Physicochemical, Functional and Structural Properties of Native Malawian Cocoyam and Sweetpotato Starches

By

Davies Emmanuel Mweta

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BLOEMFONTEIN

SOUTH AFRICA

Supervisor: Prof. Maryke T. Labuschagne

Co-supervisors: Dr. Susanna Bonnet
Prof. John D.K. Saka

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DECLARATION

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Davies Emmanuel Mweta Date

DEDICATION

I dedicate this thesis to my late father, Emmanuel Dymon Mweta who believed in ‘education for the better future’ for his children against all the financial hardships that prevailed then. Sadly, he never lived long enough to see his dreams come true and enjoy the fruits of his labour. I also dedicate this thesis to my mother Ellen for the motherly love that brought me into this world and moulded me into who I am today. More specially to my wife Agnes, son Davies Jnr., and daughter Agnes-Alinafe who endured the pain of separation and did not get much attention and love from me for the three years of this study.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
ΔH_G	Enthalpy of gelatinization
ΔH_R	Enthalpy of retrogradation
μL	micro litre
μm	micro meter
λ_{max}	Wavelength of iodine maximum absorption
%	Percentage
%T	Percentage transmittance
% w/w	Percentage by weight
% w/v	Percentage weight by volume
ANOVA	Analysis of variance
BV	Blue value
Ca	Calcium
Con	Concavanalin
cps	Centipoises
db	dry basis
Da	Daltons
DMSO	Dimethyl sulphoxide
DP_n	Degree of polymerization by number
DS	Degree of substitution
DSC	Differential scanning calorimetry
ELSD	Evaporative light scattering detector
ELSD-LT	Low temperature evaporative light scattering detector
FDA	Food and Drug Administration
Fe	Iron
g	gram
$g\ g^{-1}$	gram per gram
g	gravity
GPC	gel permeation chromatography
GOPOD	glucose peroxidase
GSD	Average granule diameter

GSM	Modal granule diameter
h	hour
ha	hectare
H_2O	Water
HCl	Hydrochloric acid
HPLC	High pressure liquid chromatography
HPSEC	High-performance size-exclusion chromatography
H_2SO_4	Sulphuric acid
I_2	Iodine
ISO	International Standards Organization
J g^{-1}	Joules per gram
K	Potassium
KCN	Potassium cyanide
KI	Potassium iodide
LALLS	Low-angle laser light scattering
LSD	Least significant difference
M	Molar
MALLS	Multi-angle laser light scattering
MC	Moisture content
Mg	Magnesium
mg	milligram
mg kg^{-1}	milligram per kilogram
min	minutes
mL	millilitre
mM	millimolar
Mn	Manganese
M_n	Number-average molecular weight
M_w	Average molecular weight
Na	Sodium
Na_2CO_3	Sodium Carbonate
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
P	Phosphorus
PAD	Pulsed amperometric detector

PDI	Polydispersity index
PHI	Peak height index
R	Temperature range
RC	Reducing capacity
RI	Refractive index
rpm	Revolutions per minute
RVA	Rapid Visco-Analyzer
SB	Solubility
sec	Seconds
SEC	Size exclusion chromatography
SP	Swelling power
T_o	Onset temperature
ton	tonnes
T_p	Peak temperature
T_c	Conclusion temperature
v/v	volume by volume
WAC	Water absorption capacity
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

Tropical root and tuber crops, of which cassava, sweetpotato and cocoyam are important representatives, constitute an under exploited resource of developing countries. Many of the developing world's poorest farmers and food insecure people are highly dependent on root and tuber crops as a contributing, if not the principal, source of food, nutrition, and cash income (Scott *et al*., 2000). The principal component of these tropical root and tuber crops is starch, which is increasingly becoming an important raw material for the food and non food industries worldwide. Despite being rich in starch, tropical root and tuber crops have remained underutilized, though starch from these crops could be used in different industrial applications (Wickramasinghe, 2009). The current industrial demand for starch is being met by a restricted number of crops mainly corn, potato and wheat (Ellis *et al*., 1998). Consequently, the world starch market is dominated by starches from these three crops. In order to increase the competitiveness of starches from tropical root and tuber crops on the world markets, unveiling of the characteristic properties of starches from these crops is required (FAO, 1998).

Starch is one of the most important products to man. It is an essential component of food providing a large proportion of daily calorie intake for both humans and livestock. Starch alone accounts for 60-70% of calorie intake of humans (Lawton, 2004). Besides its nutritive value, starch is a very versatile raw material with a wide range of applications in food, feed, pharmaceutical, textile, paper, cosmetic and construction industries. In the food industry, starch is used as a thickener, filler contributing to the solid content of soups, a binder to consolidate the mass of food and prevent it from drying out during cooking, and as a stabilizer. Non-food applications of starch include; adhesives in the paper and packaging industry, match-head binders in explosives, concrete block binders and plywood adhesive in the construction industry, fabric finishing and printing in the textile industry, pill coating and dispersing agents in pharmaceuticals, sintered metal adhesive and foundry core binders in metals, and manufacture of biodegradable plastics and dry cell batteries (Lawton, 2004; Burrell, 2003; Moorthy, 2002; FAO, 1998; Ellis *et*

al., 1998). These applications depend on the functional properties of the starches such as gelatinization, pasting, retrogradation, water absorption capacity, swelling power, and solubility which vary considerably from one botanical source to another (Yuan *et al*., 2007; Peroni *et al*., 2006; Perez *et al*., 2005), and with variety and environmental conditions (Shujun *et al*., 2006; Riley *et al*., 2006; Amani *et al*., 2004; Chen *et al*., 2003; Sefa-Dedeh and Sackey, 2002,). The functional properties are also dependent on composition and structures of the starches which include amylose/amylopectin ratio, phosphorus content, granular size, molecular weight of the starches and chain length distribution of amylopectin (Lu *et al*., 2005; Sasaki and Matsuki, 1998; Fredriksson *et al*., 1998; Shibanuma *et al*., 1996; Jane and Chen, 1992; Tian *et al*., 1991). Therefore, unravelling the potential of starches for use in the food and non-food industries calls for a better understanding of their unique physicochemical, functional and structural properties.

In Malawi, starch is used in the manufacture of various products such as food, textiles, pharmaceuticals, dry cells and adhesives. The industry uses starches, dextrins and cassava substitutes which are imported from Zimbabwe, South Africa, the Netherlands, United Kingdom and Tanzania. The imported starches constitute those of maize, potato and wheat (Masumbu, 2002; Munthali, 2001; Itaye, 2001; Fungulani and Maseko, 2001; NSO, 1999). The importation of starch, dextrins, and cold setting adhesives has lead to loss of large amounts of foreign currency and increased unemployment (Masumbu, 2002; NSO, 1999). Increased costs, supply capacity (transportation), availability, late deliveries and transit damages have also been some of the major challenges facing the industries due to starch importation (Itaye, 2008). Therefore, there is need to investigate new botanical sources of starch for the industry. Exploitation of indigenous crops locally grown by subsistence farmers would ease some of the problems the industry is currently facing and help bring direct economic benefits to those who need it most.

Efforts to find alternative sources of starch for the Malawian industry have led to the development of starch research on tuber and root crops grown in Malawi. Previous studies have focused on starch isolated from cassava (Benesi, 2006; Masumbu, 2002). Masumbu (2002) studied production of cold-setting adhesives using starch and dextrins from cassava. He found that cassava based adhesives have less solid content than commercial ones and their formulation requires less ingredients than commercial ones,

making the cassava-based glues less costly. Benesi (2006) investigated the effect of genotype, location and season on cassava extraction and also the effect of genotype and pyroconversion on physicochemical and functional properties of cassava starch. Starches from different varieties of cassava were analyzed for pH, protein, ash and moisture contents, granule size, shape and functional properties. He found that starch content varied with genotype and season. Mkondezi, Silira, Mbundumali and CH92/08 were high starch yielding varieties and high amounts of starches were extracted during the months October/November and March/April. The quality characteristics of the starches i.e. proteins pH, ash, and moisture content were within the industrial requirements of starch. Light microscopy revealed that the cassava starch granules were mostly round or oval in shape and granular size ranged from medium to small. Upon dextrinization of starch, he found that Silira, 81/00015, Mbundumali and Sauti starches were easily dextrinized and 80% solubility was achieved within 60 min of dextrinisation at 100^{o}C after acidification with 0.1M HCl. Pyrodextrin of Mkondezi cassava starch had similar functional properties to amyl maize starch used in industries. Cassava starches exhibited lower gelatinization temperatures desired for hot-setting adhesives, which leads to energy saving. Differential scanning calorimetry analysis revealed that native starch and pyrodextrins from Malawi cassava genotypes are diverse in functional properties which can meet both general and specialized uses. Native cassava starch from 83350 genotype exhibited functional properties different from the rest of the genotypes but comparable to those of amyl maize starch.

Sweetpotato and cocoyam are two other important root and tuber crops grown in Malawi. Sweetpotato (*Ipomoea batatas* Lam.) is a creeping dicotyledonous plant belonging to the family of *Convolvulaceae*. It is ranked as the 7th most important food crop worldwide and 5th in less developed countries (Kays, 2005). In Malawi, sweetpotato is the second most important root crop after cassava which supplements maize, the staple crop. It is widely grown throughout the country for its sweet tasting tuberous roots and young leaves which are important vegetables. Sweetpotato is currently being promoted in the country because of its low production cost, ability to do well even on marginal soils and semi-drought conditions, highly flexible planting dates and short growing cycle (Chipungu *et al*., 1999). Sweetpotato root tubers have high moisture content and a relatively low dry matter content of around 30%. Approximately 80-90% of the tuber dry matter is carbohydrate,

mainly starch, making sweetpotato roots a good raw material for the starch industry (Wheatley and Bofu, 2000; Woolfe 1992; Tian *et al*., 1991;). Cocoyam (*Colocasia esculenta* L. Schott), a member of the Araceae family, is one of the oldest crops grown for its edible corms and leaves, and as an ornamental planting (Ozerol, 1984). Ranking as the fourteenth most consumed vegetable worldwide; cocoyam is widely grown in tropical and subtropical countries (FAO, 2003). About 60% of the world cocoyam production (5.7 million ton) is in Africa and most of the remaining 40% in Asia and the Pacific (Mitra *et al*., 2007). In Malawi, cocoyam forms part of the diet, but to a lesser extent. Locally known as 'coco' in most parts of Malawi, cocoyam has remained a very minor crop produced by few farmers in selected locations. At times, cocoyams are planted around homesteads as ornamental crops (Sandifolo, 2002). Despite being grown on a smaller scale in Malawi, cocoyam offers an opportunity as a new source of starch for the Malawian industry. The corms of cocoyam are known to have a high content of tiny, easily digestible, starch grains ranging in content between 22 and 40%, making it a good source of starch (Adane *et al*., 2006; Moorthy *et al*., 1993).

Until now, characteristic properties of starches from Malawian sweetpotato and cocoyam have not been determined. This lack of knowledge has limited the use of starch from these crops in various industrial applications. If these crops are to be considered as new sources of starch for the Malawian industry, there is need to investigate and evaluate their physicochemical, functional and structural properties. Such knowledge would unravel the opportunities offered by these root crops and facilitate the utilization of starches from these crops in the industry. Further, a detailed knowledge of the characteristics of these starches would enable tailoring of the properties by physical and/or chemical modification and help Malawi compete effectively on the markets. In the long run, utilization of these starches will save foreign currency, create employment opportunities and bring economic benefit to the local Malawians.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Starch is a naturally occurring polymer of α-D glucose. It is the main energy reservoir of higher plants, and also a major source of dietary energy for humans and animals. Starch is found in leaves of all green plants, in seeds, fruits, stems, roots and tubers of most plants. Starch granules are formed in amyloplasts of higher plants. They are also formed in chloroplasts where they serve as temporary store of energy and carbon (Robyt, 2008; Lawton, 2004; Sivak and Preiss, 1997). Besides its nutritive value, starch is a very useful raw material with a wide range of applications in both the food and non-food industries. Starch application in industrial related products dates back to ancient times. Around 4000 B.C., Egyptians used wheat starch to body papyrus, the earliest writing material, and increase its ability to hold ink. The Chinese started using starch for similar purpose around 100 A.D. The Romans used starch to whiten cloth and powder hair as early as 100 B.C., and around 300 A.D., starch was used to stiffen cloth and was mixed with dyes to colour cloth (Robyt, 2008). Since then, the applications of starch in industries have rapidly increased, increasing its commercial value. Today, some of starch uses include; food additive to control consistency and texture of sauces and soups, to resist the breakdown of gel during processing and increase shelf life of an end product in the food industry, laundry sizing of fine fabrics and skin cosmetics in the textile and cosmetic industry, enhancing paper strength and printing properties in the paper industry, tablet fillers in pharmaceutical industry, and binders in the packaging industry. The most common sources of starch for the food and non-food industry worldwide are maize, potato, wheat and to some extent tapioca (Vaclavik and Christian, 2008; Robyt, 2008; Lawton, 2004; Jobling, 2004; FAO, 1998; Ellis *et al*., 1998). With increasing industrial demand for starches, there is need to explore new and alternative sources of starch. Tropical root and tuber crops could offer this opportunity as these crops are rich in starch (Wickramasinghe, 2009; Hoover, 2001). However, for long their role has mostly been

that of staple food for the world's hot and humid regions and the tropics, and food security crops in the developing countries (Scott *et al*., 2000; FAO, 1998).

The use of starch in various products and manufacturing processes is determined by its functional properties such as gelatinization, pasting, retrogradation, viscosity, swelling and solubility which vary considerably from crop to crop and with ecological and agronomic influences (Yuan *et al*., 2007; Peroni *et al*., 2006; Pèrez *et al*., 2005; Sefa-Dedeh and Sackey, 2002, Shujun *et al*., 2006, Riley *et al*., 2006, Amani *et al*., 2004; Chen *et al*., 2003). The starch functional properties are dependent on composition and molecular structures of the starches which include amylose/amylopectin ratio, phosphorus content, granular size, molecular weight of the starches and chain length distribution of amylopectin (Sasaki and Matsuki, 1998; Lu *et al*., 2005; Fredriksson *et al*., 1998; Shibanuma *et al*., 1996; Jane and Chen, 1992; Tian *et al*., 1991). Therefore characterization of starches for their physicochemical, functional and structural properties is essential in order to unravel their potential for use in the food and non-food industries.

2.2. Chemical composition of starch

Starch consists of two types of molecules, amylose and amylopectin. Normal starches contain 20-30% amylose, the difference being made up by amylopectin. Waxy and high amylose starches contain less than 15% and greater than 40% amylose, respectively (Van Hung *et al*., 2006; Tester *et al*., 2004). However, the relative proportion of amylose to amylopectin may vary from crop to crop and with variety (Shujun *et al*., 2006; Peroni *et al*., 2006; Jane *et al*, 1992). The amylose content values ranging from 13.6-23.8% for cassava, 20-25% for sweetpotato, and 3-43% cocoyam starches have been reported depending on variety (Moorthy, 2002; Tian *et al*., 1991). Peroni *et al*. (2006) found higher levels of amylose in yam (32.6%), canna (31.7%) and ginger (26.5%) starches than in cassava (19.8%), arrowroot (20.8%) and sweetpotato (22.6%) starches. Amylose content of five varieties of taro determined by iodine potentiometric titration and gel permeation chromatography ranged from 18 to 22% and 19 to 24% respectively. Dasheen and Bun-long taro starches gave the highest amylose contents while Hawaii White and Hawaii Red had the lowest (Jane *et al*., 1992). Shujun *et al*. (2006) found amylose

contents ranging from 20.74-25.94% for four different varieties of Chinese yam (*Dioscorea opposita* Thunb.).

The amylose content of starch is one important characteristic that affects its functionality. An increase in amylose content of starch has been found to lower swelling power and solubility of cocoyam and wheat starches (Lu *et al*., 2005; Sasaki and Matsuki, 1998). Collado *et al*. (1999) studied pasting profiles of sweetpotato starches using a Rapid Visco-Analyzer (RVA). They found that higher levels of amylose of sweetpotato starches were associated with low peak viscosity and hot paste viscosity of 11% starch pastes. Fredriksson *et al*. (1998) found that onset and peak temperatures of gelatinization increased with decrease in amylose content. Waduge *et al*. (2006) reported that high amylose barley starches exhibited different responses towards annealing due to differences in the amylose/amylopectin ratio and packing arrangement of the starch chains within the amorphous and crystalline regions of the native granule.

In addition to amylose and amylopectin, starch granules also contain minor non-carbohydrate components: ash (minerals and salts) up to 0.5%; lipids from 0.01 to 0.80%, and proteins, from 0.10 to 0.40%. The most common minerals found in starches are calcium, magnesium, phosphorus, potassium and sodium. These minerals are found in relatively small quantities (<0.4%) and most of these are of little functional significance except phosphorus (Tester *et al*., 2004). Phosphorus is found in three major forms: phosphate monoesters, phospholipids and inorganic phosphates. Root and tuber starches contain phosphorus in the form of mono phosphate esters covalently bonded to starch while phospholipids are predominant in cereal starches. Phosphorus affects starch functional properties as paste clarity, viscosity consistency and paste stability (Jane *et al*., 1996). Higher swelling power and stability of starches observed in potato starches is attributed to higher levels of phosphates (Karim *et al*., 2007). Phospholipids form helical complexes with starches reducing water binding capacity and increasing opaqueness of clarity while phosphate monoesters promote hydrophilic nature, increasing binding capacity, swelling power and paste clarity (Swinkels, 1985). Phosphorus content of starches varies with botanical source. Phosphorus content ranging from 0.09 to 0.025% has been reported for sweetpotato and cocoyam starches (Moorthy, 2002; Jane *et al*., 1992). Peroni *et al*. (2006) reported lower levels of phosphorus in cassava (0.007%) and

ginger (0.007%) starches than sweetpotato (0.014%) and arrowroot starches (0.018%). Yam and canna starches showed higher phosphorous content (0.022 and 0.031%, respectively) than cassava, sweetpotato, ginger and arrowroot starches. Lipids are another component of starch granules that has an influence on starch functional properties as swelling, solubility, paste viscosity and pasting characteristics. High contents of lipids are observed in cereal starches: 0.8-1.2 and 0.6-0.8% for wheat and normal maize, respectively. Root and tuber crops contain very low levels of lipids (Moorthy, 2002; Buléon *et al*., 1998).

In addition to amylose and amylopectin, and non-carbohydrate components, starch absorbs water when in equilibrium with its environment. Starch usually contains 10-15% (w/w) water of hydration. However, moisture content ranging from 6-16% has been reported in literature. The differences in the moisture content have been attributed to the extent of drying. Nevertheless, despite this variation, lower moisture contents are required for safe storage as higher moisture contents can lead to microbial damage and subsequent deterioration in quality (Moorthy, 2002).

2.3 Molecular structure

Both amylose and amylopectin contain polymers of α-D-glucose units and differ in degree of polymerization and branch frequency. Amylose is mainly found as linear chains of about 1500 units of α-D-glucopyranosyl residues linked by α-(1→ 4) units. However, it has also been established that some molecules found in the amylose fraction do contain a few branches [α-(1→ 6 linkages)]. These branches have no influence on the hydrodynamic behaviour of amylose (Wang *et al*, 1998; Sivak and Preiss, 1997). Amylose has a molecular mass of approximately $10^5 – 10^6$ Da, a degree of polymerization (DP) by number of (DP_n) 324-4920 with around 9-20 branching points equivalent to 3-11 chains per molecule. Each chain contains approximately 200-700 glucose residues equivalent to a molecular weight of 32400-113400 Da. The size and structure of amylose molecules vary considerably depending on the botanical source of the starch (Hoover, 2001).

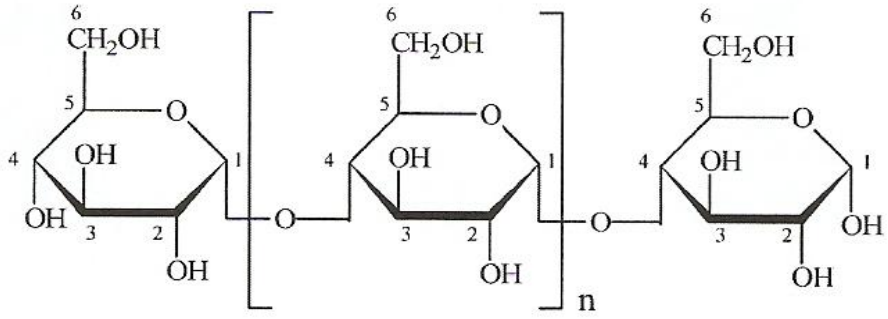

Figure 2.1 The chemical structure of amylose (Herrero-Martinez *et al*., 2004)

Amylopectin comprises 70-80% of starch and is a much larger molecule with a molecular mass ranging from 10^6 to 10^8 Da. Amylopectin has a heavily branched structure built from about 95% (1→4)-α- and 5% (1→6)-α- linkages (Robyt, 2008; Jobling, 2004). Amylopectin chains are relatively short compared to amylose; usually about 18-25 units long on average, and have a broad distribution profile. The presence of branching points allows the short linear chains to pack together efficiently as parallel left-handed double helices, giving rise to the crystalline nature of a starch granule. The DP_n of an amylopectin molecule is within the range 9600-15000 but has three major categories having a DP_n ranging from 13400-26500, 4400-8400, and 700-2100. There are three broad categories of amylopectin chains; A, B and C. The A chains are the shortest, and B chains the longest. The A chains are chains whose reducing ends attach to other B or C chains but do not carry any other chain. The B chains have their reducing ends attached to other B or C chains, and also carry other A or B chains while the C chain is the only chain of the molecule carrying a reducing end. The B chains have different chain lengths and are subdivided into B1-3 groups with B3 group containing the longest chains. Like amylose, the molecular size, shape, structure and polydispersity varies with botanical origin (Tester *et al*., 2004; Jobling, 2004; Jane, 2003; Hoover, 2001; Wang *et al*., 1998; Ellis *et al*, 1998).

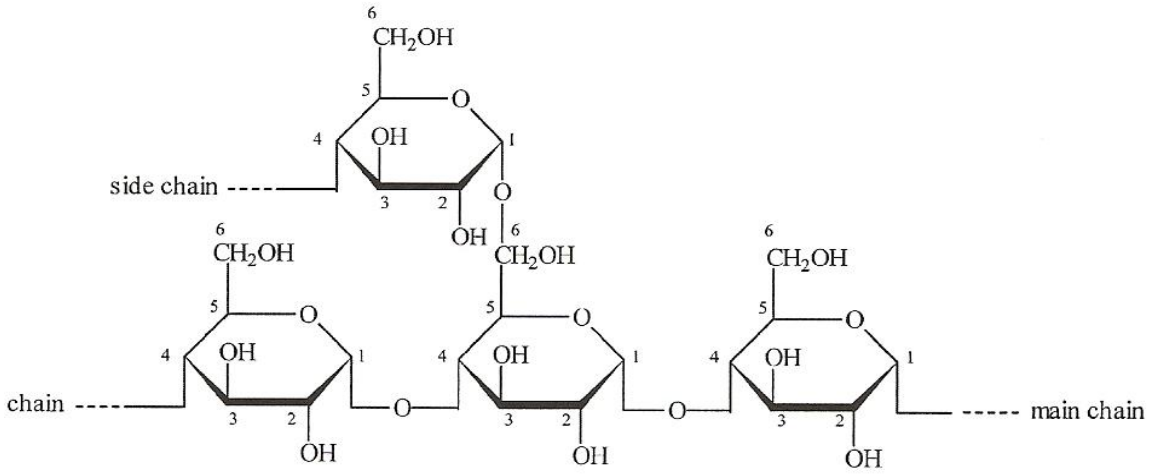

Figure 2.2 The chemical structure of amylopectin molecule (Herrero-Martinez *et al*., 2004)

High-performance size-exclusion chromatography is widely used to determine the molecular mass distribution of amylose and amylopectin, and chain length distribution of amylopectin. Size-exclusion chromatography is a chromatographic technique that separates molecules in solution according to their sizes (Meyer, 2004). High-performance size-exclusion with refractive index (RI), laser light-scattering, and fluorescent labelling2 technique detectors has been used to examine molecular weight distribution of whole and debranched starch, and chain length distribution of amylopectin (Leong *et al*., 2007; Charoenkul *et al*., 2006; Millan-Testa *et al*., 2005; Yoo and Jane, 2002a, b; Bradbury and Bello, 1993; Lehtonen, 1988). Branch-chain length of amylopectin has also been studied using high-performance anion exchange chromatography (HPAEC) coupled with a pulsed amperometric (PAD) detector (Kim *et al*., 2007; Yoo and Jane, 2002a). The refractive index detector is by far the most widely used detector, its main advantage being that any polymer solution generates a response. However, low sensitivity and high sensitivity towards pressure, flow and temperature fluctuations are its main disadvantages. The laser light scattering is a more specialized detector which gives a response that is proportional to molecular weight and concentration. Forms of light scattering detectors include low angle (LALLS), multi-angle (MALLS) and evaporative light scattering (ELSD) detectors (Kazakevich and Lobrutto, 2006).

Studies on molecular structures of starch have shown that the molecular mass of amylose molecules and chain length distributions of amylopectin vary with botanical source. Peroni *et al*. (2006) found that cassava, sweetpotato and arrowroot starches displayed amylose molecules of higher molecular mass than those of yam, canna and ginger starches and canna starch had a higher proportion of longer branch chains of amylopectin than the other starches. Millan-Testa *et al*. (2005) studied the molecular characteristics of okenia, mango, and banana starches. They found that okenia and mango starches had higher molar mass and gyration radii than banana starch. Tetchi *et al*. (2007a) compared macromolecular characteristics of cocoyam, sweetpotato, cassava, sweetpotato, and ginger with those of cereal, legume and other tuber starches. They reported significant differences in average molecular mass and gyration radii of the starches with normal maize, and wheat starch displaying the higher molecular mass, and potato starch the lowest. Ginger and rice starches displayed the lowest gyration radii and normal maize, smooth pea and wheat starches the highest. *Dioscorea esculenta* starches gave higher proportions of short branched (dp 6-12), medium chains (25-36), and average chain length of amylopectin than *Dioscorea alata* starches (Jayakody *et al*., 2007). Analysis of molecular weight and chain-length distribution of amylopectin from waxy, amylose-reduced, and normal hard red winter wheat revealed that waxy wheat amylopectin had the largest molecular weight while hard red winter wheat displayed the highest proportion of long branch-chain amylopectin (Yoo and Jane, 2002). Varietal differences and seasonal variations can also affect molecular structures of starches. Jane *et al*. (1992) found that starches from five varieties of taro exhibited varying peak chain lengths of long-chain and short-chain branches of amylopectin, with Bun-Long variety having the shortest branch chains. Amylopectin polydispersity of apple fruit starch varied significantly between different cultivars with Gala variety having the highest and Granny Smith the lowest (Stevenson *et al*., 2006). Cocoyam starches planted in summer showed significantly higher ratios of short-to-long chains of amylopectin of lower average degrees of polymerization of the chain length than those in winter and spring (Lu *et al*., 2005).

2.4 Morphological characteristics of starch granules

Granule size and size distribution of starch are unique properties of starch that have an influence on the functionality of the starches. For example, rice starches are used for laundry sizing of fine fabrics and for skin cosmetics for their small granule size. Cocoyam starches are used as fillers in biodegradable plastics, and in aerosols because of their small size as well (FAO, 2000). Smaller granules are reported to have higher solubility and water absorption capacity (Tian *et al*., 1991). Smaller granule size was associated with lower RVA peak viscosity temperature while increase in granule size increased higher peak viscosity, breakdown and setback temperatures of potato starch (Zaidul *et al*., 2007a, b).

Microscopic analysis, light and scanning electron microscopy, has been used to study the morphological characteristics of the starch granule. Light microscopy is used for identifying type of starch, and general size and shape of granules from different sources can be observed. Scanning electron microscopy allows the shape and surface of starch granules to be viewed in three dimensions (Thomas and Atwell, 1999). The size and shape of starch granules vary considerably with botanical source. Mishra and Rai (2006) studied the morphology of commercial native corn, potato and tapioca starches using light and scanning electron microscopy. Granule sizes ranged from 14.3-53.6 μm, 3.6-14.3 μm, and 7.1-25.0 μm for potato, tapioca and corn starches, respectively. Potato starch granules were oval/flattened and ellipsoid in shape, while those of corn were polyhedral and those of tapioca were spherical and truncated. A comparison of *Dioscorea nipponica* Makino starch with tapioca and potato starch revealed that *D. nipponica* starch had smaller granule size (9.5±0.2 μm) than tapioca (14.7±0.3 μm) and potato (30.5±0.5 μm) starch (Yuan *et al*., 2007). *D. nipponica* starch displayed mostly oval shaped granules with some sausage shaped while tapioca starch granules were mostly spherical. Bello-Pérez *et al*. (2005) reported mostly lenticular shapes for banana starch granules with an average size of 39 μm. Peruvian carrot starch exhibited spherical and truncated-egg shaped granules with size ranging between 4 and 26 μm (Pérez *et al*., 1999).

Most morphological studies of starches from root and tuber crops have reported cassava starches to be round with a flat surface on one side containing a conical pit which extends

to a well-defined eccentric hilum, truncated, cylindrical, oval, and spherical or compound with granule size ranging from 4 to 43μm. Sweetpotato starch granules usually exhibit round, polygonal, oval, and bell shapes, and their average granule size ranges from 2 to 72 μm. Cocoyam starch has smaller granule size compared to cassava and sweetpotato starches ranging from 1-10 μm. The granules are usually round in shape though polygonal and irregular shapes have also been reported. The surfaces of granules when observed under scanning electron microscopy appear to be smooth (Wickramasinghe *et al*., 2009; Nwokocha *et al*., 2009; Aboubakar *et al*., 2008; Chen *et al*., 2003; Moorthy, 2002; Hoover, 2001; Noda *et al*., 1995). However, size and distribution of starch granules also vary with variety. Goering and DeHaas (1972) observed two distinctly different size ranges of granule size in different varieties of *Colocasia esculenta* starches. This observation was also supported by Moorthy (1993) who reported significant differences in average granule sizes for ten varieties of *Colocasia*. Morphological studies of starches from three Chinese sweetpotato varieties, XuShu18, SuShu2 and SuShu8, also revealed variations in granule size and particle size distributions (Chen *et al*., 2003). XuShu18 starch exhibited the highest granule size (11.6±0.42 μm) and SuShu8 starch the lowest (8.4±0.42 μm). Thus, biological origin influences the morphology of starch granules.

Starch granule size may also be influenced by season. Starches from cocoyam (*Xanthosoma sagittifolium*) and taro (*Colocasia esculenta*) tubers planted in summer exhibited larger granule sizes than those of starches from tubers planted in spring and winter (Lu *et al*., 2008; Lu *et al*., 2005). The exact mechanism affecting the size of starch granules is not very clear (Lu *et al*., 2005), however, the differences in average granule size in different seasons could be attributed to significant differences in soil temperature (Noda *et al*., 2001).

2.5 Crystalline structure

Starch has a definite crystalline nature which is attributed to the well-ordered structure of the amylopectin granules inside the granules. Starch molecules exist as helices and these helices can have different packing arrangements giving rise to different crystalline patterns. Using X- ray diffractometry, the crystalline nature and levels of crystallinity of the starches can be determined. The position of the diffraction peaks defines the crystalline patterns while levels of crystallinity can be obtained by separating and integrating the areas under the diffraction peaks (Zobel, 1988). The two principal crystalline patterns of native starches based on the X-ray diffraction patterns have been classified as A or B. The A-type starches are mainly found in cereals while B- type starches are found mainly in tubers and high amylose starches. A third type of crystalline pattern has been classified as C- type and this pattern is proposed to be a mixture of both A and B types. The C-type pattern is further divided into C_A and C_B. The C_A pattern is type C which is closer or near A while C_B is type C pattern that is closer to B. The C- type starches are mainly found in legumes (Lopez-Rubio *et al*., 2008). A-type starches contain shorter average branch-chain lengths than the C- and B- type starches (Hizukuri, 1985). Native starch granules have absolute crystallinity ranging from 15 to 45%. Type A-starches have higher levels of crystallinity (33-44%) and gelatinization temperatures than B which shows levels ranging from 15-28% and lower gelatinization temperatures (Tian *et al*., 1991).

Starches from different botanical sources exhibit different crystalline patterns (Stevenson *et al*., 2006; Singh *et al*., 2006; Millan-Testa *et al*., 2005; McPherson and Jane, 1999; Hoover *et al*., 1995). Cereal grain starches, such as maize, wheat, and rice usually show typical A-patterns while most root and tuber starches exhibit B-patterns. A-type starches show peaks at 15°, 17°, 18° and 22° 2θ angles while B-type has four main reflection intensities at 5.5°, 17°, 22° and 24 2θ angles. The B-type X-ray pattern of starch is usually characterized by the position and relative peak intensity in the range of 2θ = 5 - 6°, while the absence of the peak of 2θ = 5 - 6° is characteristic of A-type starch. The C-type X-ray pattern reflects at 5.5°, 17.0°, 18.0°, 20.0° and 23.5° 2θ, which is believed to be a superposition of the A- and B-type patterns (Zobel *et al*., 1988). Cassava starch possesses A, C, or a mixed pattern with three major peaks at 2θ = 15.3º, 17.1º and 23.5º.

Sweetpotato starch shows variable X-ray patterns between C and A. Cocoyam starch also exhibits A-type pattern (Moorthy, 2002; Hoover, 2001). Variations in crystalline nature of starches from the same crops have been attributed to variety, sample preparation, growth conditions and maturity of the plant at the time of harvest (Noda *et al*., 1995; Sugimoto *et al*., 1987).

2.6 Functional properties

Application of starch in industries is primarily governed by its functional properties such as viscosity, gelatinization and retrogradation, pasting, freeze-thaw stability, solubility and swelling. According to the FAO (1998), starch can be viewed as a set of functional properties suited to a particular application. It is these functional properties that are major assets to starch marketing. The functional properties of starches vary considerably among starch from different sources and are therefore unique for each starch.

Starch structural characteristics such as molecular weight of amylose and amylopectin, and chain length distribution of the amylopectin also affect the functional properties of the starches. Larger molecular weight of amylose and amylopectin resulted in higher pasting peak viscosity in wheat starches (Shibanuma *et al*., 1996). Jane and Chen (1992) reported that the long-branch chain-length of amylopectin and the intermediate size of amylose produced the greatest synergistic effect on pasting viscosity of reconstituted starch. Lu *et al*. (2005) found that taro starch with a high proportion of short chains and long average chain length of long-chain fraction of amylopectin displayed high elasticity and strong gel during heating. Jane *et al*. (1999) studied the effect of amylopectin branch chain length and amylose content on gelatinization and pasting properties of starches from different botanical sources. They reported low gelatinization temperatures for starches with short average amylopectin branch chain lengths and large proportions of short branch chains. A smaller proportion of long amylopectin chains in tef starch compared to maize starch resulted in lower swelling, percentage crystallinity, and gelatinization temperatures as detected by differential scanning calorimetry (Bultosa and Taylor, 2003) for the tef starch.

2.6.1 Gelatinization and retrogradation

Gelatinization and retrogradation are among the most important functional properties of starches that govern its application. Gelatinization is critical in industrial application of starch as textile resizing industry and for industrial starch hydrolysis as it affects rheology and viscosity properties of the system which makes starch more accessible to enzymatic degradation (Ellis *et al*., 1998). It is also responsible for the thickening of food systems (Vaclavik and Christian, 2008). Gelatinization occurs when starch is heated progressively in excess water. This is common in processed foods where starch is heated in the presence of water resulting in starch gelatinization. A number of stages occur during this process; (i) granules hydrate progressively, (ii) double helices undo as hydrogen bonds are ruptured resulting in crystalline regions being converted into amorphous regions, (iii) granules continue to imbibe water and swell and, (v) ultimately the granule swells so much that granular form is lost and they tend towards gelation and/or solubilization. During this stage, some short chains of amylose come out of the starch granules and a viscous paste is formed (Tester and Karkalas, 2004). The temperature at which starch begins to undergo these changes is known as gelatinization temperature. Gelatinization process is endothermic i.e. requires energy input and the energy requirement varies between granules. Consequently gelatinization occurs over a range of temperatures. On an industrial scale, this energy input for gelatinization is a significant part of processing costs (Ellis *et al*., 1998).

Upon cooling of the starch paste, gelation and retrogradation occur. Gelation refers to the process whereby the amylose component of the starch paste sets and forms gel while retrogradation refers to the occurrence where starch reverts or retrogrades to a more crystalline structure. The gel network is formed as a result of reduced energy resulting in subsequent formation of intermittent hydrogen cross bonds among amylose and reassociation of amylose molecules at random intervals (Vaclavik and Christian, 2008). Retrogradation involves the re-association of molecules resulting in formation of crystalline aggregates and a gelled structure. This involves two or more starch chains forming a simple juncture in the initial stage, and then the chain develops extensively, and the glucose polymer chains in the gelatinized starch start to reassociate in an ordered structure (Thomas and Atwell, 1999). Retrogradation of starch is an important property to

be considered especially when formulating food products, as it affects quality, acceptability and shelf-life of starch containing foods because during retrogradation precipitation, gelation, and changes in consistency and opacity occur (Biliaderis, 1991).

The botanical source of the starch has an influence on the thermal properties of native and retrograded starches. Peroni *et al*. (2006) compared properties of cassava, arrowroot, sweetpotato, yam, canna, and ginger starches. Cassava, arrowroot and sweetpotato starches exhibited lower onset gelatinization temperatures (61.5, 62.6 and 62.8°C, respectively) and enthalpy changes (10.4, 11.3 and 12.9 J g^{-1}, respectively), whereas yam and ginger starches gave the highest onset temperatures (70.7 and 82.4°C, respectively) and enthalpy changes (14.3 and 15.9 J g^{-1}, respectively). Study of retrogradation by DSC revealed higher levels of retrogradation for yam (74.1%) and ginger starches (68.6%) than canna (55.6%), sweetpotato (49.6%), arrowroot (43.3%) and cassava (26.0%) starches. Pérez *et al*. (1998) reported lower onset (56°C), peak (60°C) and conclusion temperatures (73°C) for Peruvian carrot than cocoyam (74, 78 and 87°C, respectively) and potato (66, 69 and 80°C, respectively) starches. However, cocoyam starch exhibited lower gelatinization enthalpy (3.98 J g^{-1}) than Peruvian carrot (4.19 J g^{-1}) and potato (4.64 J g^{-1}) starches. Yuan *et al*. (2007) compared thermal properties of *Dioscorea nipponica* Makino, tapioca and potato starches. They reported higher gelatinization temperatures for *Dioscorea nipponica* Makino starch (67.4, 76.0 and 81°C for onset, peak and conclusion temperatures, respectively) than those of tapioca (64.9, 69.1 and 75.9°C) and potato starches (59.2, 64.1 and 73.0°C). Tapioca starch had lower enthalpy of gelatinization (14.8 J g^{-1}) than *D. nipponica* Makino starch (18.6 J g^{-1}) while potato starch had the highest energy change (23.4 J g^{-1}). Van Hung and Morita (2005) compared starch from edible canna with cassava, potato and sweetpotato starches grown in Vietnam. They reported higher transition enthalpy for edible canna (14.5 J g^{-1}) starch than potato (14.1 J g^{-1}), cassava (12.4 J g^{-1}) and sweetpotato (12.3 J g^{-1}) starches. Canna starch had a gelatinization temperature range (67.4-76.1°C) similar to that of cassava (66.9-77.0°C) and potato (64.9-76.4°C) starches but higher than that of sweetpotato starch (57.4-74.5°C).

Thermal properties of both native and retrograded starches also vary considerably with variety and environmental conditions. Jane *et al*. (1992) found varying thermal properties

of native and retrograded starches from five different varieties of taro (Bun-long, dasheen, Hawaii red, Hawaii white and Niu'e) with Bun-long showing consistently lowest gelatinization temperatures both in its native and retrograded forms. Chen *et al*. (2003) reported differing retrogradation tendencies for the three varieties of Chinese sweetpotato starches. Singh *et al*. (2004a) reported different gelatinization temperatures and enthalpy of gelatinization for six different varieties of Indian chick pea (*Cicer arietinum* L.). These differences were attributed to differences in amylose content (amylose/amylopectin ratio), size, form and distribution (compactness) of starch granules, size of the starch molecules, and internal arrangement of starch fractions within the granule. Early planting and harvesting increased onset and peak temperatures for sweetpotato starches indicating the influence of environmental conditions (Noda *et al*., 1997). Cocoyam starches planted in the summer season exhibited lower values of onset and peak temperatures and enthalpies of gelatinization than those planted in winter and spring (Lu *et al*., 2005).

Differential scanning calorimetry (DSC) has been used extensively to determine the gelatinization characteristics of starches. Gelatinization temperatures of starches depend on starch/water ratio as well as heating rate (Calzetta-Resio *et al*., 2000). Excess water is essential for complete gelatinization of the starches. To this end, usually a starch:water ratio of 1:3 is used to reach maximum value of enthalpy of gelatinization (Resio and Suarez, 2001). Using DSC, thermal properties, onset (T_o), peak (T_p) and conclusion temperatures (T_c), and gelatinization enthalpy (ΔH_g) of starches can be determined. Gelatinization temperature provides a qualitative measure of crystalline structure and is characteristic of genotype of the plant. Gelatinization temperature may also be influenced by environmental conditions. It is known to vary with amylose/amylopectin ratio, crystallinity, granule size and distribution, and quantity of smaller components like phosphorus, lipids and proteins (Goñi *et al*., 2008; Pérez *et al*., 2005). Starch retrogradation can also be studied using DSC. This involves gelatinization of starch using a DSC heating programme to measure the enthalpy of gelatinization, then storing gelatinized samples under retrogradation conditions, normally at 4°C (refrigeration). The samples are then rescanned at periodic intervals using the same heating programme as for the gelatinization. Degree of retrogradation is determined by calculating the ratio of the enthalpy of retrogradation to that of gelatinization, usually expressed as a percentage. Other methods to determine starch retrogradation include turbidometry and measurement

of syneresis. In turbidometry, a change in light transmittance of stored starch gels is measured with time. The turbidity arises from molecular associations that occur in the early stages of retrogradation. When starch gel is stored for a long time either refrigerated or frozen, there is a gradual increase in rigidity as crystallites begin to form, and phase separation between polymer and solvent occurs. This phenomenon is known as syneresis, otherwise known as 'weeping' or 'watering out' (Karim *et al*., 2000). Freeze-thaw stability expressed as percentage syneresis is also used as an indicator of starch retrogradation. Measurement of freeze-thaw stability involves freezing starch gel for a particular period, during which phase separation occurs. The frozen gel is then thawed and the water expelled from the gel is measured gravimetrically and expressed as percentage of the starch gel (Karim *et al*., 2000; Zheng and Sosulski, 1998). The freeze-thaw stability has been of interest since it plays critical roles in stability of frozen starch based foods (Thomas and Atwell, 1999). Freeze-thaw stability provides a measure of the ability of a product to withstand cold temperature cycling and/or prolonged storage at low temperatures of starch gels.

Retrogradation studies by DSC is considered advantageous over other methods as it is relatively simple, applicable over a wide range of water content allowing no change in water content over time as the sample pans are perfectly sealed, not time consuming and can be used with smaller sample sizes. It allows direct determination of energy required to melt retrograded starch. High initial capital and running costs are its major disadvantages (Karim *et al*., 2000; Nakazawa *et al*., 1985).

2.6.2 Paste clarity and viscosity

Clarity, stability and viscosity of starch pastes are very important characteristic of starches which determine their application in the food, textile, paper and the adhesive industry (Moorthy, 2002). Where starches are used as ingredients of starch-based food products for example as thickeners, gel clarity becomes a desirable function as it directly influences brightness and opacity in foods. For example, starches used to thicken fruit pies are preferably transparent while those in salad dressings are opaque. Starches with clear pastes are also preferred for combining with other colouring agents (Craig *et al*., 1989). Stability of starch paste is also important attribute of starch as it assists in

controlling consistency and texture of food products such as sauces and soups thereby extending shelf life of the products (FAO, 1998).

Paste clarity, just like other functional properties of starch, varies with its source (Tetchi *et al*., 2007b; Singh *et al*., 2003; Craig *et al*., 1989). Potato starches have higher paste clarity (96%T) than corn (31%T), wheat (28%T) and rice (24%). Craig *et al*. (1989) compared clarity of potato, tapioca, wheat, and corn starch pastes among others. They reported higher paste clarity for potato (96%T), than for tapioca (73%T), wheat (62%T) and corn (41%T) starches. Tetchi *et al*. (2007b) also found that potato starch pastes were more transparent (79%T) than cassava (47%T), sweetpotato (17%T) and cocoyam (16%T) starch pastes. These differences in paste clarity have been attributed to differences in chemical composition such as phosphate and amylose. Potato starch pastes have higher paste clarity than cereal starches due to high content of phosphate monoesters as opposed to higher contents of phospholipids in cereal starches. Phospholipids present in starches form complexes with amylose and long chain fractions of amylopectin resulting in limited swelling and hence lower light transmittance. On the other hand, phosphate monoesters covalently bond to amylopectin fraction and due to repulsions between phosphate groups on adjacent amylopectin groups swelling is enhanced and hence light transmittance (Singh *et al*., 2003; Craig *et al*., 1989). Amylose reorganisation forms aggregates that reduce light transmittance of starch pastes (Tetchi *et al*., 2007b). High amylose starches reassociate more readily amylopectin starches thereby resulting in more opacity (Bultosa *et al*., 2002).

One of the major functions of starch in the food industry is to impart viscosity to food products. This makes viscosity an important characteristic to be considered in processing of food products. Viscosity is temperature dependent, decreases by heating and increases when the starch pastes are cooled. Starches from different sources differ in their viscosity characteristics. Oladebeye *et al*. (2009) found that sweetpotato starch paste exhibited higher viscosity values than that of red cocoyam. Yuan *et al*. (2007) compared viscosity of starches from yam (*Dioscorea nipponica* Makino), cassava and potato using a Brabender viscograph. They observed lower peak viscosity, higher setback and lower breakdown viscosity for yam starch compared to potato and cassava starch. Like paste clarity of the starches, differences in chemical composition account for the variations in

paste viscosity of different starches. High phosphate monoester content is known to increase paste viscosity while an increase in phospholipids results in lower paste viscosity (Singh *et al*., 2003). Viscosity variations also exist between different vareties of crops. Sefa-Dedeh and Sackey (2002) reported varying paste characterisics between three varieties of cocoyam (*Colocasia esculenta*, red and white *Xanthosoma sagittifolium*). *Colocasia esculenta* starch exhibited lower hot paste viscosity but higher thermal stability when compared to *Xanthosoma* starches. *Colocasia esculenta* starches from Hawaii Red and Hawaii White varieties gave the highest peak viscosities, whereas Bun-long starch had the lowest (Jane *et al*., 1992).

2.6.3 Swelling and solubility

Swelling and solubility provide evidence of non-covalent bonding between starch molecules and therefore allow comparison of relative bond strength at specific temperatures (Moorthy, 2002). Swelling and solubility of starch occurs when starch is heated in excess water resulting in a disruption of crystalline structure due to breaking of hydrogen bonds. The hydroxyl groups of amylose and amylopectin are then exposed and the water molecules become bonded to these hydroxyl groups through hydrogen bonding resulting in an increase in granule swelling and solubility (Hoover, 2001). Thus, swelling and solubility of starch is temperature dependent, increasing with increasing temperature due to weakening of internal associative forces maintaining the granular structure (Peroni *et al*., 2006). Swelling and solubility of starch vary with botanical source. Peroni *et al*. (2006) reported lower swelling power for yam and ginger starches compared to that of cassava, arrowroot and sweetpotato starches. The swelling capacity and solubility of *Disocorea nipponica* starch was reported to be lower than that of tapioca and potato starches (Yuan *et al*., 2007) while that of red cocoyam starch was higher than that of sweetpotato starch (Oladeyebe *et al*., 2009). Swelling and solubility are influenced by chemical composition such as amylose/amylopectin ratio, phosphate and lipid contents, granular morphology, and the structural characteristics of amylose and amylopectin. Swelling power is reported to increase with increasing long chains of amylopectin and decreasing amylose content (Sasaki and Matsuki, 1998). Amylose in the presence of lipids forms insoluble complexes limiting swelling and solubility of the starch (Tester and Karkalas, 1996). High contents of phosphate monoesters result in increased swelling and

solubility of starches due to increased repulsions between phosphate groups on the adjacent amylopectin molecules (Singh *et al*., 2003). Starches with large granules also display higher swelling power but lower solubility than starches with smaller sized granules (Kaur *et al*., 2002).

2.7 Starch modification

Starches in their native form have limited application in industries due to extreme conditions like pH, temperature and shear during processing. Native starches have low shear stress resistance, thermal decomposition, high retrogradation, and syneresis (Fleche, 1985). In order to promote utilization of starches and widen their application, there is a need to improve their functional properties. Such improvement can be achieved through physical or chemical modification. Modification changes starch properties and greatly extends the range of starch applications. Modified starches usually have functional properties that native starches do not provide (Jobling, 2004).

2.7.1 Physical modification

Physical modification of starch is achieved without the use of chemicals. There are two types of physical modifications: pregelatinization and heat moisture treatment. Pregelatinized starch is starch that has been gelatinized and then dried. Pregelatinization methods include roll drying, extrusion and spray drying. Pregelatinized starches are used as thickeners in foods that require minimal heating. Two types of heat-moisture treatments exist; heat-moisture and annealing. Heat-moisture treatment involves heating starch at above its gelatinization temperatures but with insufficient moisture levels (<35%) to cause gelatinization, while annealing involves heating starch in excess water (>60%) for prolonged periods but at temperatures below gelatinization (Vaclavik and Christian, 2008; Thomas and Atwell, 1999).

Both annealing and heat-moisture treatment have been shown to change functional properties of native starches. Annealing and heat-moisture treatment decreased swelling and solubility of red sorghum starch but increased water absorption capacity and pasting temperature of the starch (Adebowale *et al*., 2005). Physical modifications can also change structural, thermal and pasting properties of starch. Heat-moisture treatment of

potato and corn starches decreased granular crystallinity of potato and corn starch and increased enthalpy and onset temperature of the relaxation endotherm. However, no significant differences in degree of retrogradation were observed (Lim *et al*., 2001). Heat-moisture treatment of sago starch resulted in a change of the X-ray diffraction pattern from C-type to A-type, a decrease of Rapid Visco-analyzer peak and breakdown viscosity, and an increase in final viscosity pasting temperature, DSC onset, peak and conclusion temperatures of gelatinization (Pukkahuta and Varavinit, 2007). Singh *et al*. (2005) also found a decrease in peak viscosity and an increase in cold paste viscosity following heat-moisture treatment of sweetpotato starches. However, there was a decrease in onset and gelatinization enthalpy. This indicates that different starches may respond differently to physical modification. Jayakody *et al*. (2009) found that starches from different cultivars of yam (*Dioscorea* spp) responded differently towards annealing. Annealing caused a decrease in crystallinity and increase in set-back viscosity in Nattala cultivar but an increase in crystallinity and a decrease in setback viscosity in Raja-ala cultivar. These differences were influenced by the different chemical composition and molecular structure of the starches.

2.7.2 Chemical modification

Chemical modification of starches involves three processes; cross linking, stabilization (substitution), and conversion. Cross linking improves process tolerance such as heating, stirring, pumping and packing. Cross linked starches are prepared by reacting an alkaline slurry (pH 7.5-12) of granular starch (30-45%) with a cross-linking reagent, phosphorus oxychloride or sodium trimetaphosphate, in the presence of a salt at temperatures between 25 and 50°C. Stabilization helps reduce retrogradation tendencies of starch. Stabilized starches are prepared by acetylation (esterification) and hydroxypropylation. Acetylated starch is prepared by slow addition of acetic anhydride or vinyl acetate to a starch slurry at pH 7.5-9.0, while hydroxypropylation involves the reaction between propylene oxide with starch in an aqueous alkaline slurry. After the reactions the pH of acetylated and hydroxypropylated starch slurries is adjusted to 4.5 and 5.5 respectively and starch is recovered by filtration and washed.

Conversion is mainly employed to produce starches with reduced molecular weight, hence reduced viscosity of starch pastes. Three types of reactions are involved in this process; acid hydrolysis, oxidation and pyroconversion or dextrinization. Acid hydrolysis involves addition of an aqueous solution of an acid, usually sulphuric (H_2SO_4) or hydrochloric acid (HCl), to starch and incubating for a certain period of time. During this process α-1,-4 and α-1,-6 glycosidic linkages are hydrolyzed. Oxidation of starch is carried out by slowly adding an oxidizing agent, sodium hypochlorite, to alkaline starch slurry. After completion of the reaction the starch slurry is neutralized and washed. Pyroconversion or dextrinization involves dry roasting of acidified starch (Thomas and Atwell 1999; Rutenberg and Solarek, 1984).

Chemical modification alters properties of native starches and the changes in starch properties depend on the modification method employed. Aziz *et al*. (2004) studied changes in properties following etherification and esterification. They found that acetylation increased gelatinization temperature but reduced enthalpy of gelatinization while hydroxypropylation reduced both gelatinization temperature and enthalpy. Chemical modification of Nigerian new cocoyam (*Xanthosoma sagittifolium*) starch by oxidation, acetylation and acid thinning improved the paste clarity of the starches due to chemical substitution of the hydroxyl groups in starch molecules by the acetyl moiety, carbonyl and carboxyl functional groups. This causes repulsion between adjacent starch molecules and apparently reduces interchain association, which facilitates improved light transmittance. Gelation capacity, pasting temperature, set-back tendency, differential scanning peak temperature, enthalpy of gelatinization and retrogradation tendency decreased after acetylation and oxidation reduced but increased after acid thinning. Acetylation improved swelling capacity and reduced solubility while the opposite was observed for oxidation and acid-thinning (Lawal, 2004). Acetylation, oxidation and acid thinning of bean (*Canavalia ensiformis*) starch reduced ash content, protein, fat, fibre, amylose and pH. These reductions were attributed to degradations of starch during the chemical modification process. Trends in changes in enthalpy of gelatinization, retrogradation tendency and swelling power were similar to those observed for modification of new cocoyam, however, chemical modification of jack bean starches by all methods improved solubility (Lawal and Adebowale, 2005). Dihydroxypropylation of cassava starch grown in Brazil increased the swelling power, viscosity and freeze-thaw

stability, and reduced retrogradation tendencies of the starches, thus improving their application in the food industry (Schmitz *et al*., 2006). Hydroxypropylation of banana starch also increased water binding capacity, improved paste clarity and decreased temperature of gelatinization, while phosphorylation improved the freeze-thaw stability (Waliszewski *et al*., 2003).

Starches from different sources may also respond differently to chemical modification. Acid hydrolysis of corn, potato and rice starches decreased the longer chain fractions and increased short chain fraction for corn and rice starches, but increased the longer chain and decreased the shorter chain fractions for potato starches. Gel strength was higher for potato and lower for corn and rice starches (Wang and Wang 2001). Acid hydrolysis of cassava starch from Nigeria increased pasting (gelatinization) temperature, viscosity of cassava starches and produced more stable pastes (Ahmed *et al*., 2005) while acid modification of starch from *Canavalia ensiformis*, a Mexican jack bean, resulted in reduction of viscosity, swelling power and retrogradation tendency, and an increase in solubility. No effect on gelatinization temperatures was observed (Betancur and Chel, 1997). Onset temperature and enthalpy of gelatinization of sweetpotato starches grown in India significantly decreased following acid hydrolysis but peak viscosity and cold paste viscosities of the starches remained unchanged (Singh *et al*., 2005), while increases in onset, peak, and conclusion temperatures but decrease in enthalpy were observed in acid-hydrolyzed rice starches (Thirathumthavorn and Charoenrein, 2005). Though acetylation of corn and potato starches decreased transition temperatures and enthalpy of the starches, greater changes were observed in potato starch compared to corn starch (Singh *et al*., 2004b).

2.8 Uses and sources of starch

2.8.1 Industrial uses of starch

Starch is increasingly becoming an important raw material for the food and non-food industry. Some of the starch industrial uses are listed in Table 2.1.

Table 2.1 Some industrial applications of starch

Food Applications	
Products	**Use**
Frozen foods	Resist breakdown of gel during processing and long shelf-life of end product. Stabilizer due to high water-holding capacity.
Dressings, soups and sauces	Gelling agents in producing jellies (thickeners). Opacity agents to control consistency and texture.
Cereals and snacks	Oil retention agents to create crisp, hamper penetration of cooking oils leading to low fat-intake.
Confectionery	Manufacture of gums, pastes, and other types of sweets.
Non-food applications	
Adhesives	Hot-melt glues, stamps, bookbinding, envelopes, labels.
Explosives	Match-head binder.
Paper	Paper coating, disposable diapers.
Metals	Sintered metal adhesive, foundry core binder.
Textiles	Fabric finishing, printing; stiffening agent.
Cosmetics	Make-up, face creams.
Pharmaceuticals	Pill coating, dispersing agents.
Mining	Ore floatation and sedimentation.
Others	Biodegradable plastic film, dry cell batteries.

FAO (1998)

Utilization of starch in both the food and non-food industries depends on its physical, chemical and functional properties. These properties are unique for different crops and

varieties. Therefore, understanding the physicochemical and functional properties of starch from different sources can help in utilization of starch for the different applications.

2.8.2 World sources of starch

There has been an increasing demand for starch in food and non-food applications worldwide, hence starch production has followed suit. Worldwide, starch production is estimated to have increased three-fold over the last 20 years; 54% goes into food applications while the rest (46%) finds its way into non-food applications. The paper industry is the largest non-food end-user of starch (de Bragança and Fowler, 2004). The most common sources of commercial starches are corn (maize), wheat and potato. By far, maize (*Zea mays* L.) is the most important source of starch for industrial applications accounting for more than 80% of the worldwide produced starch, most of which is produced in the USA (Lawton, 2004). The USA contributes more than 50% of the world starch. Europe is the major producer of wheat and potato starches, contributing about 17% of the world starch. Cassava or tapioca is also another important source of starch, mainly produced in Asia (Srinivas, 2007; Jobling, 2004).

2.8.3 Starch demand, sources and constraints in Malawi

In Malawi, starch has found its application in the paper, food, confectionery and adhesive industries. There is an increasing demand for starch in the Malawian industry and currently the demand is estimated at 1000 metric tons annually (Itaye, 2008). Until now, industries relied on imported starch from Zimbabwe, South Africa, the Netherlands, United Kingdom and Tanzania. These imported starches largely constitute those from maize, potato and wheat. This importation has led to loss of foreign currency and economic benefit for the local Malawians (Masumbu, 2002; Munthali, 2001; Itaye, 2001; Fungulani and Maseko, 2001; NSO, 1999). Increased costs, supply capacity (transportation), availability, late deliveries and transit damages have also been some of the major constraints facing the industries due to starch importation (Itaye, 2008). Therefore there is a need to find new sources of starch for the Malawian industry.

2.8.4 Starch research in Malawi

Efforts to find alternative sources of starch for the Malawian industry have led to the development of starch research in Malawi. Previous studies have focused on starch from cassava (Benesi, 2006; Masumbu, 2002). Masumbu (2002) studied the production of cold-setting adhesives using starch and dextrins from cassava. He found that cassava based adhesives have less solid contents than commercial ones and their formulation requires less ingredients than commercial ones, making the cassava-based glues less costly. Benesi (2006) investigated the effect of genotype, location and season on cassava extraction and also the effect of genotype and pyroconversion on physicochemical and functional properties of cassava starch. Starches from different varieties of cassava were analyzed for pH, protein, ash and moisture contents, granule size, shape and functional properties. He found that starch content varied with genotype and season. Mkondezi, Silira, Mbundumali and CH92/08 were high starch yielding varieties and a percentage of starches were extracted during the months October/November and March/April. No proteins were detected, pH ranged from 5.0 to 5.5, ash content from 0.11 to 0.20% and moisture content from 10.47 to 12.83%. Ash and moisture contents were lower than the recommended values (0.5% and 14% respectively) while the pH was within the recommended values (4.5 to 7.0). The size of the granules ranged from medium to small and they were round or oval in shape. He further reported that Silira, 81/00015, Mbundumali and Sauti were easily dextrinized and 80% solubility was achieved within 60 min of dextrinisation at 100^{o}C after acidification with 0.1M HCl. Pyrodextrin prepared from Mkondezi starch after 40 min had similar functional properties to amyl maize starch used in industries. Cassava starches had lower gelatinization temperatures desired for hot-setting adhesives since less heating is required to gelatinize cassava starch, which leads to energy saving. Differential scanning calorimetry analysis revealed that native cassava starch and cassava pyrodextrins from Malawi cassava genotypes are diverse in functional properties which can meet both general and specialized uses. Functional properties of 83350 native starches were different from that of the rest of the genotypes, but were very close to amyl maize starch being used in Malawian industries.

Following these studies, there have been attempts to use cassava starches in the Malawian industries. However, production capacity has remained very low (IITA-SARRNET, 2008) failing to meet the industrial demand. If starch production is to be increased to

meet the industrial demand, and bring economic benefit to Malawians, there is a need to explore other sources of starch which could supplement the existing sources. Sweetpotato and cocoyam offer this opportunity as alternative sources of starch for the Malawian industry.

2.9 Background information of sweetpotato and cocoyam plants

2.9.1 Sweetpotato

Sweetpotato (*Ipomoea batatas* Lam.) is a creeping dicotyledonous plant belonging to the family of *Convolvulaceae*. It is one of the world's most important food crops with an annual production of about 120 million tons. Sweetpotato is ranked as the 7th most important food crop worldwide and 5th in the less developed countries. High yield, nutritional value, production geography, length of production cycle and resistance to production stresses like high temperature and water deficit are some of its positive attributes when contrasted with other major staple food crops (Kays, 2005). Sweetpotato is thought to have originated from Central or South America. It is mainly cultivated in developing countries in Asia, Africa and Latin America with China accounting for about 85% of total world production (Woolfe 1992).

In Malawi, sweetpotato is the second most important root crop after cassava, which supplements maize, the staple crop. It is widely grown throughout the country for its sweet tasting tuberous roots and young leaves, which are important vegetables. Sweetpotato is currently being promoted in the country because of its low production costs, ability to do well even on marginal soils and semi-drought conditions, highly flexible planting dates and short growth cycle (Chipungu *et al*., 1999). Apart from being used as food crop, sweetpotato roots have found their way to produce markets in rural, urban and peri-urban centres, thus acting as a source of income for the local Malawians (Sandifolo, 2002).

Sweetpotato root tubers have a high moisture content and a relatively low dry matter content of around 30%. Approximately 80-90% of the tuber dry matter is carbohydrate, mainly starch. This makes sweetpotato roots a good raw material for the starch industry

(Wheatley and Bofu, 2000; Woolfe, 1992; Tian *et al*., 1991). Sweetpotato starch has unique characteristics and is mostly used by the food industry as an ingredient in products such as cakes, breads, biscuits, cookies and noodles. The starch is also processed into glucose syrups and various chemicals through enzymatic, microbial and chemical processes. Sweetpotato starch has also found application in non-food industries. It is used for sizing textiles and papers, for the manufacture of adhesives and in laundries (Ellis *et al*., 1998).

2.9.2 Cocoyam ('coco')

Cocoyam (*Colocasia esculenta* L. Schott), a member of the Araceae family, is one of the oldest crops grown for its edible corms and leaves, and as an ornamental plant (Ozerol, 1984). It ranks fourteenth as vegetable worldwide and is widely grown in tropical and subtropical countries. According to FAO (2003), 9.22 million ton of cocoyam were produced from 1.57 million hectares covering South East Asia, Pacific Islands, Hawaii, Philippines, Africa, West Indies and certain areas of South America (Sajeev *et al*., 2003). About 60% of the world production (5.7 million ton) is in Africa and most of the remaining 40% in Asia and the Pacific (Mitra *et al*., 2007). It is believed to have originated from Asia and was taken to the Mediterranean region in biblical times, and then spread to West Africa. It is an important staple crop throughout the Pacific region (commonly known as taro or dasheen), and in the West Indies and West Africa where corms are roasted, baked, or boiled (Hill, 2008).

Cocoyam is another root crop of Malawi forming part of the diet of Malawians but to a lesser extent. It is locally known as 'coco' in most parts of Malawi, however it is also known by other names in other parts of the country most probably due to different tribes and languages; for example it is known as 'Masimbi' in the north of Malawi which is dominated by the Tumbuka tribe and 'Zigumbwa', and 'Dumbe' among the Yao speaking people of Mangochi and Machinga districts. In Malawi, the corm is usually boiled for food. In other parts of northern Malawi, the young cocoyam leaves are eaten as a vegetable known as 'Ntembe'. Cocoyam has remained a very minor crop produced by a few farmers in selected locations. At times cocoyams are planted around homesteads as ornamental crops (Sandifolo, 2002). There is little information about cocoyam in Malawi

as research on this plant is in its primary stage. Despite being grown on a smaller scale in Malawi, cocoyam offers an opportunity as a new source of starch for the Malawian industry. The corms of cocoyam are known to have high content of tiny, easily digestible starch grains ranging between 22 and 40% and therefore make it a good source of starch (Adane *et al*., 2006; Moorthy *et al*., 1993).

2.10 Study area

Malawi lies south of the equator between latitude 9°45' and 17°16' south and between longitude 33° and 36° east. It is a land-locked country and shares boundaries with Tanzania in the north and north-east, Mozambique in the East, South and South-West and Zambia in the West (Leipizig, 1996). Malawi has a population of 13.1 million, 85% of which live in rural areas.

Malawi has two main seasons, the dry season, between May to October and wet season which occurs from November to April with rainfall between 635 mm to 3050 mm. Low rainfall is observed in the low lying areas and high rainfall on high altitude and plateau areas. Most soils in Malawi are leached and they are classified as ferralitic, ferruginous, ferisols and lithosols. Agriculture forms the backbone of the country's economy contributing over 40% of the Gross Domestic Product (GDP) and accounting for about 80% of the foreign exchange. Tobacco, sugarcane and tea, which are the three main cash crops, account for over 80% of the total exports. Maize is the most important staple food crop and occupies 68% of the crop land. Sorghum, cassava, rice and millets are the other important staple food crops.

Two main systems of agriculture prevail in the country: smallholders occupy 70% of the cultivated area under customary land tenure and the estate sector under the leasehold land tenure utilizes about 5% of the cultivated area. Family land holdings range from 0.5 to 2.5 ha. The smallholder sector produces about 80% of the total agricultural products mostly for subsistence, while the estate sector produces mostly cash crops. Of late, the country has been experiencing drought spells and smallholder farmers are encouraged to grow drought-tolerant crops like cassava, sweet potato and sorghum to counter the very erratic rainfall.

2.11 Justification for the research project

Starch is a very versatile raw material with a wide range of applications from dietary needs for humans to industries (food, plastic, cosmetics, pharmaceutical, confectionery, textile, paper and adhesive). The different applications of starch and its products in industry largely depend on the physicochemical, functional and structural properties of starch and its products. There is great diversity in the structure and characteristics of native starch granules. Botanical source, variety and environmental conditions all play great role in influencing these starch properties.

For years, the Malawian industry has relied on imported maize, potato and wheat starches for use in various applications. This importation has led to loss of large amounts of foreign currency and employment opportunities for the local Malawians. Though demand for starch is ever increasing, the Malawian industries face problems due to increased costs, supply capacity, availability and late deliveries. There is a need therefore, to explore indigenous crops locally grown by subsistence farmers, as alternative sources of starch. Such attempts have so far focussed on cassava. Sweetpotato and cocoyam, just like cassava, have great potential for this purpose, yet their utilization in diversified forms has been very limited due to lack of information on its characteristic properties. Therefore, evaluation of the functional, structural and physicochemical properties of the starches from Malawi sweetpotato and cocoyam is a major step in the positive direction. A detailed knowledge of the characteristics of these starches would facilitate their utilization in industries; enable tailoring of the properties by physical and/ or chemical modification to specific applications and bringing economic benefit to the local Malawians in the long run. The aim of this study was therefore to determine the physicochemical, functional and structural properties, and establishing the structure-function relationship of the starches from sweetpotato and cocoyam grown in Malawi. Furthermore, the study was also aimed at evaluating the functional properties of acid-thinned, acetylated and annealed starches from Malawi sweetpotato and cocoyam.

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CHAPTER 3

GRANULAR MORPHOLOGY AND CRYSTALLINE NATURE OF NATIVE MALAWIAN COCOYAM AND SWEETPOTATO STARCHES

3.1 Introduction

Starch granule shape, surface, size and size distribution are important factors that influence functional properties of starch such as gel clarity, swelling power, water binding capacity and solubility (Singh *et al*., 2003; Singh and Singh, 2001; Tian *et al*., 1991), and therefore are important for specific industrial application (FAO, 1998). Starch granules differ in shape and size depending on botanical source, variety as well as environmental conditions (Wickramasinghe *et al*., 2009; Lu *et al*., 2008; Yuan *et al*., 2007; Peroni *et al*., 2006; Noda *et al*., 2001; Tester and Karkalas, 2001). Generally, most starch granules are oval, round, spherical, polygonal and irregular in shape. The starch granule size may range from 1 to 110μm (Jobling, 2004; Moorthy, 2002; Hoover, 2001), however, there are no precise categories of granule size (Lindeboom *et al*., 2004).

Using X-ray diffraction technique, starches from different botanical sources can possess A-, B- or C- type of X-ray diffraction pattern. The C-pattern is considered to be a mixture of A- and B-types. The A-type is characteristic of cereals while B-type is characteristic of tubers. The C-type has been observed in arrowroot, pea and tapioca starch (Chandrasekaran, 1998; French, 1984). Another type is the V-crystalline nature that arises from single amylose helices, some of which complex with granular lipids (Lopez-Rubio *et al*., 2008). The A-type shows refletions centered 15°, 17°, 18°, 20° and 23° while reflections at 5.5-5.6°, 14.1°, 15°, 17.0°, 19.7° and 22° are characteristic of B type (Ohwada *et al*., 2003). A- and B-type crystal patterns share some peaks (2θ = 15°, 17° and 20°), however in such instances the absence of a peak at 5.5-5.6 2θ° is indicative of the A-type pattern. The C-type crystallinity is characterized by peaks at 2θ = 15.2°, 17.2° and 23.2° while peaks at 2θ = 20°, 7° and 13° indicate the V-type crystallinity (Jayakody

et al., 2007; Huang *et al*., 2007; Godet *et al*., 1995). Botanical source, variety, envronment and stage of maturity are known to influence the crystalline nature of starch granules (Stevenson *et al*., 2006; Singh *et al*., 2006; Pérez *et al*., 2005; McPherson and Jane, 1999; Noda *et al*., 1995; Hoover *et al*., 1995; Sugimoto *et al*., 1987).

Since botanical source, variety and environment have an influence on granular morphology and structure organization, starch from Malawian cocoyam, sweetpotato and cassava may not necessarily have the same granule morphological characteristics as those reported by other researchers for cassava, cocoyam and sweetpotato. Thus it is important to evaluate these characteristics so as to unravel the potential of starch for various applications. The objective of the study in this chapter was to determine the granular morphology and the crystalline nature of the starches isolated from sweetpotato and cocoyam grown in Malawi using light microscopy, scanning electron microscopy and X-ray diffraction.

3.2 Materials and methods

3.2.1 Materials

Fifteen sweetpotato genotypes (A45, Babache, Kakoma, Kamchiputu, Kenya, Lunyangwa, LU96/303, LU96/304, Mafutha, Mugamba, Mugande, Salera, Semusa, Tainoni and Zondeni) and seven cocoyam accessions were used in this study. These genotypes were grown at Makoka Research Station in the Zomba district in the 2007 growing season and the cocoyam accessions were obtained from seven different cocoyam growing districts in Malawi [Chitipa, Mzuzu (Mzimba), Nkhotakota, Machinga, Mulanje, Thyolo, and Zomba] and are identified in this chapter with the same district names from which they were collected (Figure 3.1). Starches from five cassava genotypes (Gomani, Maunjili, Mbundumali, Mkondezi and Sauti) from previous starch studies carried out by Benesi (2006) were used for comparison.

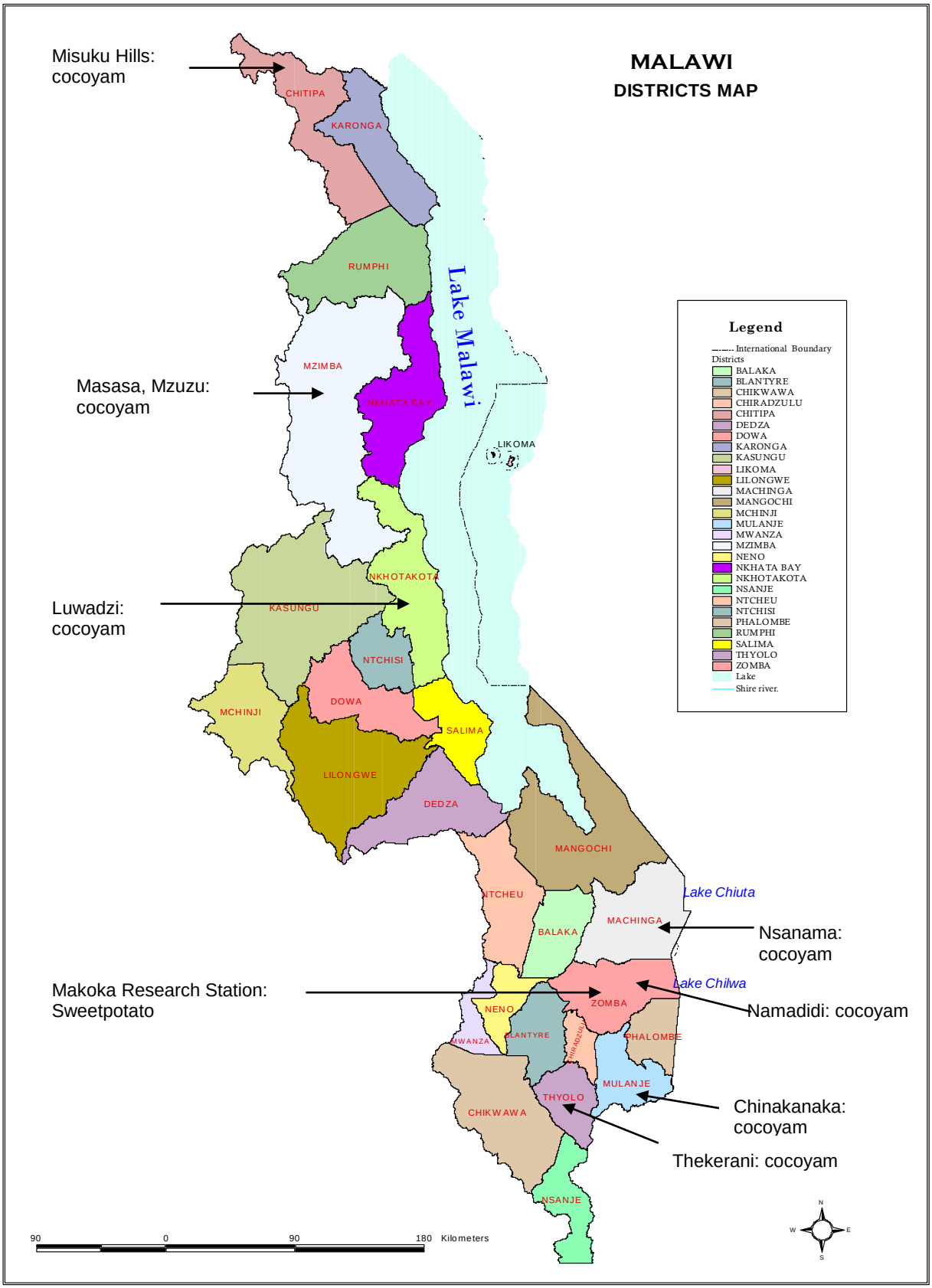


Figure 3.1 Map of Malawi showing areas where sweetpotato and cocoyam were grown and collected

3.2.2 Starch isolation

Starch was isolated from the fresh tubers as described previously (Benesi, 2006). Fresh tuberous roots were washed with tap water, peeled, washed again, chopped to about 1 cm^3 cubes and about 500g of the cubes were transferred into a heavy duty blender (Warring Commercial, model CBCSA 33BL34). One litre of distilled water was added to the cubes and pulverized at high speed for 5 min. The resultant suspension was filtered through a 250 µm sieve; the filtrate was allowed to stand for 4 h to facilitate starch sedimentation and the top liquid was decanted as waste. The sediment was resuspended in 1 L of water and the whole process was repeated three times. The final sediment was air-dried for 48 h, ground and stored in polyethylene bottles prior to analysis.

3.2.3 Light Microscopy (LM)

The granular size and shape of the native cocoyam, sweetpotato and cassava starch were examined using a Confocal/Bino light microscope. Two drops of distilled water were placed on a clean slide, a small amount of starch (about 2 mg) was dispersed into the water while making sure that the starch grains settled down, and were thinly spread onto the slide. The slide was examined under a Nikon Eclipse TE2000-E microscope and starch granules with a scale of granule sizes were photographed.

3.2.4 Estimation of granular size distribution

The range of the granule size was determined by measuring the length and width of 150 granules from light microscope pictures. In the case of non-spherical granules, the length of the major axis of the starch granule was measured. Size distributions of the starches were estimated by classifying the size of starch granules into four groups: large (>25 µm), medium (10-25 µm), small (5-10 µm) and very small (<5 µm) (Lindeboom *et al*., 2004).

3.2.5 Scanning Electron Microscopy (SEM)

The granule surface characteristics were examined using a Jeol Scanning Microscope (JSM-6400, Tokyo, Japan). Starch samples were mounted on circular aluminium stubs using adhesive and then coated with a thin layer of gold using a Bio-Rad sputter coating

system. The samples were then examined and photographed at an accelerating voltage of 5 kV and several magnifications (x 1000- x 3000).

3.2.6 X-ray diffraction

Starches were equilibrated above a saturated potassium sulphate solution (K_2SO_4) in a desiccator for two weeks. The hydrated starch powders were packed tightly in a circular aluminium cell and patterns were measured using a Shimadzu X-ray diffractometer (Model X6000, Shimadzu Co., Tokyo, Japan) by exposing the samples to the X-ray beam from an X-ray generator running at 40 kV and 30 mA. The scanning regions of the diffraction angle 2θ were 3-30°. Other operation conditions included: step interval 0.03, scan rate 2 °/min, Sollet and divergence slit, 1°, receiving slit, 0.60 mm, and scattering slit, 1°. Measurements were made at room temperature and in duplicate. From the resulting X-ray patterns, peak positions were identified using the instruments' software and these peak positions were used to determine the crystalline nature (Jayakody *et al*., 2007).

3.2.7 Data analysis

Light and scanning electron microscopic pictures were used to describe shape and surface characteristics of the starch granules. The mean granular diameter of the starch granules was subjected to analysis of variance (ANOVA) using Statistix 8 for Windows (Analytical software, Tallahassee, USA) to compare the sizes of granules of starch. When statistical differences were found, the least significant difference (LSD) was used to separate means at the 5% significance level. For the X-ray diffractogram, the position and intensity of the diffraction peaks were used to deduce the type of crystalline nature by comparing it with known diffraction patterns in literature.

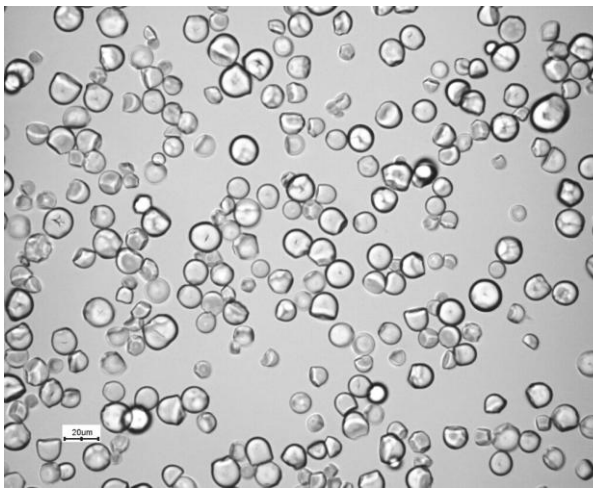
3.3 Results and discussion

3.3.1 Size and shapes of starch granules

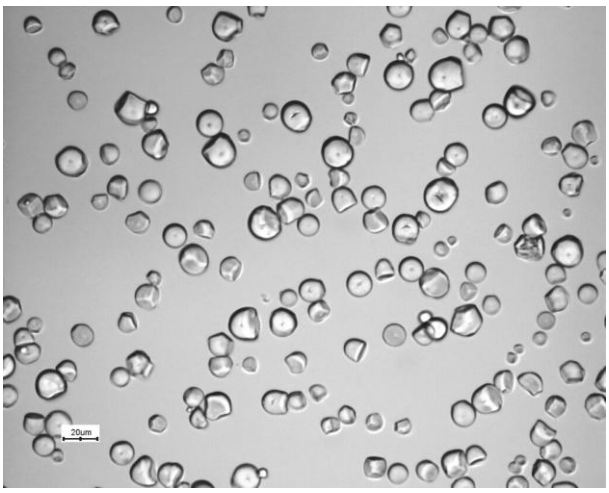
The shapes and surface features of cassava, cocoyam and sweetpotato starches as revealed by light (LM) and scanning electron (SEM) microscopy are presented in Figures 3.2-3.7. A summary of granule size (range, mode, mean diameter) and shape is provided in Table 3.1.

Both LM and SEM revealed that the cassava, cocoyam and sweetpotato starches differed in shapes. Cassava starch granules were mostly rounded (spherical) as earlier described by Benesi (2006). However, irregular with oval and truncated ellipsoidal-granules were also observed (Figure 3.2). SEM revealed mostly smooth surfaces with some portions being irregular, indicating fissures (Figure 3.5). These observations are similar to those made by Peroni *et al*. (2006), Gunaratne and Hoover (2002) and Sriroth *et al*. (1999) on cassava starches. They observed irregular with oval and round truncated granuled for cassava starches. Cocoyam starches exhibited round/spherical as well as polyhedral (polygonal) granules (Figures 3.3 and 3.6). Truncated granules were also observed in the cocoyam starch samples. This is in agreement with Pérez *et al*. (2005) who reported small rounded, medium ellipsoidal-truncated and large polyhedral shaped starch granules for *Colocasia esculenta* starch. However contrasting observations were made by Huang *et al*. (2008), Sefa-Dedeh *et al*. (2002) and Jane *et al*. (1992). Huang *et al*. (2008) reported mostly polygonal shapes while Sefa-Dedeh *et al*. (2002) reported 5- to 6-sided polygonal shapes for the cocoyam starches. Jane *et al*. (1992) reported polygonal and irregular shapes for five varieties of cocoyam (taro) starch.

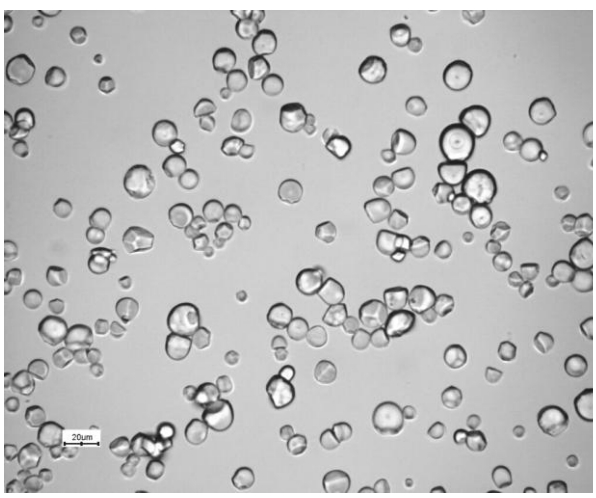
Most of the sweetpotato starch granules were polygonal in shape (Figures 3.4), however round and elongated truncated granules were also found within the sweetpotato starch samples. The presence of rounded granules was more pronounced in Kenya, LU96/303, Mugande, Salera, and Semusa starch samples while few elongated and truncated granules were observed in Mugamba, Mugande and Salera starch samples (Figure 3.4). These starches had mostly irregular surfaces as revealed by SEM (Figure 3.7). Polygonal shape for sweetpotato starch granules have also been reported by Peroni *et al*. (2006) and Huang (2008).



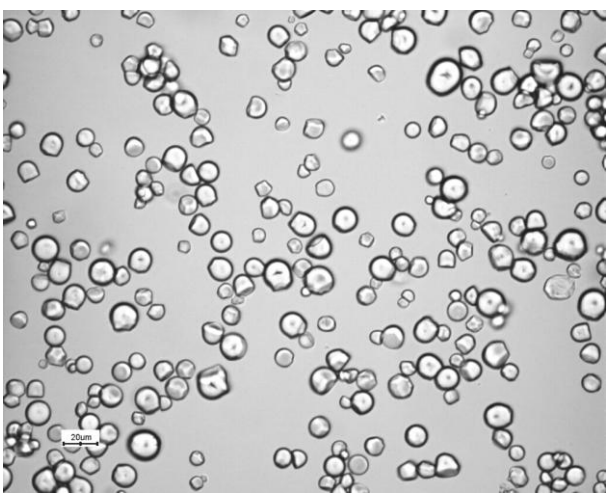
(a) Gomani



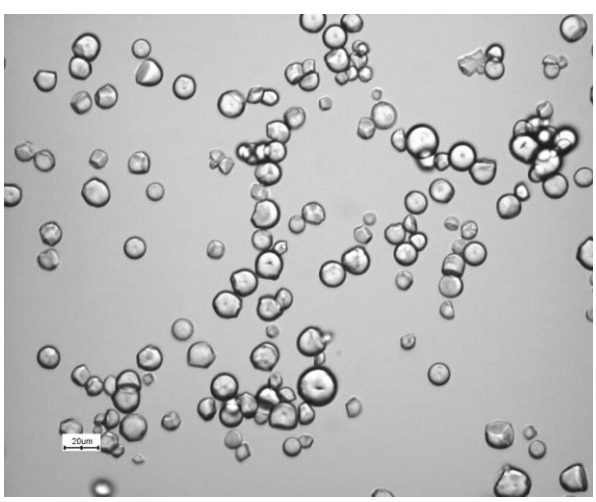
(d) Mkondezi



(b) Maunjili

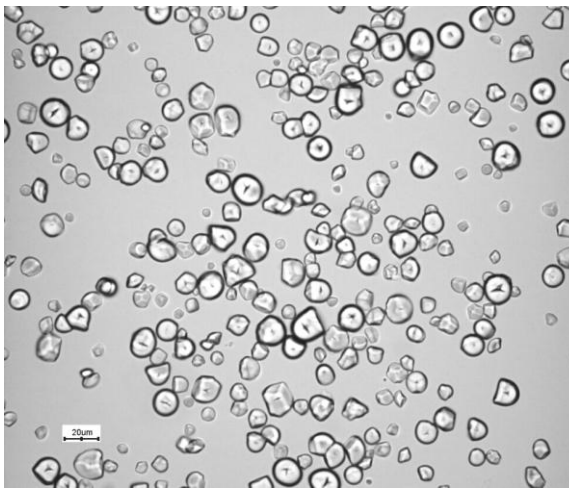


(e) Sauti

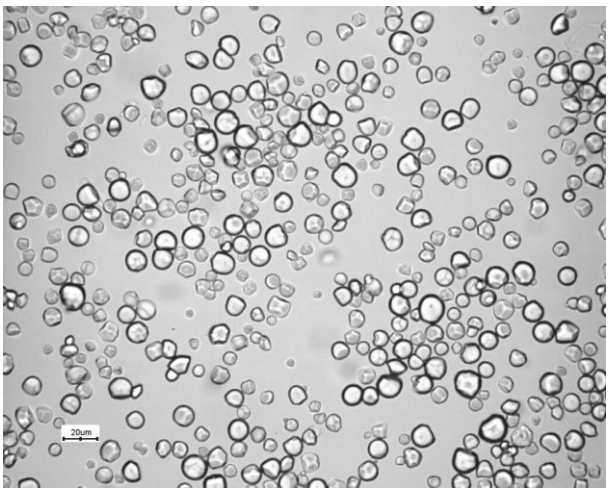


(c) Mbundumali

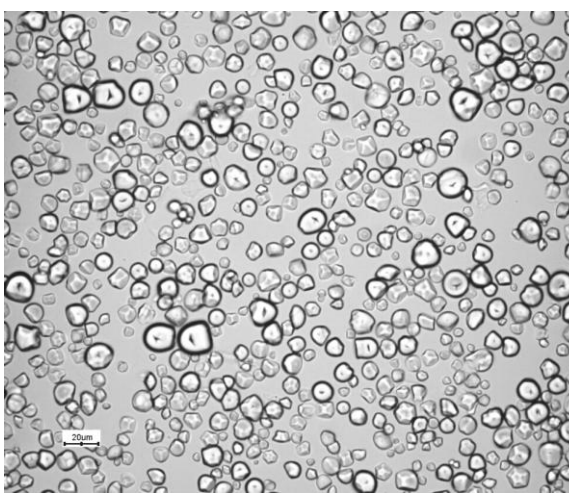
Figure 3.2 Light micrographs of starch granules from five cassava genotypes



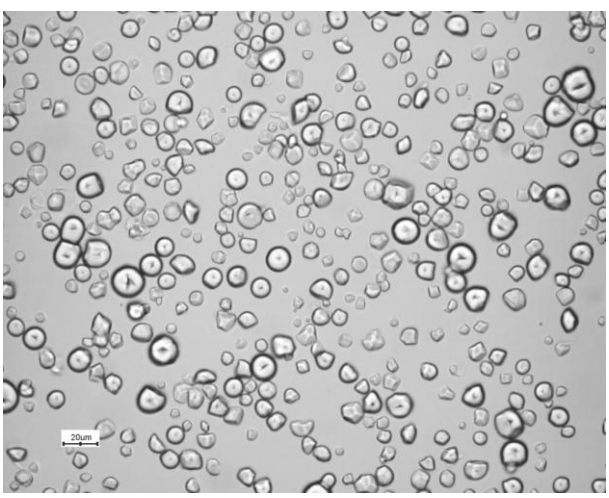
(a) Chitipa



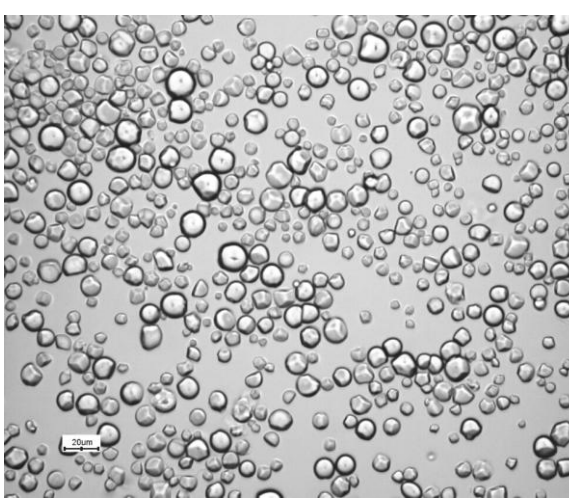
(d) Nkhotakota



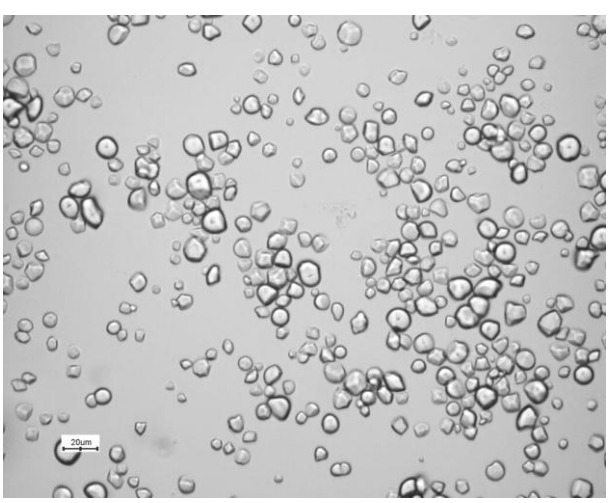
(b) Machinga



(e) Thyolo

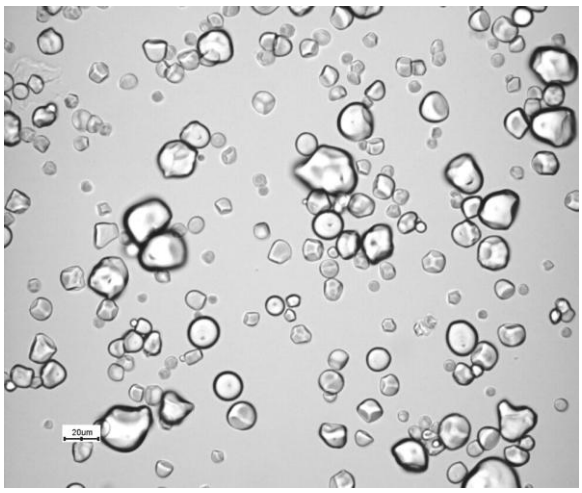


(c) Mzuzu

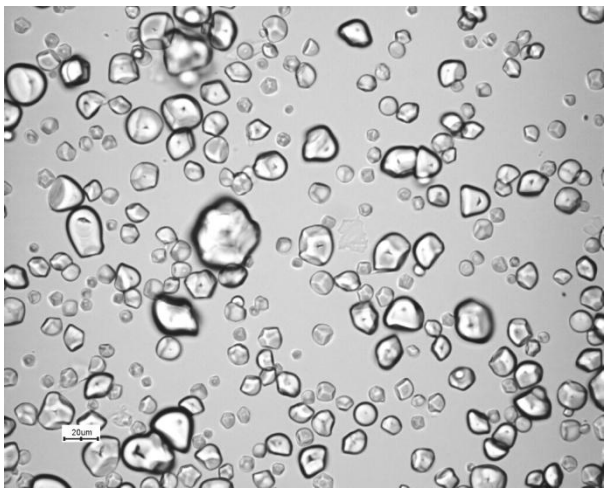


(f) Zomba

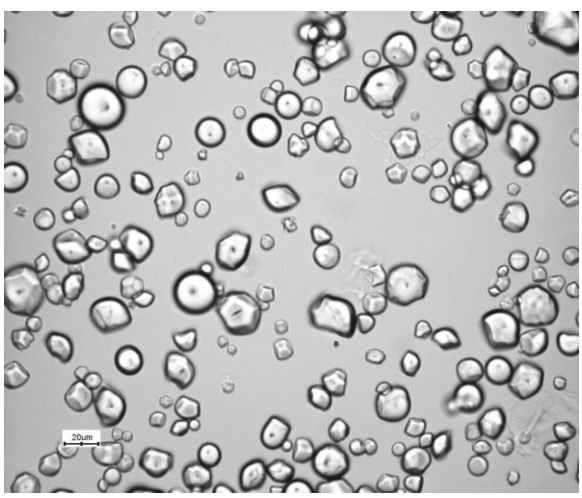
Figure 3.3 Light micrographs of starch granules from cocoyam accessions



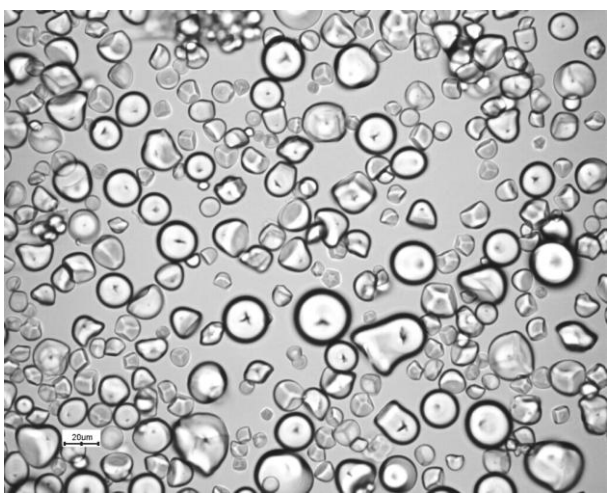
(a) A45



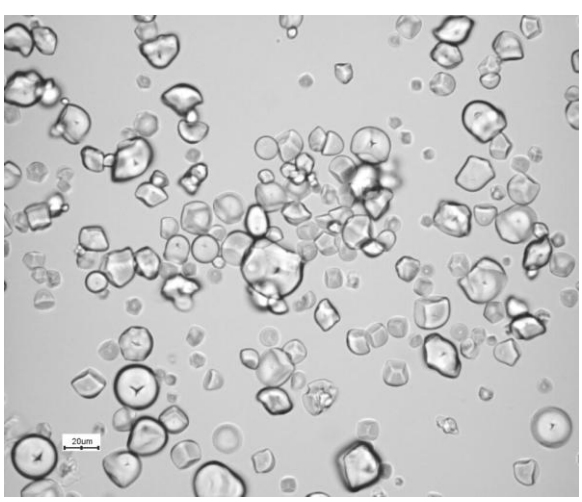
(d) Kamchiputu



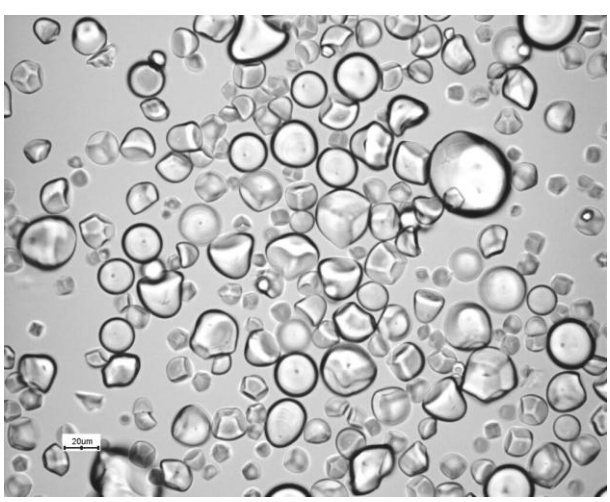
(b) Babache



(e) Kenya

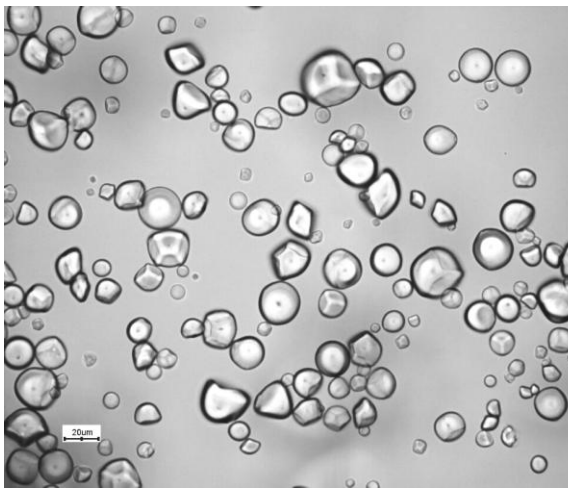


(c) Kakoma

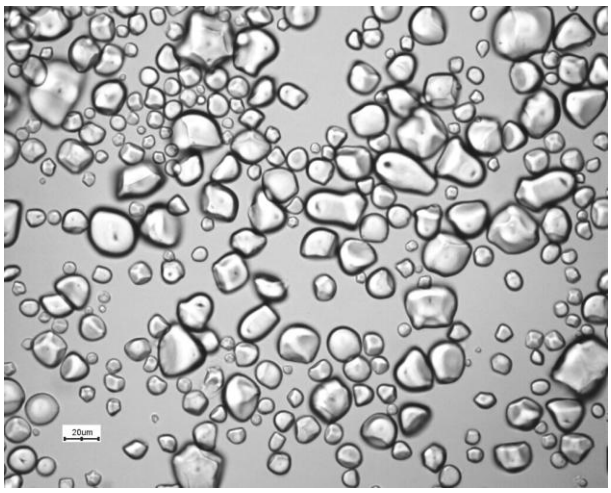


(f) LU96/303

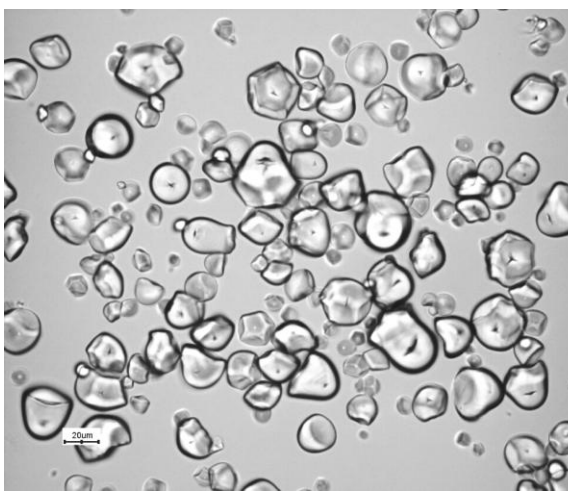
Figure 3.4 Light microscopy photographs of sweetpotato starch granules



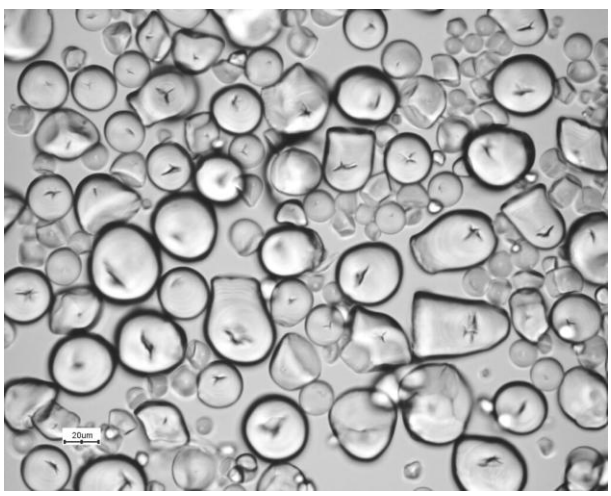
(g) LU96/304



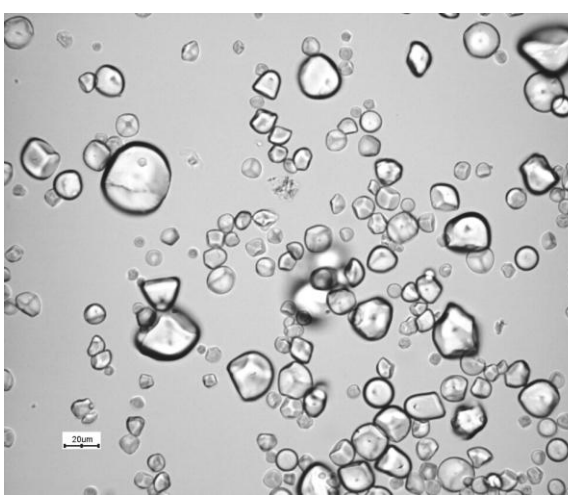
(j) Mugamba



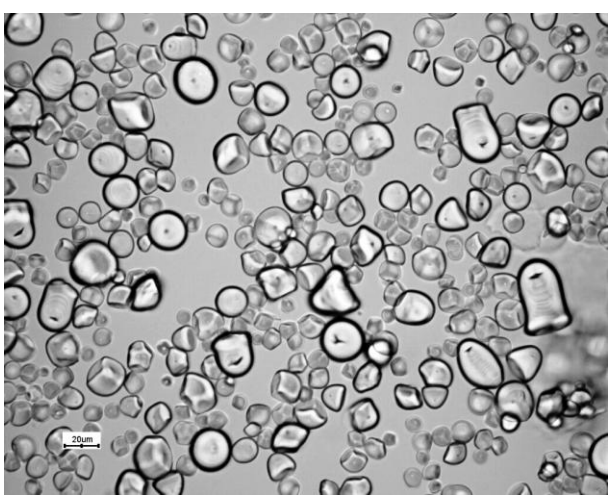
(h) Lunyangwa



(k) Mugande

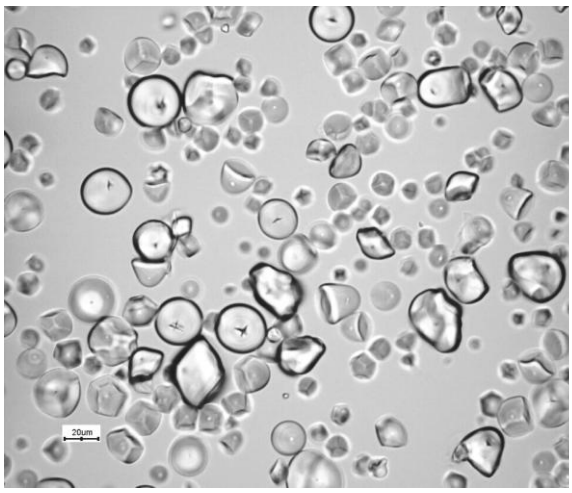


(i) Mafutha

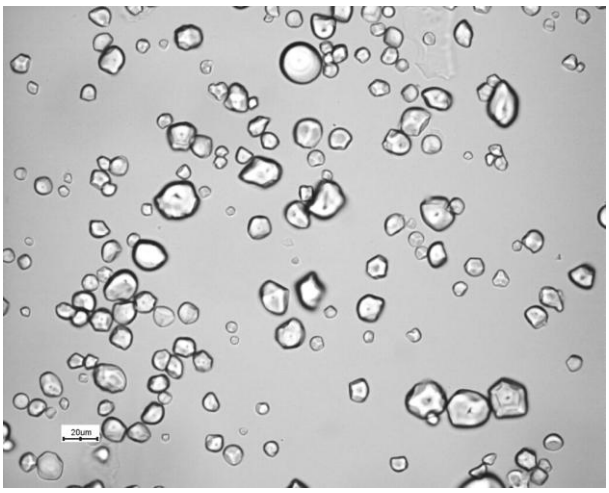


(l) Salera

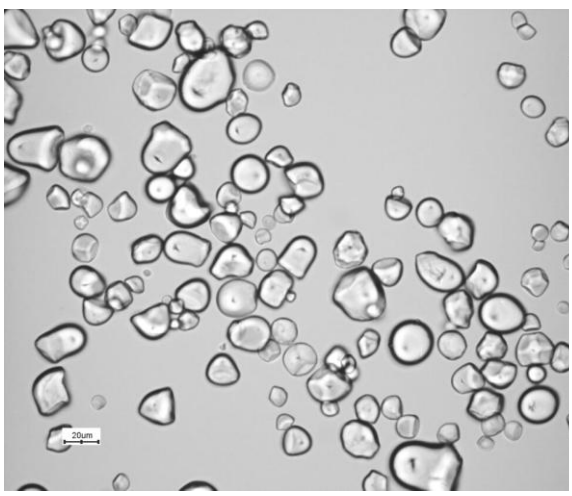
Figure 3.4cont'd. Light microscopy photographs of sweetpotato starch granules



(m) Semusa

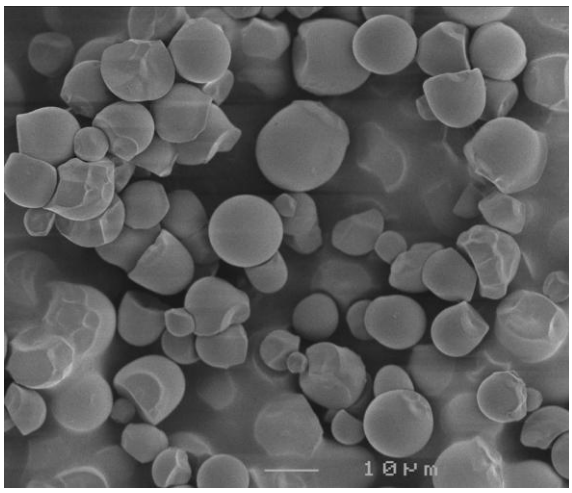


(o) Zondeni

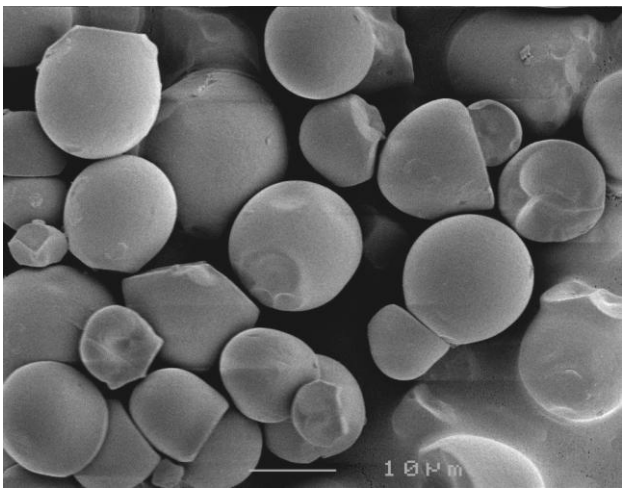


(n) Tainoni

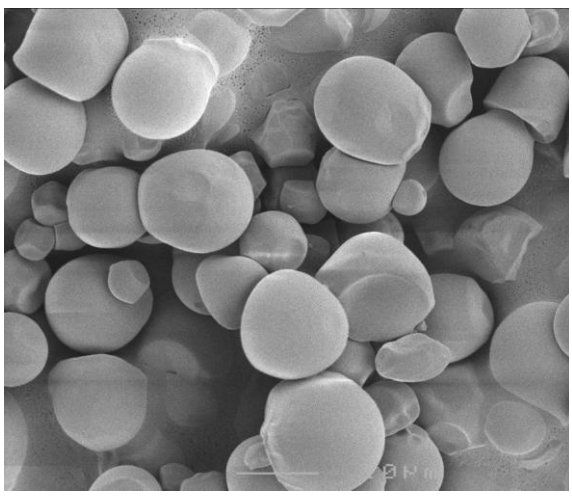
Figure 3.4 cont'd. Light microscopy photographs of sweetpotato starch granules



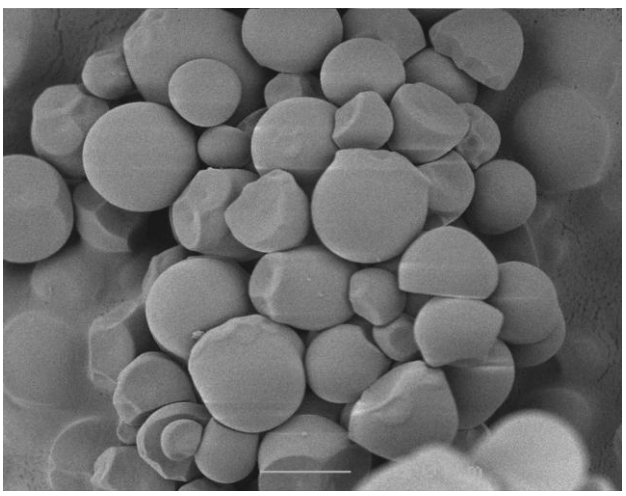
(a) Gomani



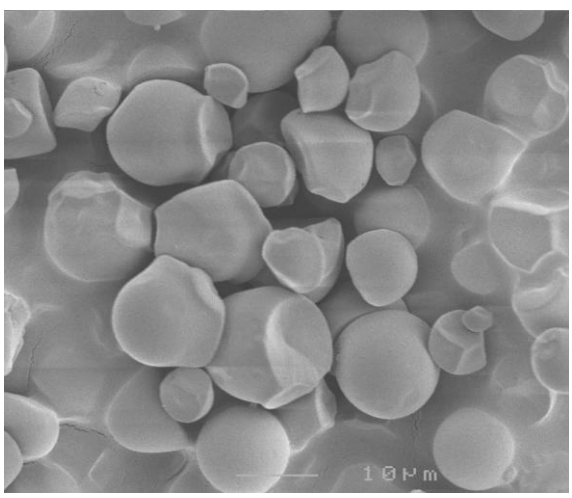
(d) Mkondezi



(b) Maunjili

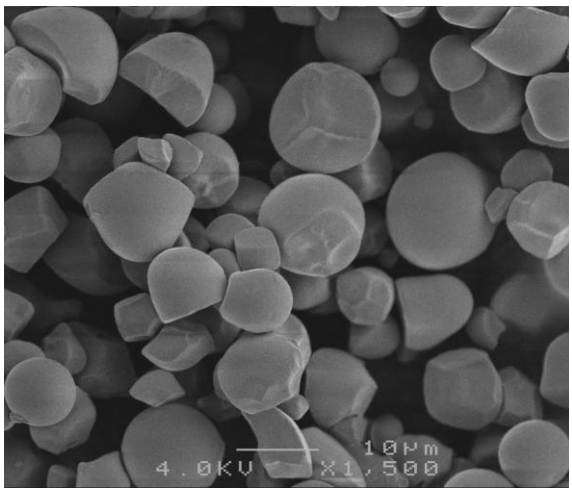


(e) Sauti

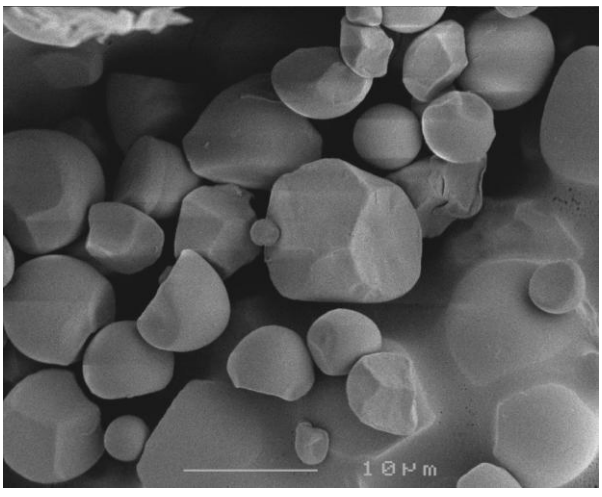


(c) Mbundumali

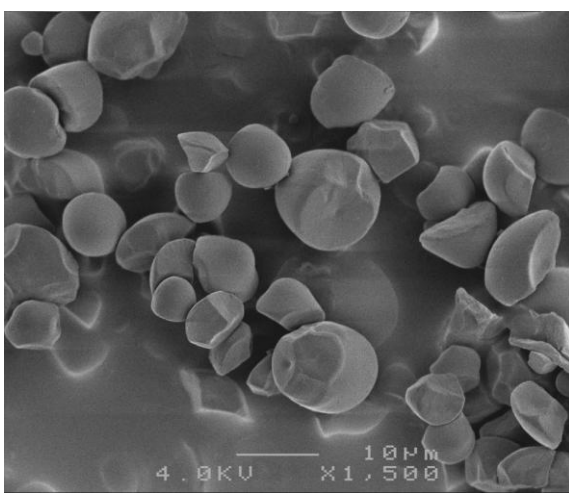
Figure 3.5 Scanning electron micrographs of cassava starch granules



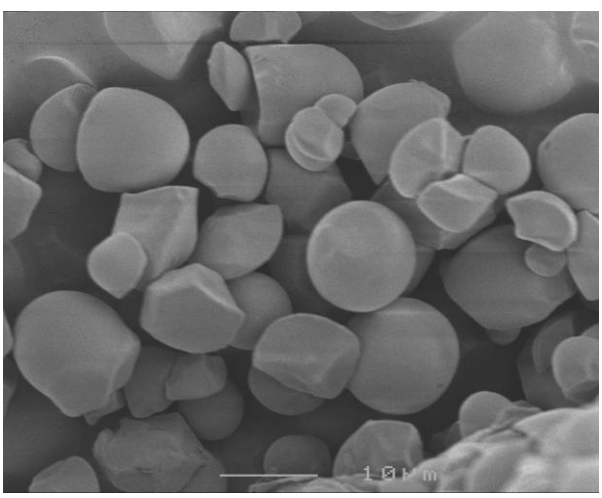
(a) Chitipa



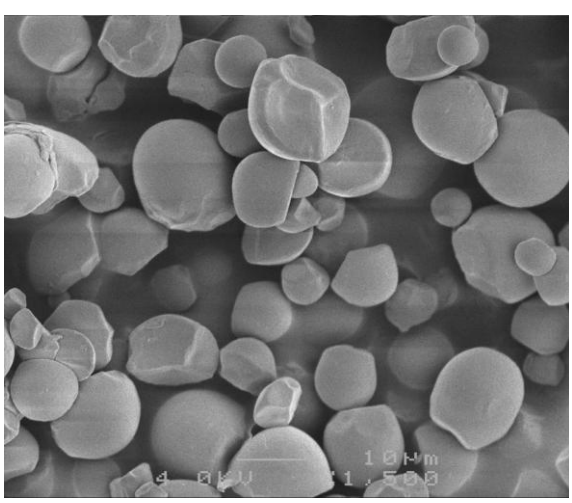
(d) Nkhotakota



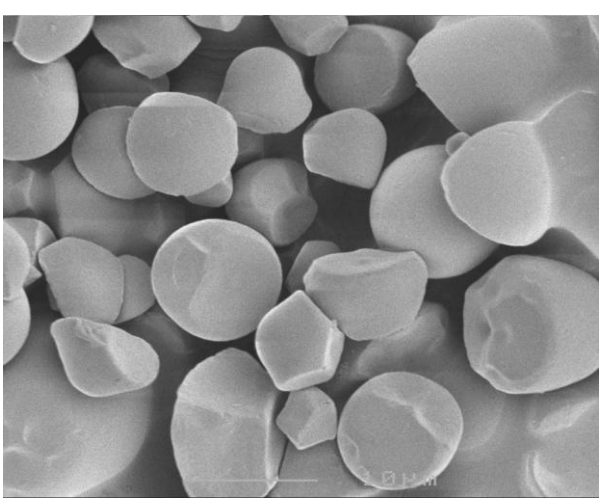
(b) Machinga



(e) Thyolo

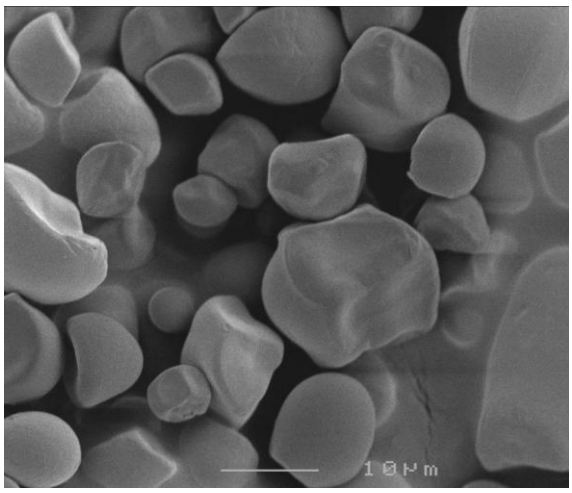


(c) Mzuzu

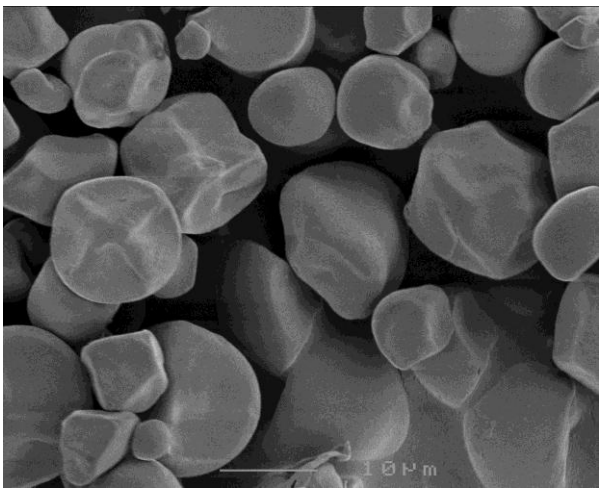


(f) Zomba

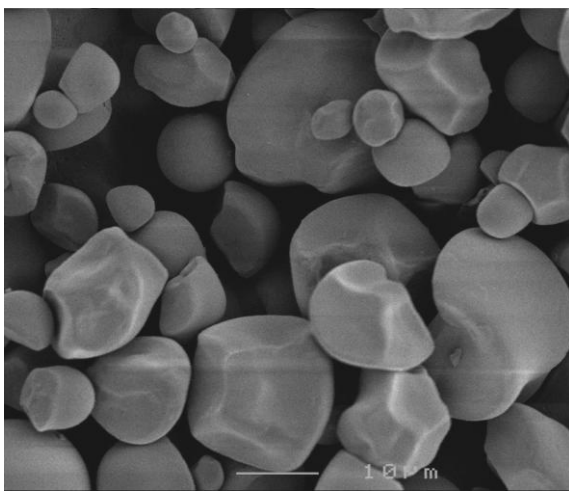
Figure 3.6 Scanning electron micrographs of cocoyam starch granules



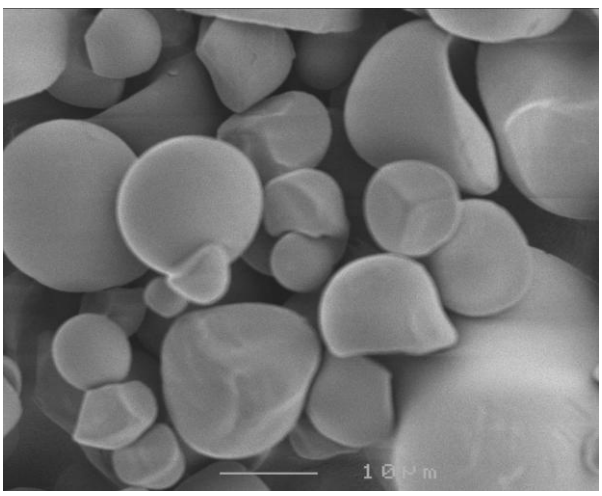
(a) A45



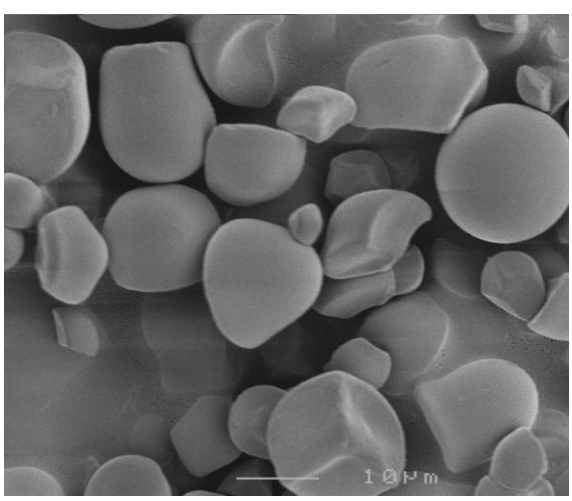
(d) Kamchiputu



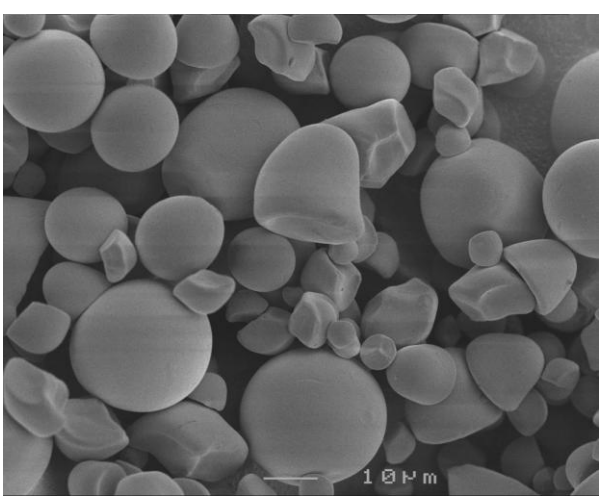
(b) Babache



(e) Kenya

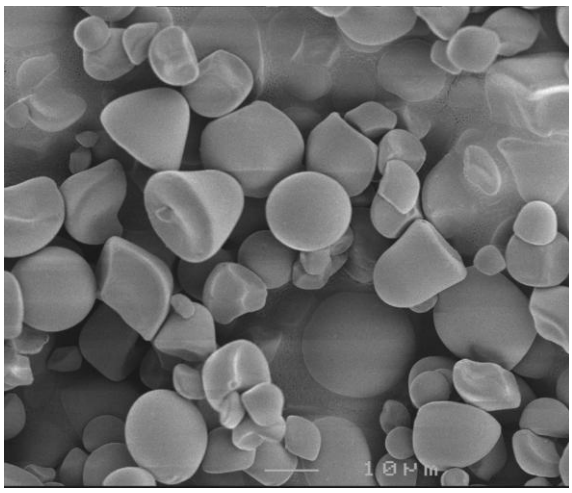


(c) Kakoma

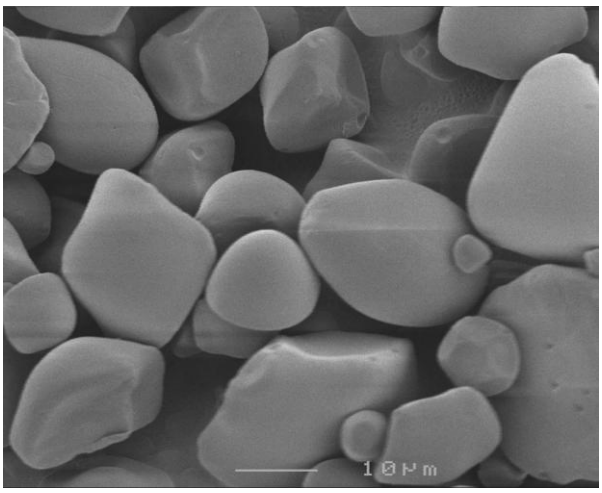


(f) LU96/303

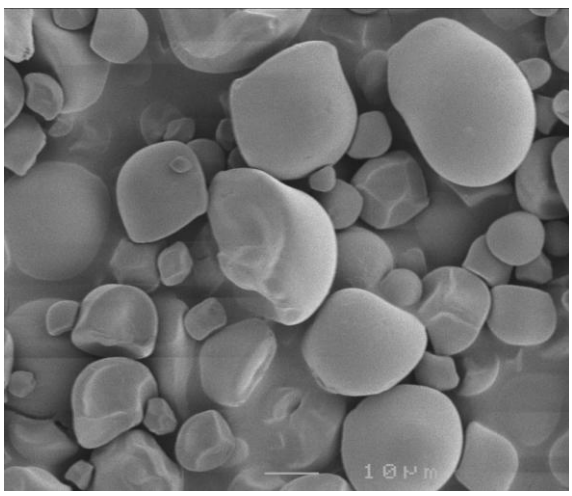
Figure 3.7 Scanning electron micrographs of sweetpotato starch granules



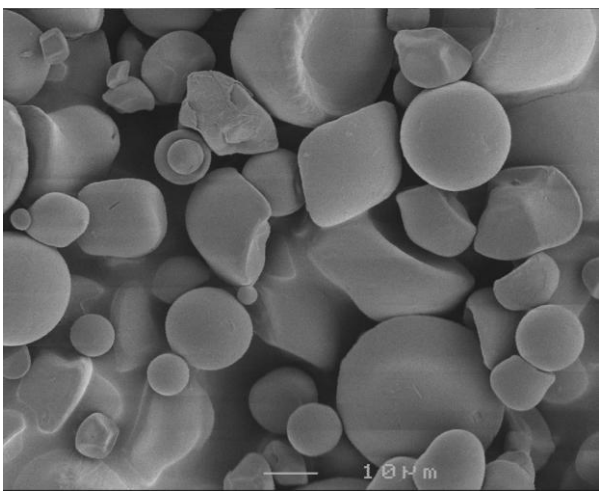
(g) LU96/304



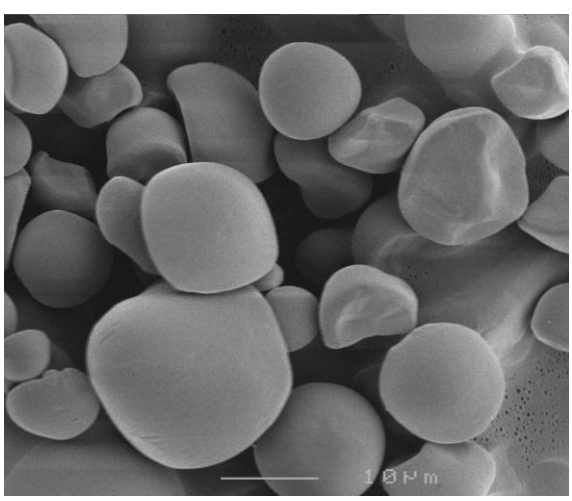
(j) Mugamba



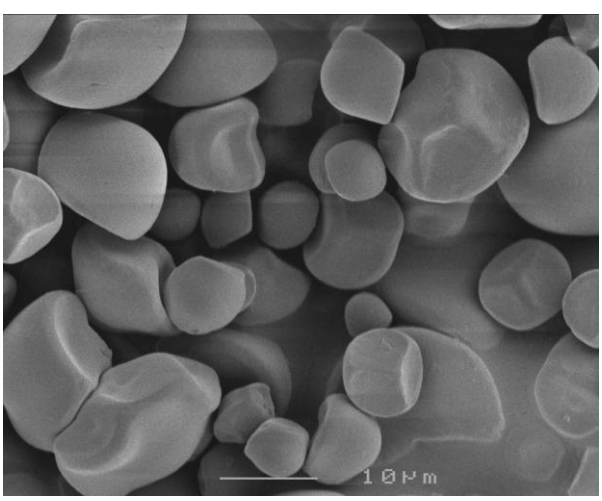
(h) Lunyangwa



(k) Mugande

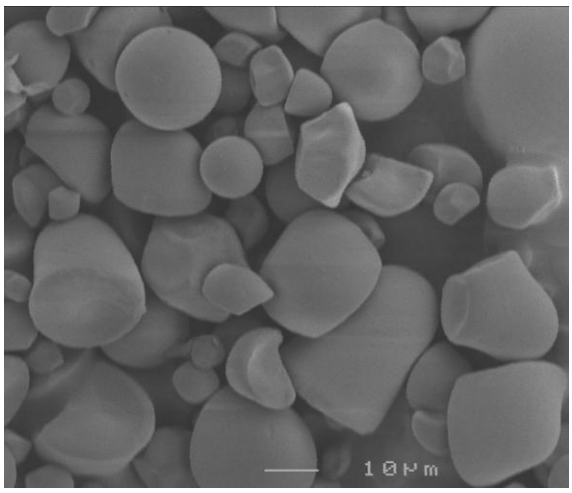


(i) Mafutha

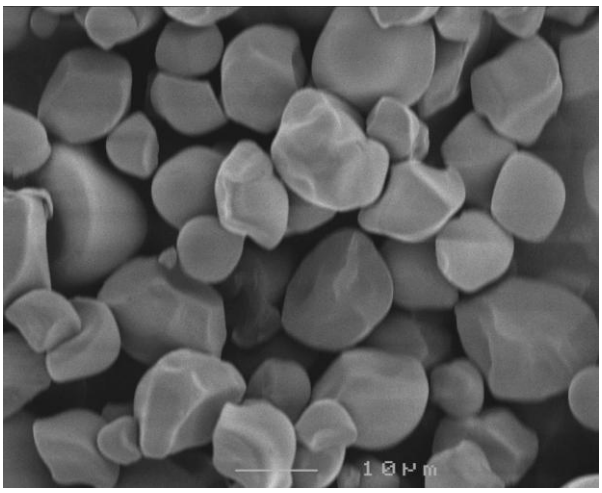


(l) Salera

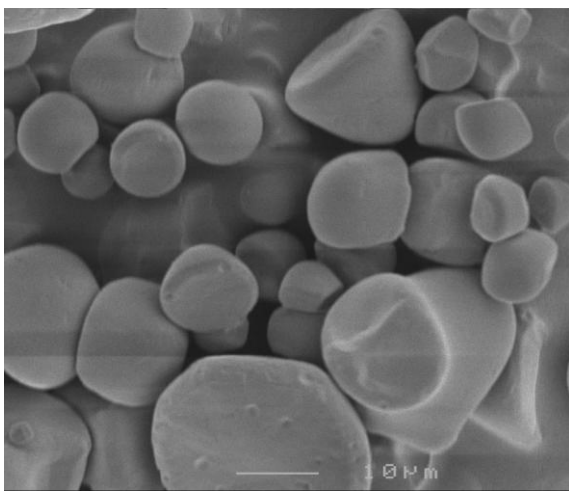
Figure 3.7 cont'd. Scanning electron micrographs of sweetpotato starch granules



(m) Semusa



(h) Zondeni



(n) Tainoni

Figure 3.7 cont'd. Scanning electron micrographs of sweetpotato starch granules

Table 3.1 Granule size and shape of the starches as determined by light microscopy

Botanical Source	Genotype/ Accession	Size range (μm)	Mode (μm)	Mean diameter (μm)	Shape description
Cocoyam	Chitipa	4.0-18.7	9.3	10.2^{j}	round/ spherical, irregular, truncated
	Machinga	4.0-16.0	8.0	10.1^{j}	round/ spherical, irregular, truncated
	Mulanje	5.3-16.0	10.7	10.4^{ij}	round/ spherical, irregular, truncated
	Mzuzu	5.3-16.0	8.0	9.6^{j}	round/ spherical, irregular, truncated
	Nkhotakota	5.3-17.3	9.3	9.4^{j}	round/ spherical, irregular, truncated
	Thyolo	5.3-17.3	10.7	10.1^{j}	round/ spherical, irregular, truncated
	Zomba	5.3-18.7	9.3	10.3^{ij}	round/ spherical, irregular, truncated
	Overall	4.0-18.7	9.3	10.0	
Sweetpotato	A45	5.3-33.3	6.7	11.8^{gh}	Round, polygonal, truncated
	Babache	5.3-30.7	13.3	14.0^{de}	Round and polygonal
	Kakoma	5.3-28.0	13.3	14.6^{d}	Mostly polygonal, round
	Kamchiputu	5.3-36.0	9.3	12.9^{fg}	Polygonal and truncated
	Kenya	5.3-34.7	13.3	16.0^{c}	Round and polygonal
	LU96/303	6.7-40.0	13.3	17.0^{c}	Round and polygonal
	LU96/304	4.0-30.7	13.3	14.3^{d}	Round and polygonal
	Lunyangwa	6.7-38.7	12.0	16.4^{c}	Most polygonal, round, truncated
	Mafutha	5.3-24.0	13.3	12.5^{fg}	Polygonal, round
	Mugamba	6.7-37.3	13.3	14.8^{d}	Polygonal, elongated
	Mugande	6.7-48.0	18.7	21.3^{a}	Round, elongated truncated, polygonal
	Salera	4.0-36.0	12.0	14.0^{de}	Round, elongated, truncated, polygonal,
	Semusa	8.0-40.0	14.7	19.4^{b}	Round, polygonal
	Tainoni	6.7-45.3	13.3	14.5^{d}	Round, polygonal, elongated truncated
	Zondeni	4.0-26.7	13.3	11.9^{fgh}	Polygonal
	Overall	4.0-48.0	13.3	15.0	
Cassava	Gomani	6.7-20.0	13.3	11.8^{gh}	Round/truncated
	Maunjili	5.3-21.3	13.3	12.0^{fgh}	Round/truncated
	Mbundumali	6.7-20.0	12.0	12.9^{ef}	Round/truncated
	Mkondezi	6.7-22.7	13.3	12.7^{fg}	Round/truncated
	Sauti	5.3-20.0	10.7	11.4^{hi}	Round/truncated
	Overall	5.3-22.7	13.3	12.2	

NB: Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Starch granule sizes of cocoyam, sweetpotato and cassava ranged from 4.0-18.7 μm, 4.0-48 μm and 5.3-22.7 μm, respectively, with average diameters of 10.0 μm, 15 μm and 12.2 μm. The modal diameters were 9.3 μm for cocoyam, 13.3 μm for sweetpotato, and 13.3 μm for cassava starch (Table 3.1). Generally, sweetpotato starch granules exhibited the largest granules as revealed by the mean (15.0 μm), mode (13.3 μm) as well as range (4.0-48 μm) of starch granule size (Table 4.1). Cocoyam starch exhibited the lowest mean diameter (10.0 μm), modal diameter (9.3 μm) and granule size range (4.0-18.7 μm). Cassava starch granules were of intermediate size between sweetpotato and cocoyam starch. Variations in granular size were observed among sweetpotato starch from the different genotypes. Starch from Mugande, Semusa, LU96/303, Lunyangwa and Kenya sweetpotato genotypes exhibited the largest granule size while A45 and Zondeni had the smallest. Mugande sweetpotato starch also displayed a higher granule size range (6.7-48 μm) than the rest of the sweetpotato starch, while Kakoma and Zondeni had the lowest granule size range. Among the cassava genotypes, starch from Mbundumali exhibited the highest granule size and Sauti the lowest (Table 3.1). Little variation was observed in granule size of starch from different cocoyam accessions.

The size of the starch granules is known to vary with botanical sources. Granule sizes ranging from 0.05 - 0.08 μm, 2.96 - 5.19 μm and 0.5 to 5.0 μm (Pérez *et al*., 2005; Maeda *et al*., 2004; Jobling, 2004; Sefa-Dedeh and Kofi-Agyir Sackey, 2002; Moorthy *et al*.,1993) have been reported reported for cocoyam starches. The granule size of sweetpotato starch ranges from 2-72 μm (Chen *et al*., 2003; Moorthy, 2002; Hoover, 2001; Walter *et al*., 2000; Tian *et al*., 1991), while cassava starches ranges from 4 to 25.0 μm (Mishra and Rai, 2006; Pérez *et al*., 2005; Gunaratne and Hoover, 2002; Sriroth *et al*.,1999). The granular size of cocoyam starch is comparatively larger (4.0-18.7μm) than most reported values but closer to that reported for *C. esculenta* starch (mean diameter of 13 μm) from Ethiopia (Adane *et al*., 2006). A comparison of starch granule sizes has reaveled that sweetpotato starch has larger granules than cassava and cocoyam starches (Wickramasinghe *et al*., 2009; Nwokocha *et al*., 2009). Different environments/growing conditions also affect the size of the starch granules (Le *et al*., 2008; 2005). Thus, variations in starch granule size observed in this study could be be due to genetic variations and/or different environmental conditions (Tester and Karkalas, 2001; Noda *et al*., 2001).

3.3.2 Granular size distribution of the starch granules

The results of granular size distribution from very small (<5 μm), small (5-10 μm), medium (10-25 μm) and large (>25 μm) are presented in Table 3.2.

Table 3.2 Granular size distribution of the cocoyam, sweetpotato and cassava starches

Botanical Source	Genotype/ Accession	Size distribution (%)			
		0-5 μm	5-10 μm	10-25 μm	>25 μm
Cocoyam	Chitipa	3	45	52	0
	Machinga	1	51	48	0
	Mulanje	0	43	57	0
	Mzuzu	0	56	44	0
	Nkhotakota	0	65	35	0
	Thyolo	0	51	49	0
	Zomba	0	51	49	0
Overall		0.5	51.8	47.7	0
Sweetpotato	A45	0	47	48	5
	Babache	0	28	67	5
	Kakoma	0	15	80	5
	Kamchiputu	0	39	56	5
	Kenya	0	15	77	8
	LU96/303	0	10	77	13
	LU96/304	1	25	79	4
	Lunyangwa	0	14	73	13
	Mafutha	0	29	71	0
	Mugamba	0	25	65	11
	Mugande	0	9	55	36
	Salera	1	20	76	3
	Semusa	0	6	71	23
	Tainoni	0	21	75	5
	Zondeni	3	39	56	3
Overall		0.3	22.7	67.7	9.2
Cassava	Gomani	0	27	73	0
	Maunjili	0	27	73	0
	Mbundumali	0	17	83	0
	Mkondezi	0	23	77	0
	Sauti	0	33	67	0
Overall		0	25.3	74.7	0

Starch from cocoyam, sweetpotato and cassava displayed variable granule size distribution. Most sweetpotato starches had granule sizes ranging from 10-25 μm (>60%). Starch granules from Kakoma sweetpotato genotype gave the highest proportion (80%) of starch granules within the 10-25 μm size range despite having a narrower granule size range (5.3-28 μm) than the rest of the sweetpotato starch. Kenya, LU96/303, LU96/304, Lunyangwa, Mafutha, Salera, Semusa and Tainoni sweetpotato starch had more than 70% of its granules in this size range. A substantial fraction (9%) of the sweetpotato starch granules had a large size (>25 μm). In this size range, starch from Mugande displayed the largest proportion (36%) of its granules followed by starches from Semusa (23%), LU96/303 (13%), and Lunyangwa (13%). No granules from Mafutha sweetpotato starch were found in the >25 μm size range despite being among sweetpotato starches that had a higher percentage of large granules (10-25 μm). Most of the cassava starch granules (75%) fell within the large size group (10-25 μm). Starch from Mbundumali genotype gave the highest proportion (83%) of its granules within this size range followed by Mkondezi (77%), and Gomani and Maunjili (73%). However, no cassava starch granules fell within the >25 μm group. Cocoyam starch had almost equal size distribution of small sized (52%) and medium sized granules (48%). Nkhotakota cocoyam starch had a larger fraction of its granules in the small size range (65%). All cocoyam starches gave little or no fraction of the very small sized starch granules while none had granules within the >25 μm size range. The high fraction of sweetpotato and cassava starch granules in the 10-25 μm size range compared to cocoyam starch clearly indicate that both sweetpotato and cassava starch have larger sized granules compared to cocoyam starch. Furthermore, the presence of sweetpotato starch granules within the >25 μm size range confirms that sweetpotato have larger granules than both cassava and cocoyam starch.

3.3.3 X-ray patterns

The results of X-ray diffraction patterns for cocoyam, sweetpotato and cassava starches showing the position of the strongest peaks and their relative intensities are presented in Table 3.3. The actual X-ray diffraction patterns for all the starches are presented in Appendices 1-3.

Table 3.3 X-ray diffraction data of the cocoyam, sweetpotato and cassava starches

Botanical source	Genotype/ Accession	Strongest peaks											
		Peak 1			Peak 2			Peak 3			Peak 4		
		angle (2θ °)	d-spacing	Relative intensity (%)	angle (2θ °)	d-spacing	Relative intensity (%)	angle (2θ °)	d-spacing	Relative intensity (%)	angle (2θ °)	d-spacing	Relative intensity (%)
Cocoyam	Chitipa	5.4	16.3	100	7.8	11.3	60	8.8	10.0	20	13.2	6.7	12
	Machinga	5.5	16.1	100	7.6	11.5	67	8.9	10.0	48	19.0	4.6	14
	Mulanje	5.5	16.2	100	7.9	11.2	63	8.9	10.0	26	13.8	6.4	15
	Mzuzu	5.5	16.2	100	7.9	11.2	64	9.1	9.8	27	13.1	6.8	14
	Nkhotakota	5.5	16.0	100	7.9	11.2	71	9.2	9.6	33	10.6	8.3	21
	Thyolo	5.4	16.3	100	7.9	11.2	56	8.9	10.0	28	12.4	7.2	12
	Zomba	5.5	16.2	100	7.9	11.2	71	9.5	9.3	21	13.1	6.7	13
Sweetpotato	A45	5.4	16.2	100	7.9	11.2	71	9.1	9.7	29	11.2	7.9	17
	Babache	5.5	16.3	100	7.9	11.2	65	8.5	10.4	43	7.2	12.3	35
	Kakoma	5.4	16.4	100	7.8	11.3	48	4.7	18.6	34	6.2	14.3	31
	Kamchiputu	5.5	16.0	100	7.9	11.2	73	8.7	10.2	38	10.5	8.4	12
	Kenya	5.4	16.3	100	7.8	11.3	45	8.8	10.0	32	19.3	4.6	18
	LU96/303	5.4	16.3	100	7.9	11.2	59	4.6	19.4	24	13.8	6.4	14
	LU96/304	5.5	16.2	100	7.9	11.2	63	8.7	10.1	25	13.3	6.7	14
	Lunyangwa	5.4	16.4	100	7.9	11.2	62	8.6	10.3	31	17.6	5.0	19
	Mafutha	5.5	16.1	100	7.8	11.3	56	8.8	10.0	16	11.9	7.4	16
	Mugamba	5.4	16.3	100	8.9	9.9	41	7.8	11.3	34	7.2	12.3	24
	Mugande	5.5	16.1	100	7.9	11.2	68	8.8	10.0	36	10.4	8.5	14
	Salera	5.4	16.2	100	7.8	11.3	56	8.8	10.0	32	9.9	8.9	12
	Semusa	5.4	16.5	100	7.9	11.2	57	8.9	10.0	18	9.8	9.1	14
	Tainoni	5.5	16.0	100	7.9	11.2	54	9.0	9.82	25	14.3	6.19	8
	Zondeni	5.5	16.0	100	7.9	11.1	77	12.2	7.3	19	19.2	4.6	15
Cassava	Gomani	5.4	16.5	100	7.9	11.2	63	9.0	9.8	21	13.5	6.6	21
	Maunjili	5.5	16.0	100	8.0	11.1	60	9.0	9.9	20	13.5	6.6	12
	Mbundumali	5.4	16.3	100	7.9	11.2	44	10.6	8.3	12	13.7	6.4	8
	Mkondezi	5.4	16.3	100	7.8	11.3	56	17.3	5.1	12	13.1	6.8	8
	Sauti	5.4	16.4	100	7.9	11.2	64	22.2	4.0	11	11.8	7.5	7

Starches from different botanical sources can possess A-, B- or C-type X-ray diffraction pattern. Starches from cocoyam, sweetpotato and cassava gave very strong reflections in the region of 5.4° to 15° 2θ angles with other weak peaks at angles beyond 15° 2θ (Table 3.3 and Appendices 1-3). All starches gave the strongest and broadest diffraction peak centered at an angle of 2θ=5.4-5.6°. This reflection indicates the presence of a B-type crystalline nature. However, the reflections at 17° 2θ angles were weak for most starches, not expected expected of tuber and root starches that exhibit B-type X-ray patterns. These starches exhibit maximum X-ray diffraction peaks at 17º 2θ (Hoover, 2001). The second strong reflection was observed at 2θ=7.6-7.9° for all starches. Reflections of relatively stronger intensities were also observed between 8.5-10.6° 2θ except for starch from LU96/303 and Zondeni sweetpotato genotypes and Mkondezi and Sauti cassava genotypes. Starch from Kakoma, LU96/303 and Zondeni showed a reflection at 4.6°, 4.6° and 12.2° 2θ angles, respectively. Cassava starch from Gomani and Sauti genotypes gave a third strongest reflection at 17.3° and 22.2° 2θ angles, respectively. The fourth strongest diffraction peak appeared at different positions for the different starch: starch from Chitipa, Mulanje, Mzuzu and Zomba cocoyam accessions, LU96/303 and LU96/304 sweetpotato genotypes, and, Gomani, Maunjili, Mbundumali and Sauti cassava genotypes had the fourth reflection at 2θ=13.1-13.8°. Zondeni sweetpotato and Machinga cocoyam starch had the reflection at 2θ = 19°. The other starches exhibited the reflection at 2θ=9.4-12.0° except for Kakoma, Babache, and Mugamba sweetpotato starch that had the reflection at 6.2° and 7.2° 2θ. The presence of strong diffraction peaks at 7.6-7.8° 2θ angles seem to suggest the presence of crystallites arising from amylose single helices.These single helical conformations in granular starches give rise to V-type crystallinity which is characterized by a peak 2θ=20° and two other reflections at 7° and 13° 2θ angles (Godet *et al*., 1995).

Several studies have reported different X-ray diffraction pattern for cassava, sweetpotato and cocoyam starches. Cassava starches have been reported to possess A- type pattern with most intense peaks at 15°, 17°, 18°, 22°, 23.3° and 25.4° 2θ angles (Srichuwong *et al*., 2005; Bertolini *et al*., 2001) Sweetpotato starch possess C_A-type diffraction patterns with characteristic peaks at 9.9°, 10.9°, 15.1° and 17.1° 2θ angles (Ramesh *et al*., 2006), and

15.4°, 17.2°, 18.3° and 23.4° 2θ angles (Osundahunsi *et al*., 2003; Noda *et al*., 1995). McPherson and Jane (1999) reported a C-type for for sweetpotato starch. Different X-ray patterns have been reported for cocoyam starch. Mostly A- type patterns have ben reported for cocoyam (taro, Colocasia esculenta) starches (Srichuwong *et al*., 2005; Moorthy, 2002; Jane *et al*., 1992). However, Adane *et al*. (2006) reported B- type pattern with maximum X-ray diffraction peaks at 17º 2θ for Ethiopian Godare (*Colocasia esculenta*) with other significant peaks centred at 4.5, 19.5, 22.0 and 24.5º 2θ. The X-ray diffraction patterns observed for cassava, cocoyam and sweetpotato starches observed in this study therefore disagree with those reported. The presence of the most intense peak at 5.5 2θ angles suggests B-type crystalline pattern.

3.4 Conclusions

Results of this study have revealed a diversity of starch granular size and shapes from Malawian cocoyam, sweetpotato and cassava starch. Sweetpotato starch showed mostly polygonal granules that ranged between 4.0 and 48 μm. Cocoyam starch granules exhibited mostly round/spherical shapes similar to that of cassava starch and granule size ranged from 4.0-18.7 μm. A comparison of granule size distribution has shown that cocoyam starch had smaller sized granules than sweetpotato and cassava starch. Most of the sweetpotato and cassava starch granules had sizes ranging from 10-25 μm. With respect to granule size distribution, starch isolated from Kakoma, Kenya, LU96/303, LU96/304, Lunyangwa, Mafutha, Salera, Semusa and Tainoni sweetpotato genotypes had the largest granule size. Starch from cocoyam, sweetpotato and cassava exhibited B-type crystallinity with a distinctive major diffraction peak at 5.4-5.6° 2θ angles. However, relatively strong peaks at 7.6-7.8° 2θ angles also suggested the presence of single helical conformations. Since functionality of starches also depends on granule size and particle size distribution, sweetpotato and cocoyam starch will show markedly different functionalities.

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CHAPTER 4

CHEMICAL COMPOSITION OF STARCHES FROM COCOYAM AND SWEETPOTATO STARCHES GROWN IN MALAWI

4.1 Introduction

Starch granules constitute two major components: amylose and amylopectin polymers, and minor non-carbohydrate components: ash (minerals and salts), lipids and proteins (Moorthy, 2002). These components vary from one crop to another and with variety and environment (Shujun *et al*., 2006; Peroni *et al*., 2006; Lu *et al*., 2005; Tester *et al*., 2004; Jane *et al*., 1996, 1992). The amylose/amylopectin ratio greatly influences the functionality of the starch such as swelling power and solubility (Lu *et al*., 2005; Sasaki and Matsuki, 1998), peak and hot paste viscosity (Collado *et al*., (1999), onset and peak temperatures of gelatinization (Fredriksson *et al*., 1998), and retrogradation tendencies (Fredriksson *et al*., 1999). The most common minerals found in starch are calcium, magnesium, phosphorus, potassium and sodium. However, these minerals are found in relatively small quantities (<0.4%) and most of them are of little functional significance except phosphorus (Tester *et al*., 2004) though, sodium, potassium and calcium have been shown to influence on RVA pasting properties of potato starches (Zaidul *et al*., 2007). Phosphorus affects starch functional properties such as paste clarity, viscosity consistency, paste stability, swelling power, gel strength and gelatinization temperatures (Noda *et al*., 2007; Jane *et al*., 1996; Moorthy and Nair, 1989). Lipids also influence starch functional properties such as swelling, water binding capacity, solubility, paste viscosity and pasting characteristics (Radhika *et al*., 2008; Shimelis *et al*., 2006).

Until now, no research has been conducted to evaluate the chemical composition and mineral profile of starches isolated from sweetpotato and cocoyam grown in Malawi. Availability of such information is vital if the starches from these crops are to be exploited for the Malawi industry. The objective of this study in this chapter was therefore to determine the chemical composition of starches isolated from sweetpotato and cocoyam grown in Malawi.

4.2 Materials and methods

4.2.1 Materials

Fifteen sweetpotato genotypes (A45, Babache, Kakoma, Kamchiputu, Kenya, Lunyangwa, LU96/303, LU96/304, Mafutha, Mugamba, Mugande, Salera, Semusa, Tainoni and Zondeni), seven cocoyam accessions from seven different cocoyam growing districts in Malawi [Chitipa, Mzuzu (Mzimba), Nkhotakota, Machinga, Mulanje, Thyolo, and Zomba], and five cassava genotypes (Gomani, Maunjili, Mbundumali, Mkondezi and Sauti) were used for this study.

4.2.2 Moisture content

Moisture content was determined according to the ISO method (Benesi, 2006). Crucibles were cleaned and dried in an oven at 105°C overnight, cooled to room temperature in a desiccator with dry Silica gel for 40 min, and weighed to the nearest 1 mg (W_0). Triplicate starch samples of 2-3 g were weighed into the cooled crucibles (W_1). The crucibles and samples were dried for 24 h at 105°C, and then dried samples and their containers were cooled down in a desiccator with dry Silica gel for 1 h and weighed immediately after removal from the desiccator to the nearest 1 mg (W_2). The moisture content in percent (% MC) of the samples (average results of three replicates) was calculated as follows:

$$\% \text{ Moisture} = 100 - \left\{ \frac{(W_2 - W_0)}{(W_1 - W_0)} \times 100 \right\}$$

4.2.3 Ash content

Clean, dry ashing crucibles were heated for half an hour in a muffle furnace at 550°C, cooled in a desiccator containing a drying agent (Silica gel) to room temperature for 30 min, and weighed on a balance to the nearest 0.1 mg (W_0). Starch samples of 2-3 g were weighed in triplicate to the nearest 0.1 mg and put in crucibles (W_1), placed in a muffle furnace at 525°C

for 5 h, then cooled in a desiccator and weighed immediately to the nearest 0.1 mg (W_2). Ash content was determined by weight difference and expressed as a percentage as follows:

$$\text{Ash content (\%)} = \left\{\frac{(W_2 - W_0)}{(W_1 - W_0)} \times 100\right\}$$

4.2.4 Protein content

The starch protein content was analyzed using an automatic Protein/Nitrogen Determinator LECO FP-528. Starch samples of about 3 g were dried in an oven at 105°C for 24 h, and then cooled in a desiccator containing dry Silica gel for 1 h. The dried starch samples (0.30 g) in triplicate were weighed immediately after removal from the desiccator and then loaded into the protein analyzer. The protein was calculated by multiplying the total nitrogen with 6.25, a conversion factor obtained from the calibration of the instrument using high purity ethylenediamminetetraacetic acid (EDTA).

4.2.5 Fat content

Fat content of the starches was determined using Soxhlet extraction. Starch samples of about 3 g were dried in an air oven at 105°C overnight and cooled in a desiccator. Clean extraction pots were also dried in an oven at 105°C overnight, cooled in a desiccator, and weighed (W_0). The dried starch samples of about 3 g (W_1) were weighed in triplicate on a Whatman No. 1 filter paper, wrapped lightly and plugged with defatted cotton wool down the extraction thimble. Fat was extracted from the sample using 150 mL hexane for 3 h, after which the samples were removed from the extractor and most of the solvent was removed by evaporation. The extraction pots were then dried at 105°C overnight, cooled in a desiccator, and reweighed (W_1). The fat content of the starches was calculated as follows:

$$\text{Fat (\%)} = \frac{(W_2 - W_0)}{W_1} \times 100$$

4.2.6 pH

Triplicate starch samples, 5g dry basis (db), were weighed into a beaker and mixed with 20 mL of distilled water. The resulting suspension was stirred for 5 min and left to settle for 10 min. The pH of the water phase was measured using a calibrated pH meter (AACC, 2000).

4.2.7 Amylose content

Amylose content was determined using an Amylose/Amylopectin Assay Kit (Megazyme International Ireland Ltd., Bray, Ireland). In this method, starch samples are completely dispersed by heating in dimethyl sulphoxide, and lipids are removed by precipitating the starch in ethanol. The precipitated starch is recovered by centrifugation and dissolved in acetate buffer solution. Amylopectin is specifically precipitated by addition of concavanalin (Con A) and removed by centrifugation. Amylose in the aliquot is determined using glucose/peroxidase reagent (GOPOD) after enzymatic hydrolysis to glucose. Total starch in a separate aliquot is determined using GOPOD reagent after hydrolysis to glucose and the concentration of amylose is estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of Con A precipitated sample, to that of total starch sample (Gibson *et al*., 1997). Duplicate starch samples of 25 mg (db) were weighed to the nearest 0.1 mg in 10 mL centrifuge tubes and 1 mL of DMSO was added while stirring at low speed. The tubes were then capped and the starch suspension heated in a boiling water bath for 1 min after which the contents were vigorously mixed and then heated for 15 min with constant stirring. After 15 min, the tubes were removed from the boiling water bath, cooled to room temperature for 5 min and 6 mL of 95% ethanol added. The tubes were left to stand for 1 h to precipitate the starch. After 1 h the tubes were centrifuged at 12000 x *g* for 5 min and the supernatant was decanted. The tubes were then drained on tissue paper for 10 min to ensure all ethanol was removed. To the starch pellet, 1 ml DMSO was added with gentle mixing and the mixture heated in a boiling water bath for 15 min with constant stirring. After the heating period, 2 mL of diluted Con A solvent was added and the mixture transferred quantitatively to a 25 mL volumetric flask and made up to the mark with Con A solvent.

The resulting starch solution was used to precipitate amylopectin, determine amylose and total starch as follows: amylopectin was precipitated from the starch solution by mixing 1 mL of the starch solution with 0.50 mL of Con A solution (4 mg mL^{-1}) in a 2.0 mL Eppendorf micro centrifuge tube. The mixture was left to stand for 1 h at room temperature and then centrifuged at 20000 x *g* for 10 min. Aliquots (1 mL) of the supernatant were transferred into the screw-capped centrifuge tubes and 3 mL of 0.1 M sodium acetate buffer (pH 4.5) added. The resulting mixture was heated in a boiling water bath for 5 min to denature the Con A. After 5 min, the tubes were placed in a water bath at 40°C for 5 min, and 0.1 mL of amyloglucosidase/α-amylase enzyme mixture added and the mixture was incubated at 40°C for 30 min. The resultant mixture was susbsequently centrifuged at 12000 x *g* for 5 min. Amylose was determined by transferring 1 mL aliquots of the supernatant in triplicate into glass test tubes, adding 4 mL of the GOPOD reagent and incubating the mixture at 40°C for 20 min. The absorbance of the samples and standard solution was measured at 510 nm against a reagent blank using a UV-Visible spectrophotometer (Spectronic Unicam, Heλios, Cambridge, United Kingdom). The blank solution contained 1 mL of sodium acetate buffer and 4.0 mL of the GOPOD reagent, incubated for the same period as the samples. A standard control contained 0.1 mL of glucose standard solution (1 mg mL^{-1}), 0.9 mL of sodium acetate buffer and 4 mL of GOPOD reagent treated in the same way as the sample solutions. In order to determine total starch, 0.5 mL aliquots of the diluted starch solution were placed in clean test tubes, mixed with 4 mL of 0.1 M sodium acetate buffer (pH 4.5) and 0.1 mL of amyloglucosidase/α-amylase enzyme mixture and the mixture was incubated at 40°C for 10 min. Aliquots (1 mL) of the digested solution were transferred in triplicate into glass tubes and 4 mL of GOPOD reagent was added. The mixture was incubated at 40°C for 20 min. The incubation was performed concurrently with samples and standards for amylose determination and the absorbance also read at 510 nm together with the amylose determination samples.

The amylose (% w/w) was calculated using the following equation:

$$\text{Amylose \%} = \frac{\text{Absorbance Con A supernatant}}{\text{Absorbance Total Starch aliquot}} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

$$= \frac{\text{Absorbance Con A supernatant}}{\text{Absorbance Total Starch aliquot}} \times 66.8$$

Where 6.15 and 9.2 are dilution factors for the Con A and Total Starch extracts respectively (Megazyme International Ireland Limited, 2006).

4.2.8 Determination of mineral composition

4.2.8.1 Digestion of starch samples

The mineral composition of the starches was determined by a modified method of Njoku and Ohia (2007). Starch samples (2-3 g) were weighed into 150 mL ceramic beakers in triplicate, ashed at 550°C for 5 h, cooled in a desiccator, and mixed with 10 mL of 1.0 M HNO_3 to digest the ash. The digest was quantitatively transferred to a 100 mL volumetric flask with distilled water and made up to the mark. The diluted digest was then used to determine the mineral contents of the starches.

4.2.8.2 Determination of phosphorus (P)

Phosphorus content of the starch samples was determined from the diluted digest by the ascorbic acid colorimetric method (Murphy and Riley, 1958). Triplicate aliquots (1 mL) were transferred to test tubes and mixed with 9 mL of Murphy-Riley solution. The mixture was left to stand for 15 min and absorbance at 880 nm read against a reagent blank. The Murphy-Riley solution was prepared by dissolving separately 0.40 g of antimony potassium tartrate, 4.3 g ammonium molybdate in 400 mL and 100 mL of distilled water, respectively, and then mixing the solutions in a 1000 mL volumetric flask. The flask was then placed in an ice bath and 54 mL of concentrated sulphuric acid was added slowly with stirring. The solution was cooled and made up to the 1000 mL mark with distilled water. An aliquot (100 mL) of this solution was mixed with 500 mL of distilled water and 0.526 g ascorbic acid and made up to

1000 mL with distilled water to make the final Murphy-Riley working solution. A calibration curve was prepared using phosphorus standards ranging from 0.01 to 2.0 mg P/L.

4.2.8.3 Determination of metals: calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K) and Zinc (Zn)

The metal ions were analyzed on a Varian SpectrAA 300 spectrometer (Varian Techtron Pty. Limited, Mulgrave Victoria, Australia) at the following wavelengths; 589.0 nm for sodium (Na), 766.5 nm for potassium (K), 248.3 nm for iron (Fe), 214.0 nm for Zinc (Zn), 285.2 nm for Magnesium (Mg), 279.6 nm for Manganese (Mn), and 422.7 nm for calcium (Ca) using an air-acetylene flame.

4.2.9 Data analysis

Univariate analysis

The data obtained was subjected to analysis of variance (ANOVA) using Statistix 8 for Windows software (Analytical software, Tallahassee, USA, 1985) to compare the variation in chemical composition of the starches with botanical source and within the same plant.

Multivariate analysis

Principal component analysis (PCA) was performed on the chemical composition of the starches using NCSS 2004 statistical software (Hintze, 2001). The first and second PCs were used to show pattern of the data.

4.3 Results and discussion

4.3.1 Proximate composition of cocoyam, sweetpotato and cassava starches

The results of the proximate analysis of the cocoyam, sweetpotato and cassava starches are presented in Tables 4.1 and 4.2.

4.3.1.1 Moisture content and pH

The moisture content (MC) of the tested starches ranged from 12.4-13.0% for cassava, 10.4-13.2% for cocoyam and 7.9-11.6% for sweetpotato starches. These moisture levels are in agreement with those reported for root and tuber starches (Nwokocha *et al.*, 2009; Pérez *et al*., 2005; Chen *et al*., 2003; Moorthy, 2002; Tian *et al*., 1991). The moisture levels of the starches also fall within the recommended moisture levels for safe storage (maximum of 13%) except for Chitipa cocoyam starch (13.2%). Higher levels of MC can lead to microbial damage and subsequent deterioration in quality (Moorthy, 2002). Generally, cassava starch gave higher moisture content (12.8±0.2%) values than cocoyam (11.8±1.0%) and sweetpotato (9.9±1.0%) starch. The moisture content levels differed significantly ($p \leq 0.001$) among the different starches of cocoyam, sweetpotato and cassava starches. The highest moisture levels were exhibited by Chitipa cocoyam starch, and Sauti, Mkondezi and Maunjili cassava starches while Salera sweetpotato starch gave the lowest. The differences in moisture content of the starches could be due to the extent of drying (Moorhty, 2002).

Table 4.1 Average values and mean separation of moisture, ash, protein and fat contents and pH of cocoyam, sweetpotato and cassava starches

Botanical source	Genotype/ Accession	MC (%)	pH	Ash (%)	Protein (%)	Fat (%)
Cocoyam	Chitipa	13.2^{a}	6.4^{de}	0.137^{ghi}	0.55^{d}	0.150^{ab}
	Machinga	11.1^{g}	6.3^{e}	0.153^{fg}	0.71^{b}	0.135^{cde}
	Mulanje	10.9^{ghi}	6.6^{c}	0.133^{ghi}	0.48^{e}	0.160^{a}
	Mzuzu	12.6^{bcd}	6.5^{cd}	0.150^{fgh}	0.45^{f}	0.135^{cde}
	Nkhotakota	12.1^{e}	6.7^{b}	0.133^{ghi}	0.70^{b}	0.105^{ijk}
	Thyolo	10.4^{jkl}	5.2^{k}	0.157^{efg}	0.84^{a}	0.145^{bc}
	Zomba	12.2^{de}	7.0^{a}	0.143^{ghi}	0.49^{e}	0.130^{def}
Mean value ± SD		11.8±1.0	6.4±0.1	0.14±0.02	0.60±0.14	0.14±0.02
Sweetpotato	A45	10.0^{lm}	5.0^{l}	0.243^{b}	0.36^{jk}	0.125^{efg}
	Babache	8.4^{p}	5.2^{k}	0.277^{a}	0.39^{ghij}	0.130^{def}
	Kakoma	10.3^{kl}	5.5^{j}	0.203^{c}	0.41^{g}	0.145^{bc}
	Kamchiputu	9.7^{mn}	4.9^{l}	0.253^{ab}	0.53^{d}	0.110^{hij}
	Kenya	9.6^{n}	5.7^{gh}	0.200^{c}	0.53^{d}	0.120^{fgh}
	Lunyangwa	10.5^{jk}	5.5^{j}	0.163^{def}	0.39^{ghi}	0.105^{ijk}
	LU96/303	11.6^{f}	5.2^{k}	0.183^{cd}	0.50^{e}	0.115^{ghi}
	LU96/304	10.4^{jkl}	5.4^{j}	0.137^{ghi}	0.36^{k}	0.100^{jkl}
	Mafutha	10.1^{l}	5.1^{k}	0.157^{efg}	0.39^{ghij}	0.125^{efg}
	Mugamba	10.6^{jkl}	5.6^{hi}	0.147^{fgh}	0.39^{ghi}	0.150^{ab}
	Mugande	11.1^{gh}	5.9^{f}	0.180^{cde}	0.66^{c}	0.130^{def}
	Salera	7.9^{q}	4.9^{l}	0.147^{fgh}	0.36^{k}	0.100^{jkl}
	Semusa	9.5^{n}	5.9^{f}	0.187^{cd}	0.40^{g}	0.095^{klm}
	Tainoni	10.7^{hij}	4.9^{l}	0.230^{b}	0.49^{e}	0.150^{ab}
	Zondeni	9.0^{o}	4.7^{m}	0.153^{fg}	0.45^{f}	0.105^{ijk}
Mean value ± SD		9.9±1.0	5.3±0.1	0.19±0.04	0.44±0.08	0.12±0.02
Cassava	Gomani	12.4^{cde}	5.5^{ij}	0.133^{ghi}	0.37^{ijk}	0.085^{m}
	Maunjili	12.9^{ab}	4.9^{l}	0.120^{i}	0.37^{hijk}	0.140^{bcd}
	Mbundumali	12.7^{bc}	5.4^{j}	0.127^{hi}	0.35^{k}	0.110^{hij}
	Mkondezi	12.8^{abc}	5.6^{ghi}	0.030^{j}	0.39^{ghi}	0.110^{hij}
	Sauti	13.0^{ab}	5.7^{g}	0.133^{ghi}	0.36^{k}	0.090^{lm}
Mean value ± SD		12.8±0.2	5.4±0.1	0.11±0.04	0.37±0.02	0.11±0.02
LSD (P=0.05)		0.23	0.12	0.02	0.01	0.01
CV (%)		1.29	1.27	8.9	1.92	4.45

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

The pH values ranged from 4.9-5.7, 5.2-7.0 and 4.7-5.9 with average values of 5.4, 6.4 and 5.3 for cassava, cocoyam and sweetpotato starches, respectively. Generally, cocoyam starches exhibited higher pH values than sweetpotato and cassava starches. The pH values of sweetpotato and cassava starches were comparable. The pH varied considerably among the different starches from the cocoyam accessions, sweetpotato and cassava genotypes. The highest pH was displayed by Zomba cocoyam starch (7.0) while Zondeni sweetpotato starch gave the loswet (4.7). Very few studies have reported pH values of starches from root and tuber crops. Nwokocha *et al*. (2009) reported pH values of 6.56 and 6.76 for starches from Nigerian cassava and cocoyam, respectively. Mbougueng *et al*. (2008) reported pH values ranging from 5.43-6.56 for three cassava cultivars grown in Cameroon. Thus, the pH values of the starches obtained in this study are in agreement reported in literature. The pH of the starches is also within the acceptable range for low acid food starches (Thomas and Atwell, 1999; Moore *et al*., 1984).

4.3.1.2 Ash, protein and fat content

Ash, protein and fat content varied significantly ($p \leq 0.001$) among the different starches. Generally, sweetpotato starches gave higher ash content (0.19±0.04%) than cocoyam (0.14±0.02%) and cassava starches (0.11±0.04%). The ash content values ranged from 0.133-0.157%, 0.137-0.277% and 0.030-0.133% for cocoyam, sweetpotato and cassava starches, respectively. The starches exhibited low levels of protein and fat (<1%). Nonetheless, higher protein levels were observed in cocoyam starches (0.60±0.14%) than in sweetpotato (0.44±0.08%) and cassava starches (0.37±0.02%). A similar trend was observed in fat contents of the starches. Cocoyam starches gave higher levels of fat (0.14±0.02%) than sweetpotato (0.12±0.02%) and cassava starches (0.11±0.02%). Both protein and fat contents varied significantly ($p \leq 0.001$) among the cocoyam accessions and within the sweetpotato and cassava genotypes. Protein content of the starches ranged from 0.490-0.835% for cocoyam, 0.359-0.659% for sweetpotato, and 0.352-0.391% for cassava starches. The values of fat content ranged from 0.105-0.160%, 0.095-0.150% and 0.085-0.140% for cocoyam, sweetpotato and cassava starches, respectively. Higher levels of protein were obtained in Thyolo, Nkhotakota and Machinga cocoyam accessions. Protein content was lower in Mzuzu

cocoyam starch. Mulanje and Chitipa cocoyam starches gave the highest levels of fat content while Nkhotakota had the lowest. Starch from Mugande sweetpotato genotype had the highest protein content among the sweetpotato starches and Salera and LU96/304 had the lowest. Tainoni and Kakoma sweetpotato starches gave the highest levels of fat among the sweetpotato starches, and Salera and Semusa the lowest. Protein content did not vary much among the cassava genotypes; however, Mkondezi had higher protein content than the rest of cassava starches while Mbundumali had the lowest. Maunjili cassava starch had the highest fat content, and Sauti and Gomani the lowest. Studies have shown that protein, ash and lipid content of the starches vary with botanical source. Peroni *et al*. (2006) studied the chemical composition of starches of six different crops; cassava, arrowroot, sweetpotato, yam, canna and ginger. Significant variations in protein, ash and lipid content were observed; cassava starch exhibited higher protein (0.20±0.03%), and lipid (0.15±0.03%) levels than sweetpotato starch (0.14±0.03%, 0.14±0.01% respectively). Both sweetpotato and cassava starch gave the same ash content levels (0.21±0.01%) which were lower than that of ginger (0.35±0.03%), arrowroot (0.28±0.03%) and canna starches (0.24±0.03%). Pérez *et al*. (2005) compared properties of cocoyam and cassava starches. They reported higher levels of ash (0.31%) and protein (0.53%) in cocoyam starch than in cassava starch (0.12%). No proteins were detected in cassava starch. Ash and lipid contents ranging from 0.81-0.92%, 0.39% for cocoyam starch, 0.05-1.3% and 0.006-0.6% for sweetpotato starch, and 0.01-0.8% and 0.07-1.54% for cassava starch, respectively, have been reported (Moorthy, 2002; Hoover, 2001). Ash and fat content of the sweetpotato and cassava starches found in this study fall within those ranges reported. However, those of cocoyam starches are lower. These differences in ash, fat and protein content could therefore be attributed to botanical and cultivar variations.

Table 4.2 Average values and mean separation of amylose and amylopectin contents of cocoyam, sweetpotato and cassava starches

Botanical source	Genotype/Accession	Amylose (%)	Amylopectin (%)
Cocoyam	Chitipa	16.1^{hij}	83.9^{fgh}
	Machinga	13.6^{klm}	86.4^{cde}
	Mulanje	11.1^{no}	88.9^{ab}
	Mzuzu	15.5^{ijk}	84.5^{efg}
	Nkhotakota	10.6^{o}	89.4^{a}
	Thyolo	16.1^{hij}	83.9^{fgh}
	Zomba	21.0^{ef}	79.0^{jk}
Mean value ± SD		14.8±3.4	85.2±3.4
Sweetpotato	A45	18.1^{gh}	81.9^{hi}
	Babache	22.9^{cde}	77.1^{klm}
	Kakoma	17.4^{ghi}	82.6^{ghi}
	Kamchiputu	18.9^{fg}	81.1^{ij}
	Kenya	14.8^{jkl}	85.2^{def}
	Lunyangwa	25.0^{bc}	75.0^{mn}
	LU96/303	12.0^{mno}	88.0^{abc}
	LU96/304	34.4^{a}	65.6^{o}
	Mafutha	19.4^{fg}	80.6^{ij}
	Mugamba	12.6^{lmno}	87.4^{abcd}
	Mugande	25.0^{bc}	75.0^{mn}
	Salera	25.9^{b}	74.1^{n}
	Semusa	17.5^{ghi}	82.5^{ghi}
	Tainoni	25.7^{b}	74.3^{n}
	Zondeni	22.5^{de}	77.6^{kl}
Mean value ± SD		20.8±5.9	79.2±5.2
Cassava	Gomani	23.7^{bcd}	76.3^{lmn}
	Maunjili	17.7^{ghi}	82.3^{ghi}
	Mbundumali	19.6^{fg}	80.4^{ij}
	Mkondezi	19.4^{fg}	80.6^{ij}
	Sauti	13.3^{klmn}	86.7^{bcd}
Mean value ± SD		18.8±3.6	81.2±3.6
LSD (P=0.05)		1.38	1.38
CV (%)		4.46	1.04

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

4.3.3.1.3 Amylose and amylopectin

The amylose content of the starches ranged from 10.6 to 34.4% (Table 4.2) and varied significantly ($p \leq 0.001$) between cocoyam, sweetpotato and cassava starches. These levels of amylose are within those of normal starches i.e. not less than 10% and not more than 30% (Noda *et al*., 1998), except for starch from LU96/304 sweetpotato genotype that gave the highest value of amylose content (34.4%). Generally, starches from the sweetpotato genotypes exhibited higher levels of amylose (20.8±5.9%) than those of the cocoyam accessions (14.8±3.4%). Starches from cassava genotypes displayed amylose levels (18.8±3.6%) comparable to those of sweetpotato. The amylose/amylopectin ratio of tropical root and tuber crops has been shown to vary with botanical source. Wickramasinghe *et al*. (2009) reported higher levels of amylose in cassava starches (25.4-28.8%) than in starches from sweetpotato (16.6-23.5%) grown in Sri Lanka. Peroni *et al*. (2006) reported the opposite for tropical root and tuber crops of Brazil. Sweetpotato starches displayed higher levels of amylose (22.6%) than cassava starches (19.8%). Tetchi *et al*. (2007) found higher levels of amylose in cocoyam starch (26.7%) compared to sweetpotato (24.0%) and cassava starches (21.6%) produced in the Ivory Coast. Nwokocha *et al*. (2009) also reported higher levels of amylose in cocoyam starch (33.3%) than in cassava starch (29.3%). The results of this study therefore confirm that botanical source has significant influence on the amylose/amylopectin ratio of the starches. Different trends in levels of cocoyam, sweetpotato and cocoyam starches may be attributed to genotypic variations.

The amylose content of the starches varied significantly ($p \leq 0.001$) among the different cocoyam accessions as well as within the sweetpotato and cassava genotypes. Amylose content of the cocoyam starches ranged from 10.6-21.0%. Amylopectin content ranged from 79.0-89.4%. Starch from Zomba cocoyam accession gave the highest levels of amylose (21.0%) and that from Nkhotakota cocoyam accession the lowest (10.6%). Different studies have reported different amylose contents for cocoyam starches. These differences have been attributed to different cultivars as well as methods employed. Jane *et al*. (1992) reported amylose contents ranging from 18 to 22% as determined by iodine affinity for taro grown in Hawaii. Moorthy *et al*. (1993) also determined amylose content of ten taro varieties in India

by iodine affinity, and found amylose contents ranging from 14 to 19%. Recent studies have reported amylose contents ranging from 14.7 to 30.8% for six varieties of Cameroon taro starches determined by the DSC method (Aboubakar *et al*., 2008). Amylose content of starches from three taro varieties grown in Taiwan during winter, spring and summer seasons determined by iodine potentiometric titration ranged from 8.7 to 14.9% (Lu *et al*., 2008). Thus, amylose content of cocoyam starches found in this study fall within the reported ranges.

The amylose and amylopectin contents of the starches from the 15 sweetpotato genotypes ranged from 11.4-34.4% and 65.6-80.6%, respectively. Starch from LU96/304 sweetpotato genotype had the highest amylose content (34.4%) while LU96/303 gave the lowest (12.0%). Tainoni, Mugande and Lunyangwa sweetpotato starches also gave relatively high amounts of amylose. Starches from cassava genotypes had amylose and amylopectin contents ranging from 13.3-23.7% and 76.3-86.7%, respectively. Gomani had the highest level of amylose (23.7%) and Sauti the lowest (13.3%). The amylose contents of sweetpotato and cassava starches obtained in this study fall within ranges reported in the literature (Moorthy, 2002; Hoover, 2001; Tian *et al*., 1991). The differences in amylose contents of the cocoyam, sweetpotato, and cassava starches may therefore be attributed to botanical and cultivar variations (Sefa-Dedeh & Sackey, 2002; Katayama *et al*., 1999; Moorthy *et al*., 1993). This therefore indicates that these starches will display different functional properties.

4.3.2 Mineral composition

The mineral composition of the starches (Tables 4.3 and 4.4) revealed the presence of higher levels of potassium, calcium and phosphorus than those of magnesium, iron, manganese, sodium and zinc. Magnesium and sodium were present in intermediate levels while iron, zinc and manganese were present in very low amounts with manganese being the lowest. Levels of K, Ca, P, Mg, Fe, Mn, Na, and Zn were 345±42, 36±14, 109±13, 35±11, 8±2, 0.5±0.1, 34±12, and 1.9±0.5 mg kg^{-1}, respectively, for cocoyam starches, 254±80, 196±93, 118±22, 50±12, 14±2, 1.1±0.4, 38±12, and 0.5±0.2 mg kg^{-1}, respectively, for sweetpotato starches and 44±14, 106±37, 90±21, 44±11, 13±4, 0.5±0.2, 38±12, and 1.7±0.4 mg kg^{-1} respectively for

cassava starches. Mineral levels of the starches varied significantly ($p \leq 0.001$) from one crop to another. Generally cocoyam starches gave significantly higher ($p \leq 0.001$) levels of potassium than sweetpotato and cassava starches, but sweetpotato starches displayed higher levels of phosphorus (118±22 mg kg^{-1}) and calcium (196±93 mg kg^{-1}) than cocoyam (109±13 mg P kg^{-1}, 36±14 mg Ca kg^{-1}) and cassava (90±21 mg P kg^{-1}, 106±37 mg Ca kg^{-1}). Though Mg, Fe, Zn, Mn and Na were present in lower levels compared to K, Ca and P, sweetpotato starches generally showed higher levels of Mg, Fe, Zn and Mn than cocoyam and cassava starches. Sodium levels were similar in cocoyam, sweetpotato and cassava starches.

The mineral levels also varied significantly ($p \leq 0.001$) within the crop though no consistent trends were observed. Among the starches from the cocoyam accessions, Zomba and Chitipa gave the highest levels of phosphorus (120, 117 mg kg^{-1}, respectively) and Mulanje the lowest (99 mg kg^{-1}). The highest levels of potassium were observed in starches from Machinga (382 mg kg^{-1}) and Nkhotakota (377 mg kg^{-1}) starches, and the lowest in Zomba starch (288 mg kg^{-1}). Zomba cocoyam starch displayed the highest levels of calcium (51 mg kg^{-1}) and Mzuzu the lowest (26 mg kg^{-1}). Among sweetpotato starches, the highest level of phosphorus was observed in Kakoma (146 mg kg^{-1}) starch and the lowest in Zondeni starch (87 mg kg^{-1}). Kenya starch gave the highest level of potassium and Zondeni the lowest (140 mg kg^{-1}). Kamchiputu starch gave the highest level of calcium (392 mg kg^{-1}) and Mugande the lowest (90 mg kg^{-1}). Among the cassava starches, Gomani gave the highest levels of phosphorus (115 mg kg^{-1}) and potassium (64 mg kg^{-1}) while Mbundumali had the highest calcium content (138 mg kg^{-1}). The lowest levels of phosphorus, potassium and calcium were found in Mkondezi starch (67, 32, and 51 mg kg^{-1}, respectively).

Table 4.3 Average values and mean separation of mineral composition: P, Ca, Mg, K, and Na of the cocoyam, sweetpotato and cassava starches

Botanical Source	Genotype/ Accession	Mineral concentration (mg kg^{-1})				
		P	**Ca**	**Mg**	**K**	**Na**
Cocoyam	Chitipa	117^{bcd}	48^{klm}	30^{jk}	369^{bc}	27.0^{ijkl}
	Machinga	110^{bcde}	43^{klm}	40^{hi}	382^{b}	21.6^{kl}
	Mulanje	99^{efgh}	29^{lm}	31^{ij}	294^{f}	31.5^{fgh}
	Mzuzu	108^{cde}	26^{m}	21^{k}	346^{cd}	29.1^{ghi}
	Nkhotakota	102^{efg}	29^{lm}	27^{jk}	377^{b}	26.0^{jkl}
	Thyolo	103^{def}	37^{klm}	54^{bcd}	357^{bc}	51.3^{bc}
	Zomba	120^{bcd}	51^{kl}	31^{ij}	288^{f}	51.9^{bc}
Mean ± SD		109±13	36±14	34.5±11	345±42	34.1±12.1
Sweetpotato	A45	116^{bcd}	293^{c}	48^{def}	302^{ef}	44.8^{cd}
	Babache	110^{bcde}	343^{b}	75^{a}	242^{g}	30.9^{fgh}
	Kakoma	146^{a}	231^{d}	58^{bc}	246^{g}	35.3^{efg}
	Kamchiputu	113^{bcde}	392^{a}	56^{bcd}	197^{hi}	33.9^{efgh}
	Kenya	133^{abc}	124^{gh}	50^{cde}	438^{a}	67.5^{a}
	Lunyangwa	113^{bcde}	145^{fg}	44^{fgh}	184^{ij}	34.3^{efgh}
	LU96/303	130^{abc}	169^{e}	49^{cde}	324de	35.0^{efg}
	LU96/304	137^{ab}	112^{hi}	52^{bcd}	310^{ef}	36.3^{ef}
	Mafutha	119^{bcd}	152^{ef}	49^{cde}	198^{hi}	28.5^{ghi}
	Mugamba	119^{bcd}	96^{ij}	41^{gh}	347^{cd}	32.1^{fgh}
	Mugande	109^{cde}	90^{ij}	31^{ij}	302^{ef}	26.5^{jkl}
	Salera	89^{ghi}	133^{fgh}	31^{ij}	167^{jk}	27.4^{hij}
	Semusa	132^{abc}	149^{ef}	54^{bcd}	224^{gh}	39.5^{de}
	Tainoni	119^{bcd}	282^{c}	61^{b}	187^{ij}	51.9^{dc}
	Zondeni	87^{ij}	229^{d}	56^{bcd}	140^{k}	21.0^{l}
Mean ± SD		118±22	196±93	50±12	254±80	36.4±11.7
Cassava	Gomani	115^{bcde}	124^{gh}	55^{bcd}	64^{l}	57.2^{b}
	Maunjili	87^{ij}	86^{j}	47^{def}	38^{lm}	36.9^{ef}
	Mbundumali	92^{fgh}	138^{fg}	46^{efg}	40^{lm}	32.3^{fgh}
	Mkondezi	67^{j}	51^{kl}	25^{jk}	32^{m}	28.9^{fghi}
	Sauti	89^{hij}	132^{fgh}	47^{def}	43^{lm}	31.4^{fghi}
Mean ± SD		90±21	106±37	44.1±11.2	44±14	37.5±12.1
LSD (P=0.05)		27.1	22.7	9.6	29.0	7.1
CV (%)		15.0	10.0	13.0	7.4	12.1

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

Table 4.4 Average values and mean separation of mineral composition: Fe, Mn, and Zn of the cocoyam, sweetpotato and cassava starches

Botanical Source	Genotype/ Accession	Mineral concentration (mg kg^{-1})		
		Fe	**Mn**	**Zn**
Cocoyam	Chitipa	9.3^{gh}	0.41^{mn}	2.09^{ijk}
	Machinga	8.5^{ghi}	0.43^{mn}	2.35^{hi}
	Mulanje	6.2^{ij}	0.39^{n}	1.29^{m}
	Mzuzu	10.7^{efg}	0.43^{mn}	1.78^{jkl}
	Nkhotakota	7.5^{hi}	0.39^{n}	1.76^{kl}
	Thyolo	4.4^{j}	0.39^{n}	2.54^{gh}
	Zomba	7.8^{hi}	0.79^{ijk}	1.56^{lm}
Mean ± SD		7.8±2.3	0.5±0.1	1.9±0.5
Sweetpotato	A45	16.8^{ab}	0.92^{fgh}	1.56^{lm}
	Babache	14.7^{bcd}	1.89^{b}	3.51^{d}
	Kakoma	14.6^{bcd}	1.23^{d}	5.57^{a}
	Kamchiputu	14.6^{bcd}	1.04^{ef}	4.24^{c}
	Kenya	12.5^{def}	0.66^{l}	2.08^{ijk}
	Lunyangwa	15.6^{abc}	1.01^{efg}	3.01^{ef}
	LU96/303	13.8^{cd}	1.45^{c}	2.79^{fg}
	LU96/304	14.4^{bcd}	0.74^{jkl}	3.84^{d}
	Mafutha	13.7^{cd}	0.89^{ghi}	3.16^{e}
	Mugamba	14.2^{bcd}	0.71^{kl}	2.12^{ij}
	Mugande	8.9^{ghi}	0.85^{hij}	1.41^{m}
	Salera	13.5^{cde}	0.83^{hij}	1.85^{jkl}
	Semusa	14.1^{bcd}	2.22^{a}	4.58^{b}
	Tainoni	13.7^{cd}	1.05^{e}	4.28^{bc}
	Zondeni	12.8^{de}	1.30^{d}	2.67^{fgh}
Mean ± SD		13.9±2.2	1.1±0.4	3.1±1.2
Cassava	Gomani	17.8^{a}	0.75^{jkl}	2.10^{ijk}
	Maunjili	12.2^{def}	0.37^{n}	1.56^{lm}
	Mbundumali	10.0^{fgh}	0.39^{n}	2.06^{ijk}
	Mkondezi	7.9^{hi}	0.35^{n}	1.32^{m}
	Sauti	16.7^{ab}	0.53^{m}	1.42^{m}
Mean ± SD		12.9±4.2	0.5±0.2	1.7±0.4
LSD (P=0.05)		2.8	0.1	0.3
CV (%)		14.1	8.9	8.3

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$.

Phosphorus remains the most evaluated mineral in starches due to its established influence on functional properties. Swelling power, paste clarity and stability, peak viscosity and breakdown, and onset and peak gelatinization temperatures have been reported to increase with increasing levels of phosphates (Noda *et al*., 2007; Karim *et al*., 2007). Higher gel strength of *Dioscorea* starch paste compared to cassava starch was attributed to phosphate linkages in the starch granules (Moorthy and Nair, 1989). Phosphorus levels in starch vary with botanical source. Moorthy and Nair (1989) found that *Dioscorea* starch had higher phosphorus content (three times as much) than cassava starch but lower than potato starch. Peroni *et al*. (2006) reported the highest phosphorus content in yam and canna starches (0.022%, 0.031%, respectively) and the lowest in cassava and ginger starches (0.07%). Sweetpotato had twice the phosphorus levels (0.014±0.001%) of cassava starches. In most studies, phosphorus content of the starches has been reported to vary between 0.007-0.012% for cassava, 0.009-0.022% for sweetpotato and 0.006-0.025% for cocoyam starches (Moorthy, 2002; Hoover, 2001; Jane *et al*., 1992; Tian *et al*., 1991). The phosphorus content of the starches obtained in this study; 99-120 mg kg^{-1} (0.010- 0.012%) for cocoyam, 87-146 mg kg^{-1} (0.009-0.015%) for sweetpotato and 67-115 mg kg^{-1} (0.007-0.012%) for cassava starches fall within those ranges reported.

The contents of mineral elements such as potassium, calcium, magnesium, zinc and iron in starches have received little attention compared to phosphorus. However, starches from different botanical sources may contain significant amounts of these elements. Pérez and Lares (2005) found higher levels of sodium, potassium, phosphorus and magnesium than those of iron, calcium and zinc in canna and arrowroot starches. They reported sodium, potassium, phosphorus and magnesium levels of 442.9, 272.0, 779.1 and 203.7 mg kg^{-1} respectively for canna starches and 572.6, 286.0, 751.0 and 122.4 mg kg^{-1} respectively for arrowroot starches. Calcium, zinc and iron levels were 24.0, 22.1 and 47.9 mg kg^{-1} respectively for canna starches and 27.9, 38.2 and 142.7 mg kg^{-1} respectively for arrowroot starches. Clearly, levels of mineral elements obtained for cocoyam, sweetpotato and cassava starches in this study are lower those reported for canna and arrowroot starches except for potassium levels of cocoyam and sweetpotato starches that are higher. Walter *et al*. (2000) reported calcium, potassium, sodium and magnesium levels ranging from 71-583, 7.5-42.5,

8.5-12.0 and 12.0-26.3 mg kg^{-1}, respecively for starches from different sweetpotato cultivars. The levels of calcium obtained in this study for sweetpotato studies fall within those reported by Walter et al. (2000), however, potassium, sodium and magnesium levels are lower.

The presence of these minerals may also have a significant influence on the functional properties of the starches. Calcium, potassium and sodium contents were found to affect pasting properties of potato starches (Zaidul *et al*., 2007). High calcium content was associated with a decrease in peak viscosity and an increase in breakdown, setback viscosity and peak viscosity temperatures, whereas higher potassium content was associated with increasing peak viscosity, breakdown, and setback viscosity and peak viscosity temperature. High sodium content was associated with high peak viscosity and setback viscosity but low breakdown and peak viscosity temperature. The results of this study indicate that sweetpotato starches had relatively higher phosphorus and calcium contents than cocoyam and cassava starches whereas cocoyam starches exhibited higher levels of potassium than sweetpotato and cassava starches. However, the association of the different mineral elements with functional properties of the starches needs to be established.

4.3.3 Principal component analysis (PCA)

The results of the principal component analysis (PCA) performed on the chemical composition of the cocoyam, sweetpotato and cassava starches revealed that the first four principal components explained 75% of the variability (Table 4.5). Principal component 1 (PC1) accounted for 39.0% of the variability, while principal components 2 (PC2), 3 (PC3), and 4 (PC4) accounted for 19.9, 8.8 and 7.3% of the variability.

Eigenvectors of PC1 had large positive weights for calcium, magnesium, manganese, iron, zinc and ash, and large negative weights for pH and moisture. Eigenvectors of PC2 had large positive weights for potassium, protein, phosphorus, fat and ash while those of PC3 had large positive weights for sodium and phosphorus. Eigen vector for PC4 had large positive weighting for sodium (Table 4.5).

Table 4.5 Principal component analysis of 14 chemical composition parameters of starches from seven cocoyam accessions, 15 sweetpotato genotypes and five cassava genotypes

	Eigenvectors			
Variable	**PC1**	**PC2**	**PC3**	**PC4**
Amylose	0.20	-0.21	0.07	0.03
Ash	0.32	0.30	-0.11	-0.09
Fat	-0.10	0.34	-0.25	-0.36
Moisture (MC)	-0.29	-0.14	0.34	-0.14
pH	-0.30	0.22	0.27	0.36
Protein	-0.17	0.39	-0.25	-0.16
Phosphorus (P)	0.20	0.39	0.45	0.21
Calcium (Ca)	0.38	-0.02	-0.20	-0.12
Iron (Fe)	0.32	-0.20	0.28	0.11
Potassium (K)	-0.07	0.53	0.04	0.18
Magnesium (Mg)	0.36	0.04	-0.03	-0.29
Manganese (Mn)	0.34	0.07	-0.04	0.36
Sodium (Na)	0.10	0.14	0.58	-0.59
Zinc (Zn)	0.32	0.16	0.00	0.17
Eigen values	5.5	2.8	1.2	1.0
Individual %	39.0	19.9	8.8	7.3
Cumulative %	39.0	58.9	67.8	75.1

The loading plot of the chemical composition of the starches is presented in Figure 4.1 and the correlation coefficients among the different properties of the starches are presented in Table 4.6. Both the loading plot and the correlation matrix showed that ash, Zn, Mg, Mn and Ca were close to each other. These variables were positively correlated. There was significant negative correlation between amylose and pH of the starches. Ash content was also

negatively correlated with moisture content while moisture content was positively correlated with pH but negatively correlated with the mineral elements (Ca, Mg, Mn and Zn). No correlation was observed between amylose and phosphorus contents of the starches. This disagrees with findings of Singh *et al*. (2006) and Karim *et al*. (2007) who have reported significant negative correlation between phosphorus and amylose for potato starches as increase in phosphorus content is associated with increase in amylopectin fraction.

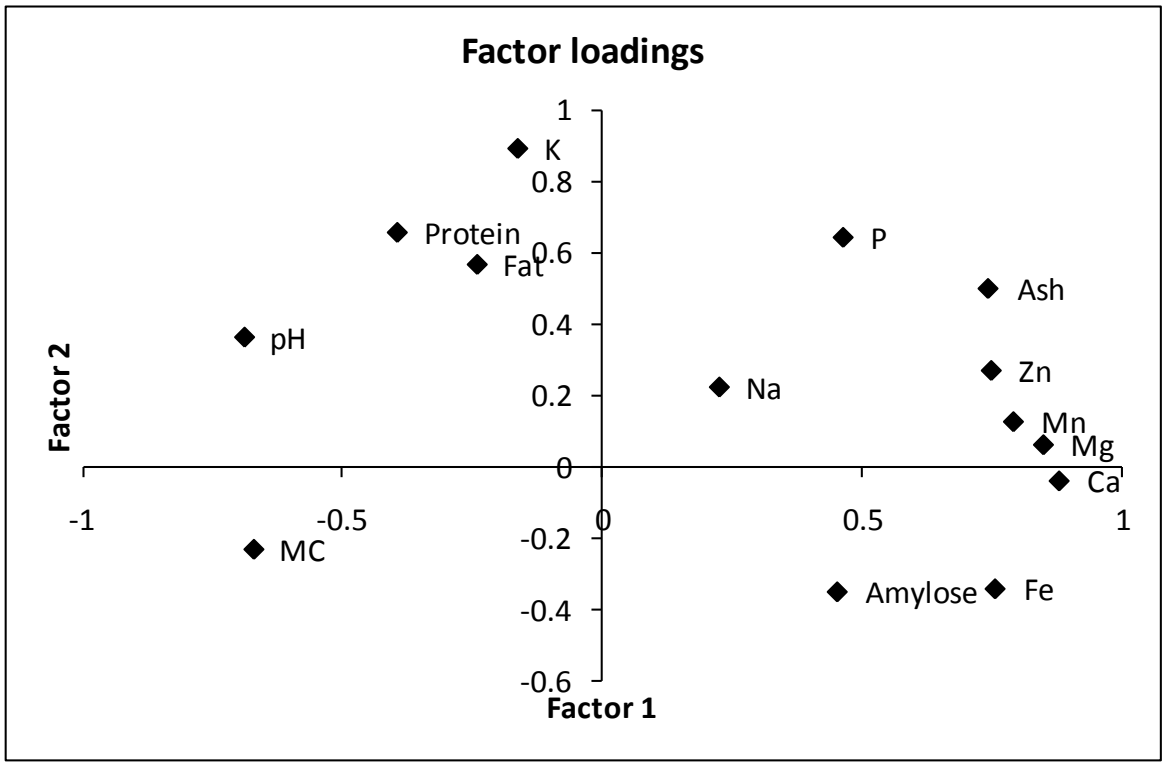

Figure 4.1 PCA loading plot for chemical composition of the cocoyam, sweetpotato and cassava starches

Table 4.6 Correlation coefficients between the chemical composition parameters of the cocoyam, sweetpotato and cassava starches

	Amylose	Ash	Fat	MC	pH	Protein	P	Ca	Fe	K	Mg	Mn	Na
Ash	0.09												
Fat	-0.31	0.16											
MC	-0.32	-0.59**	0.07										
pH	-0.41*	-0.32	0.19	0.49*									
Protein	-0.33	0.06	0.33	0.05	0.34								
P	0.07	0.54**	0.12	-0.26	0.09	0.04							
Ca	0.30	0.79**	-0.13	-0.55**	-0.66**	-0.32	0.22						
Fe	0.32	0.42*	-0.44*	-0.29	-0.55**	-0.69**	0.33	0.64**					
K	-0.30	0.31	0.41*	-0.20	0.44*	0.60**	-0.33	-0.22	-0.33				
Mg	0.28	0.64**	-0.10	-0.48*	-0.64**	-0.18	0.55**	0.74**	0.55**	-0.18			
Mn	0.23	0.61**	-0.24	-0.61**	-0.34	-0.27	0.50**	0.63**	0.50**	-0.02	0.61**		
Na	0.10	0.24	0.00	-0.02	-0.08	0.02	0.15	0.09	0.15	-0.10	0.32	0.03	
Zn	0.29	0.54**	-0.02	-0.46*	-0.36	-0.10	0.62**	0.59**	0.39*	0.03	0.67**	0.65**	0.08

*, ** significant at p = 0.05 and p = 0.01, respectively

The plot of factor scores of PC1 and PC2 grouped the cocoyam, sweetpotato and cassava starches into four quadrants (Figure 4.2). The bottom right quadrant contained five sweetpotato genotypes (Mafutha, L96/304, Lunyangwa, Zondeni and Salera) and one cassava genotype (Gomani). The bottom left quadrant consisted of the four cassava genotypes, Mbundumali, Maunjili, Mkondezi and Sauti. The top left quadrant contained all the cocoyam accessions, and Mugande and Mugamba sweetpotato genotypes. The top right quadrant contained eight sweetpotato genotypes (Kenya, Kakoma, LU96/303, Tainoni, Babache, Kamchiputu, Semusa and A45.

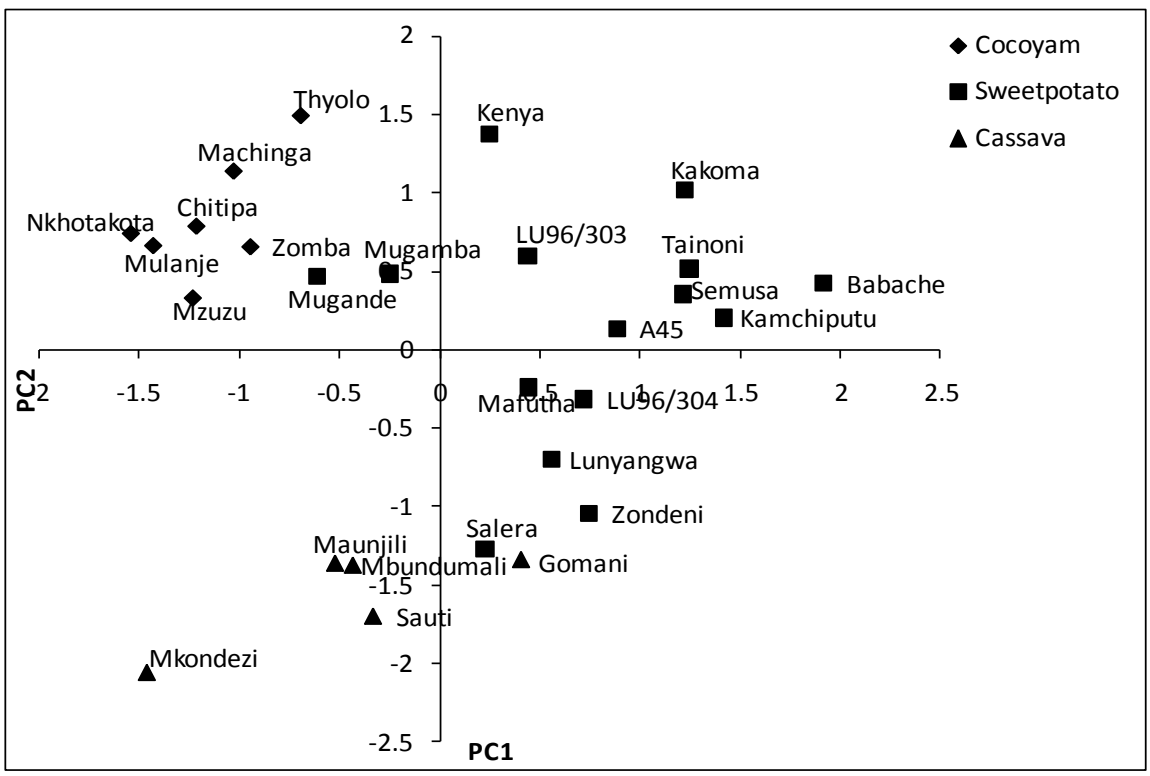

Figure 4.2 Plot of principal components 1 and 2 of cocoyam, sweetpotato and cassava starches using 13 chemical composition parameters.

The plot of factors scores for PC1 and PC2 also revealed that under PC2 cassava starches were all in the negative sector while all cocoyam starches were in the positive sector. Sweetpotato starches were found in both the negative (Mafutha, LU96/304, Lunyangwa, and Zondeni) and positive sector (A45, Babache, Kakoma, Kamchiputu, Kenya, LU96/303, Mugamba, Mugande, Semusa and Tainoni). A plot of PC1 revealed that mostly sweetpotato starches had the highest positive scores. These were discriminated in terms of high ash and mineral (Zn, Mn, Mg, Ca and Fe) contents, and lower moisture

content and pH. The plot also showed that that cocoyam starches had the lowest negative scores. These starches were discriminated in terms of their high pH, protein, fat and K contents. The cassava starches were discriminated mostly for high moisture content.

4.4 Conclusions

The results have revealed significant variation in chemical composition and mineral profile among the different cocoyam, sweetpotato and cassava starches. Generally, cocoyam starches displayed higher pH, and protein levels, but lower ash and amylose contents than sweetpotato and cassava starches. The phosphorus content of cocoyam starches was similar to that of sweetpotato starches but higher than that of cassava starches. Sweetpotato and cassava gave comparable levels of amylose. Amylose content was the lowest in cocoyam starches. The results of elemental analysis have shown that cocoyam, sweetpotato and cassava starches contain higher levels of potassium, calcium and magnesium than iron, zinc, manganese and sodium. The levels of potassium, phosphorus and calcium differed significantly from crop to crop and within the different genotypes and accessions. These differences in chemical composition of the starches therefore indicate that differences in structural peoperties exist between the different starches studied and hence the functionality of the starches. The structural and functional properties of the starches will be dealt within the next chapters.

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CHAPTER 5

MOLECULAR CHARACTERISTICS OF STARCHES FROM MALAWIAN COCOYAM AND SWEETPOTATO

5.1 Introduction

Starch granules contain two macromolecules; amylose and amylopectin, which differ both in size and structure. Amylose is a relatively long, linear polymer α-glucan containing around 99% (1→4)-α- and 1% (1→6) linkages, while amylopectin is a much larger and heavily branched polymer with about 95% (1→4)-α- and 5% (1→6)-α-linkages (Sivak and Preiss, 1997). Amylose has molecular weight ranging from 1 x 10^5 to 1 x 10^6 Da while the average molecular weight amylopectin varies from 1 x 10^6 to 5.0 x 10^8 Da (Robyt, 2008; Buléon *et al*., 1998). The degree of polymerization by number (DP_n) of amylose ranges from 324 to 4920, while that of amylopectin falls within the range 9600-15,000 and comprises three major species with DP_n 13400-26500, 4400-8400, and 700-2100. Amylopectin chains are relatively shorter than that of amylose and have a broad distribution profile. The average length of the amylopectin chain is about 18-25 α-D-glucose units while that of amylose is about 1500 units (Tester *et al*., 2004; Wang *et al*, 1998; Ellis *et al*., 1998; Sivak and Preiss, 1997).

The molecular weight and degree of polymerization of amylose and amylopectin molecules and chain length distributions of amylopectin vary with botanical source, varietal and seasonal differences (Peroni *et al*., 2006; Millan-Testa *et al*., 2005; Lu *et al*., 2005; Jane *et al*., 1992). These structural differences have varying effects on the starch functionality such as pasting viscosity, gel elasticity and strength, gelatinization temperatures and, swelling power and solubility (Lu *et al*., 2008; Tattiyakul *et al*., 2007; Lu *et al*., 2005; Charles *et al*., 2005; Jane *et al*., 1999; Sasaki and Matsuki, 1998; Noda *et al*., 1998; Shibanuma *et al*., 1996). It is thus important to investigate molecular characteristics of starch from different botanical sources to explore its full functionality. No information exists on the molecular characteristics of starches from Malawian cocoyam and sweetpotato. Therefore, this study was undertaken to determine the

molecular properties of starches isolated from sweetpotato and cocoyam grown in Malawi. This chapter concerns the iodine binding spectra, reducing capacity, extent of acid hydrolysis and molecular weight distribution of whole and debranched starch for the three root crops.

5.2 Materials and methods

5.2.1 Materials

Starch was isolated from 15 sweetpotato genotypes (A45, Babache, Kakoma, Kamchiputu, Kenya, Lunyangwa, LU96/303, LU96/304, Mafutha, Mugamba, Mugande, Salera, Semusa, Tainoni and Zondeni) and seven cocoyam accessions. The 15 sweetpotato genotypes were grown at Makoka Research Station in the Zomba district in the 2007 growing season. The seven cocoyam accessions were obtained from seven different cocoyam growing districts in Malawi; Chitipa, Mzimba (Mzuzu), Nkhotakota, Machinga, Mulanje, Thyolo and Zomba and therefore named by district of origin. Starches from five cassava genotypes (Gomani, Maunjili, Mbundumali, Mkondezi and Sauti) were used for comparison (Benesi 2006).

5.2.2 Reducing capacity

Starch samples were prepared by the method of Singh *et al*. (2005). Triplicate starch samples (50 mg dry basis) were suspended in 1.5 mL deionized water and kept at 50 °C for 1 h in a water bath with constant shaking. The suspension was centrifuged at 1870 x *g* for 10 min at 25 °C. The reducing capacity of the starch samples was determined (as glucose equivalent) following the method of Park and Johnson (1949) as modified by Hizukuri *et al*. (1981). Aliquots of the supernatant (1 mL) were transferred into clean tubes and 0.5 mL of sodium carbonate-sodium hydrogen carbonate buffer containing potassium cyanide (4.8 g of Na_2CO_3, 9.2 g of $NaHCO_3$ and 0.65 g of KCN L^{-1} water) added. The mixture was heated for 15 min in a vigorously boiling water bath and cooled for 10 min in running tap water. After cooling, 2.5 mL of ferric ammonium sulphate solution (3 g L^{-1} of 50 mM H_2SO_4) was added under effective ventilation and the mixture was kept for 20 min at room temperature. The absorbance of the mixture was read at 715 nm against a water blank. A reference solution containing 1 mg mL^{-1} of D-glucose was

used and the reducing capacity was calculated from the absorbance of the samples and standard solution.

$$\text{Concentration of D-glucose in aliquot (mg mL}^{-1)} = \frac{\text{Sample absorbance x concentration of standard}}{\text{Absorbance of the standard}}$$

$$\text{Reducing capacity (mg g}^{-1)} = \frac{\text{concentration of glucose (mg mL}^{-1}\text{) x 1.5}}{\text{sample mass (g)}}$$

5.2.3 Iodine absorption spectra and blue value

Iodine spectra of the native starches were determined by the DMSO-Urea method (Van Hung and Morita, 2005). Triplicate starch samples (40 mg) were dispersed in 10 mL of 10%-urea containing dimethyl sulphoxide (90% DMSO, 10% 6 M urea) by vigorous vortexing, heated in a boiling water bath for 20 min and cooled to room temperature. Aliquots (1 mL) of the dispersion were transferred in triplicate to 100 mL volumetric flasks, mixed with about 95 mL of distilled water and 2 mL of iodine-potassium iodide solution (200 mg I_2 and 2 g KI in 100 mL distilled water), and filled to the mark. The mixture was allowed to stand at room temperture for 20 min and an absorption curve measured from 500 to 800 nm using a spectrophotometer (Spectronic Unicam, Heλios, Cambridge, United Kingdom). The blue value (BV) of the starches was determined as absorbance measured at 680 nm.

5.2.4 Extent of acid hydrolysis

Acid hydrolysis of cocoyam, sweetpotato and cassava starch was carried out over a period of 12 days at 35°C (Jayakody *et al*., 2007). Starch (0.25 g dry basis), in triplicate, was suspended in 10 mL of 2.2 M HCl in a 15 mL screw capped centrifuge tube and the suspensions were gently shaken daily to resuspend the deposited starch granules. After a specific period (1, 2, 3, 4, 6, 8 and 12 days), the starch suspension was centrifuged at 1870 x *g* for 10 min. An aliquot of the digest (25 µL) was taken in triplicate and neutralized by adding an equal volume of 2.2 M NaOH. The total carbohydrate in the aliquot was determined by using the anthrone method (Brook *et al*., 1986) as follows: 3 mL of freshly prepared anthrone reagent [0.1% w/v anthrone in diluted sulphuric acid (2.3:1.0 v/v sulphuric acid:water)] was added to the neutralized digest and the resulting

mixture heated in a boiling water bath for 5 min. The heated samples were cooled in cold water and absorbance read at 630 nm at 15-35 min after heating using the spectrophotometer (Spectronic Unicam, Heλios, Cambridge, United Kingdom). A standard solution containing 1mg of D-glucose per mL was used as a reference. The extent of hydrolysis was determined by expressing the total carbohydrates as a percentage of the initial starch.

5.2.5 Molecular weight distribution of whole and debranched starch

5.2.5.1 Preparation of starch solution

A starch solution was prepared by dissolving the defatted starch in boiling distilled water by modifying the method of Chung and Lai (2006). Starch sampes (60 mg, db) in duplicate were accurately weighed into 15 mL screw-capped centrifuge tubes and wetted with 0.6 mL of distilled water and dispersed in 5.4 mL of dimethyl sulphoxide (DMSO). The starch dispersion was heated in a boiling water bath for 15 min with constant stirring and then continuously stirred on a rocking shaker for 1 h. Aliquots (2.4 mL) of the starch dispersion were transferred into clean centrifuge tubes and four volumes (9.6 mL) of absolute ethanol were added to precipitate the starch. The tubes were left to stand overnight and then centrifuged at 1550 x *g* for 10 min to separate the starch. The supernatant was decanted and the starch pellet washed twice with absolute ethanol. The precipitated starch was dissolved in boiling water (4 mL) and subsequently heated in boiling water for 30 min with constant stirring. After 30 min, the starch solution was filtered through a 25 mm 0.45 μm syringe filter (Acrodisc, PALL Life Sciences, Ann Arbor, MI, USA) and then 20 μL of the filtrate was injected into an HPSEC system.

5.2.5.2 Starch debranching

Starch samples were debranched following the method of Tester and Qi (2004). Starch samples (10 mg, db) were accurately weighed in duplicate into 15 mL screw-capped centrifuge tubes and dispersed in 1 mL of 90 % DMSO. The starch dispersion was heated in a boiling water bath for 15 min with constant stirring to completely dissolve the starch, after which the starch solutions were cooled to room temperature. Five volumes of absolute ethanol (5 mL) were added to the starch solution to precipitate the starch and then the tubes were centrifuged at 1550 x *g* for 10 min to separate the starch. The resulting starch pellet was dissolved in 4 mL of 0.1 M acetate buffer (pH 3.8) by heating in a boiling water bath

for 15 min. The sample solutions were placed in a water bath at 37°C for 5 min to equilibrate the starch solutions and then 30 μL of 0.1M acetate buffer containing isoamylase (590 units) was added and mixed. The mixture was incubated at 37°C for 24 h, after which the mixture was heated in a boiling water bath for 10 min to terminate the reaction. The sample solutions were then filtered through the 0.45 μm syringe filter and then 20 μL of the filtrate was injected into the HPSEC system.

5.2.5.3 HPSEC system

The HPSEC system consisted of a HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a Low Temperature Evaporative Light Scattering (ELSD) detector (ELSD-LT II, Shimadzu Corporation) set at 80°C and a series of TSK gel columns. The chromatographic columns were TSK-PWH guard, TSK gel G5000PW, TSK gel G3000PW and TSK gel G2500PW (TSK gel, TOSOH Bioscience GmBH, Stuttgart, Germany) connected in series and maintained at 40°C in an oven. The columns were eluted with 500 mM ammonium acetate (pH 6.5) at a flow rate of 1.0 mL min^{-1}.

5.2.5.4 Calibration of the HPSEC system

The HPSEC system was calibrated with Pullulan standards (Polymer Standards services, Sigma-Aldrich Production); P5 (Mw = 5900, Mp = 5600, Mn = 5400, PDI = 1.09), P10 (Mw = 11800, Mp = 11200, Mn = 10700, PDI = 1.10), P20 (Mw = 22800, Mp = 22000, Mn = 21300, PDI = 1.07), P50 (Mw = 47300, Mp = 45900, Mn = 44600, PDI = 1.06), P100 (Mw = 112000, Mp = 106000, Mn = 100000, PDI = 1.12), P200 (Mw = 212000, Mp = 200000, Mn = 188500, PDI = 1.13), P400 (Mw = 404000, Mp = 380000, Mn = 358000, PDI = 1.13) and P800 (Mw = 788000, Mp = 710000, Mn = 641000, PDI = 1.23). From the resulting elution profiles the calibration curve of log molecular weight vs. elution time was drawn. The retention time at the maximum height of each peak was taken to represent the retention time for that particular molecular weight (Stone and Krasowskl, 1981).

5.2.5.5 Determination of molecular weight averages

The average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity indices (PDI) were determined as described by Lehtonen (1988). The chromatogram of the starch samples was divided into slices of 5 sec width and the area under each section of the chromatogram was obtained through integration using the

instrument's software (LC solutions, Shimadzu Corporation, Kyoto, Japan). The molecular weight of each slice was determined using the calibration curve of the system. The following equations were used to calculate the molecular weight averages and the polydispersity of the starch samples:

$$M_w = \Sigma (Area_i \times M_i) / \Sigma Area_i \quad (1)$$

$$M_n = \Sigma Area_i / \Sigma (Area_i / M_i) \quad (2)$$

$$PDI = M_w/M_n \quad (3)$$

5.2.6 Data analysis

Data obtained for reducing capacity, wavelength of maximum iodine absorption, blue value, extent of acid hydrolysis, molecular weight averages and degree of polymerization were subjected to analysis of variance (ANOVA) using Statistix 8 for Windows software (Analytical software, Tallahassee, USA, 1985).

The PCA analysis was performed using NCSS 2004 (Hintze, 2001). Twelve (12) molecular properties of the starches, blue value (BV), wavelength of maximum iodine absorption (wavemax), reducing capacity (RC), acid hydrolysis after 12 days (AH), average molecular weight of amylose (AMMw) and amylopectin (AMPMw), average molecular weight of fractions I, II and III (FrIMw, FRIIMw and FRIIIMw) and percentage areas of fractions I, II and III (FrIArea, FRIIArea and FRIIIArea) were used in the analysis to group the starches according to their similarity/differences in molecular characteristics.

5.3 Results and discussion

5.3.1 Reducing capacity, maximum wavelength of iodine absorption (λ_{max}) and blue value (BV)

Table 5.1 The average values and mean separation of reducing capacity (RC) and iodine binding spectra (wavelength of maximum absorption, λ_{max} and blue value) of the starches

Botanical source	Genotype/ accession	RC (mg glucose/g starch)	λ_{max} (nm)	BV (Abs)
Cocoyam	Chitipa	5.2^{lm}	606^{a}	0.366^{b}
	Machinga	7.0^{ij}	596^{b}	0.356^{bc}
	Mulanje	6.5^{ijk}	603^{a}	0.373^{b}
	Mzuzu	5.0^{m}	595^{b}	0.333^{cd}
	Nkhotakota	7.4^{hi}	588^{c}	0.334^{c}
	Thyolo	13.5^{c}	603^{a}	0.372^{b}
	Zomba	5.6^{klm}	607^{a}	0.408^{a}
Mean value ± SD		7.2±2.8	600±7	0.363±0.026
Sweetpotato	A45	21.4^{a}	579^{def}	0.297^{ef}
	Babache	6.8^{ij}	579^{defg}	0.297^{ef}
	Kakoma	6.2^{jkl}	578^{defg}	0.302^{ef}
	Kamchiputu	8.8^{fg}	579^{def}	0.309^{de}
	Kenya	11.0^{d}	579^{defg}	0.303^{ef}
	Lunyangwa	7.0^{ij}	582^{d}	0.254^{jk}
	LU96/303	8.3^{gh}	577^{defg}	0.297^{ef}
	LU96/304	5.2^{lm}	570^{gh}	0.279^{fghi}
	Mafutha	8.5^{g}	572^{hij}	0.240^{kl}
	Mugamba	11.0^{d}	576^{efgh}	0.264^{hijk}
	Mugande	9.8^{ef}	575^{fghi}	0.258^{hijk}
	Salera	10.6^{de}	570^{j}	0.256^{ijk}
	Semusa	13.2^{c}	576^{efgh}	0.271^{ghij}
	Tainoni	5.4^{lm}	577^{defg}	0.282^{fgh}
	Zondeni	8.6^{g}	578^{defg}	0.281^{fgh}
Mean value ± SD		9.5±3.9	576±4	0.279±0.022
Cassava	Gomani	10.8^{de}	574^{ghij}	0.251^{jkl}
	Maunjili	6.6^{ij}	578^{defg}	0.265^{hij}
	Mbundumali	17.0^{b}	580^{de}	0.290^{efg}
	Mkondezi	9.3^{fg}	577^{efgh}	0.255^{ijk}
	Sauti	16.9^{b}	570^{ij}	0.226^{l}
Mean value ± SD		12.1±4.4	576±4	0.257±0.024
LSD (P = 0.05)		1.02	2.76	0.014
CV		6.66	0.29	2.87

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$

The reducing or alkaline number measures the number of aldehyde groups present and is inversely related to the molecular weight (Robyt, 2008). The greater the number of glucose units combined, the less the reducing power. Reducing capacity of the starches expressed as mg glucose per g starch ranged from 5.0-21.4 (Table 5.1) and varied significantly ($p \leq 0.001$) with botanical source. Generally, starch from cassava exhibited higher values of reducing capacity (12.1±4.4 mg g^{-1}) than that of sweetpotato (9.5±3.9 mg. g^{-1}) and cocoyam (7.2±2.8 mg. g^{-1}). The reducing capacity values also varied significantly ($p \leq 0.001$) within sweetpotato and cassava genotypes and among the different cocoyam accessions. Starch from Mbundumali and Sauti cassava genotypes gave the highest reducing capacity values and Maunjili the lowest. Among the sweetpotato genotypes, A45 starch had the highest reducing capacity value while Tainoni and LU96/304 starch had the lowest. Within the cocoyam accessions, Thyolo starch had the highest reducing capacity value and that of Mzuzu and Chitipa the lowest. These results indicate molecular structural differences among the starches. The generally lower values of reducing capacity for cocoyam starch suggest that the cocoyam starch had higher molecular weights than sweetpotato and cassava starch. The significant variations in reducing capacity values of starch from the different cocoyam accessions and sweetpotato and cassava genotypes therefore indicate that structural differences exist within the starch from these crops.

The wavelength of maximum absorption of the starches (λ_{max}) ranged from 588-607 nm for cocoyam, 570-581 nm for sweetpotato and 570-580 nm for cassava (Table 5.1). The values of λ_{max} obtained for sweetpotato starch (576±4 nm) was similar to that of cassava starch (576±4 nm) but lower than that of cocoyam starch (600±7 nm). Among the cocoyam accessions, starch from Thyolo, Zomba and Chitipa gave the highest values of λ_{max} and Nkhotakota the lowest. Cassava starch from Mbundumali gave the highest λ_{max} value among the cassava starches while Sauti gave the lowest. Most sweetpotato starches displayed similar λ_{max} values; however, Salera and LU96/304 had the lowest value while Lunyangwa had the highest. The blue value (BV) of the starches showed similar trends as the wavelengths of maximum absorption. Cocoyam starch, on average, gave the higher BV (0.363±0.026) than either sweetpotato (0.0279±0.022) or cassava (0.257±0.024) starch. Starch from Zomba, Thyolo and Chitipa cocoyam accessions gave higher BV than other cocoyam starches while Mzuzu gave the lowest. Among the sweetpotato genotypes,

starch from Kamchiputu, Kenya, and Kakoma gave higher BV values while Mafutha had the lowest. Mbundumali cassava starch exhibited the highest BV among the cassava genotypes and Sauti the lowest. The value of λ_{max} for the starch-iodine complex is related to the chain length of the starch molecules. As the chain length increases, so does the value of λ_{max} (Tetchi *et al*., 2007). The results therefore indicated the presence of longer chain molecules in cocoyam starch than in sweetpotato and cassava starches. This is consistent with the lower reducing capacity values of the starches. Cocoyam starches gave lower reducing capacity values, indicating higher molecular weights and therefore longer chain molecules.

5.3.2 Acid hydrolysis

The acid solubilization patterns of cocoyam, sweetpotato and cassava starch are presented in Table 5.2. Acid hydrolysis of starches is known to occur in two stages; first the destruction of the amorphous region characterized by a higher hydrolysis rate and secondly the degradation of the crystalline phase characterized by a lower rate of hydrolysis. The fast destruction of the amorphous region occurs in the first 8 days and the slow degradation of the crystalline region between 7 and 12 days (Hoover, 2000). However, a steady increase in acid hydrolysis was observed in the first 8 days with a large increase observed between 8 and 12 days.

Table 5.2 The average values of the extent (%) of hydrolysis of native cocoyam, sweetpotato and cassava starches

Botanical source	Genotype/ accession	Number of days					
		1	2	4	6	8	12
Cocoyam	Chitipa	2.3±0.1	3.8±0.6	5.0±0.5	7.8±0.3	9.2±0.8	23.1±2.3
	Machinga	2.7±0.0	4.9±0.4	6.1±0.1	7.0±0.8	10.9±0.2	23.6±3.0
	Mulanje	2.3±0.6	4.2±0.3	4.9±0.3	6.0±0.2	9.8±0.1	21.3±0.5
	Mzuzu	1.7±0.2	4.0±0.2	5.5±0.2	8.2±0.3	10.1±0.3	20.9±0.6
	Nkhotakota	1.9±0.9	4.3±0.4	5.0±0.8	7.0±0.5	10.4±0.8	20.2±1.3
	Thyolo	2.9±0.6	3.6±0.3	4.8±0.9	7.9±0.4	10.9±0.7	19.3±1.8
	Zomba	1.1±0.1	3.9±0.1	4.5±0.1	5.9±1.2	8.5±0.8	19.7±3.2
Mean value ± SD		2.1±0.7	4.1±0.5	5.1±0.6	7.1±1.0	10.0±1.0	21.2±2.3
Sweetpotato	A45	1.6±0.5	2.9±0.1	4.5±1.0	6.4±0.9	9.0±0.3	22.8±6.4
	Babache	2.3±0.6	3.1±0.3	3.9±0.5	7.0±1.0	9.0±0.3	19.8±2.4
	Kakoma	2.0±0.7	3.2±0.7	5.2±1.1	7.5±0.2	9.8±0.3	20.3±1.7
	Kamchiputu	3.2±1.1	3.9±0.8	4.6±0.3	9.2±1.0	10.4±0.5	24.2±3.7
	Kenya	1.7±0.3	3.1±0.5	4.3±0.5	5.9±0.2	8.8±0.2	21.3±4.0
	Lunyangwa	2.2±0.3	2.7±0.2	5.9±0.8	8.7±0.8	10.1±0.8	19.5±0.1
	LU96/303	2.3±0.6	3.3±0.2	4.3±0.4	7.5±0.5	10.5±0.1	24.1±1.8
	LU96/304	1.8±0.5	2.6±.06	5.1±0.1	7.6±0.2	9.4±0.9	18.0±4.0
	Mafutha	1.9±0.4	2.6±0.2	5.2±0.2	8.8±0.3	9.4±0.3	18.5±2.1
	Mugamba	1.0±0.1	3.1±0.4	4.2±0.1	8.7±0.3	9.4±0.4	18.2±0.9
	Mugande	1.1±0.1	2.5±0.1	5.1±0.2	8.4±0.3	9.1±0.3	17.8±1.1
	Salera	2.1±0.5	2.8±0.5	5.0±0.4	7.8±0.4	9.6±0.6	20.5±1.2
	Semusa	1.8±0.1	2.8±0.1	5.4±0.4	9.0±0.1	11.7±1.5	19.1±2.5
	Tainoni	1.8±0.1	2.7±0.3	5.2±0.3	10.2±0.4	11.0±0.37	21.0±2.1
	Zondeni	1.4±0.1	3.2±1.0	5.0±0.4	8.4±0.6	9.4±0.3	17.8±1.1
Mean value ± SD		1.9±0.6	3.0±0.5	4.9±0.7	8.1±1.2	9.8±0.9	20.2±3.1
Cassava	Gomani	1.1±0.1	2.7±0.1	3.9±0.6	6.2±0.1	7.9±0.4	17.3±0.6
	Maunjili	2.0±0.3	3.1±0.3	3.5±0.1	5.7±0.4	7.6±0.3	15.7±0.4
	Mbundumali	0.9±0.0	3.3±0.2	4.0±0.4	6.5±0.5	8.0±1.1	17.3±1.0
	Mkondezi	0.9±0.1	3.0±0.1	3.7±0.4	6.3±0.3	8.2±0.3	18.6±0.8
	Sauti	3.2±0.4	4.9±0.4	7.1±0.3	8.3±0.5	9.3±0.6	21.5±2.0
Mean value ± SD		1.6±1.0	3.4±0.8	4.4±1.4	6.6±1.0	8.2±0.8	18.1±2.2

Values expressed as mean ±SD

The extent of hydrolysis of the cocoyam, sweetpotato and cassava starches differed significantly ($p \leq 0.001$) after 2, 6, 8 and 12 days. Generally, cocoyam and sweetpotato starches showed higher solubilization in 2.2 M HCl than cassava starches. After 2 days, cocoyam starches displayed a higher extent of hydrolysis (4.1±0.5%) than cassava (3.4±0.8%) and sweetpotato (3.0±0.5%) starches. After 6 days, sweetpotato starches exhibited higher levels of hydrolysis (8.1±1.2%) than cocoyam (7.1±1.0%) and cassava (6.6±1.0%) starches. Cocoyam and sweetpotato starch gave similar values of the extent of hydrolysis after 8 and 12 days which were higher than those of cassava starches. Cassava and cocoyam starch gave similar values of the extent of acid hydrolysis after 6 days. At the end of 12 days of hydrolysis, the extent of acid hydrolysis of cocoyam, sweetpotato and cassava starches were 21±2, 20±3 and 18±2%, respectively. The comparable values of the extent of acid hydrolysis for cocoyam and sweetpotato starches indicate the identical packing and orientation of starch chains in amorphous regions (Ratnayake *et al*., 2001). The higher degree of acid hydrolysis of cocoyam and sweetpotato starch compared to cassava starch suggests that the amorphous regions of cocoyam and sweetpotato starch are less compactly packed than those of cassava starch (Hoover and Manuel, 1995).

Over the 12-day period of hydrolysis, significant differences in the extent of hydrolysis were observed within starch from different cocoyam accessions and, sweetpotato and cassava genotypes. Among the starch from cocoyam accessions, Machinga displayed the greatest extent of hydrolysis after 1, 2, 4, 8 and 12 days. Other cocoyam starches that gave comparatively high values of extent of hydrolysis were Thyolo (1, 6 and 8 days), Mulanje (1, 2 and 12 days), Chitipa (1, 6 and 12 days), Mzuzu (2, 4, 6 and 12 days) and Nkhotakota (2 days). Starch from Zomba cocoyam accession displayed the lowest values of extent of hydrolysis. Starch from Kamchiputu (1, 2, 6, 8, and 12 days), Semusa (4, 6, and 8 days), Tainoni (4, 6, and 8 days), Lunyangwa (4 and 6 days), and LU96/303 (1, 2, 8 and 12 days) sweetpotato genotypes displayed relatively higher susceptibility to acid hydrolysis than other sweetpotato genotypes. No sweetpotato starch displayed consistently lower susceptibility to acid hydrolysis over the hydrolysis period. However, starch from Mugamba, Kenya, Mugande, Babache and Zondeni showed higher resistance to acid hydrolysis at different days. In contrast to sweetpotato starches, some consistency was observed in the susceptibility of cassava starch to acid hydrolysis. Sauti cassava starch exhibited the highest susceptibility to acid hydrolysis and Maunjili the lowest over the 12-day period. These differences in the extent of acid hydrolysis therefore confirm the

differences in the extent of chain packing in the amorphous regions of the starch granules. Thus, based on the extent of acid hydrolysis, starches from Machinga, Chitipa and Thyolo cocoyam accessions, Kamchiputu, Semusa, Tainoni, Lunyangwa and LU96/303 sweetpotato genotypes, and Sauti cassava genotype have more compact amorphous regions than the rest.

Compared to other root and tuber crops, starches in this study have shown higher resistance to acid degradation. Gunaratne and Hoover (2002) have reported the extent of hydrolysis ranging from 60% to 82% for yam, taro, new cocoyam and potato starches after 12 days. Jayakody *et al*. (2005) reported values of 40% to 45% for two cultivars of Chinese potato. The lower values of acid hydrolysis were attributed to starch chain interactions of higher order of magnitude in the amorphous and crystalline regions. Therefore, the much lower values obtained in this study signify much stronger chain interactions in the Malawian sweetpotato, cocoyam and cassava starches than in other root and tuber crop starches reported.

5.3.3 Molecular weight distribution of whole and debranched starches

5.3.3.1 Calibrartion of the size exclusion column system

The elution profiles of different Pullulan standards and the resulting calibration curve are shown in Figures 5.1 and 5.2. The results indicated that retention time of the Pullulan standards decreased with increasing molecular weight and the associated calibration curve was strongly linear (r^2 = 0.9972). The resulting linear regression equation was therefore used to determine the molecular weight distribution of the various starches.

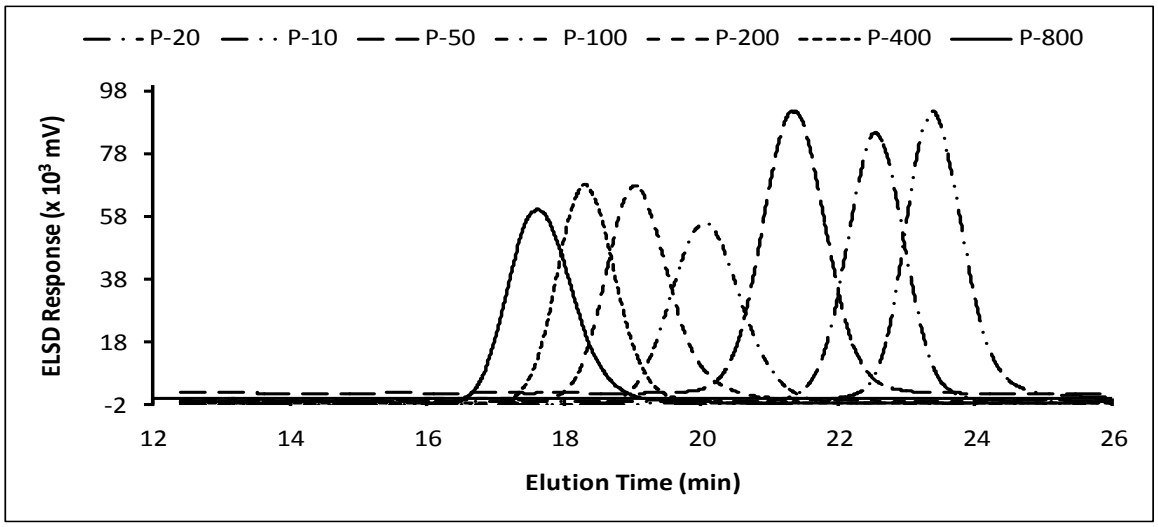

Figure 5.1 Elution profiles of Pullulan standards, P-10, P-20, P-50, P-100, P-200, P-400 and P-800 of molecular weight ranging from 11,800 to 788,000

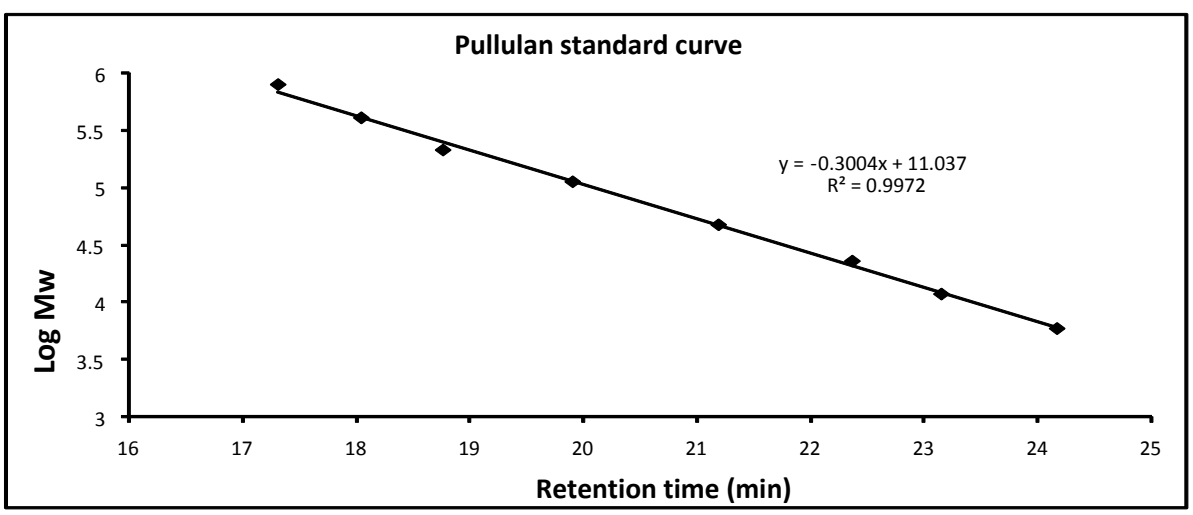

Figure 5.2 Log molecular weight vs. retention time plot for the Pullulan standards

5.3.3.2 Molecular weight distribution of the starches

The carbohydrate distribution of native starch of cassava, cocoyam and sweetpotato are presented in Figures 5.3-5.5. The chromatograms showed that the starches consisted of mainly two fractions; a higher molecular weight fraction mainly amylopectin with elution time between 15 and 17 min, and, a lower molecular weight fraction, mainly amylose, eluted between 17 min and 20 min. The molecular weight averages; average molecular weight (M_w), number-average molecular weight (M_n), and polydipersity indexes (PDI) for the amylopectin and amylose fractions calculated using the calibration curve are presented in Tables 5.3 and 5.4.

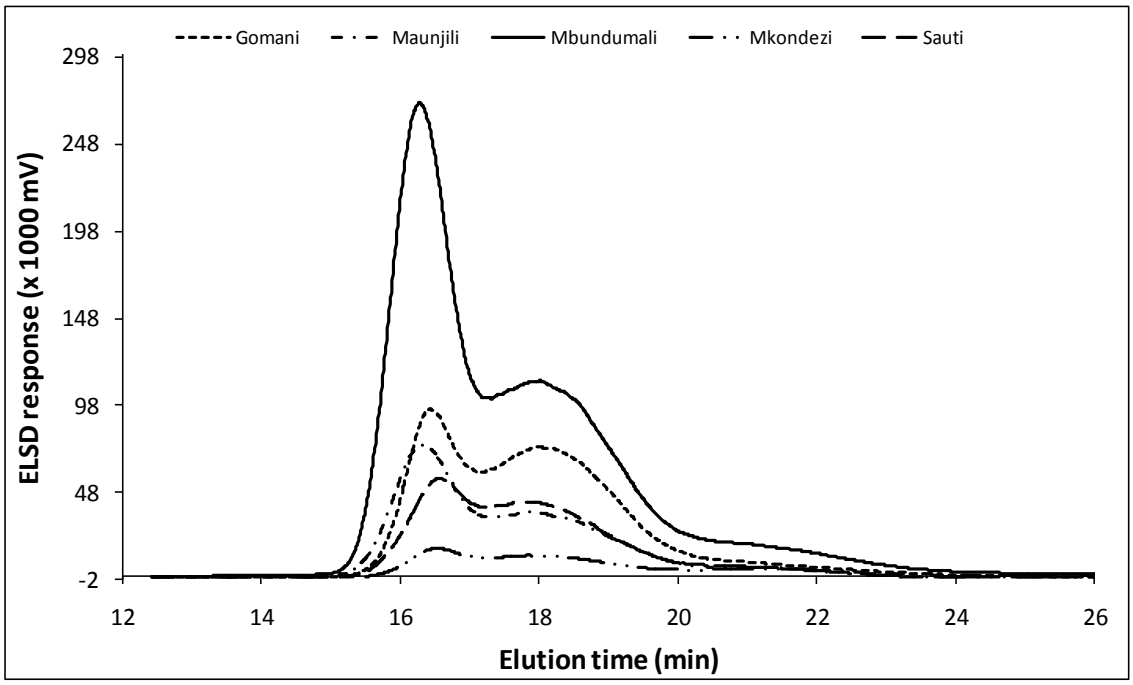

Figure 5.3 HPSEC chromatograms of unbranched cassava starch

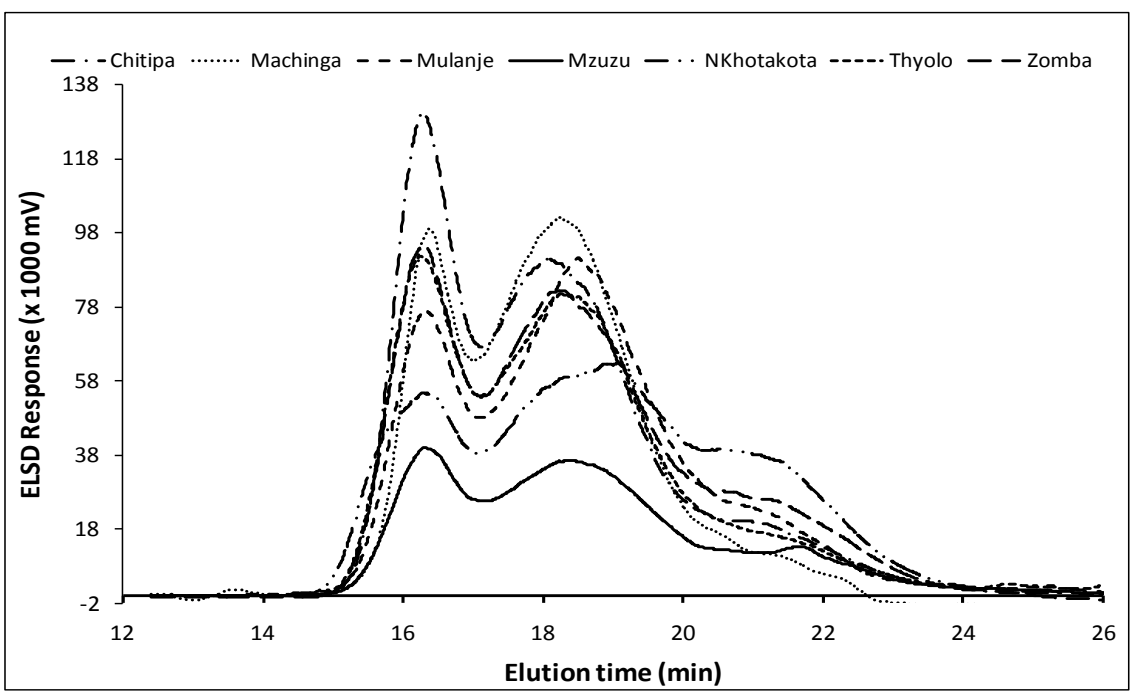

Figure 5.4 HPSEC chromatograms of unbranched cocoyam starch

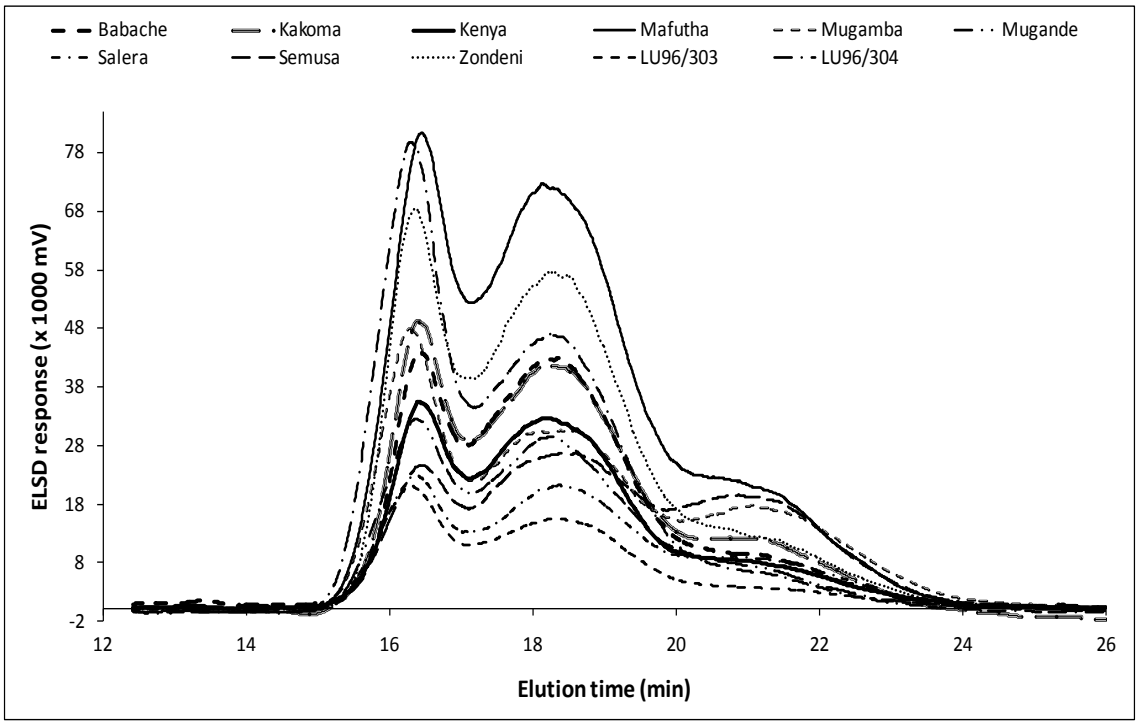

Figure 5.5. HPSEC chromatograms of some unbranched sweetpotato starch

Table 5.3 The average values and mean separation of average molecular weight (M_w), number-average molecular weight (M_n), and polydipersity index (PDI) of the amylopectin for the cocoyam, sweetpotato and cassava starches

Botanical source	Genotype/ accession	Fraction I (Amylopectin)		
		M_w (x 10^6 Da)	M_n (x 10^6 Da)	PDI
Cocoyam	Chitipa	1.67^{a}±0.11	1.59^{ab}±0.11	1.05^{ab}±0.01
	Machinga	1.54^{ab}±0.06	1.49^{ab}±0.05	1.03^{ab}±0.01
	Mulanje	1.53^{ab}±0.10	1.47^{ab}±0.08	1.04^{ab}±0.02
	Mzuzu	1.49^{ab}±0.07	1.44^{b}±0.09	1.04^{ab}±0.01
	Nkhotakota	1.50^{ab}±0.09	1.56^{ab}±0.19	1.04^{ab}±0.03
	Thyolo	1.50^{ab}±0.16	1.51^{ab}±0.14	1.04^{ab}±0.02
	Zomba	1.59^{ab}±0.13	1.55^{ab}±0.10	1.02^{ab}±0.02
Mean value (cocoyam)		1.54±0.10	1.51±0.11	1.04±0.02
Sweetpotato	A45	1.60^{ab}±0.06	1.57^{ab}±0.04	1.02^{ab}±0.01
	Babache	1.47^{ab}±0.04	1.44^{b}±0.04	1.02^{ab}±0.01
	Kakoma	1.55^{ab}±0.05	1.51^{ab}±0.06	1.03^{ab}±0.01
	Kamchiputu	1.42^{b} ±0.04	1.40^{b}±0.04	1.01^{b}±0.01
	Kenya	1.49^{ab}±0.05	1.45^{b}±0.08	1.03^{ab}±0.02
	LU96/303	1.59^{ab}±0.04	1.55^{ab}±0.06	1.02^{ab}±0.01
	LU96/304	1.52^{ab}±0.08	1.48^{ab}±0.14	1.03^{ab}±0.03
	Lunyangwa	1.63^{ab}±0.08	1.86^{a}±0.10	1.03^{ab}±0.01
	Mafutha	1.53^{ab}±0.12	1.50^{ab}±0.11	1.02^{ab}±0.01
	Mugamba	1.53^{ab}±0.06	1.49^{ab}±0.06	1.03^{ab}±0.01
	Mugande	1.59^{ab}±0.04	1.53^{ab}±0.05	1.04^{ab}±0.02
	Salera	1.62^{ab}±0.03	1.56^{ab}±0.04	1.03^{ab}±0.01
	Semusa	1.51^{ab}±0.06	1.46^{ab}±0.07	1.03^{ab}±0.01
	Tainoni	1.55^{ab}±0.05	1.50^{ab}±0.07	1.03^{ab}±0.01
	Zondeni	1.50^{ab}±0.04	1.46^{ab}±0.06	1.03^{ab}±0.02
Mean value (sweetpotato)		1.54±0.78	1.52±0.18	1.03±0.01
Cassava	Gomani	1.42^{b}±0.07	1.39^{b}±0.07	1.02^{ab}±0.01
	Maunjili	1.46^{ab}±0.16	1.42^{b}±0.10	1.03^{ab}±0.01
	Mbundumali	1.42^{b}±0.16	1.44^{b}±0.16	1.02^{ab}±0.01
	Mkondezi	1.53^{ab}±0.11	1.44^{b}±0.20	1.06^{a}±0.02
	Sauti	1.48^{ab}±0.14	1.44^{b}±0.14	1.03^{ab}±0.01
Mean value (cassava)		1.46±0.11	1.43±0.13	1.03±0.02
LSD (P = 0.05)		0.21	0.41	0.04
CV (%)		5.35	10.18	1.54

Values expressed as mean ±SD; Means followed by the same letter in the same column are not significantly different at p≤0.05

Table 5.4 The average values and mean separation of average molecular weight (M_w), number-average molecular weight (M_n), and polydipersity index (PDI) of amylose for the cocoyam, sweetpotato and cassava starches

Botanical source	Genotype/ accession	Fraction II (Amylose)		
		M_w (x 10^5 Da)	M_n (x 10^5 Da)	PDI
Cocoyam	Chitipa	4.08^{ab}±0.22	3.31^{ab}±0.08	1.24^{ab}±0.08
	Machinga	4.07^{ab}±0.30	3.29^{ab}±0.16	1.24^{ab}±0.06
	Mulanje	3.88^{b}±0.41	3.16^{b}±0.39	1.23^{ab}±0.07
	Mzuzu	3.86^{b}±0.31	3.12^{b}±0.20	1.24^{ab}±0.06
	Nkhotakota	3.79^{b}±0.26	3.09^{b}±0.41	1.32^{a}±0.11
	Thyolo	4.11^{ab}±0.34	3.51^{ab}±0.45	1.21^{ab}±0.07
	Zomba	4.08^{ab}±0.21	3.35^{ab}±0.33	1.27^{ab}±0.09
Mean value (cocoyam)		3.98±0.29	3.26±0.31	1.25±0.07
Sweetpotato	A45	4.22^{ab}±0.40	3.79^{ab}±0.39	1.12^{b}±0.05
	Babache	4.14^{ab}±0.2+	3.44^{ab}±0.20	1.20^{ab}±0.06
	Kakoma	4.02^{ab}±0.10	3.25^{ab}±0.22	1.24^{ab}±0.09
	Kamchiputu	4.22^{ab}±0.12	3.43^{ab}±0.17	1.24^{ab}±0.09
	Kenya	4.03^{ab}±0.31	3.37^{ab}±0.21	1.20^{ab}±0.07
	LU96/303	3.83^{ab}±0.23	3.14^{b}±0.09	1.22^{ab}±0.07
	LU96/304	3.89^{b}±0.26	3.16^{b}±0.19	1.23^{ab}±0.07
	Lunyangwa	4.30^{ab}±0.12	3.57^{ab}±0.16	1.21^{ab}±0.05
	Mafutha	4.14^{ab}±0.18	3.38^{ab}±0.10	1.23^{ab}±0.08
	Mugamba	4.17^{ab}±0.10	3.45^{ab}±0.25	1.21^{ab}±0.08
	Mugande	4.21^{ab}±0.10	3.52^{ab}±0.21	1.20^{ab}±0.08
	Salera	4.13^{ab}±0.25	3.59^{ab}±0.30	1.20^{ab}±0.07
	Semusa	4.33^{ab}±0.38	3.72^{ab}±0.55	1.21^{ab}±0.10
	Tainoni	3.96^{ab}±0.19	3.19^{b}±0.08	1.24^{ab}±0.07
	Zondeni	4.03^{ab}±0.25	3.29^{ab}±0.08	1.22^{ab}±0.06
Mean value (sweetpotato)		4.11±0.25	3.42±0.29	1.21±0.03
Cassava	Gomani	4.59^{a} ±0.29	3.89^{a}±0.24	1.18^{ab}±0.05
	Maunjili	4.39^{ab}±0.28	3.72^{ab}±0.28	1.18^{ab}±0.05
	Mbundumali	4.42^{ab}±0.24	3.65^{ab}±0.05	1.21^{ab}±0.06
	Mkondezi	4.35^{ab}±0.18	3.76^{ab}±0.25	1.16^{ab}±0.04
	Sauti	4.41^{ab}±0.14	3.68^{ab}±0.06	1.20^{ab}±0.04
Mean value (cassava)		4.43±0.23	3.74±0.20	1.18±0.05
LSD (P = 0.05)		0.69	0.70	0.19
CV (%)		6.19	7.54	5.72

Values expressed as mean ±SD; Means followed by the same letter in the same column are not significantly different at p≤0.05

The molecular weight averages; the average- and number average molecular weight (M_w and M_n) varied with the botanical origin of the starch. However, no significant variation was observed in the polydispersity values. The average molecular weight (M_w) of the amylopectin fraction ranged from 1.49-1.67 x 10^6, 1.42-1.62 x 10^6 and 1.42-1.53 x 10^6 Da for cocoyam, sweetpotato and cassava starch, respectively. Generally, cocoyam (1.54 x 10^6 Da) and sweetpotato (1.54 x 10^6 Da) starch gave similar M_w values that were significantly higher ($p \leq 0.001$) than those of cassava starch (1.46 x 10^6 Da). These values are within the range of average molecular weight of amylopectins (Robyt, 2008) but lower than those reported for similar starches (Aberle *et al*., 1994). Such differences could be attributed to different detection techniques employed (Fishman *et al*., 1996; Ong *et al*., 1994). The higher M_w and M_n values for cocoyam and sweetpotato starches than those of cassava starches therefore indicate that cocoyam and sweetpotato starches have higher molecular weights. This accounts for observed differences in reducing capacity values of the different starches (Section 5.3.1). The polydispersity index provides a measure of the width of the molecular weight distribution of a polymer (Lehtonen, 1988). The lack of variation in PDI values for the higher molecular weight fraction indicates a similar range of molecular weight distributions for the high molecular fraction of starch from the three crops. When the starches from different cassava and sweetpotato genotypes, and cocoyam accessions were compared, significant variations in M_w ($p \leq 0.001$) and M_n ($p \leq 0.01$) were observed. Starch from Chitipa cocoyam exhibited higher M_w and M_n values while starch from Mzuzu gave the lowest values. Lunyangwa sweetpotato starch displayed the highest M_w and M_n values and Kamchiputu the lowest. Among the starch from cassava genotypes, Mkondezi gave higher M_w and M_n values while Maunjili and Gomani exhibited lower values. Thus, structural differences exist between starch from different cocoyam accessions, and sweetpotato and cassava genotypes.

In the lower molecular weight fraction (amylose), M_w and M_n values also varied significantly ($p \leq 0.001$) with botanical source. Cassava starch gave the highest M_w (4.43 x 10^5 Da) and M_n (3.74 x 10^5 Da) values, while cocoyam starch had the lowest M_w (3.98 x 10^5 Da), and M_n (3.26 x 10^5 Da), and sweetpotato starch intermediate values (4.11 x 10^5 and 3.42 x 10^5 Da respectively). Thus, cassava starch contained higher molecular weight amylose than sweetpotato and cocoyam starch. Polydispersity index (PDI) values also varied ($p \leq 0.05$) among the starches. Cocoyam and sweetpotato starch exhibited similar values which were higher than those of cassava starch. This indicates that sweetpotato and

cocoyam starch contained molecules with a broader range of molecular weights than cassava starch. The starch from different cocoyam accessions also displayed significantly ($p \leq 0.001$) different M_w and M_n values. Starch from Thyolo accession exhibited the highest M_w and M_n values and Nkhotakota the lowest. Semusa, Lunyangwa, A45, and Salera starch gave comparatively higher M_w and M_n values than other sweetpotato starch while LU96/303 and LU96/304 had lower values. Little variation was observed in M_w and M_n values among starch from the different cassava genotypes.

5.3.3.3 Molecular weight distribution of debranched starches

The HPSEC chromatograms of the isoamylase debranched native cocoyam, sweetpotato and cassava starches are presented in Figures 5.6-5.8. Generally trimodal distribution was obtained: Fraction I consisted of carbohydrates of high molecular weight, Fraction II of intermediate molecular weight and Fraction III of lower molecular weight carbohydrates. When compared with HPSEC chromatograms of whole starch samples, it is apparent that fraction I consisted of amylose molecules, while Fractions II and III consisted of long and short chains of amylopectin respectively (Wang *et al*., 2003) as the isoamylase enzyme specifically hydrolyzes the α-(1→6)-D- glycosidic chain linkages (Ong *et al*., 1994).

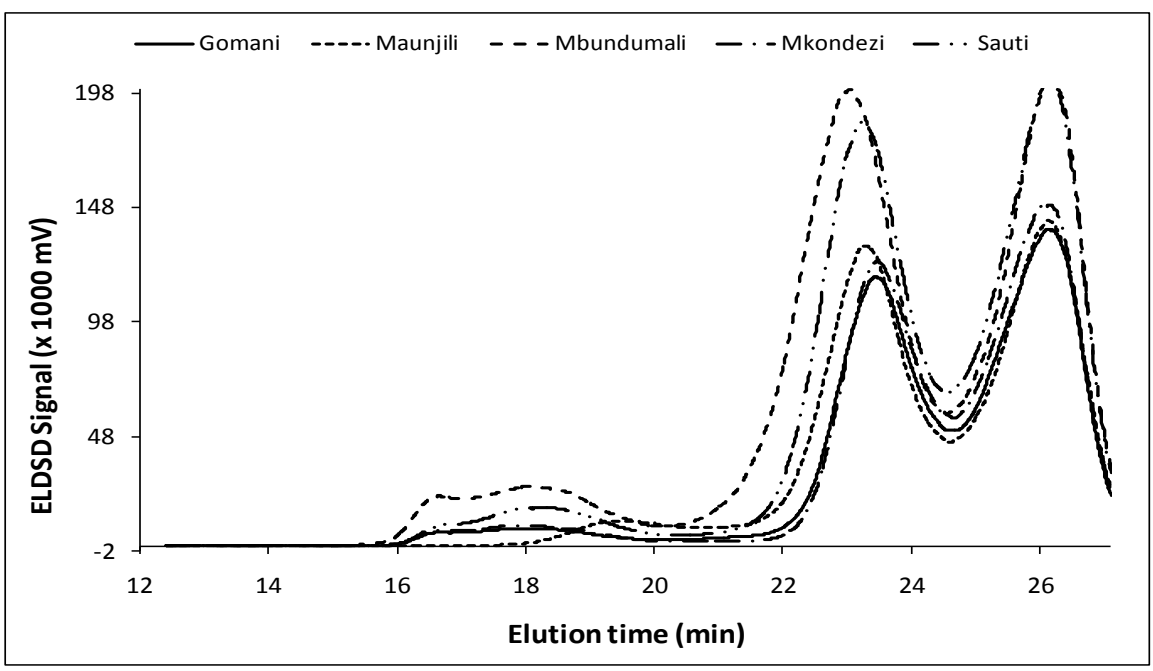

Figure 5.6 HPSEC chromatograms of debranched cassava starch

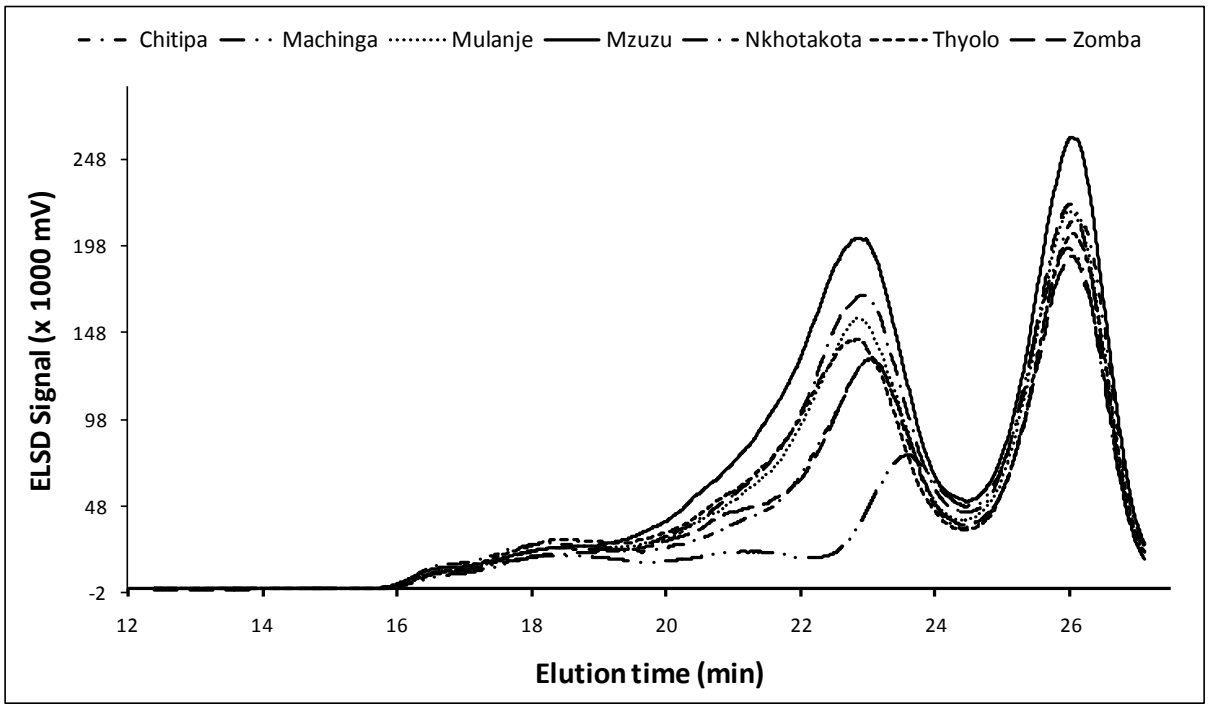

Figure 5.7 HPSEC chromatograms of debranched cocoyam starch

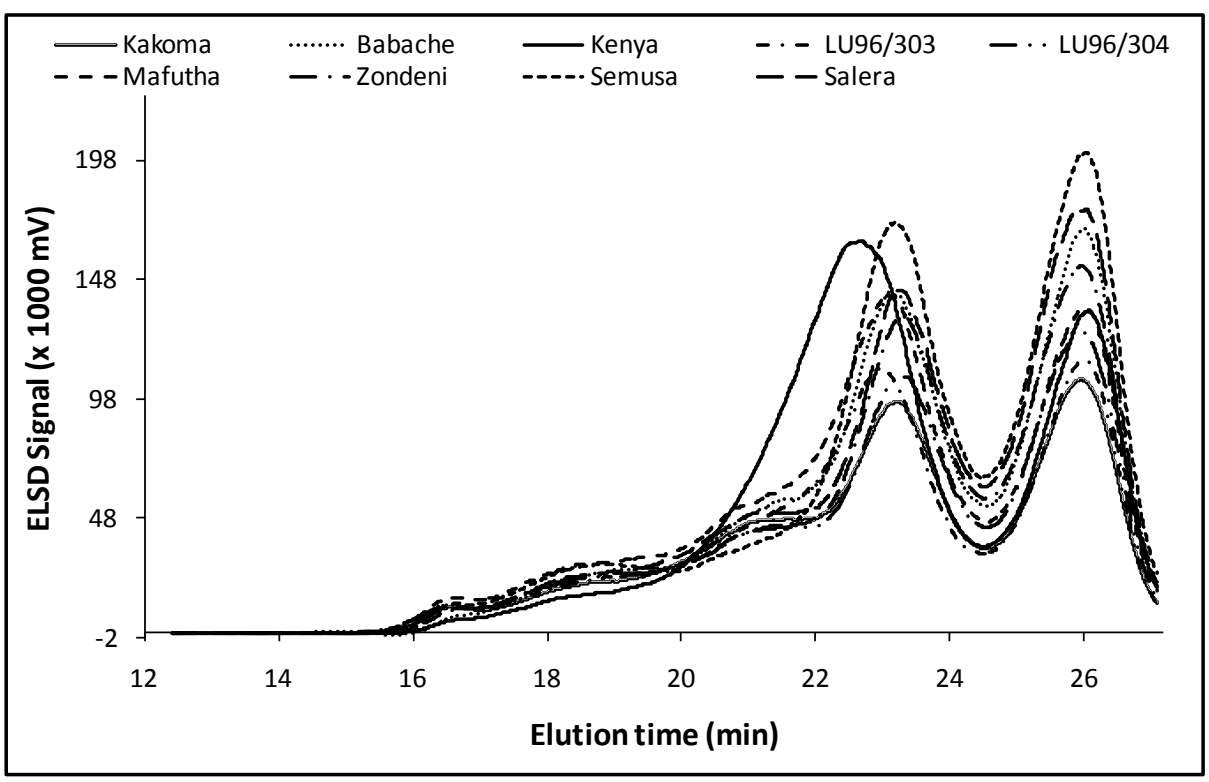

Figure 5.8 HPSEC chromatograms of debranched sweetpotato starch

The average molecular weight and relative area of the fraction are presented in Table 5.5a and b.

Table 5.5a The average vaules of average molecular weight, M_w and relative area of the isoamylase debranched cocoyam, sweetpotato and cassava starches

Botanical Source	Accession/ Genotype	Fraction I		Fraction II	
		M_w ($x10^5$ Da)	Area (%)	M_w ($x10^4$ Da)	Area (%)
Cocoyam	Chitipa	5.73^{cdef}	6.5^{cdefg}	3.08^{abc}	29.5^{g}
	Machinga	4.89^{fgh}	7.0^{bcdefg}	2.25^{fg}	25.0^{g}
	Mulanje	5.21^{defgh}	6.5^{cdefg}	2.97^{abcde}	29.5^{g}
	Mzuzu	6.51^{abc}	4.5^{efg}	3.01^{abcde}	54.0^{a}
	Nkhotakota	5.88^{cdef}	3.5^{g}	2.79^{bcdefg}	32.5^{fg}
	Thyolo	4.61^{gh}	8.0^{bcdef}	2.29^{fg}	24.5^{g}
	Zomba	5.33^{defgh}	6.5^{cdefg}	3.05^{abcd}	30.0^{g}
Mean value (cocoyam)		5.45±0.78	6.1±3.0	2.78±0.44	32.1±11.1
Sweetpotato	A45	6.11^{bcde}	8.5^{abcde}	2.83^{bcdef}	49.0^{abcd}
	Babache	4.46^{h}	6.5^{cdefg}	2.41^{efg}	46.0^{abcde}
	Kakoma	5.86^{cdef}	9.5^{abc}	2.65^{bcdefg}	48.0^{abcde}
	Kamchiputu	5.03^{fgh}	6.0^{cdefg}	2.23^{fg}	45.5^{bcde}
	Kenya	6.23^{abcd}	8.0^{bcdef}	2.69^{bcdefg}	47.5^{bcde}
	Lunyangwa	6.47^{abc}	12.5^{a}	3.13^{ab}	53.0^{ab}
	LU96/303	5.71^{cdef}	9.5^{abc}	2.48^{cdefg}	48.5^{abcd}
	LU96/304	5.69^{cdef}	10.0^{abc}	2.30^{fg}	44.0^{cde}
	Mafutha	6.14^{bcde}	9.0^{abcd}	2.58^{bcdefg}	52.0^{abc}
	Mugamba	7.11^{ab}	6.5^{cdefg}	3.11^{ab}	51.0^{abc}
	Mugande	7.26^{a}	11.0^{ab}	3.47^{a}	47.0^{abcde}
	Salera	5.82^{cdef}	6.5^{cdefg}	2.46^{cdefg}	44.5^{cde}
	Semusa	4.92^{fgh}	8.0^{bcdef}	2.18^{g}	42.5^{de}
	Tainoni	4.94^{fgh}	5.0^{defg}	2.44^{defg}	44.5^{cde}
	Zondeni	5.15^{efgh}	7.5^{bcdefg}	2.31^{fg}	44.5^{cde}
Mean value (sweetpotato)		5.79±1.10	8.3±3.6	2.62±0.60	47.2±6.5
Cassava	Gomani	5.62^{cdefg}	3.5^{g}	1.15^{h}	41.5^{de}
	Maunjili	1.84^{i}	3.5^{g}	1.33^{h}	47.0^{abcde}
	Mbundumali	6.59^{abc}	7.0^{bcdefg}	2.45^{defg}	52.0^{abc}
	Mkondezi	6.14^{bcde}	4.0^{fg}	1.12^{h}	40.0^{ef}
	Sauti	5.29^{defgh}	5.0^{defg}	1.36^{h}	45.5^{bcde}
Mean value (cassava)		5.09±1.76	4.6±1.8	1.48±0.53	45.2±4.5
LSD (P = 0.05)		1.03	4.2	0.63	8.4
CV (%)		13.18	17.2	18.32	13.94

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$

Table 5.5b The average values of average molecular weight, M_w and relative area of the isoamylase debranched cocoyam, sweetpotato and cassava starches

Botanical Source	Accession/ Genotype	Fraction III	
		M_w ($x10^3$ Da)	Area (%)
Cocoyam	Chitipa	2.23^{abcd}	64.5^{ab}
	Machinga	2.28^{abcd}	68.0^{a}
	Mulanje	2.31^{abc}	64.0^{abc}
	Mzuzu	2.16^{bcde}	42.5^{efgh}
	Nkhotakota	2.33^{a}	64.0^{abc}
	Thyolo	2.30^{abcd}	68.0^{a}
	Zomba	2.28^{abcd}	64.0^{abc}
Mean value (cocoyam)		2.27±0.10	62.1±11.7
Sweetpotato	A45	2.31^{abc}	43.0^{defgh}
	Babache	2.26^{abcd}	47.5^{defg}
	Kakoma	2.31^{ab}	42.5^{efgh}
	Kamchiputu	2.33^{a}	48.5^{defg}
	Kenya	2.31^{abc}	45.0^{defg}
	Lunyangwa	2.27^{abcd}	31.5^{hi}
	LU96/303	2.30^{abcd}	41.5^{fgh}
	LU96/304	2.30^{abcd}	46.0^{defg}
	Mafutha	2.28^{abcd}	39.0^{ghi}
	Mugamba	2.28^{abcd}	42.5^{efgh}
	Mugande	2.35^{a}	41.5^{fgh}
	Salera	2.34^{a}	53.5^{bcdef}
	Semusa	2.29^{abcd}	55.0^{abcde}
	Tainoni	2.35^{a}	51.0^{cdefg}
	Zondeni	2.35^{a}	48.0^{defg}
Mean value (sweetpotato)		2.31±0.11	45.1±10.1
Cassava	Gomani	2.14^{cde}	55.0^{abcde}
	Maunjili	2.01^{ef}	49.5^{defg}
	Mbundumali	1.95^{f}	26.0^{i}
	Mkondezi	2.14^{de}	56.0^{abcd}
	Sauti	2.15^{cde}	49.5^{defg}
Mean value (cassava)		2.08±0.13	47.2±14.0
LSD (P = 0.05)		0.16	13.3
CV (%)		5.12	19.0

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$

The results showed that the average molecular weight of Fraction I molecules for sweetpotato starch (5.79 x 10^5Da) was on average similar to that of cocoyam (5.45 x 10^5Da) starch but higher than that of cassava (5.09 x 10^5Da) starch (Table 5.5a). Mugande and Mugamba sweetpotato starches exhibited the highest average molecular weights (7.26 x 10^5Da and 7.11 x 10^5Da, respectively) while Maunjili cassava starch gave the lowest (1.84 x 10^5Da). The average molecular weight ranged from 1.84 - 6.59 x 10^5 Da, 4.61 - 6.51 x 10^5 Da, and 4.46 - 7.26 x 10^5 Da, for cassava, cocoyam and sweetpotato starches, respectively. These values are consistent with those reported for molecular weight of amylose (Hoover, 2001; Ellis *et al*., 1998). However, average molecular weights found for the amylose fraction of sweetpotato and cassava starch are less than those reported by Ong *et al*. (1994). This is because besides botanical origin of starch and the method employed, the technique used to determine molecular weight also has an influence on the molecular weight of amylose and amylopectin. The average molecular weights of the amylose fraction in the branched starches were also found to be higher than those obtained for the unbranched starch (Table 5.4). This may be due to the differences in elution times observed for the corresponding peaks. The lower molecular weight fraction in unbranched starch eluted between 17 and 20 min, while in branched starch the higher molecular weight fraction eluted between 15 and 20 min (Figure 6.6-6.8).

The results of the molecular weight distribution of Fraction II carbohydrates showed a similar trend as exhibited by Fraction I carbohydrates. Carbohydrates of sweetpotato and cocoyam starches generally exhibited similar molecular weight (2.62 x 10^4 Da and 2.78 x 10^4 Da, respectively) but higher than that of cassava (1.48 x 10^4 Da) starch (Table 5.5a). Since Fraction II contains long chain amylopectins, the results indicate that the long amylopectin chains of cocoyam and sweetpotato starches are longer than those of cassava starch. The molecular weight of long amylopectin chains varied significantly among the different sweetpotato, cocoyam and cassava starches. Starch from Mugande sweetpotato genotypes gave the highest molecular weight in the long chain amylopectin fraction while Gomani, Maunjili, Sauti and Mkondezi had the lowest. Thus, structural differences exist among the different cocoyam, sweetpotato and cassava starches. Further, sweetpotato and cassava starch had a larger fraction of long chain amylopectins (47 and 45%, respectively) than cocoyam starch (32%).

The molecular weight distribution of the short amylopectin chains (Fraction III) also varied from crop to crop. On average, the molecular weight of sweetpotato starch (2.31 x 10^3 Da) was similar to that of cocoyam starch (2.27 x 10^3 Da) but higher than that of cassava (2.08 x 10^3 Da) starch (Table 5.5b). The average molecular weight values ranged from 2.16-2.33 x 10^3, 2.26-2.35 x 10^3, and 1.95-2.15 x 10^3 Da for cocoyam, sweetpotato and cassava starch, respectively. The average molecular weight of the short amylopectin chains varied significantly among the different sweetpotato, cocoyam and cassava starches. Mugande, Salera, Tainoni, Zondeni and Kamchiputu sweetpotato starches, and Nkhotakota cocoyam starch exhibited the highest average molecular weight while Mbundumali cassava starch gave the lowest. There was significant variation ($p \leq 0.001$) in the area of the fraction between the different starches. The area of Fraction III for cocoyam starch (62%) was the highest, while that of cassava and sweetpotato starch were 47% and 45%, respectively. Thus a much larger proportion of short-chain amylopectin branches occurred in cocoyam starch than sweetpotato and cassava starch. Machinga and Thyolo cocoyam starches displayed the highest area (68%) and Mbundumali cassava starch the lowest (26%).

Several authors have reported the influence of molecular structural properties of starch on its functional properties. The larger proportion of amylopectin long-branch chains have resulted in higher gelatinization enthalpy (Yoo *et al*., 2009), swelling (Sasaki and Matsuki, 1998), and viscosity of starch (Lu *et al*., 2008; Charles *et al*., 2005; Shibanuma *et al*., 1996). Starch with larger proportions of short chains has also been reported to display higher gelatinization temperatures and enthalpy (Lu *et al*., 2005; Jane *et al*., 1999; Noda *et al*., 1998). Results of this study have shown that cassava starch has a larger proportion of long-chain amylopectins while cocoyam starch has a larger proportion of short chain amylopectins. Thus, these different structural properties will have varying effects on the functional property of the starches.

5.4 Principal component analysis (PCA)

The PCA analysis performed using 12 molecular properties of the starches, blue value (BV), wavelength of maximum iodine absorption (wavemax), reducing capacity (RC), acid hydrolysis after 12 days (AH), average molecular weight of amylose (AMMw) and amylopectin (AMPMw), average molecular weight of fractions I, II and III (FrIMw, FRIIMw and FRIIIMw) and percentage areas of fractions I, II and III (FrIArea, FRIIArea and FRIIIArea) are presented in Table 5.6.

Table 5.6 Principal component analysis of the molecular properties of the cassava, cocoyam and sweetpotato starches

	Eigenvectors			
Variable	PC1	PC2	PC3	PC4
AH	0.29	-0.07	-0.19	-0.52
RC	0.21	0.02	0.60	0.02
BV	-0.45	0.00	0.03	0.11
Wavemax	-0.43	-0.04	0.08	0.18
AMPMw	0.08	-0.40	-0.47	0.17
AMMw	0.31	-0.20	0.32	-0.18
FrIMw	0.06	0.47	0.32	0.14
FrIArea	0.08	0.41	-0.18	-0.26
FrIIMw	-0.18	0.45	-0.19	0.13
FrIIArea	0.39	0.19	-0.22	0.32
FrIIIMw	-0.18	0.33	-0.14	-0.53
FrIIIArea	-0.36	-0.26	0.18	-0.36
Eigen values	4.15	3.00	1.31	1.17
Individual %	34.6	25.0	10.9	9.8
Cumulative %	34.6	59.6	70.5	80.3

PC1, PC2, PC3 and PC4 accounted for 80% of the total variability, while PC1 and PC2 alone accounted for 35% and 25% of the variabilty resectively. Eigen vectora of PC1 had large negative weights for BV and wavemax, and large positive weigths for FIIArea and AMMw. Eigenvectors of PC2 had large postive weights for FrIMw, FrIArea and FrIIIMw, and lsrge negative weight for AMPMw.

The factor loading lot of the molecular characteristics of the starches is presented in Figure 5.9. and the correlation coefficients among the properties are presented in Table 5.7. Both the loading plot and correlation matrix showed that BV and Wavemax were close to each other. These two variables were highly positively correlated. BV and Wavemax were also highly positively correlated with the FrIIIArea and negatively correlated with FrIIIArea indicating the influence of the chain length of amylopectin on the iodine binding spectra of the starches.

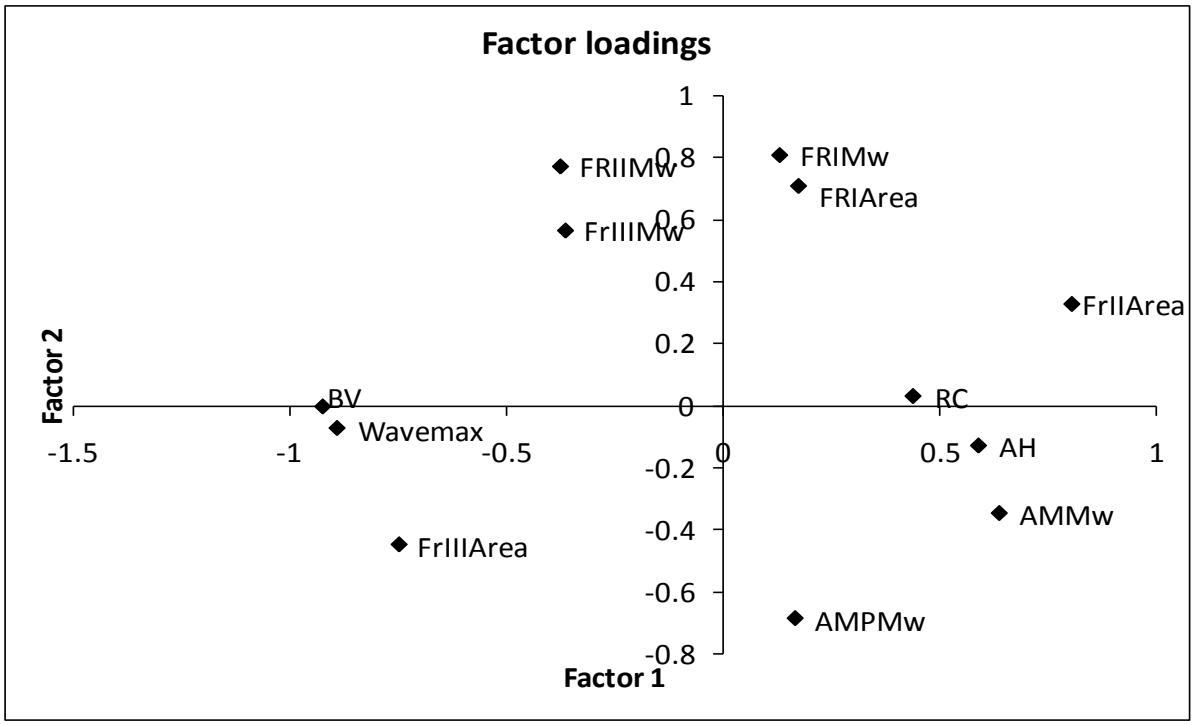

Figure 5.9 PCA loading plot for molecular properties of the cocoyam, sweetpotato and cassava starches

Table 5.7 Correlation coefficients between the molecular characteristics of the cocoyam, sweetpotato and cassava starches

	AH	RC	BV	Wavemax	AMPMw	AMMw	FrIMw	FrIArea	FrIIMw	FrIIArea	FrIIIMw
RC	0.11										
BV	-0.51**	-0.30									
Wavemax	-0.53**	-0.30	0.93**								
AMPMw	0.20	-0.15	-0.12	-0.06							
AMMw	0.46*	0.43*	-0.55**	-0.41*	0.17						
FrIMw	-0.13	0.19	-0.14	-0.11	-0.71**	-0.07					
FrIArea	0.22	0.06	-0.11	-0.14	-0.30	-0.05	0.38*				
FrIIMw	-0.25	-0.19	0.41*	0.38	-0.33	-0.49**	0.52**	0.50**			
FrIIArea	0.26	0.19	-0.72**	-0.73**	0.07	0.21	0.30	0.25	0.04		
FrIIIMw	-0.09	-0.21	0.19	0.05	-0.46*	-0.43*	0.21	0.41*	-0.49**	-0.20	
FrIIIArea	-0.24	-0.24	0.60**	0.61**	0.02	-0.19	-0.40*	-0.44*	-0.16	-0.93**	0.25

*, ** significant at p = 0.05 and p = 0.01, respectively

The factor score plot of PC1 and PC2 is presented in Figure 5.10.

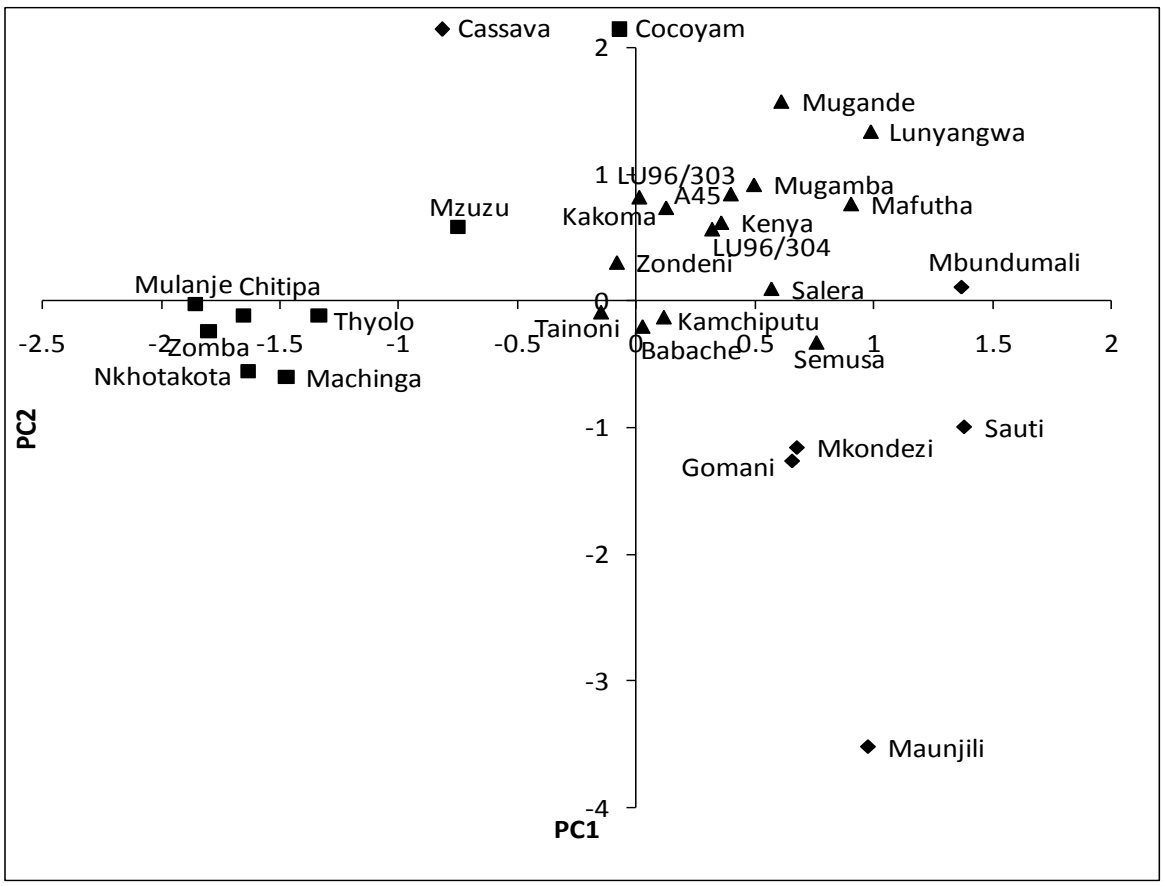

Figure 5.10 PC1 and PC2 plot for cocoyam, sweetpotato and cassava starches using 12 molecular properties

The factor score plot grouped the starches into four quadrants. Bottom right quadrant contained Babache, Kamchiputu and Semusa sweetpotato genotypes, and Gomani, Mkondezi, Sauti and Maunjili cassava genotypes. All the cocoyam accessions except Mzuzu were in the bottom left quadrant together with Tainoni sweetpotato genotype. The starches of these cocoyam accession and sweetpotato genotype were identifiable with high BV and Wavemax values, and higher percentage of short chain amylopectin molecules (FrIIIArea). The top left quadrant consisted of Mzuzu cocoyam and Zondeni sweetpotato starches while the top right quadrant consisted of A45, Kakoma, Kenya, LU96/304, LU96/304, Lunyangwa, Mafutha, Mugamba, Mugande, and Salera sweetpotato starches, and Mbundumali cassava starch. These starches differed in the reducing capacity (RC), average molecular weigth and area of Fraction I (FrIMw and

FrIArea) but displated similar values of long chain amylopectin molecule content (FrIIArea).

5.5 Conclusions

The results of this study have shown that starch from cocoyam, sweetpotato and cassava have different structural properties. Cocoyam starches displayed higher blue values and λ_{max} but lower reducing capacity values than sweetpotato and cassava starches. Based on the blue values, $\lambda_{max,}$ and reducing capacity values, cocoyam starches consisted of molecules of higher molecular weight and a larger proportion of short chain amylopectins than sweetpotato and cassava starches. The acid solubilization of the starches showed a steady increase between day 1 and 8 of acid hydrolysis and a larger increase from day 8 and 12. Sweetpotato and cocoyam starch exhibited similar values of the extent of acid hydrolysis after 12 days (20.2 and 21.2%, respectively) which were higher than that of cassava starch (18.1%). This means that packing and orientation of chains in the amorphous regions of sweetpotato and cocoyam starch are similar but different from that of cassava starch.

The HPSEC analysis of the unbranched starch has revealed that cocoyam and sweetpotato starch contain amylopectin molecules with similar average- and number average molecular weights while cassava contains amylopectin molecules of lower molecular weight. Cocoyam starch contained amylose molecules of lower molecular weight than sweetpotato and cassava starches. The isoamylase debranched starches gave a trimodal distribution profile corresponding to the higher molecular weight amylose fraction, long chain and short chain amylopectin fractions. Sweetpotato and cocoyam starches gave higher molecular weight amylopectin fractions than cassava starch. Sweetpotato starch exhibited the largest fraction of long-chain amylopectins and the lowest fraction of short-chain amylopectins, while cocoyam starch exhibited the highest fraction of short chain amylopectins and smallest proportion of large chain amylopectins. Cassava starch gave intermediate values. Therefore the observed structural differences indicate that cocoyam, sweetpotato and cassava starches will exhibit different functional proprties. The functional properties are discussed in the next chapter.

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CHAPTER 6

FUNCTIONAL PROPERTIES OF MALAWIAN COCOYAM AND SWEETPOTATO STARCHES

6.1 Introduction

The commercial value of starch is rapidly increasing due to its high industrial demand as a raw material in food, feed, pharmaceutical, textile, paper, cosmetic and construction industries (Vaclavik and Christian, 2008; Lawton, 2004). The application of starch in industries is primarily governed by its functional properties such as viscosity, gelatinization and retrogradation, pasting, freeze-thaw stability, swelling and solubility which vary with botanical source (Wickramasinghe *et al*., 2009, Nwokocha *et al*., 2009; Yuan *et al*., 2007). The variations in starch functionality depend on the their composition and molecular structures which include amylose/amylopectin ratio, phosphorus content, molecular weight distribution of starches and chain length distribution of amylopectin (Karim *et al*., 2007, Jane *et al*., 1999; Fredriksson *et al*., 1998).

The demand for starch in industries worldwide is currently being met by a restricted number of crops mainly corn, potato and wheat (Ellis *et al*., 1998) and consequently, starches from these crops have dominated the world market. Tropical root and tuber crops, such as cassava, cocoyam and sweetpotato, remain underexploited sources of starch for the industry worldwide despite being rich in starch. There is need to fully understand the functional properties of the starches from Malawian sweetpotato and cocoyam if these starches are to be exploited in both food and non-food industrial applications. However, information on functional properties of these starches is lacking. The non-availability of such information has limited the application of starches from these crops in the Malawian industry. Therefore this study was undertaken to determine the functional properties of native starches from Malawian sweetpotato and cocoyam, more specifically water binding capacity, swelling power, solubility, starch paste clarity and stability, viscosity, syneresis, and thermal properties of native and retrograded starches.

6.2 Materials and methods

6.2.1 Materials

Starch from 15 genotypes of sweetpotato (A45, Babache, Kakoma, Kamchiputu, Kenya, Lunyangwa, LU96/303, LU96/304, Mafutha, Mugamba, Mugande, Salera, Semusa, Tainoni and Zondeni), seven cocoyam accessions collected from different cocoyam growing districts of Malawi [Chitipa, Mzimba (Mzuzu), Nkhotakota, Machinga, Mulanje, Thyolo and Zomba] and five genotypes of cassava (Gomani, Maunjili, Mbundumali, Mkondezi and Sauti) were used for this study. Starch was isolated and processed from the sweetpotato and cocoyam tubers as outlined in section 3.2.1.

6.2.2 Water absorption capacity (WAC)

Water absorption capacity (WAC) of the starches was determined in triplicate using 2.5% starch suspensions at four temperatures; 30, 50, 70 and 90°C. Dried starch samples (0.125 g) were weighed into pre-weighed centrifuge tubes and 5 mL of distilled water added. The samples were heated at each of the above temperatures for 1 hour with constant shaking and thereafter centrifuged for 15 min at 1500 × *g*. The free water was decanted and the tubes allowed to drain for 10 min at a 45° angle. Subsequently the sample tubes were weighed, and the gain in weight used to calculate the water absorption capacity. Water absorption capacity was calculated using the following equation:
WAC (g H_2O g^{-1} starch) = (mass of wet starch - mass of dry starch)/ mass of dry starch (Mishra and Rai, 2006)

6.2.3 Swelling and solubility

Swelling power and solubility of the starches were determined in triplicate following the procedure of Kojima *et al*. (2006). Dried starch samples (0.1 g) were mixed with 5 mL of distilled water in 10 mL centrifuge tubes, heated for 1 h at 50, 70 or 90°C while shaking every 5 min. The slurry was centrifuged for 30 min at 1500 × *g*, and the weight of the sediment in gram (B) determined. The supernatant (A) was diluted with water until the total volume was 10 mL, and the amount of starch in it was determined by the anthrone-

sulphuric acid method (Brook *et al*., 1986). The solubility and the swelling power were calculated using the following equations, where (S) is the sample weight in gram.

Solubility (%) = (100 x A)/S ; Swelling power (g. g^{-1}) = B/(S-A)

6.2.4 Clarity and stability of starch pastes

Paste clarity of the starches was determined in triplicate as described by Craig *et al*. (1989). A 1% aqueous suspension of starch was made by adding 10 mL of distilled water to exactly 0.1 g of starch (dry basis) in a centrifuge tube with screw caps and vortex mixed. The suspension was heated in a boiling water bath for 30 min with constant stirring every 5 min and thereafter cooled to room temperature for 1 h. The percentage transmittance (%T) was measured at 650 nm against a water blank on a spectrophotometer (Spectronic Unicam, Heλios, Cambridge, United Kingdom).

The stability of the starch pastes was determined by placing the triplicate starch paste samples prepared above in disposable cuvettes and storing it for 5 days at 4°C in a refrigerator. Turbidity was determined every 24 h by measuring absorbance at 640 nm against a water blank (Sandhu and Singh, 2007; Jacobson *et al*., 1997). Normalized absorbance was calculated as:
$A_{norm} = (A_x - A_0)/(A_5-A_0)$, where A_0, A_x, and A_5 are absorbance of fresh paste, paste after X days and paste after 5 days.

6.2.5 Syneresis

Syneresi was determined in triplicate using a 5% aqueous starch suspension made by adding 5 mL of distilled water to 0.25 g (db) starch in screw-capped centrifuge tube. The suspension was heated in a boiling water bath for 30 min with constant stirring and then cooled rapidly to room temperature in an ice bath. After cooling, the tubes with the starch pastes were reweighed to determine the amount of starch paste and then placed in a still-air freezer at -20°C for 48 h. After the freezing period, the samples were placed in a 40°C water bath for 1.5 h to thaw and equilibrate. Syneresis was measured in triplicate as percentage of water released after centrifuging at 1500 x *g* for 30 min (Singh *et al*., 2004).

6.2.6 Viscosity

The viscosity of the starch pastes was determined according to the ISI method 17-1e (International Starch Institute, 2002) with modifications. To 10 g of dried starch samples in a 600 mL beaker, distilled water was added to bring the total weight of starch and water to 500 g. The starch suspension was heated in a water bath at 95°C for 30 min with constant stirring. The resulting gelatinized starch was weighed and water added to replace the evaporated water until a gross weight of 500 g. The starch gel was cooled to 50°C in a water bath while stirring, and viscosity measured in centipoises (cps) with spindle no. 2 at 100 rpm using a Brookfield Digital Viscometer model RTDV II (Brookfield Engineering Laboratories Inc, Stoughton MA 02072, USA).

6.2.7 Thermal properties: gelatinization and retrogradation

Thermal properties of raw starches were obtained using differential scanning calorimetry (DSC 822e, Mettler, Toledo, Switzerland). Exactly 3.0 mg, in triplicate, of dried starch was weighed into DSC aluminium pans and distilled water added using a transfer pipette to make a starch:water ratio of 1:3. The pans were hermetically sealed and samples were left to stand for 1 h at room temperature for moisture equilibration. The sealed pans were heated from 20°C to 95°C under nitrogen gas at a heating rate of 10°C/min to gelatinize the starch samples. From the DSC thermograms, onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_G) were determined using instrument software (STARe SW 9.00). The temperature range and peak height index (PHI) were also calculated as $T_c - T_o$ and as the ratio $\Delta H_G/(T_p-T_o)$, respectively (Peroni *et al*., 2006). The analysis was performed in triplicate.

The gelatinized samples were then stored at 4°C (refrigerator) for a period of 7 days, equilibrated at room temperature for 2 h, and then rescanned in the DSC from 20 to 95°C at 10°C/min to measure the retrogradation transition temperatures and enthalpy. The degree of retrogradation was determined as the ratio of enthalpy change of retrograded starch to enthalpy change of gelatinized starch (Gunaratne and Hoover, 2002).

6.2.8 Data analysis

Data obtained was subjected to analysis of variance (ANOVA) using Statistix 8 for Windows software (Analytical software, Tallahassee, USA, 1985). Mean separation was done using Least Significance Difference (LSD).

Principal component analysis (PCA) was also performed on the functional properties of the starches using NCSS 2004 statistical software (Hintze, 2001). The parameters analyses were paste clarity, thermal properties, onset temperature (To), peak temperature (Tp), conclusion temperature (Tc) and gelatinization enthalpy (ΔH), degree of retrogradation (Retro), water absorption capacity at 50, 70 and 90ºC (WAC50, WAC70 and WAC 90), swelling power at 50, 70 and 90ºC (SP50, SP70 and SP 90), viscosity and syneresis. The first and second PCs were used to show pattern of the data. Correlation analyses between physicochemical and functional properties were also performed using NCSS 2004 (Hintze, 2001).

6.3 Results and discussion

6.3.1 Water absorption capacity (WAC)

The results of water binding capacity of cocoyam, sweetpotato and cassava starches determined at 30, 50, 70 and 90°C are provided in Table 6.1.

Table 6.1 The average values and mean separation of water binding capacity of the cocoyam, sweetpotato and cassava starches

Botanical Source	Genotype/ Accession	Water absorption capacity (g H_2O g^{-1} starch)			
		30°C	50°C	70°C	90°C
Cocoyam	Chitipa	1.23^{bcd}	1.50^{efg}	13.42^{def}	30.47^{c}
	Machinga	1.34^{b}	1.46^{fgh}	6.07^{j}	24.35^{d}
	Mulanje	1.24^{bcd}	1.39^{h}	6.31^{j}	22.17^{def}
	Mzuzu	1.13^{fg}	1.39^{h}	10.91^{gh}	23.83^{d}
	Nkhotakota	1.19^{cde}	1.58^{cde}	2.35^{k}	24.70^{d}
	Thyolo	1.59^{a}	1.60^{cde}	10.98^{gh}	24.27^{d}
	Zomba	1.14^{efg}	1.60^{cde}	6.04^{j}	23.28^{de}
Mean value ± SD		1.3±0.2	1.5±0.2	8.0±3.7	24.7±3.5
Sweetpotato	A45	1.21^{bcd}	1.80^{c}	8.22^{i}	20.58^{efg}
	Babache	1.18^{cde}	1.66^{cd}	8.09^{i}	20.05^{fg}
	Kakoma	1.27^{bcd}	1.70^{cd}	11.96^{fg}	19.37^{fg}
	Kamchiputu	1.21^{bcd}	1.71^{cd}	13.67^{de}	19.96^{fg}
	Kenya	1.17^{cde}	1.48^{efg}	13.46^{def}	19.54^{fg}
	Lunyangwa	1.24^{bcd}	1.51^{efg}	13.74^{de}	17.70^{g}
	LU96/303	1.19^{cde}	1.57^{de}	11.90^{fg}	18.41^{g}
	LU96/304	1.23^{bcd}	1.57^{de}	13.07^{ef}	19.28^{fg}
	Mafutha	1.18^{cde}	1.60^{cde}	13.09^{ef}	19.96^{fg}
	Mugamba	1.27^{bcd}	1.63^{cde}	14.84^{cd}	20.31^{efg}
	Mugande	1.24^{bcd}	1.77^{c}	12.75^{ef}	20.17^{fg}
	Salera	1.28^{bcd}	1.47^{efg}	13.72^{de}	21.77^{def}
	Semusa	1.15^{def}	1.44^{gh}	9.98^{h}	18.14^{g}
	Tainoni	1.19^{cde}	1.65^{cd}	12.84^{ef}	24.72^{d}
	Zondeni	1.30^{bc}	1.77^{c}	10.01^{h}	20.49^{efg}
Mean value ± SD		1.2±0.1	1.6±0.2	12.1±2.3	20.0±1.8
Cassava	Gomani	1.13^{fg}	1.82^{c}	15.43^{bc}	34.92^{b}
	Maunjili	1.29^{bcd}	2.35^{b}	17.53^{a}	34.07^{b}
	Mbundumali	1.16^{cde}	1.49^{efg}	15.88^{bc}	38.30^{a}
	Mkondezi	1.03^{g}	1.65^{cd}	16.17^{abc}	37.99^{a}
	Sauti	1.23^{bcd}	3.29^{a}	16.74^{ab}	38.46^{a}
Mean value ± SD		1.2±0.2	2.1±0.7	16.4±0.8	36.7±2.6
LSD (P = 0.05)		0.10	0.13	0.70	0.95
CV (%)		5.2	4.74	3.63	2.39

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

The results showed significant increase in water holding capacity of the starches with temperature. High increases were observed between 50°C to 70°C while lower increases were observed from 70 to 90°C. Even lower increases in WAC values were observed from 30 to 50°C. Generally, WAC increased eight times for cassava and cocoyam starches and, 10 times for sweetpotato starches from 50°C to 70°C. Water absorption capacity differed significantly between cocoyam, sweetpotato and cassava starches. Cassava starches exhibited higher WAC values than sweetpotato and cocoyam starches at 50, 70 and 90°C. At 30°C, there was little variation in WAC values of the starches. Average WAC values at 50, 70 and 90°C for cassava starches were 2.12, 16.32, and 36.64 g H_2O g^{-1} compared to 1.50, 8.01, and 24.42 g H_2O g^{-1} for cocoyam and 1.62, 11.92 and 20.03 g H_2O g^{-1} for sweetpotato starches at 50, 70 and 90°C, respectively. At 50°C, sweetpotato and cocoyam starches gave similar WAC values, but at 70°C sweetpotato starch showed higher WAC than cocoyam. However, starches from Chitipa, Mzuzu and Thyolo cocoyam accessions exhibited WAC values similar to those obtained for sweetpotato starches. At 90°C cocoyam starches displayed higher WAC values than sweetpotato starches. Chitipa cocoyam starch gave the highest WAC value while Tainoni sweetpotato starch exhibited WAC value similar to most of the cocoyam starches.

Variations in WAC values were also observed within starches from the same botanical source. WAC of the cocoyam starches ranged between 1.13-1.59, 1.16- 1.80 g H_2O g^{-1}, 1.69-14.08 g H_2O g^{-1} and 20.37-37.65 g H_2O g^{-1} at 30, 50, 70 and 90°C respectively. The WAC of the starches from the sweetpotato genotypes ranged between 1.28- 2.05 g H_2O g^{-1}, 7.47-18.60 g H_2O g^{-1} and 17.11-25.28 g H_2O g^{-1} at 50, 70 and 90°C respectively. No clear trends in WAC values for individual starches were observed, however, starch from Chitipa cocoyam accession exhibited higher WAC values at 70 and 90°C than the other cocoyam starches. Within the sweetpotato genotypes, starches from Mugamba and Tainoni exhibited higher WAC at 70°C and 90°C respectively. Lunyangwa, Kamchiputu, Kenya, Mafutha, LU96/304, Tainoni and Mugande sweetpotato starches also displayed WAC at 70°C. Among the five cassava genotypes, Sauti starch displayed higher WAC values among cassava starches at 50, 70 and 90°C. Mbundumali and Mkondezi also gave higher WAC values at 90°C while Maunjili had higher WAC values at 50 and 70°C. Gomani displayed relatively lower WAC values among the cassava starches. The observed differences in WAC of the starches are due to various factors such as particle

size, amylose/amylopectin ratio and molecular structure. The larger the granular size, the greater the WAC, while the higher the amylose levels, the lower the water binding capacity of starches (Akalu *et al*., 1998; Wotton and Bamunuarachi, 1978). The variations in WAC values also indicate differences in the degree of engagement to form hydrogen and covalent bonds between starch chains and the degree of availability of water binding sites among the starches (Hoover and Sosulski, 1986). Earlier results have shown that sweetpotato starches have higher amylose content and larger granule size than cassava starches, while cocoyam starches have lower levels of amylose and smaller granular size than cassava starches (Chapters 3 and 4). Thus, differences in water binding capacity of the starches could largely be due to molecular structures of the starches. Higher water holding capacity of cassava starches compared to cocoyam and sweetpotato starches suggest the presence of weaker intermolecular hydrogen bonds and/or more water binding sites resulting in lower swelling of starch granules of cocoyam and sweetpotato.

6.3.2 Swelling power

The results of the analysis of the swelling power of the starches at different temperatures are presented in Table 6.2. The swelling power of the starches increased when temperature was raised from 50 to 90°C. The swelling power of the starches at 50, 70 and 90°C ranged from 2.50-4.44, 17.23-19.43 and 37.36-43.81 g g^{-1} for cassava starches, 2.29-2.61, 3.36-16.08 and 26.63-34.10 g g^{-1} for cocoyam starches and, 2.49-2.89, 9.35-16.83 and 19.96-28.22 g g^{-1} for sweetpotato starches. Generally, the cocoyam and sweetpotato starches exhibited lower swelling power than cassava starches at all the above temperatures. The swelling power of sweetpotato starches was significantly higher than that of cocoyam at 70°C but lower at 90°C.

Table 6.2 The average values and mean separation of **s**welling power of the cocoyam, sweetpotato and cassava starches

Botanical Source	**Genotype/ Accession**	**Swelling power (g g^{-1} starch)**		
		50°C	**70°C**	**90°C**
Cocoyam	Chitipa	2.50^{jklm}	16.08^{ef}	34.10^{c}
	Machinga	2.32^{n}	7.54^{q}	29.39^{de}
	Mulanje	2.40^{mn}	7.74^{q}	26.63^{gh}
	Mzuzu	2.29^{n}	12.79^{mn}	27.57^{fg}
	Nkhotakota	2.50^{klm}	3.36^{r}	28.16^{ef}
	Thyolo	2.51^{jklm}	13.03^{lmn}	29.95^{d}
	Zomba	2.61^{ghijk}	7.44^{q}	28.52^{ef}
Mean value ± SD		2.44±0.13	9.71±4.18	29.19±2.46
Sweetpotato	A45	2.70^{efg}	9.50^{p}	23.12^{ij}
	Babache	2.67^{fgh}	9.35^{p}	22.42^{jk}
	Kakoma	2.85^{c}	13.72^{kl}	21.77^{kl}
	Kamchiputu	2.73^{def}	15.63^{fg}	22.89^{ijk}
	Kenya	2.49^{lm}	15.17^{ghi}	22.13^{jk}
	Lunyangwa	2.52^{jkl}	15.46^{fgh}	19.96^{m}
	LU96/303	2.58^{hijkl}	13.45^{lm}	20.67^{lm}
	LU96/304	2.51^{jklm}	14.73^{hij}	21.61^{kl}
	Mafutha	2.62^{fghij}	14.88^{ghij}	22.61^{ijk}
	Mugamba	2.64^{fghi}	16.83^{de}	23.22^{ij}
	Mugande	2.89^{c}	14.35^{jk}	22.73^{ijk}
	Salera	2.49^{lm}	12.58^{n}	25.99^{h}
	Semusa	2.54^{ijkl}	11.34^{o}	20.81^{lm}
	Tainoni	2.66^{fghi}	14.48^{ijk}	28.22^{ef}
	Zondeni	2.79^{cde}	11.39^{o}	23.81^{i}
Mean value ± SD		2.64±0.14	13.52±2.23	22.80±2.11
Cassava	Gomani	2.84^{cd}	17.28^{cd}	37.36^{b}
	Maunjili	3.24^{b}	19.43^{a}	38.48^{b}
	Mbundumali	2.50^{jklm}	17.96^{bc}	43.44^{a}
	Mkondezi	2.79^{cde}	18.32^{b}	43.81^{a}
	Sauti	4.44^{a}	18.76^{ab}	43.39^{a}
Mean value ± SD		3.16±0.71	18.35±0.84	41.30±2.95
LSD (P = 0.05)		0.12	0.80	1.31
CV (%)		2.67	3.66	2.87

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Larger increases in the swelling power of the starches were observed from 50 to 70°C than the corresponding increases between 70 and 90°C. On average, the swelling power of the cocoyam starches increased four times as much from 44±0.13 g g^{-1} at 50°C to 9.71±4.18 g g^{-1} at 70°C. The swelling power of the sweetpotato starches increased five times as much from 2.64±0.14 g g^{-1} at 50°C to 13.52±2.23 g g^{-1} at 70°C while that of cassava starches increased six times as much from 3.16±0.71 g g^{-1} at 50°C to 18.35±0.84 g g^{-1} at 70°C. From 70 to 90°C, the swelling power of cocoyam starches increased three times as much, while that of sweetpotato and cassava increased twice as much. The swelling power of the starches also varied significantly ($p \leq 0.001$) among the different sweetpotato and cassava genotypes, and cocoyam accessions. Highest swelling power was observed for Maunjili and Sauti cassava starches at 50 and 70°C while Sauti, Mkondezi and Mbundumali starches displayed highest solubility at 90°C. Gomani exhibited the lowest swelling power among the cassava starches at all temperatures. Chitipa cocoyam starch gave the highest swelling power at both 70 and 90°C, while Nkhotakota and Mulanje gave the lowest values at 70 and 90°C, respectively. Mugamba and Tainoni sweetpotato starches gave the highest swelling power values while A45, Babache, and Lunyangwa gave the lowest at 70 and 90°C, respectively.

Swelling power of starch depends on the capacity of starch molecules to hold water through hydrogen bonding. After gelatinization these hydrogen bonds between the starch molecules are broken and replaced by hydrogen bonds with water. Thus, rapid swelling of the starches due to the breaking of intermolecular hydrogen bonds in the amorphous areas occurred at temperatures below 70°C. This explains the increase in swelling power as well as water absorption capacity as earlier observed (De la Torre-Gutiérrez *et al*., 2008). The differences in the extent of swelling also indicate structural differences among starches. Swelling power is influenced by a strong bonded micellar network, amylopectin molecular structure and amylose content (Tang *et al*., 2005; Gujska *et al*., 1994). Crystallite formation by the association between long amylopectin chains increases granular stability, thereby reducing the extent of granular swelling (Singh *et al*., 2004). Swelling power also increases with increasing long chains of amylopectin and decreasing amylose content (Srichuwong *et al*., 2005; Sasaki and Matsuki 1998). In this study, amylose content was higher in sweetpotato (20.8±5.9%) than cassava (18.8±3.6) and cocoyam starches (14.8±3.4%). Thus the difference in amylose content could explain the differences in swelling power between cassava and sweetpotato. However, cocoyams that

generally had lower amylose content, exhibited lower swelling power than sweetpotato and cassava starches. Therefore, other factors such as molecular structure could be playing a significant role in the swelling power of the starches.

6.3.3 Solubility patterns

The solubility of the starches exhibited similar patterns to that of swelling power, increasing with increasing temperature (Table 6.3), and differed significantly ($p \leq 0.001$) among the cocoyam, sweetpotato and cocoyam starches. Cocoyam starches generally displayed higher solubility than cassava and sweetpotato starches. Sweetpotato starches showed the lowest solubility, except at 50°C where solubility was higher than that of cocoyam starches. At 50°C the solubility of cocoyam starches was similar to that of cassava starches. The solubility values at 50, 70 and 90°C ranged from 0.36-0.62%, 4.61-6.30% and 8.86-10.11% for cassava, 0.22-0.58%, 4.46-10.30% and 7.45-15.68% for cocoyam, and, 0.41-0.64%, 2.86-6.13% and 5.72-9.43% for sweetpotato starches. Except at 50°C, starch from Nkhotakota exhibited the lowest solubility among the cocoyam accessions, while starch from Chitipa gave the highest solubility. Among the sweetpotato genotypes, Babache and A45 starches displayed the lowest solubility, while Kamchiputu and Zondeni exhibited the highest solubility at 70 and 90°C, respectively. Solubility also varied significantly among cassava starches but just like cocoyam and sweetpotato starches, no consistent trends were observed for the individual starches. Gomani had higher solubility at 50 and 90°C while Mbundumali and Mkondezi gave higher solubility at 70°C. Maunjili cassava starch had the lowest solubility among cassava starches both at 70 and 90°C. At 70°C, Mbundumali and Mkondezi cassava starches exhibited solubility similar to those of cocoyam starches. The differences in solubility of the starches could largely be due to structural differences. Differences in chain length distributions in the starches cause differences in solubility (Bello-Pérez *et al*., 2000). Granular size also affects solubility of the starches. The smaller the granule size, the higher the solubility (Tian *et al*., 1991). This could also probably explain the higher solubility of cocoyam starches, as they had smaller sized granules compared to cassava and sweetpotato starches.

Table 6.3 The average values and mean separation of **s**olubility of the cocoyam, sweetpotato and cassava starches

Botanical Source	**Genotype/ Accession**	**Solubility (%**		
		50°C	**70°C**	**90°C**
Cocoyam	Chitipa	0.22^{o}	10.30^{a}	15.68^{a}
	Machinga	0.31^{mn}	6.19^{d}	14.32^{b}
	Mulanje	0.30^{n}	6.11^{d}	12.48^{c}
	Mzuzu	0.55^{defgh}	6.85^{c}	10.66^{e}
	Nkhotakota	0.51^{fghij}	4.46^{jkl}	7.45^{l}
	Thyolo	0.54^{efghi}	8.05^{b}	11.69^{d}
	Zomba	0.58^{abcde}	5.91^{de}	14.08^{b}
Mean value ± SD		0.43±0.15	6.84±1.79	12.34±2.61
Sweetpotato	A45	0.41^{kl}	2.93^{m}	6.43^{no}
	Babache	0.47^{jk}	2.86^{m}	5.95^{qr}
	Kakoma	0.54^{efghi}	5.55^{ef}	6.70^{mn}
	Kamchiputu	0.58^{abcde}	6.13^{d}	9.15^{hi}
	Kenya	0.52^{efghij}	4.98^{gh}	7.98^{k}
	Lunyangwa	0.50^{ghij}	4.20^{kl}	6.31^{op}
	LU96/303	0.53^{efghij}	4.07^{l}	6.06^{pq}
	LU96/304	0.61^{abcd}	4.53^{ijk}	5.72^{r}
	Mafutha	0.56^{cdefg}	5.29^{fg}	7.29^{l}
	Mugamba	0.48^{ij}	5.91^{de}	8.48^{j}
	Mugande	0.63^{ab}	4.14^{l}	6.69^{mn}
	Salera	0.50^{hij}	4.07^{l}	6.98^{m}
	Semusa	0.57^{bcdef}	3.13^{m}	7.52^{l}
	Tainoni	0.64^{a}	4.46^{jkl}	8.23^{jk}
	Zondeni	0.58^{abcde}	2.93^{m}	9.43^{gh}
Mean value ± SD		0.54±0.07	4.35±1.07	7.26±1.15
Cassava	Gomani	0.62^{abc}	4.90^{hi}	10.11^{f}
	Maunjili	0.48^{ij}	4.61^{hij}	8.86^{i}
	Mbundumali	0.36^{lm}	6.30^{d}	9.54^{g}
	Mkondezi	0.38^{lm}	6.27^{d}	9.46^{g}
	Sauti	0.56^{cdefg}	5.39^{f}	9.06^{i}
Mean value ± SD		0.48±0.11	5.49±0.74	9.41±0.45
LSD (P = 0.05)		0.06	0.39	0.30
CV (%)		7.82	4.62	2.03

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

6.3.4 Clarity and stability of starch pastes

The results of paste clarity determined as percentage transmittance on day 1 and changes in paste clarity throughout a 5-day storage period are presented in Table 6.4. For changes in paste clarity upon storage, Table 6.4 gives the absolute values of percentage transmittance of the starch pastes over a period of five days while Figure 6.1 displays normalized absorbance, i.e., as the relative rates of turbidity development between pastes over the 5-day period of analysis for some starches.

Paste clarity varied significantly ($p \leq 0.001$) among cassava, sweetpotato and cocoyam starches. The values of paste clarity ranged from 23.5-40.9% for cassava, 10.9-20.4% for cocoyam and 10.9-18.7% for sweetpotato starches (Table 6.4). Generally cassava starches had higher paste clarity (33.8±6.0%) than sweetpotato (16.1±2.5%) and cocoyam (15.9±3.5%) starches. The paste clarity of the cocoyam and sweetpotato starches was comparable. Paste clarity of the starches varied significantly ($p \leq 0.001$) with genotype among cassava and sweetpotato starches. Different accessions of cocoyams also exhibited varying paste clarity. Among the cassava genotypes, Maunjili gave highest paste clarity while Gomani starch had the lowest. The starches from Mugande, Semusa and Kenya sweetpotato genotypes exhibited higher paste clarity than other starches. A45 starch gave the lowest paste clarity. Chitipa and Mzuzu starch provided the highest clarity among the cocoyam accessions while Thyolo starch displayed the lowest paste clarity. Though in general cocoyam starches displayed lower paste clarity than sweetpotato and cassava starches, starches from Chitipa and Mzuzu had higher paste clarity than sweetpotato starches. Amylose and phosphorus contents are known to influence clarity of starch pastes. High amylose content may result in more opaque starch pastes (Schmitz *et al*., 2006). Presence of high amounts of phosphate groups tends to increase starch paste clarity (Jane *et al*., 1996). However, differences in amylose and phosphorus contents cannot explain the differences in starch paste clarity in this study as cassava starches that gave the highest pastes clarity had relatively higher amylose content and lower phosphorus content. Also, cocoyam starches that gave lower paste clarity had lower amylose content and a higher amount of phosphorus than cassava starch. Lower paste clarity of cocoyam and sweetpotato starches may therefore be attributed to the presence of amylose molecules of high susceptibility to retrogradation (Mélo *et al*., 2003). When starch is heated in water, amylose leaches out into solution. Upon cooling, microcrystals

begin to form and cause turbidity (Craig *et al*. 1989). Thus starches that retrograde faster will have lower paste clarity. This would be the case with sweetpotato and cocoyam starches in this study.

Table 6.4 The average values and mean separation of **c**larity and stability of the starch pastes

Botanical Source	**Genotype/ Accession**	**Paste clarity and stability at 4°C (%T)**				
		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Cocoyam	Chitipa	20.4^{e}	10.0^{ef}	8.4^{ghi}	7.6^{fg}	7.0^{ef}
	Machinga	13.6^{lm}	5.9^{klmn}	4.8^{no}	4.1^{mno}	2.8^{l}
	Mulanje	16.8^{ghij}	7.6^{hijk}	6.2^{kl}	4.9^{klm}	4.2^{jk}
	Mzuzu	19.8^{ef}	10.5^{e}	9.2^{f}	8.2^{f}	7.4^{ef}
	Nkhotakota	12.6^{mn}	5.5^{lmn}	3.9^{pq}	3.0^{p}	2.5^{l}
	Thyolo	10.9^{n}	4.7^{mn}	4.1^{0pq}	3.5^{op}	2.9^{l}
	Zomba	17.1^{hij}	9.1^{efghi}	6.6^{k}	5.1^{jkl}	4.5^{ij}
Mean value ± SD		15.9±3.5	7.6±2.2	6.2±2.0	5.2±1.9	4.5±1.9
Sweetpotato	A45	10.9^{n}	4.5^{n}	3.6^{q}	2.9^{p}	2.5^{l}
	Babache	15.8^{ijk}	7.5^{hijk}	6.6^{k}	5.7^{ij}	5.1^{hij}
	Kakoma	16.2^{hijk}	8.6^{fghij}	6.5^{kl}	5.9^{i}	5.5^{ghi}
	Kamchiputu	17.6^{ghi}	8.0^{ghij}	5.2^{mn}	4.8^{klm}	4.6^{hij}
	Kenya	18.0^{fgh}	9.4^{efgh}	8.8^{fgh}	8.2^{f}	7.8^{e}
	Lunyangwa	15.8^{ijk}	8.1^{fghij}	6.6^{k}	6.1^{i}	5.6^{gh}
	LU96/303	16.4^{hijk}	9.2^{efghi}	8.1^{hij}	7.0^{g}	6.5^{fg}
	LU96/304	15.4^{jkl}	8.0^{ghij}	7.6^{j}	6.2^{hi}	5.5^{ghi}
	Mafutha	17.6^{ghi}	8.5^{fghij}	7.7^{ij}	7.0^{gh}	6.6^{fg}
	Mugamba	17.7^{ghi}	9.8^{efg}	8.9^{fg}	8.1^{f}	7.7^{e}
	Mugande	18.7^{efg}	9.6^{efg}	8.7^{fgh}	7.6^{fg}	7.1^{ef}
	Salera	15.2^{jkl}	7.3^{ijkl}	6.5^{kl}	5.6^{ijk}	5.0^{hij}
	Semusa	18.7^{efg}	10.5^{e}	9.2^{f}	8.0^{f}	7.1^{ef}
	Tainoni	14.4^{klm}	6.6^{jklm}	5.8^{lm}	4.7^{lmn}	4.1^{jk}
	Zondeni	12.9^{mn}	5.2^{mn}	4.6nop	3.9^{no}	3.3^{kl}
Mean value ± SD		16.1±2.5	8.0±2.1	7.0±1.7	6.1±1.6	5.6±1.5
Cassava	Gomani	23.5^{d}	20.5^{d}	18.4^{e}	18.0^{e}	16.8^{d}
	Maunjili	40.9^{a}	35.9^{a}	34.0^{a}	32.8^{a}	33.0^{a}
	Mbundumali	33.1^{c}	28.6^{c}	27.2^{b}	25.4^{d}	23.6^{c}
	Mkondezi	36.6^{b}	33.0^{b}	31.5^{b}	29.0^{b}	27.1^{b}
	Sauti	35.0^{bc}	31.8^{b}	30.5^{c}	27.6^{c}	26.4^{b}
Mean value ± SD		33.8±6.0	30.0±5.5	28.3±5.7	26.6±5.1	25.4±5.6
LSD (P = 0.05)		2.09	1.94	0.76	0.84	1.11
CV (%)		6.60	9.89	4.31	5.29	7.57

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

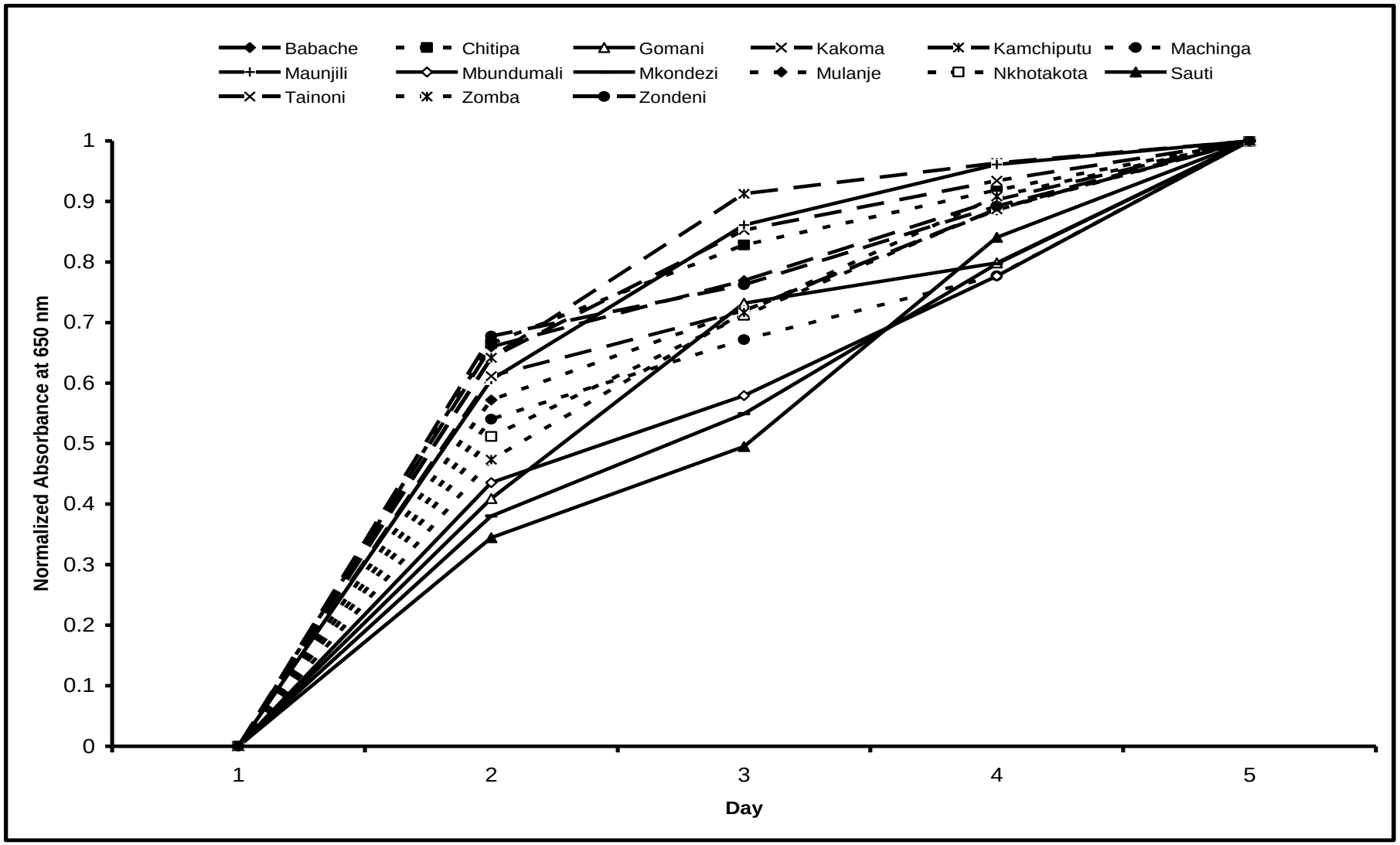

Figure 6.1 Normalized turbidities of pastes of five varieties of cassava starch, and some sweetpotato and cocoyam starches

For the five days of refrigerated storage, opacity of the cocoyam, sweetpotato and cassava starch paste increased. Greater increases in opacity were observed in cocoyam and sweetpotato starches than in cassava starches. This indicates that pastes of cocoyam and sweetpotato are less stable compared to those of cassava starches. Thus, higher retrogradation occurred in cocoyam and sweetpotato starches than in cassava starches. Rapid increase in opacity was observed in cocoyam and sweetpotato starches after one day and thereafter a steady increase was observed. In cassava, starch paste continued to show higher paste clarity over the storage period. Turbidity development in starches during storage is due to the interaction of several factors, such as granule swelling, granule remnants, leached amylose and amylopectin, amylose and amylopectin chain length, intra or interbonding, and lipid (Jacobson *et al*., 1997). Differences in paste clarity and rates of turbidity development indicate that the starches have different structures. Since unaltered transparency during storage is a much desired characteristic of starches used in food products (Schmitz *et al*., 2006), cassava starch would be more suitable for this application as there were small increases in opacity of the starches upon storage.

6.3.5 Paste viscosity and syneresis

The viscosity values of the starch pastes ranged from 5333-8733 cps, 6067-12733 cps and 10140-14033 cps for cocoyam, sweetpotato and cassava starch pastes, respectively (Table 6.5). The viscosity values of cassava starch pastes were higher when compared with those of sweetpotato and cocoyam starch pastes. Cocoyam starch pastes had lower viscosity than sweetpotato starches except for Mzuzu and Nkhotakota starches which had paste viscosity similar to most of the sweetpotato starches. Starch pastes from the same botanical source also differed significantly ($p \leq 0.001$) in their viscosity values. Starches of cocoyam accessions obtained from Mzuzu exhibited higher viscosity values while those from Machinga and Thyolo had the lowest. Genotypic differences in starch paste viscosity were observed among the sweetpotato and cassava starches. Semusa sweetpotato starch gave the highest paste viscosity followed by LU/96/303, Mugande and Mugamba, while Kakoma, Kamchiputu and Zondeni had the lowest viscosity. The paste viscosity of Semusa, LU96/303, Mugamba, and Mugande sweetpotato starches were similar to those of cassava starches. Mkondezi and Gomani had the highest viscosity among cassava starches while Maunjili had the lowest.

Since starches are used in industries to impart viscosity, the use of cassava, sweetpotato or cocoyam starches will be determined by the desired viscosity of the end product.

Table 6.5 The average values and mean separation of **v**iscosity and degree of syneresis of the starch pastes

Botanical Source	**Genotype/ Accession**	**Syneresis (%)**	**Viscosity (cps)**
Cocoyam	Chitipa	46.5^{a}	5640^{nop}
	Machinga	49.2^{a}	5453^{op}
	Mulanje	47.1^{a}	5733^{nop}
	Mzuzu	47.2^{a}	8733^{hij}
	Nkhotakota	28.9^{cde}	7067^{kl}
	Thyolo	32.9^{bc}	5333^{p}
	Zomba	32.9^{bc}	6240^{mno}
Mean value ± SD		40.7±8.6	6314±1251
Sweetpotato	A45	26.4^{de}	8160^{j}
	Babache	24.5^{e}	7320^{k}
	Kakoma	32.3^{cd}	6307^{lmn}
	Kamchiputu	14.4^{fg}	6560^{klm}
	Kenya	11.5^{g}	9800^{efg}
	Lunyangwa	18.0^{f}	9053^{ghi}
	LU96/303	33.8^{bc}	11700^{c}
	LU96/304	30.0^{cd}	9080^{ghi}
	Mafutha	26.8^{de}	9507^{fgh}
	Mugamba	32.8^{bc}	10373^{de}
	Mugande	36.4^{b}	10633^{d}
	Salera	36.3^{b}	8547^{ij}
	Semusa	29.9^{cd}	12733^{b}
	Tainoni	33.0^{bc}	8600^{ij}
	Zondeni	36.7^{b}	6067^{mnop}
Mean value ± SD		28.1±8.3	8963±1927
Cassava	Gomani	0.0^{h}	13000^{b}
	Maunjili	0.0^{h}	10140^{def}
	Mbundumali	0.0^{h}	12267^{bc}
	Mkondezi	0.0^{h}	14033^{a}
	Sauti	0.0^{h}	11733^{c}
Mean value ± SD		0.00±0.0	12235±1393

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

The degree of syneresis ranged from 28.9-49.2% and 11.5-37.7% for cocoyam and sweetpotato, respectively. Cassava starches gave zero values. These results indicate that cassava starch is more stable to freeze-thawing (lower degree of retrogradation) than cocoyam and sweetpotato starch. Cassava starch could therefore be better suited for use in frozen products than cocoyam and sweetpotato starches (Vaclavik and Christian, 2008). The degree of syneresis of cocoyam starches was significantly ($p \leq 0.001$) higher than that of sweetpotato starches. The amount of water released from the frozen cocoyam differed significantly ($p \leq 0.001$) among the different accessions. Starches from Machinga, Mzuzu, Mulanje, and Chitipa cocoyam accessions showed the highest tendency for water loss. The Nkhotakota starch released the lowest amount of water. The degree of syneresis for Nkhotakota, Thyolo and Zomba cocoyam starches was comparable to that of most sweetpotato starches. Among sweetpotato genotypes, starches from Zondeni, Mugande and Salera showed greater tendency to lose water while Lunyangwa, Kenya and Kamchiputu lost the least. Higher tendencies to lose water for sweetpotato and cocoyam starches indicate that they will have limited application in frozen food products. In order to overcome this challenge, resistance of the starches to retrogradation could be improved through modification.

6.3.6 Thermal properties

6.3.6.1 Gelatinization

The Differential Scanning Calorimetry thermographs, gelatinization temperatures and enthalpy values for cocoyam, sweetpotato and cassava starches are presented in Figures 6.2-6.4 and Table 6.6 respectively.

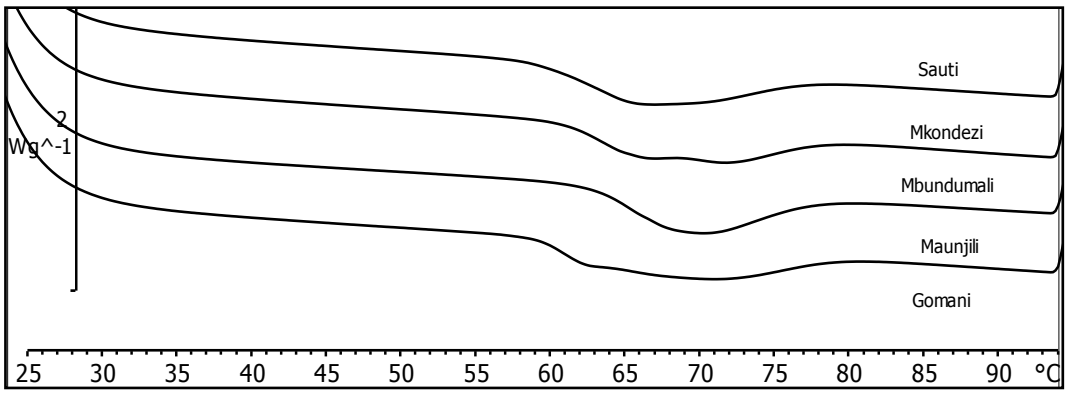

Figure 6.2 DSC thermographs of native cassava starches

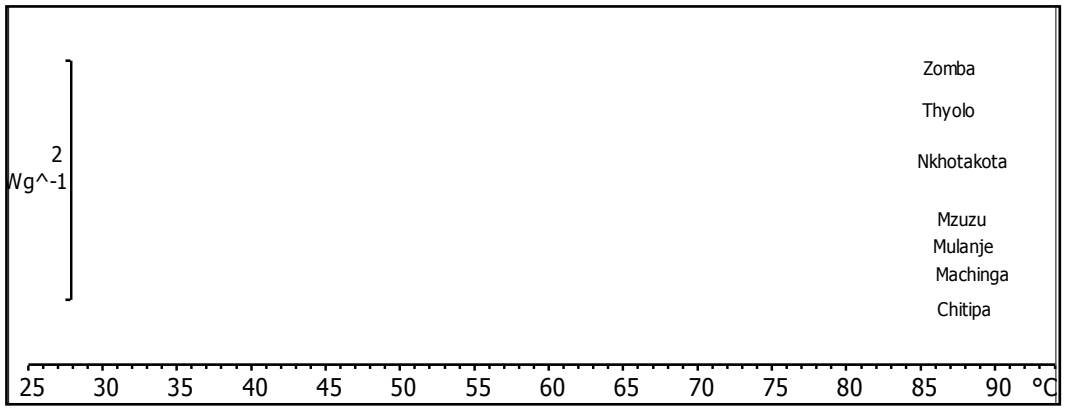

Figure 6.3 DSC thermographs of native cocoyam starches

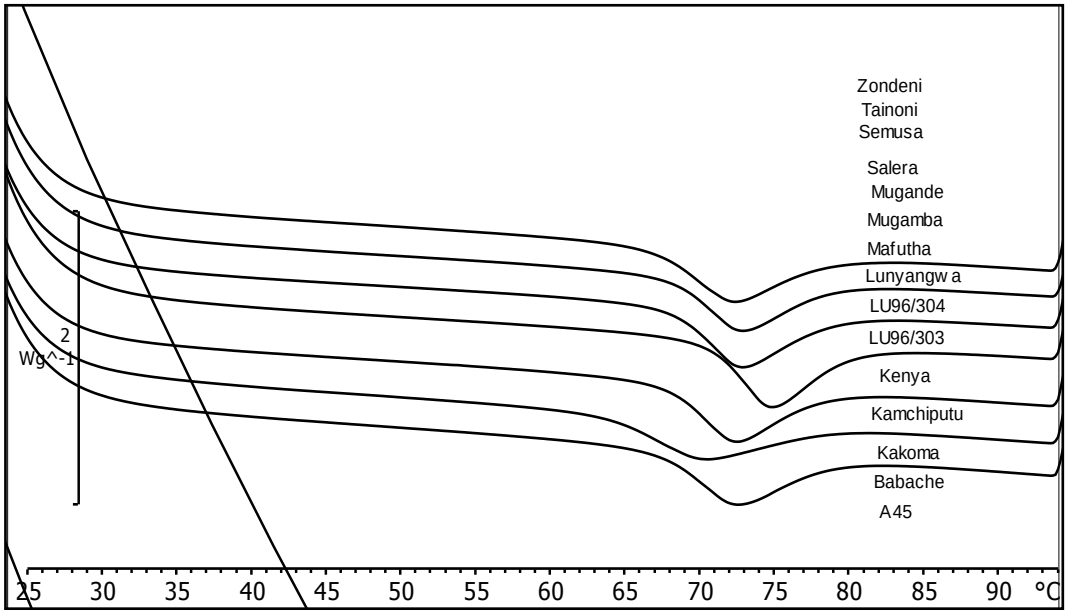

Figure 6.4 DSC thermographs of native sweetpotato starches

All the starches from cocoyam, sweetpotato and cassava showed similar thermal behaviour when heated in the presence of excess water. A single endothermic peak was observed between 60°C and 81°C (Figures 6.2, 6.3 and 6.4). Cassava starches exhibited broader endothermic peaks. Gelatinization temperatures and enthalpy differed significantly ($p \leq 0.001$) among the starches. The gelatinization temperatures ranged from 69.4-81.4°C, 68.5-79.2°C and 60.1-76.8°C for cocoyam, sweetpotato and cassava starches respectively. Generally, cocoyam starch exhibited higher onset, peak, and conclusion temperatures than sweetpotato and cassava starches. This is in agreement with findings of Pérez *et al*. (2005). They reported higher onset, peak and conclusion temperature values for *C. esculenta* (taro) starch than cassava. The enthalpy of gelatinization of cocoyam starches (13.4±2.1 J/g) was higher than that of sweetpotato starches (12.3±1.1 J/g) but similar to that of cassava starches (14.0±0.8 J/g). Peroni *et al*. (2006) reported gelatinization temperatures ranging from 61.55-72.94°C for cassava and 62.85-77.91°C for sweetpotato starches using a starch:water ratio of 1:3. They also obtained enthalpy values of 10.4 J/g and 12.9 J/g for cassava and sweetpotato starches respectively. Collado *et al*. (1999) reported a gelatinization temperature range of 64.6 -84.6°C and transition enthalpy of 12.9 J/g for 44 genotypes of sweetpotato from Philippines. Jane *et al*. (1999) reported a gelatinization range of 43.0-63.3°C and enthalpy change of 6.8 J/g for five varieties of taro (*C. esculenta*) using DSC and the same starch: water ratio of 1:3. Gelatinization temperatures of cassava and sweetpotato starches obtained in this study are therefore in agreement with those reported, while those of cocoyam starch are higher. Gelatinization enthalpy of sweetpotato starches is also within the range reported but that of cassava and cocoyam starches is higher. Unlike findings of Peroni *et al*. (2006), in this study cassava starches exhibited lower gelatinization temperatures but higher enthalpy than sweetpotato starches.

Table 6.6 The average values and mean separation of the thermal properties: onset (T_o), peak (T_p) and conclusion (T_c) temperatures, temperature range (R), peak height index (PHI) and transition energy (ΔH_G) of the native starches

Botanical Source	Genotype/ Accession	T_o (°C)	T_p (°C)	T_c (°C)	R (°C)	PHI	ΔH_G (J/g)
Cocoyam	Chitipa	61.8^{k}	69.4^{l}	78.6^{hi}	16.8^{b}	2.1^{n}	14.2^{bcd}
	Machinga	71.4^{b}	76.7^{b}	82.4^{bc}	11.1^{fg}	2.4^{jk}	11.4^{hijk}
	Mulanje	70.2^{c}	75.7^{c}	82.6^{b}	12.5^{e}	2.6^{def}	14.0^{bcd}
	Mzuzu	68.2^{gh}	73.1^{ghi}	79.2^{efgh}	11.0^{fgh}	1.7^{o}	8.3^{n}
	Nkhotakota	75.6^{a}	78.7^{a}	83.7^{a}	8.1^{m}	4.8^{a}	14.7^{ab}
	Thyolo	67.8^{h}	73.3^{g}	80.9^{cd}	13.1^{e}	2.7^{de}	14.6^{abc}
	Zomba	71.3^{b}	76.4^{b}	82.5^{b}	11.2^{fg}	2.7^{de}	13.8^{cde}
Mean value ± SD		69.4±4.0	74.8±2.9	81.4±1.8	12.0±2.5	2.7±1.0	13.4±2.1
Sweetpotato	A45	69.8^{cd}	74.9^{de}	81.4^{c}	11.6^{f}	2.5^{ghi}	12.91^{fghi}
	Babache	69.8^{cd}	74.6^{e}	80.6^{d}	10.8^{ghi}	2.6^{def}	12.4^{ghijk}
	Kakoma	68.0^{h}	73.0^{hi}	78.6^{hi}	10.6^{ghi}	2.0^{n}	10.5^{m}
	Kamchiputu	64.3^{j}	70.9^{k}	78.9^{ghi}	14.5^{d}	1.8^{o}	11.6^{kl}
	Kenya	68.2^{gh}	73.1^{ghi}	79.0^{ghi}	10.8^{ghij}	2.5^{efg}	12.5^{abcd}
	LU96/303	70.9^{b}	75.1^{d}	80.7^{d}	9.7^{kl}	3.5^{b}	14.0^{bcde}
	LU96/304	68.7^{ef}	73.4^{fg}	79.2^{fgh}	10.4^{hij}	2.5^{hij}	11.7^{kl}
	Lunyangwa	68.8^{ef}	73.3^{gh}	78.6^{ghi}	9.9^{jkl}	2.5^{ijk}	11.1^{lm}
	Mafutha	67.9^{h}	72.8^{i}	78.5^{i}	10.4^{ghi}	2.5^{ijk}	12.1^{ijk}
	Mugamba	68.5^{fg}	72.9^{i}	78.8^{ghi}	10.3^{ijk}	2.8^{d}	12.0^{jk}
	Mugande	69.1^{e}	73.7^{f}	79.5^{ef}	10.4^{hij}	2.7^{de}	12.2^{hijk}
	Salera	68.6^{fg}	73.4^{fg}	79.7^{e}	11.1^{fg}	2.5^{ijk}	12.0^{jk}
	Semusa	69.7^{d}	73.2^{gh}	79.2^{efg}	9.5^{l}	3.2^{c}	12.9^{fgh}
	Tainoni	68.0^{h}	72.0^{j}	77.5^{j}	9.5^{l}	3.3^{c}	12.6^{ghij}
	Zondeni	66.8^{i}	71.8^{j}	77.8^{j}	11.0^{fgh}	2.3^{kl}	11.6^{kl}
Mean value ± SD		68.5±1.5	73.2±1.1	79.2±1.0	10.7±1.2	2.6±0.4	12.3±1.1
Cassava	Gomani	60.8^{l}	65.6^{o}	76.9^{k}	16.1^{c}	3.1^{c}	14.0^{bcd}
	Maunjili	58.2^{o}	67.4^{n}	77.5^{j}	19.3^{a}	1.6^{o}	15.1^{a}
	Mbundumali	62.1^{k}	68.6^{m}	76.2^{l}	14.1^{d}	2.2^{lm}	13.4^{def}
	Mkondezi	60.2^{m}	65.7^{o}	76.8^{k}	16.6^{bc}	2.5^{fgh}	13.1^{efg}
	Sauti	59.1^{n}	65.4^{o}	76.4^{kl}	17.3^{b}	2.1^{mn}	14.1^{bcd}
Mean value ± SD		60.1±1.4	66.5±1.3	76.8±0.6	16.7±1.8	2.3±0.5	14.0±0.8
LSD (P = 0.05)		0.45	0.34	0.58	0.66	0.25	0.83
CV (%)		0.41	0.28	0.45	3.29	6.05	3.99

NB: Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

The differences in gelatinization properties of starches are largely due to the molecular structure of amylopectin, and granular architecture (Gunaratne and Hoover, 2002). Gelatinization temperature is considered to be a reflection of the degree of orderly arrangement of molecules in the starch granule while gelatinization enthalpy reflects the loss of molecular order. Higher gelatinization temperatures and enthalpy indicate the presence of strong bonding forces within the granule interior i.e. more orderly arrangement of molecules and thus higher degree of crystallinity (Peroni *et al*., 2006; Tester and Morrison, 1990). The onset temperature is influenced by short amylopectin branch-chains and low gelatinization temperatures are characteristic of starches with larger proportions of short amylopectin branch chains (Jane *et al*., 1999). Peak temperature is an indication of granular architecture (crystalline quality) and a high peak temperature might be due to a higher proportion of longer chains in the amylopectin as these require higher temperatures to dissociate completely than required for shorter double helices (Karim *et al*., 2000). Thus, higher gelatinization temperatures and enthalpy of cocoyam starches compared to cassava and sweetpotato starches suggest a more orderly arrangement of molecules and the presence of higher proportions of long amylopectin chains in cocoyam starch.

Gelatinization temperatures are also influenced by starch composition. Sasaki (2005) reported a significant negative correlation between conclusion temperature and endothermic enthalpy with amylose content of starches indicating that starch with more amylose has a more amorphous region and is less crystalline and thus has lower gelatinization temperature and endothermic enthalpies. Pérez *et al*. (2005) also reported a significant relationship between amylose and gelatinization profile, and enthalpic change and ash. The differences in gelatinization temperatures and enthalpy could also be partly due to differences in amylose contents of the starches. Composition analysis of the starches revealed that cocoyam starches have lower levels of amylose (14.8±3.4%) than cassava (18.8±3.6%) and sweetpotato (20.8±5.8%) starches.

Gelatinization of cassava starch occurred over a wider range of temperatures compared to cocoyam and sweetpotato starches as shown by a higher temperature range. Temperature range values of 16.7±1.8, 12.0±2.5 and 10.7±1.2°C were obtained for cassava, cocoyam and

sweetpotato starches, respectively. Pérez *et al*. (2005) also found a wider gelatinization profile in cassava starch compared to taro starch. Peroni *et al*. (2006) found that sweetpotato had a wider gelatinization profile than cassava starches. In this study, cassava starch exhibited a wider profile than cocoyam and sweetpotato starch. Peak height index measures uniformity in gelatinization (Sandhu *et al*., 2005). The peak height index values of the cassava, sweetpotato and cocoyam starches fell within the same range. The peak height index values ranged from 1.6-3.1, 1.8-3.5 and 1.7-4.8 for cassava, sweetpotato and cocoyam starches. The peak height index values differed significantly ($p \leq 0.001$) within starches from sweetpotato and cassava genotypes, and cocoyam accessions. Starches of LU96/303, Semusa and Tainoni sweetpotato genotypes had the highest PHI values while Kamchiputu had the lowest. Among the cassava starches, Gomani had the highest PHI value while Maunjili had the lowest. Starch from Nkhotakota cocoyam had the highest PHI value while Mzuzu gave the lowest. Temperature range of gelatinization and peak height index gives an indication of the distribution of starch granules. The more heterogeneous the granules, the broader the temperature range and the lower the PHI (Sasaki, 2005; Yamin *et al*., 1999; Knutson *et al*., 1982). Higher amylopectin content can also lead to the narrowing of temperature range of gelatinization (Krueger, 1987). Thus heterogeneity and differences in amylose/amylopectin ratio could probably explain the differences in gelatinization temperature range and PHI of cassava, sweetpotato and cocoyam starches.

6.3.6.2 Retrogradation

The Differential Scanning Calorimetry thermographs, gelatinization temperatures and enthalpy values of the retrograded cocoyam, sweetpotato and cassava starches are presented in Figures 6.5-6.7 and Table 6.7 respectively. Compared with thermal properties of native starches (Table 6.6), the retrograded starches displayed lower gelatinization temperatures (T_o, T_p, and T_c) and smaller enthalpies than raw starches. This indicates that retrograded starches have weaker crystallinity (Sasaki, 2005).

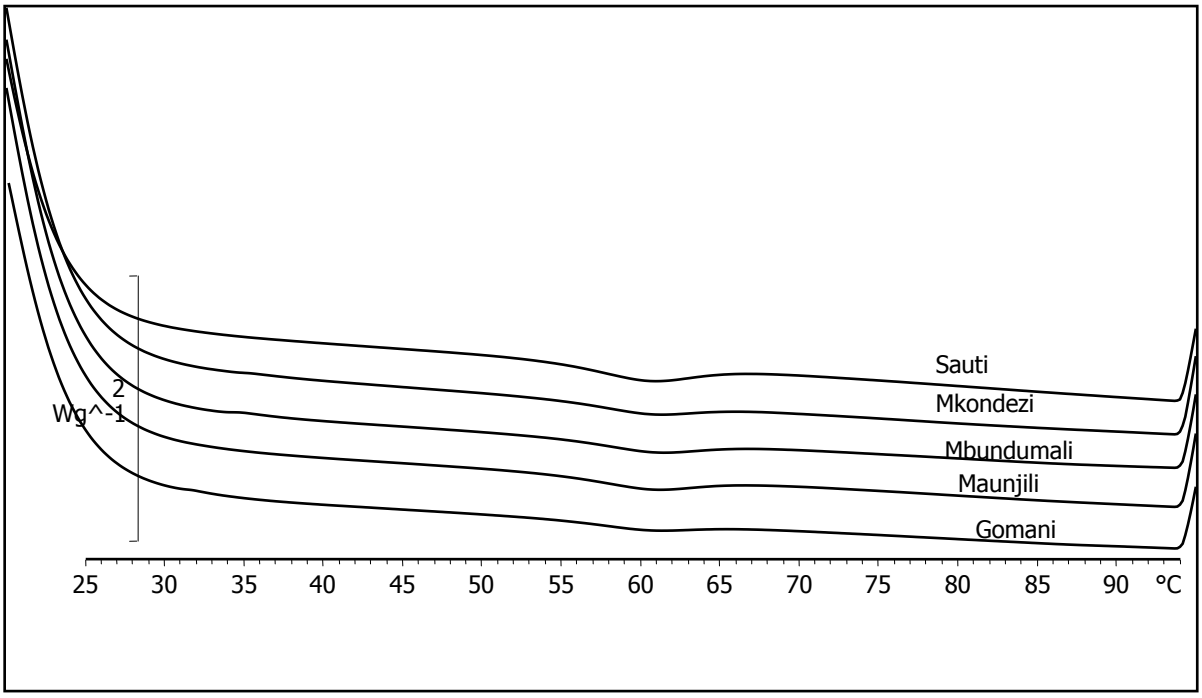

Figure 6.5 DSC thermographs of retrograded cassava starches

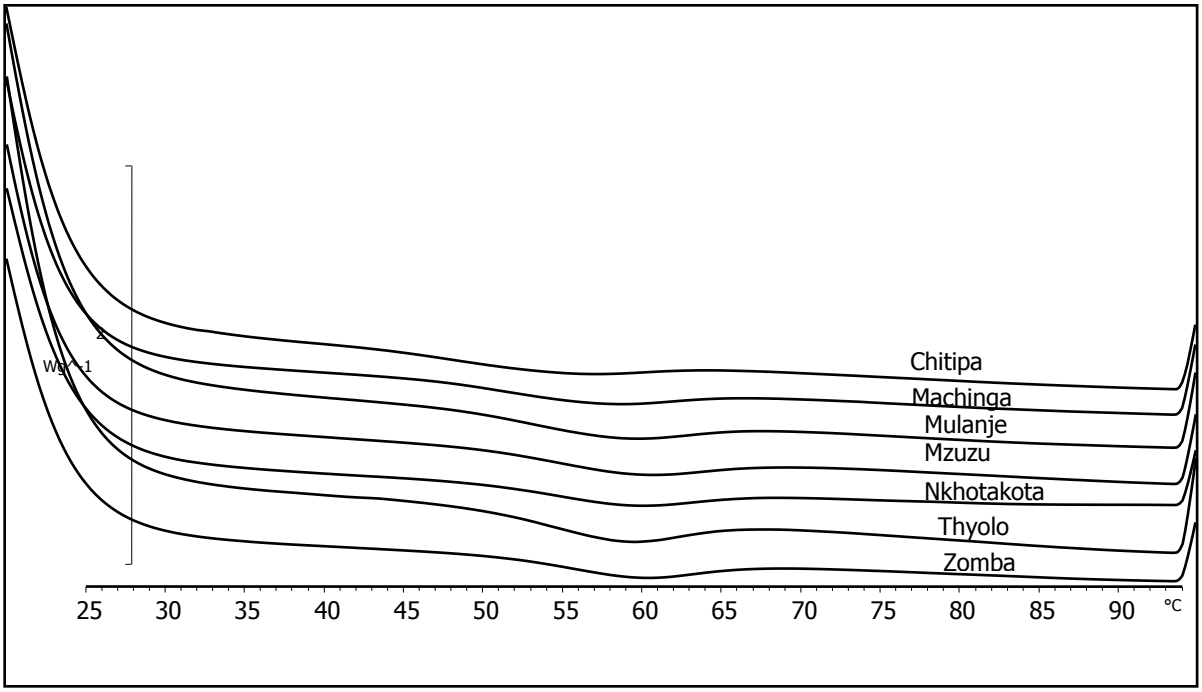

Figure 6.6 DSC thermographs of retrograded cocoyam starches

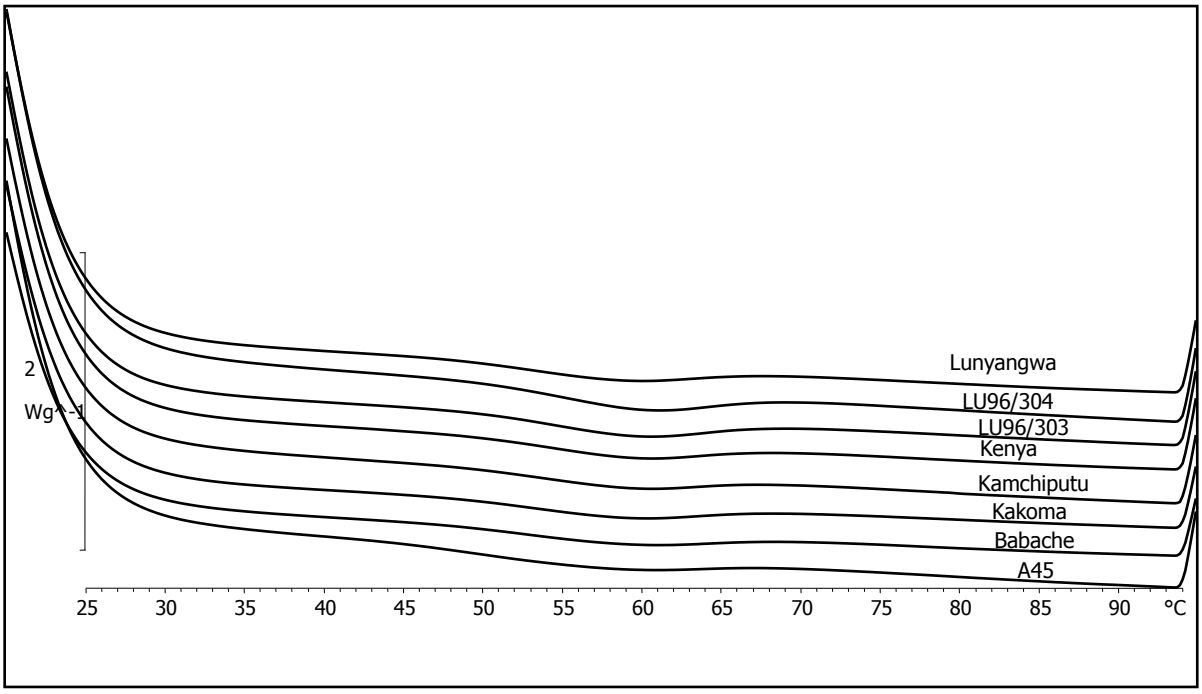

Figure 6.7a DSC thermographs of retrograded sweetpotato starches

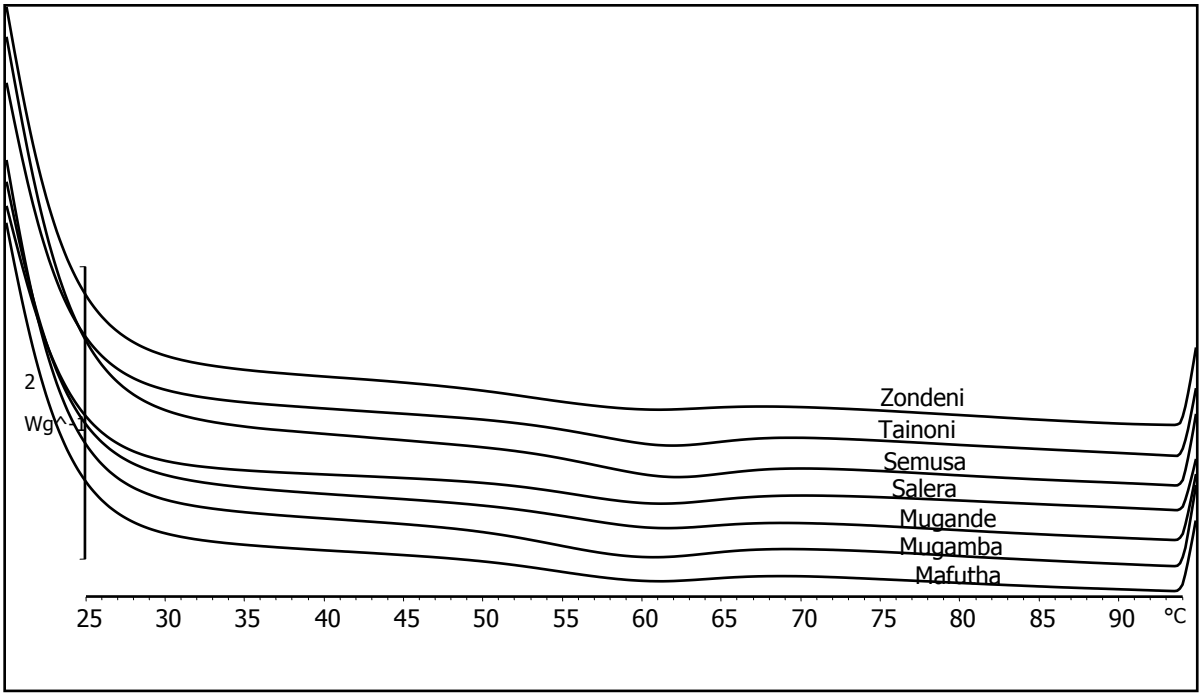

Figure 6.7b DSC thermographs of retrograded sweetpotato starches

Table 6.7 The average values and mean separation of the thermal properties of the retrograded starches: onset (T_o), peak (T_p) and conclusion (T_c) temperatures, temperature range (R), and transition energy (ΔH_G)

Botanical Source	Genotype/ Accession	T_o (°C)	T_p (°C)	T_c (°C)	R (°C)	ΔH_G (J/g)	Retrog. (%)
Cocoyam	Chitipa	45.9^{m}	56.1^{p}	62.5^{j}	16.6^{ab}	3.5^{hij}	23.4^{l}
	Machinga	49.7^{kl}	58.6^{n}	65.2^{h}	15.5^{def}	5.0^{b}	41.6^{b}
	Mulanje	51.2^{j}	59.3^{jkl}	66.2^{def}	15.0^{defg}	4.4^{cde}	31.2^{efg}
	Mzuzu	52.0^{fgh}	59.0^{m}	65.3^{gh}	13.3^{j}	3.8^{g}	45.3^{a}
	Nkhotakota	51.8^{fghi}	59.7^{hi}	66.0^{defg}	14.2^{ghij}	5.4^{a}	37.0^{c}
	Thyolo	52.5^{ef}	59.3^{klm}	65.8^{fgh}	13.3^{j}	4.2^{def}	27.4^{ij}
	Zomba	52.9^{e}	60.4^{de}	66.1^{def}	13.3^{j}	4.8^{b}	35.1^{cd}
Mean value ± SD		50.8±2.3	58.9±1.3	65.3±1.3	14.4±1.3	4.4±0.7	34.5±7.4
Sweetpotato	A45	49.2^{l}	58.2^{o}	65.7^{fgh}	16.5^{abc}	3.2^{k}	24.9^{l}
	Babache	50.0^{k}	59.1^{lm}	66.8^{bcd}	16.8^{a}	4.1^{f}	32.9^{def}
	Kakoma	51.3^{ij}	59.6^{ij}	66.0^{efgh}	14.6^{fghi}	3.6^{ghi}	36.2^{c}
	Kamchiputu	52.1^{fgh}	59.7^{hi}	65.8^{fgh}	13.7^{ij}	2.9^{l}	25.2^{kl}
	Kenya	52.2^{fg}	60.0^{fg}	66.3^{cdef}	14.1^{ghij}	3.4^{ghi}	27.3^{jk}
	LU96/303	52.0^{fgh}	60.2^{ef}	66.8^{bcd}	14.8^{efgh}	4.4^{cde}	31.5^{efg}
	LU96/304	51.4^{hij}	60.6^{d}	67.0^{bc}	15.6^{cdef}	4.2^{ef}	36.0^{c}
	Lunyangwa	49.5^{kl}	58.5^{n}	65.5^{fgh}	16.0^{abcd}	3.3^{jk}	30.0^{ghi}
	Mafutha	51.8^{ghij}	59.9^{gh}	66.6^{bcde}	14.8^{efgh}	3.6^{ghi}	30.2^{fg}
	Mugamba	52.4^{efg}	60.5^{d}	67.1^{b}	14.7^{fghi}	4.4^{cd}	37.0^{c}
	Mugande	53.9^{cd}	61.4^{b}	67.4^{ab}	13.5^{j}	3.6^{ghi}	29.9^{ghi}
	Salera	53.6^{d}	61.1^{c}	67.4^{ab}	13.8^{ij}	3.7^{gh}	30.7^{fg}
	Semusa	54.1^{bcd}	61.7^{a}	68.0^{a}	13.9^{hij}	4.5^{c}	33.4^{de}
	Tainoni	54.8^{a}	61.6^{ab}	66.8^{bcd}	12.0^{k}	3.5^{ghi}	28.1^{hij}
	Zondeni	50.0^{k}	59.4^{jk}	65.7^{fgh}	15.7^{bcde}	3.4^{ijk}	29.5^{ghij}
Mean value ± SD		51.8±1.7	60.1±1.1	66.6±0.8	14.7±1.4	3.7±0.5	30.9±3.8
Cassava	Gomani	54.3^{abc}	59.3^{jkl}	63.4^{i}	9.1^{l}	1.1^{n}	7.9^{n}
	Maunjili	54.6^{ab}	59.7^{hi}	63.9^{i}	9.2^{l}	1.6^{m}	10.6^{m}
	Mbundumali	54.4^{abc}	59.7^{hi}	64.0^{i}	9.2^{l}	1.6^{m}	11.9^{m}
	Mkondezi	54.7^{ab}	59.7^{hi}	63.7^{i}	9.0^{l}	1.4^{m}	10.5^{m}
	Sauti	54.6^{ab}	59.5^{ijk}	63.8^{i}	9.2^{l}	1.1^{n}	8.0^{n}
Mean value ± SD		54.5±0.2	59.6±0.2	63.8±0.4	9.2±0.4	1.4±0.2	9.8±1.8
LSD (P = 0.05)		0.63	0.27	0.81	0.99	0.24	2.34
CV (%)		0.74	0.27	0.75	4.44	4.29	5.12

NB: Means followed by the same letter within the same row are not significantly different from each other ($p \leq 0.05$)

The gelatinization temperatures of the retrograded starches ranged from 50.8-65.3°C for cocoyam, 51.8-66.6°C for sweetpotato, and 54.5-63.8°C for cassava starches. Enthalpy of retrogradation was significantly higher ($p \leq 0.001$) for cocoyam starches (4.4±0.7 J/g) than both sweetpotato (3.7±0.5 J/g) and cassava starches (1.4±0.2 J/g) resulting in higher levels of retrogradation. The degree of retrogradation varied significantly ($p \leq 0.001$) with starch source. The degree of retrogradation values ranged from 23.4-45.3%, 24.9-37.0% and 7.9-11.9% for cocoyam, sweetpotato and cassava starches, respectively. Generally, cocoyam starches exhibited a higher degree of retrogradation (34.5±7.4%) than sweetpotato (30.9±3.8%) and cassava starches (9.8±1.8%) indicating rapid retrogradation for cocoyam starches. Among the cocoyam starches, Mzuzu and Machinga, which exhibited lower gelatinization enthalpies, displayed the highest degree of retrogradation (45.3% and 41.6%, respectively). Chitipa and Thyolo cocoyam starches gave lower values for the degree of retrogradation (23.4 and 27.4%, respectively). Apparently both starches were among cocoyam starches that gave higher gelatinization enthalpies. Among the sweetpotato genotypes, starches from Kakoma, Mugamba, LU96/304 and Babache gave the highest degree of retrogradation while A45 gave the lowest. No significant differences on the levels of retrogradation for cassava starches were observed among the cassava starches. Peroni *et al*. (2006) reported gelatinization temperature ranges of 42.73-61.98°C and 42.04-66.28°C for retrograded cassava and sweetpotato starches respectively. The enthalpy values were higher in sweetpotato (6.4 J/g) than cassava starch (2.7 J/g). They further reported higher levels of retrogradation for ginger and yam starch compared to canna, sweetpotato, arrowroot and cassava starches. Sweetpotato starch had a higher degree of retrogradation (49.6%) compared to cassava starch (26.0%). The gelatinization temperatures of the retrograded sweetpotato and cassava starches in this study are within the reported range. However, enthalpies of retrogradation are lower resulting in lower levels of retrogradation.

Retrogradation properties of starch paste are often related to the structures of amylose and amylopectin. Higher retrogradation tendencies are attributed to crystallization involving small amylose molecules and long chain amylopectin (Peroni *et al*., 2006; Gudmundsson, 1994) whereas lower degree of retrogradation is attributed to higher content of short branches of amylopectin chains and long amylose molecules (Spence and Jane, 1999). The

greater degree of retrogradation in cocoyam starches could therefore be attributed to higher contents of short amylopectin branches (Shi and Seib, 1992). Cassava starches had the lowest enthalpies of retrogradation, consistent with lower levels of retrogradation. It is well established that starches with higher enthalpies of gelatinization exhibit lower levels of retrogradation and vice versa. This means that a higher degree of crystallinity in starches is associated with higher transition enthalpies resulting in lower levels of retrogradation due to greater perfectness of the amylopectin crystals (Spence and Jane, 1999).

6.4 Principal component analysis (PCA): Functional properties

The results of PCA are shown in Tables 6.8 and Figures 6.9, 6.8 and 6.9. The first three principal components explained 85% of the tortal variability. Principal components 1, 2, and 3 accounted for 63.4, 13.6, and 8.3% of the variability. Eigenvectors of PC1 had large negative weights for peak, onset and conclusion gelatinization temperatures, degree of retrogradation and syneresis but large positive for paste, WAC50, WAC70, WAC90, SP50, SP70, SP90 and viscosity. Eigenvectors of PC2 had large positive weights for enthalpy of gelatinization and conclusion gelatinization temperature, and negative weighting for WAC70 and SP70 while those of PC3 had large negative weights for SP50 and WAC50 (Table 6.8). Both the loading plot (Figure 6.8) and the correlation matrix (Table 6.9) showed that SP50 and WAC50, SP90 and WAC90, SP70 and WAC70, Viscosity and paste clarity were closely related. These variables were positively correlated. On the other hand To, Tp and syneresis were also close to each other. Kong *et al*. (2009) also reported significant positive correlation between gelatinization temperatures, To, Tp, and Tc and the enthalpy. There was significant negative correlation between SP50, SP70, SP90, WAC50, WAC70, WAC 90, viscosity and paste clarity, and To, Tp, Tc, retrogradation and syneresis. This is in agreement with results of Singh *et al*. (2006), who reported a significant correlation between swelling power and gelatinization temperatures, and syneresis. However, swelling power was negatively correlated with setback viscosity. Singh *et al*. (2004) also reported positive correlation between swelling power and light transmittance, peak and conclusion gelatinization temperatures but a negative correlation with water binding capacity.

Table 6.8 Principal component analysis of the functional properties of the starches from seven cocoyam accessions, 15 sweetpotato genotypes and five cassava genotypes

	Eigenvectors		
Variable	PC1	PC2	PC3
Paste	0.30	0.07	0.12
To	-0.31	0.05	-0.07
Tp	-0.32	0.10	-0.06
Tc	-0.28	0.36	0.00
ΔH	0.11	0.57	0.11
Retro	-0.31	-0.17	-0.07
WAC50	0.23	0.16	-0.64
WAC70	0.28	-0.35	0.01
WAC90	0.27	0.30	0.25
SP50	0.23	0.16	-0.63
SP70	0.28	-0.34	0.03
SP90	0.26	0.31	0.27
Viscosity	0.22	-0.16	0.12
Syneresis	-0.27	-0.03	-0.02
Eigen values	8.9	1.9	1.2
Individual %	63.4	13.6	8.3
Cumulative %	63.4	77.0	85.3

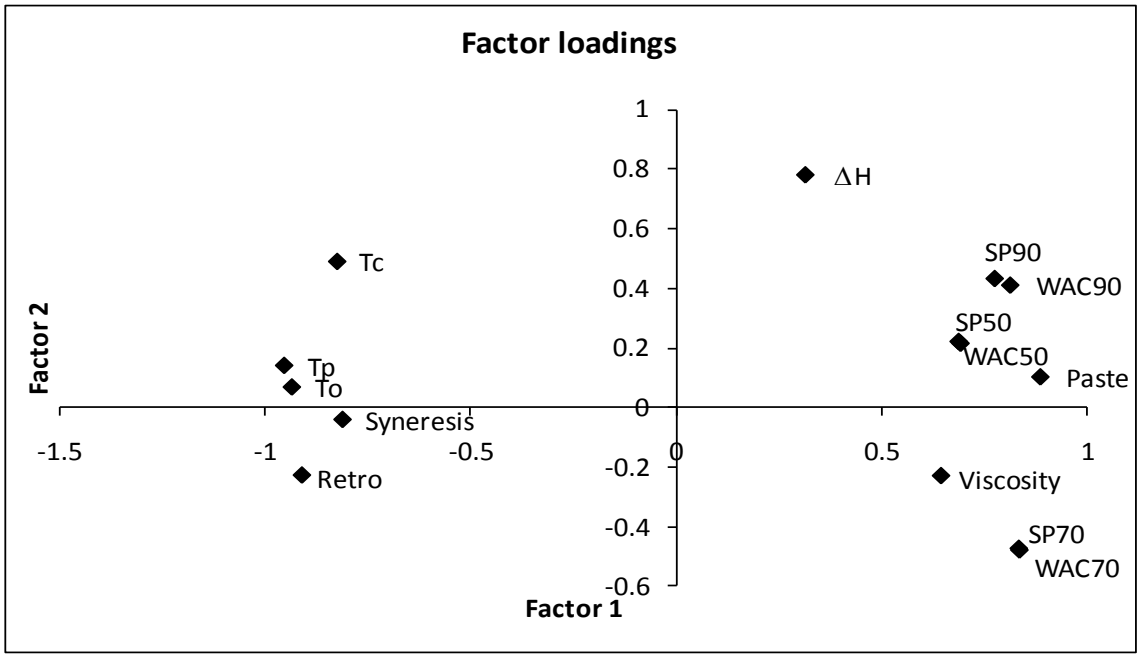

Figure 6.8 PCA loading plot for functional properties of the cocoyam, sweetpotato and cassava starches

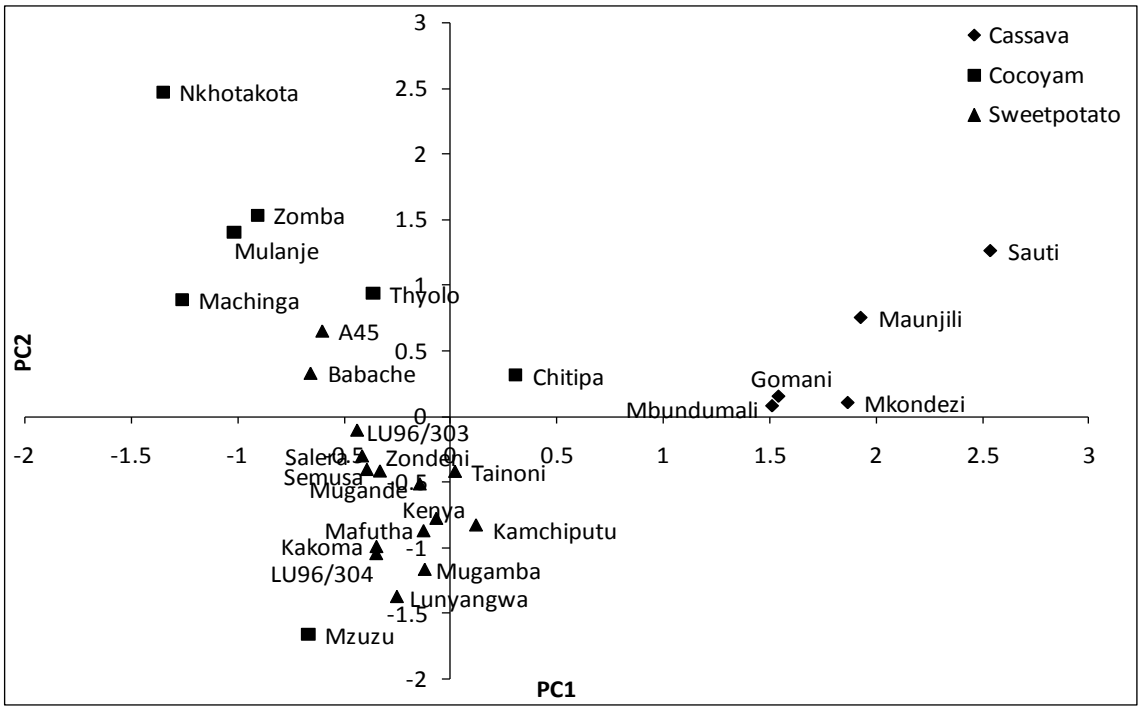

Figure 6.9 PC1 and PC2 plot for cocoyam, sweetpotato and cassava starches using 14 functional properties

Table 6.9 Correlation coefficients between the functional parameters of the cocoyam, sweetpotato and cassava starches

	Paste	To	Tp	Tc	ΔH	Retro	WAC50	WAC70	WA90	SP50	SP70	SP90	Viscosity
To	-0.83**												
Tp	-0.80**	0.97**											
Tc	-0.64**	0.82**	0.88**										
ΔH	0.30	-0.23	-0.17	0.13									
Retro	-0.76**	0.85**	0.86**	0.65**	-0.54**								
WAC50	0.55**	-0.57**	-0.58**	-0.46*	0.28	-0.61**							
WAC70	0.67**	-0.82**	-0.85**	-0.90**	-0.01	-0.66**	0.45						
WAC90	0.81**	-0.76**	-0.75**	-0.49**	0.48*	-0.80**	0.49*	0.46					
SP50	0.56**	-0.58**	-0.59**	-0.47*	0.27	-0.60**	1.0**	0.46	0.49**				
SP70	0.67**	-0.83**	-0.85**	-0.90**	-0.01	-0.65**	0.46	1.0**	0.46	0.44			
SP90	0.80**	-0.74**	-0.72**	-0.45*	0.47*	-0.76**	0.49**	0.42	0.99**	0.46	0.43		
Viscosity	0.62**	-0.43	-0.57**	-0.59**	0.11	-0.53**	0.3	0.60**	0.42	0.32	0.59**	0.36	
Syneresis	-0.71**	0.66**	0.70**	0.61**	-0.33	0.81**	-0.53**	-0.64**	-0.61**	-0.53**	-0.62**	-0.54**	-0.65**

*, ** significant at p = 0.05 and p = 0.01, respectively

The principal component analysis grouped the cocoyam, sweetpotato and cassava starches into four quadrants (Figure 6.9). The bottom right quadrant contained two sweetpotato genotypes (Kamchiputu and Tainoni). The bottom left quadrant consisted of the 11 sweetpotato genotypes (LU96/303, Salera, Semusa, Zondeni, Mugande, Kenya, Mafutha, Kakoma, LU96/304, Mugamba and Lunyangwa) and one cocoyam accession, Mzuzu. The top left quadrant contained five cocoyam accessions (Nkhotakota Zomba, Mulanje, Machinga and Thyolo) and two sweetpotato genotypes (A45 and Babache) while the top right quadrant contained all the five cassava genotypes and one cocoyam accession (Chitipa). Under PC2, sweetpotato starches were mainly in the negative sector except for A45 and Babache. All cassava and cocoyam starches were in the positive sector except for Mzuzu cocoyam starch. A plot of PC1 revealed that cassava starches had the highest positive scores. These were discriminated in terms of high paste clarity, viscosity, water absorption capacity and swelling power, lower values of onset, peak and conclusion temperatures of gelatinization and lower degree of retrogradation and syneresis. The plot also showed that that cocoyam starches (Nkhotakota, Zomba, Mulanje and Machinga) had the lowest negative scores. These starches were discriminated in terms of their higher degree of syneresis and retrogradation, higher gelatinization temperatures and lower water absorption capacity, swelling power, viscosity and paste clarity. Most sweetpotato starches exhibited intermediate scores between the cassava and cocoyam starches.

6.5 Correlations between physicochemical and functional properties

The correlations between the functional and the physicochemical properties are presented in Table 6.10. Granule diameter (GDiam) exhibited a positive relationship with viscosity and negative relationship with water absorption capacity, swelling power and solubility confirming that small granule sized starches exhibit low swelling power and high solubility (Tian *et al*., 1991). Water absorption capacity and and swelling power at 90ºC (WAC90 and SP90) were negatively correlated with phosphorus content of the starches. Karim *et al*. (2007) have reported positive correlation between phosphorus (P) and swelling power and attributed this to weak internal organisation of starch granules caused by negatively charged phosphate esters. The negative correlation between phosphorus and swelling power in this study could indicate the presence of phospholipids in the starch which inhibit granular swelling (Jane *et al*., 1996).

Table 6.10 Correlation coefficients between the physicochemical and functional parameters of the starches

		Paste	WAC50	WAC70	WAC90	SP50	SP70	SP90	SB50	SB70	SB90
Granule size (GDiam)		-0.05	0.00	0.27	-0.40*	0.01	0.25	-0.45*	0.38*	-0.63**	-0.63**
Size distribution	<5 µm	-0.17	-0.12	-0.06	-0.04	-0.11	-0.04	-0.00	-0.31	0.22	0.37
	5-0 µm	-0.24	-0.13	-0.55**	0.10	-0.13	-0.54**	0.16	-0.26	0.39*	0.58**
	10-25µm	0.40*	0.17	0.65**	0.15	0.17	0.63**	0.09	0.16	-0.24	-0.47*
Chemical composition	Ash	-0.54**	-0.08	-0.24	-0.60**	-0.11	-0.25	-0.63**	0.26	-0.38	-0.36
	Fat	-0.18	-0.25	-0.17	-0.17	-0.27	-0.15	-0.10	-0.35	0.43*	0.38
	Protein	-0.42*	-0.30	-0.45*	-0.16	-0.29	-0.43*	-0.12	-0.06	0.36	0.39*
	Fat	-0.09	-0.06	0.24	-0.11	-0.06	0.22	-0.15	0.42*	-0.33	-0.35
	Amylose	-0.08	-0.05	0.24	-0.11	-0.06	0.22	-0.14	0.43*	-0.33	-0.35
	P	-0.52**	-0.29	-0.16	-0.65**	-0.29	-0.15	-0.66**	0.29	-0.08	-0.22
	Ca	-0.21	0.17	0.09	-0.30	0.14	0.07	-0.35	0.30	-0.49*	-0.49**
	Fe	0.03	0.38*	0.39*	-0.15	0.37	0.36	-0.22	0.39*	-0.52**	-0.59**
	K	-0.70**	-0.54**	-0.57**	-0.64**	-0.55**	-0.56**	-0.60**	-0.13	0.21	0.15
	Mg	-0.16	0.19	0.17	-0.20	0.18	0.15	-0.26	-0.36	-0.41**	-0.40*
	Mn	-0.33	-0.04	-0.15	-0.56**	-0.05	-0.17	-0.59**	0.38	-0.63**	-0.50**
	Na	-0.06	-0.01	0.14	-0.01	-0.03	0.13	-0.02	0.34	-0.03	-0.01
	Zn	-0.34	-0.15	0.02	-0.46*	-0.15	0.02	-0.49**	0.36	-0.19	-0.36
Molecular properties	BV	-0.39*	-0.48*	-0.64**	-0.18	-0.48*	-0.60**	-0.08	-0.38*	0.51**	0.68**
	Wavemax	-0.24	-0.40*	-0.50**	-0.02	-0.39*	-0.46*	0.08	-0.47*	0.64**	0.81**
	RC	0.15	0.36	0.18	0.28	0.34	0.17	0.24	-0.04	-0.19	-0.20
	AMMw	0.57**	0.47*	0.58**	0.45*	0.48*	0.58**	0.40*	0.10	-0.10	-0.12
	AMPMw	0.53**	0.26	0.29	0.28	0.26	0.28	0.27	-0.05	-0.06	0.00
	FRIMw	-0.24	-0.24	0.01	-0.20	-0.24	0.02	-0.21	0.01	0.08	-0.18
	FRIIMw	-0.62**	-0.51**	-0.41*	-0.66**	-0.51**	-0.43*	-0.62**	-0.10	0.09	0.01
	FRIIIMw	-0.85**	-0.28	-0.53**	-0.79**	-0.29	-0.54**	-0.79**	0.27	-0.26	-0.19
	FRIArea	-0.43*	-0.21	-0.01	-0.63**	0.21	-0.01	-0.63**	0.10	-0.21	-0.38
	FrIIArea	0.21	0.19	0.51**	-0.15	0.19	0.48*	-0.21	0.38*	-0.46*	-0.72**
	FrIIIArea	-0.18	-0.10	-0.49*	0.13	-0.09	-0.47	0.18	-0.24	0.36	0.64**

*, ** significant at p = 0.05 and p = 0.01, respectively

Table 6.10 cont'd Correlation coefficients between the physicochemical and functional properties of the starches

		Viscosity	Syneresis	To	Tp	Tc	ΔHg	Retrogradation
Granule size (GDiam)		0.47*	-0.09	0.14	0.04	-0.22	-0.20	0.08
Size distribution	<5 µm	-0.39*	0.39*	-0.11	-0.06	-0.08	-0.04	0.07
	5 -10 µm	-0.62**	0.35	0.20	0.29	0.53**	0.11	0.23
	10 -25 µm	0.59**	-0.53**	-0.39*	-0.46*	-0.64**	-0.04	0.39*
Chemical composition	Ash	-0.33	0.20	0.38	0.39*	0.21	-0.26	0.33
	Fat	-0.52**	0.52**	0.17	0.29	0.28	-0.02	0.33
	Protein	-0.50**	0.42*	0.40*	0.45*	0.55**	0.22	0.33
	Fat	0.11	-0.14	-0.09	-0.15	-0.32	-0.28	-0.06
	Amylose	0.11	-0.14	-0.09	-0.15	-0.32	-0.28	-0.06
	P	-0.15	0.28	0.44*	0.42*	0.21	-0.26	0.50**
	Ca	-0.06	-0.18	-0.01	0.04	-0.21	-0.24	-0.06
	Fe	0.36	-0.36	-0.20	-0.29	-0.45*	-0.28	-0.19
	K	-0.52**	0.69**	0.72**	0.76**	0.70**	-0.16	0.76**
	Mg	-0.01	-0.28	-0.06	-0.10	-0.25	0.01	-0.13
	Mn	0.10	0.10	0.30	0.21	0.00	-0.22	0.26
	Na	0.14	-0.33	-0.05	-0.09	-0.05	0.23	-0.21
	Zn	-0.14	0.08	0.18	0.12	-0.14	-0.37	0.30
Molecular properties	BV	-0.71**	0.54**	0.40*	0.53**	0.69**	0.15	0.43*
	Wavemax	-0.64**	0.52**	0.24	0.36	0.56**	0.19	0.30
	RC	0.37	-0.40*	-0.20	-0.24	-0.18	0.24	-0.44*
	AMMw	0.57**	-0.30	-0.61**	-0.66**	-0.57**	0.21	-0.67**
	AMPMw	0.08	-0.64**	-0.40**	-0.26	-0.15	0.34	-0.32
	FRIMw	0.21	0.17	0.27	0.14	-0.03	-0.42*	0.24
	FRIIMw	-0.46*	0.69**	0.66**	0.70**	0.49**	-0.35	0.69**
	FRIIIMw	-0.49*	0.62**	0.71**	0.66**	0.52**	-0.23	0.63**
	FRIArea	-0.09	0.23	0.36	0.35	0.09	-0.31	0.34
	FrIIArea	0.52**	-0.35	-0.17	-0.24	-0.54	-0.51**	-0.09
	FrIIIArea	-0.45*	0.34	0.13	0.19	0.51**	0.46*	0.10

*, ** significant at p = 0.05 and p = 0.01, respectively

Paste clarity was significantly influenced with granule size as well as phosphorus and ash contents. A positive correlation was observed between paste clarity and percentage of large granules (10-25µm), whereas phosphorus (P) and ash contents exhibited a negative association with paste clarity. Unlike the results of this study, a significant positive correlation has been reported between paste clarity and ash content (Singh *et al*., 2004). Paste clarity also exhibited a positive relationship with average molecular weights of amylose (AMMw) and amylopectin (AMPMw), and a negative relationship with average molecular weigths of long- and short-chain amylopectin molecules (FrIIMw and FrIIIMw) indicating the influence of molecular structure. Thermal properties of the starches were mostly influenced by granule size, phosphorus and protein contents, blue value (BV), the molecular weigth of amylose, long chain and short chain amylopectin molecules confirming the influence of starch composition, granule architecture and molecular structure on thermal properties (Gunaratne and Hoover, 2002). Gelatinization temperatures (To, Tp and Tc), showed positive association with percentage of small granules (5-10 µm), phosphorus and protein contents and BV, but negative association with AMMw. Enthalpy of gelatinization (ΔHg) was positively correlated to the fraction of short chain amaylopectin molecules (FrIIIArea) and negatively correlated to the fraction of long chain amylopectin molecules (FrIIArea). Proportions of short chains to long chains of amylopectin are reported to influence gelatinization temperatures and enthalpy (Yoo *et al*., 2009; Lu *et al*., 2005; Jane *et al*., 1999; Noda *et al*., 1998)

Though the influence of minerals on functional properties of the starches is not well established, potassium (K) was negatively associated with paste clarity, water absorption capacity, swelling power and viscosity, and positively correlated with gelatinization temperatures (To, Tp, and Tc), retrogradation and syneresis. Viscosity of the starches was associated with granule size distribution, fat and protein contents, and the molecular characteristics (BV and wavemax). A negative correlation was observed between viscosity and the percentage of small granules, and fat and protein contents. Wickramasinghe *et al*. (2009) reported a positive relationship of peak viscosity and median particle size of the starch granule. Thus starches with large granules displayed high paste peak viscosity. Viscosity also had a significant positive correlation with the fraction of long chain amylopectin molecules and a negative correlation with short chain amylopectin molecules. The larger proportion of amylopectin long-branch chains have been reported to increase viscosity of starch paste (Lu *et al*., 2008; Charles *et al*., 2005;

Shibanuma *et al*., 1996). The results of this study therefore indicate that the functional properties of the cassava, cocoyam and sweetpotato starches in this study were influenced by the differences in the physicochemical and structural properties of the starches.

6.6 Conclusions

The results of this study have revealed differences in functional properties of starches from different botanical sources and among different genotypes and accessions. Increasing temperature resulted in increased water absorption capacity, swelling power and solubility of the starches. Cassava starches exhibited higher water absorption capacity, swelling power, paste clarity, resistance to retrogradation and viscosity than cocoyam and sweetpotato starches. Thermal analysis of the starches by DSC has revealed higher gelatinization temperatures for cocoyam starches than sweetpotato and cassava starches. Gelatinization enthalpies of cocoyam and cassava starches were similar but higher than those of sweetpotato starches. Exploration of sweetpotato starches would reduce industrial energy costs due to lower gelatinization energy. Retrogradation studies by DSC and turbidometry have shown that cocoyam starches have higher tendency towards retrogradation than sweetpotato and cassava starches. Cassava starches provided the least tendency towards retrogradation. The results clearly indicate that structural differences do exist among starches and within different genotypes and accessions of cassava, sweetpotato and cocoyam starches.

In applications of starch like the food industry where swelling power, paste clarity and resistance to retrogradation are desired functionalities, cassava starches would be more suitable. The higher degree of syneresis for cocoyam and sweetpotato starches compared to cassava starches would limit their application in frozen food products. Hence, value addition to increase the commercial value of the cocoyam and sweetpotato through physical and chemical modification should be exploited.

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CHAPTER 7

EFFECT OF ANNEALING, ACETYLATION AND ACID HYDROLYSIS ON FUNCTIONAL PROPERTIES OF MALAWIAN COCOYAM AND SWEETPOTATO STARCHES

7.1 Introduction

Native starches often have limited application and industrial use due to their inability to withstand extreme processing conditions such as pH, temperature and shear. This is because they have low shear stress resistance, thermal resistance, thermal decomposition and high retrogradation tendencies (Fleche, 1985). In order to improve their functional characteristics and hence their commercial value, native starches are modified by either physical or chemical means. Starches can be modified physically by annealing and heat-moisture treatment, and chemically by acetylation and acid-hydrolysis(Jobling, 2004; Gunaratne and Hoover, 2002; Thomas and Atwell, 1999; Eliasson and Gudmundsson, 1996).

The physical and chemical modification can alter functional properties of starch depending on the botanical source and method employed. Annealing and heat-moisture treatment increased the water absorption capacity, but decreased swelling power and solubility of red sorghum (Adebowale *et al*., 2005a), fermented and unfermented cassava (Gomes *et al*., 2005; 2004), and normal, waxy and high amylose wheat bread (Lan *et al*., 2008) starch. Lorenz and Kulp (1981) found that heat-moisture treatment increased solubility of cereal (barley, triticale and red millet) starches but decreased that of tuber starches (arrowroot and cassava). Heat-moisture treatment of sweetpotato starch reduced peak viscosity, onset temperature and gelatinization enthalpy but increased cold paste viscosity (Singh *et al*., 2005), while annealing increased gelatinization temperatures and enthalpy of yam (Jayakody *et al*., 2009), barley (Waduge *et al*., 2006), and unfermented and fermented cassava (Gomes *et al*., 2005; 2004) starch.

Both acetylation and acid thinning improved paste clarity of Nigerian new cocoyam (*Xanthosoma sagittifolium*) starch. However, gelation capacity, pasting temperature, set-back tendency, gelatinization peak temperature and enthalpy, and retrogradation tendency

were reduced after acetylation but increased after acid thinning. Acetylation increased swelling capacity but decreased solubility of the starch, while acid thinning reduced swelling power and increased solubility (Lawal, 2004). Both acetylation and acid hydrolysis of jackbean (*Canavalia ensiformis*) starch increased solubility of the starch. However, acetylation resulted in increased swelling power, water absorption capacity, viscosity, and paste clarity while acid-thinning reduced them. The gelatinization temperature range, enthalpy of gelatinization and retrogradation tendency were increased following acid thinning, but reduced after acetylation (Lawal and Adebowale, 2005; Betancur *et al*., 1997). The acid hydrolysis of jackbean starch, however, also resulted in reduced retrogradation tendency and produced no effect on gelatinization temperatures (Betancur and Chel, 1997). Acid hydrolysis of Indian sweetpotato starches decreased the onset temperature but increased enthalpy of gelatinization while the pasting profile remained unchanged (Singh *et al*., 2005). Acetylation has also resulted in decreased transition temperatures, enthalpy of gelatinization and retrogradation tendency, but increased swelling, solubility and light transmittance of corn and potato starches (Singh *et al*., 2004a; b).

It is clear that changes in starch properties following modification depend on the source of starch and method of modification employed. A study was therefore undertaken to determine some functional characteristics of physically (annealed) and chemically (acid hydrolyzed and acetylated) modified starches from Malawian sweetpotato and cocoyam in order to understand how starch properties of Malawian cocoyam and sweetpotato starch could be affected and improved.

7.2 Materials and methods

7.2.1 Materials

Starches from three sweetpotato genotypes (Babache, Mugande, and Tainoni), and three accessions of cocoyam (Mzuzu, Mulanje, and Zomba) were used. These starches were subjected to physical modifications by annealing and chemical modifications by acid hydrolysis and acetylation as described below.

7.2.2 Annealing of the starches

Starch suspensions (1:2 w/v) were prepared by adding 200 mL of distilled water to 100 g starch (dry basis) in a sealable container. The container was sealed and the suspension heated at 50°C in a water bath for 24 h. After this the suspension was filtered through Whatman No. 1 filter paper and dried in an air convection oven at 30°C for 48 h. The dried sample was ground and stored in a polyethylene bag prior to analysis (Atichokudomchai *et al*., 2002).

7.2.3 Acetylation of the starches

Starch samples (100 g, db) were dispersed in 500 mL of distilled water and stirred magnetically for 20 min. The pH of the suspension was adjusted to 8.0 using 1 M NaOH followed by the slow addition of 10.2 g of acetic anhydride while maintaining constant stirring and monitoring pH in the range of 8.0-8.5. After addition, the reaction was allowed to proceed for 5 min after which the pH of the slurry was adjusted to 4.5 using 0.5 M HCl. Starch was recovered by filtering through Whatman no. 4 filter paper. The recovered starch was washed four times with excess distilled water and dried at 30°C for 48 h (Sathe and Salunkhe, 1981).

7.2.3.1 Degree of acetylation of the starches

Samples of acetylated starches (5 g) were weighed into a 250 mL flask and 50 mL of distilled water was added. A few drops of phenolphthalein indicator were added and the starch suspension titrated with 0.1 M NaOH solution to a permanent pink end-point after which 25 ml 0.45 M NaOH was added. The mixture was sealed tightly with a rubber stopper and shaken vigorously for 30 min. After 30 min the stopper was removed and the starch washed down from the walls of the container with distilled water. The slurry was subsequently titrated with 0.2 M HCl solution until the disappearance of the phenolphthalein colour. Native starch was treated in a similar manner for blank determination. The degree of acetylation and substitution were determined using the following equations:

$$\% \text{ acetyl (db)} = \frac{(\text{Blank titre} - \text{Sample titre}) \text{ ml} \times \text{acid molarity} \times 0.043 \times 100}{\text{Sample weight in g (db)}}$$

$$\text{Degree of substitution (DS)} = \frac{162 \text{ x } \% \text{ acetyl}}{4300 - (42 \text{ x } \% \text{ acetyl})}$$

7.2.4 Acid hydrolysis

Starch samples were hydrolyzed by suspending 100 g (dry basis) of starch in 150 ml of 6% HCl solution at 25 °C for 192 h without stirring. After hydrolysis, the suspension was neutralized with 10 % (w/v) NaOH solution and filtered. The hydrolyzed starch was then washed three times with distilled water and the water was removed by vacuum filtration. The acid-modified starch was dried in an air convection oven at 30°C for 48 h and ground (Atichokudomchai, 2002).

7.2.4.1 The extent of acid hydrolysis

The extent of acid hydrolysis was determined by measuring the amount of the carbohydrate in the filtrate using the Anthrone method (Brook *et al*., 1986). The acid hydrolyzed starch samples were solubilized to yield approximately 0.8-1.0 mg glucose per mL by heating it in screw tubes to prevent evaporation. Aliquots (25 μL) of the samples were transferred to clean test tubes and 3 mL of freshly prepared anthrone reagent [0.1% w/v in diluted sulphuric acid (2.3:1 v/v sulphuric acid:water)] was added. The mixture was heated in a boiling bath for 5 min and cooled in cold water. After 15-35 min, the absorbance was read at 630 nm on a spectrophotometer (Spectronic Unicam, Heλios, Cambridge, United Kingdom). A standard curve of 0 to 50 mg per mL glucose was run simultaneously.

7.2.4 Determination of functional properties

7.2.4.1 Water absorption capacity, swelling power and solubility

Water absorption capacity, swelling and solubility of the starches were determined following the procedure of Kojima *et al*. (2006): Starch samples (0.1 g, db) in triplicate were weighed into 10 mL centrifuge tubes, mixed with 5 mL of distilled water and heated for 1 h at 50°C, 70°C or 90°C with shaking every 5 min. The slurry was centrifuged for 30 min at 1500 × *g*, and the weight of the sediment in gram (B) was determined. The

supernatant (A) was diluted with water until the total volume was 10 mL, and the amount of starch in it was determined by the anthrone-sulphuric acid method (Brook *et al.*, 1986). The water absorption capacity, solubility and the swelling power were calculated using the following equations (Mishra and Rai, 2006).

$$\text{WAC (g } H_2O \text{ g}^{-1}) = \frac{\text{Mass of wet starch - Mass of dry starch}}{\text{Mass of dry starch}}$$

$$\text{Solubility (\%)} = \frac{100 \times A}{S}$$

$$\text{Swelling Power (g g}^{-1}) = \frac{B}{S-A}$$

where (S) is the sample weight in grams.

7.2.4.2 Paste clarity and stability

Paste clarity of the starches was determined as described by Craig *et al*. (1989). A 1% aqueous suspension of starch was made by adding 10 mL of distilled water to 0.1 g of starch (dry basis) in a centrifuge tube and vortex mixed. The suspension was heated in a boiling water bath for 30 min with constant stirring and then cooled to room temperature. The percentage transmittance (%T) was measured at 650 nm against a water blank on a spectrophotometer (Spectronic Unicam, Heλios, Cambridge, United Kingdom).

The stability of the starch pastes was determined by placing triplicate samples (prepared above) in disposable cuvettes and storing for 10 days at 4°C. Turbidity was determined every 24 h by measuring absorbance at 640 nm against a water blank (Sandhu and Singh, 2007; Jacobson *et al*., 1997).

7.2.4.3 Viscosity

The viscosity of the starch pastes was determined using a modification of the ISI method 17-1e (International Starch Institute, 2002). To dried starch samples (10 g) in a 600 mL beaker, distilled water was added to bring to the total weight of starch and water to 500 g and the starch suspension heated in a water bath at 95°C for 30 min with constant stirring. The resultant gelatinized starch was weighed and water added to replace the evaporated

water up to a gross weight of 500 g. The starch gel was cooled to 50°C in a water bath at 50°C while stirring and viscosity was measured in centipoises (cps) with spindle no. 2 at 100 rpm using a Brookfield Digital Viscometer model RTDV II (Brookfield Engineering Laboratories Inc, Stoughton MA 02072, USA).

7.2.4.4 Gelatinization and retrogradation

Gelatinization and retrogradation of the starch samples were determined using differential scanning calorimetry (DSC 822e, Mettler, Toledo, Switzerland) and a starch:water ratio of 1:3. Starch samples of 3.0 mg in triplicates were weighed into DSC aluminium pans. Distilled water was added to the starch sample using a transfer pipette to establish a starch:water ratio of 1:3. The pans were then hermetically sealed and the samples were left to stand for 1 h at room temperature for moisture equilibration. The sealed pans were heated from 20°C to 95°C under nitrogen gas at a heating rate of 10°C/min to gelatinize the starch samples. From the DSC thermograms onset temperature (T_o), peak temperature (T_p), conclusion temperature (Tc) and enthalpy of gelatinization (ΔH_G) were determined using instruments software (STARe SW 9.00). Temperature range and peak height index (PHI) were also calculated as T_c - T_o and as the ratio $\Delta H_G/(T_p-T_o)$, respectively. The gelatinized samples were stored at 4°C for a period of 7 days, after which the samples were equilibrated at room temperature for 2 h, and rescanned in the DSC using the previous heating programme to measure the retrogradation transition temperatures and enthalpy. The degree of retrogradation was determined as the ratio of the enthalpy change of retrograded starch to the enthalpy change of gelatinized starch (Gunaratne and Hoover, 2002).

7.2.5 Data analysis

Data was subjected to analysis of variance (ANOVA) using Statistix 8 for Windows software (Analytical software, Tallahassee, USA).

7.3 Results and discussion

7.3.1 Degree of acetylation and acid hydrolysis

The results of the extent of acetylation and acid hydrolysis of the cocoyam and sweetpotato starch are presented in Table 7.1.

Table 7.1 The average values and mean separtion of degree of acetylation, substitution (DS) and acid hydrolysis of cocoyam and sweetpotato starch

Botanical source	**Accession/ Genotype**	**Acetylation (%)**	**DS**	**Acid hydrolysis (%)**
Cocoyam	Mulanje	2.5^{a}	0.095^{ab}	2.61^{ab}
	Mzuzu	2.4^{a}	0.090^{ab}	3.11^{a}
	Zomba	2.5^{a}	0.095^{ab}	2.68^{ab}
	Mean	**2.45±0.18**	**0.093±0.005**	**2.80±0.40**
Sweetpotato	Kamchiputu	2.6^{a}	0.105^{a}	1.82^{b}
	Mugande	2.3^{a}	0.090^{ab}	1.79^{b}
	Tainoni	2.1^{a}	0.080^{b}	2.10^{b}
	Mean	**2.33±0.29**	**0.092±0.013**	**1.90±0.28**

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$); Means for botanical source are expressed as mean ± standard deviation

The degree of acetylation and substitution ranged from 2.4-2.5% and 0.09-0.95%, respectively for cocoyam starch, and 2.1-2.6% and 0.080-0.105%, respectively, for sweetpotato starch. Most of the starches exhibited similar values of the degree of substitution except for Tainoni sweetpotato starch which displayed the lowest values for both degree of acetylation and substitution. Acetylated starch is usually used in food applications owing to its paste stability and clarity and the set limit by the FDA is 2.5% acetyl content (Thomas and Atwell, 1999; Rutenberg and Solarek, 1984). Therefore, the acetyl content of the acetylated starches produced in this study is within the prescribed limit, making them suitable for use in food products. A significant variation ($p \leq 0.05$) was

observed in the degree of acid hydrolysis of the starch. Cocoyam starch exhibited a higher degree of hydrolysis than sweetpotato starch: Mzuzu cocoyam starch exhibited the highest degree of acid hydrolysis (3.11%) while Mugande sweetpotato starch gave the lowest (1.79%).

7.3.2 Water absorption capacity, swelling and solubility

The water absorption capacity, swelling power and solubility studies of acetylated, acid-hydrolyzed and annealed cocoyam and sweetpotato starch are presented in Tables 7.2 and 7.3. Water absorption capacity, swelling power and solubility of the modified starches were temperature dependent, increasing with rising temperature. The increase in swelling power and water absorption capacity is a result of weakening intragranular binding forces with increased temperature, facilitating less restricted swelling (Lawal and Adebowale, 2005).

Modification of cocoyam and sweetpotato starch significantly ($p \leq 0.001$) influenced the water absorption capacity of starch. The effect was more pronounced at 70 and 90°C. Generally, acetylation of cocoyam and sweetpotato starch resulted in increased water absorption capacity of the starch, while acid-hydrolysis reduced it. On average, annealing had little influence on water binding capacity. Considering individual starches, there was a mixed response towards annealing. Water absorption capacity of all starches significantly decreased at 70°C after annealing, except for Zomba cocoyam starch where no significant change was observed. Mulanje cocoyam and Tainoni sweetpotato starch displayed markedly lower water absorption capacity at 90°C while Mzuzu cocoyam and Kamchiputu sweetpotato starch showed an increase at the same temperature. Acetylated starch displayed the highest water binding capacity at all temperatures and acidified starch the lowest. Generally, the water binding capacity of the starch was in the following order: acetylated > annealed ~ native > acidified. On average, acetylation of cocoyam starch increased the water absorption capacity from 9.3 to 18.8 g H_2O g^{-1} starch and from 19.8 to 25.0 g H_2O g^{-1} at 70 and 90°C respectively, while acid hydrolysis reduced the water absorption capacity to 3.3 and 9.9 g H_2O g^{-1} at 70 and 90°C, respectively. Acetylation of sweetpotato starch increased the water absorption capacity from 13.6 to 21.4 g H_2O g^{-1} at 70°C and from 19.0 to 22.4 g H_2O g^{-1} at 90°C, while acid hydrolysis reduced it to 5.5 and 6.0 g H_2O g^{-1} at 70 and 90°C, respectively.

Table 7.2 The average values and mean separtion of water absorption capacity, swelling power and solubility of modified and native cocoyam starches

Accession	Modification	Water absorption capacity (g H_2O g^{-1} starch)			Swelling power (g g^{-1} starch)			Solubility (%)		
		50°C	70°C	90°C	50°C	70°C	90°C	50°C	70°C	90°C
Mulanje	Acetylated	2.6^{b}	16.4^{bc}	22.8^{bc}	3.6^{b}	18.1^{c}	29.0^{b}	2.3^{bc}	3.9^{bc}	17.8^{ef}
	Acidified	2.0^{b}	2.8^{h}	2.6^{g}	3.1^{b}	4.0^{i}	7.3^{g}	2.6^{ab}	3.4^{c}	50.9^{a}
	Annealed	2.1^{b}	7.2^{ef}	18.4^{e}	3.1^{b}	8.4^{fg}	24.2^{de}	1.4^{e}	1.5^{e}	5.6^{i}
	Native	2.3^{b}	7.6^{e}	22.6^{c}	3.3^{b}	8.8^{f}	27.6^{bc}	0.9^{f}	1.8^{e}	14.7^{fg}
Mzuzu	Acetylated	3.4^{a}	18.3^{b}	29.3^{a}	4.6^{a}	20.2^{b}	30.5^{a}	3.1^{a}	4.4^{b}	33.7^{c}
	Acidified	2.2^{b}	4.2^{gh}	24.1^{b}	3.3^{b}	5.6^{hi}	28.4^{b}	2.6^{ab}	6.1^{a}	49.8^{a}
	Annealed	2.4^{b}	12.1^{d}	23.8^{bc}	3.4^{b}	13.3^{e}	27.2^{bc}	1.3^{ef}	1.4^{e}	8.9^{hi}
	Native	1.2^{c}	14.7^{c}	19.9^{d}	2.2^{c}	15.9^{d}	26.1^{cd}	0.8^{f}	1.9^{e}	19.7^{e}
Zomba	Acetylated	2.4^{b}	21.7^{a}	22.9^{bc}	3.5^{b}	23.3^{a}	23.0^{e}	2.0^{cd}	2.7^{d}	27.0^{d}
	Acidified	2.2^{b}	2.9^{h}	3.0^{g}	3.3^{b}	4.0^{i}	7.2^{g}	2.7^{ab}	2.7^{d}	44.4^{b}
	Annealed	2.1^{b}	5.7^{efg}	17.0^{ef}	3.1^{b}	6.8^{fgh}	20.4^{f}	1.6^{de}	1.8^{e}	11.8^{gh}
	Native	2.2^{b}	5.6^{fg}	16.9^{f}	3.2^{b}	6.7^{gh}	22.6^{e}	1.1^{ef}	1.6^{e}	20.8^{e}
Mean	Acetylated	2.8	18.8	25.0	3.9	20.5	27.5	2.5	3.6	26.2
	Acidified	2.1	3.3	9.9	3.2	4.5	14.3	2.6	4.1	48.4
	Annealed	2.2	8.3	19.7	3.2	9.5	24.0	1.4	1.5	8.7
	Native	1.9	9.3	19.8	2.9	10.5	25.4	0.9	1.8	18.4
LSD (modification)		0.5	3.7	7.6	0.6	3.7	7.6	0.4	1.1	5.8

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Table 7.3 The average values and mean separtion of water absorption capacity, swelling power and solubility of modified and native sweetpotato starches

Genotype	Modification	Water absorption capacity (g H_2O g^{-1} starch)			Swelling power (g. g^{-1} starch)			Solubility (%)		
		50°C	70°C	90°C	50°C	70°C	90°C	50°C	70°C	90°C
Kamchiputu	Acetylated	3.2^{a}	19.0^{c}	21.4^{b}	4.3^{a}	20.5^{c}	24.4^{b}	1.5^{bc}	2.1^{d}	8.3^{e}
	Acidified	1.5^{e}	7.1^{g}	5.9^{f}	2.6^{e}	8.7^{g}	13.8^{g}	1.8^{ab}	6.8^{a}	49.6^{b}
	Annealed	1.5^{e}	12.6^{e}	18.5^{de}	2.5^{e}	13.8^{e}	21.2^{de}	1.2^{cd}	1.4^{efg}	8.2^{e}
	Native	1.6^{e}	14.6^{d}	17.8^{e}	2.7^{e}	15.9^{d}	20.5^{e}	1.0^{d}	1.5^{defg}	8.2^{e}
Mugande	Acetylated	2.4^{cd}	23.4^{a}	22.3^{b}	3.4^{cd}	24.9^{a}	26.9^{a}	1.2^{cd}	1.7^{def}	13.2^{d}
	Acidified	1.5^{e}	5.5^{h}	5.7^{f}	2.5^{e}	6.8^{h}	16.4^{f}	1.8^{ab}	4.3^{b}	59.4^{a}
	Annealed	2.2^{cde}	12.6^{e}	20.1^{c}	3.2^{cde}	13.7^{e}	22.8^{c}	1.2^{cd}	1.0^{g}	7.6^{e}
	Native	1.8^{cde}	14.1^{d}	20.0^{c}	2.8^{cde}	15.2^{d}	22.8^{c}	0.9^{d}	1.0^{fg}	7.7^{e}
Tainoni	Acetylated	3.1^{ab}	21.8^{b}	23.6^{a}	4.2^{ab}	23.2^{b}	25.3^{b}	1.7^{ab}	2.0^{de}	27.6^{c}
	Acidified	2.4^{bc}	4.0^{i}	6.3^{f}	3.5^{bc}	5.1^{i}	14.4^{g}	2.1^{a}	3.0^{c}	49.3^{b}
	Annealed	2.5^{bc}	11.1^{f}	18.8^{de}	3.5^{c}	12.3^{f}	21.5^{de}	1.1^{d}	1.5^{defg}	7.8^{e}
	Native	1.7^{de}	12.1^{e}	19.3^{cd}	2.7^{de}	13.4^{e}	21.8^{cd}	0.9^{d}	1.8^{de}	7.0^{e}
Mean	Acetylated	2.5	21.4	22.4	4.0	22.9	25.5	1.5	1.9	16.4
	Acidified	1.8	5.5	6.0	2.9	6.9	14.9	1.9	4.7	52.8
	Annealed	2.0	12.1	19.2	3.1	13.3	21.9	1.1	1.3	7.9
	Native	1.7	13.6	19.0	2.7	14.8	21.7	0.9	1.5	7.2
LSD (modification)		0.6	1.8	1.1	0.6	1.8	1.4	0.3	1.1	6.7

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Swelling power of the cocoyam and sweetpotato starch was significantly influenced ($p \leq 0.001$) by modification. Compared to native starch, acetylation significantly increased the swelling power of the starch significantly while acid hydrolysis decreased it. The swelling power of annealed starch did not vary significantly from that of native starch. Generally, acetylation of cocoyam starch increased swelling power from 2.9 to 3.9 g g^{-1} starch, 10.5 to 20.5 g g^{-1} starch and from 25.4 to 27.5 g g^{-1} at 50, 70 and 90°C, respectively. Acid hydrolysis of cocoyam starch reduced swelling power to 4.5 and 14.3 g g^{-1} at 70 and 90°C, respectively. Acetylation of sweetpotato starch increased swelling power from 2.7 to 4.0 g g^{-1} starch, 14.8 to 22.9 g g^{-1} starch and from 21.7 to 25.5 g g^{-1} at 50, 70 and 90°C, respectively while acid hydrolysis reduced it to 6.9 and 14.9 g g^{-1} at 70 and 90°C, respectively. Among the acetylated starch, Mzuzu cocoyam starch displayed the highest swelling power (4.6 g g^{-1}) similar to that of Kamchiputu (4.3 g g^{-1}) and Tainoni (4.2 g g^{-1}) acetylated sweetpotato starch at 50°C. Acetylated Mugande sweetpotato starch exhibited the highest swelling power (24.9 g g^{-1}) at 70°C, while acetylated Mzuzu (30.5 g g^{-1}) and Mulanje (29.0 g g^{-1}) cocoyam starch displayed the highest swelling power at 90°C.

Generally, both acetylation and acid hydrolysis significantly ($p \leq 0.001$) increased the solubility of the cocoyam and sweetpotato starch, and annealing slightly reduced it. Solubility was the highest in acid hydrolyzed starches. Acid hydrolysis of cocoyam starch increased solubility from 0.9 to 2.6%, 1.8 to 4.1% and 18.4 to 48.4%, while acetylation increased it to 2.5, 3.6 and 26.2% at 50, 70 and 90°C, respectively. Acid hydrolysis of sweetpotato starch increased solubility from 0.9 to 1.9%, 1.5 to 4.7% and 7.2 to 52.8%, while acetylation increased it to 1.5, 1.9 and 16.4% at 50, 70 and 90°C, respectively. Both acetylated and acid hydrolyzed starch from different cocoyam accessions and sweetpotato genotypes displayed different solubility. Acid hydrolyzed Mzuzu cocoyam starch showed the highest solubility at both 70 and 90°C while acid hydrolyzed Mulanje cocoyam starch displayed the highest solubility at 90°C as well. Acetylated Mzuzu cocoyam starch also showed a higher solubility at all temperatures among the acetylated cocoyam starch. However, after annealing, Mzuzu cocoyam starch displayed the lowest solubility. Among the acid hydrolyzed sweetpotato starch, Mugande displayed the highest solubility at 90°C while Kamchiputu had the highest solubility at 70°C. In the acetylated form, Tainoni

sweetpotato starch exhibited the highest solubility and Kamchiputu the lowest. Annealed Mugande starch displayed the lowest solubility among the annealed sweetpotato starch.

The increase in water absorption capacity, swelling power and solubility after acetylation of starch obtained in this study is due to introduction of acetyl groups in the starch molecules. The introduction of acetyl groups on the starch molecules causes electrostatic repulsions among starch molecules, lowering interchain binding and thereby facilitating access of water into the starch matrices (Lawal and Adebowale, 2005; Liu *et al*., 1999). The presence of acetyl groups also produces a network of chain exudates from starch granules thereby increasing the water retention capacity. These acetyl groups allow retention of water molecules and enable better dispersion of starch in aqueous systems due to their ability to form hydrogen bonds and obstruct chain associations, hence increasing swelling power and solubility of the starch (Bello-Pérez *et al*., 2000; Betancur *et al*., 1997). Acetylation has been reported to increase water binding capacity, swelling power and solubility of banana starch (Bello-Pérez *et al*., 2000) and jack bean starch (Lawal and Adebowale, 2005), swelling power and solubility of corn, potato, normal rice and *Canavalia ensiformis* starch (Singh *et al*., 2004a; b; Liu *et al*., 1999; Betancur *et al*., 1997), and swelling power of new cocoyam (Lawal, 2004) starch. Contrarily, acetylation has also been reported to decrease swelling power of waxy rice starch (Liu *et al*., 1999), and solubility of breadfruit and new cocoyam starch (Adebowale *et al*., 2005b; Lawal, 2004). The differences in the extent of responses could be due to the composition and molecular structure of the starch.

Acid-catalyzed hydrolysis occurs preferentially in the amorphous region of the starch granule, leaving starch granules with a more crystalline structure (Thomas and Atwell, 1999). The increase in relative crystallinity is responsible for the increased restricted swelling of acid hydrolyzed starch (Hoover, 2000). Owing to the reduction of the amorphous region of the starch granule, the number of binding sites for starch granules decreases, consequently reducing the water absorption capacity of the starch (Lawal, 2004). Acid hydrolysis diminishes molar mass, thereby increasing the lower molecular weight fraction containing hydroxyl groups (Singh and Ali, 2000). This results in increased solubilization of the starch (Sandhu *et al*., 2007). Since acid hydrolyzed starch granules are fragmented in a radial way upon heating and subsequent cooling without reaching an organized structure, the granules cannot retain water inside the structure.

Consequently, swelling power of the starch decreases (Betancur and Chel, 1997). Therefore, the decreased swelling power and water absorption capacity of acid hydrolyzed cocoyam and sweetpotato starch observed in this study are due to the increase in the relative crystallinity of the starch after hydrolysis, whereas the increased solubility resulted from the increased lower molecular weight fraction. This trend has also been obtained for other starches; an increase in solubility but decrease in swelling power of starch was observed after acid hydrolysis of arrow root, jack bean, and normal and waxy corn starches (Sandhu *et al*., 2007; John *et al*., 2002; Betancur and Chel, 1997).

Modification of starch by annealing has also been shown to reduce swelling power and solubility of red sorghum, but increase its water absorption capacity (Adebowale *et al*., 2005). Annealing also reduced swelling power and solubility of unfermented and fermented cassava, and normal, waxy and high amylose wheat bread starch (Lan *et al*., 2008; Gomes *et al*., 2005; 2004). This change is due to the rearrangement of starch molecules, resulting in a more stable structure (Gomes *et al*., 2004). The slight differences in water absorption capacity, swelling power and solubility between native and annealed starch observed in this study suggest that this rearrangement might have taken place to a lesser extent in the annealed starch.

7.3.3 Paste clarity and viscosity

The paste clarities of annealed, acetylated and acid hydrolyzed sweetpotato and cocoyam starches are presented in Table 7.4. Significant changes in paste clarity ($p \leq 0.001$) were observed following modification of the starch; paste clarity increased after acid hydrolysis and acetylation, and decreased after annealing. Significantly greater increases in paste clarity were observed following acid hydrolysis as compared to acetylation. On average, acid hydrolysis increased paste clarity three-fold or more in both cocoyam and sweetpotato starch. Paste clarity ranged from 43.5-50.8% and 50.5-70.6% for acidified cocoyam and sweetpotato starch, respectively. Paste clarity of acetylated starch ranged from 25.4-34.9% for cocoyam and 20.4-28.2% for sweetpotato starch. Thus, acid hydrolyzed sweetpotato starch gave higher paste clarity than their cocoyam counterparts.

The opposite was observed after acetylation; acetylated cocoyam starch had higher paste clarity than that of acetylated sweetpotato starch. Most notably, among acid hydrolyzed sweetpotato starches, Mugande gave higher paste clarity than the rest while Mzuzu and Zomba displayed the highest clarity among the acid hydrolyzed cocoyam starch.

Acetylated cocoyam and sweetpotato starch exhibited higher paste viscosity than their corresponding acid hydrolyzed, annealed and native starches. Compared to native starch, acetylation generally increased the paste viscosity of the starch while both acid hydrolysis and annealing reduced the paste viscosity compared to native starches. Acid hydrolyzed starches gave the lowest paste viscosity. In all cases of modified starches, the following pattern was observed: acetylated > native > annealed > acid hydrolyzed. On average, acetylation of cocoyam starch increased paste viscosity from 5367 to 8093 cps, while acid hydrolysis and annealing reduced it to 1530 and 3120 cps, respectively. For sweetpotato starch, acetylation increased paste viscosity from 8580 to 9327 cps, and acid hydrolysis and annealing reduced it to 2040 and 3587, cps respectively.

Table 7.4 The average values and mean separtion of paste clarity and viscosity of modified and native cocoyam and sweetpotato starches

Property	Modification	Cocoyam				Sweetpotato			
		Mulanje	Mzuzu	Zomba	Mean	Kamchiputu	Mugande	Tainoni	Mean
Paste clarity (%T)	Acetylated	25.4[b]	34.5[b]	34.9[b]	**31.6[b]**	20.4[b]	28.2[b]	22.6[b]	**23.7[b]**
	Acidified	43.5[a]	50.8[a]	50.7[a]	**48.3[a]**	50.5[a]	70.6[a]	62.4[a]	**61.1[a]**
	Annealed	13.1[d]	11.9[d]	13.3[d]	**12.8[d]**	9.6[d]	13.7[d]	10.6[d]	**11.3[c]**
	Native	16.6[c]	16.3[c]	16.0[c]	**16.3[c]**	15.2[c]	21.5[c]	14.6[c]	**17.1[c]**
Viscosity (cps)	Acetylated	7040[a]	9940[a]	7300[a]	**8093[a]**	7120[a]	11900[a]	8960[a]	**9327[a]**
	Acidified	1430[d]	1540[d]	1620[d]	**1530[d]**	1880[d]	2300[d]	1940[d]	**2040[d]**
	Annealed	2400[c]	3600[c]	3360[c]	**3120[c]**	3060[c]	4040[c]	3660[c]	**3587[c]**
	Native	5200[b]	6880[b]	4020[b]	**5367[b]**	6460[b]	10700[b]	8580[b]	**8580[b]**

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Paste clarity and viscosity are two important attributes of starch that determine its application particularly in the textile, paper, adhesive and food industries (Sivak and Preiss, 1997). Acetylation increases both paste clarity and viscosity while acid hydrolysis improves paste clarity but reduces paste viscosity of starch (Sandhu *et al*., 2007; Singh *et al*., 2004; Lawal, 2004; Bello-Pérez *et al*., 2000; Liu *et al*., 1999; Betancur *et al*., 1997; Betancur and Chel, 1997). Annealing of starch reduces both paste clarity and viscosity. Results obtained in this study are therefore in agreement with above reports. Opacity of starch paste arises from associative bonding forces between starch molecules. In acetylated starch, the presence of acetyl groups causes repulsion between starch molecules, thereby reducing interchain associations and hence improved percentage transmittance (Lawal, 2004). Increased paste clarity of acetylated starch may also be due to increased swelling (Singh *et al*., 2004). The increase in paste viscosity after acetylation is not consistent with the role of acetylation in weakening interchain associations (Liu *et al*., 1999), though under controlled reaction conditions, incorporation of acetyl groups can facilitate capture and retention of water molecules and development of more organized structures, which show high resistance to deformation (Betancur *et al*., 1997). During acid hydrolysis of starch, the glycoside link ruptures resulting in production of shorter chains, which is responsible for the increased paste clarity as associative forces will be weaker than in native starch. The same applies to changes in viscosity of acid hydrolyzed starch (Sandhu *et al*., 2007; Betancur and Chel, 1997). Reduced granular swelling, amylose leaching and increased interaction between starch chains during annealing are responsible for the observed changes in viscosity and paste clarity (Jayakody and Hoover, 2008). Earlier in this study (Chapter 6), it was observed that native cassava starch had higher paste clarity than viscosity of native cocoyam and sweetpotato starch. The paste clarity of cassava starch ranged from 23.5-40.9% while the viscosity was in the range of 10140 to 14033 cps. The ranges of paste clarity obtained in this study indicate that paste clarity of native cocoyam and sweetpotato starch can be enhanced through acetylation and acid hydrolysis to match those of cassava. Better paste clarity, however, can be obtained through acid hydrolysis while higher paste viscosity can be achieved through acetylation.

7.3.4 Stability of starch pastes

The effect of storage on paste clarity of modified cocoyam and sweetpotato starch is shown in Figures 7.1 and 7.2.

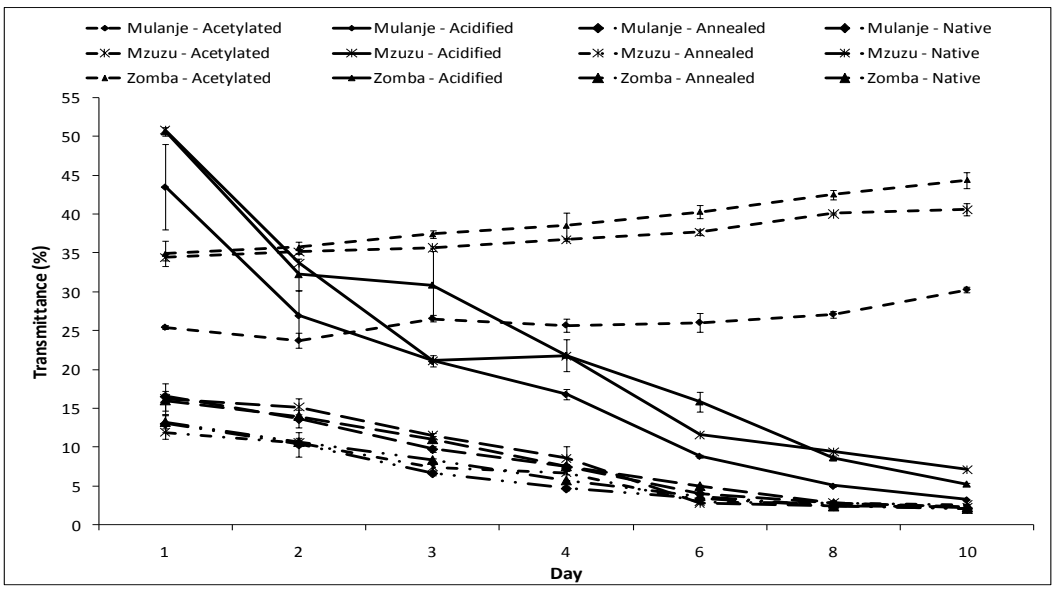

Figure 7.1 Effect of storage on gel light transmittance of acetylated, acid hydrolyzed, annealed and native cocoyam starch

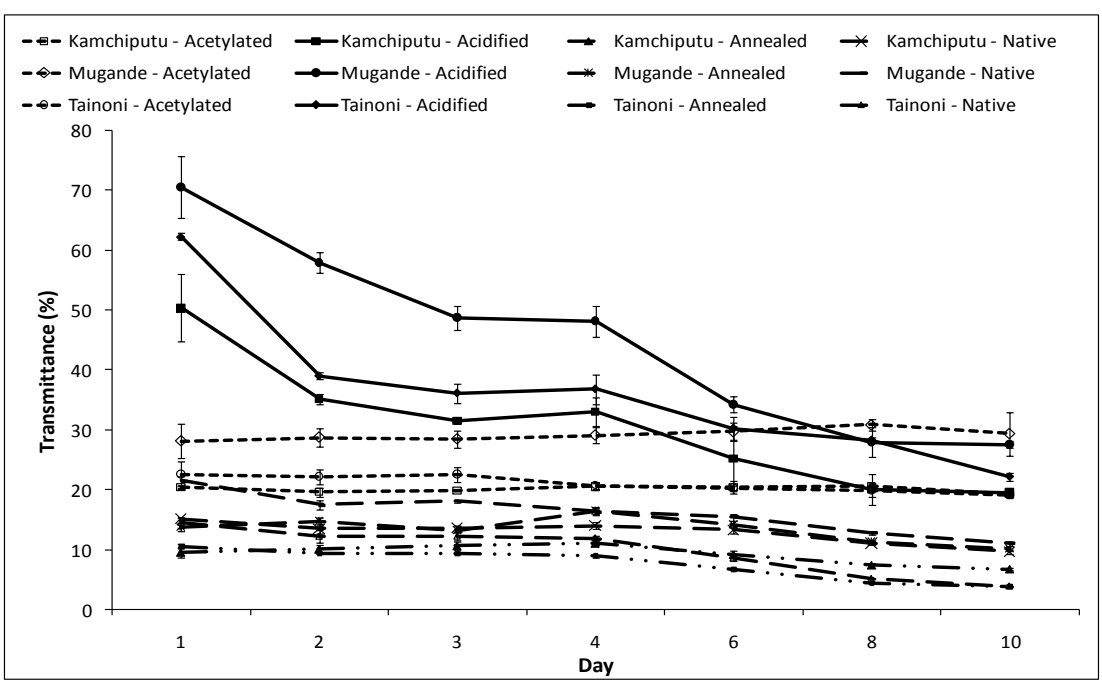

Figure 7.2 Effect of storage on gel light transmittance of acetylated, acid hydrolyzed, annealed and native sweetpotato starch

The opacity of acidified, annealed and native cocoyam and sweetpotato starches increased as evidenced by the decrease in percentage transmittance of the starch pastes. Greater increases in opacity were observed in acid hydrolyzed starches than in annealed and native starches. However, annealed starches still exhibited the highest opacity throughout the period of storage. The transmittance of acetylated cocoyam starch pastes slightly increased over the storage period (Figure 7.1); acetylated sweetpotato starch showed no significant change (Figure 7.2). Thus, acetylated cocoyam and sweetpotato starch pastes displayed higher stability than acid hydrolyzed and annealed starches. The increase in opacity of starch gel is indicative of a retrogradation process taking place. Therefore, acetylated cocoyam and sweetpotato starch displayed higher resistance to retrogradation compared to acidified and annealed starch. The higher increases in opacity of the starch gels observed in acid hydrolyzed cocoyam and sweetpotato starch than their annealed counterparts indicate instability and higher retrogradation tendency for the acid hydrolyzed starch.

7.3.5 Gelatinization and retrogradation

The DSC results of raw and retrograded annealed, acetylated, acid hydrolyzed and native cocoyam and sweetpotato starch are presented in Tables 7.5 and 7.6, respectively. Starch modification significantly influenced the gelatinization temperatures and enthalpy of the starch ($p \leq 0.001$).

On average, acid hydrolysis of cocoyam starch increased the onset, peak and conclusion temperatures from 66.8 to 69.4°C, 75.8 to 78.4°C and 83.0°C to 86.0°C respectively. Gelatinization enthalpy was reduced from 14.3 to 13.3 J/g (Table 7.6). Among the acid hydrolyzed cocoyam starch, Zomba exhibited the highest onset temperature (72.4°C) and gelatinization enthalpy (13.6 J/g). Mulanje starch displayed the highest peak (80.7°C) and conclusion gelatinization temperature (88.3°C) but the lowest enthalpy (12.9 J/g). Acid hydrolysis of sweetpotato starch increased the onset temperature from 68.8 to 71.0°C, peak temperature from 73.0°C to 74.9°C and conclusion temperature from 79.0°C to 80.7°C.

Table 7.5 The average values and mean separtion of the thermal properties of modified cocoyam starches

Accession	Modification	Raw starch						Retrograded starch						Degree retro. (%)
		Onset (T_o)	Peak (T_p)	End (T_c)	Range	PHI	ΔH_G (J/g)	Onset (T_o)	Peak (T_p)	End (T_c)	Range	PHI	ΔH_R (J/g)	
Mulanje	Acetylated	63.4^{g}	71.0^{g}	79.2^{ef}	15.8^{bc}	1.5^{de}	11.2^{f}	55.9^{a}	59.9^{ab}	64.7^{c}	8.6^{c}	0.9^{a}	2.3^{f}	20.9^{e}
	Acidified	70.1^{c}	80.7^{a}	88.3^{a}	18.3^{a}	1.2^{f}	12.9^{cd}	52.7^{b}	60.6^{a}	66.6^{a}	13.6^{ab}	0.7^{abc}	5.7^{cd}	42.3^{b}
	Annealed	70.8^{b}	77.4^{d}	84.9^{c}	14.1^{d}	2.2^{b}	14.6^{ab}	53.2^{b}	60.4^{ab}	66.4^{a}	13.2^{ab}	0.7^{abc}	5.2^{d}	35.8^{c}
	Native	67.8^{e}	77.7^{cd}	84.8^{c}	17.0^{ab}	1.3^{ef}	12.8^{de}	52.5^{b}	60.1^{ab}	66.1^{a}	13.6^{ab}	0.8^{ab}	4.1^{e}	32.0^{d}
Mzuzu	Acetylated	60.1^{i}	66.4^{h}	74.2^{g}	14.1^{d}	1.4^{ef}	8.6^{g}	53.8^{ab}	59.7^{b}	64.1^{c}	10.3^{c}	0.3^{c}	1.9^{g}	22.4^{e}
	Acidified	65.6^{f}	74.8^{e}	83.1^{d}	17.5^{a}	1.5^{de}	13.5^{bc}	52.4^{b}	59.8^{ab}	66.1^{a}	13.6^{ab}	0.8^{ab}	5.9^{bc}	44.1^{b}
	Annealed	69.1^{d}	74.4^{e}	80.2^{e}	11.1^{e}	2.5^{a}	13.3^{bc}	52.4^{b}	59.8^{ab}	66.1^{a}	13.8^{ab}	0.9^{a}	6.7^{a}	50.4^{a}
	Native	62.5^{h}	72.6^{f}	80.0^{e}	17.5^{a}	1.5^{de}	15.2^{a}	52.4^{b}	59.7^{ab}	65.8^{ab}	13.4^{ab}	0.9^{a}	6.4^{ab}	42.4^{b}
Zomba	Acetylated	63.3^{g}	70.6^{g}	78.2^{f}	14.9^{cd}	1.6^{cd}	11.9^{ef}	53.6^{b}	60.2^{ab}	64.9^{bc}	11.3^{bc}	0.4^{bc}	2.9^{f}	24.1^{e}
	Acidified	72.4^{a}	79.7^{b}	86.7^{b}	14.3^{d}	1.7^{c}	13.6^{bc}	52.8^{b}	60.5^{ab}	66.7^{a}	13.8^{ab}	0.8^{ab}	6.0^{bc}	44.5^{b}
	Annealed	72.7^{a}	78.1^{c}	83.4^{d}	10.7^{e}	2.6^{a}	14.1^{ab}	52.9^{b}	60.4^{ab}	66.6^{a}	13.7^{ab}	0.7^{abc}	5.6^{cd}	40.0^{bc}
	Native	70.3^{bc}	77.2^{d}	84.2^{cd}	14.0^{d}	2.1^{b}	14.7^{ab}	52.0^{b}	59.8^{ab}	66.5^{a}	14.5^{a}	0.8^{ab}	6.0^{bc}	40.6^{bc}
Mean	Acetylated	62.3	69.3	77.2	14.9	1.5	10.6	54.4	59.9	64.5	10.1	0.5	2.4	22.4
	Acidified	69.4	78.4	86.0	16.7	1.5	13.3	52.7	60.3	66.5	13.8	0.8	5.9	44.3
	Annealed	70.9	76.6	82.8	11.9	2.5	14.0	52.8	60.2	66.4	13.5	0.8	5.9	42.1
	Native	66.8	75.8	83.0	16.2	1.6	14.3	52.3	59.8	66.1	13.8	0.8	5.5	38.3
LSD (modification)		3.3	2.9	2.9	2.1	0.3	1.4	1.2	0.6	0.6	1.3	0.3	0.8	5.5

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Table 7.6 The average values and mean separtion of the thermal properties of modified sweetpotato starches

Genotype	Modification	Raw starch						Retrograded starch						Degree retro. (%)
		Onset (T_o)	Peak (T_p)	End (T_c)	Range	PHI	ΔH_G (J/g)	Onset (T_o)	Peak (T_p)	End (T_c)	Range	PHI	ΔH_R (J/g)	
Kamchiputu	Acetylated	61.2^{i}	66.0^{g}	72.4^{i}	11.3^{bc}	1.7^{f}	8.4^{f}	55.5^{a}	61.7^{ab}	66.3^{de}	10.8^{cd}	0.2^{f}	1.0^{g}	11.4^{f}
	Acidified	68.3^{f}	73.0^{d}	80.0^{b}	11.7^{b}	2.4^{de}	11.3^{e}	55.8^{a}	61.3^{bc}	66.4^{cde}	10.6^{d}	0.4^{e}	2.2^{g}	19.5^{e}
	Annealed	71.2^{c}	73.8^{c}	79.0^{ef}	7.8^{g}	5.5^{a}	14.2^{b}	55.7^{a}	62.3^{a}	67.5^{ab}	11.8^{c}	0.5^{cd}	3.6^{f}	25.0^{d}
	Native	65.0^{g}	71.4^{e}	78.6^{f}	13.5^{a}	2.0^{ef}	12.6^{d}	53.4^{c}	60.8^{de}	66.9^{bc}	13.5^{ab}	0.6^{ab}	4.8^{bc}	37.9^{b}
Mugande	Acetylated	64.2^{h}	68.7^{f}	75.1^{g}	10.9^{c}	2.9^{cd}	12.6^{d}	55.8^{a}	62.0^{ab}	66.3^{de}	10.4^{d}	0.2^{f}	1.3^{g}	10.6^{f}
	Acidified	71.8^{b}	75.6^{b}	81.0^{a}	9.2^{f}	3.8^{b}	14.4^{b}	52.7^{c}	60.0^{h}	65.8^{ef}	13.2^{ab}	0.5^{de}	3.6^{ef}	25.2^{d}
	Annealed	72.9^{a}	75.6^{b}	79.8^{bc}	6.9^{h}	5.6^{a}	15.1^{a}	53.2^{c}	61.1^{cd}	66.9^{bc}	13.7^{ab}	0.6^{bc}	4.6^{cd}	30.1^{c}
	Native	69.3^{e}	73.8^{c}	79.4^{cde}	10.1^{de}	2.8^{cd}	12.6^{d}	53.7^{bc}	61.4^{bc}	67.9^{a}	14.2^{a}	0.7^{a}	5.5^{a}	44.0^{a}
Tainoni	Acetylated	64.1^{h}	68.7^{f}	74.6^{h}	10.6^{cd}	2.5^{de}	11.7^{e}	54.3^{b}	60.7^{ef}	65.2^{f}	10.9^{cd}	0.2^{f}	1.6^{g}	13.4^{f}
	Acidified	72.8^{a}	76.1^{a}	81.2^{a}	8.4^{g}	4.0^{b}	13.3^{cd}	53.4^{bc}	60.5^{fg}	66.4cde	13.0^{b}	0.5^{cd}	3.9^{de}	29.0^{cd}
	Annealed	73.2^{a}	75.7^{b}	79.6^{bcd}	6.4^{h}	5.3^{a}	13.4^{c}	53.0^{c}	60.4^{gh}	66.7^{cd}	13.7^{ab}	0.6^{ab}	4.9^{bc}	36.8^{b}
	Native	69.8^{d}	73.9^{c}	79.4^{de}	9.5^{ef}	3.1^{c}	12.7^{cd}	53.7^{bc}	61.6^{ab}	67.7^{a}	14.1^{a}	0.7^{a}	5.1^{ab}	40.3^{ab}
Mean	Acetylated	63.2	67.8	74.1	10.9	2.4	10.9	55.2	61.5	65.9	10.7	0.2	1.3	11.8
	Acidified	71.0	74.9	80.7	9.8	3.4	13.0	54.2	61.3	66.3	12.0	0.5	3.1	24.6
	Annealed	72.0	75.0	79.5	7.0	5.5	14.2	54.0	61.3	67.0	13.0	0.6	4.3	30.6
	Native	68.8	73.0	79.0	11.1	2.6	12.7	53.6	60.7	67.5	13.9	0.7	5.1	40.7
LSD, 0.05 (modification)		2.3	1.6	1.0	1.6	0.7	1.6	1.3	0.8	0.6	1.1	0.1	0.8	4.8

Means followed by the same letter within the same column are not significantly different from each other ($p \leq 0.05$)

Gelatinization enthalpy slightly increased from 12.7 to 13.0 J/g. Acid hydrolyzed Tainoni starch gave the highest onset (72.8°C), peak (76.1°C) and conclusion (81.2°C) temperatures while Mugande starch had the highest gelatinization enthalpy (14.4 J/g). Kamchiputu starch displayed the lowest gelatinization temperatures and enthalpy among the acid hydrolyzed sweetpotato starch. An increase in onset and peak temperatures but lowered enthalpy of starch following acid hydrolysis has been reported for corn (Sandhu *et al*., 2007), rice (Thirathumthavorn and Charoenrein, 2005) and cassava starch (Ahmed *et al*., 2005). Acid hydrolysis has also been shown to increase gelatinization temperatures and gelatinization enthalpy of potato starch (Wang and Wang, 2001), and new cocoyam starch (Lawal, 2004). The increases in gelatinization temperatures and enthalpy are due to the increases in relative crystallinity after acid hydrolysis (Wang and Wang, 2001). Gelatinization is known to occur first in the amorphous regions as opposed to crystalline regions, due to weakened hydrogen bonding in these areas (Singh *et al*., 2003). This is the region which is preferentially attacked by the acid during hydrolysis. Further, when acid attacks the amorphous region, crystallites are decoupled from the amorphous parts and are no longer destabilized by them (Sandhu *et al*., 2007). This results in higher melting temperatures of starch. The decrease in gelatinization enthalpy following acid hydrolysis which was observed in Mzuzu and Zomba cocoyam starch and Kamchiputu sweetpotato starch may be attributed to a loss in some degree of order in the amorphous region before gelatinization, weakening the granule matrix (Muhr *et al*., 1984).

Gelatinization temperatures and enthalpy of cocoyam and sweetpotato decreased upon acetylation of the starch. The acetylation of cocoyam starch generally reduced the onset temperature from 66.8 to 62.3°C, the peak temperature from 75.8°C to 69.3°C and the conclusion temperature from 83.0°C to 77.2°C. The gelatinization enthalpy was significantly reduced from 14.3 to 10.6 J/g. Acetylated Mulanje and Zomba cocoyam starch exhibited similar gelatinization temperatures and enthalpy values which were higher than those of acetylated Mzuzu starch. Acetylation of sweetpotato starch reduced the onset temperature from 68.8 to 63.2°C, the peak temperature from 73.0 to 67.8°C, the conclusion temperature from 79.0 74.1°C and the gelatinization from 12.7 to 10.9J/g. Mugande acetylated starch displayed the highest gelatinization temperatures and enthalpy, and Kamchiputu the lowest. The reduced gelatinization temperatures and enthalpy of cocoyam and sweetpotato starch indicates that some loss of structural order

may have occurred during the modification. Acetylation is known to cause early rupture of amylopectin double helices resulting in lower gelatinization temperatures (Lawal and Adebowale, 2005; Aziz *et al*., 2004; Lawal 2004; Singh *et al*., 2004b; Adebowale and Lawal, 2003; Betancur *et al*., 1997). However, Aziz *et al*. (2004) reported an increase in peak temperature of sago starch after acetylation. They attributed this to the strengthened integrity of starch granules due to the higher levels of acetylation achieved (8.3%).

Annealed cocoyam and sweetpotato starch showed higher gelatinization temperatures and enthalpy. Annealing of cocoyam increased the onset temperature from 66.8 to 70.9°C and the peak temperature from 75.8°C to 76.6°C. The conclusion temperature and gelatinization enthalpy of the annealed starch (82.8°C, 14.0 J/g) was similar to that of native starch (83.0°C, 14.3 J/g). Annealing of sweetpotato starch increased the onset, and conclusion temperatures from 68.8 to 72.0°C, 73.0°C to 75.0°C and 79.0°C to 79.5°C, respectively. The gelatinization enthalpy increased from 12.7 to 14.2 J/g. The increase in the gelatinization temperatures and enthalpy indicates increased perfection of crystals due to increased reinforcement of helical packing, or increased interactions with amylose, or a combination of these events. The more crystalline the sample, the larger the amount of energy required to melt those crystals. Annealing has been shown to increase the relative crystallinity of starch (Lan *et al*., 2008; Waduge *et al*., 2006). Increased gelatinization temperatures and enthalpy have also been reported for yam (Jayakody *et al*., 2009), barley (Waduge *et al*., 2006), and unfermented and fermented cassava (Gomes *et al*., 2005; 2004) starch after annealing.

The extent of the influence of starch modification on gelatinization temperatures and enthalpy of the starches from different cocoyam accessions and sweetpotato genotypes also varied significantly ($p \leq 0.001$). Annealed Zomba cocoyam starch displayed the highest onset (72.7°C) and peak (78.1°C) gelatinization temperatures and Mzuzu starch the lowest (60.1°C, 74.4°C). The highest conclusion temperature was displayed by Mulanje starch (84.9°C) while Mzuzu acetylated starch gave the lowest value (80.2°C). Annealed Mulanje and Zomba cocoyam starch exhibited the highest and lowest gelatinization enthalpy values respectively. Among sweetpotato starch, annealed Tainoni and Mugande gave the highest onset (73.2, and 72.9°C, respectively) and peak (75.7, and 75.6°C, respectively) temperatures, and Kamchiputu starch the lowest (71.2°C, 73.8°C).

Annealed Mugande had the highest gelatinization enthalpy (15.1 J/g) and Tainoni the lowest (13.4 J/g). The different responses towards annealing may be due to differences in chemical composition (amylose/amylopectin ratio) and structural arrangements of starch chains in amorphous and crystalline regions (Waduge *et al*., 2006).

DSC analysis of retrograded starch revealed that annealed and acidified cocoyam starch exhibited similar onset and peak temperatures, and retrogradation enthalpy values as those of retrograded native starch. Retrograded acetylated starch gave a significantly higher onset temperature but a lower retrogradation enthalpy than native starch ($p \leq 0.001$). The degree of retrogradation of acid hydrolyzed starch (44.3%) was similar to that of annealed starch (42.1%), but significantly higher than that of native (39.6%) and acetylated starch (22.5%). Acetylated starch exhibited the lowest degree of retrogradation. In contrast, retrograded sweetpotato starch did not reveal any significant differences in the onset and peak temperatures of modified sweetpotato starch compared to its native starch, except for enthalpy of retrogradation. The enthalpy of retrogradation was the highest in retrograded native starch (5.4 J/g) and the lowest in acetylated starch (1.4 J/g). The order of enthalpy of retrogradation for sweetpotato starch was: native > annealed > acidified > acetylated. The degree of retrogradation was the highest in native sweetpotato starch (40.7%) and the lowest in acetylated starch (11.8%). However, unlike the modified cocoyam starch, degree of retrogradation for native sweetpotato starch was reduced to 30.6 and 24.6% after annealing and acid hydrolysis, respectively (Table 7.7). This is in agreement with findings of others who have reported reduced retrogradation tendencies after acid thinning (Sandhu *et al*., 2007; Betancur and Chel, 1997), acetylation (Lawal and Adebowale, 2005; Lawal, 2004; Betancur *et al*., 1997), and annealing (Jayakody *et al*., 2009) of starch, though an increase in retrogradation tendency after acid-thinning has also been reported (Lawal and Adebowale, 2005; Lawal, 2004). The decrease in enthalpy and degree of retrogradation in annealed starch is attributed to weaker interactions between amylopectin chains and/or decreased amylopectin chain mobility during gel storage (Jayakody *et al*., 2009). Starch retrogradation involves reaggregation of starch molecules and hydrogen bonding plays an important role in this process. Following acetylation, the presence of acetyl groups gives rise to intra and intermolecular electrostatic repulsions in the starch molecules restricting hydrogen bonding (Lawal and Adebowale, 2005). This is responsible for the reduction of enthalpy and extent of retrogradation. Since acid hydrolysis produces a high proportion of short

linear chains, it would be expected that these molecules would reassociate rapidly, resulting in a higher retrogradation enthalpy (Thirathumthavorn and Charoenrein, 2005). This would probably explain the increase in degree of retrogradation of cocoyam starch after acid hydrolysis, since cocoyam starch exhibited a higher degree of acid hydrolysis than sweetpotato starch (Table 7.1). The reduced enthalpy and extent of retrogradation observed after acid hydrolysis of sweetpotato is most probably due to the weaker interaction between the starch molecules.

7.4 Conclusions

The results of this study have revealed that functional properties of cocoyam and sweetpotato starch can be altered through physical and chemical modifications. The acetylation of cocoyam and sweetpotato starch increased the water absorption capacity and swelling power, while acid hydrolysis reduced them. The solubility of the native cocoyam and sweetpotato starches was enhanced following acetylation and acid hydrolysis, but acid hydrolyzed starch displayed higher solubility than their acetylated counterparts. There were slight changes in water absorption capacity, swelling power and solubility after annealing, suggesting a lesser extent of rearrangement of starch molecules. Paste clarity and viscosity increased after acetylation and decreased after annealing. Acid hydrolysis also improved the paste clarity but decreased the paste viscosity. A higher paste clarity was achieved through acid hydrolysis than acetylation. During storage, the pastes of the acetylated starch showed greater stability than pastes of acid hydrolyzed and annealed starch.

Further, acid hydrolysis and annealing increased the thermal stability of cocoyam and sweetpotato starches as evidenced by the increased gelatinization temperatures. However, enthalpy of gelatinization was reduced. The acetylation of cocoyam and sweetpotato starch resulted in reduced gelatinization temperatures as well as enthalpy. Acetylation reduced the retrogradation tendencies of both cocoyam and sweetpotato starches, most probably due to weaker interactions between starch molecules arising from electrostatic repulsions brought about by the presence of acetyl groups. Acid hydrolysis and annealing increased the retrogradation tendencies of cocoyam starch, but decreased the same for sweetpotato starches. Thus, varying strengths of interactions of cocoyam and sweetpotato starch molecules are obtained after acid hydrolysis and annealing.

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CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The demand for starch as a raw material for the food and non-food industry in Malawi is rapidly increasing. This demand is currently being met by largely imported starch from South Africa, the United Kingdom, the Netherlands, Tanzania and Zimbabwe. Increased costs, supply capacity, availability, late deliveries and transit damages are some of the major challenges the industries are facing due to this starch importation. These problems could be overcome by exploiting locally grown crops as alternative sources of starch. Cassava, sweetpotato and cocoyam grown in Malawi mainly as subsistence crops could offer this opportunity. This study was undertaken to unravel the unique characteristics of starches from these crops. The chemical composition, physicochemical, functional and structural properties of the starches were investigated and compared. Further physical and chemical modifications were carried out on cocoyam and sweetpotato starches to evaluate how starches from these crops respond to modification.

Using starches from seven cocoyam accessions, 15 sweetpotato genotypes and five cassava genotypes, the study revealed significant variation in composition, mineral profiles, granular morphology, functional and molecular properties among the different starches. The functional properties of the starches also varied significantly after physical modification by annealing and chemical modification by acetylation and acid hydrolysis.

Proximate analysis of the starch revealed pH ranging from 5.2-7.0, 4.7-5.9, and 4.9-5.7, moisture content from 10.4-13.2%, 7.9-11.6% and 12.4-13.0%, ash contents from 0.13-0.16%, 0.15-0.28% and 0.03-0.13%, protein levels of 0.45-0.84%, 0.36-0.53% and 0.35-0.39%, fat contents from 0.10-0.16%, 0.10-0.15% and 0.08-0.14% and amylose contents from 10.6-21.0%, 12.0-34.4% and 13.3-23.7% for cocoyam, sweetpotato and cassava starches, respectively. The pH was higher in cocoyam than cassava and sweetpotato starches. The pH levels of the starches obtained suggest that the starches could be ideal for low acid foods. The moisture content level of the starches was below the maximum limit (13%) required for prolonged storage of the starches. Higher levels of moisture

content can lead to microbial damage. Ash and amylose content were higher in sweetpotato starch while pH, protein and fat levels were higher in cocoyam starch compared to other starches. All starches exhibited higher levels potassium (32-438 mg kg^{-1}), followed by calcium (26-392 mg kg^{-1}) phosphorus (67-146 mg kg^{-1}), magnesium (21-75 mg kg^{-1}) and sodium (21-67.5 mg kg^{-1}) than iron (4.4-17.8 mg kg^{-1}) , manganese (0.35-2.2 mg kg^{-1}) and zinc (1.32-5.57 mg kg^{-1}) . All these elements were comparatively higher in sweetpotato than in cocoyam and cassava starches. The principal component analysis of the different starches showed that sweetpotato starches differed mainly from cocoyam and cassava starches in terms of Ca, Mg, Mn, Zn, Na, pH, protein and fat contents. Sweetpotato starches exhibited high Ca, Mg, Mn, Zn and Na contents but were low in pH, protein and fat contents. Cocoyam starches exhibited high pH, protein and fat contents while cassava starches gave intermediate values.

There was diversity of starch granular size and shapes from Malawian cocoyam, sweetpotato and cassava starches. Cocoyam starch granules exhibited mostly round/spherical shapes similar to that of cassava starch and granule size ranged from 4.0 to 18.7 μm. Sweetpotato starch showed mostly polygonal granules that ranged between 4.0 and 48 μm in size. A comparison of granule size distribution revealed that cocoyam starch had smaller sized granules than sweetpotato and cassava starch. Most of the sweetpotato and cassava starch granules had sizes ranging from 10 to 25 μm. With respect to granule size distribution, starch isolated from Kakoma, Kenya, LU96/303, LU96/304, Lunyangwa, Mafutha, Salera, Semusa and Tainoni sweetpotato genotypes had the largest granule size. All starches exhibited B-type crystallinity with distinctive major diffraction peaks at 5.4-5.6° 2θ angles, however relatively strong peaks at 7.6-7.8° 2θ angles suggesting the presence of single helical conformations.

Application of starch in the food and non-food industries relies largely on its functional properties. Investigation on functional properties of cocoyam, sweetpotato and cassava starches revealed variation in water absorption capacity, swelling power, solubility, paste clarity, viscosity, syneresis, gelatinization temperatures and enthalpy, and retrogradation tendencies of the starches. Increasing temperature resulted in increased water absorption capacity, swelling power and solubility of the starches. Cassava starches exhibited higher water absorption capacity, swelling power, paste clarity, resistance to retrogradation and

viscosity than cocoyam and sweetpotato starches. Gelatinization temperatures ranged from 69.4±4.4 to 81.4±1.8°C for cocoyam, 68.5±1.5 to 79.2±1.0°C for sweetpotato and 60.1±1.4 to 76.8±0.6°C for cassava starches. Generally, cocoyam starches exhibited higher gelatinization temperatures than sweetpotato and cassava starches. Gelatinization enthalpies of cocoyam (13.4±2.1 J/g) and cassava starches (14.0±0.8 J/g) were similar, but higher than those of sweetpotato starches (12.3±1.1 J/g). Retrogradation studies showed that cocoyam starches have higher tendency (34.5±7.4%) towards retrogradation than sweetpotato (30.9±3.8%) and cassava starches (9.8±1.8%). Cassava starches provided the least tendency towards retrogradation. This implies that in applications of starch like the food industry where swelling power, paste clarity and resistance to retrogradation are desired functionalities, cassava starches would be more suitable. Lower paste clarity and high retrogradation tendencies of cocoyam and sweetpotato starches would limit their use in food products. However such setbacks could be overcome through modification. In terms of energy requirements, exploration of sweetpotato starches would result in reduced industrial energy costs due to lower gelatinization energy.

Studies on molecular characteristics of the starches have shown that starch from cocoyam, sweetpotato and cassava have different structural properties. Cocoyam starches displayed higher blue values (0.363±0.026) and λ_{max} (600±7 nm) but lower reducing capacity (7.2±2.8 mg g^{-1}) values than sweetpotato (0.279±0.022, 576±4 nm, 9.5±3.9 mg g^{-1}, respectively) and cassava starches (0.257±0.024, 576±4 nm, 12.1±4.4 mg g^{-1}, respectively). Based on blue values, $\lambda_{max,}$ and reducing capacity values, starch from cocoyam consisted of molecules of higher molecular weight and a larger proportion of short chain amylopectins than sweetpotato and cassava starch. After 12 days of acid hydrolysis, sweetpotato and cocoyam starch exhibited similar values of the extent of acid hydrolysis (20.2±3.1%, 21.2±2.3%, respectively) which were higher than that of cassava starch (18.1±2.2%). This indicates similarity of packing and orientation of chains in the amorphous regions of sweetpotato and cocoyam starch which may be different from that of cassava starch. HPSEC analysis of the unbranched starch has revealed that cocoyam and sweetpotato starch contain amylopectin molecules with similar average molecular weights (1.54±0.10 x 10^6 Da and 1.54±0.78 x 10^6 Da, respectively) while cassava contains amylopectin molecules of lower molecular weight (1.46±0.11 x 10^6 Da).

Cocoyam starch contained amylose molecules of lower molecular weight (3.98±0.31 x 10^5 Da) than sweetpotato (4.11±0.25 x 10^6 Da) and cassava starches (4.43±0.23 x 10^6 Da). Isoamylase debranched starches showed a trimodal distribution profile corresponding to the higher molecular weight amylose fraction, long chain and short chain amylopectin fractions. Sweetpotato starch gave the largest fraction of long-chain amylopectins (47%) and the lowest fraction of short-chain amylopectins (45%), while cocoyam starch exhibited the largest fraction of short chain amylopectins (62%) and smallest proportion of large chain amylopectins (32%). The observed structural differences could therefore explain the different functional properties displayed by cocoyam, sweetpotato and cassava starch.

Acetylation, acid hydrolysis and annealing of cocoyam and sweetpotato starch produced starches that varied in functional properties. Acetylation enhanced water absorption capacity, swelling power and solubility of the starches. Acid hydrolysis tended to reduce the water absorption capacity and swelling power but improved the solubility and paste clarity of starch. Annealing reduced water absorption capacity, swelling power, solubility and paste clarity. Pastes of acetylated starch were more stable than those of acid-hydrolyzed, native and annealed starches. Acid hydrolysis and annealing of the starches produced starches with high thermal stability as indicated by increase in gelatinization temperatures and enthalpy. Acetylation resulted in reduced gelatinization temperatures, gelatinization enthalpy and retrogradation tendency.

Despite cocoyam and sweetpotato starches exhibiting lower water absorption capacity, swelling power, paste clarity, viscosity and higher gelatinization temperatures, some individual starches could be singled out for displaying functional properties similar or closer to those of cassava starches. These included; starches of Mugamba, Kamchiputu, Salera and Zondeni sweetpotato genotypes, and Chitipa, Thyolo and Machinga cocoyam accessions for higher swelling power and water absorption capacity, starches of Semusa, Mugande and Kenya sweetpotato genotypes, and Chitipa and Mzuzu cocoyam accessions for better paste clarity, starches of Zondeni and Kamchiputu sweetpotato genotypes and of Chitipa and Mzuzu cocoyam accessions for lower gelatinization temperatures and enthalpies, starches of Chitipa and Thyolo cocoyam, and for Kamchiputu and A45 and Kenya lower retrogradation tendencies, starches of Semusa, Mugande, Mugamba, and LU96/303 sweetpotato, and Mzuzu and Nkhotakota cocoyam for higher viscosity than the

rest of sweetpotato and cocoyam starches. These starches could be explored further for various industrial applications and modification. In food applications where starches are used due to their thickening ability, paste clarity and stability, acetylated sweetpotato and cocoyam starches could be exploited.

This study has unravelled the unique characteristics of starches from cocoyam and sweetpotato grown in Malawi. Data obtained in this study can guide the end product use of starch from these crops. It will also help in the tailoring of the starch to meet specialized use in the food and non-food industries. Therefore, there is need to test the suitability of the industries in Malawi. There is also need to investigate further the changes in functional and structural properties of the Malawian sweetpotato and cocoyam starches upon modification.

ABSTRACT

Key words: cocoyam, sweetpotato, cassava, starch, chemical composition, physicochemical, functional, structural

Starches isolated from Malawian sweetpotato, cocoyam and cassava were comparatively studied and evaluated for physicochemical, functional and structural properties in order to unveil their characteristic properties and unravel their potential for industrial application. Further, sweetpotato and cocoyam starches were modified by physical and chemical means, and changes in their functional properties were determined. The results showed that cocoyam, sweetpotato and cassava starch exhibited properties that were unique to botanical source.

The pH of the starches ranged within the 4.7 to 7.0 range required for use in low acid foods while moisture content was below the maximum limit required for prolonged storage of the starches. Ash and amylose content of the starches ranged from 0.03 to 0.28%, and 10.6 to 34.4%, respectively while protein and fat levels ranged from 0.35 to 0.84%, and 0.085 to 0.160%, respectively. All starches exhibited high levels of potassium, followed by calcium, phosphorus, magnesium and sodium. Iron, manganese and zinc were present in very low levels compared to the other elements.

Cocoyam starch granules exhibited mostly round/spherical shapes similar to that of cassava starch, while sweetpotato starch granules were mostly polygonal in shape. Granule size ranged from 4.0 to 18.7 μm, 4.0 to 48.0 μm and 5.3 to 22.7 μm for cocoyam, sweetpotato and cassava starches, respectively. Cocoyam starch had a larger fraction of smaller sized (0-10 μm) granules than sweetpotato and cassava starch. Sweetpotato and cassava starches had mostly medium sized (10-25 μm) granules. All starches exhibited B-type crystallinity with distinctive major diffraction peaks at 5.4-5.6° 2θ angles, however relatively strong peaks at 7.6-7.8° 2θ angles suggesting the presence of single helical conformations.

Water absorption capacity, swelling power and solubility of all starches increased with increasing temperature. Cassava starches exhibited higher water absorption capacity, swelling power, paste clarity, resistance to retrogradation and viscosity than cocoyam and

sweetpotato starches. Cocoyam starches exhibited higher gelatinization temperatures than sweetpotato and cassava starches. Cocoyam and cassava starches gave similar gelatinization enthalpies which were higher than those of sweetpotato starches. Cocoyam starches displayed higher retrogradation tendencies than sweetpotato and cassava starches.

Cocoyam starches displayed higher blue values and wavelength of maximum absorption but lower reducing capacity values than sweetpotato and cassava starches. There was similarity in the packing and orientation of chains in the amorphous regions of sweetpotato and cocoyam starches as evidenced by similar values of the extent of acid hydrolysis of the starches for 12 days. Average molecular weight of amylopectin molecules of the starches studied by HPSEC ranged from 1.42×10^6 to 1.67×10^6 Da. Cocoyam starch contained amylose molecules of lower molecular weight than sweetpotato and cassava starches. Analysis of isoamylase debranched starches showed that sweetpotato starch had the largest fraction of long-chain amylopectins (47%) and the lowest fraction of short-chain amylopectins (45%), while cocoyam starch exhibited the largest fraction of short chain amylopectins (62%) and smallest proportion of large chain amylopectins (32%).

Acetylation enhanced water absorption capacity, swelling power and solubility of the starches, but reduced gelatinization temperatures, gelatinization enthalpy and retrogradation tendencies of the starches. Acid hydrolysis reduced the water absorption capacity and swelling power but improved the solubility and paste clarity of starch, and increased gelatinization temperatures and enthalpy. Annealing reduced water absorption capacity, swelling power, solubility and pastes clarity but increased gelatinization temperatures and enthalpy. Due to their differences in physicochemical, functional and structural properties, starches from Malawian cocoyam, sweetpotato and cassava can play different roles in the Malawian industry.

OPSOMMING

Sleutelwoorde: amadumbie, soetpatats, cassava, stysel, chemiese samestelling, fisies-chemies, funksioneel, struktureel

Stysel is geïsoleer van Malawiese soetpatats, amadumbies en cassava om vergelykend met mekaar te bestudeer. Dit is geëvalueer vir fisies-chemiese, funksionele en strukturele eienskappe om hulle kenmerkende eienskappe en potensiële industriële toepassing te bepaal. Verder is soetpatat en amadumbie stysels deur fisiese en chemiese metodes gemodifiseer, en veranderings in hulle funksionele eienskappe is bepaal. Die resultate het getoon dat amadumbie, soetpatat en cassava stysel eienskappe uniek aan die botaniese bron het.

Die pH van die stysel het tussen 4.7 en 7.0 gewissel, wat binne die aanvaarbare waardes vir gebruik in laesuurvoedsel val, en die voginhoud was laer as die maksimum limiet nodig vir die langtermyn storing van stysel. As en amilose inhoud van die stysels het gewissel tussen 0.03 en 0.28%, en 10.6 en 34.4%, onderskeidelik, terwyl proteïen en vetvlakke tussen 0.35 en 0.84%, en 0.085 en 0.160%, onderskeidelik gewissel het. Alle stysel het die hoogste vlak van kalium gehad, gevolg deur kalsium, fosfaat, magnesium en natrium. Yster, mangaan en sink was in baie laer konsentrasies teenwoordig as die ander elemente.

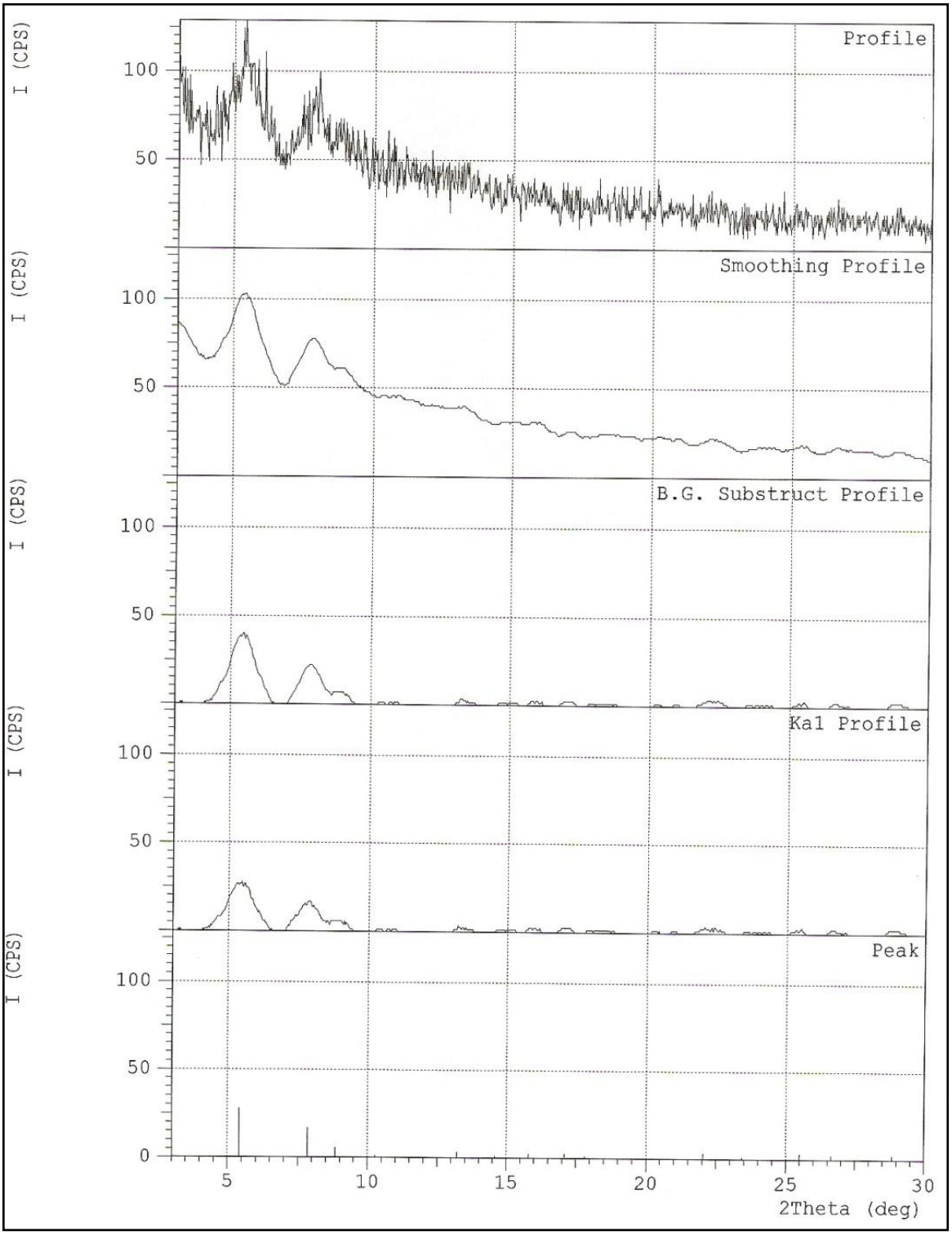
Amadumbie styselgranules was meestal ‘n ronde/sferiese vorm, baie dieselfde as in cassava stysel terwyl soetpatat granules meestal ‘n poligonale vorm gehad het. Granule grootte het gewissel van 4.0 tot 18.7 μm, 4.0 tot 48.0 μm en 5.3 tot 22.7 μm vir amadumbie, soetpatats en cassava stysel onderskeidelik. Amadumbie stysel het ‘n groter fraksie van die kleiner grootte (0-10 μm) granules as in soetpatat en cassava stysel gehad. Soetpatat en cassava stysel het meestal medium grootte (10-25 μm) granules gehad. Alle stysels het B-tipe kristaliniteit gehad met duidelike hoof diffraksiepieke by 5.4-5.6° 2θ hoeke, maar relatiewe sterk pieke by 7.6-7.8° 2θ hoeke wat die teenwoordigheid van enkel heliese konformasies voorstel.

Waterabsorpsiekapasiteit, swellings vermoë en oplosbaarheid van alle stysel het toegeneem met toenemende temperatuur. Cassava stysel het hoër waterabsorpsiekapasiteit, swellingsvermoë, pastahelderheid, weerstand teen retrogradering en viskositeit getoon as die stysel van amadumbie en soetpatats. Amadumbie stysel het hoër gellingstemperature as die van soetpatats en cassava getoon. Amadumbie en cassava stysel het ooreenstemmende gellingsentalpie gehad, en dit was hoër as vir soetpatat stysel. Amadumbie stysel het hoër retrograderingtendense gehad as soetpatat en cassava stysel.

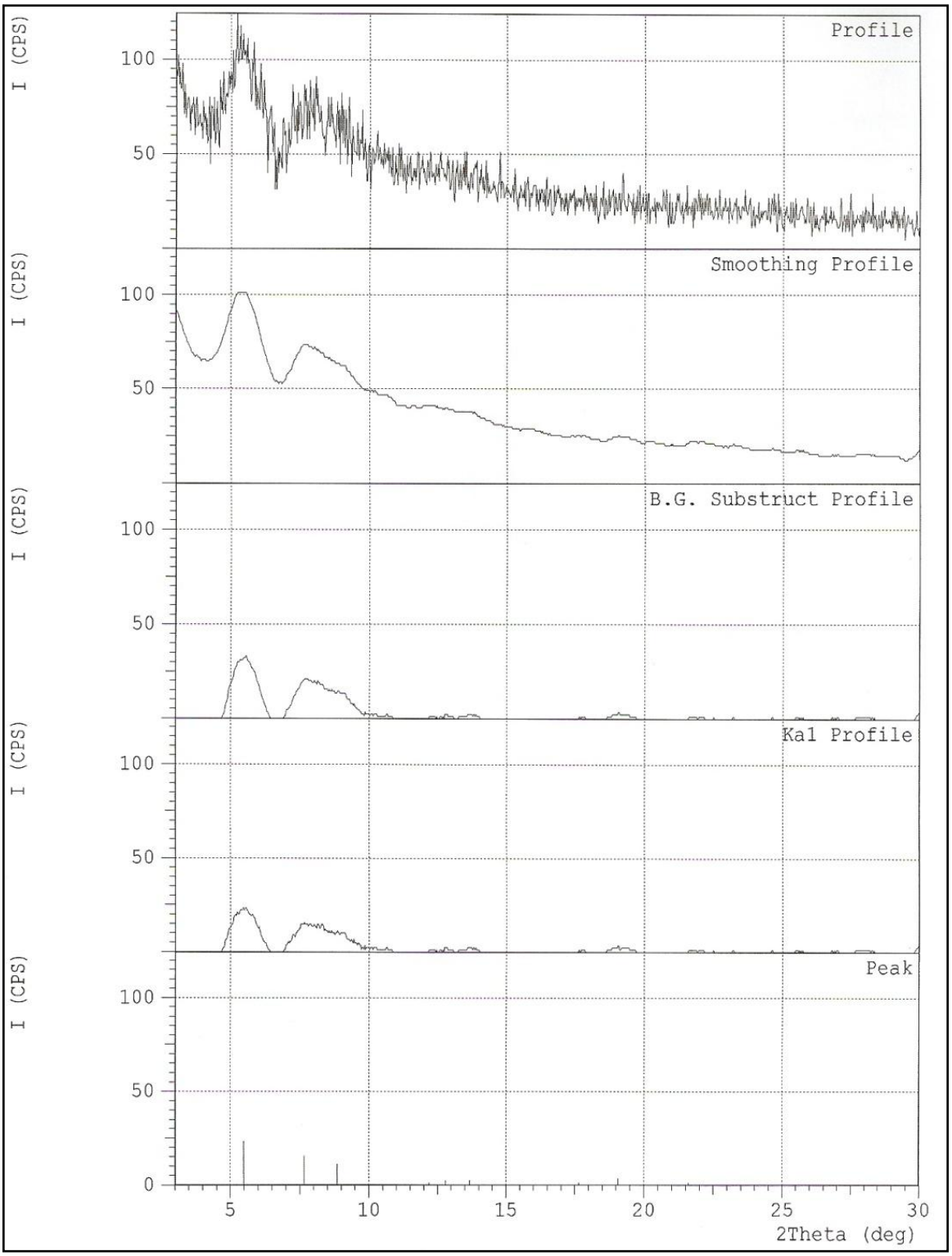
Amadumbie stysel het hoër "blou" waardes en golflengte van maksimum absorpsie gehad, maar laer reduseringskapasiteit waardes as soetpatat en cassava stysel. Daar was ooreenkomste in die pak en oriëntasie van kettings in die amorfe areas van soetpatat en amadumbie stysel, wat gesien kon word in die ooreenstemmende waardes van die hoeveelhed suurhidrolise in stysel na 12 dae. Gemiddelde molekulêre massa van amilopektien molekules van stysel wat met HPSEC ondersoek is, het gewissel van 1.42 x 10^6 tot 1.66 x 10^6 Da. Amadumbie stysel het amilose molekules van laer molekulêre massa gehad as die van soetpatat en cassava stysel. Analise van isoamilase ontkettingde stysel het getoon dat cassava stysel die grootste fraksie lang ketting amilopektien en die laagste fraksie kort ketting amilopektien het, terwyl amadumbie stysel die grootste fraksie kort ketting amilopektien en die kleinste proporsie lang ketting amilopektien het.

Asetilering het waterabsorpsiekapasiteit, swellingsvermoë en oplosbaarheid van stysel verhoog, maar het gelatineringstemperature, gelatineringsentalpie en retrogradering van stysel verlaag. Suurhidrolisering het waterabsorpsiekapasiteit en swellingsvermoë verlaag, maar het oplosbaarheid en pastahelderheid vergroot, en gelatineringstemperature en entalpie verhoog. Verbinding het die waterabsorpsiekapasiteit, swellingsvermoë, oplosbaarheid en pastahelderheid verlaag, maar gelatineringstemperatuur en entalpie verhoog. Omdat daar groot variasie is in die fisies-chemiese, funksionele en strukturele eienskappe van stysel van Malawiese amadumbie, soetpatat en cassava, kan hierdie stysels verskillende rolle speel in industrië in Malawi.

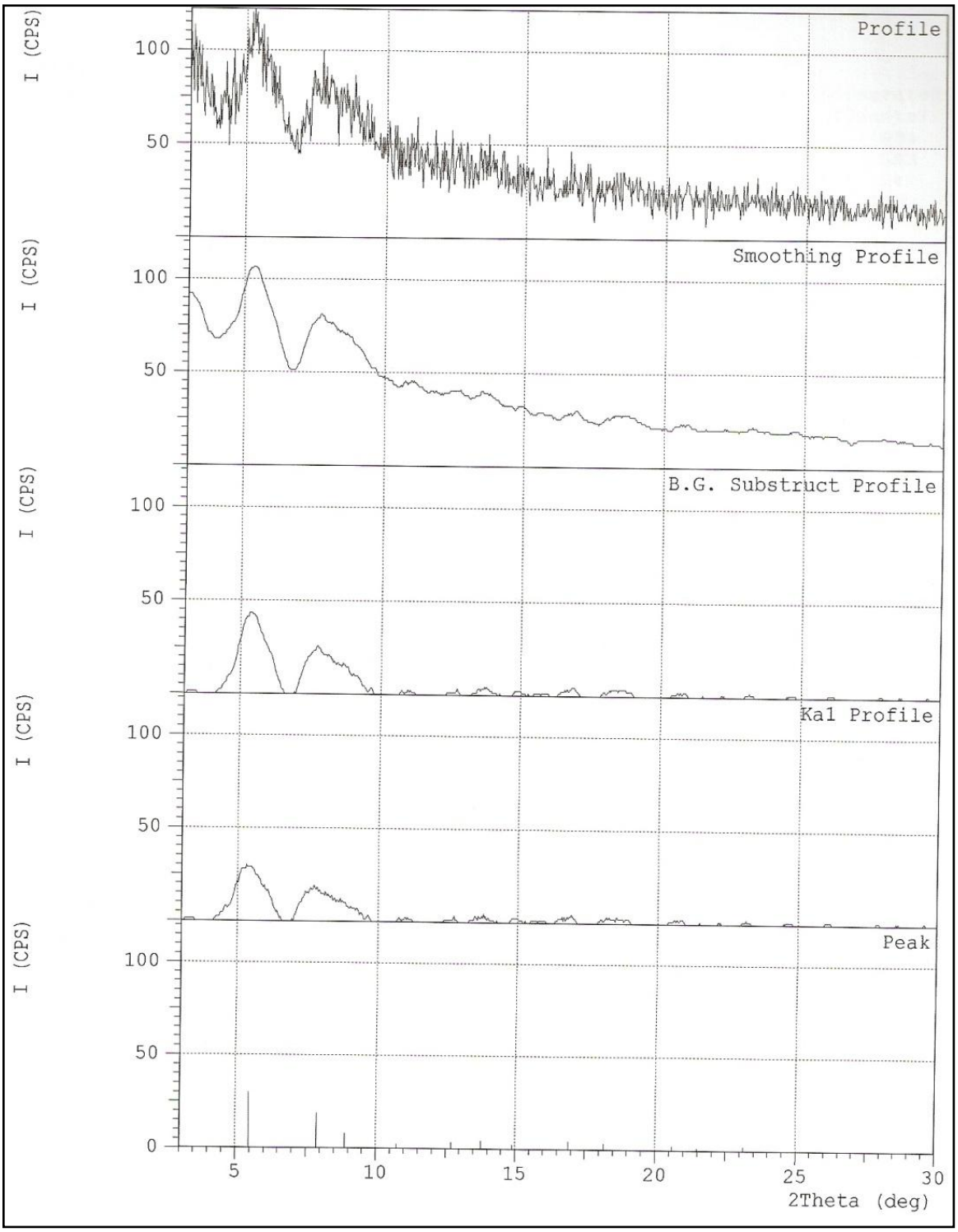
APPENDICES



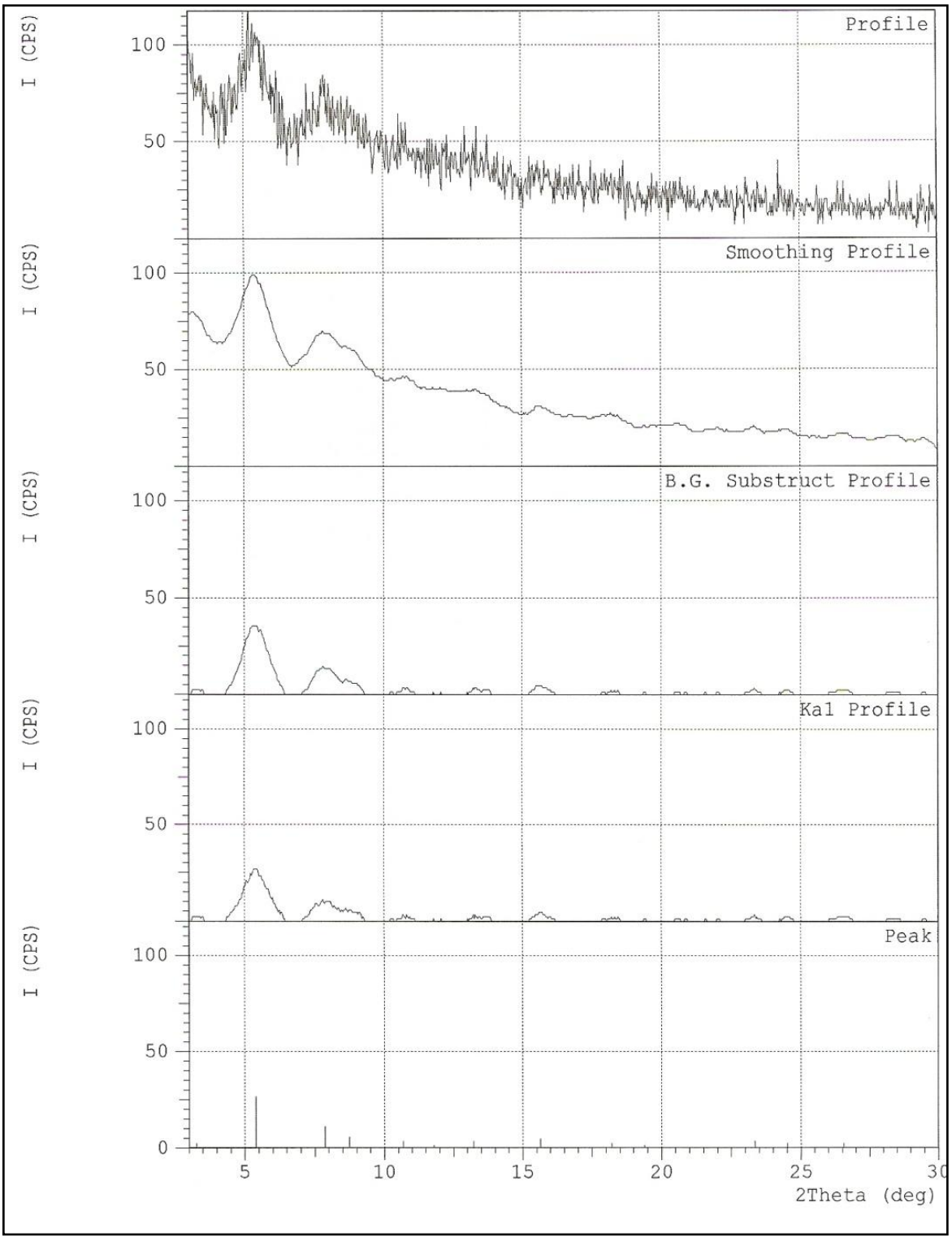
Appendix 1a X-ray diffraction pattern of Chitipa cocoyam starch



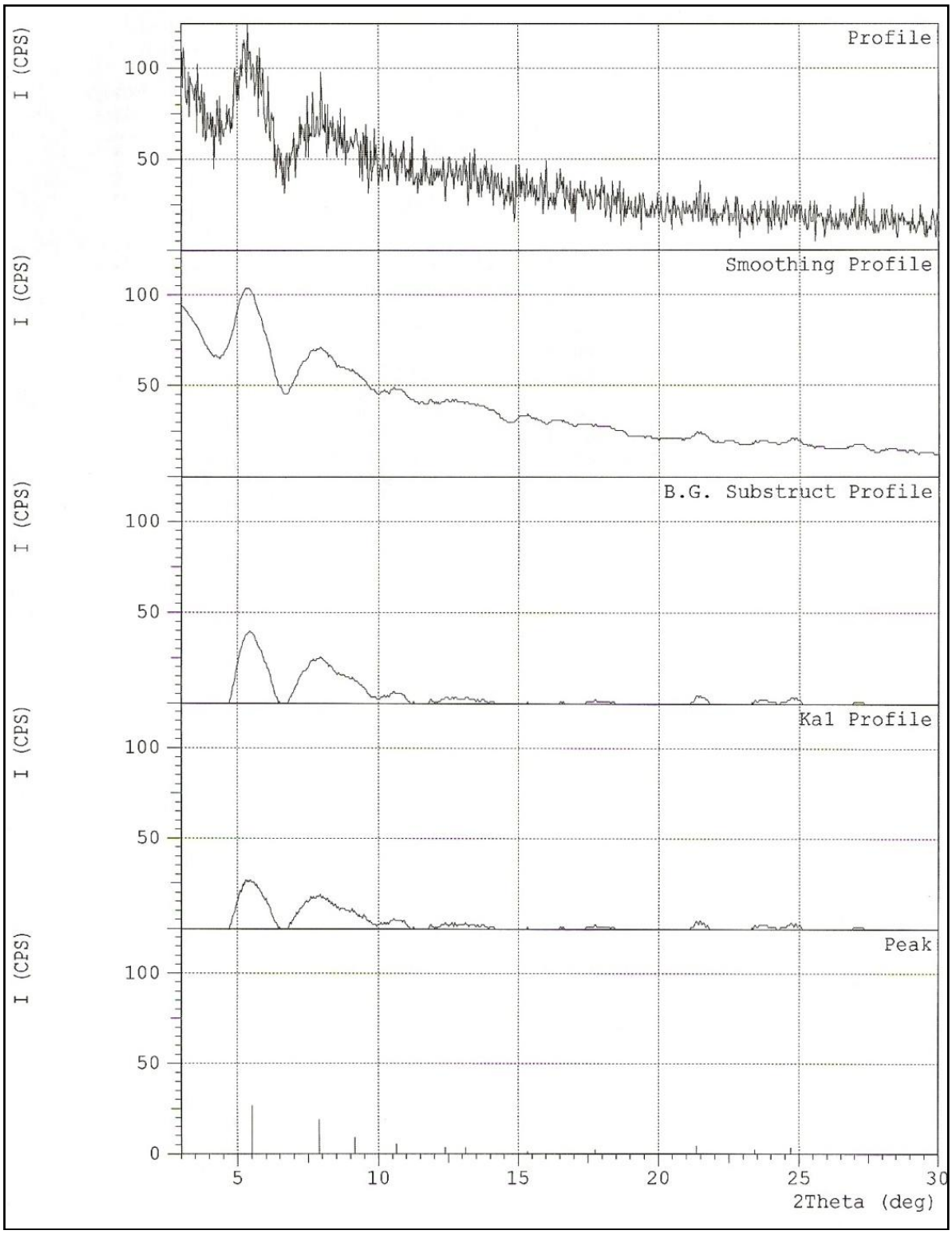
Appendix 1b X-ray diffraction pattern of Machinga cocoyam starch



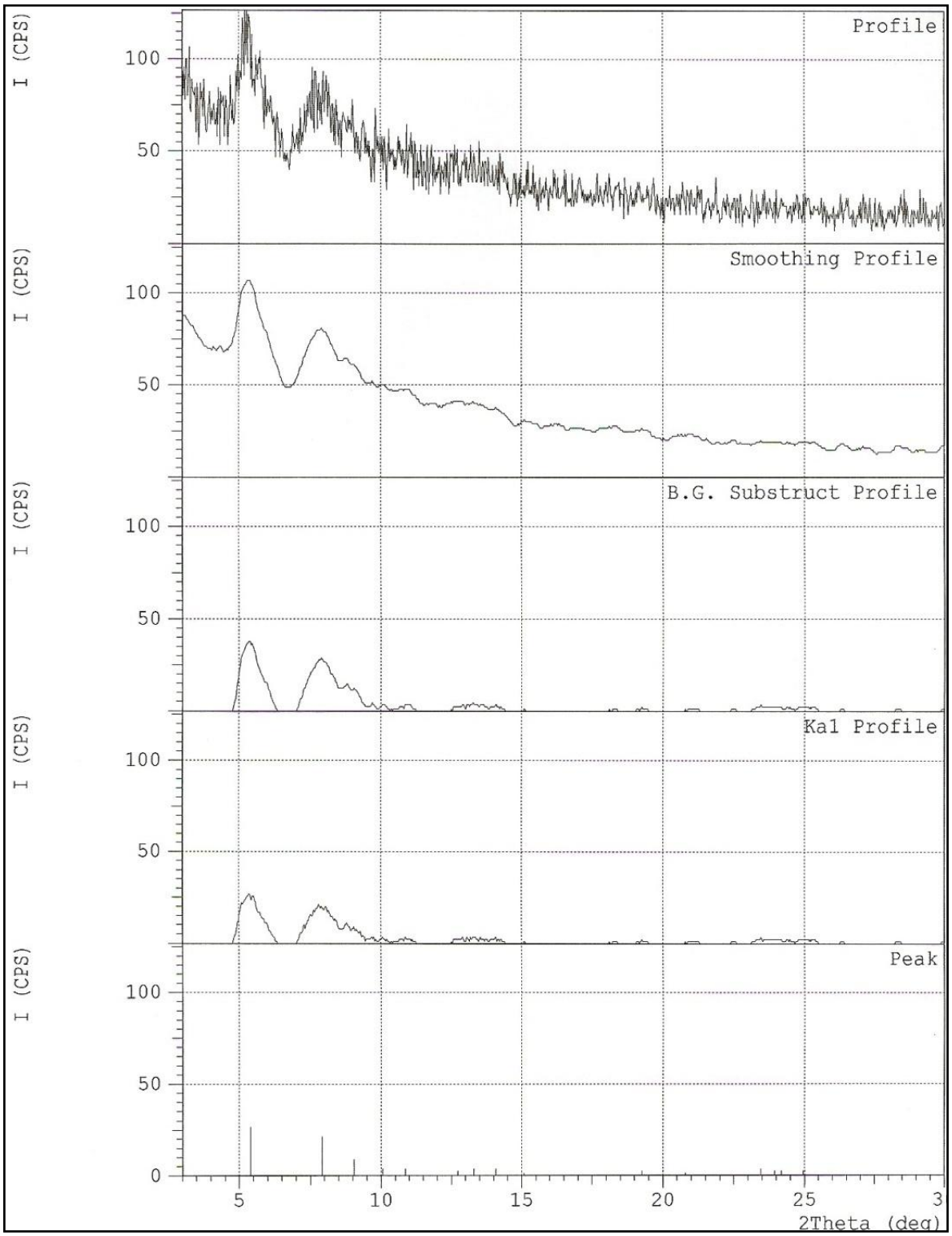
Appendix 1c X-ray diffraction pattern of Mulanje cocoyam starch



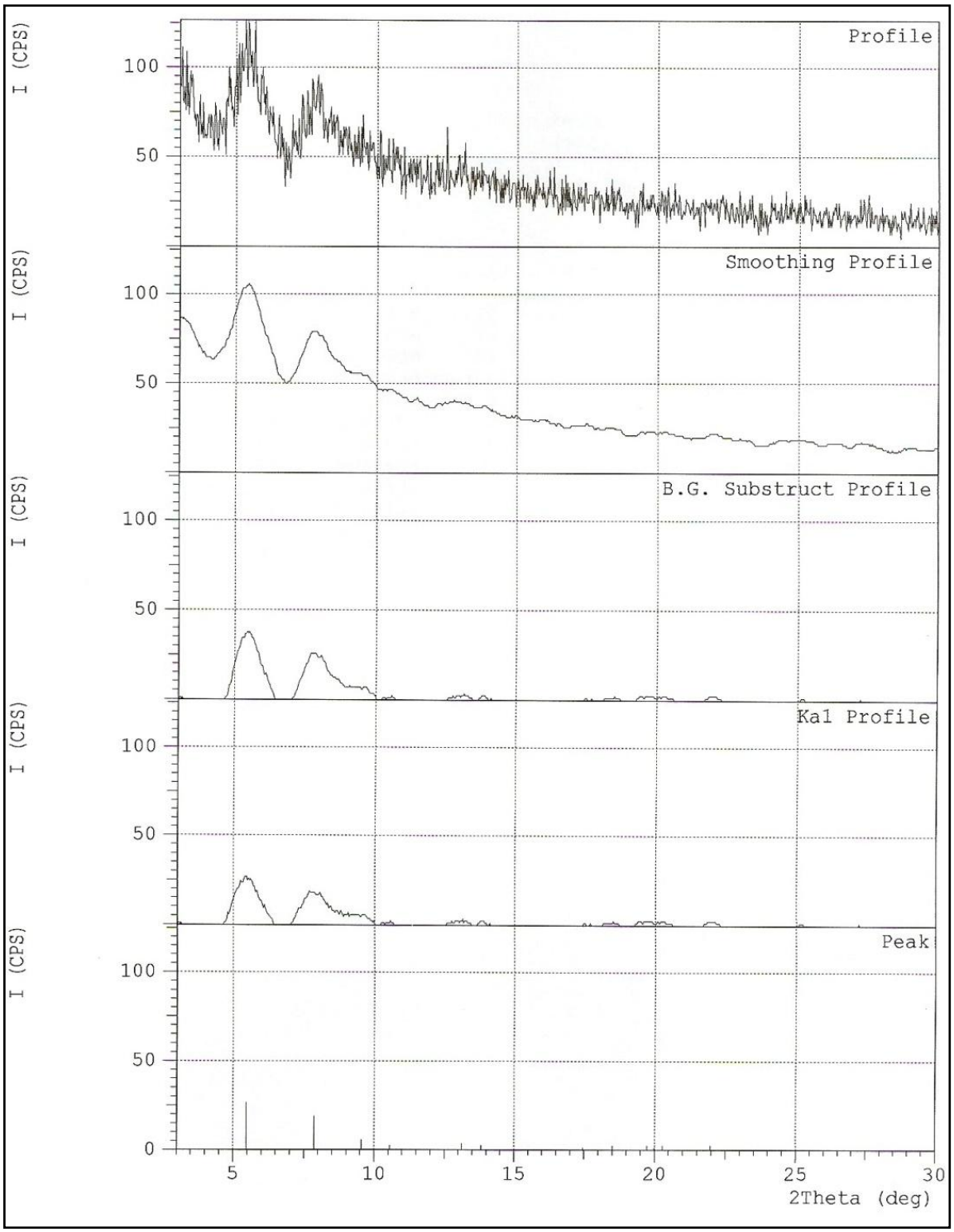
Appendix 1d X-ray diffraction pattern of Mzuzu cocoyam starch



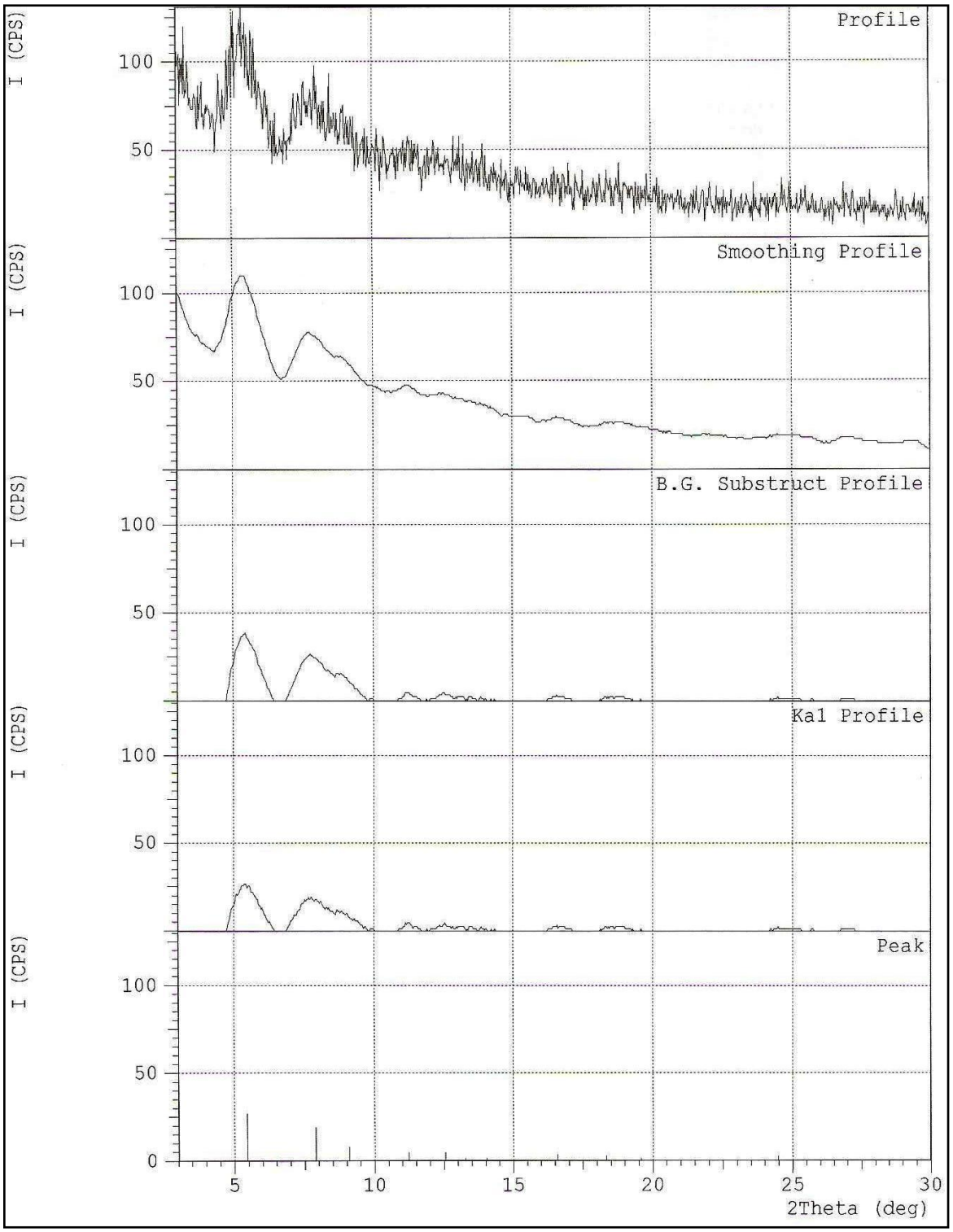
Appendix 1e X-ray diffraction pattern of Nkhotakota cocoyam starch



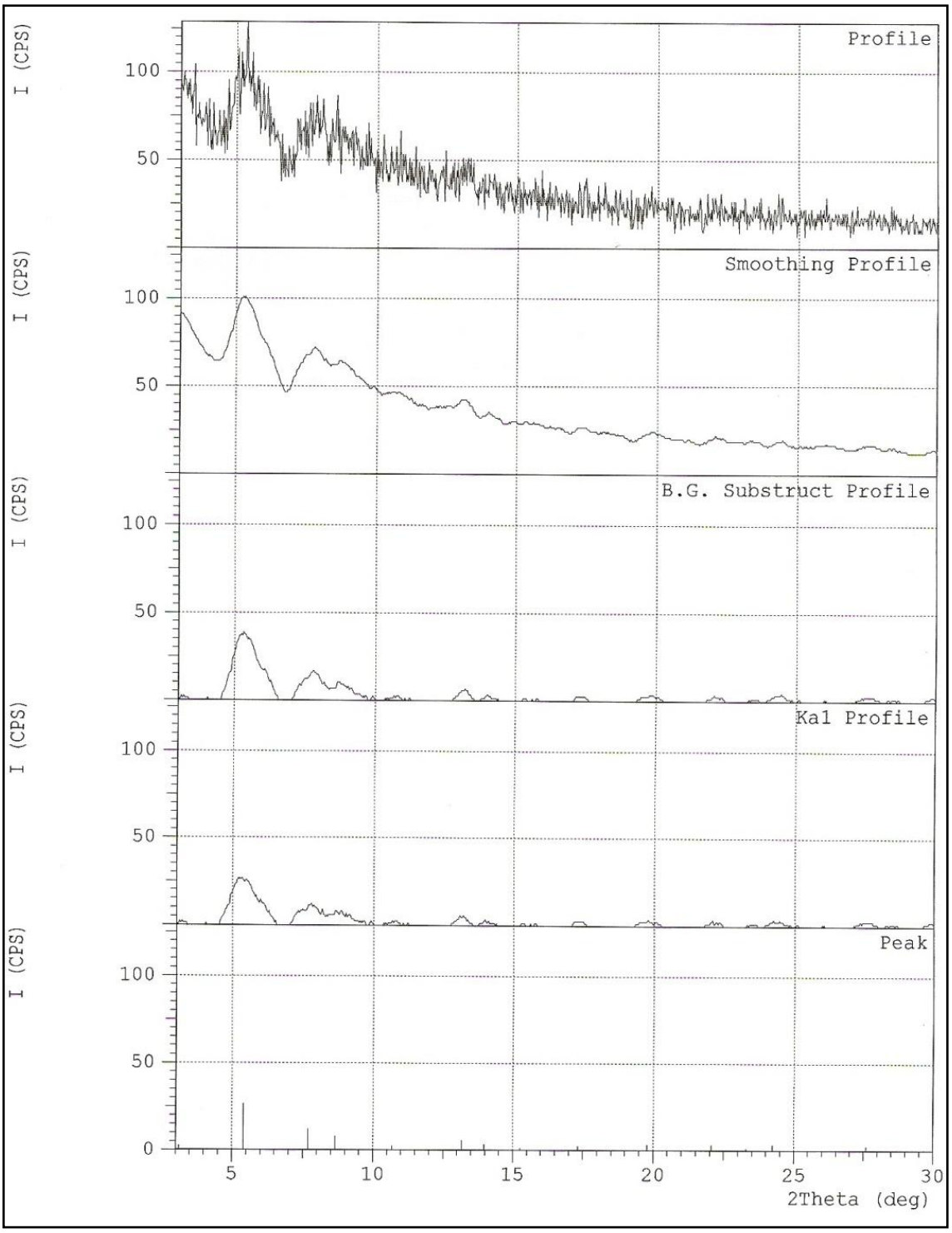
Appendix 1f X-ray diffraction pattern of Thyolo cocoyam starch



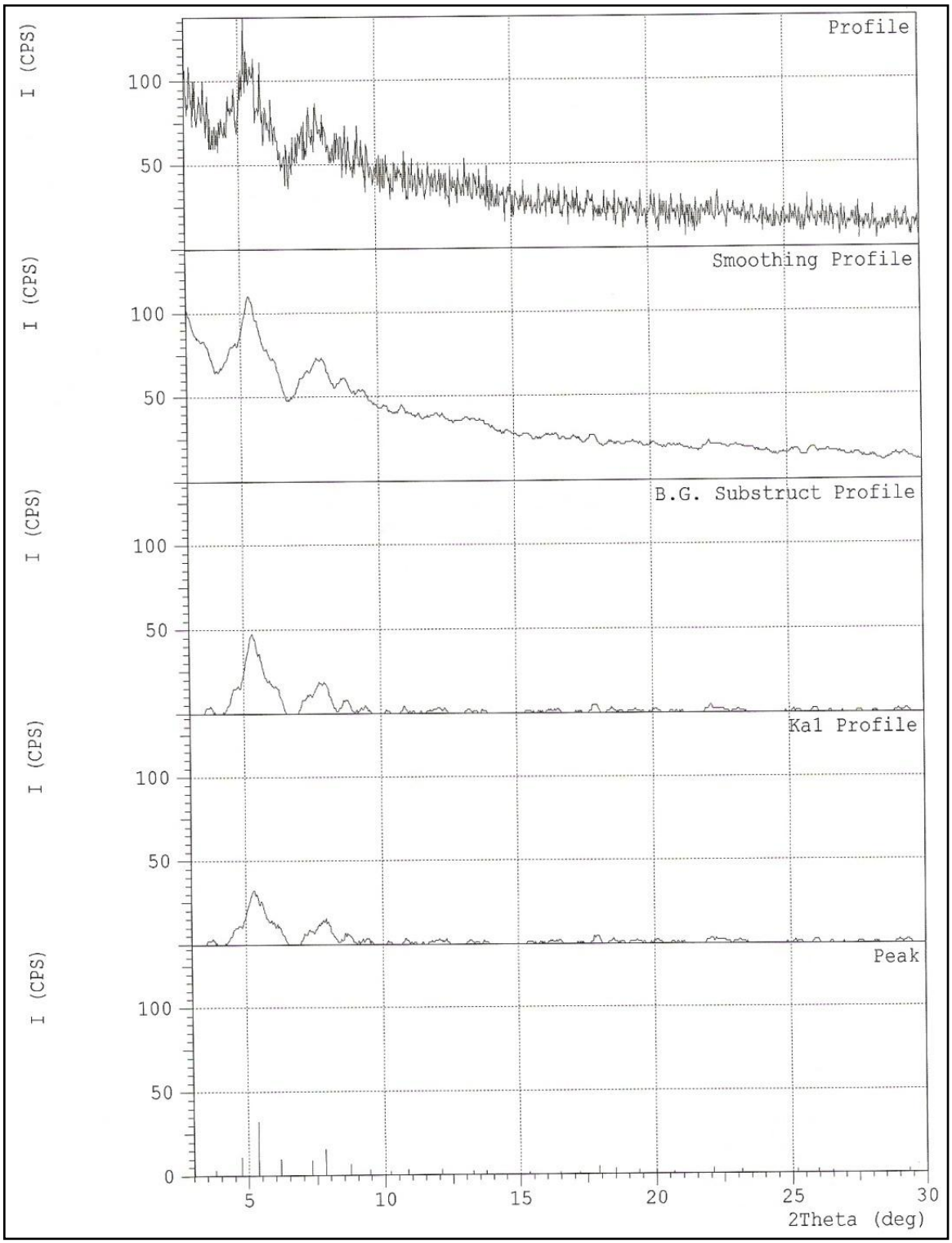
Appendix 1g X-ray diffraction pattern of Zomba cocoyam starch



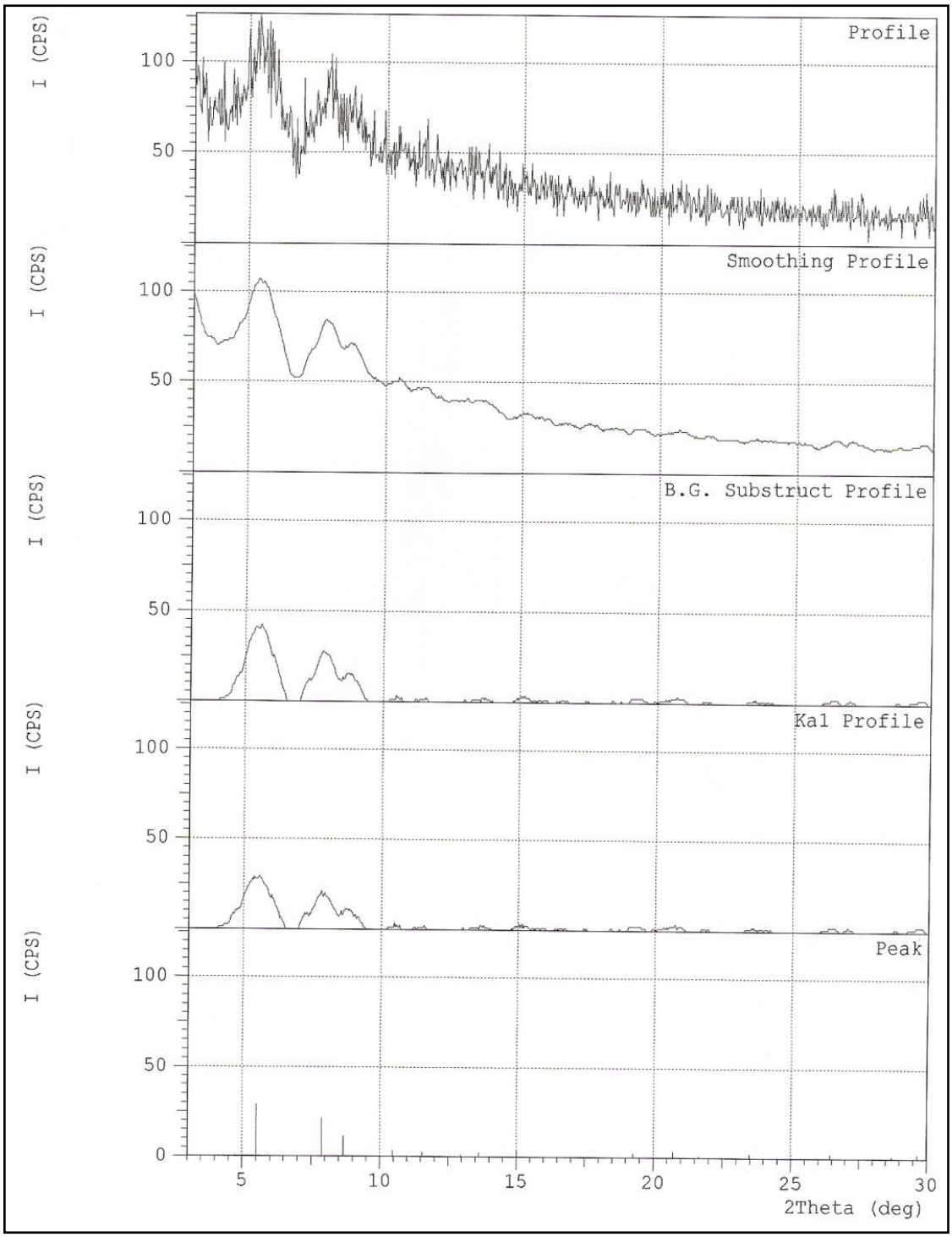
Appendix 2a X-ray diffraction pattern of A45 sweetpotato starch



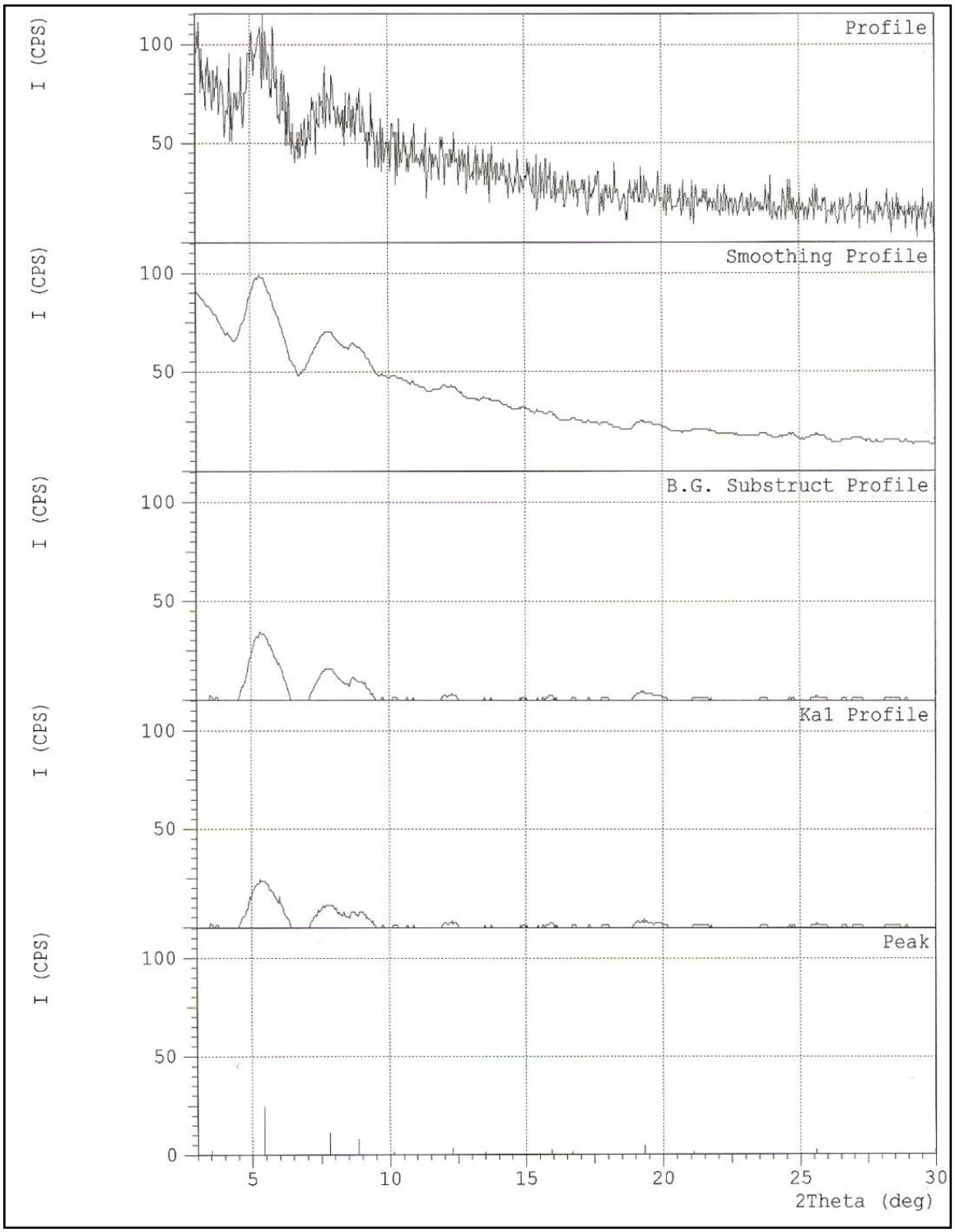
Appendix 2b X-ray diffraction pattern of Babache sweetpotato starch



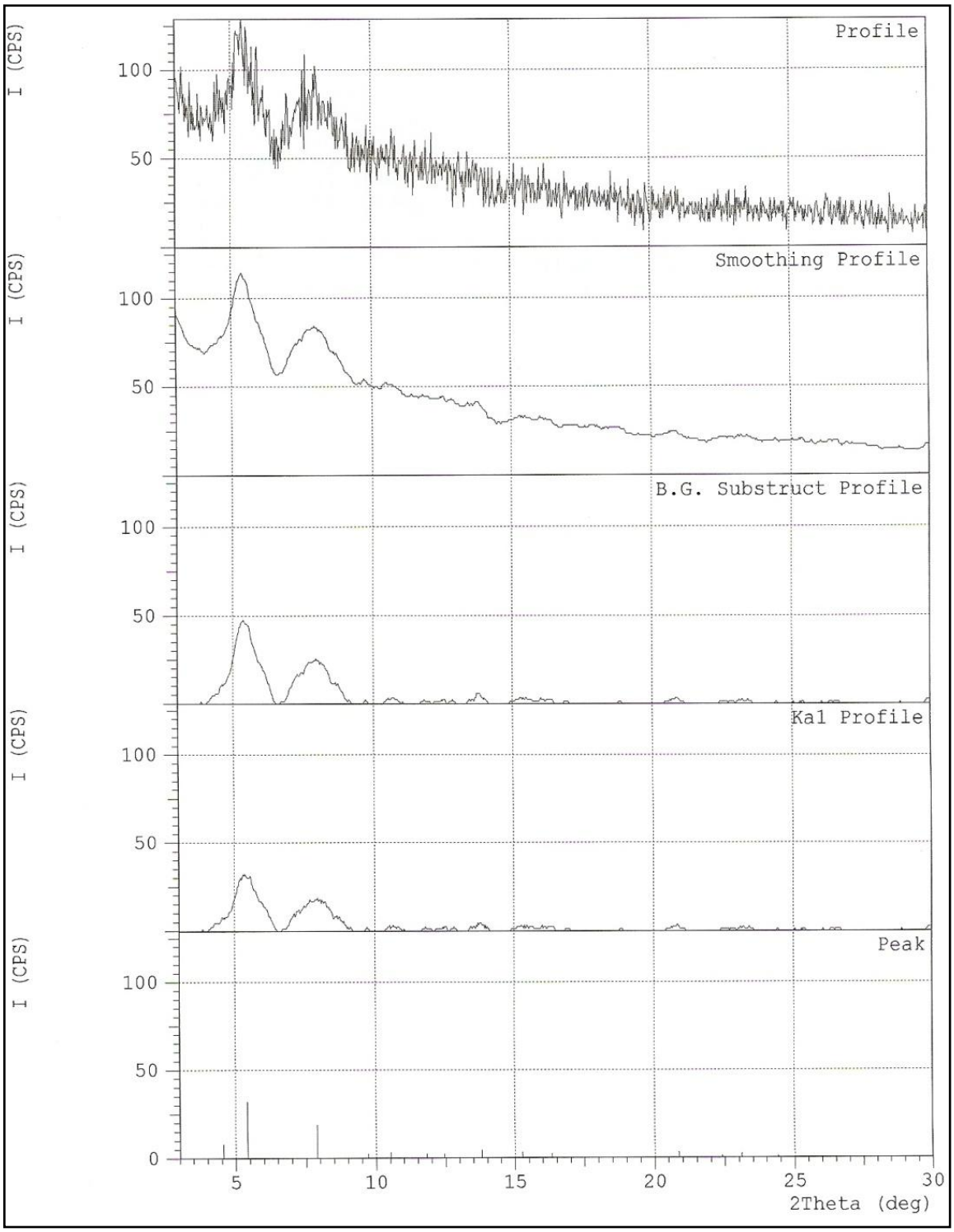
Appendix 2c X-ray diffraction pattern of Kakoma sweetpotato starch



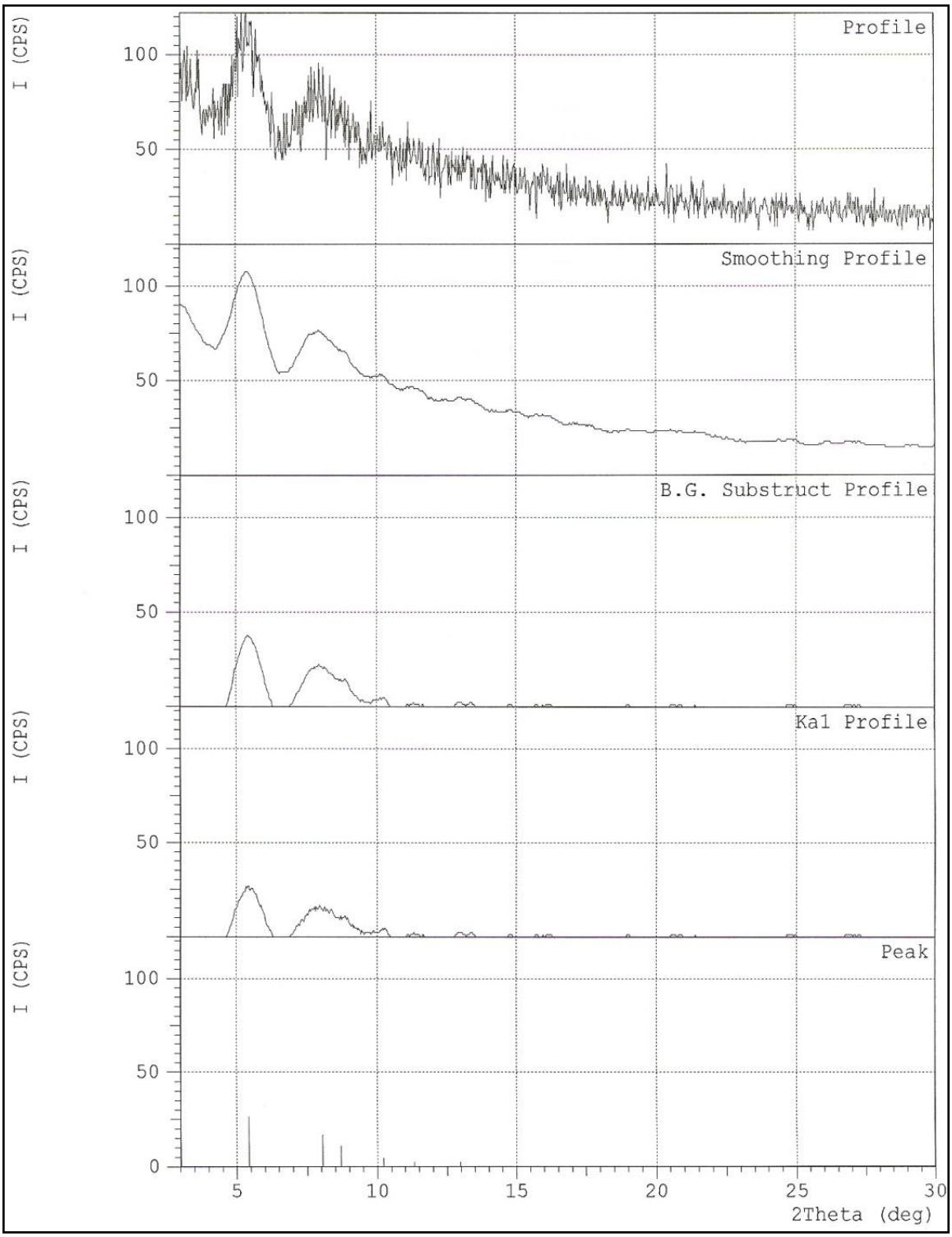
Appendix 2d X-ray diffraction pattern of Kamchiputu sweetpotato starch



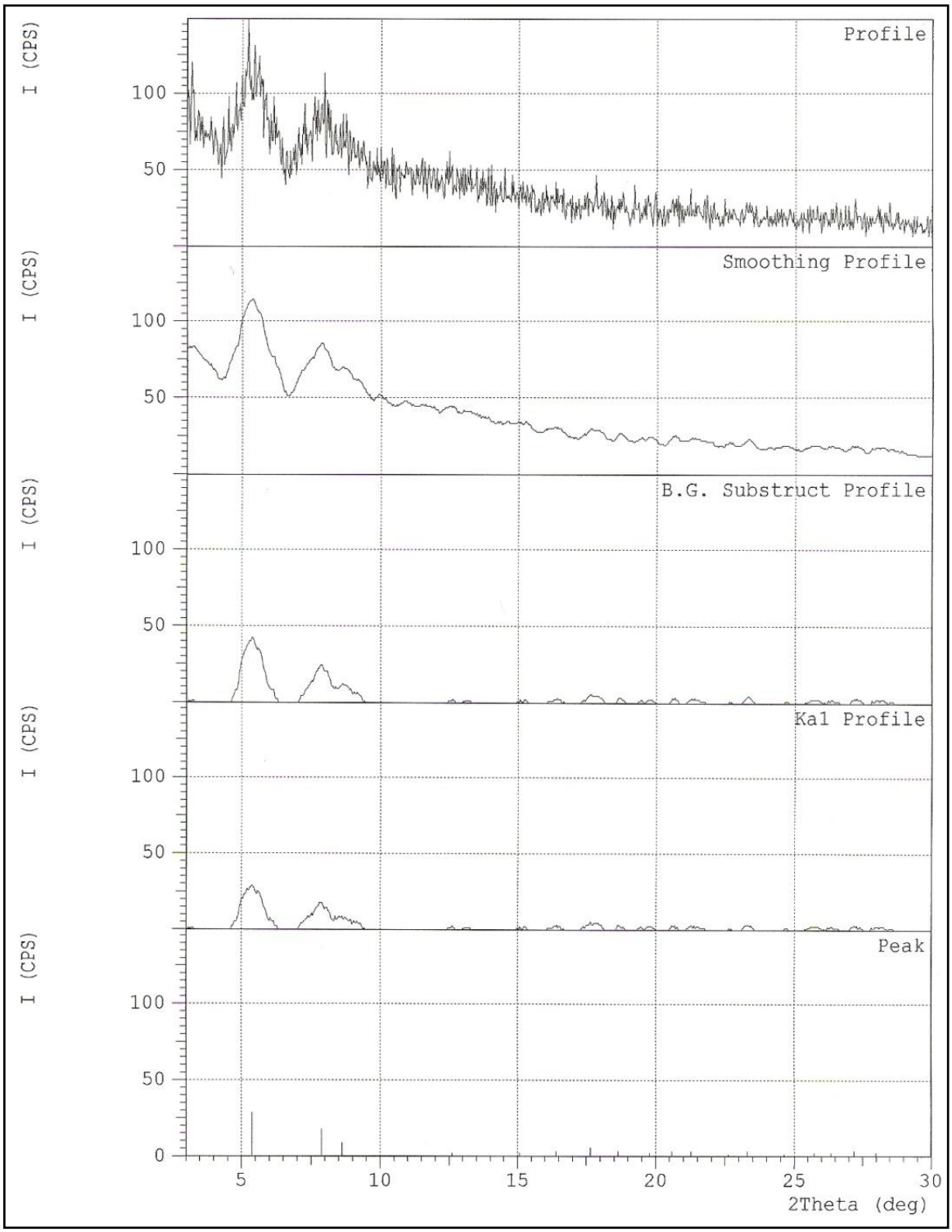
Appendix 2e X-ray diffraction pattern of Kenya sweetpotato starch



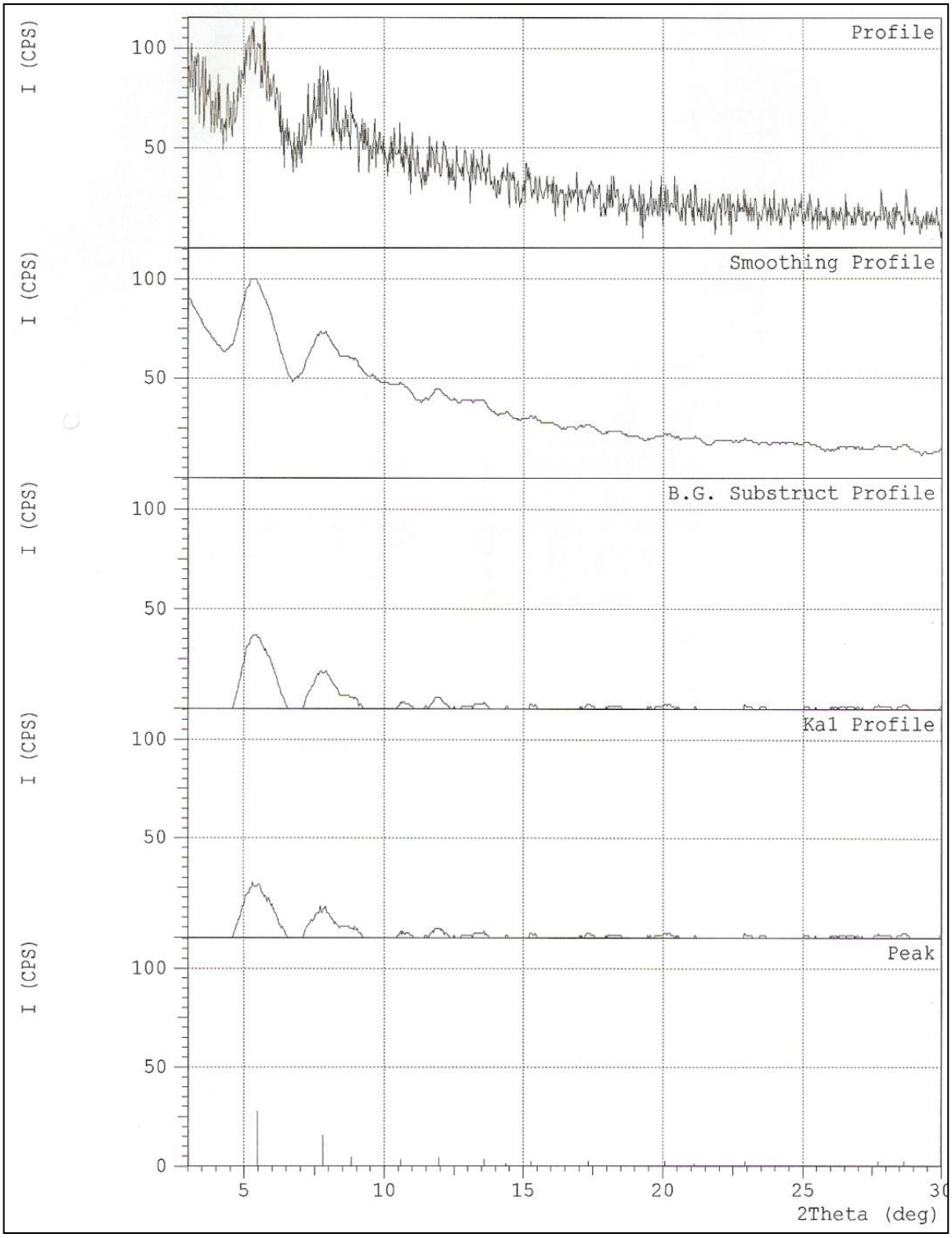
Appendix 2f X-ray diffraction pattern of LU96/303 sweetpotato starch



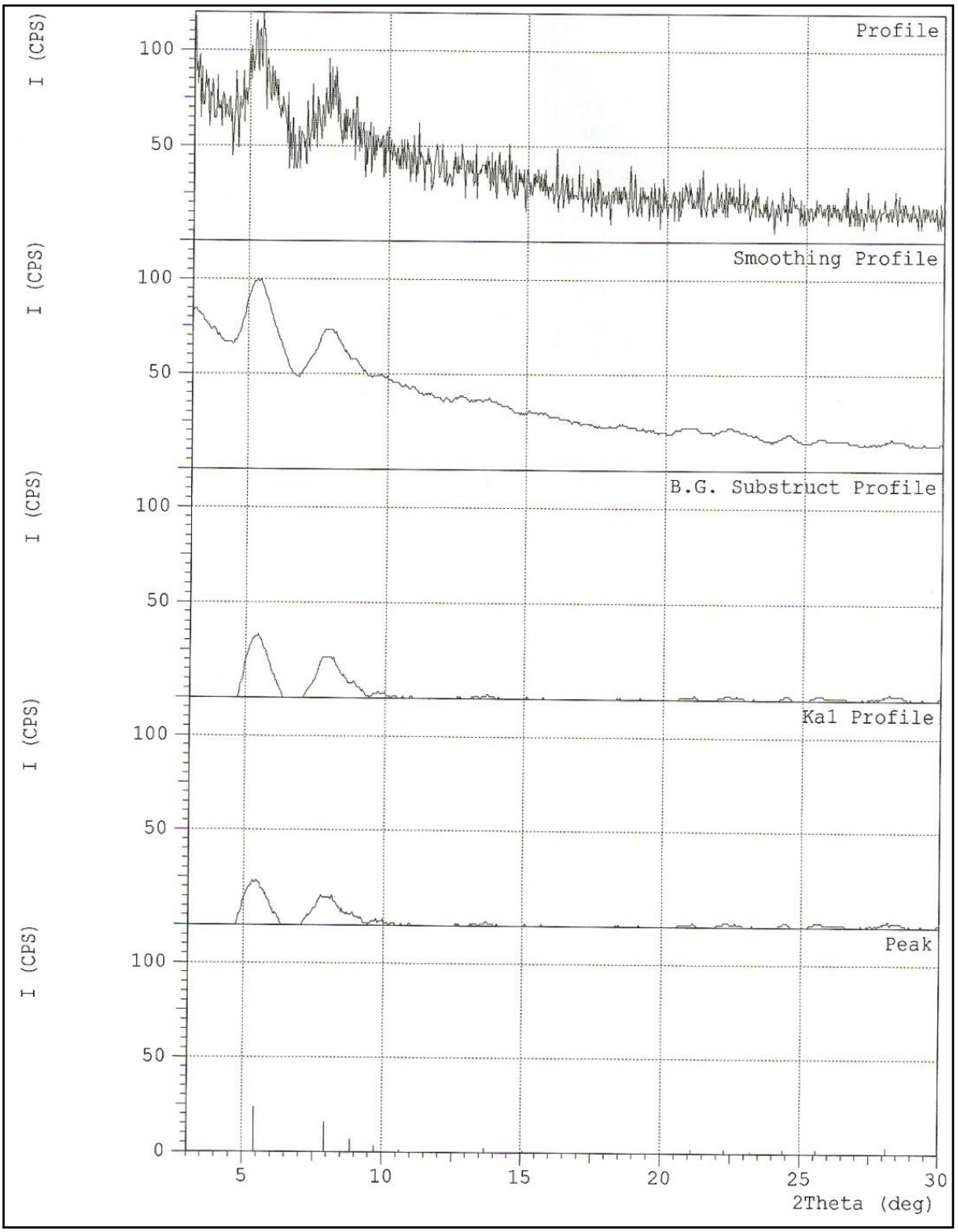
Appendix 2g X-ray diffraction pattern of LU96/304 sweetpotato starch



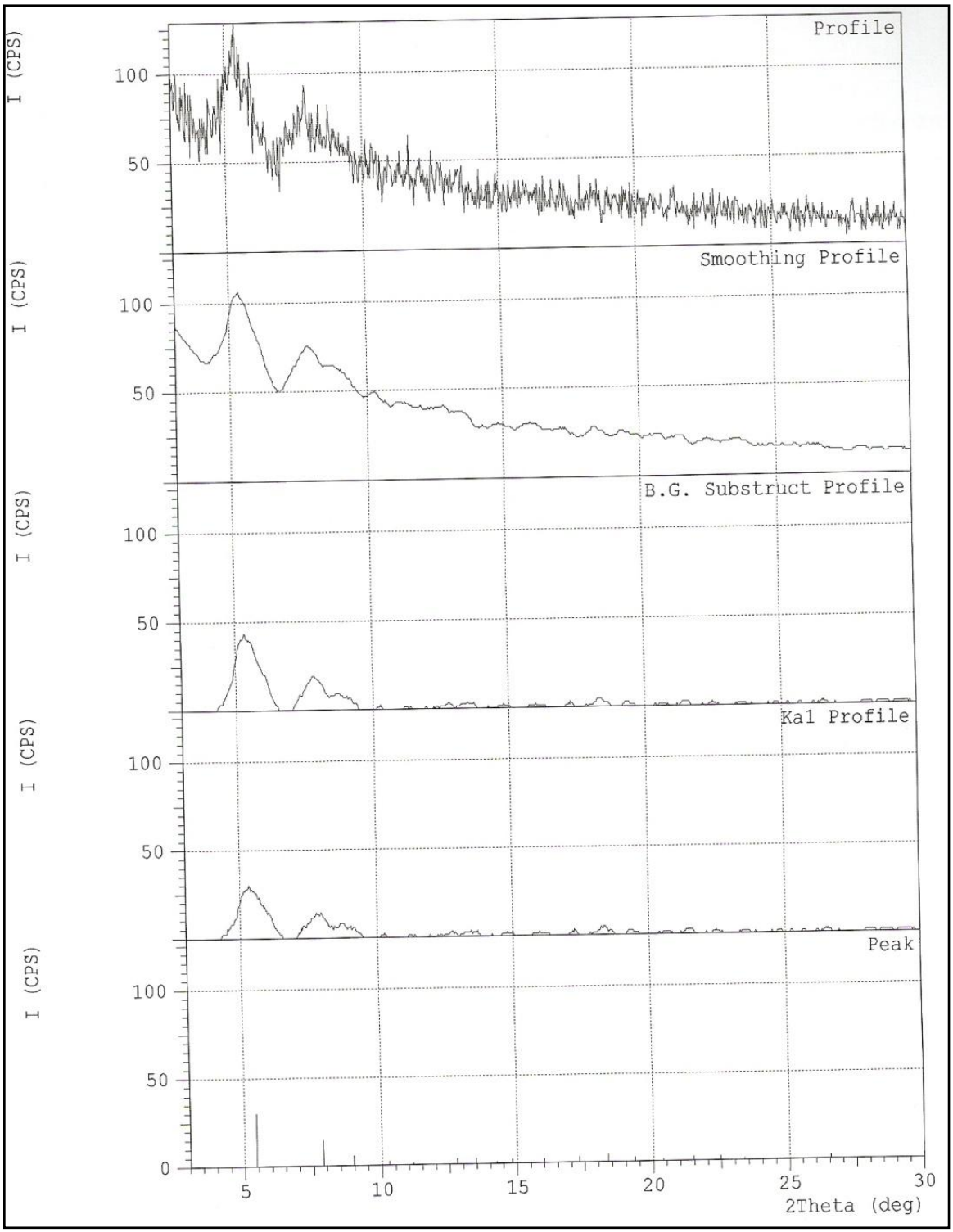
Appendix 2h X-ray diffraction pattern of Lunyangwa sweetpotato starch



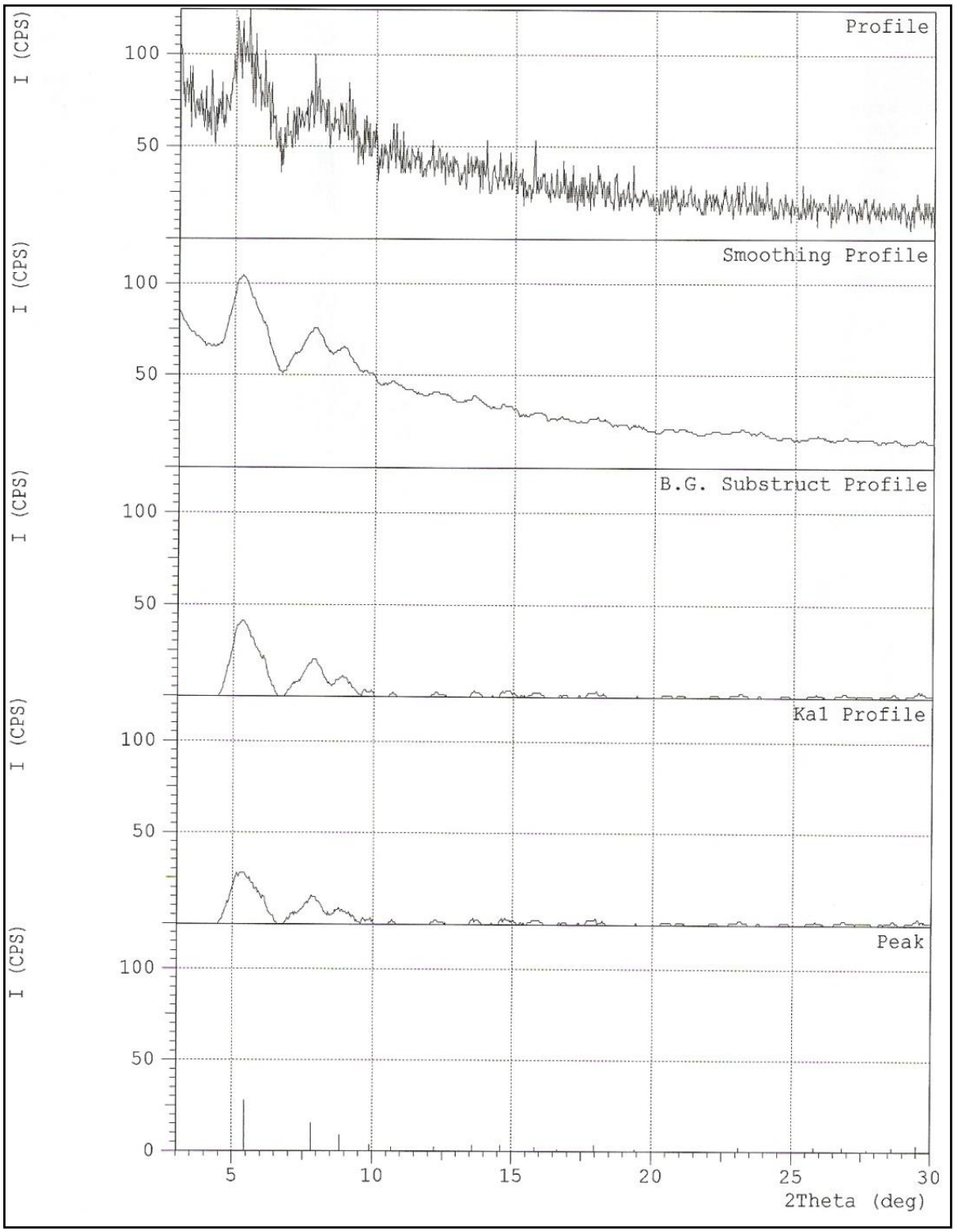
Appendix 2i X-ray diffraction pattern of Mafutha sweetpotato starch



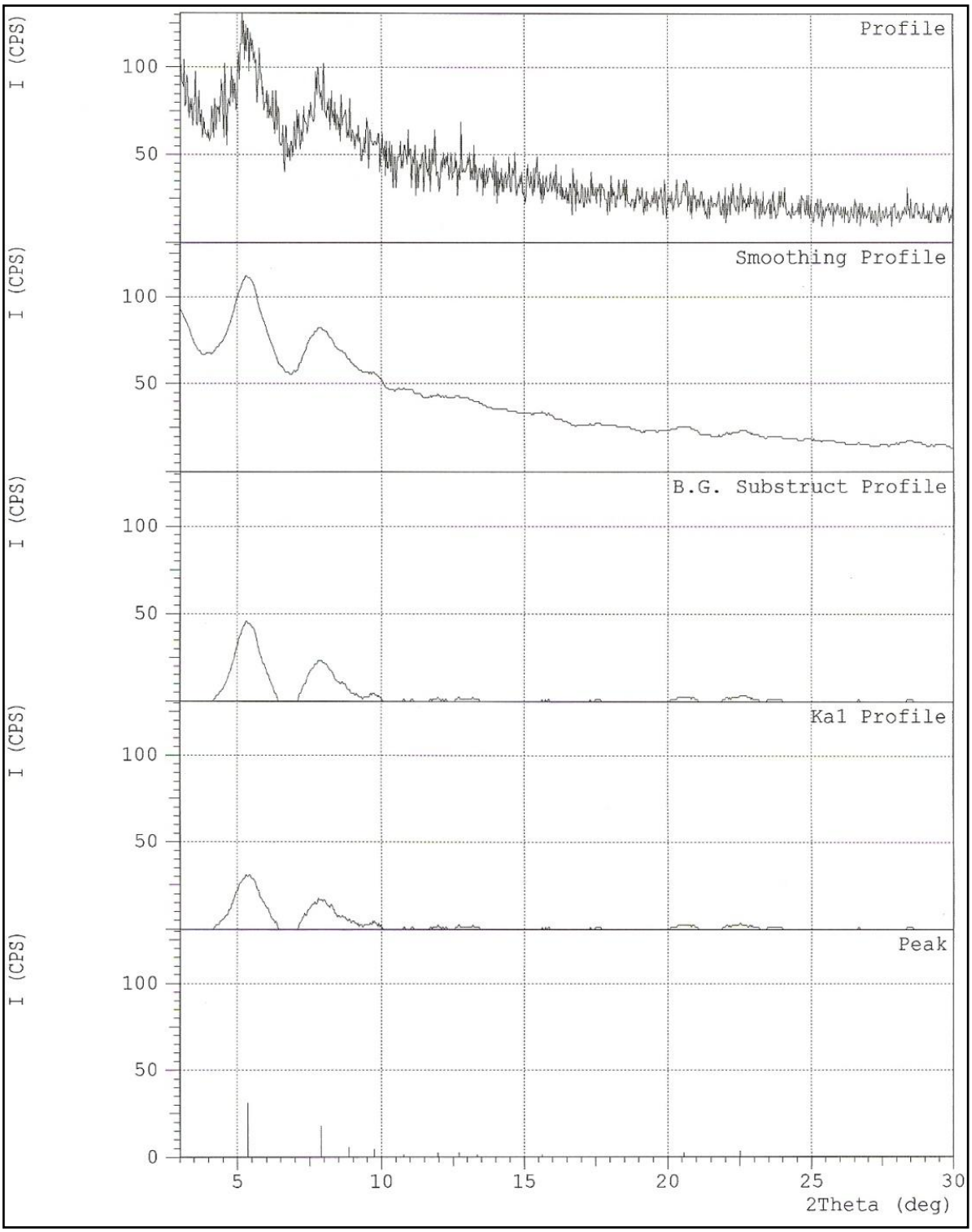
Appendix 2j X-ray diffraction pattern of Mugamba sweetpotato starch



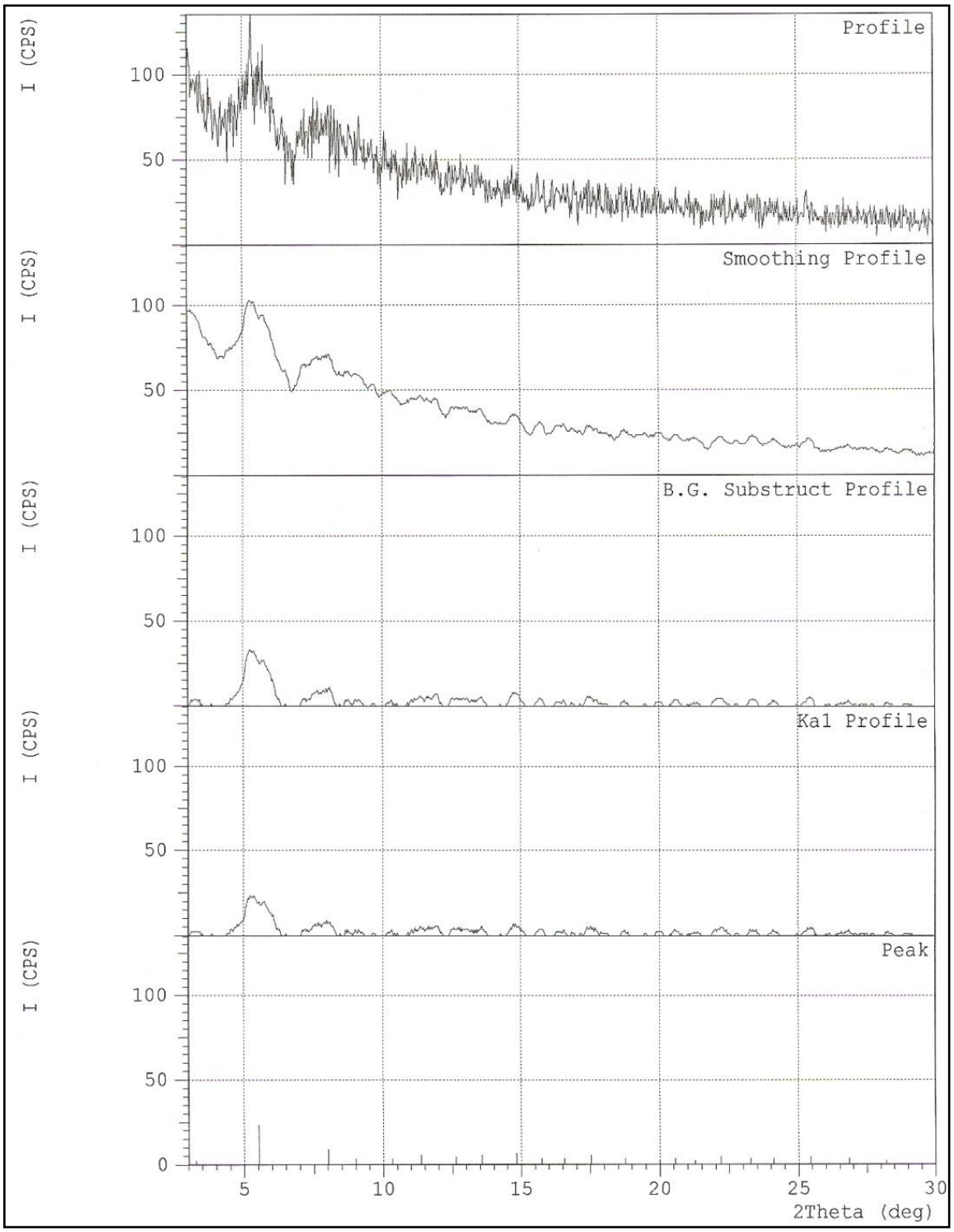
Appendix 2k X-ray diffraction pattern of Mugande sweetpotato starch



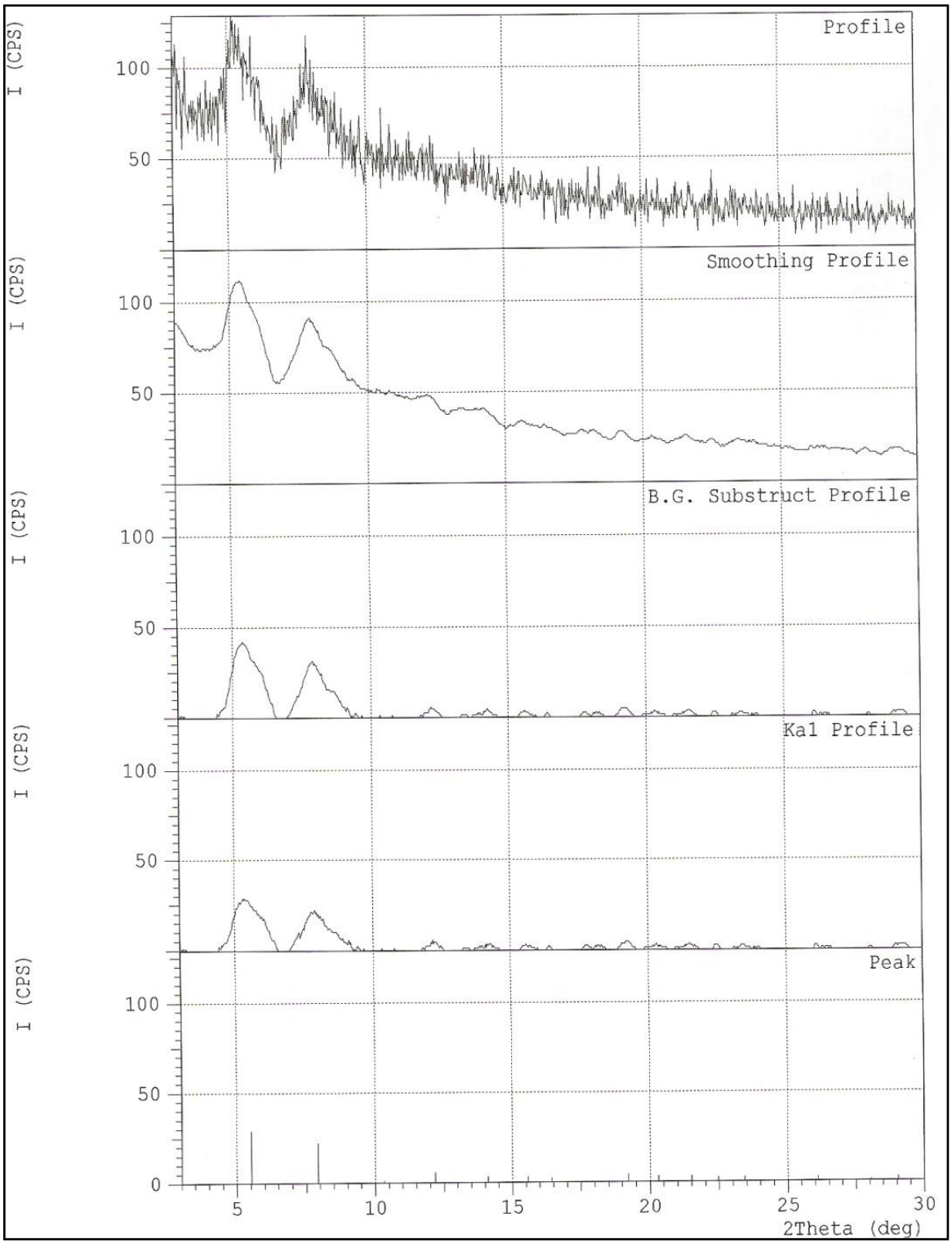
Appendix 2l X-ray diffraction pattern of Salera sweetpotato starch



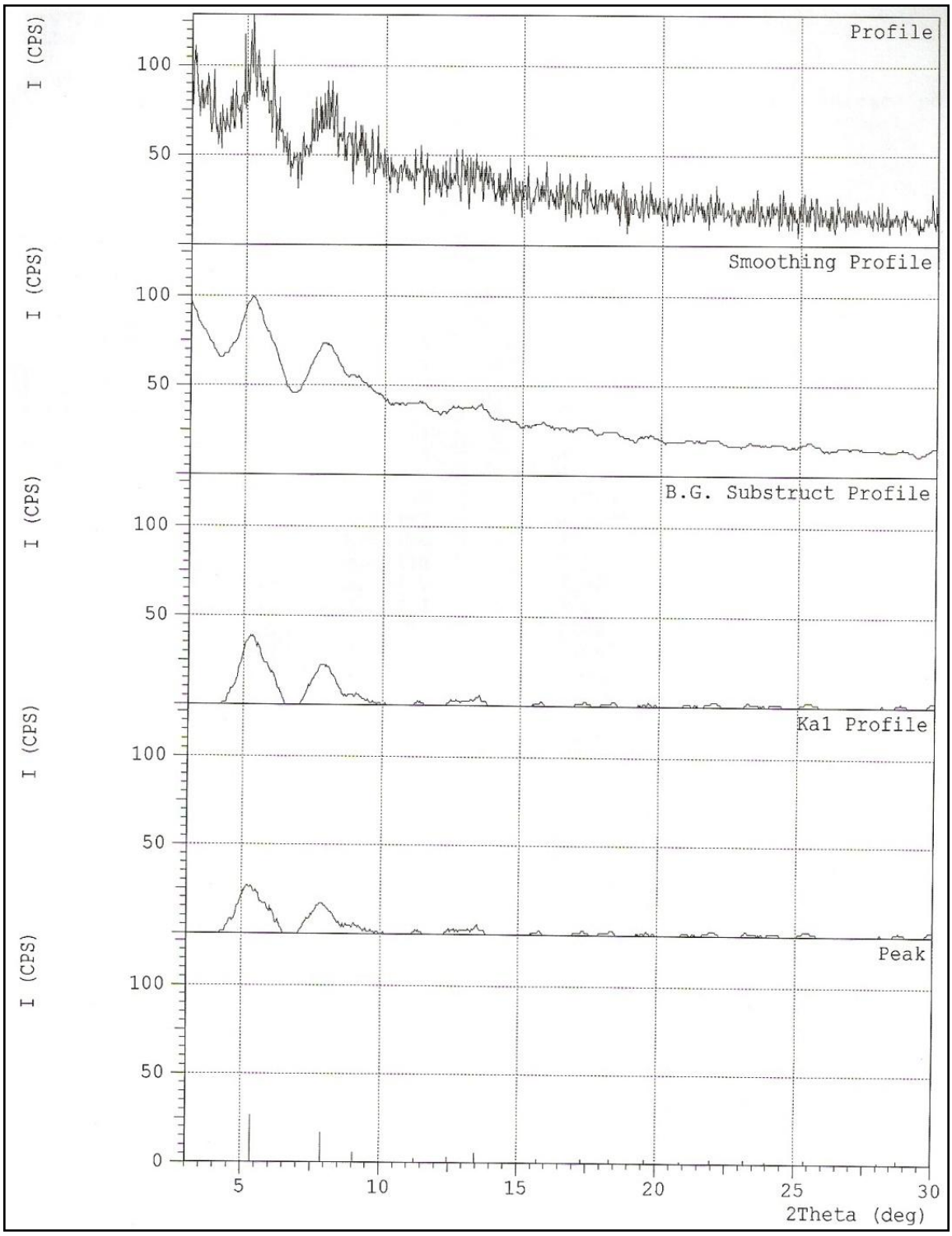
Appendix 2m X-ray diffraction pattern of Semusa sweetpotato starch



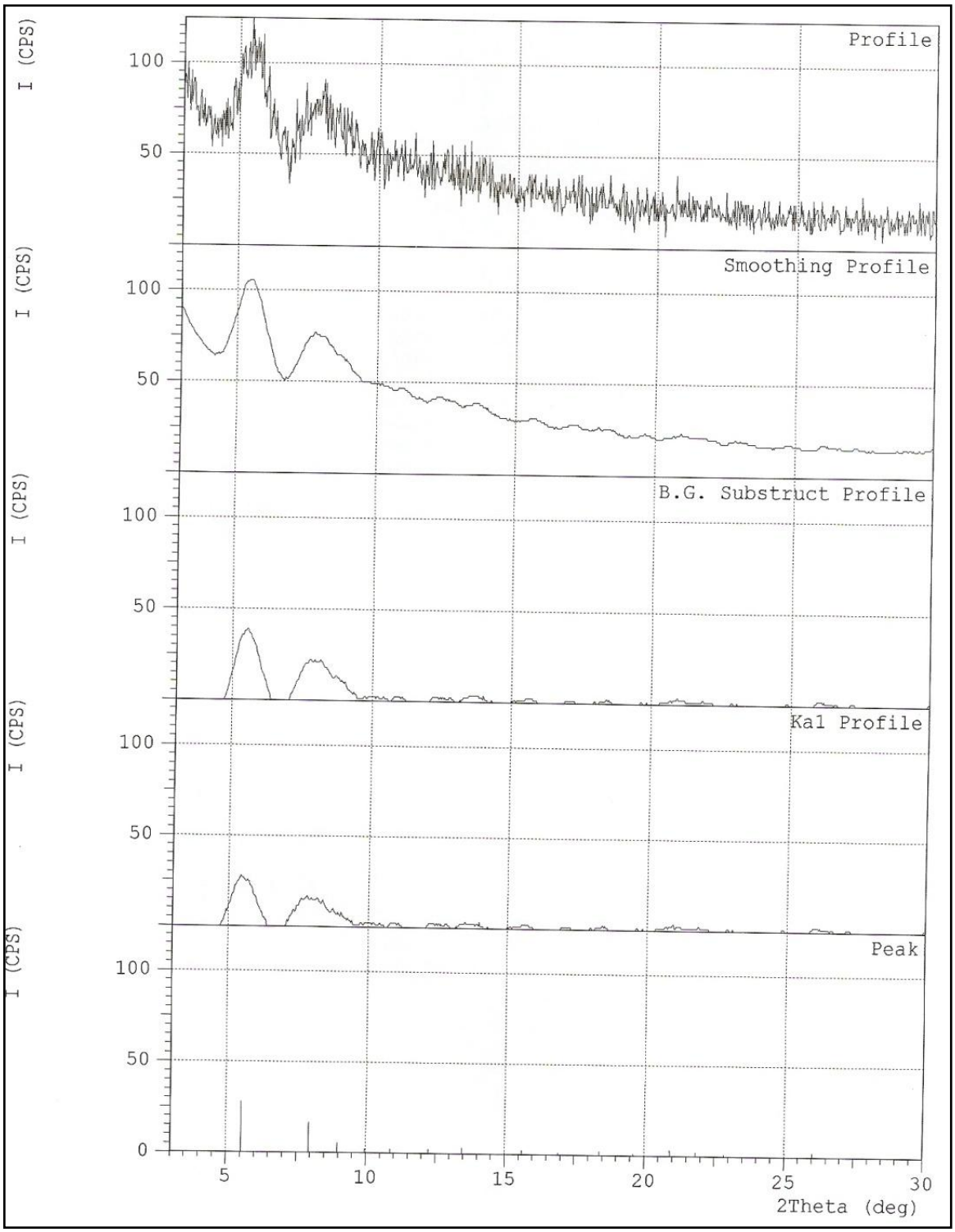
Appendix 2n X-ray diffraction pattern of Tainoni sweetpotato starch



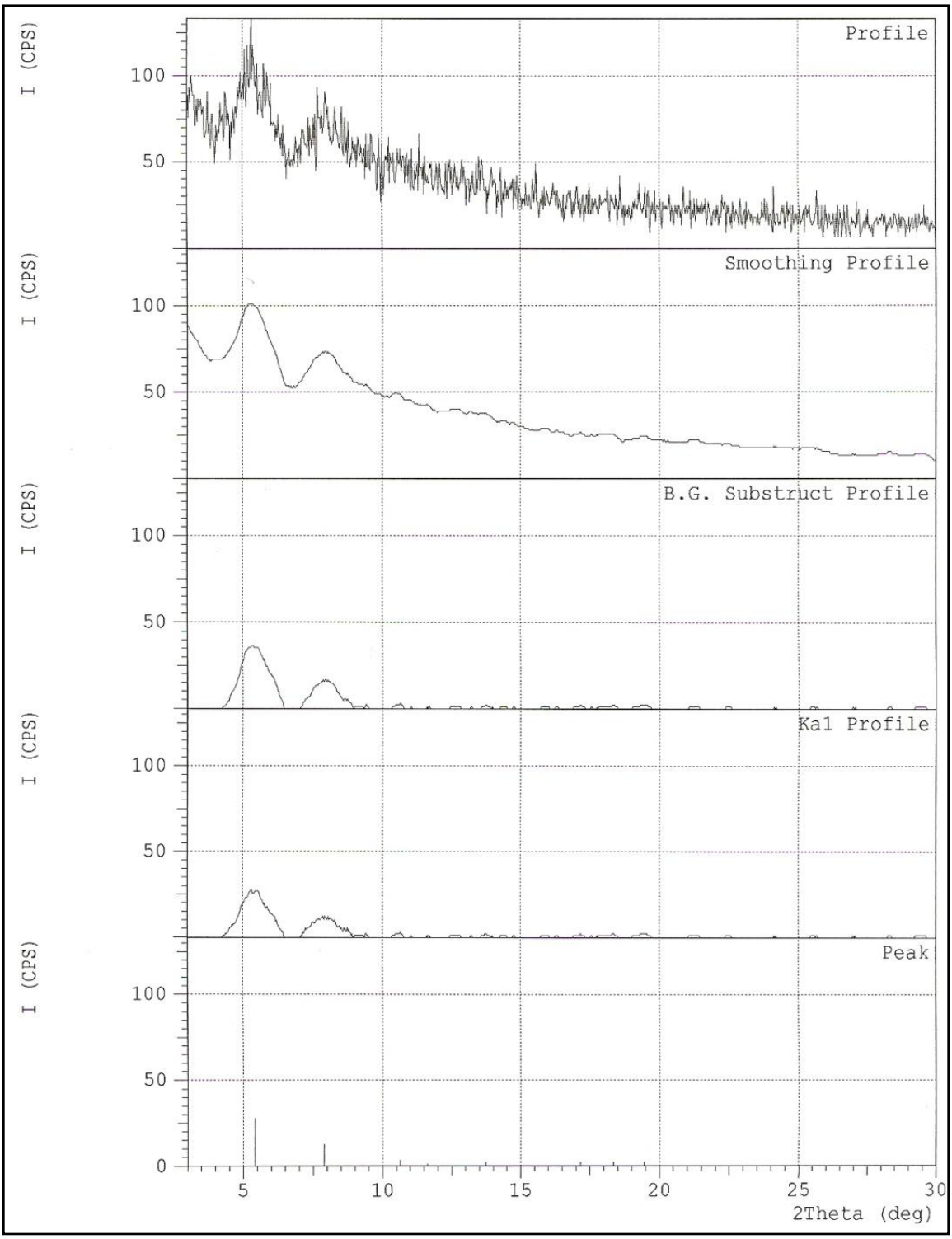
Appendix 2o X-ray diffraction pattern of Zondeni sweetpotato starch



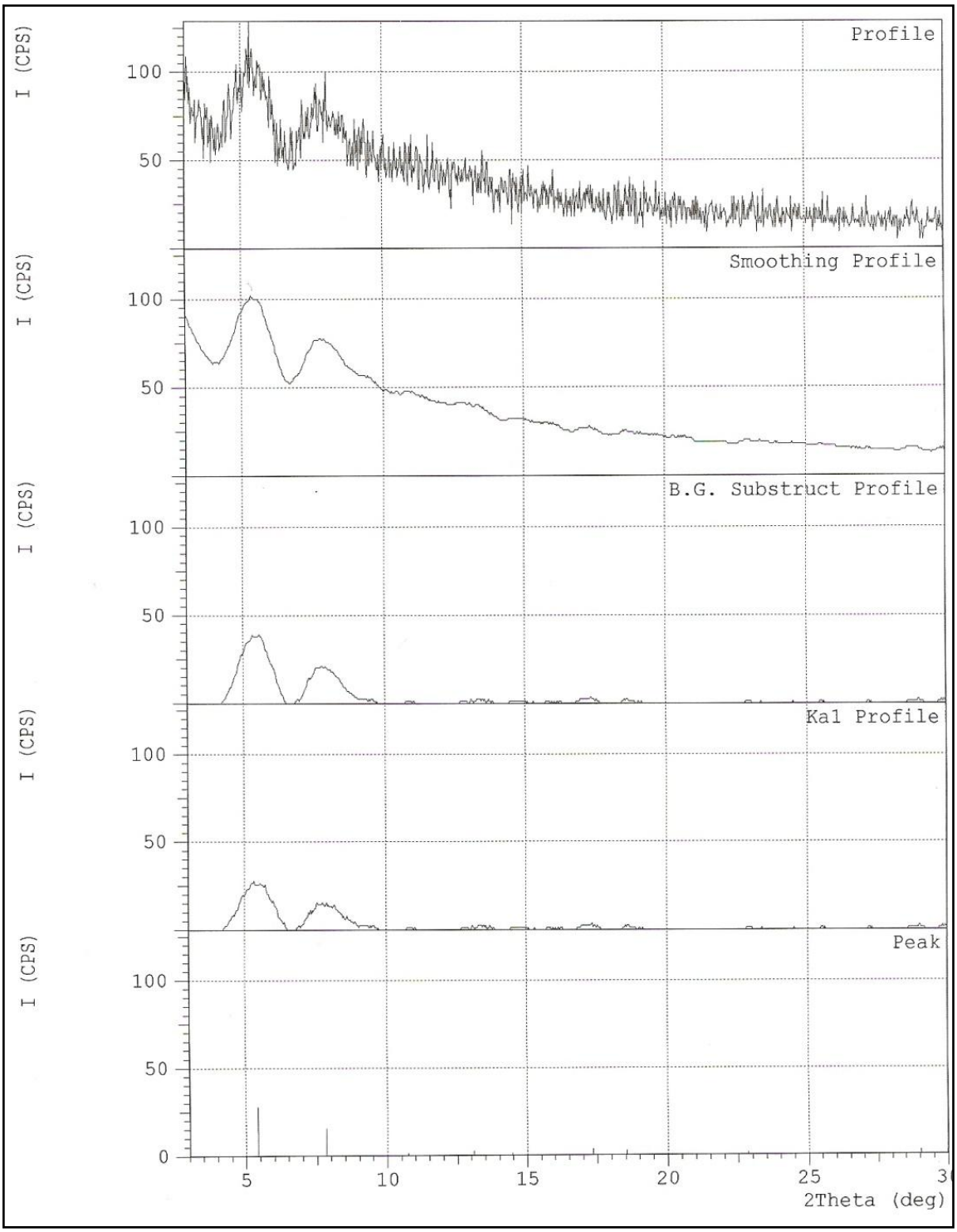
Appendix 3a X-ray diffraction pattern of Gomani cassava starch



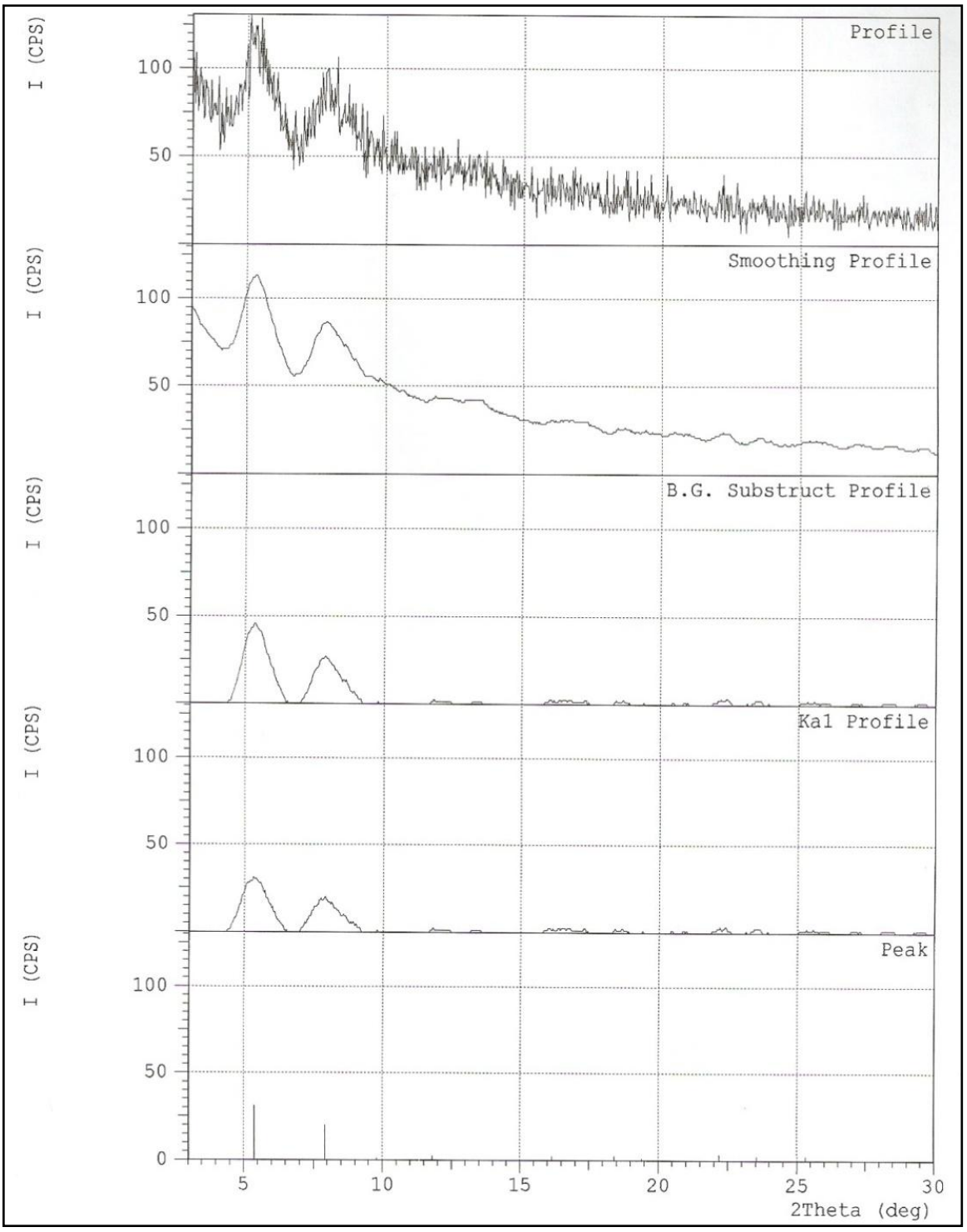
Appendix 3b X-ray diffraction pattern of Maunjili cassava starch



Appendix 3c X-ray diffraction pattern of Mbundumali cassava starch



Appendix 3d X-ray diffraction pattern of Mkondezi cassava starch



Appendix 3e X-ray diffraction pattern of Sauti cassava starch