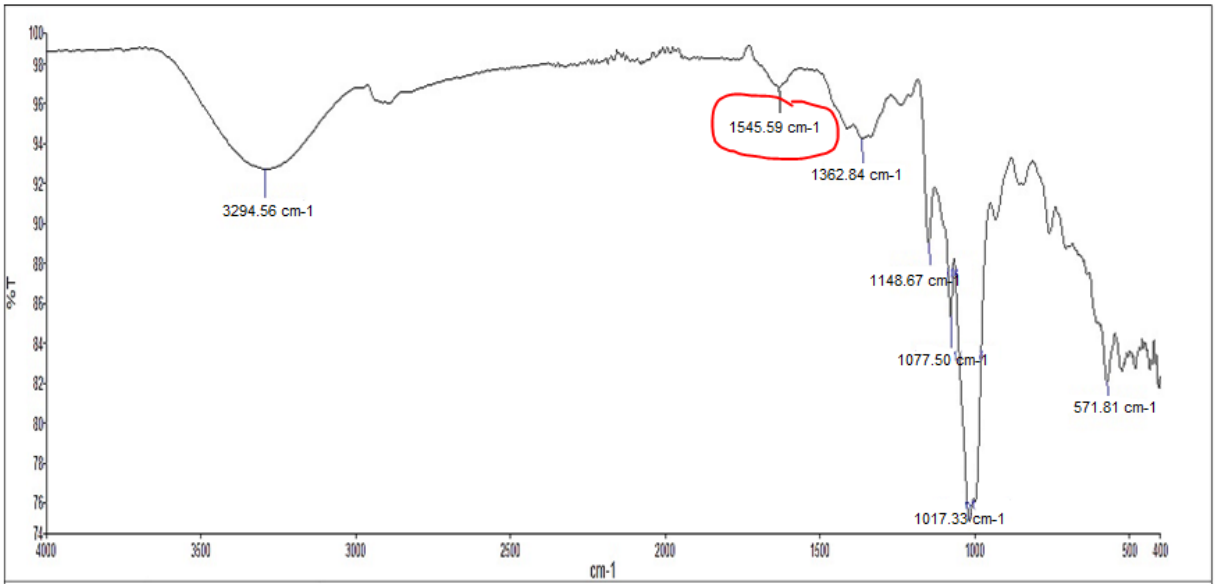

Figure 3.22 FTIR spectrum of BGN starch-catechin complex modified through chemical modification

Structure of modified BGN starch by complexation

Co-evaporation

Co-evaporation resulted in a shift in some spectral bands of the native BGN starch. Complexed BGN starches using beta cyclodextrin increased the broad peak at 3285 cm^{-1} in native BGN starch to 3294 cm^{-1} and 3298 cm^{-1} (with catechin) in complexed BGN starches. The spectral band at 1149 cm^{-1} in the native BGN starch which is a characteristic C-O, C-C and C-O-H stretching shifted to 1151 cm^{-1} in all complexed BGN starches. In addition, the characteristic hydroglucose ring O-C stretch at 1077 cm^{-1} in the native BGN starch decreased to 1075 cm^{-1} in alpha cyclodextrin complexed BGN starches. There was a disappearance of the 860 cm^{-1} peak in beta cyclodextrin complexed BGN starches. On the other hand, new peaks were observed in the complexed BGN starches in comparison to the

native BGN starch. The new peaks ranged from 1020 to 1025 cm^{-1} which are found in the amorphous regions of the complexed BGN starches. In addition, new peaks at low wavelengths were also observed and they ranged from 521 to 526 cm^{-1} which is attributed to skeletal mode vibrations within the starch molecules. Lastly, the peaks at 1521.25 cm^{-1} (Figure 3.23) and 1526.36 cm^{-1} (Figure 3.24) found in the alpha and beta cyclodextrin catechin complexed BGN starches with catechin, respectively, were characteristic of the C-C stretching of catechin aromatic ring. These peaks gave an indication that catechin was successfully grafted to the BGN starch resulting in the formation of new complexes.

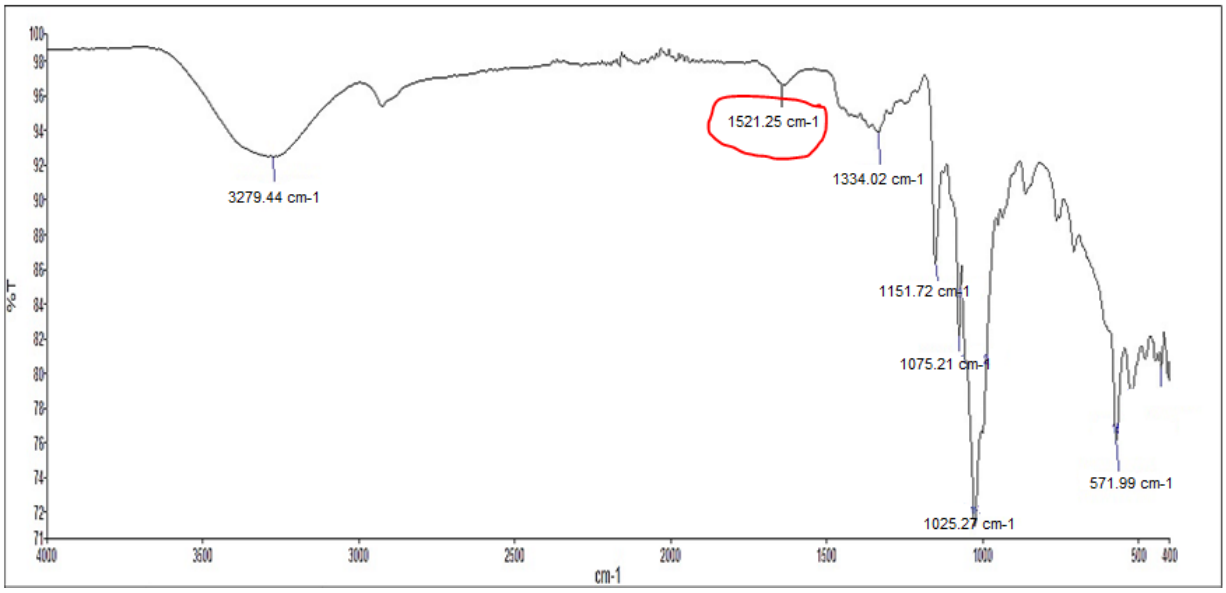

Figure 3.23 FTIR spectrum of BGN starch-catechin complex modified through co-evaporation method using alpha cyclodextrin

Microwave

Microwave as a complexation method resulted in some spectral bands within the native BGN starch shifting in the complexed BGN starches as well as the formation of new peaks indicating that BGN starch was complexed with cyclodextrin (alpha and beta) and catechin in others. The broad peak at 3285 cm^{-1} in native BGN starch decreased to 3276 cm^{-1} in alpha cyclodextrin + catechin complexed BGN starch while it increased to 3288 and 3298 cm^{-1} in beta cyclodextrin and beta cyclodextrin + catechin complexed BGN starches, respectively. It was speculated that the addition of catechin and cyclodextrin (alpha and beta) to the BGN starch contributed to the vibrational stretches within the intra and intermolecular bound hydroxyl groups making up the gross structure of starch. The 860 cm^{-1} peak which was

found in the native BGN starch was not observed in all complexed BGN starches modified through the microwave method. Some peaks in the native BGN starch shifted in the complexed BGN starch.

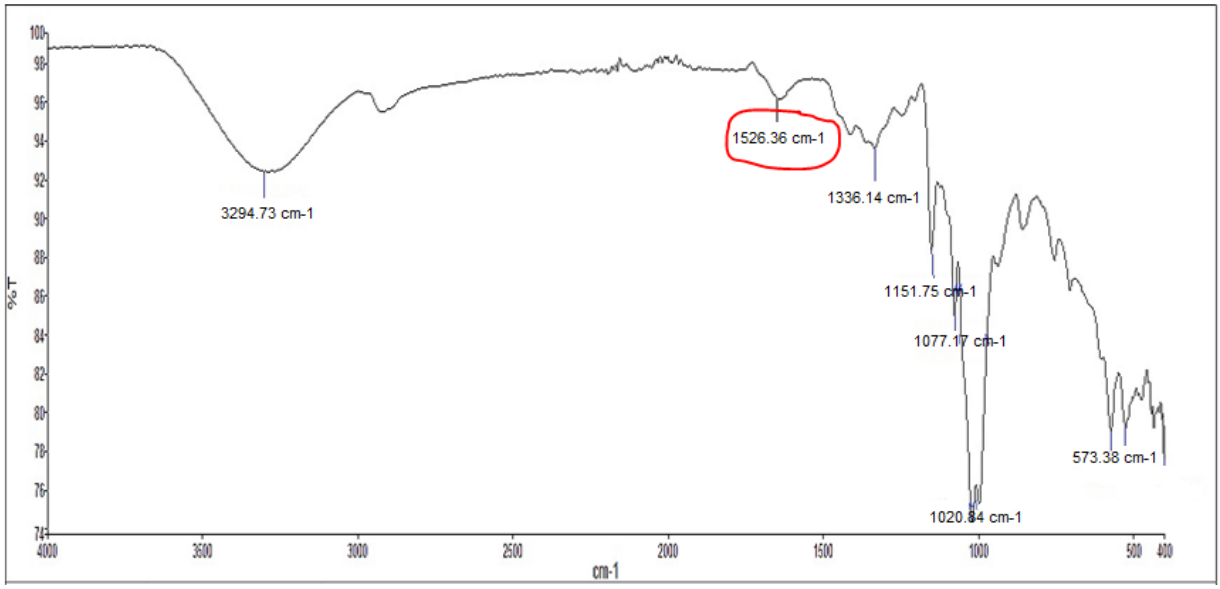

Figure 3.24 FTIR spectrum of BGN starch-catechin complex modified through co-evaporation method using beta cyclodextrin

The 1149 cm^{-1} peak in native BGN starch which is attributed to the C-O, C-C and C-O-H stretching appeared at 1151 cm^{-1} in complexed BGN starches. Furthermore, the characteristic hydroglucose ring O-C stretch at 1077 cm^{-1} in native starch appeared at 1075 cm^{-1} in alpha cyclodextrin complexed starches. In addition, new peaks were observed in the complexed BGN starches which gave an indication that new complexes were formed. The peaks at low wavelengths attributed to the skeletal mode vibrations ranged from 404 to 526 cm^{-1}. The peaks in amorphous regions of complexed BGN starches were in the range of 1020 to 1023 cm^{-1}. Finally, the new peaks observed at 1528.51 (Figure 3.25) and 1530.08 cm^{-1} (Figure 3.26) within the alpha and beta cyclodextrin catechin complexed BGN starches is a characteristic C-C stretching of catechins' aromatic ring. This gave an indication of catechin grafting to the BGN starch.

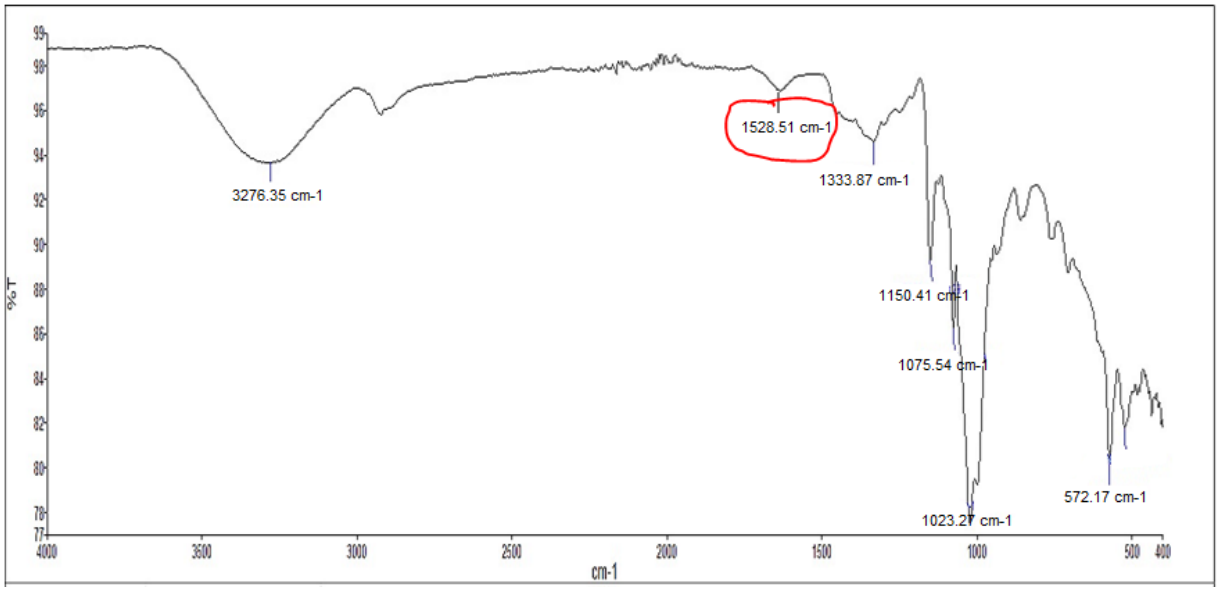

Figure 3.25 FTIR spectrum of BGN starch-catechin complex modified through microwave method using alpha cyclodextrin

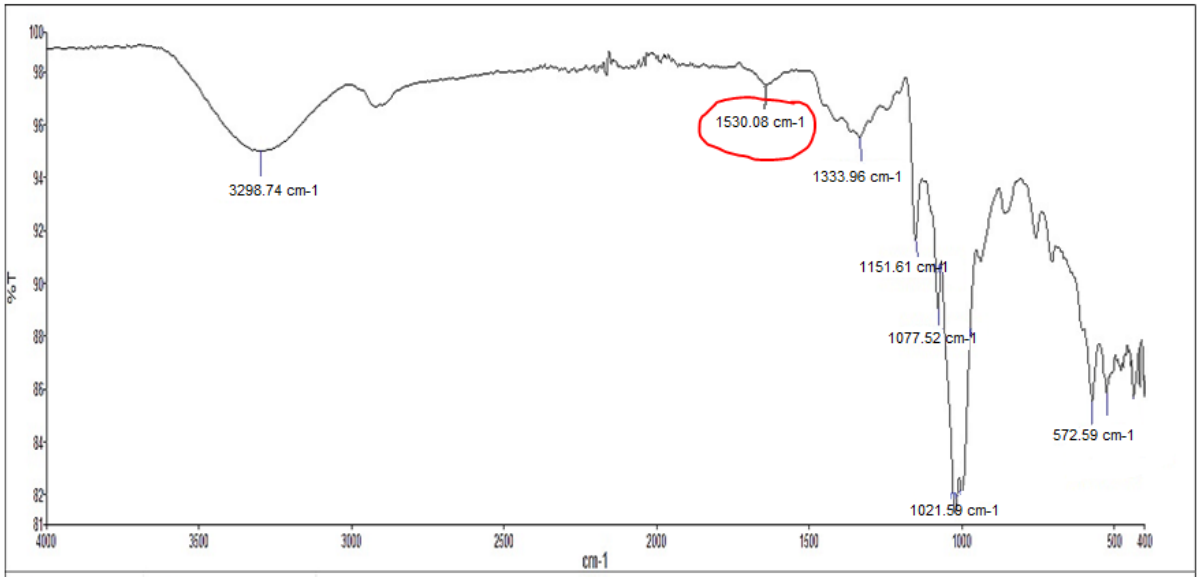

Figure 3.26 FTIR spectrum of BGN starch-catechin complex modified through microwave method using beta cyclodextrin

Kneading

Kneading also resulted in a shift in some spectral bands in the native BGN starch as well as the appearance of new peaks in the complexed BGN starches. The broad peak at 3285 cm^{-1} in the native BGN starch reduced to 3269 and 3275 cm^{-1} (with catechin) in alpha cyclodextrin complexed BGN. However, in the beta cyclodextrin complexed BGN starches it increased to 3292 and 3295 cm^{-1} (with catechin). The 860 cm^{-1} peak found in the native BGN starch disappeared in all complexed BGN starches through the kneading method with exception of the alpha cyclodextrin + catechin complexed BGN starch. New peaks at lower wavelengths were observed in the range of 520 to 528 cm^{-1} and these are known to be due to the skeletal mode vibrations within the starch molecule. Furthermore, new peaks in the range of 1022 to 1024 cm^{-1} were also observed and these are found in the amorphous regions of the complexed BGN starches. The characteristic hydroglucose ring O-C stretch peak at 1075 cm^{-1} in the native BGN starch appeared at 1077 cm^{-1} in the alpha cyclodextrin complexed BGN starches. The 1149 cm^{-1} peak in native starch just like in other complexation methods appeared at 1151 cm^{-1} in complexed BGN starches. Lastly, the new peaks observed at 1520.25 (Figure 3.27) and 1522.25 cm^{-1} (Figure 3.28) within the alpha and beta cyclodextrin catechin complexed BGN starches were a characteristic of catechin aromatic ring C-C stretch. These gave an indication that catechin was grafted to the BGN starch.

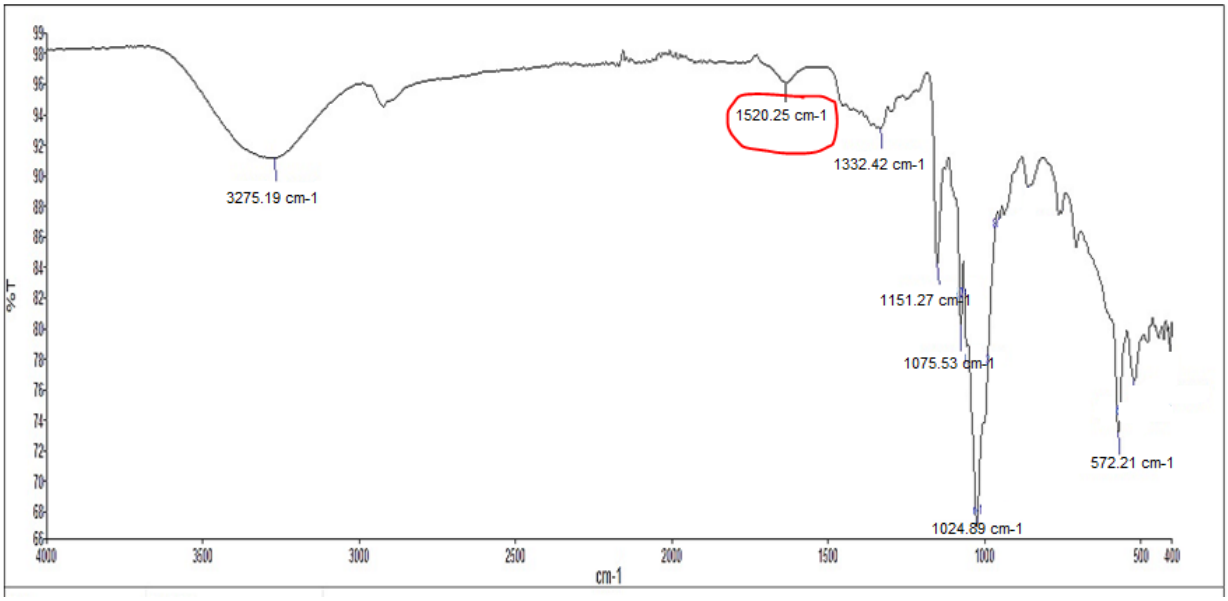

Figure 3.27 FTIR spectrum of BGN starch-catechin complex modified through kneading method using alpha cyclodextrin

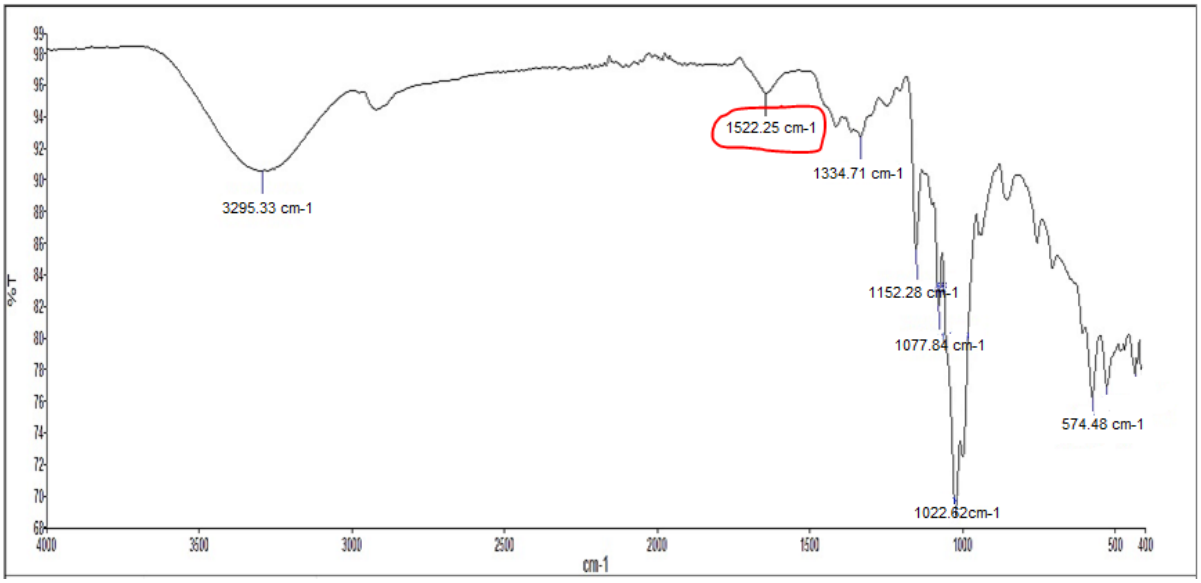

Figure 3.28 FTIR spectrum of BGN starch-catechin complex modified through kneading method using beta cyclodextrin

3.6.5 Starch crystallinity

The powder X-ray diffraction patterns of native and modified BGN starches are presented in Figure 3.29 to Figure 3.32. Native BGN starch had diffraction peaks at 2θ values of 15^{o}, 17^{o} and 23^{o} exhibiting a typical C type crystallinity. This means that BGN starch (legume starch) has lower digestibility in comparison to cereal starches. It therefore promotes moderate and slow insulin and postprandial glucose responses (Sandhu & Lim, 2008). Furthermore, the lowest digestibility and low GI values of BGN starch makes it suitable for diabetic patients. Type C starch crystallinity has been reported to be a mixture of A- and B- types. The A- and B- types can be differentiated by the packing arrangement of double helices formed from short chains within the amylopectin molecule as well as their level of hydration. The A-type is closely packed with water molecules between each double helix, while the B-type is less densely packed and more hydrated in the central cavity (Oyeyinka *et al.*, 2015). In addition type C is characteristic for legume starches (Hoover *et al.*, 2010). This result concurs with the reports made by Afolabi (2012) and Oyeyinka *et al.* (2015) of native BGN starch exhibiting type C crystallinity. However, some researchers reported the A-type crystallinity for some varieties of BGN (Sirivongpaisal, 2008; Kaptso *et al.*, 2015).

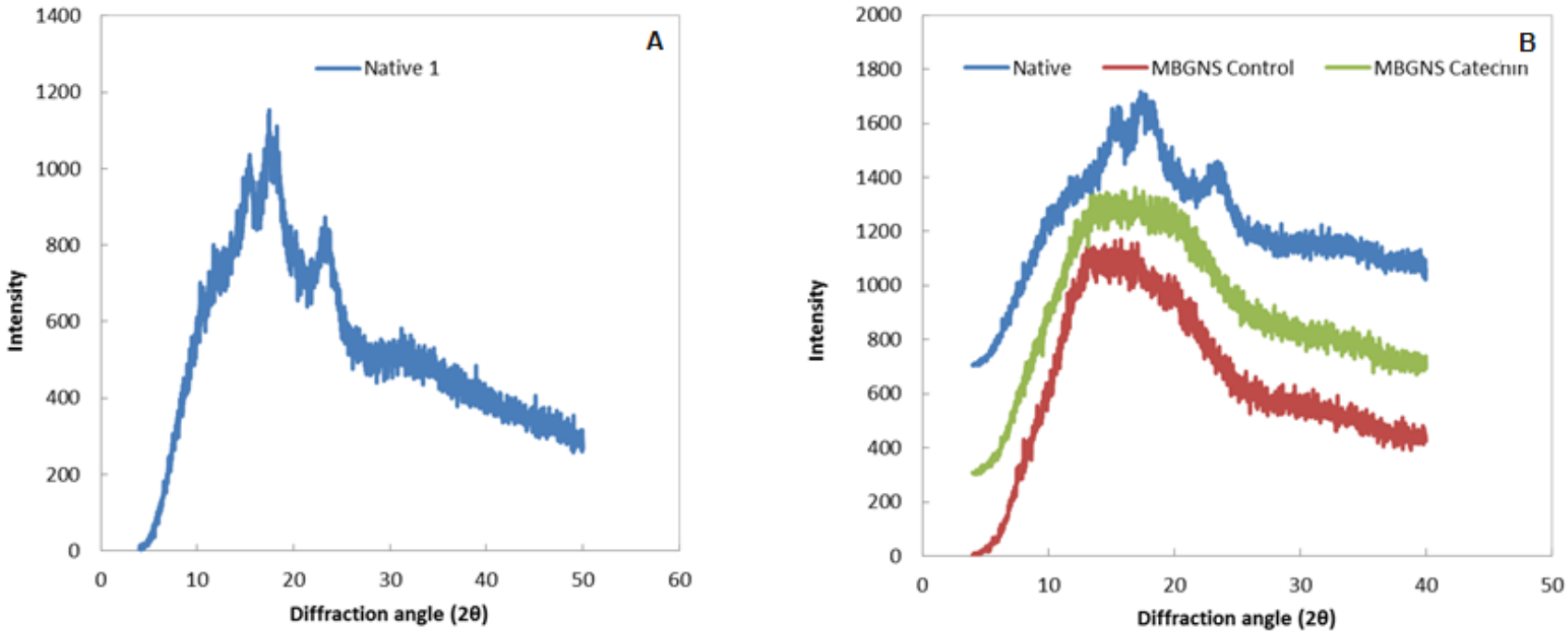

Figure 3.29 Powder X-ray diffraction patterns of (**A**) native BGN starch and (**B**) Chemically modified BGN starch. BGN: Bambara groundnut; MBGNS: Modified Bambara groundnut starch

Following chemical modification, starch crystallinity was lost as evidenced by the amorphous region (Figure 3.29b). Similar results of starch crystallinity loss following chemical modification were reported for carboxymethylated BGN starch and mung bean starch (Kittipongpatana *et al.*, 2006; Afolabi, 2012). The X-ray diffraction peaks are an indication of starch crystallinity, their disappearance thereafter will be an indication of loss or reduction in starch crystallinity within the starch molecules (Kittipongpatana *et al.*, 2006; Afolabi, 2012). Kittipongpatana *et al.* (2006) attributed the loss of starch crystallinity to the rupturing of the starch granules in the presence of heat and water thereby resulting in the broken chemical bonds within the starch molecules. The amorphicity of chemically modified starches better explains its cold water solubility in comparison to its native form. Amorphous materials have been reported to have high water absorption capacities. This is in agreement with the water absorption capacity of chemically modified BGN starches (2.98 g/g) obtained in Chapter 4 section 4.7.1.

There was an increase in the starch crystallinity in all complexed BGN starches (Figure 3.30 to Figure 3.32) irrespective of the methods (co-evaporation, microwave, kneading). This was evidenced by an increase in the X-ray diffraction peaks in comparison to those of native BGN starch. Sharper peaks were an indication of increased starch crystallinity following complexation. In addition, Sapana & Shashikant (2014) reported the appearance of new peaks, shifting of some peaks as well as the sharpening of X-ray diffraction peaks due to the formation of complexes. Beta cyclodextrin exhibited diffraction peaks at 2θ values of 4.7^{o}, 9.2^{o}, 12.9^{o}, 13.6^{o}, 15.0^{o}, 18.3^{o}, 19.0^{o}, 21.5^{o}, 22.9^{o}, 25.8^{o}, 27.2^{o} and 35.0^{o} (Figure 3.33b). On the other hand, alpha cyclodextrin exhibited diffraction peaks at 2θ values of 9.6^{o}, 11.9^{o}, 13.5^{o}, 14.2^{o}, 15.8^{o}, 18.1^{o}, 19.9^{o} and 21.6^{o} (Figure 3.33a). The X-ray diffraction patterns of complexed BGN starches had (1) sharper peaks in comparison to the native BGN starch, (2) new peaks displayed and (3) some peaks shifted from the original existing ones prior to complexation. This is a confirmation that native BGN starch was successfully complexed with cyclodextrins (alpha and beta). The highly ordered crystalline structures of the complexed BGN starches were because of the intra- and intermolecular hydrogen bonds within the starch molecules.

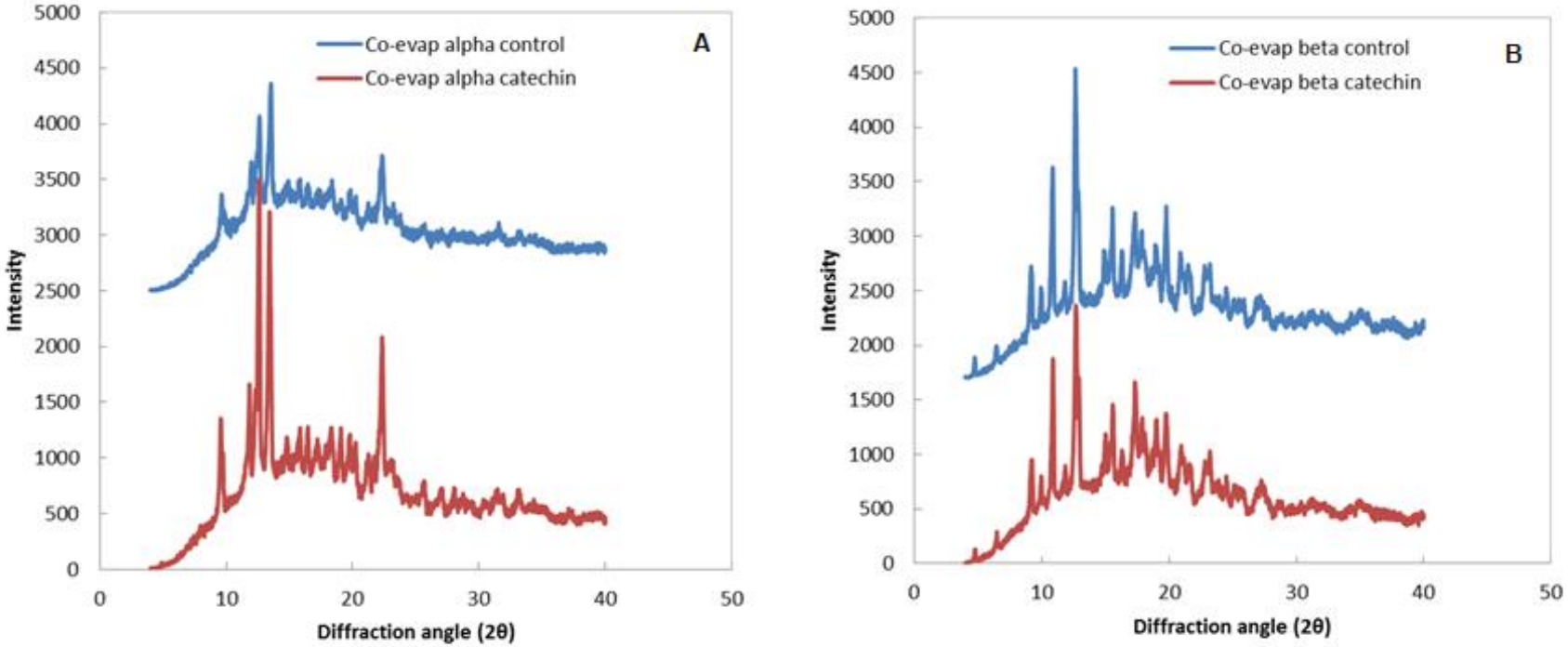

Figure 3.30 Powder X-ray diffraction patterns of modified BGN starches through co-evaporation method (**A**) alpha cyclodextrin and (**B**) beta cyclodextrin. BGN: Bambara groundnut

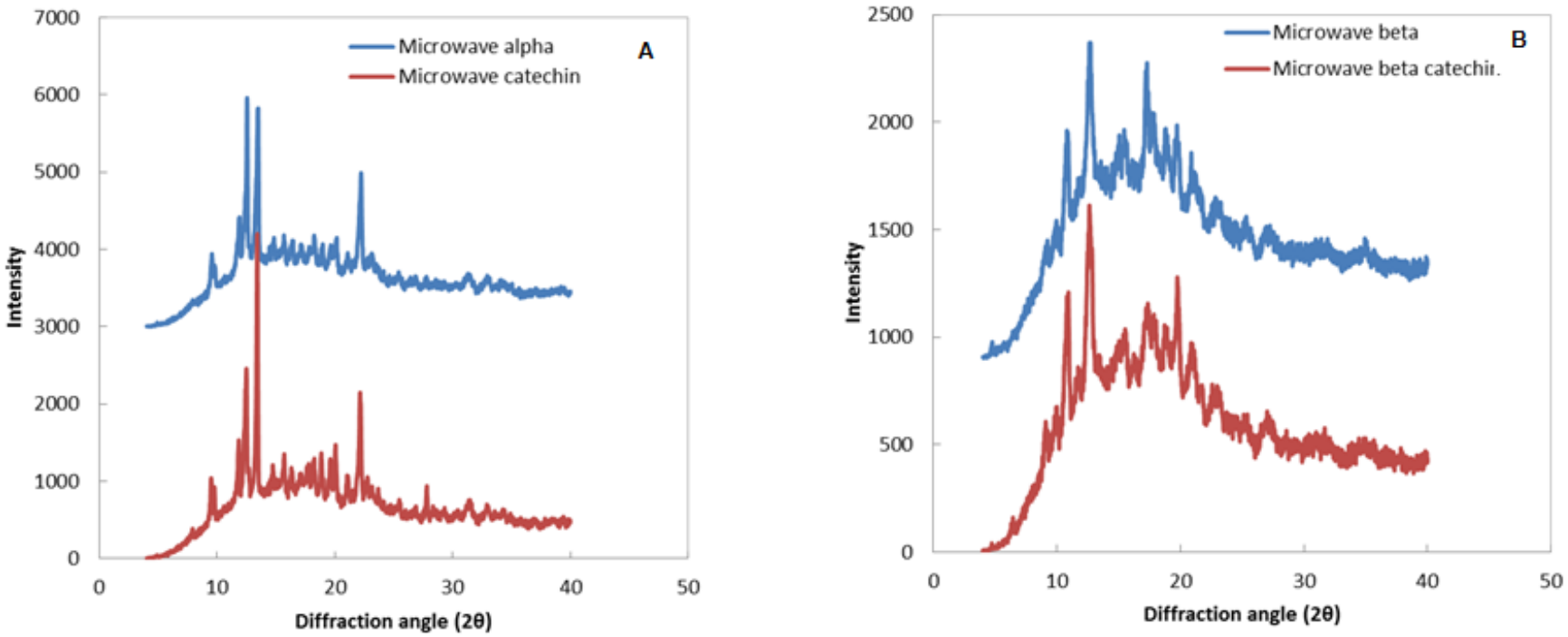

Figure 3.31 Powder X-ray diffraction patterns of modified BGN starches through microwave method **(A)** alpha cyclodextrin and (**B**) beta cyclodextrin. BGN: Bambara groundnut

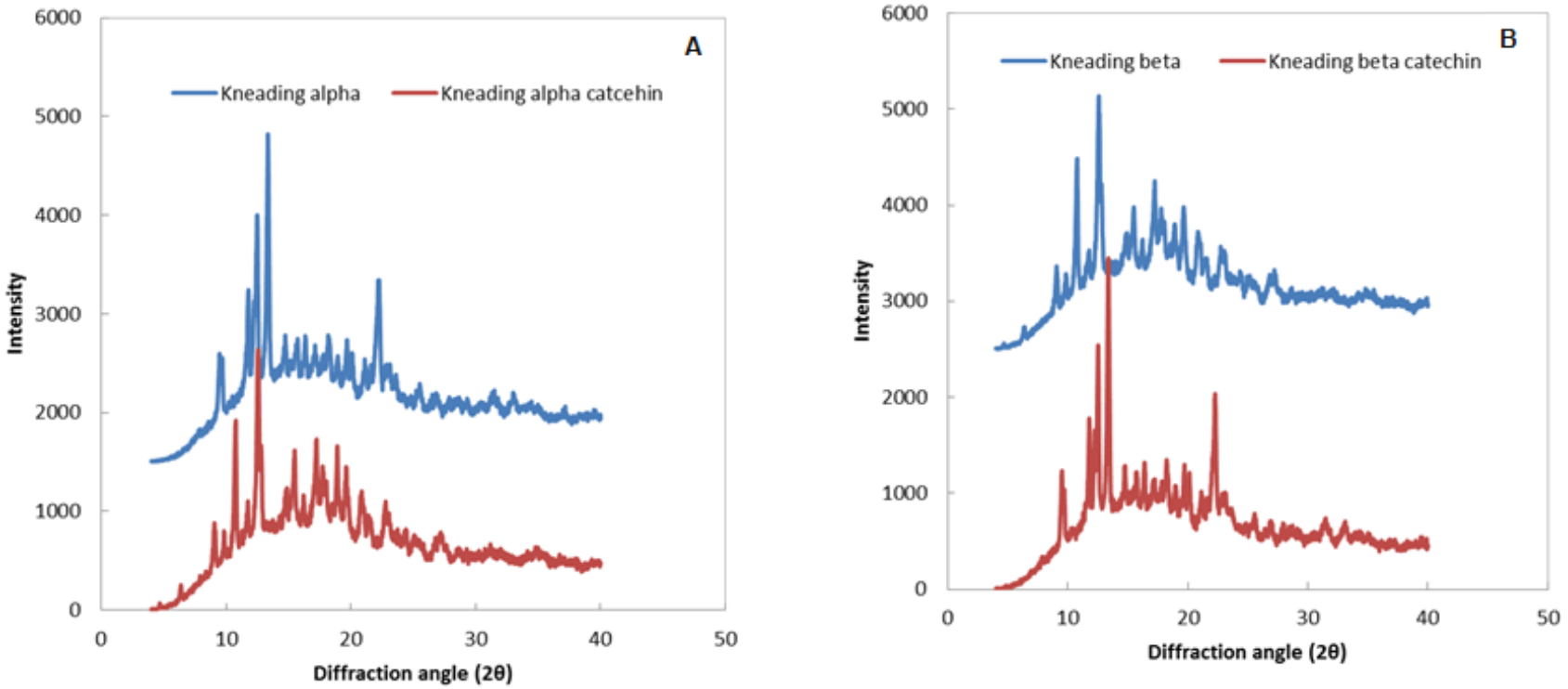

Figure 3.32 Powder X-ray diffraction patterns of modified BGN starches through kneading method **(A)** alpha cyclodextrin and (**B**) beta cyclodextrin. BGN: Bambara groundnut

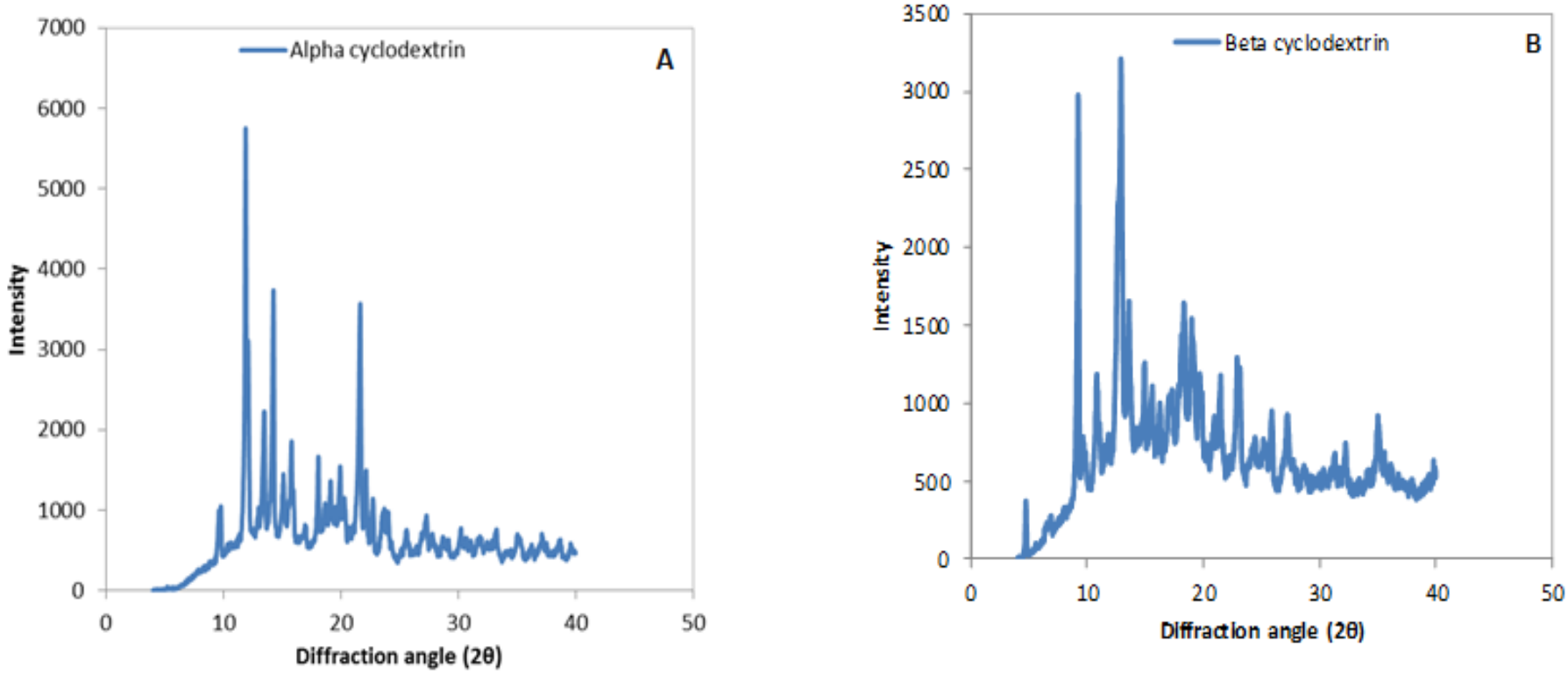

Figure 3.33 Powder X-ray diffraction patterns of pure cyclodextrins: (**A**) alpha cyclodextrin and (**B**) beta cyclodextrin

3.7 Antioxidant activity of modified BGN starches

3.7.1 Scavenging activity on DPPH radicals

The antioxidant scavenging activity on DPPH radicals ranging from 2.26 to 38.31 µmol TE/g is presented in Figure 3.34. BGN starch-catechin complex modified through kneading (beta cyclodextrin + catechin) had the highest antioxidant activity of 38.31 µmol TE/g and BGN starch modified through kneading (alpha cyclodextrin) had the lowest antioxidant activity of 2.26 µmol TE/g. The antioxidant activity of starches with catechin was significantly ($p \leq 0.05$) higher in comparison to the ones without catechin. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical which gets reduced to diphenyl-picryl hydrazine with a colour change from purple to yellow (Curcio *et al.*, 2009; Scherer & Godoy, 2009; Uyanzindile, 2014). The reduction is caused by the hydrogen donating ability of catechin. Curcio *et al.* (2009) reported that catechin's hydrogen donating ability is the main antioxidant mechanism. Modified BGN starch-catechin complexes had higher antioxidant properties in comparison to the chemically modified BGN starch-catechin complex. This may be attributed to the intermolecular hydrogen bonding that occurs between cyclodextrin (alpha and beta) and catechin which then increased the antioxidant capacity of catechin in inclusion complexes. The intermolecular hydrogen bonding is brought about by the interaction of cyclodextrin's (alpha and beta) hydroxyl group with catechin. This result concurs with reports made by Uyanzindile (2014) where gamma cyclodextrin inclusion complexes with catechins increased the antioxidant capacity. Furthermore, the cyclodextrin-catechin complexes enhanced the ability of catechin to reduce DPPH radical and thus the higher antioxidant capacity in complexed BGN starch-catechin.

DPPH assay mechanism is based on the electron transfer reaction whereby, DPPH a free radical reacts when acted upon by an antioxidant. The antioxidant will reduce DPPH which is observable by a colour change; the antioxidant achieves this by donating an electron to the unpaired DPPH electron thereby resulting in a colour change (Zulueta *et al.*, 2009; Uyanzindile, 2014). The antioxidant capacity is then correlated with the degree of colour change.

3.7.2 Total antioxidant activity using ORAC assay

The total antioxidant activity of modified BGN starches is presented in Figure 3.35, and they ranged from 0.07 to 126.71 µmol TE/g. Chemically modified BGN starch-catechin complex had the highest antioxidant activity of 126.71 µmol TE/g while BGN starch modified through kneading (alpha cyclodextrin) had the lowest antioxidant activity of 0.07 µmol TE/g. The total antioxidant activity of the modified BGN starch-catechin complexes was significantly ($p \leq 0.05$) higher than the ones without catechin. This gave an indication that catechin was successfully grafted onto the BGN starch molecule.

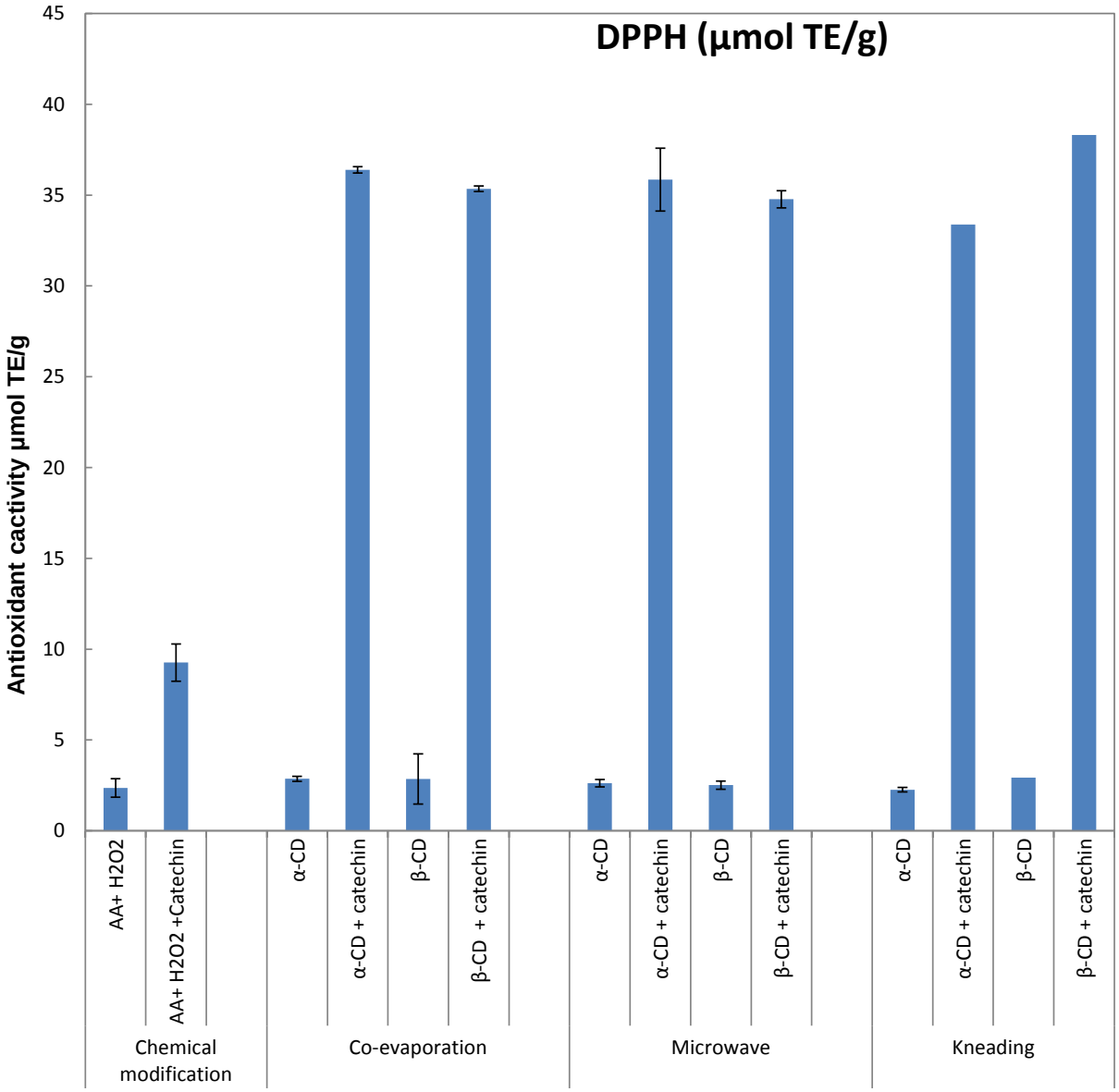

Figure 3.34 Antioxidant scavenging activity on DPPH radicals

Chemically modified BGN starch-catechin complex had the highest antioxidant activity in comparison to the complexed BGN starch-catechin complexes. This may be attributed to the reaction mechanism of ORAC assay. ORAC assay reaction based on the hydrogen atom transfer mechanism where the oxygen radical will abstract a hydrogen atom from the antioxidant leading to a stable antioxidant radical being formed (Ou *et al.*, 2002).

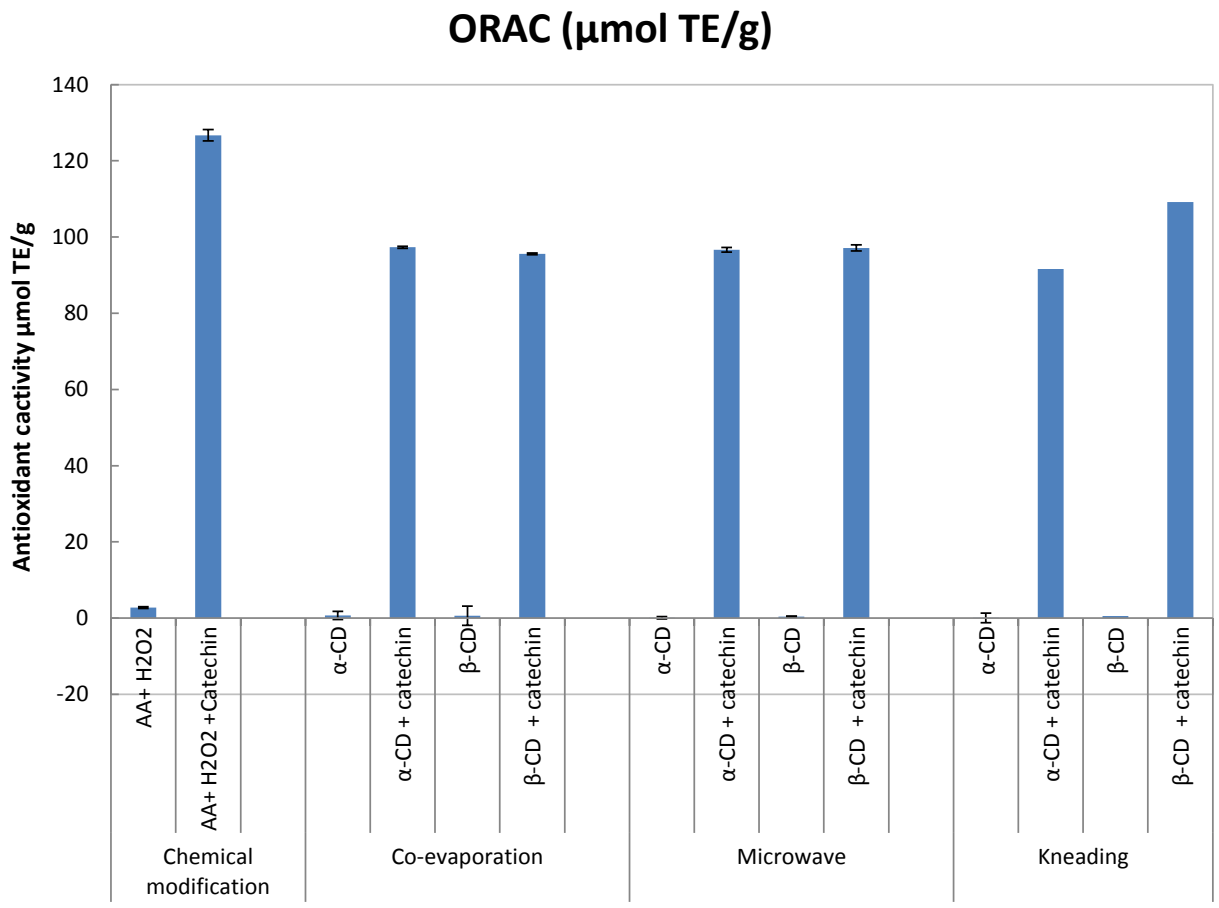

Figure 3.35 Total antioxidant activity of modified BGN starches

The starch chemical modification method using ascorbic acid and hydrogen peroxide as a redox pair initiator exposed the reactive starch functional groups consequently allowing the covalent binding of catechin. The interaction mechanism of redox agents involves the oxidation of ascorbic acid by hydrogen peroxide at room temperature, which leads to the formation of hydroxyl and ascorbate radical intermediates that initiate the reaction (Spizzirri *et al.*, 2010). Hydrogen peroxide is a free radical may have interfered with the hydrogen atom transfer mechanism of ORAC assay thereby influencing the antioxidant mechanism leading to the higher antioxidant capacity of chemically modified BGN starch-catechin complex. Furthermore, ORAC assay activity has been reported to be based on the oxidation of the fluorescence probe (which is vulnerable to free radicals) by peroxyl radicals (Cell Biolabs, 2014). The free radical initiator produces the peroxyl radicals which in turn act on the fluorescence probe over time. The antioxidant (catechin) will therefore inhibit/block the peroxyl radical oxidation of the fluorescent probe till the antioxidant activity is complete.

Following that, the remaining peroxyl radical will then destroy the fluorescent probe until the assay is completed. Consequently, this assay measured both the antioxidant inhibition as well as the percentage inhibition of free radical damage thus giving the total antioxidant capacity of a compound. Therefore explains the higher antioxidant capacity values of modified BGN starch-catechin complexes obtained using the ORAC assay in comparison to the DPPH assay.

3.7.3 Effect of the modification method on antioxidant activity

The effect of modification methods on the antioxidant activity of BGN starches is presented in Table 3.2. Within the DPPH method, the chemical modification method significantly ($p \leq 0.05$) differed from the complexation methods. The complexation methods (co-evaporation, microwave and kneading) did not significantly ($p > 0.05$) differ from each other. The antioxidant activity ranged from 5.81 to 19.36 µmol TE/g. The co-evaporation method had the highest antioxidant activity of 19.36 µmol TE/g while the chemical modification method had the lowest antioxidant activity of 5.81 µmolTE/g. This result concurs with the fluorescence emission spectra of starch-catechin complexes modified through co-evaporation, which had the highest intensity of catechin (Figure 3.18).

Within the ORAC assay method, a similar trend was observed where chemical modification significantly ($p \leq 0.05$) differed from the complexation methods. However, the complexation methods did not significantly ($p > 0.05$) differ from each other. The total antioxidant activity ranged from 48.56 to 64.74 µmol TE/g. Chemical modification had the highest total antioxidant activity (64.74 µmol TE/g) while the co-evaporation method had the lowest total antioxidant activity (48.56 µmol TE/g). ORAC assay uses the hydrogen atom transfer mechanism whereby the hydrogen donating ability of an antioxidant is measured; while the DPPH assay uses the electron transfer mechanism. The difference between the ORAC and DPPH assays, therefore explains the variation in the antioxidant activity of modified BGN starch-catechin complexes.

Table 3.2 Effect of starch modification method on antioxidant activity

Modification method	DPPH (µmol TE/g)	ORAC (µmol TE/g)
Chemical modification	5.81 ± 3.85^{a}	64.74 ± 67.90^{a}
Co-evaporation	19.36 ± 17.26^{b}	48.56 ± 50.05^{b}
Microwave	18.94 ± 17.12^{b}	48.57 ± 50.53^{b}
Kneading	19.22 ± 17.47^{b}	50.34 ± 52.67^{b}

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; ORAC: Oxygen Radical Absorbance Capacity

3.8 Proposed grafting reaction mechanism

Ascorbic acid and hydrogen peroxide were used as redox bio-compatible initiators for the chemical modification of BGN starch. The reaction mechanism (as shown in Figure 3.36) of the two redox initiators involved ascorbic acid oxidation by hydrogen peroxide at room temperature leading to the formation of ascorbate and hydroxyl radical intermediates that initiate the reaction (Spizzirri *et al.*, 2010). This occurred in two steps; firstly there was activation of the polysaccharide chain towards radical reaction followed by the covalent binding of the antioxidant (catechin) molecule to the preformed macro ascorbate and hydroxyl radicals. It is speculated that the proposed BGN starch-catechin reaction mechanism followed the same path as the one proposed by Spizzirri *et al.* (2010). The hydroxyl radicals generated by the initiator system will abstract the H-atoms from the hydroxyl groups of the polysaccharides. This then leads to the formation of the free radical sites and the insertion of the new functional group (catechin). Spizzirri *et al.* (2010) hypothesised that the insertion of antioxidant (catechin) on the polysaccharide chains occurred in positions 2', 5' (B ring) and 6, 8 (A ring) for catechin. Figure 3.37 presents the proposed reaction mechanism and insertion of catechin to alginate and inulin as reported by Spizzirri *et al.* (2010). Since alginate and inulin are both polysaccharide like starch it is therefore speculated that the grafting of catechin to BGN starch will follow the same pathway as shown in Figure 3.37.

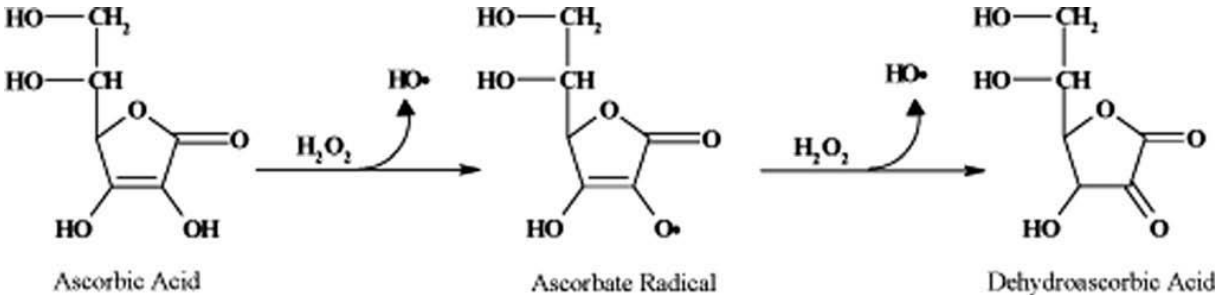

Figure 3.36 Interaction mechanism between ascorbic acid and hydrogen peroxide (Spizzirri et al., 2010)

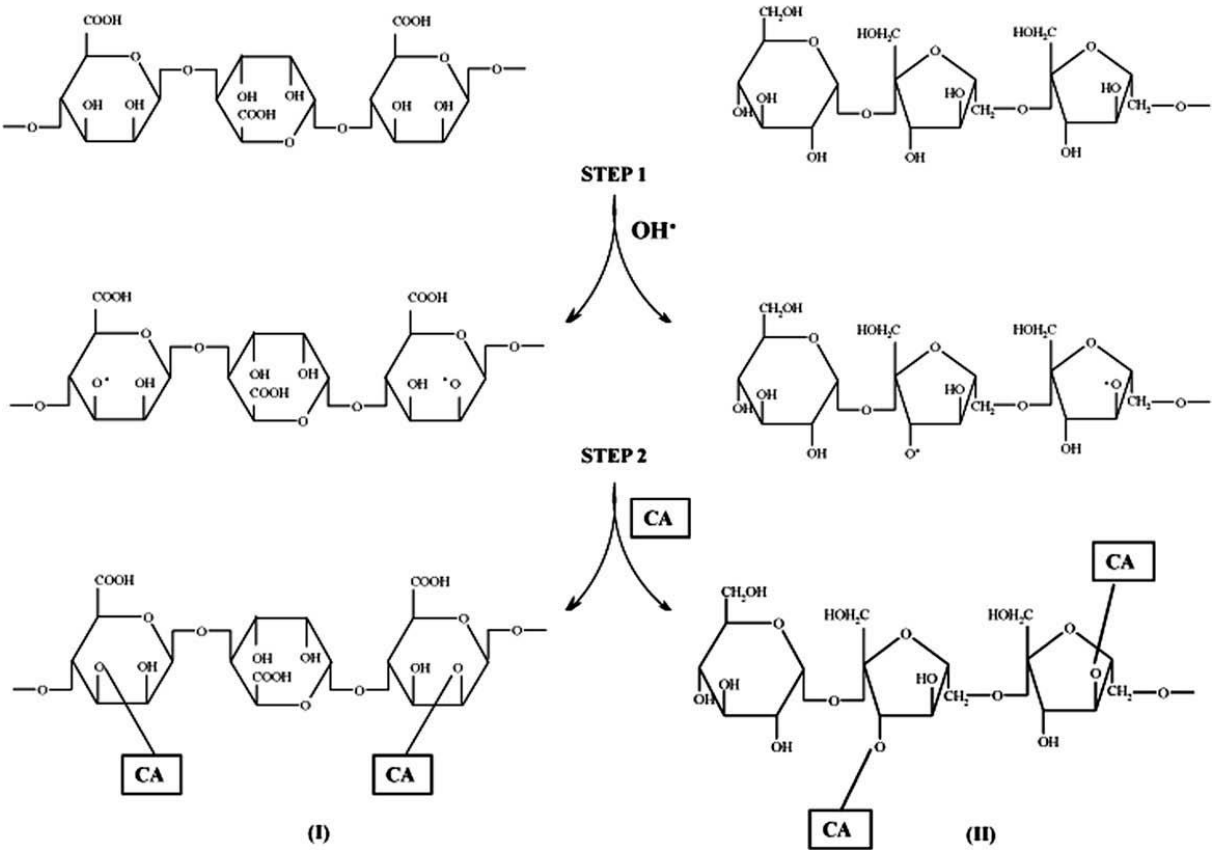

Figure 3.37 Insertion of catechin to polysaccharide chains (Spizzirri et al., 2010)

3.9 Conclusion

Bambara groundnut starch yield was 32% and this suggests that the legume has potential to be used as a source of starch. Catechin was successfully grafted onto the BGN starch molecule using chemical and complexing methods. Furthermore, the successful grafting of catechin onto the BGN starch suggests that BGN starch molecule is a potential antioxidant carrier, for producing starch possessing antioxidant properties. It is hoped that the produced BGN starch-catechin complex will reduce starch digestibility with the view to combat against chronic diseases like diabetes, obesity and cardiovascular diseases.

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CHAPTER FOUR

FUNCTIONAL AND RHEOLOGICAL CHARACTERISATION OF BAMBARA GROUNDNUT STARCH-CATECHIN COMPLEX FORMED USING CHEMICALS AND CYCLODEXTRINS AS INITIATORS

Abstract

The functional, thermal and rheological properties of native and modified BGN starches were assessed. The swelling capacity of BGN starches increased with an increase in temperature. The swelling capacities of BGN starches ranged from 0.36 g/g (BGN starch modified through the co-evaporation method, beta cyclodextrin + catechin) to 5.64 g/g (chemically modified BGN starch). Chemical modification significantly increased the swelling capacity of native BGN. Complexation methods significantly reduced the swelling capacity of native BGN starch. Temperatures had a significant ($p \leq 0.05$) effect on the swelling capacity of BGN starches. The solubility of BGN starches ranged from 0.61 g/100g (native BGN starch) to 46.04 g/100g (BGN starch modified through kneading method, alpha cyclodextrin). The water absorption of native and modified BGN starches ranged from 0.23 to 3.10 g/g. Water absorption capacity of chemically modified BGN starches was significantly higher than the water absorption capacity of native BGN starch (1.17 g/g). Complexation methods significantly ($p \leq 0.05$) reduced the water absorption of native BGN starch. Starches complexed with β-cyclodextrin showed increased water absorption capacity in comparison to the starches complexed with alpha cyclodextrin. The modification methods had a significant ($p \leq 0.05$) effect on the water absorption capacities of BGN starches. There was no significant difference ($p > 0.05$) in the oil absorption capacities of BGN starches and they ranged from 1.02 to 1.07 g/g. A rapid visco analyser (RVA) was used to assess the pasting properties of BGN starches. Starch modification lowered breakdown viscosity, setback viscosity and final viscosity. Starch modification methods significantly ($p \leq 0.05$) increased the gelatinisation temperatures of native BGN starch. Microwave and chemically modified starches displayed a shear thinning behaviour while those modified through co-evaporation and kneading methods displayed a shear thickening behaviour.

4.1 Introduction

Starch is an important functional food biopolymer which contributes to the characteristic properties of food products (Singh & Kaur, 2004). Over the years starches have been used either in their natural or modified form as processing components in the food industries. Functional properties of starch and their uniqueness in different food products differ depending on the botanical source (Ancona *et al.*, 2001; Singh *et al.*, 2007). The fundamental functional properties of native starches are their great thickening and gelling properties, thus making them an excellent ingredient for the manufacture of foods such as custards, sausages and cookies (Ancona *et al.*, 2001). Depending on the desired properties of the food product in production, various food products make distinctive demands on the starches to use in their formulations (Adebowale & Lawal, 2002). Functional properties affect the sensory characteristics of foods. Furthermore, they play an important role in the physical behaviour of food or its ingredients during preparation, processing and storage (Adebowale & Lawal, 2004). These properties include foaming, emulsification, texture, gelation, water and oil absorption capacity and viscosity. A few studies have been reported on the functional and rheological properties of bambara groundnut (BGN) starch. Adebowale *et al.* (2002) reported that the swelling capacity of BGN starch increased with an increase in temperature. When starch is heated in water, its granules absorb water, swell and lose their molecular order thus resulting in a process known as gelatinisation. Gelatinisation causes a very big change in the rheological properties of starch suspensions (Eliasson & Gudmundsson, 2006).

Starch is a major dietary carbohydrate, hence it is a source of energy. However, starchy diets can result in higher glycemic response which then leads to different chronic diseases like diabetes, cardiovascular diseases and obesity. One way of overcoming this, could be the use of phenolic compounds such as catechin, to reduce starch digestibility (Guzar, 2012). There are reports from previous studies on how complexes of starch and antioxidants affected the functionality of starch. It is reported that the addition of catechins significantly affected the pasting, rheological and thermal properties of rice starch. Wu *et al.* (2015) reported that catechin significantly altered the viscoelastic properties of rice starch and influenced the structure of rice gels. Addition of catechins facilitated the hydration, delayed short and long-term retrogradation of rice starch, and weakened rice starch gels firmness and strength to different extents. Zhu (2015) reported that addition of catechin decreased hot paste viscosity (HPV) of rice starch (A-type polymorph) and increased that of potato starch (B-type polymorph). However, there is no documented study that reports the use of BGN starch to form complexes with catechin. In the previous chapter it was shown that BGN starch-catechin complex was obtained through graft polymerisation. Hence, the need to investigate the effect of initiators (hydrogen peroxide and ascorbic acid) and cyclodextrin on the functional and rheological properties of the BGN starch-catechin

complex. The objective of this study was to establish the functional, rheological and thermal properties of the new BGN starch-catechin complex.

4.2 Materials and Methods

4.2.1 Source of materials

Bambara groundnuts were purchased from Triotrade, Johannesburg, Gauteng province, South Africa. Catechin was purchased from Sigma Aldrich. All other materials and equipment were from the Department of Food Science and Technology laboratories of the Cape Peninsula University of Technology, Bellville. Chemical reagents were of analytical grade. The major equipment were; the centrifuge (high speed), Variwhirl mixer (Model A901. Salver Chem, Chicago, USA), rapid visco analyser (RVA 4500 Perten instruments, SIN 214 31208-45A, Australia), differential scanning calorimeter (Perkin Elmer) and a rheometer (Rheolab Anton Paar, CPTD 180/AIR/QC, Austria). Figure 4.1 outlines the overview of this chapter.

4.3 Evaluation of the Functional properties of BGN Starch-catechin Complex

4.3.1 Effect of temperature on solubility and swelling

The method reported by Adebowale & Lawal (2002); Adebowale *et al.* (2002); Adebowale & Lawal (2003) and Lawal *et al.* (2004) was employed to establish the effect of temperature on solubility and swelling of modified BGN starch and BGN starch-catechin complex obtained by chemical grafting. A 1 g sample was accurately weighed and quantitatively transferred into a clean dried test tube which was then re-weighed (W1). Then 50 ml of distilled water was used to dissolve the starch until slurry was obtained. The slurry was then heated at temperatures of 60^{o}C, 70^{o}C, 80^{o}C and 90^{o}C for 30 min in a temperature-regulated water bath. The mixture was then cooled to room temperature and centrifuged at 500 rpm, for 15 min. Thereafter, 5 ml of the supernatant was dried to a constant weight at 110^{o}C. The residue obtained after drying the supernatant represented the amount of starch solubilised in water. Solubility was calculated as g per 100 g of sample on dry weight basis. The residue obtained after centrifugation with the water it retained was quantitatively transferred to a clean dried test tube which was weighed and recorded as W2. Swelling capacity was calculated by dividing the difference between W2 and W1 by the original sample weight and was expressed as g/g as shown by equation 4.1.

$$\text{Swelling capacity } \left(\frac{g}{g}\right) = \frac{W2\text{-}W1}{\text{Original sample weight}} \qquad \textit{Equation 4.1}$$

Where, W1 = weight of sample plus test tube

W2 = weight of residue plus test tube.

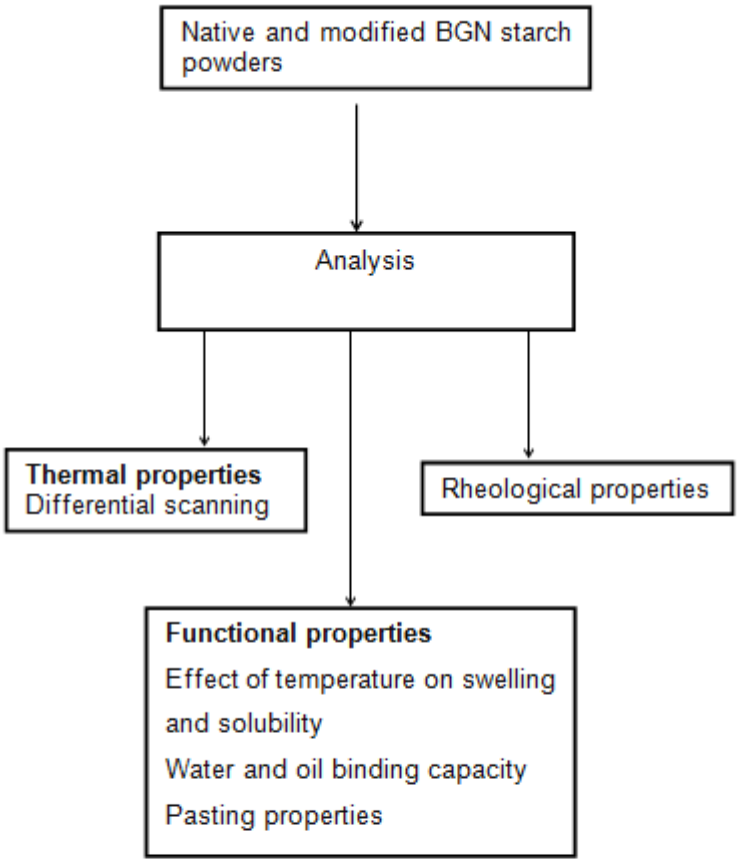

Figure 4.1 Chapter 4 outline

4.3.2 Oil and water absorption capacities

Oil and water absorption capacities were determined by the method reported by Adebowale & Lawal (2002); Adebowale & Lawal (2003); Adebowale & Lawal (2004) and Lawal *et al.* (2004). A 10 ml aliquot of distilled oil or water was added to 1 g sample (native or modified BGN starches). The mixture was then mixed thoroughly in a Variwhirl mixer (fast mixer) for 30 s. This was then allowed to stand at room temperature for 30 min, after which it was centrifuged at 5000 rpm for a min. Graduated 10 ml cylinder was used to measure the volume of the supernatant. Absorbed oil or water mass was expressed as gg^{-1} starch on a dry weight basis.

4.3.3 Pasting properties

The pasting properties of modified BGN starch and BGN starch-catechin complex were determined using a rapid visco analyser (RVA). The method adapted from Afolabi (2012); Jan *et al.* (2013) was used to determine the pasting properties. A suspension of 3 g starch in 25 ml distilled water was subjected under controlled heating and cooling cycles under constant shear. During this cycle it was held at 50^{o}C for a minute, heated from 50 to 95^{o}C at 6^{o}C/min, held at 95^{o}C for 5 minutes, cooled to 50^{o}C at 6^{o}C/min, and held at 50^{o}C for 5 minutes. The pasting temperature, peak, trough, breakdown, final and setback viscosities were obtained from the RVA curves and viscosities were expressed as centipoise units (Cp).

4.3.4 Differential scanning calorimetry (DSC) analysis

The method reported by Afolabi (2012) was used for the determination of thermal properties of native and modified BGN starches. The thermal properties of the starch were studied with the aid of a differential scanning calorimeter. About 12 µL of distilled water was added to 3 mg of starch in the DSC pans. The pans were then sealed, reweighed and allowed to stand for 3 h at room temperature before the DSC analysis. This was done to allow the sample to have an even distribution of water. The scanning temperature ranged from 30–140^{o}C, while the heating rate was 10^{o}C per min. An empty aluminium pan was used as the reference. The transition temperatures reported were the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy of gelatinisation (ΔH) was estimated by integrating the area between the thermogram and a base-line under the peak and was expressed in terms of Joules per gram of dry starch.

4.4 Assessment of rheological properties of BGN starches

The method adapted from Kim & Yoo (2006) was used to evaluate the rheological properties of modified BGN starch and BGN starch-catechin complex. Small amplitude oscillatory rheological measurements were made from BGN starches with a dynamic rheometer

equipped with a parallel plate system (4 cm diameter). Each BGN starch sample was transferred to the rheometer plate. Steady shear (shear stress and shear rate) data were obtained over a shear rate range of 1.0 to 1000 s^{-1} at 25^{o}C. The data was fitted to the power law model (Izidoro *et al.*, 2009), which is extensively used to describe the flow properties of non-Newtonian fluids. This was done so as to describe the variation in the rheological properties of BGN starch samples under steady shear.

4.5 Statistical Analysis

All results were reported as means ± standard deviation of three independent trials. Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan's multiple range tests was used to separate means where significant difference existed (IBM SPSS version 22, 2013). Principal Component Analysis (PCA) was used to extract the components that explained the variability in the functional properties of native and modified BGN starches (Unscrambler ver 10.4).

4.6 Results and Discussion

4.6.1 Effect of temperature on native and modified BGN starch swelling capacity

The effect of temperature on swelling power of Bambara groundnut (BGN) starches (native and modified) indicates the different molecular organisation within starch granules. The swelling of starches was found to be a function of temperature (Figure 4.2). The swelling power of native and modified BGN starches increased with an increase in temperature. A similar trend of increased swelling power with increase in temperature was reported for BGN, taro, acha and rice starches (Adebowale & Lawal, 2002; Adebowale *et al.*, 2002; Alam & Hasnain, 2009; Olu-Owolabi *et al.*, 2014; Bhat & Riar, 2016). This trend observed in the swelling of BGN starches (native and modified) concurs with the reports of Adebowale *et al.* (2002); Adebowale & Lawal (2003) that the swelling power of legume starches increases with an increase in temperature. The swelling power of BGN starches ranged from 0.36 – 5.64 g/g. At 60^{o}C, all starches showed slight swelling with the chemically modified starches having the highest swelling power. Increased swelling power was highest at 80^{o}C and 90^{o}C because starch granules gradually swell as the temperature increases. This could be attributed to the rupturing of the intermolecular hydrogen bonds of the amorphous regions in the starch granule thereby resulting in irreversible and continuous absorption of water.

Chemical modification significantly increased the swelling power of the native BGN starch by 7 fold from 0.55 to 4.57 g/g and this could be attributed to the loss of starch crystallinity due to the incorporation of the functional groups onto the starch molecule and the rapid increase in the amorphous region [confirmed by the starch powder X-ray diffraction pattern, Chapter 3, section 3.6.5] (Adebowale & Lawal, 2003; Afolabi, 2012).

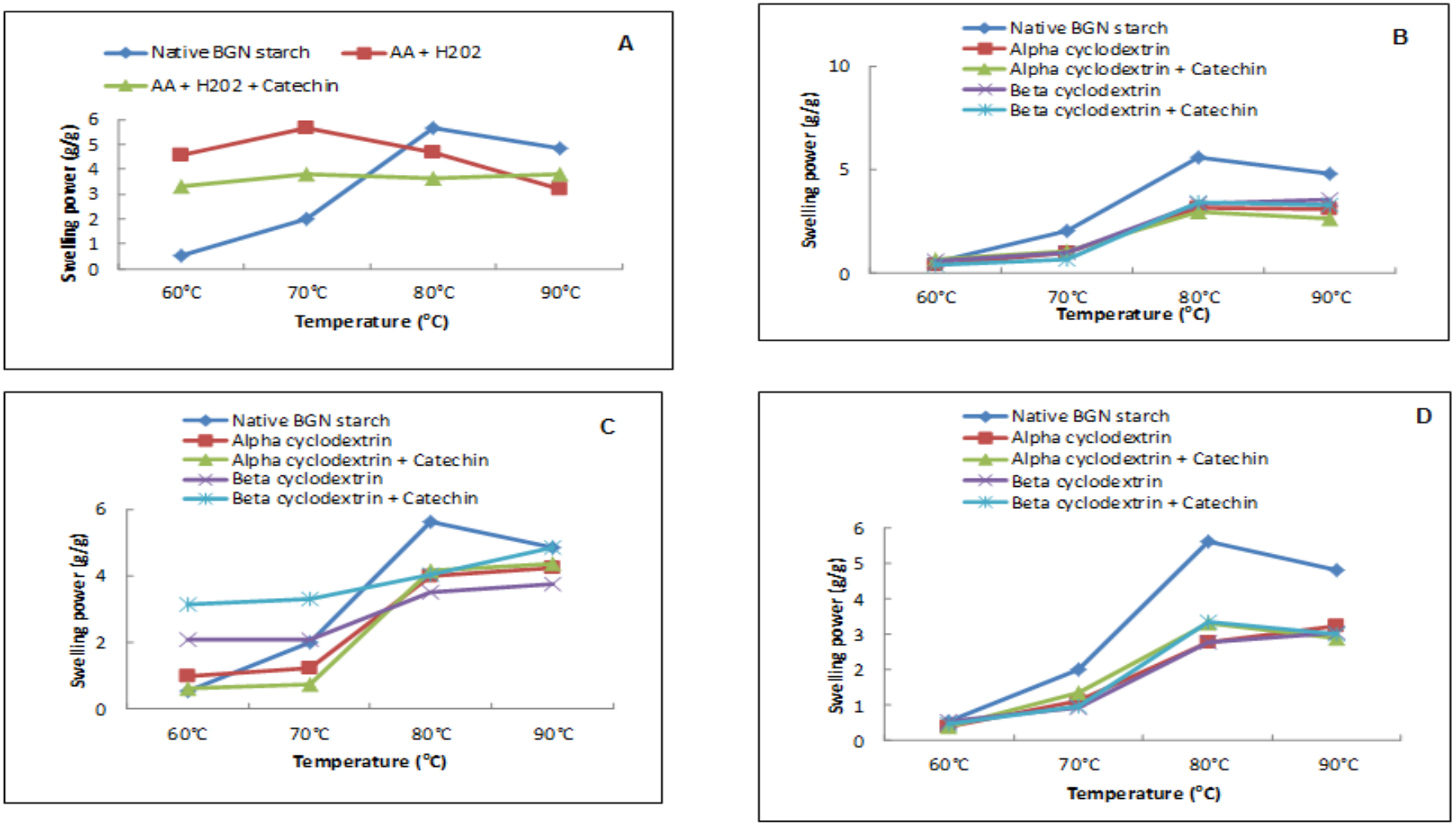

Figure 4.2 Effect of temperature on swelling of native and modified Bambara groundnut starches. **A**: Chemical modification, **B**: Co-evaporation, **C**: Microwave, **D**: Kneading. AA: Ascorbic acid, H2O2: Hydrogen peroxide

Furthermore, it has been reported that amorphous materials have a high capacity to absorb water thus explaining the increase in swelling power of the chemically modified starches. On the other hand, the complexation methods (co-evaporation, microwave and kneading) reduced the swelling power of native BGN starch. This reduction may be due to the increased crystallinity of those starches as crystalline granules limit starch swelling capacity (Adebowale & Lawal, 2002). This increased starch crystallinity is confirmed by the sharper X-ray diffraction peaks of these starches in comparison to those of native BGN starch as discussed in Chapter 3, section 3.6.5.

In comparison to BGN native starch, all modification methods had a significant effect ($p \leq 0.05$) on the starch swelling capacity. However, the kneading and co-evaporation methods did not give significantly different results ($p > 0.05$). All initiators had a significant impact on the swelling capacity of BGN starches (native and modified). Furthermore, the initiators significantly differed ($p \leq 0.05$) from each other with the exception of alpha cyclodextrin which did not differ significantly ($p > 0.05$) from beta cyclodextrin and alpha cyclodextrin + catechin. Temperatures had a significant effect ($p \leq 0.05$) on the starch swelling capacity and they significantly differed from each other. However, high temperatures ($80^{o}C$ and $90^{o}C$) did not significantly differ in the swelling power of native and modified BGN starches. This may be due to gelatinisation when the starch granules are heated and they absorb water and gradually swell; however at temperatures higher than gelatinisation temperature the starch granules do not continue to swell because the starch granule structure will be essentially lost.

4.6.2 Effect of temperature on native and modified BGN starch solubility

Starch solubility was a function of temperature (Figure 4.3) increasing as the temperature increased for chemically modified BGN starches. However, native BGN starch and the ones modified through complexation methods (co-evaporation, microwave and kneading) indicated solubility to be inversely proportional to temperature. For these starches, solubility was maximal at lower temperature ($60^{o}C$) and it decreased with an increase in temperature. The solubility of BGN starches ranged from 0.61 – 46.04 g/100 g. Native BGN starch had the least solubility (0.61 g/100 g) and BGN starch modified through the kneading method with alpha cyclodextrin had the maximum solubility (46.04 g/100 g). All modification methods significantly increased starch solubility at all studied temperatures relative to the native BGN starch. Increasing temperature resulted in the weakening of the starch intramolecular binding forces thereby enhancing the leaching of starch granular particles which led to increased solubility (Adebowale & Lawal, 2002; Lawal *et al.*, 2004). Within the complexation methods, starches complexed with alpha cyclodextrin had higher solubility when compared to those complexed with beta cyclodextrin. This is because beta cyclodextrin is less soluble than alpha cyclodextrin

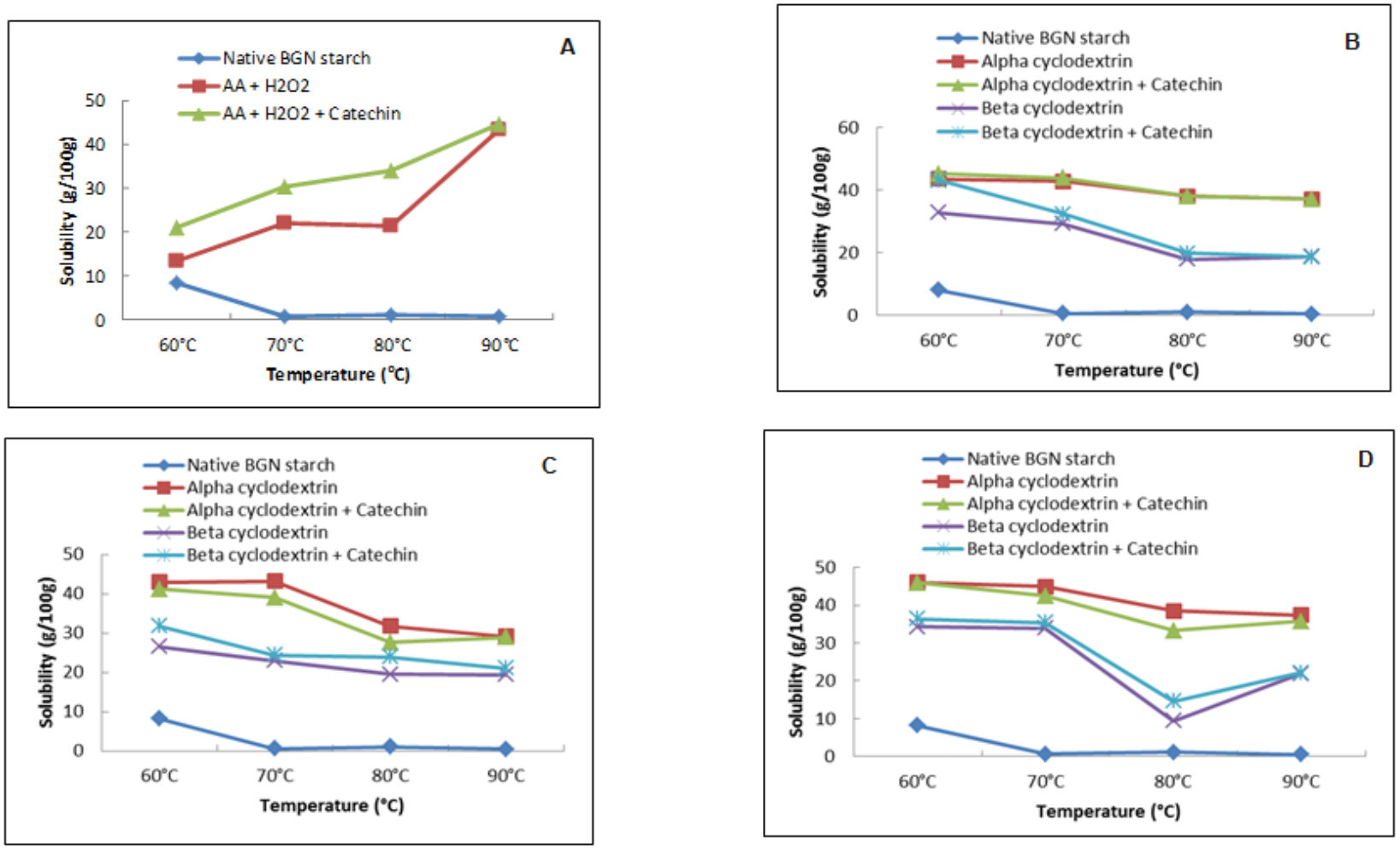

Figure 4.3 Effect of temperature on solubility of native and modified Bambara groundnut starches. **A**: Chemical modification, **B**: Co-evaporation, **C**: Microwave, **D**: Kneading. AA: Ascorbic acid, H2O2: Hydrogen peroxide

Del Valle (2004) reported beta cyclodextrin to be at least 9 times less soluble than other cyclodextrins. The lower solubility of beta cyclodextrin in water is driven by the less favourable entropy.

The different modification methods had a significant impact on the starch solubility. In comparison to BGN native starch, all modification methods had a significant effect ($p \leq 0.05$) on the starch solubility. However, kneading and co-evaporation methods did not differ significantly ($p > 0.05$) from each other as well as chemical modification and microwave method. The initiators all had a significant impact on the starch solubility. Furthermore, the initiators significantly differed ($p \leq 0.05$) from each other with the exception of alpha cyclodextrin which did not differ significantly ($p > 0.05$) from alpha cyclodextrin + catechin. Ascorbic acid + hydrogen peroxide did not differ significantly ($p > 0.05$) from beta cyclodextrin and beta cyclodextrin + catechin. At higher temperatures there was enhanced starch mobility which results in increased dispersion of starch granules thus leading to increased starch solubility. Starches modified through chemical modification and the microwave methods were more soluble in water when compared to the ones modified through co-evaporation and kneading methods. This is because co-evaporated and kneaded methods did not involve heat hence the starch granules were not completely gelatinised thus restricting their solubility in cold water.

4.7 Water and oil absorption capacities of native and modified BGN starches

Water absorption capacity is a function of water holding ability of the starch sample. Water absorption properties of native and modified BGN starch ranged from 0.23 – 3.10 g/g (Table 4.1). BGN starch-catechin complex modified through chemical modification absorbed more water (3.10 g/g) while the one modified through co-evaporation (alpha cyclodextrin + catechin) absorbed the least water (0.23 g/g). The chemical modification method significantly ($p < 0.05$) increased the water absorption capacity of native starch. This was because chemical modification incorporates the hydroxyl groups onto the starch molecules thereby resulting in starch with enhanced binding capacities more than the native starch. Native BGN starch absorbed 1.17 g/g water and this was lower than that reported by Adebowale *et al.* (2002) of 2.0 g/g. This could be attributed to the differences in the way BGN seeds were prepared before starch extraction. Unlike Adebowale *et al.* (2002) a dry milling method was used in this research project with flour hydration compared to the wet milling method reported by the authors. Among the chemical modifications treatments, BGN starch-catechin complex absorbed more water (3.10 g/g) than the one modified without catechin (2.86 g/g) though they do not differ significantly. However, the complexation methods significantly ($p \leq 0.05$) reduced the water absorption capacity of the native BGN starch.

Among the complexation methods, BGN starch complexed with beta cyclodextrin absorbed more water when compared to the ones complexed with alpha cyclodextrin. This may probably be due to the higher hydrophilicity of beta cyclodextrin in comparison to alpha cyclodextrin.

Table 4.1 Water and oil absorption capacities of native and modified Bambara groundnut starch[1, 2]

Modification method	Initiators	Water absorbed (g/g)	Oil absorbed (g/g)
Native BGN starch	Native starch	1.17 ± 0.71[a]	1.06 ± 0.03[a]
Chemical modification	AA + H_2O_2	2.86 ± 0.01[a]	1.03 ± 0.01[a]
	AA + H_2O_2 +catechin	3.10 ± 0.23[a]	1.05 ± 0.02[a]
Microwave	Alpha cyclodextrin	0.58 ± 0.05[a]	1.09 ± 0.01[a]
	Alpha cyclodextrin + catechin	0.44 ± 0.04[b]	1.04 ± 0.03[a]
	Beta cyclodextrin	0.91 ± 0.02[c]	1.07 ± 0.02[a]
	Beta cyclodextrin + catechin	1.02 ± 0.04[d]	1.07 ± 0.03[a]
Co-evaporation	Alpha cyclodextrin	0.24 ± 0.03[a]	1.07 ± 0.03[a]
	Alpha cyclodextrin + catechin	0.23 ± 0.04[a]	1.05 ± 0.04[a]
	Beta cyclodextrin	0.62 ± 0.18[b]	1.03 ± 0.02[a]
	Beta cyclodextrin + catechin	0.76 ± 0.04[b]	1.02 ± 0.02[a]
Kneading	Alpha cyclodextrin	0.44 ± 0.07[a]	1.03 ± 0.02[a]
	Alpha cyclodextrin + catechin	0.46 ± 0.11[a]	1.05 ± 0.03[a]
	Beta cyclodextrin	0.73 ± 0.03[b]	1.05 ± 0.02[a]
	Beta cyclodextrin + catechin	0.69 ± 0.01[b]	1.06 ± 0.04[a]

[1]AA: Ascorbic acid; H_2O_2: Hydrogen peroxide
[2]Mean values of triplicate determinations ± standard deviation. Means within a column (under the same row heading) followed by the same superscript are not significantly ($p > 0.05$) different.

Furthermore, most BGN starches complexed with cyclodextrin + catechin absorbed more water than those without catechin. This may be because catechin hydroxyl groups increased the complexes' hydrophilicity property hence an increase in the water absorption capacity. The initiators within each method were significantly ($p \leq 0.05$) different. In the microwave method, all initiators significantly ($p \leq 0.05$) differed from each other. BGN starches modified through beta cyclodextrin complexation absorbed more water. In addition, beta cyclodextrin + catechin complex had the maximal water absorption (1.02 g/g) while alpha cyclodextrin + catechin complex absorbed the least (0.44 g/g).

Among the co-evaporation method, beta cyclodextrin + catechin complex had the highest water absorption (0.76 g/g) while alpha cyclodextrin + catechin had the lowest (0.23 g/g). The initiators used significantly differed from each other. However, alpha cyclodextrin initiators did not differ significantly ($p > 0.05$) from each other; and this trend applies to the beta cyclodextrin initiators.

Among the kneading method, beta cyclodextrin complex had the highest water absorption (0.73 g/g) while alpha cyclodextrin had the lowest (0.44 g/g). The initiators significantly differed from each other. However, alpha cyclodextrin initiators did not differ significantly ($p > 0.05$) from each other; and this trend applies to the beta cyclodextrin initiators.

Oil absorption capacity works on the principle of physically entrapping oil through capillary attraction. Oil absorption capacity plays an important role in food products by retaining flavour, improving palatability, as well as extending the shelf life of the food product especially in meat and bakery products. The oil absorption properties of BGN starches (native and modified) ranged from 1.02 – 1.07 g/g. BGN starch complexes (beta cyclodextrin and beta cyclodextrin + catechin) in the microwave method and BGN starch complex (alpha cyclodextrin) in the co-evaporation methods had the maximal oil absorption capacity of 1.07 g/g. On the other hand, BGN starch complex (beta cyclodextrin + catechin) in the co-evaporation method absorbed the least oil at 1.02 g/g. Native BGN starch absorbed 1.06 g/g oil and this is lower than 1.76 g/g reported by Adebowale *et al.* (2002). However, it was higher than 1.01 g/g reported by Sirivongpaisal (2008). These variations could be attributed to the differences in the way BGN seeds were prepared prior to starch extraction as well as the differences in the methods used to extract the starch from BGN flour. For this research study, dry milling of whole seeds was used while Adebowale *et al.* (2002) and Sirivongpaisal (2008) employed the wet milling method and they used dehulled seeds. There was no significant difference ($p > 0.05$) in the oil absorbed in all BGN starches (native and modified) irrespective of the modification used. The oil absorption capacities for the chemical modification method were lower than the water absorption capacities. This therefore, gives an indication that the chemically modified starches had a higher level of hydrophilicity compared to hydrophobicity. The oil absorption capacities within the complexation methods

(microwave, co-evaporation and kneading) were higher than water absorption capacities. This may be due to the higher level of hydrophobicity compared to hydrophilicity in the complexed starches. The oil absorption trend for native BGN starch concurs with the observations reported by Adebowale *et al.* (2002) and Sirivongpaisal (2008) for native BGN starches.

4.7.1 Effect of modification method on the functional properties of native and modified BGN starches

Modification methods had a significant ($p \leq 0.05$) effect on the water absorption capacities of BGN starch, differing ($p < 0.05$) from each other except for the kneading method which did not differ from the microwave or co-evaporation method (Figure 4.4). Chemical modification significantly ($p \leq 0.05$) increased the water absorption of the native starch. Hydrogen peroxide and ascorbic acid were the two initiators used to chemically modify BGN starch by allowing the incorporation of new functional groups (hydroxyl groups) into the starch molecule. The interaction mechanism of redox agents involves the oxidation of ascorbic acid by hydrogen peroxide at room temperature, which leads to the formation of hydroxyl radical and ascorbate radical intermediates that initiate the reaction (Spizzirri *et al.*, 2010). It is therefore speculated that it is the formed hydroxyl groups that increased the water absorption capacity of BGN starch. Furthermore, an increase in water absorption within the chemical modification might be due to the weakened intermolecular bonds within the starch granules thus allowing more water to be absorbed. The water absorption capacities ranged from 0.46 – 2.98 g/g. The chemical modification method resulted in the highest amount of absorbed water (2.98 g/g) while the co-evaporation method showed the least amount of absorbed water (0.46 g/g). Complexation methods significantly ($p \leq 0.05$) reduced BGN native starch tendency to absorb water. However, among the complexation methods, the microwave gave the most absorbed water (0.74 g/g); this might be because the method involved heating of the starch granules. When starch granules are heated in water, the water molecules will begin to vibrate causing a disruption on the starch granules and when starch granules are disrupted, they begin to absorb more water and eventually they begin to swell. On the other hand, the co-evaporation and kneading methods demonstrated low water absorption capacities. This might be due to the higher levels of hydrophobicity than hydrophilicity in the starches. In addition, since these two methods did not involve any heating it is believed that the starch intermolecular bonds were not fully broken (rather only partially disrupted) to allow the starch to absorb more water.

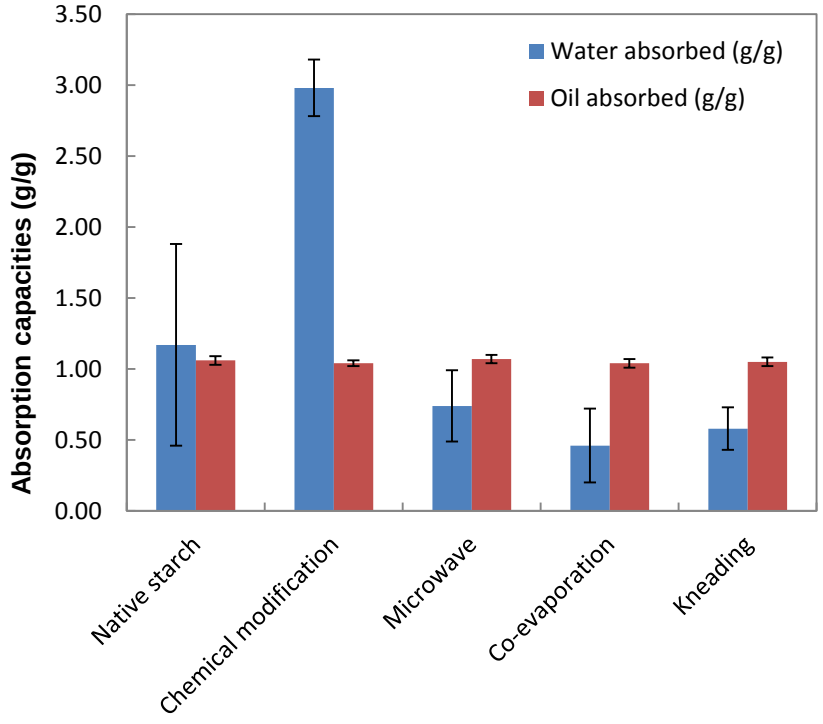

Figure 4.4 Effect of the modification method on the water and oil absorption properties of BGN starch

Modification methods did not differ significantly ($p > 0.05$) from each other in the oil absorption capacities of native and modified BGN starches. Chemically modified starch had higher water absorption capacity than oil absorption capacity. This result gives an indication that chemical modification increased the hydrophilicity of BGN starch more than its hydrophobicity. Conversely, after complexation the oil absorption values increased significantly in comparison to their water absorption capacity values. This might be due to the increased levels of starches hydrophobicity levels thereby allowing the starch granules to absorb more oil than water.

4.8 Pasting properties of native and modified BGN starches

The pasting characteristics of native and modified BGN starches are presented in Table 4.2. The maximum viscosity obtained during starch gelatinisation is known as peak viscosity. Peak viscosity indicates the water binding capacity of starch granules, it therefore is an important parameter used in the processing of starch. The peak viscosity ranged from 344.3 to 4767.0 cP. The peak viscosity (4767.0 cP) of native BGN starch was significantly higher than the modified starches. Starch modification significantly reduced the peak viscosity of native starch, with BGN starch modified through the microwave method (alpha cyclodextrin + catechin) having the least peak viscosity of 344.3 cP. The peak viscosity of chemically

modified starches did not differ significantly from each other. Within the microwave method, the peak viscosity significantly ($p \leq 0.05$) differed while that of alpha cyclodextrin and beta cyclodextrin + catechin did not differ significantly ($p > 0.05$) from each other. The initiators within the co-evaporation method had a significant effect ($p \leq 0.05$) on the starches' peak viscosity. Among the kneading method, the initiators significantly differed from each other with exception of alpha cyclodextrin + catechin and beta cyclodextrin that did not have a significant ($p > 0.05$) effect on the peak viscosity. The higher peak viscosity of native BGN starch in comparison to modified starches could be attributed to the lack of new functional groups and the native starch's unrestricted swelling property (Adebowale *et al.*, 2002). The results show that the peak viscosity of all BGN starches (native and modified) differs from each other and this might be due to the differences in the rate at which starch granules swell and absorb water when heated (Falade *et al.*, 2014; Wu *et al.*, 2015). According to Wu *et al.* (2015), high peak viscosity is an indication that the starch has high water binding capacity; therefore it can be concluded that native BGN starch can swell easily and form a paste in comparison to the modified BGN starches. Furthermore, Falade *et al.* (2014) and Sanni *et al*. (2001) reported that there is a strong correlation between peak viscosity and starch damage, high starch damages results in high peak viscosity.

Breakdown viscosity measures the starch granules susceptibility to disintegrate (Jan *et al.*, 2013; Falade *et al.*, 2014; Wu *et al.*, 2015). It gives an indication of the starch organisation structure. The breakdown viscosity of native and modified BGN starches ranged from 2.3 to 1654.0 cP. Native BGN starch had a significantly higher breakdown viscosity (1654 cP) while BGN starch modified through kneading with alpha cyclodextrin had the lowest breakdown viscosity (2.3 cP). All modification methods were observed to reduce native starch breakdown viscosity. The breakdown viscosity of chemically modified starches did not significantly differ ($p > 0.05$) from each other. Among the microwave method, all initiators had a significant ($p \leq 0.05$) effect on the breakdown viscosity of starch. Within the co-evaporation method, all initiators significantly differed ($p \leq 0.05$) from each other with exception of alpha cyclodextrin and alpha cyclodextrin + catechin which did not differ significantly ($p > 0.05$) from each other. Within the kneading method, all initiators had a significant ($p \leq 0.05$) effect on the starches' breakdown viscosity. However, beta cyclodextrin did not differ significantly ($p > 0.05$) either from alpha cyclodextrin or beta cyclodextrin + catechin. Complexation methods were observed to have a significant effect in reducing the breakdown viscosity of BGN native starch more especially the kneading method. According to Falade *et al.* (2014), higher breakdown viscosity means that the starch has lower ability to withstand heating and shear stress during cooking. Low breakdown viscosity indicates thermal stability while high values indicate relatively low heat stability.

Table 4.2 Pasting properties of native and modified Bambara groundnut starch[1, 2]

Modification method	Initiators	Peak Viscosity	Trough viscosity	Breakdown viscosity	Final viscosity	Set back	Peak time (min)	Pasting temp (T°C)
Native	Native starch	4767.00 ±177.49^{a}	3013.00 ±8.72^{a}	1654.00 ±22.61^{a}	4876.33 ±180.27^{a}	1956.67 ±16.50^{a}	4.70 ±0.03^{a}	79.90 ±0.00^{a}
Chemical	AA +H_2O_2	643.77 ±109.13^{a}	150.33 ±18.01^{a}	502.00 ±132.02^{a}	445.67 ±26.31^{a}	294.00 ±9.64^{a}	1.09 ±0.04^{a}	57.23 ±9.06^{a}
	AA + H_2O_2 + Catechin	457.33 ±62.16^{a}	88.67 (6.66)^{b}	368.67 ±59.94^{a}	221.33 ±120.43^{a}	132.67 ±127.08^{a}	2.88 ±2.03^{a}	50.85 ±1.13^{a}
Microwave	α-cyclodextrin	562.00 ±21.52a	463.33 ±11.93^{a}	111.00 ±1.73^{a}	974.33 ±20.98^{a}	514.33 ±9.29^{a}	7.00 ±0.00^{a}	85.67 ±0.03^{a}
	α-cyclodextrin + Catechin	344.33 ±10.41^{b}	266.67 ±10.79^{b}	75.67 ±1.53^{b}	603.33 ±17.21^{b}	326.00 ±10.58^{b}	7.00 ±0.00^{a}	85.70 ±0.00^{a}
	β-cyclodextrin	704.00 ±74.63^{c}	641.33 ±83.46^{c}	88.00 ±1.00^{c}	1114.00 ±72.69^{c}	472.67 ±24.09^{c}	6.98 ±0.04^{a}	84.57 ±0.58^{b}
	β-cyclodextrin + Catechin	543.00 ±9.85^{a}	447.67 ±10.50^{a}	97.33 ±1.15^{d}	893.33 ±12.50^{d}	442.00 ±2.00^{d}	7.00 ±0.00^{a}	85.10 ±0.58^{a,b}
Co-evaporation	α-cyclodextrin	812.33 ±2.52^{a}	801.33 ±3.21^{a}	10.33 ±0.58^{a}	1429.67 ±7.51^{a}	627.67 ±4.51^{a}	5.36 ±0.15^{a}	82.48 ±0.08^{a}
	α-cyclodextrin + Catechin	713.67 ±4.73^{b}	692.00 ±21.38^{b}	12.00 ±1.73^{a}	1249.00 ±11.36^{b}	558.33 ±16.65^{b}	6.96 ±0.04^{b}	81.62 ±0.03^{b}
	β-cyclodextrin	882.33 ±11.72^{c}	831.00 ±14.18^{c}	54.67 ±0.58^{c}	1473.00 ±16.82^{c}	644.67 ±5.51^{a}	5.20 ±0.00^{c}	81.85 ±0.52^{b}
	β-cyclodextrin + Catechin	678.00 ±4.58^{d}	647.00 (10.54)^{d}	36.00 ±0.00^{d}	1151.33 ±4.51^{d}	510.00 ±0.00^{c}	7.00 ±0.00^{b}	82.43 ±0.06^{a}
Kneading	α-cyclodextrin	863.33 ±21.01^{a}	866.67 ±24.01^{a}	2.33 ±0.58^{a}	1573.67 ±45.65^{a}	729.67 ±23.12^{a}	6.12 ±0.36^{a}	81.55 ±0.00^{a}
	α-cyclodextrin + Catechin	906.67 ±6.11^{b}	890.67 ±0.58^{a}	15.33 ±5.51^{b}	1654.67 ±10.07^{b}	764.67 ±9.71^{b}	5.74 ±0.14^{b}	80.62 ±0.03^{b}
	β-cyclodextrin	893.67 ±9.45^{b}	886.33 ±10.21^{a}	3.33 ±0.58^{a,c}	1647.00 ±15.13^{b}	757.00 ±5.29^{a,b}	6.21 ±0.13^{a}	81.50 ±0.00^{c}
	β-cyclodextrin + Catechin	808.67 ±14.05^{c}	799.33 ±14.50^{b}	8.33 ±0.58^{c}	1456.67 ±34.21^{c}	651.00 ±18.00^{c}	6.93 ±0.07^{c}	80.65 ±0.00^{d}

[1]AA: Ascorbic acid; H_2O_2: Hydrogen peroxide. [2]Mean values of triplicate determinations ±standard deviation. Means within a column (under the same row heading) followed by the same superscript are not significantly ($p > 0.05$)

Therefore, native BGN showing higher breakdown viscosity will have a lower ability to withstand shear stress and heating during cooking in comparison to starches with lower breakdown viscosity (chemically modified starches and all complexed starches). Therefore, modified starches are preferred at higher temperatures due to their high thermal stabilities. In addition, higher breakdown viscosity gives an indication of starch which has undergone a higher degree of swelling and subsequent disintegration.

When starch pastes starts to cool down there is re-association of the solubilised amylose and amylopectin molecules which results in increased viscosity. This rise in viscosity is known as the setback viscosity. Setback viscosity measures the starch tendency towards retrogradation (Jan *et al.*, 2013; Falade *et al.*, 2014; Olu-Owolabi *et al.*, 2014). The setback viscosity of native and modified BGN starches ranged from 132.7 to 1956.7 cP. Native BGN starch had the highest setback viscosity (1956.7 cP) while BGN starch-catechin complex modified through chemical modification had the lowest setback value of 132.7 cP. Native BGN starch had a significantly higher setback viscosity when compared to modified starches. This shows that native BGN starch has a high tendency towards retrogradation. Furthermore, this is an indication that there was nothing inhibiting the re-association of the amylose and amylopectin molecules. On the other hand, modification methods decreased the setback viscosity of native starch. This is because modification processes involve the introduction of functional groups which then prevent the starch molecules from re-associating after cooling thereby resulting in lower setback viscosity (Adebowale *et al.*, 2002; Adebowale & Lawal, 2003; Lawal *et al.*, 2004). In addition, reduction in setback viscosity after modification could be due to depolymerisation that happens during starch modification. The setback viscosity of chemically modified starches did not differ significantly ($p > 0.05$) from each other. Among the microwave method, the initiators had a significant effect on the setback viscosity as they all significantly ($p \leq 0.05$) differed from each other. Within the co-evaporation method, the initiators significantly ($p \leq 0.05$) differed from each other; however, alpha and beta cyclodextrin did not differ significantly ($p > 0.05$). Within the kneading method, the initiators significantly ($p \leq 0.05$) differed from each other except for beta cyclodextrin which did not differ significantly ($p > 0.05$) either from alpha cyclodextrin or alpha cyclodextrin + catechin. Among the modification methods, chemical modification significantly reduced the setback viscosity of native BGN starch. This is because of the introduction of the new functional groups (hydroxyl groups) to the starch molecule which restricts the tendency of starch molecules to realign after cooling. This result is in agreement with reports from Adebowale *et al.* (2002), Lawal *et al.* (2004) and Afolabi (2012) that chemical modification significantly reduced the setback viscosity of native BGN starches. The setback viscosity of native BGN and chemically modified starches observed in this study followed a similar trend to that reported by Afolabi (2012). During starch modification, hydrogen bonds are formed due to the introduction of hydroxyl groups on the starch molecules; as a result

realignment or re-association of the starch molecules is prevented leading to lower setback viscosity. Therefore, using the same reasoning this explains the lower setback viscosity for all modified BGN starches in comparison to the native BGN starch. BGN starch-catechin modified starches all had lower setback viscosity due to the addition of catechin. This might probably be due to the interference of starch-catechin complexes with the alignment of starch molecules thereby resulting in lower setback viscosities. This indicated that inclusion complexes were able to stabilise starch pastes. This result concurs with the reports made by Beta & Corke (2004) and Wu *et al.* (2015) that addition of catechin significantly reduced the setback viscosities of maize and rice starches. Food products such as soups and sauces usually lose viscosity and precipitate due to retrogradation and as a result using modified starches with lower setback viscosities such as chemically modified starches will be an advantage. Chemically modified starches can therefore be used in soups and sauces because of their low setback viscosities.

The temperature at which starch granules start to swell is known as the pasting temperature. Pasting temperature gives an indication of the minimum temperature required to initiate starch gelatinisation, it also indicates energy costs involved as well as stability of other components (Araujo-Farro *et al.*, 2005; Shimelis *et al.*, 2006; Jan *et al.*, 2013). The pasting temperature of native and modified BGN starches ranged from 50.9^{o}C to 85.7^{o}C. BGN starch modified through microwave (alpha cyclodextrin + catechin) had the highest pasting temperature (85.7^{o}C) while chemically modified BGN starch-catechin complex has the lowest pasting temperature (50.9^{o}C). The pasting temperature of native BGN starch was 79.9^{o}C which is higher than 77.5^{o}C of starch from chenopodium album grains reported by Jan *et al.* (2013). However, it is lower than 84^{o}C for BGN starch, 82^{o}C for Mucuna bean starch and 83.20^{o}C for BGN starch reported by Adebowale *et al.* (2002); Adebowale & Lawal (2003) and Afolabi (2012), respectively. Chemical modification significantly reduced the pasting temperature of native BGN starch and the addition of catechin had a further reduction from 79.9 to 50.9^{o}C. However, within the chemical modification the effect of initiators on the starch pasting temperature did not differ significantly ($p > 0.05$) from each other. Starch chemical modification results in the disruption of intermolecular bonds within the starch granules thus causing starch to lose birefringence and respond to heat at much lower temperatures in comparison to native starch (Adebowale & Lawal, 2003; Lawal & Adebowale, 2005). This explains the reduction of pasting temperature following chemical modification. Similar results have been reported that pasting temperature of native starch reduced following chemical modification (Adebowale & Lawal, 2003). The low pasting temperatures of chemically modified starches indicated that their crystalline structure had significantly reduced to an amorphous form; this concurs with the X-ray diffraction patterns of these starches which showed amorphous regions (chapter 3, section 3.6.5). On the other hand, all complexation methods were observed to increase the pasting temperature of native

BGN starch. This increase may be attributed to cyclodextrin's tendency to increase the crystallinity region due to the reorientation of starch granules. This resulted in strengthened starch intramolecular bonds allowing starch to require more heat before paste formation and structural disintegration (Olu-Owolabi *et al.*, 2014). In addition, higher pasting temperatures are an indication of higher resistance towards swelling and this concurs with the results of swelling ability of the complexed starches as shown in Figure 4.2.

Final viscosity gives an indication of the starch ability to form a gel or viscous paste after cooking and cooling (Araujo-Farro *et al.*, 2005; Shimelis *et al.*, 2006; Jan *et al.*, 2013). Furthermore, final viscosity is used to determine starch quality and stability of the cooked starch paste in food products. According to Falade *et al.* (2014), final viscosity plays a vital role in the rigidity and stability of the swollen granule structure. The final viscosities of native and modified BGN starches ranged from 221.3 to 4876.3 cP. Native BGN starch had the highest final viscosity of 4876.3 cP while BGN starch-catechin complex chemically modified had the lowest final viscosity value of 221.3 cP. Similar results were reported for the final viscosity of native starches. Jan *et al.* (2013) reported 4699.00 cP to be the final viscosity of starch obtained from chenopodium album grains, while Araujo-Farro *et al.* (2005) reported 4989.00 cP and 4467.00 cP to be the final viscosities of quinoa and rice starches, respectively. Modification methods reduced the final viscosity of native BGN starch, with chemical modification showing a significant reduction. When the amylopectin in the starch molecule start to breakdown they lose crystallinity thereby reducing the starch final viscosity. Addition of catechin significantly reduced the final viscosity, and this result concurs with the reports made by Beta & Corke (2004) and Wu *et al.* (2015) where addition of catechins reduced the final viscosity of maize and rice starches, respectively. Beta & Corke (2004) speculated the reduction of final viscosity after addition of catechins to be caused by the formation of starch-catechin complexes which then interfere with the starch molecule alignment.

4.8.1 Effect of modification methods on the pasting properties of native and modified BGN starches

The effect of the modification methods on the pasting properties of BGN starches is shown in Table 4.3. Native BGN starch had the highest peak viscosity of 4767.00 cP. All modification methods significantly reduced peak viscosity of native BGN starch. In addition, all these methods significantly differed ($p \leq 0.05$) from each other except for the chemical modification and microwave methods which did not differ significantly ($p > 0.05$) from each other. High peak viscosity gives an indication that starch has a high water binding capacity therefore it swells easily and forms a paste. Chemical modification significantly ($p \leq 0.05$) reduced the pasting temperature of native BGN starch from 79.90^{o}C to 54.04^{o}C. This means that chemically modified starch requires lower temperatures to initiate starch gelatinisation and

this will be an advantage in industry as it will reduce energy costs during processing due to reduced processing times. In addition, chemical modification easily weakened the starch granules thus allowing more water to penetrate the starch thereby decreasing the pasting temperature. However, complexation methods increased the pasting temperature of native BGN starch. Modification methods had a significant ($p \leq 0.05$) impact on the starch pasting temperature except for co-evaporation and kneading which did not differ significantly ($p > 0.05$) from the native starch pasting temperature. Chemical modification significantly ($p \leq 0.05$) reduced the peak time (time required to reach peak viscosity) of native starch while the complexation methods significantly ($p > 0.05$) increased the peak time. However, the peak time of co-evaporation and kneading methods did not differ significantly ($p > 0.05$) from each other.

Following modification, the setback viscosity of native BGN starch significantly ($p \leq 0.05$) reduced, this is an indication that modification processes minimised starch retrogradation. Chemical modification had the lowest setback viscosity of 213.33 cP indicating that chemically modified starch has a lower tendency to retrograde. All modification methods significantly differed ($p \leq 0.05$) from each other in terms of their setback viscosity. The setback viscosities ranged from 213.33 to 1956.67 cP with native BGN starch having the highest tendency towards retrogradation. The breakdown viscosity of BGN starches based on modification methods ranged from 7.33 (kneading method) to 1654.00 cP (native BGN starch). The modification methods differed significantly ($p \leq 0.05$) from each other in their breakdown viscosities. However, there was no significant difference ($p > 0.05$) in the breakdown viscosities obtained through co-evaporation and kneading methods. The high breakdown viscosity of native BGN starch shows that native BGN starch has relatively low heat stability therefore it cannot withstand shear stress and heat during cooking processes.

Based on the modification methods, the final viscosities of BGN starches ranged from 333.50 (chemical modification) to 4876.33 cP (native BGN starch). The low final viscosity of chemically modified starch means that the starch paste will be stable after cooling. In addition, the low viscosity might probably be due to shear thinning which is in agreement with the rheological properties of chemically modified starch as shown in Figure 4.5. High final viscosity as obtained in native BGN starch (4876.33 cP), co-evaporation (1325.75 cP) and kneading methods (1583.00 cP) are an indication that these starches will be less stable after cooling. Furthermore, the high final viscosities could be attributed to shear thickening of these starches, as shown in Figure 4.7 andFigure 4.8. In terms of final viscosity, the modifications significantly differed ($p \leq 0.05$) from each other. Lastly, the trough viscosity of BGN starches based on the modification methods ranged from 119.50 (chemical modification) to 3013.00 cP (native BGN starch). All the methods significantly differed ($p \leq 0.05$) from each other.

Table 4.3 Effect of the modification method on the pasting properties of BGN starch[1]

Modification method	Peak viscosity	Trough viscosity	Breakdown viscosity	Final Viscosity	Set back	Peak time (min)	Pasting temp (T°C)
Native	4767.00 ± 177.49[a]	3013.00 ± 8.72[a]	1654.00 ± 22.61[a]	4876.33 ± 180.27[a]	1956.67 ± 16.50[a]	4.70 ± 0.03[a]	79.90 ± 0.00[a]
Chemical	550.50 ± 129.33[b]	119.50 ± 35.89[b]	435.33 ± 117.22[b]	333.50 ± 145.52[b]	213.33 ± 119.61[b]	1.98 ± 1.61[b]	54.04 ± 6.75[b]
Microwave	538.33 ± 137.99[b]	454.75 ± 143.21[c]	93.00 ± 13.55[c]	896.25 ± 197.79[c]	438.75 ± 74.05[c]	6.99 ± 0.02[c]	85.26 ± 0.58[c]
Co-evaporation	771.58 ± 84.47[c]	742.83 ± 80.03[d]	28.25 ± 19.16[d]	1325.75 ± 137.30[d]	585.17 ± 57.05[d]	6.13 ± 0.89[d]	82.10 ± 0.45[a]
Kneading	868.08 ± 41.14[d]	860.75 ± 40.29[e]	7.33 ± 5.88[d]	1583.00 ± 86.87[e]	725.58 ± 48.84[e]	6.25 ± 0.48[d]	81.08 ± 0.47[a]

[1]Mean values of triplicate determinations ± standard deviation. Means within a column followed by the same letter are not significantly ($p > 0.05$) different

4.9 Thermal properties of Native and Modified BGN Starches

Characterisation of starch thermal properties plays a vital role in the determination of the starch cooking variables. The thermal properties of BGN starches are presented in Table 4.4. The gelatinisation temperatures ranged from 67.58^{o}C for native BGN starch onset temperature to 126.55^{o}C for chemically modified BGN conclusion temperature. In this study the following gelatinisation temperatures were reported for native BGN starch: onset temperature (67.58^{o}C), peak temperature (79.38^{o}C), conclusion temperature (90.09^{o}C), temperature range (22.51^{o}C) and enthalpy change (55.37^{o}C), and this result is similar to the results reported by Afolabi (2012) for native BGN starch. However, in this study peak temperature, conclusion temperature and temperature range shifted to lower values while the enthalpy change shifted to higher values in comparison to the results obtained by Afolabi (2012). These differences could be attributed to the different methods used for starch extraction and their effect on the degree of starch crystallinity, amylopectin content as well as the presence of crystalline regions of different strength within the starch granules (Wang *et al.*, 2011). The starch modification methods significantly increased the gelatinisation temperature of native BGN starch as shown in Table 4.4.

Within the chemical modification method, there was no significant ($p > 0.05$) difference in the gelatinisation temperatures. Chemical modification involves the introduction of the new reactive functional groups to the starch molecule which then weakens the starch granules due to the disruption of inter- and intra-molecular hydrogen bonds (Adebowale & Lawal, 2003; Afolabi, 2012; Olayinka *et al.*, 2015). Consequently, there will be an increased accessibility of water to the weakened starch granules thus leading to lower gelatinisation temperatures compared to those of native starch. Consequently, chemical modification is known to require less energy to initiate starch gelatinisation. Reports from Adebowale & Lawal (2003), Afolabi (2012) and Olayinka *et al.* (2015) concur that following chemical modification, gelatinisation temperatures of mucuna bean starch, BGN starch and yellow sorghum starch reduced in comparison to their native starches. Contrary to those reports, in this research study chemical modification was observed to have a significant increase in the starch gelatinisation temperatures. This increase might be because the amount of water was not sufficient enough to swell the starch granules as a result the starch gelatinisation peaks broadened and shifted to higher temperatures (Ai & Jane, 2015).

Complexation methods significantly ($p \leq 0.05$) increased the gelatinisation temperatures (T_o, T_p, T_c) of native BGN starch. This increase is an indication that these starches had higher levels of crystallinity and they therefore needed more energy to initiate starch gelatinisation in comparison to native BGN starch (Wang *et al.*, 2011; Bhupender *et al.*, 2013). Addition of cyclodextrins increased the gelatinisation temperatures.

Table 4.4 Thermal properties of native and modified BGN starches [1, 2]

Modification Method	Initiators	Onset Temp (T_o) ^oC	Peak Temp (T_p) ^oC	Conclusion Temp (T_c) ^oC	$\Delta T = T_c - T_o$ ^oC	ΔH (J/g)
Native	Native starch	67.58 ± 4.41[a]	79.38 ± 1.35[a]	90.09 ± 5.00[a]	22.51 ± 9.41[a]	55. 37 ± 2.00[a]
BGN starch						
Chemical	AA + H_2O_2	102.69 ± 1.90[a]	116.58 ± 0.04[a]	126.55 ± 0.60[a]	23.86 ± 2.50[a]	3834.90 ± 1073.88[a]
	AA + H_2O_2 +Catechin	101.54 ± 0.29[a]	115.59 ± 2.09[a]	126.27 ± 2.37[a]	24.73 ± 2.08[a]	4169.29 ± 591.87[a]
Co-evaporation	Alpha cyclodextrin	105.05 ± 1.52[a]	110.66 ± 1.89[a]	123.12 ± 0.95[a]	17.97 ± 0.57[a]	1377.20 ± 474.09[a,c]
	Alpha cyclodextrin + Catechin	105.30 ± 1.40[a]	111.10 ± 1.22[a]	118.06 ± 1.36[b]	12.76 ± 2.76[b]	704.86 ± 198.65[a,b]
	Beta cyclodextrin	104.73 ± 0.36[a]	114.59 ± 0.49[b]	122.45 ± 0.16[a]	17.73 ± 0.21[a]	1854.25 ± 107.48[c]
	Beta cyclodextrin + Catechin	113.28 ± 0.06[b]	116.97 ± 0.00[b]	121.24 ± 0.04[a]	7.96 ± 0.03[c]	204.63 ± 6.65[b]
Microwave	Alpha cyclodextrin	102.40 ± 0.14[a]	112.16 ± 0.23[a]	121.19 ± 1.08[a]	19.42 ± 1.22[a]	1962.93 ± 57.16[a]
	Alpha cyclodextrin + Catechin	103.67 ± 1.11[a]	112.90 ± 1.55[a]	121.82 ± 1.70[a]	18.16 ± 0.59[a]	1905.82 ± 374.39[a]
	Beta cyclodextrin	103.83 ± 1.81[a]	113.34 ± 1.42[a]	123.29 ± 0.88[a]	19.46 ± 0.93[a]	1787.90 ± 580.65[a]
	Beta cyclodextrin + Catechin	102.79 ± 0.88[a]	112.56 ± 0.33[a]	122.51 ± 0.67[a]	19.72 ± 1.56[a]	1995.16 ± 559.10[a]
Kneading	Alpha cyclodextrin	103.82 ± 0.54[a]	111.90 ± 2.09[a]	118.95 ± 0.08[a]	15.13 ± 0.45[a]	1686.95 ± 733.40[a]
	Alpha cyclodextrin + Catechin	104.57 ± 3.34[a]	111.66 ± 4.82[a]	119.61 ± 5.56[a]	15.04 ± 2.22[a]	756.23 ± 155.79[a,b]
	Beta cyclodextrin	84.84 ± 6.77[b]	95.33 ± 5.48[b]	101.59 ± 4.98[b]	16.76 ± 1.79[a]	128.71 ± 41.44[b]
	Beta cyclodextrin + Catechin	106.10 ± 8.60[a]	117.39 ± 1.61[a]	124.39 ± 1.20[a]	18.29 ± 7.40[a]	1120.19 ± 73.44[a,b]

[1]AA: Ascorbic acid; H_2O_2: Hydrogen peroxide. [2]Mean values of triplicate determinations ± standard deviation. Means within a column (under the same row heading) followed by the same superscript are not significantly ($p > 0.05$)

During complexation, alpha and beta cyclodextrin were used as initiators to form starch-cyclodextrin complexes and these inclusion complexes increased gelatinisation temperature and enthalpy change (ΔH). This is probably because when the inclusion complexes were formed, the cyclodextrins (alpha and beta) compete with starch for water by binding with water thereby reducing water's availability for starch gelatinisation. This result concurs with the reports made by Ai & Jane (2015) that inclusion complexes result in increased gelatinisation temperature as well as temperature. Furthermore, Adebowale & Lawal (2003b) attributed the increase in the gelatinisation temperatures (T_o, T_p, T_c) to structural changes within the starch granules and in this case these structural changes include amylose-amylose, amylose-beta cyclodextrin and amylose-alpha cyclodextrin interactions. The strong interactions between cyclodextrins (alpha and beta) and starch are formed by hydrogen bonds through the hydroxyl groups between cyclodextrins and starch. The shift to higher gelatinisation temperatures gives an indication that there was complete complexation. Gelatinisation enthalpy change (ΔH) measures the overall starch crystallinity and it also indicates the amount of energy required to initiate starch gelatinisation (Jan *et al.*, 2013; Olayinka *et al.*, 2013; Wu *et al.*, 2015). Furthermore, ΔH indicates the loss of molecular order within the starch granule. The gelatinisation enthalpy change temperature of native BGN starch significantly increased after starch modification. The high ΔH of complexed starches suggested that these starches have a higher degree of crystallinity and this is confirmed by sharper X-ray diffraction peaks of complexed starches discussed in chapter 3, section 3.5.2. There was a variation in the ΔH of BGN starches and this variation was due to the different hydrogen bond alignment within the starch molecules caused by differences in bonding forces in the amylopectin double helices (Bhupender *et al.*, 2013).

4.10 Rheological properties of modified BGN starches

Rheological data of modified BGN starches was fitted into a power law model, which was then used to describe the flow behaviour of BGN starches. The power law model is widely used in the characterisation of fluids and it comprises of two parameters (K and n) which are used to determine the shear stress-shear rate relationship (Adeyi, 2014). The consistency coefficient (K) describes the viscosity of the system and low values are an indication of a less viscous solution and vice versa. The flow behaviour index (n) is a measurement of a fluid's resistance to flow, where $n < 1$ the fluid is shear thinning and $n \geq 1$ the fluid is shear thickening (Kim & Yoo, 2006; Lin *et al.*, 2010; Adeyi, 2014; Oyeyinka *et al.*, 2015). The power law model parameters of modified BGN starches are shown in Table 4.5. The consistency coefficients ranged from 0.0012 to 3.8100 $Pa.s^n$. Chemical modification and microwave methods were observed to have higher consistency coefficients (K) suggesting that these starches were more viscous in comparison to others. Kneading and co-evaporation methods on the other hand had lower K indicating that these starches formed

less viscous pastes/solutions. The flow index behaviour (n) ranged from 0.0319 to 0.9188. Starches modified through chemical modification and microwave methods had the lowest flow index ranging from 0.0319 to 0.1367, thus indicating a shear thinning behaviour less than unity. Contrarily, co-evaporation and kneading modified starches had high flow behaviour indexes in the range 0.6760 to 0.9188, thus indicating a shear thickening behaviour as the values were very close to the unity. The coefficient of determination (R^2) must be close to 1 indicating the high relation between data points. Furthermore R^2 values give an indication of power law model accuracy to be employed for predicting the intrinsic rheological properties of starches (Adeyi, 2014; Gomez-Romero *et al.*, 2014). The coefficient of determination for modified BGN starches ranged from 0.5114 to 0.9590. Starches modified through chemical modification and microwave methods showed higher coefficient of determination which were close to 1 thus indicating suitability of the power law to explain the rheological behaviour.

Table 4.5 Power law model parameters for modified Bambara groundnut starches[1, 2]

Modification method	K (Pa.s^n)	n	R^2
Chemical modification			
AA + H_2O_2	1.7595 ± 0.00	0.1401 ± 0.00	0.8506
AA + H_2O_2 +Catechin	2.2031 ± 1.58	0.1367 ± 0.16	0.8375
Co-evaporation			
Alpha cyclodextrin	0.0043 ± 0.00	0.7155 ± 0.00	0.5114
Alpha cyclodextrin + Catechin	0.0038 ± 0.00	0.7922 ± 0.18	0.5574
Beta cyclodextrin	0.0057 ± 0.00	0.6760 ± 0.00	0.6037
Beta cyclodextrin + Catechin	0.0012 ± 0.00	0.9131 ± 0.08	0.6008
Microwave			
Alpha cyclodextrin	2.0320 ± 0.00	0.0589 ± 0.00	0.6562
Alpha cyclodextrin + Catechin	3.8400 ± 0.00	0.0319 ± 0.00	0.5694
Beta cyclodextrin	3.1205 ± 0.00	0.0692 ± 0.00	0.9590
Beta cyclodextrin + Catechin	3.3016 ± 0.32	0.0611 ± 0.012	0.9317
Kneading			
Alpha cyclodextrin	0.0012 ± 0.00	0.9188 ± 0.00	0.6144
Alpha cyclodextrin + Catechin	0.0064 ± 0.00	0.7132 ± 0.05	0.5993
Beta cyclodextrin	0.0037 ± 0.00	0.7621 ± 0.00	0.6566
Beta cyclodextrin + Catechin	0.0044 ± 0.00	0.7231 ±0.01	0.5701

[1]Values are mean ± standard deviation. K= consistency coefficient; n= flow behaviour index; R^2= coefficient of determination. [2]AA= Ascorbic acid; H_2O_2= Hydrogen peroxide

The rheograms (Figure 4.5 to Figure 4.8) show the flow behaviour of BGN starches. Chemical modification and microwave rheograms were all displaying a shear thinning behaviour (Figure 4.5 and Figure 4.6 respectively) by the decreasing apparent viscosities with an increase in shear rate. However, co-evaporation and kneading rheograms are all displayed a shear thickening behaviour (Figure 4.7 and Figure 4.8 respectively) by an increasing apparent viscosity with an increase in shear rate. This behaviour might probably be due to the interaction of molecular bonds that were broken during shearing as they now start to form new bonds thus causing thickening (Adeyi, 2014). Furthermore this might be because these starches (modified through co-evaporation and kneading) were not completely pre-gelatinised thus they have to be treated as native starches before use in any formulation. As shown in flow curves in Figure 4.6 and Figure 4.5, microwave and chemically modified starches displayed a pseudoplastic behaviour decreased apparent viscosity with increased shear rates. This then suggests that the starch structure was breaking down as shear stress was being applied (Oyeyinka *et al.*, 2015). Shear thinning behaviour was also reported for indica rice and japonica rice starches as well as BGN starch genotypes as reported by Lin *et al.* (2010) and Oyeyinka *et al.* (2015), respectively.

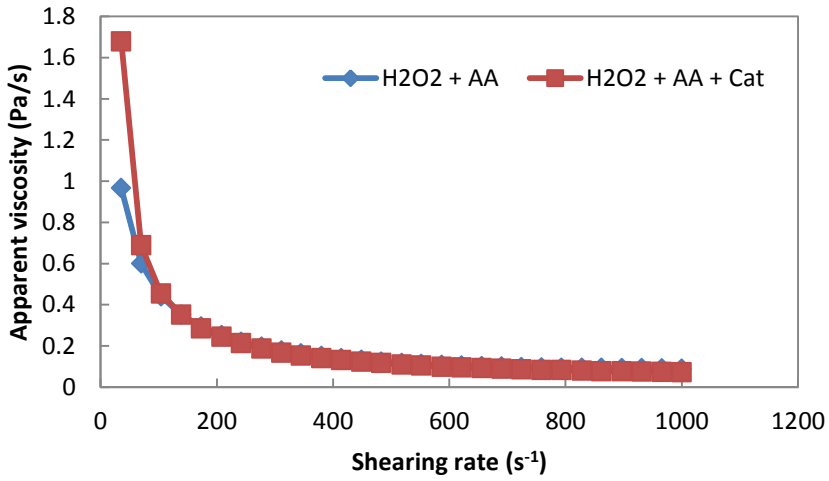

Figure 4.5 Apparent viscosity of chemically modified BGN starch. AA= Ascorbic acid; H2O2= Hydrogen peroxide, Cat= Catechin

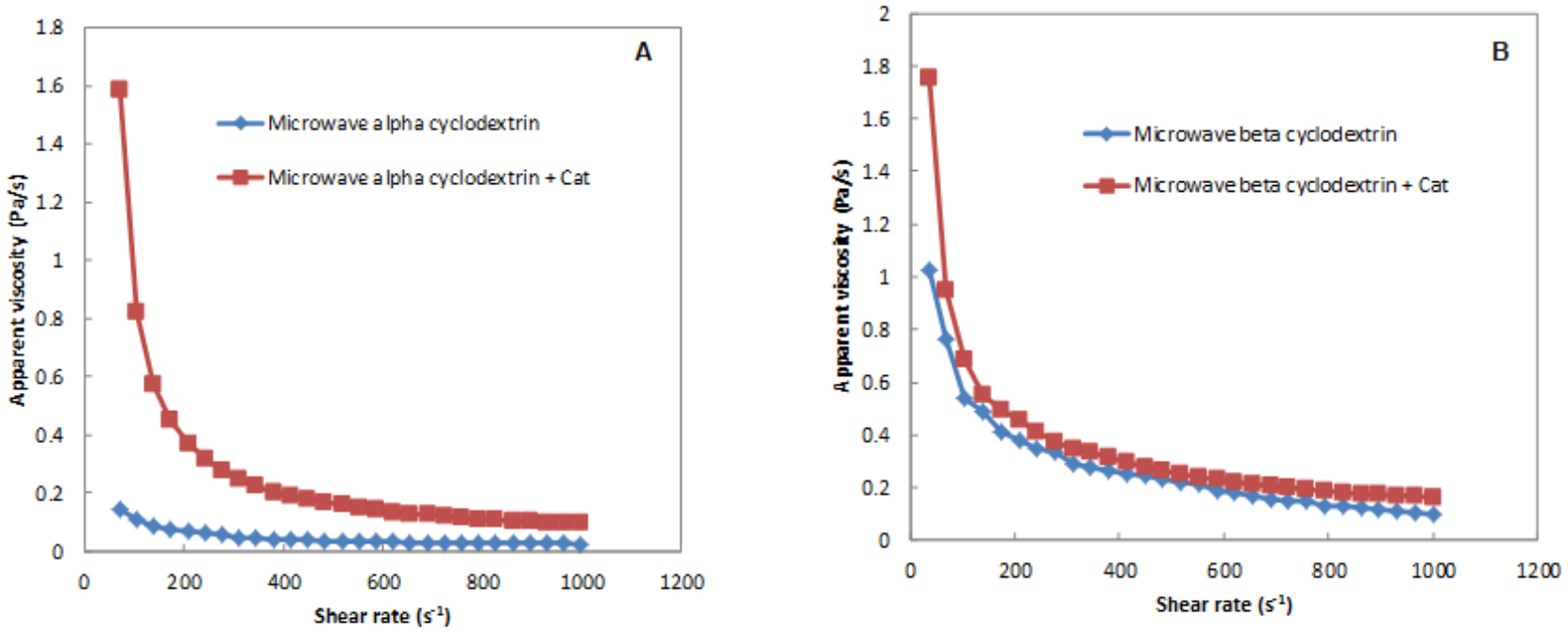

Figure 4.6 Apparent viscosity of BGN starch modified through microwave method (**A**) alpha cyclodextrin, (**B**) beta cyclodextrin. Cat= Catechin

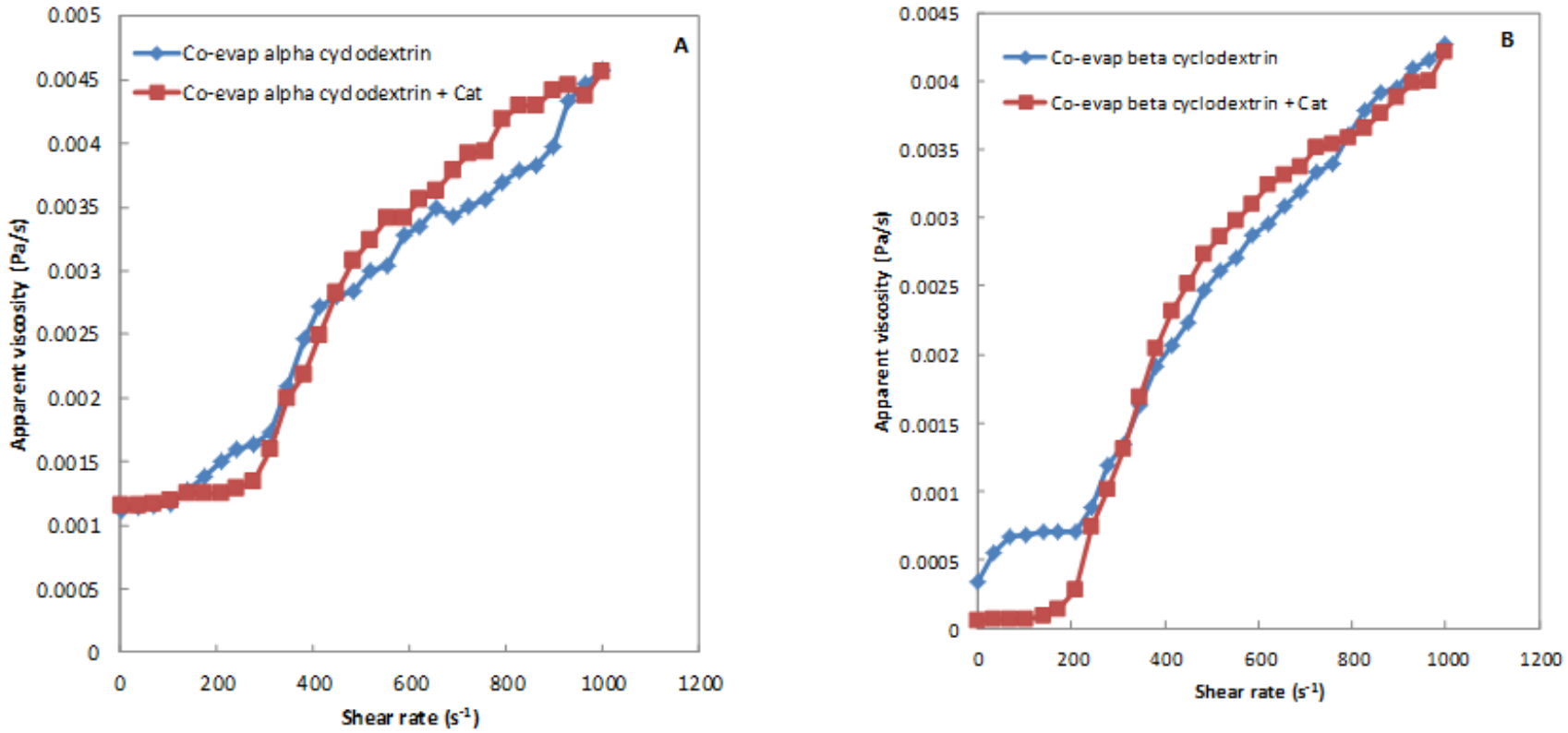

Figure 4.7 Apparent viscosity of BGN starch modified through co-evaporation method (**A**) alpha cyclodextrin, (**B**) beta cyclodextrin. Cat= Catechin

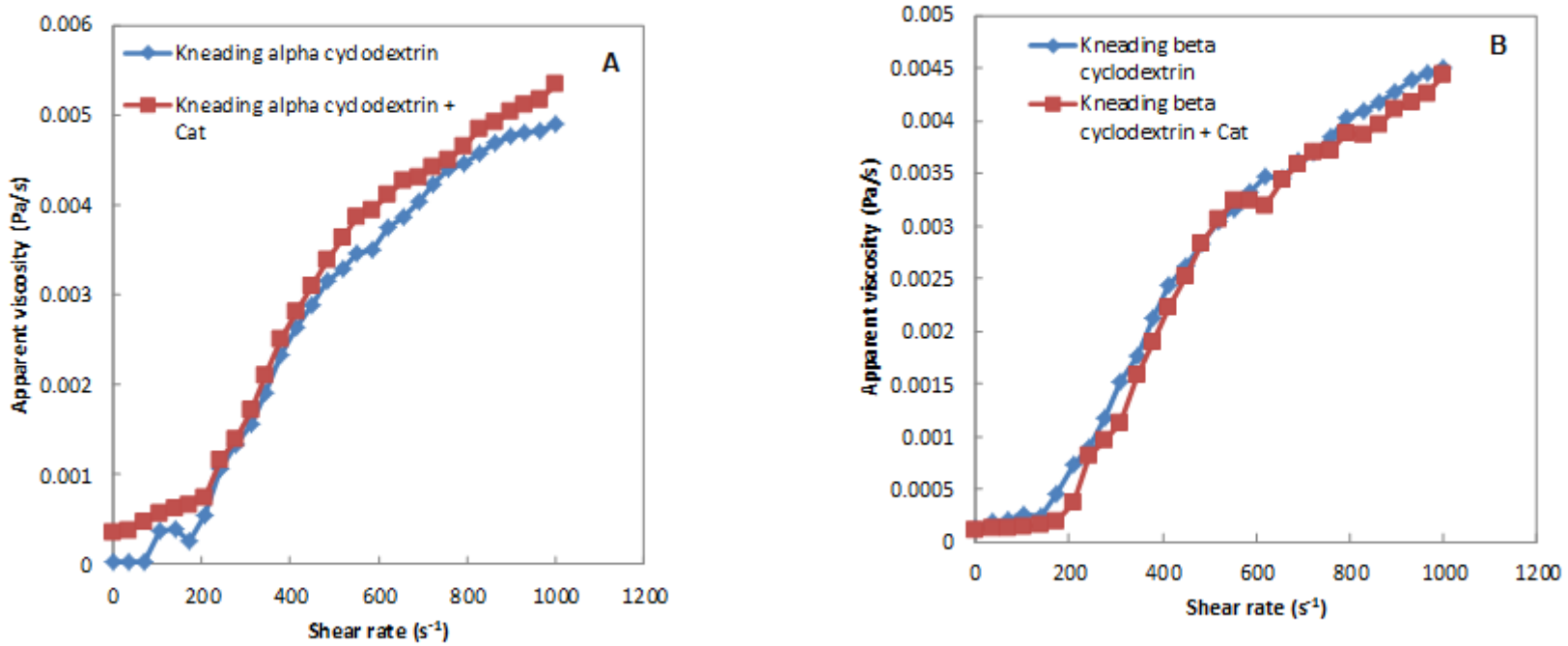

Figure 4.8 Apparent viscosity of BGN starch modified through kneading method (**A**) alpha cyclodextrin, (**B**) beta cyclodextrin. Cat= Catechin

4.11 Principal components explaining the variability in BGN starch functional properties

This variation in the functional properties of the BGN starches was explained by two components with eigenvalues exceeding one. Principal component 1 (PC1) described the greatest source of variability and it accounted for 52%, while principal component 2 (PC2) accounted for 34% of variability. The cumulative variability of the two components added up to 86%. PC1 correlates with water absorption, breakdown viscosity and peak viscosity while PC2 correlates with oil absorption, pasting temperature, peak time, final viscosity, set back, peak viscosity and trough. Figure 4.9 shows the score plot differentiating functional properties of BGN starches with respect to PC1 and PC2. The clustered variables are closely correlated. Component 1 is positively correlated with water absorption and negatively correlated with breakdown and peak viscosity. Hence, chemically modified starches had higher water absorption capacity and lower breakdown and peak viscosity when compared to other starches. From Figure 4.9, native BGN starch had a lower breakdown viscosity which suggests that it has low ability to withstand high temperatures during processing, hence, the need to modify starch to overcome its shortcomings. Hence, PC1 separates chemically modified starches from native BGN starch and the complexed starches. Component 2 is positively correlated with oil absorption, pasting temperature, peak time, final viscosity, set back and trough while it is negatively correlated with peak viscosity. Within PC2, final viscosity, set back and trough are closely correlated as they are all clustered. In addition, PC2 is highly loaded in pasting temperature and peak time. However, BGN starches cannot be separated based on their oil absorption capacities because they were all clustered together as shown in Figure 4.9. Furthermore, final viscosity, setback viscosity, trough and peak viscosity cannot be used to categorise the functional behaviour of BGN starches. The functional properties of native starch were below the average and lower than those of the modified starches. This suggests that starch was successfully modified.

4.12 Conclusion

Starch modification significantly improved functional, thermal and rheological properties of native BGN starch, with chemical modification method yielding the best results. Chemical modification increased the native starch solubility in cold water. Chemically modified BGN starches can therefore find application in the food industry as thickeners in instant food products like desserts, soups and baby foods thus owing to their ability to easily dissolve in cold water and forming pastes. The high breakdown viscosity of native BGN starch gave an indication that native BGN starch has a lower ability to withstand shear stress and heat during cooking processes. The modified starches will withstand high shear stress and temperatures during processing. Modification processes were observed to minimise starch retrogradation.

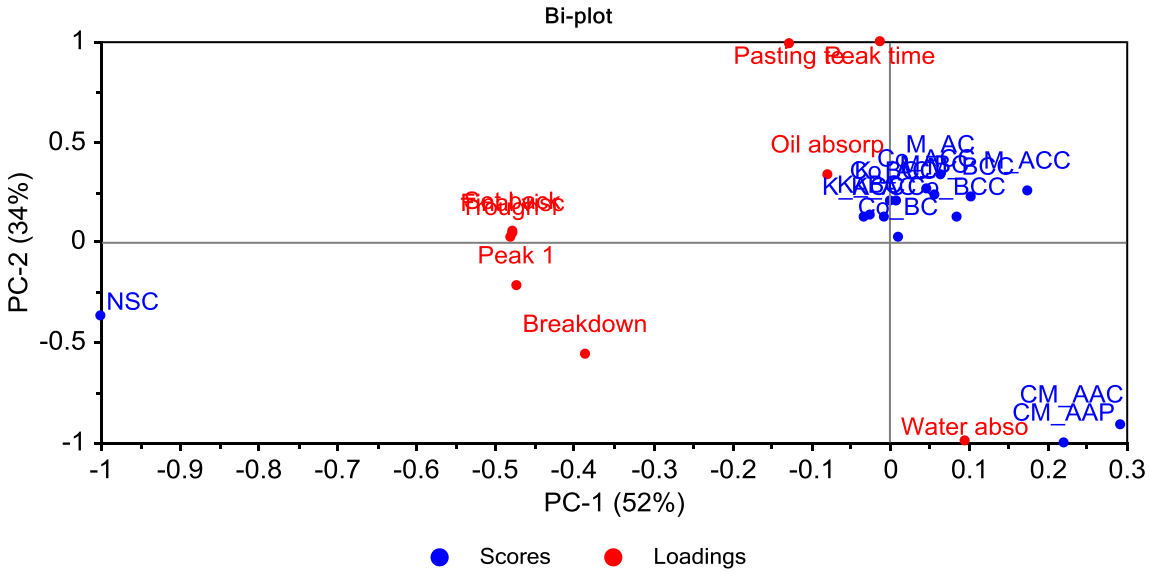

Figure 4.9 Score plot showing variation in the functional properties of BGN starches with respect to components 1 and 2

The lower setback viscosities of modified starches are an advantage in retarding retrogradation. Using chemically modified starches in the industry will be beneficial as they will reduce energy cost by using less energy during processing thus owing to their low pasting temperatures. Chemically modified starches had lower gelatinisation temperatures, hence, less energy is required to initiate starch gelatinisation and this is in agreement with the low pasting temperatures of these starches. A shear thinning behaviour was displayed by microwave and chemically modified starches. However, starches modified through co-evaporation and kneading methods displayed a shear thickening behaviour. Co-evaporation and kneading methods did not produce pre-gelatinised starches, they however, have potential. It is therefore recommended that further work be done on these two methods to obtain pre-gelatinised starch.

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CHAPTER FIVE
GENERAL SUMMARY AND CONCLUSIONS

The functional and rheological properties of Bambara groundnut (BGN) starch-catechin complex obtained by chemical grafting were investigated in this study. The aim of the study was to produce BGN starch-catechin complex using chemical initiators (ascorbic acid and hydrogen peroxide) and cyclodextrin (alpha and beta) with the view to obtain a functional ingredient for the food industry. The following objectives were identified in this study:

1. Production of BGN starch-catechin complex as a food ingredient using chemical initiators (ascorbic acid and hydrogen peroxide) and cyclodextrin (alpha and beta).
2. Establishing the structure of the new BGN starch-catechin complex.
3. Establishing functional, rheological and thermal properties of the new BGN starch-catechin complex, compared to the native starch.

The first step in achieving the first objective was to extract BGN starch from BGN flour using the dry milling method. Using dry milling method for starch extraction was found to be more beneficial as it is a more sustainable method with low costs using less water and energy when compared to the wet milling method. The starch extraction procedure yielded 32% BGN starch. The first objective was achieved as BGN starch-catechin complex was successfully produced using ascorbic acid (0.1%) and hydrogen peroxide (16.5%). In addition the use of cyclodextrin (alpha and beta) as initiators in the production of BGN starch-catechin complex was also successfully employed through the use of three different complexation techniques (co-evaporation, microwave irradiation and kneading methods). The hypothesis that a new BGN starch-catechin complex will be produced as a food ingredient using chemical initiators (ascorbic acid and hydrogen peroxide) and cyclodextrin (alpha and beta), was accepted.

The second objective was achieved by characterizing the new BGN starch-catechin complex. Characterisation of the structure of the new BGN starch-catechin complex was achieved by performing FTIR spectroscopy, fluorescence analysis and X-ray diffraction pattern analysis. The FTIR results of native BGN starch confirmed that it is made up of carbon, hydrogen and oxygen molecules which make up the gross structure of starch just like other starches. Furthermore, those results gave an indication that BGN starch is made up of glucose molecules like other starches. All modified BGN starch-catechin complexes showed new absorption peaks ranging from 1520-1560 cm^{-1} attributed to the C-C stretching found within the aromatic ring of catechin. This then suggested that catechin was successfully grafted onto the BGN starch resulting in the new structure of BGN starch-catechin complex. The fluorescence results concurred with the FTIR results thereby

confirming the successful grafting of catechin onto the BGN starch molecule. This was evidenced by a peak at 320 nm in BGN starch-catechin complexes while native BGN starch had a peak at 270 nm. The hypothesis that a new catechin functional group will be grafted onto the BGN starch molecule was accepted. Native BGN starch exhibited typical C type crystallinity implying that it has lower digestibility when compared to cereal starches. This is beneficial in that it promotes slow and moderate insulin and postprandial glucose responses.

The third objective was achieved by evaluating the functional, rheological and thermal properties of modified BGN starches, compared to the native BGN starch. The oil and water absorption capacity, swelling and solubility capacity and pasting properties of modified BGN starches revealed their potential for use in the food industry for different applications. Modified BGN starches had increased solubility in comparison to native starch; therefore, these starches can find application in the food industry as thickeners especially in instant food products such as baby foods, soups and sauces. In addition, modified BGN starches were thermally stable as they are able to withstand high shear stress and temperatures during processing. BGN starches modified through microwave and chemical modification methods displayed a shear thinning behaviour. On the other hand, those modified through co-evaporation and kneading methods displayed a shear thickening behaviour (a property required for fluids good for ammunition), this then suggests that these starches were not completely modified and as such they have to be pregelatinised before they are used in food products. The hypothesis that the new BGN starch-catechin complex will have improved functional, rheological and thermal properties, compared to the native starch was accepted.

The high antioxidant activity in modified BGN starch-catechin complexes indicates the potential of BGN starch as an antioxidant carrier. This also confirms the successful grafting of catechin to BGN starch, thus resulting in starch with antioxidant properties. Hence, the new BGN starch-catechin complex will act not just as a functional ingredient in food products but will also have health implications to the consumers. It is speculated that the produced BGN starch-catechin complex will have a significant impact in reducing starch digestibility with the view of combating against chronic diseases like obesity, cardiovascular diseases and diabetes. The following conclusions can therefore be drawn from this study:

1. BGN starch is a potential antioxidant carrier.
2. Cyclodextrin (alpha and beta) can be used as initiators in the starch modification process.
3. The functional properties of modified BGN starches make them suitable for use in the food industry as thickeners. Furthermore, the availability and relatively low cost of BGN make BGN starch suitable for use in the food industry for various applications.
4. Using modified BGN starch-catechin complex in the food industry will be beneficial as it will reduce energy costs by using less energy during processing thus owing to their low pasting temperatures, requiring low temperatures to initiate starch gelatinisation.

5. The thermal stability of modified BGN starches makes them suitable for use in food products that require high processing temperatures.
6. Chemical modified BGN starches yielded the best results, among all the methods used to modify BGN starch.
7. Using grafting method is advantageous over the existing starch modification methods, as it involves the incorporation of a new antioxidant functional group to the starch molecule.

The rheological properties of modified BGN starch through co-evaporation and kneading methods displayed a shear thickening behaviour. It is therefore, recommended that further work be done on these two complexation methods so as to yield better results. In addition, the modified BGN starches still need to be used in food systems to assess their behaviour.