INFLUENCE OF KEY CHEMICAL COMPONENTS ON HARDNESS OF INDIGENOUS COOKING AND JUICE BANANA CULTIVARS FROM UGANDA

Samuel GAFUMA
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Abstract

Bananas undergo significant postharvest losses at the farm, during handling, cooking and serving. Loss of palatability and food mass occurs during serving and consumption due to hardening of cooked banana texture. Texture affects sensory properties of bananas and is very important in determining a good cooked banana. Different banana cultivars differ in hardness and the causes for these differences are not well studied particularly among indigenous cooking and juice banana cultivars grown in Uganda.

This study examined influence of key chemical components on hardness of bananas. A total of eleven (11) cooking and three (3) juice banana cultivars grown in and endemic to Uganda, were selected as a case study. Bananas were harvested and used at green mature unripe stage. The samples were analyzed for starch, pectic substances and proximate composition using standard methods, and subsequently assessed the physico-chemical properties of starch and the pectic substances which were the major components. Hardness profile of the 14 banana cultivars was assessed using a texture analyzer. Principle Component Analysis (PCA) revealed that starch and pectic substances were highly and positively correlated with hardness of raw bananas. Then, the role of starch and pectin in influencing hardness of cooked bananas was determined. Sliced bananas were separately treated with pectin, starch and starch-pectin composite before being cooked by steaming combined with mashing and their hardness determined. Pectin was also structurally hydrolyzed from sliced bananas using pectinase (polygalacturonase) and the resulting bananas were cooked in the same way and evaluated for changes in hardness.

According to results, water, starch, and pectic substances were the main components of fresh green mature bananas. Juice bananas yielded significantly more dry matter (30 to 33%) than cooking bananas ($P<0.05$) which yielded 19 - 25.6% dry matter. Juice bananas yielded significantly more starch (19 to 25%) than cooking banana cultivars ($P<0.05$) which yielded (4 to 15.2% except one cultivar at 21%). Juice banana cultivars also yielded significantly more crude pectic substances (29.7 to 30.5%) than cooking banana cultivars ((7.5 to 21%) ($P<0.05$). Other chemical components examined included crude ash (1.97 to 4.24%), crude fibre (0.251 to 0.478%), crude protein (1.23 to 5.64%) and crude fat (0.15 to 0.58%) which did not differ significantly between cooking and juice banana cultivars ($P>0.05$). The physico-chemical properties (i.e. swelling power, solubility, amylose & resistant starch content and pasting

properties) of banana starch did not differ significantly between cooking and juice banana cultivars ($P>0.05$). However, both amylose and resistant starch were generally higher in juice bananas relative to cooking bananas. Similarly, the chemical properties of pectic substances did not differ significantly between the two banana cultivars and were all characterized by a high degree of esterification (DE) ranging between 88 and 96%.

Texture analysis indicated that juice bananas were significantly harder than cooking bananas ($P<0.05$) in raw, cooked and cooled forms regardless of cooking treatment. Hardness of raw juice bananas ranged between 36.17 to 42.43 N while that of cooking bananas ranged between 22.37 to 26.36 N. Upon cooking, all bananas rapidly softened in the first 30 min followed by a gradual decrease in hardness over the next 100 min of cooking. Upon cooling, texture of all cooked bananas significantly hardened ($P<0.05$) being rapid in the first 1 h of cooling and gradual thereafter.

Added starch significantly increased hardness of bananas upon cooking and cooling relative to the control ($P<0.05$). Hardness increased with increasing starch concentration both during cooking and cooling with regression coefficients of 99% and 91% and coefficients of determination (r^2) of 98% and 83% respectively. However, added pectin did not significantly increase hardness of bananas upon cooking relative to the control ($P>0.05$) and exhibited a low regression coefficient of 43% and a coefficient of determination of 18.5%. However, upon cooling, added pectin decreased hardness of cooked bananas significantly with increasing pectin concentration ($P<0.05$) and exhibited a strong regression coefficient of 80% and a coefficient of determination of 64%. Structural hydrolysis of pectin using pectinase (polygalacturonase) resulted in significantly harder bananas during cooking and upon cooling ($P<0.05$) implying that pectin is responsible for tenderness in cooked bananas. Hardness of cooked bananas treated with a combination of starch and pectin increased but was not significantly different from the control. Upon cooling, hardness of cooked bananas with added starch-pectin composite decreased with increasing composite concentration similar to the effect of pectin when added alone.

Results imply that starch increases hardness in bananas and could be responsible for major differences in hardness between cooking and juice banana cultivars. Pectin apparently appears to contribute to tenderness of cooked bananas particularly upon cooling. Therefore, pectin can

be added and optimized between 1 and 5% to decrease hardness in cooked bananas. Differences in physico-chemical properties of banana starch and pectin were not major and may not significantly influence hardness of bananas. Results also imply that bananas should either be boiled or “steamed combined with mashing” for a softer texture and should be eaten immediately after serving while still hot to avoid hardening of the texture that occurs upon cooling. Juice bananas are not suitable for cooking because of their extremely hard texture.

Keywords: Chemical components, cooking bananas, hardness, juice bananas, pectin, starch, texture.

Declaration by candidate

This PhD Thesis is my original work and has not been presented to any other University or Institution of higher learning for any degree award.

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Samuel Gafuma Date

Reg. No. 14/U/14225/GDFT/PE

Declaration by supervisors

We confirm that the work in this Thesis was done by the candidate under our supervision.

For and on behalf of Kyambogo University

1) …… ……. ……

Dr. George William Byarugaba-Bazirake (Ph.D) Date

Assoc. Professor,

Department of Food Technology,

Faculty of Science, Kyambogo University

2) …… ……. ……

Dr. Mugampoza Dirrisa (Ph.D) Date

Senior Lecturer,

Department of Food Technology,

Faculty of Science, Kyambogo University

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Dedication

I dedicate this work to my three sons Ivan Mulema, Ian Ssempijja and Sam Kasiita whose physical and spiritual presence gave me the drive to keep going till completion. May the Great Lord guide you in pursuing your dreams far and beyond my personal achievements. I love you so much.

I also dedicate this work to all those individuals and organizations involved in banana research in their various endeavors. This work serves as a foundation for those wishing to find solutions to the hardening phenomenon in cooked bananas and developing the much needed innovative banana products.

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LIST OF ACRONYMS

General acronyms

AAA-EA	East African Highland bananas of *Musa Acuminata* A triploid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BC	Before Christ
BCE	Before Common Era
CB	Cooking bananas
DE	Degree of Esterification
DM	Degree of methylation
EA	East Africa
FAO	Food and Agricultural Organization of the United Nations
FCNT	Food Composition and Nutrition Tables
FT	Feet
h	Hour(s)
HG	Homogalacturonan
HM	High methoxyl
IFTNet	International Tropical Fruit Network
INFOCOM	Market Information on Agricultural Commodities
JB	Juice bananas
LM	Low methoxyl
LSD	Least Square Difference
M.	*Musa*
MAE	Microwave Assisted Extraction
Mg/ml	Milligrams per milliliter

MT	Metric tones
N	Newtons
n	Chromosome number
Nc	Number of cooking banana samples
Nj	Number of juice banana samples
^{o}C	Degrees Celsius
^{o}E	Degrees East
^{o}F	Degrees Fahrenheit
OH	Hydroxyl group
^{o}N	Degrees North
^{o}W	Degrees West
RG	Rhamnogalacturonan
RTE	ready-to-eat
UNCTAD	United Nations Conference on Trade and Development
US $	United States Dollars

Acronyms of banana samples used

KAY	Kayinja
KAZ	Kazirakwe
KIB	Kibuzi
KIS	Kisansa
KISB	Kisubi
MPO	Mpologoma
MUS	Musakala
NAKB	Nakabululu
NAKT	Nakitembe

NAKW	Nakawere
NAKY	Nakyetengu
NAMD	Namande
NAMZ	Namweezi
NDI	Ndiizi

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Bananas belong to the family *Musaceae* and genus *Musa* (Pereira & Maraschin, 2015; Janssens, et al., 2016) and are considered to be the world's oldest cultivated crop. There is evidence that bananas were cultivated in the highlands of New Guinea at least 7,000 years ago and that *Musa* cultivars were being bred and grown in the Mekong Delta area of Southeast Asia as long as 10,000 years ago (Reid, 2015). According to De Langhe (2009), all bananas eaten today are descendants of two types of wild fruit and seedy species i.e. *Musa acuminta* (2n=22), a plant originally from Malaysia that produces single sweet-pickle-size green fruits that have a milky flesh and several hard peppercorn-size seeds inside; and *Musa balbisiana* (2n=22), a plant originally from India that is larger and more robust than *M. acuminata* and produces more fruits with thousands of round, button-like seeds. Cross breeding of these two species resulted in a series of diploid, triploid and tetraploid bananas utilized today. The resulting genome groups are classified as AA, AB, AAA, AAB, ABB, AABB, AAAB, ABBB with the letters A and B representing the contributions of *M. acuminata* and *M. balbisiana*, respectively (Nayar, 2017).

Bananas are the world's most popular fruit and one of the world's most important staple foods, along with rice, wheat and maize (Tripathi, Tripathi, & Vroh-Bi, 2007). Based on their end use products, bananas can be categorized into diverse types namely i) dessert bananas which are normally consumed raw when ripe and are distinguished by their sweet flavour; ii) cooking bananas (or plantains) are consumed when cooked; iii) Beer or juice bananas whose pulp is bitter and astringent and are commonly used to make juice and alcohol (Swennen & Vuylsteke, 1987). Local Ugandan examples of juice bananas include *Kayinja, Kisubi, Embidde and Ndiizi.* Annually, more than 1,000 billion bananas (including plantains) are consumed, making bananas the world's fourth most important food crop after maize, rice and wheat (Life Sciences Research Institute (VIB), 2016). They are a staple food for over 400 million people globally, representing an average of 15% to 27% of their daily calorie intake (VIB, 2016). Globally, banana production has been increasing over the recent years growing from 142 MT in 2010 to 154 MT in 2015 valued at over US $ 43 billion and produced from over 130 countries on more than 11 million hectares of land (Food and Agricultural Organization of the United Nations

Statistics (FAOSTAT), 2018). According to UNCTAD (2016), in 2013, approximately 40% of world banana production constituted of cooking banana varieties (plantain and other types) and 60% were desert varieties (Cavendish, Gros Michel and others). The top two banana producers are India, with around 27 MT (amounting to 19% of total production), and China with 12 MT (8% of total production). By 2004, Uganda was the second largest producer of bananas with an annual production of 10 MT (FAO, 2004). However, according to FAOSTAT (2018), Uganda's banana production has declined from 10.5 MT in 2002 to 3.9 MT in 2017 moving from the second to the 14th position. By the start of the last decade, about 20% global banana production was from East and Central Africa (Kabahenda & Kapiriri, 2010) with Uganda's annual production estimated at 50% (approx. 10 MT) of that of East Africa which accounted for approximately 10% of total global production (Kabahenda & Kapiriri, 2010; FAOSTAT, 2006). The global consumption of bananas is highest in the East African great lakes region where average per capita consumption ranges between 440-600 kg annually (Kabahenda & Kapiriri, 2010). Globally, Uganda is the largest producer and consumer of cooking bananas with an estimated per capita consumption between 250 - 480 kg (Kalyebara et al., 2003; Kabahenda & Kapiriri, 2010; FAOSTAT, 2013). It is also estimated that 13 million Ugandans depend on bananas for food and income (VIB, 2016).

Banana consumption is affected by a number of factors such as cultural and textural properties. Texture is one of the most important criterion when evaluating the quality and acceptability of cooking bananas (Qi et al., 2000). The textural quality of cooked bananas is strongly dependent on cultivar, cooking method used and whether the cooked bananas are hot or cold. Inherently, cell wall polymers such as pectin meshed with the insoluble cellulose and hemicellulose fibers affect texture of foods (Sila et al., 2005). In living tissues, cellulose and hemicellulose exhibit a distinctive microfibril network via hydrogen bonds, which enhances cell wall rigidity and resistance to tearing. Pectin and hemicellulose confer plasticity and the ability to stretch (Sila et al., 2005). In the middle lamella, pectin plays a primary role in intercellular adhesion (van Buren, 1979). The unique mixture of pectin, hemicellulose, and cellulose in cell walls, and the changes that occur during ripening and storage are highly implicated in the textural changes observed in plant foods (Abbott, 1999). The most important changes in texture during thermal processing are related to the structure of both protopectin and pectin (Carpita & Gibeaut 1993)

and including starch, all of which undergo gelatinization causing softening not only in bananas but other foods.

1.2 Problem Statement

Despite the high production volumes, banana processing remains limited to date. In Uganda, actors along the cooking banana value chain face risks of high postharvest losses due to the short green life of bananas and damage arising from poor postharvest handling (Nalunga et al., 2015). According to Nalunga et al (2015), during low production, on-farm postharvest losses affect about 3.3% (in form of physical losses) and 5.4% (in form of economic losses) of bananas, while during high production, postharvest losses increase to 9.6% (physical losses) and 8.1% (economic losses). These losses continue to occur up to the table when bananas are cooked and served for consumption. Once served, cooked bananas cool and the texture hardens resulting in significant loss of food mass which is undocumented. Texture is one of the most important attributes in determining a good cooked banana or plantain (Qi et al., 2000) and it affects the sensory properties of bananas. The inadequate processing of cooking bananas noted above could be partly attributed to the hardening of the cooked banana texture. When "cooking bananas" are cooked, they develop a desirable soft texture, flavor and become palatable. However, upon cooling, cooked bananas harden immediately resulting in loss of the desirable soft texture, flavor and palatability. This hardening appears to be almost irreversible such that when reheated, the bananas do not regain their original soft texture, flavor and palatability thus leading to discard of the meal. The non-cooking cultivars i.e. juice/beer and desert bananas remain extremely hard when cooked, and eating them in cooked form occurs only in rare cases such as during famine.

Despite the above, not much has been done on Ugandan banana cultivars. There has been no research to assess hardness profile of local banana cultivars grown in Uganda in raw, cooked and cooled forms and why certain banana cultivars are harder than others. The cooking and cooling behavior has not been investigated. During cooling, hardening of cooked bananas is often attributed to retrogradation of starch (Bello-Pérez, Ottenhof, Agama-Acevedo, & Farhat, 2005) which refers to the re-association or recrystallization of amylose and amylopectin in gelatinized starch. To understand the hardening phenomenon of cooked bananas, knowledge of their chemical composition is important. There is scant information on the chemical

composition of Uganda's indigenous cooking and non-cooking banana cultivars. Starch retrogradation is affected by storage temperature, food composition such as water content, sugars, lipids, salts, and anti-staling enzymes. It also occurs more readily with amylose than with amylopectin because amylose is a smaller unbranched molecule; therefore the use of waxy starches (low amylose content) in foods has been applied to decrease the level of retrogradation (Singh & Anderson, 2004). Besides Muranga (1998); and Ssonko & Muranga (2017), no other studies have been performed on the physico-chemical characterization of starch from Uganda's indigenous banana cultivars. Therefore, knowledge of starch and of its properties in local banana cultivars could be vital in understanding hardness in cooked bananas. There is scanty information on the general composition of Uganda's bananas in terms of their starch, moisture and pectin content. Pectin has a high water binding and holding capacity which may be an important factor in influencing the behavior of starch particularly in cooked bananas. The chemical properties of pectin (methoxyly content and degree of esterification) in raw green mature bananas have not been investigated which could affect water availability to banana starch during and after cooking either directly or indirectly thus affecting starch retrogradation during cooling. Most of the information about pectin is derived from ripe bananas which may not be representative of the true nature of pectic substances in bananas at green maturity. The chemical properties of pectin may influence the amount of water absorbed and extent of pectin gelatinization which may in turn affect the extent of water availability to starch during cooking and cooling. Similarly, no study has been conducted to evaluate the effect of different levels of added starch and pectin or their reduction (by hydrolysis) on hardness of cooked bananas. This could affect hardness of cooked bananas.

1.3 Justification of the study

The information generated on composition of cooking bananas, their hardness profile including role of starch & pectin will help in understanding which of these factors influence hardness of bananas particularly in cooked form. This information will be used in development of new solid-state shelf-stable cooked banana products. For example, as the global population grows, consumers may demand for ready-to-eat (RTE) pre-packed banana products such as "a cooked mixture of bananas with beans or beef" (*Katogo*) which could be microwaved in 2min before consumption. This in general will contribute to increased value addition to cooking bananas

which will boost banana productivity, marketability and subsequently contribute to reduction in farm losses and poverty among banana farmers.

1.4 Research objectives

1.4.1 General objective

To investigate the influence of key chemical components on hardness of cooking and juice banana cultivars grown in Uganda.

1.4.2 Specific objectives

1. To determine the content of starch, pectin, crude fibre, crude ash, calcium and sodium in selected cooking and juice banana cultivars.
2. To evaluate the physico-chemical properties of starch and pectin from the selected banana cultivars.
3. To evaluate hardness of selected banana cultivars when raw, cooked and cooled.
4. To determine the effect of starch, pectin and pectinase (polygalacturonase) on hardness of cooked bananas.

1.5 Null hypotheses

1. The content of starch, pectin, crude fibre, crude ash, calcium and sodium does not differ significantly between cooking and juice banana cultivars.
2. The physico-chemical properties of starch and pectin from selected cooking and juice banana cultivars do not differ significantly.
3. Hardness of cooking and juice banana cultivars does not differ significantly in raw, cooked and cooled forms.
4. Starch and pectin or hydrolysis of pectin do not increase hardness of cooked bananas.

CHAPTER 2

LITERATURE REVIEW

2.1 The history of bananas and their dispersal in Africa

The word "banana" is said to have its roots in the Arabic word "banan" which means "finger" (Cumo, 2013). Banana is a general term embracing a number of species or cultivars in the genus *Musa*. Bananas belong to the order Zingiberales and family Musaceae. Members of this family are large herbs standing 2-9 metres tall with an aerial trunk consisting of compacted leaf sheaths which grow directly from the top of the corm (Purseglove, 1972; Karamura, 1998). Most edible fruited bananas are usually seedless and belong to the species *Musa acuminata*, *Musa sapientum*, *Musa cavendishi*, *Musa paradisiaca* and many others. Other species include *Musa balbisiano colla* of southern Asia which bears a seeded fruit. *Musa basjoosieb* of Japan and *Musa ornate* from Pakistan are grown mainly as ornamental plants and for fibre. *Musa textiles Nee* of the Philippines is grown for its fibre and for making tissue thin tea bags. *Musa ensete Gmel* which belongs to the genus *Ensete* is cultivated in Ethiopia for fibre and for the foods derived from the young shoot, base of the stem and the corm (Morton, 1987).

Bananas are one of the most important and oldest food crops of humankind with evidence of cultivation dating to 4000 BC in New Guinea (Denham, Haberle, & Lentfer, 2004; Denham et al., 2003). Bananas were introduced into Africa from South and South East Asia. In the absence of direct archaeological evidence, there have been differences of opinion about the time and place of their first arrival. Banana introduction has been widely thought to have happened between 200 BCE and 500 CE, but De Langhe et al. (1994–95), De Langhe & De Maret (1999) and De Langhe, (2000) have hypothesized that this may have occurred about 3,000 years ago based on biological, linguistic, and prehistory evidences. This long-held and traditional view about the time of earliest introduction of bananas into Africa has been thrown into turmoil with the discovery of banana phytoliths from Nkang (South Cameroon) dated to first millennium BCE and from Munsa (Uganda) dated to fourth millennium BCE (Mbida et al., 2000; Lejju et al., 2006). When the European explorers first reached the West African coast in the mid 15th century, they found bananas already growing there (Reynolds, 1951). Several authors had originally proposed that bananas were introduced into Africa from India by way of Arabia and the Horn of Africa, but Simmonds (1962a) has dismissed this due to lack of archaeological evidence along the proposed routes. It is well documented that bananas were introduced into

Egypt and the Eastern Mediterranean region following Alexander the Great's conquest of India in 329 BCE, but there is no evidence of spread South to the Sub-Saharan region.

On the other hand, bananas are suggested to have come from Borneo, Sumatra, and Timor (Janick, 2009). The people from the East brought with them skills in agriculture and crops such as rice, sugarcane, bananas, and possibly, coconuts. The initial introduction of bananas to Madagascar, and hence to the East Coast of Africa, has been endorsed by several workers (Purseglove, 1976). However, De Langhe et al. (1994–1995) and De Langhe & De Maret (1999) have supported the view point advanced by Berchem in 1990, based on comparative study of cultural lexicons, that Malagasy (the native people and language of Madagascar) were certainly not the first people to introduce banana to Africa and that Bantu (the language and people of East and Central Africa) must have known the plant before they came into contact with the Malagasy.

A new perspective has now appeared about the first introduction of banana into Africa with the archaeological findings of banana phytoliths in Nkang, South Cameroon, dated to the first millennium BCE (Mbida et al., 2001, 2006) and in Munsa, Uganda dated to the fourth millennium BCE (Lejju et al., 2006). The findings by Mbida et al. (2001) of phytoliths in south Cameroon (840–370 BCE) were severely criticized by Vansina (2004) since there has been no evidence that sedentarization and cultivation occurred in East and Central Africa several centuries earlier to Mbida's date. Sedenterization and acquisition of at least some farming skills by early communities are prerequisites for taking up the cultivation of already domesticated crops like bananas. It was argued that there was no evidence of this in the region. Mbida et al. (2004, 2006) have since countered in detail all the reservations and objections raised by Vansina (2004). These reservations have become even less relevant now with the report of Lejju et al. (2006) of obtaining banana phytoliths from even an earlier period than Mbida's (First millennium BCE) from a swamp in Munsa (1220 m), Uganda, dated to the fourth millennium BCE. In the absence of more archaeological and/or genetic information, it is not possible to develop a more definitive chronology for the arrival and spread of bananas in Africa.

2.2 Botanical characteristics of bananas

Bananas are a type of fruit from herbaceous plants of the genus *Musa*. Botanical or morphological characteristics of bananas have been described by various authors. The botanical characteristics of bananas vary widely depending on environmental growth conditions (Nelson, Ploetz, & Kepler, 2006), genetics, region and growth conditions. According to Nelson et al. (2006), in terms of size, banana is large, considered as a perennial, monocotyledonous herb 2–9 m (6.6–30 ft) in height that arises from large, subterranean rhizomes (usually called "corms"). Upon flowering, the true stem or growing point emerges from the center of the tightly rolled bunch of leaves (Stover & Simmonds, 1987). This odd-looking "flower cluster" is actually an elongated, plump, purple to green "bud" (sometimes called the "bell" or "heart"), which at first displays large female flowers whose ovaries ripen into fruit. As the bud elongates, it exposes semicircular layers of female flowers, then neutral flowers, and finally small, generally non-functional (with no viable pollen) male flowers (Nelson et al., 2006; ITFNet, 2016). Each group of flowers is arranged radially on the stem in nodal clusters. Each flower cluster is borne on a prominence of the stem bearing the fruit (peduncle) and covered by a bract. About 12–20 flowers are produced per cluster. Collectively, the flowering parts and fruit are referred to as the bunch while individual clusters of fruits are known as hands, and individual fruits are known as fingers (Nelson et al., 2006). The entire above-ground portion of the plant is not a true woody trunk, as in other trees, but a "false trunk" or "false stem" that consists of leaves and their fused petiole bases, referred to as a pseudostem. The pseudostem supports a canopy consisting of 6–20 (or more) leaves (Nelson et al., 2006; ITFNet 2016). Nelson et al. (2006) further describes *Musa* fruits as variable in size, shape, and color. They are generally elongate-cylindrical, straight to strongly curved, 3–40 cm long, and 2–8 cm in diameter. The fruit apex is important in variety identification; it may be tapered, rounded, or blunt. The skin is thin and tender to thick and leathery, and silver, yellow, green, or red in color. Inside the ripe fruit, the flesh ranges from starchy to sweet, and in color from white, cream, yellow, or yellow-orange to orange (Nelson et al., 2006). Bananas also vary in peel thickness (Nelson et al., 2006; ITFNet, 2016). Some varieties have a thin peel and are more susceptible to damage in transport, whereas others have a comparably thicker peel. According to Nelson et al. (2006), all cltivated banana varieties are typically seedless). When seeds are present, they vary among species in shape and morphology. Seeds of *Musa balbisiana*, parent of many commercial edible banana

varieties, are dark brown, ovoid, about 4 mm long, with a conspicuous white, powdery endosperm. Plants have numerous fibrous roots ranging from 200 to 500. In well drained, deep, and fertile soils, roots may extend 1.5 m deep and 4.9 m laterally. In dry, shallow, or rocky soils, roots of *Musa* may not compete well.

Another important species of the banana that deserves description is *Musa Schizocarpa* which is one of a handful of wild self-peeling species of banana, whose peel of the fruits splits (i.e. is schizocarpic) at maturity, exposing the seeds (Simmonds, 1956). According to Argent (1976), *M. Schizocarpa* has a pseudostem of up to 10 m tall and its girth is 100 cm at the base. It is dark blackish brown in the lower part, gradually becoming bright non-waxy or paler waxy green in the upper part, which may be irregularly blotched dark brown. Its sap is watery. *Musa schizocarpa* has suckers emerging close to the parent stem but grow away vertically. The mats are mostly rather small. The peduncle (the part between the leaf crown and the first hand of fruits) is green and glabrous. The bunch is very dense, sometimes curved in the upper part, but the lower part and usually the whole bunch hangs vertically downwards. The fruits occasionally curve upwards but usually spread outwards through compression. The young fruits of *Musa schizocarpa* are glabrous, distinctly dark green with a purplish flush. The mature fruits are short and broad, often strongly angular, bottle-nosed, dark green and schizocarpic with the skin peeling back in a rather irregular fashion to expose the gleaming white flesh. The seeds are 6-7 mm in diameter, dark brown or blackish (Argent, 1976).

2.3 Taxonomy of bananas

Bananas belong to the genus *Musa* of the family *Musaceae* (Stover and Simmonds 1987). *Musaceae* includes only 2 genera, *Ensete* and *Musa* (Table 1). The family has not been revised after the classical work of Cheesman (1947–1950) and Simmonds (1953, 1962a), although several species have been subsequently described. The genus *Musa* was first given by Carl Linnaeus in 1753. Linnaeus had named only two *Musa* species, *M. paradisiaca* (1753) and *M. sapientum* (1759). According to Cheesman (1948), Linneaus described *M. paradisiaca* from a plantain (AAB) and *M. sapientum* from what was most probably the dessert banana, 'Silk Fig' of the West Indies (syn. 'Rasthali', 'Latundan', 'Sugar' [AAB]). Simmonds (1962a, b) observed that since Linneaus had named both *M. paradisiaca* and *M. sapientum* using sterile triploid interspecific hybrids of the genomic constitution AAB, they did not represent "species"

in any reasonable sense of the word, and the Linnaean names could not be correctly applied to any bananas of interspecific constitution. Simmonds suggested that a clone might be referred to the genus and the appropriate group: for example, *Musa* (AAA group) 'Gros Michel', *Musa* (AB group) 'Ney Poovan'.

The foundation for the classification of the genus *Musa* was laid by Sagot in 1887 while Baker in 1893 gave the first classification. However, Cheesman (1947–1950) did the comprehensive classification of *Musaceae* and delimited its two genera i.e. *Ensete* and *Musa* and described and defined the species in both of them. Cheesman listed 25 species in *Ensete* and 21 species in *Musa*. At present, over 80 species have been recognized in *Musa* by various authors. Cheesman (1947) grouped the *Musa* species into four sections: *Eumusa*, *Rhodochlamys* (both n=11), *Australimusa*, and *Callimusa* (both n=10) (Table 1). Argent (1976) erected a fifth section, *Ingentimusa* (n=7, 9, or 10) and included in it a new species from Papua New Guinea, *M. ingens*.

The number of sections was reduced to three by Wong et al. (2003) based on amplified fragment length polymorphism (AFLP) studies: *Ingentimusa*, *Callimusa* (including *Australimusa*), and *Musa* (including *Rhodochlamys*). *Musa lasiocarpa*, which Simmonds (1962a) had not grouped under any of the four series (incertae sedis), was included in a new genus *Musella* (Frachet) *Li* as *Musella lasiocarpa* by some authors. Currently, most taxonomists consider *Musella* as congeneric with *Musa* (Anderson, 1998; Hanelt, 2001). Daniells et al. (2001) listed 29 species in the genus *Musa*. GRIN Taxonomy (2007) recognized only 15 species. In a recent enumeration of all the valid *Musa* species, Haekkinen & Vaere (2008) recognized 73 species. Taking the enumerations of Cheesman (1947–1950), Simmonds (1952), and Haekkinnen and Vaere (2008) into consideration, 81 species are now recognized in the genus *Musa* but they have not been keyed.

The important characteristics used to separate the species of *Musa* include basic chromosome number, pseudostem height, sheath hairiness, petiole margin and base, peduncle hairiness, bunch posture, basal flower sex, bunch density, pedicel length, fruit skin hairiness, seed shape and the male bracts characteristics, behavior (deciduous/persistent), surface (polished/dull), rolling and color, and free sepal length (Haekkinen & Vaere, 2008).

Table 1: Taxonomy of *Musa* and *Ensete* genera of *Musaceae*

Genus	Section	Haploid chromosome no	No. species	Distribution	Uses
Ensete		9	7–8	West Africa to New Guinea	Fiber, vegetable
Musa	*Australimusa*	10	5–7	North Australia to Philippines	Fiber, fruit
	Callimusa	10	6–10	Indo-China and Indonesia	Ornamental
	Eumusa	11	13-15	Sri Lanka, peninsular India to Japan to Samoa	Predominantly as fruit & vegetable; also fiber, ornamental
	Rhodochlamys	11	5-7	India to IndoChina	Ornamental
	Ingentimusa	7[y]	1	Papua New Guinea	Wild

[y]Some authors have given the chromosome number n = 9 or 10

Source: Stover and Simmonds (1987).

Table 2: Recognized species of *Musa*

Eumusa (n=11)	**Callimusa (including Australimusa)** (n=10)	*monticola*	**Eumusa including Rhodochlamys (n=11)**
acuminata	*alinsanaya*	*muluensis*	*angcorensis*
balbisiana	*beccariiy*	*paracoccinea*	*aurantiaca*
banksii	*boman*	*pigmaea*	*cheesmanii*
basjoo	*bukensis*	*salaccensis*	*coccinea*
itinerans	*campestris*	*splendida*	*flaviflora*
nagensium	*exotica*	**Ingentimusa (n=7, 9, or 10)**	*griersonii*
paradisiaca	*fitzalanii*	i*ngens*	*mannii*
Rhodochlamys (n=11)	*flavida*		*ochracea*
laterita	*hirta*	**Ingentimusa (n=7, 9, or 10)**	*rosea*
ornata	*insularimontana*	i*ngens*	*rubra*
sanguinea	*jackeyi*	**Callimusa (n=10)**	*schizocarpa*
velutina	*johnsii*	*borneensis*	*siamea*
Australimusa (n=10)	*lawitiensis*	*erecta*	*sikkimensis*
angustigemma		*gracilis*	*thomsonii*
fehi		*violascens*	*lasiocarpa*
lolodensis			
maclayi			
peekelii			
textilis			

Sources: Cheesman (1947–1950); Simmonds (1952); Daniells et al. (2001); (Constantine, 2003); (GRIN Taxonomy, 2007) and Haekkinen (2008).

2.4 Distribution and Ecology

Musa is widely distributed geographically in the tropics, from 175^{o}E to 150^{o}W longitude and from 30^{o}N to 23^{o} S latitude. According to Nelson et al (2006), *Musa* is widely adapted, growing at elevations of 0–920 m (0–3000 ft) or more, depending on latitude with mean annual temperatures of 26–30°C (79–86°F) and annual rainfall of 2000 mm (80 in) or higher for commercial production. *Musa* grows in a wide range of soils, preferably well drained soils with pH 5.5 to 7.5. Sandy loams and sandy clay loams are ideal soils for *Musa* production. According to the Durmanov (1974) alluvial and volcanic soils are best for *Musa* cultivation.

The distribution of *Musa* is disjunct though contiguous, in that the species occur in hundreds of islands of South and Southeast Asia and west tropical Pacific Ocean, Sri Lanka, through India (peninsular, east, and northeast region), Bangladesh, south and southeast China, Myanmar, Laos, Vietnam, Cambodia, Thailand, Malaysia, Indonesia, Philippines, and New Guinea (Nayar, 2017). The distribution of *M. balbisiana* may be somewhat disjunct. Maximum species diversity occurs in peninsular Malaysia and Indonesia (Cheesman, 1947; Simmonds, 1962a; Argent, 1976). All the wild bananas are warm-region plants. The genus possesses only a limited range of temperature tolerance, although a few species, for instance *M. basjoo*, *M. ingens*, *M. nagensium*, and *taxa* may withstand cool climates. No species shows drought resistance. While *Australimusa* and *Callimusa* species are quickly and severely affected by drought, *Eumusa* species are comparatively more tolerant, with *M. balbisiana* and *M. acuminata* subspecies, *siamea* and *burmannica*, showing the most tolerance (Simmonds, 1962a). *M. acuminata* (genome A) is assumed to have contributed most to the origin of cultivated bananas. Its occurrence covers almost the entire region of distribution of the genus *Musa* and shows great variability. Simmonds (1962) identified five subspecies (*siamea, burmannica, banksii, microcarpa* and *malaccensis*) with the center of diversity as Malaya. Daniells et al. (2001) studied a more comprehensive collection of the species and recognized nine subspecies (*burmannicoides, truncata*, z*ebrina*, and *errans*, plus the five subspecies of Simmonds). *M. balbisiana* (genome B) is the second most important species involved in the origin of bananas. It has almost the same range of distribution as *M. acuminata*. According to Ude et al. (2002a,b) and Uma, et al., (2005, 2006), *M. balbisiana* possesses a good level of intraspecific variability.

Two other species implicated in the ancestry of cultivated bananas are *M. schizocarpa* (genome S, n=11) and the *Australimusa* species, *M. textilis* (genome T, n=10). *M. schizocarpa* was described in 1956 (Simmonds, 1962b). The species is characterized by its self-peeling fruits and occurs widely in New Guinea. *Musa schizocarpa* is a wild species of banana, one of a handful of self-peeling species of banana, whose peel of the fruits splits (i.e. is schizocarpic) at maturity, exposing the seeds (Daniells et al., 2001). In the Papua New Guinea Biological Foundation's banana collection, Shepherd and Ferreira (1982) recorded five AAS banana cultivars, among their total living collection of 211 accessions. Originally, there were 17 AAS collections, but the rest were lost due to disease and neglect. *M. textilis*, the fourth species implicated in the ancestry of cultivated bananas, was one of the earliest species described in the genus *Musa*. It is a native of the Philippines, where it has been used from historical times for making cords and ropes (Manila hemporabaca); its pre-eminence has been lost with the development of synthetic fibers. Along with *M. textilis*, *M. balbisiana* also occurs widely in the Philippines. In earlier years, the pseudo stems of both species used to be mixed and utilized for making cordage. *M. textilis* shows much diversity, and several cultivars have been cultivated in the Philippines (Cheesman, 1949; Spencer, 1953). Natural hybrids of *M. textilis* with *M. balbisiana*, *M. acuminata*, and the cultivated bananas have been reported by several authors (Shepherd & Ferreira, 1982; Daniells et al., 2001).

2.5 Categories of bananas

Bananas are broadly classified into dessert, juice/beer and cooking types. Dessert types are eaten raw when ripe, the juice/beer are used for juice extraction and beer making when ripe while cooking bananas are boiled, fried, powdered, or roasted before consumption. Plantains are the best known among the cooking bananas and form about one-third of total banana production (Nayar, 2017). According to Nayar (2017), there is no clear demarcation between bananas and plantains, either botanically or genetically, although plantains usually contain more dry matter mainly starch than bananas. Several of the plantain and banana cultivars are used in various countries for both dessert and/or cooking use. Plantain includes a large group of bananas that has upto 100 cultivars (Swennen, 1990). The genome of most plantain cultivars is AAB, which denotes that these bananas have three sets of chromosomes and that they are hybrids of *Musa acuminata* and *Musa balbisiana* in a proportion of roughly two to one. Even though bananas originate from the Asia-Pacific region, the diversity of plantain cultivars is

highest in Africa, especially West and Central Africa. This diversity was created locally by farmers selecting and vegetatively propagating natural mutants derived from more than one cultivar introduced to the African continent. Plantains are more starchy and less sweet; they are eaten cooked rather than raw; they have a thicker skin, which may be green, yellow or black; and they can be used at any stage of ripeness (Chiquita, 2013). Linnaeus made the same distinction between plantains and bananas when he first named two "species" of *Musa* (Valmayor et al., 2000). Members of the "plantain subgroup" of banana cultivars, most important as food in West Africa and Latin America, correspond to the Chiquita description i.e. they have long pointed fruits. They are described as "true" plantains, distinct from other cooking bananas (Ploetz, Kepler, Daniells, & Nelson, 2007). The cooking bananas of East Africa belong to a different group, the East African Highland bananas (AAA-EA) (Ploetz et al., 2007), and would not qualify as "true" plantains based on this definition. The East African Highland cooking bananas are cooked when green mature as a starchy staple but can also be eaten ripe as desert hence not true plantains.

Genetically, desert bananas are Triploid cultivars derived solely from *M. acuminata* whereas plantains are triploid cultivars derived from the hybrid between *M. acuminata* and *M. balbinosa* (in particular the plantain subgroup of the AAB Group) are "plantains (Stover & Simmonds, 1987; Qi et al., 2000). According to Valmayor et al., (2000) in Southeast Asia – which is the center of diversity for bananas, both wild and cultivated – the distinction between "bananas" and "plantains" does not work. This is because, many bananas are used both raw and cooked. There are starchy cooking bananas which are smaller than those eaten raw. The range of colors, sizes and shapes is far wider than in those grown or sold in Africa, Europe or the Americas. Southeast Asian languages do not make the distinction between "bananas" and "plantains" that is made in English. Thus both Cavendish cultivars, the classic yellow dessert bananas, and Saba cultivars, used mainly for cooking, are called *pisang* in Malaysia and Indonesia, *kluai* in Thailand and *chuoi* in Vietnam (Valmayor et al., 2000). *Fe'i* bananas, grown and eaten in the islands of the Pacific, are derived from entirely different wild species than traditional bananas and plantains. Most *Fe'i* bananas are cooked, but *Karat* bananas, which are short and squat with bright red skins are very different from the usual yellow dessert bananas and are eaten raw (Englberger, 2003). In this work, the term banana is used to mean both bananas and plantains unless otherwise stated.

2.6 Diversity of bananas in Uganda

It is estimated that the total number of banana cultivars grown in Africa is about 180; about 60 are in east Africa (coastal and highland region) and 120 in central and west Africa (De Langhe 2000; De Langhe et al., 2005). The genomic composition of the African plantain is mostly AAB and ABB. Although AAA bananas are generally eaten as fruit, some of their forms have naturally developed into cooking bananas or plantains in this region. Similarly, while most AAB bananas are used as cooking (starchy) bananas or plantains, a few edible fruit forms are present. ABB bananas are almost only used for cooking (Rowe, 1984; Swennen et al., 1995). According to Karamura (1998), there are three categories of bananas grown in Uganda: dessert bananas, plantains, and east African highland (cooking and beer) bananas. The majority of the dessert bananas are AAA triploids; the rest are AA and AB and AAB 'Prata' subgroup. The dessert and plantain (AAB) bananas are grown in backyards. Using an elaborate system for classification (238 accessions, 61 morphological characters, 3 clustering methods), Karamura (1998) identified 5 banana clone sets grown in Uganda namely *Nakabululu*, *Nfuuka*, *Nakitembe*, *Musakala*, and *Mbidde* (a beer banana). The first three clone sets have moderate affinity to each other, while the last two are grouped separately. Interestingly, this author found that some of the recent banana introductions having different genomic composition for instance *Kisubi* (AB), *'Kayinja*, and '*Kivuvu* (ABB 'Bluggoes subgroup) were also being used for beer making. The bananas grown in Uganda belong to the East African Highland Bananas (AAA-EA) which are endemic to the East African Highlands (Robertshaw, 2006). This is contrary to the argument that they were brought to the East African Coast by Arabs in about 200 BC. Uganda is currently estimated to have over 87 endemic species of bananas (Edmeades et al., 2006). According to the Regional Banana Germplasm Collection Centre of Biodiversity in Mbarara, Uganda has records of over 200 East African highland banana cultivars from Uganda, Tanzania, Congo, and Rwanda (Karamura, 1998).

2.7 Banana production and trade

Banana production has seen a steady growth over the years (Figure 1) and is expected to increase with increase in world population and demand. Based on production quantities, bananas are the most popular fresh fruits worldwide followed by apples and grapes and are second in terms of production volume after watermelons (The Portal for Statistics (Statista), 2018). Globally, banana production has been increasing over the recent years growing from

142 MT in 2010 to 154 MT in 2015 produced by over 130 countries and valued at over US $ 43 billion (FAOSTAT, 2018). This shows a 9% increase over a ten year period. According to UNCTAD INFOCOMM (2016), banana production has grown rapidly registering a performance of over 15% since 2008. This steady increase in banana production is mainly attributed to increasing consumption in the producing countries as well as the growing exports. Uganda's banana production has declined over the years reducing from 10.5 MT in 2002 to 3.9 MT in 2017 (FAOSTAT, 2018). According to FAOSTAT (2018), Uganda's banana production declined from 5.29 MT in 2010 to 3.9 MT by 2017 moving from the 7th position to the 14th position during this period (Figure 2). According to FAOSTAT (2004), bananas were second place after citrus fruits among the most produced fruits in the world with bananas registering 106.34 MT from 9.52 million hectares while citrus fruits registered 110.91 MT. Banana production is done throughout the tropics and, to a limited extent, in the subtropics, with 37% of banana production in South and Southeast Asia and the Pacific, 30% of banana production in Tropical Africa while 26% is in Central and South America and the Caribbean, and about 7% elsewhere (FAOSTAT, 2013; Nayar, 2017).

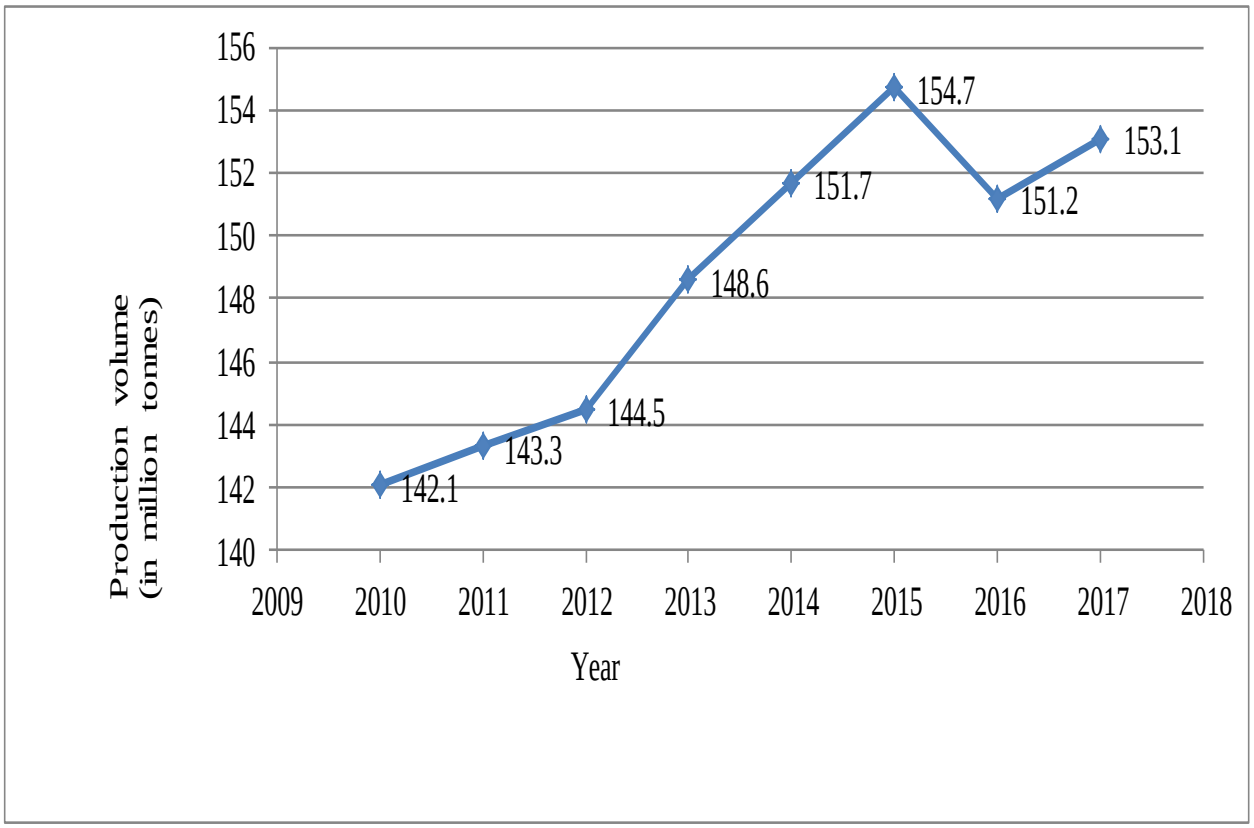


Figure 1: World banana production volumes from 2010 to 2017

Bananas constitute the primary or secondary staple food for millions of people in tropical Africa, Pacific Ocean islands, Latin America, and the Caribbean (FAOSTAT, 2013). Bananas provide 10 to 27% of the daily calorie intake of the population in several countries (FAOSTAT, 2013; Nayar, 2017). According to FAOSTAT (2013), by 2001, annual world consumption of bananas was 5.2 kg/person but was 5 times higher in 28 of 162 consumption-reporting countries. The top banana-consuming countries (kg/person per year) were Uganda (237), Burundi (215), Rwanda (180), Sao Tome and Principe (151), Gabon (139), Ecuador (112), Bermuda (107), Ghana (92), Cameroon (90), St. Lucia (83), and Samoa (82).

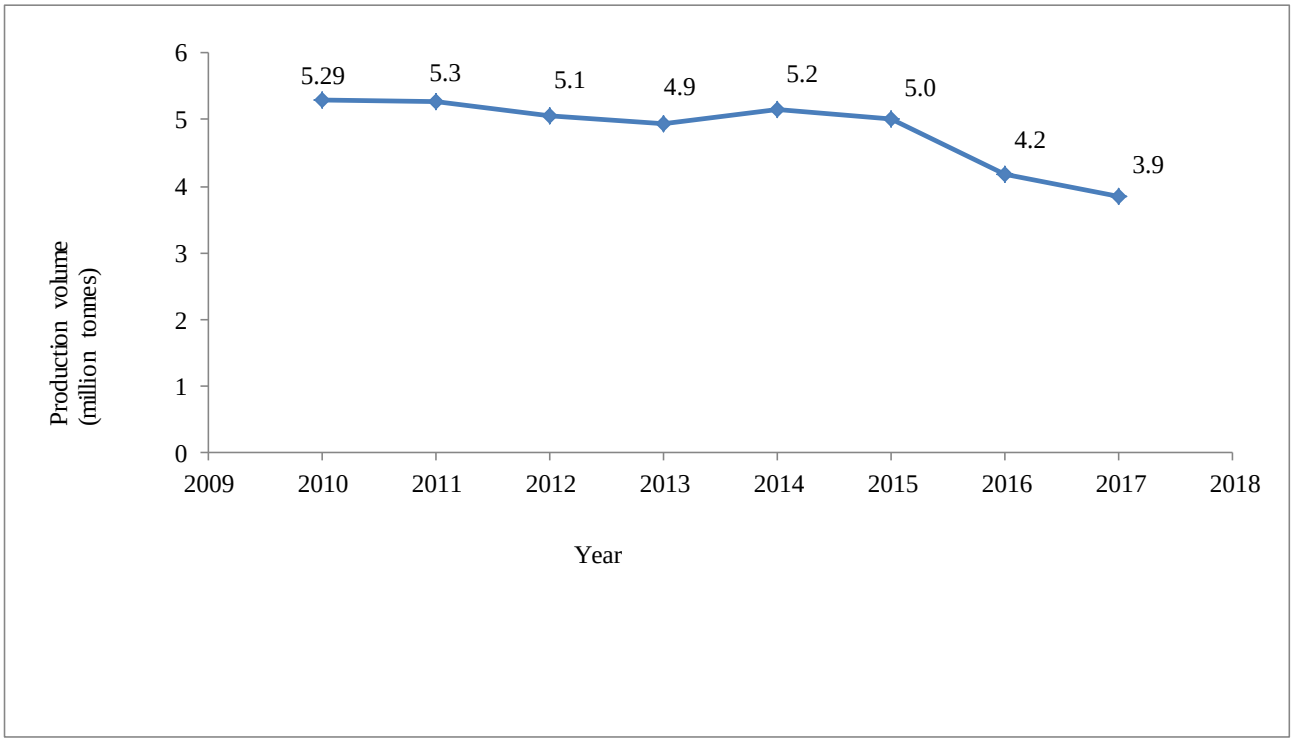


Figure 2: Uganda's banana production volumes from 2010 to 2017

Bananas possess a major advantage over other important food crops because they are harvested throughout the year, hence providing food security all-year round. The top five banana-producing countries of the world are India (15% of total production), China, Ecuador, Brazil, and Philippines (5–6% each).

Only about 15 to 20% of the total banana production in the world is traded internationally with an annual value of over US$ 6 billion. According to FAO (2003), the major exporting countries were Ecuador, Costa Rica, Philippines, Colombia, Panama, and Honduras while the major importing countries/regions were the United States, Canada, European Union, Japan, Russian Federation, and the Middle East. Nigeria, with an estimated population of 177.5 million people, gross domestic product per capita of US$3,203.3 (World Bank, 2013) is one of the largest producers in Africa recording about 2.78 MT of bananas (FAOSTAT, 2013). Africa exported about 649,000 tons of bananas in 2012 representing 3.9 percent of global export with Côte d'Ivoire and Cameroon being the two topmost exporters (FAO, 2014). Almost all banana exports are of the dessert type; however, about 10–15% of all banana production is exported, with the United States and European Union being the dominant buyers (International Tropical Fruits Network (IFTNet), 2016).

2.8 Utilization of bananas

Musa species attained a position of central importance within Pacific societies: the plant is a source of food, beverages, fermentable sugars, medicines, flavorings, cooked foods, silage, fragrance, rope, cordage, garlands, shelter, clothing, smoking material, and numerous ceremonial and religious uses (ITFNet, 2016; Rosentrater, Todey, & Persyn, 2009). Fibers from the banana plant are comparable in physical strength and cellulose content to fibers obtained from other byproducts of fibrous commodities (Uma, Kalpana, & Sathiamoorthy, 2005) and have been extensively characterized from their fruit stalk (Zuluaga, Putaux, Cruz, Vélez, Mondragon, & Ganan, 2009), pseudostem (Cherian, Pothan, Nguyen-Chung, Mennig, Kottaisamy, & Thomas, 2008) and leaves (Oliveira, Cordeiro, Evtuguin, Torresa, & Silvestre, 2007). A few studies have been published emphasizing the potential of banana fibers as the raw materials in making composite boards (Chattopadhyay, Khandal, Uppaluri, & Goshal, 2010). Although mostly consumed locally in the Pacific region, the fruit enjoys a significant worldwide export market. They are cultivated primarily for their fruit, and to a lesser extent for the production of fibre and as ornamental plants (Nelson et al, 2006). Bananas come in a variety of sizes and colors when ripe, including yellow, purple and red. Most production for local sale is of green cooking bananas, as ripe dessert bananas are easily damaged while being transported to the market. Although the wild species have fruits with numerous large, hard seeds, virtually all culinary bananas have seedless fruits (Nelson et al., 2006; ITFNet, 2016).

Bananas are classified either as dessert bananas (meaning they are eaten when yellow and fully ripe) or as green cooking bananas. Bananas can be eaten raw though some varieties are generally cooked first.

Some Asian cultures generally eat both the skin and the inside pulp when cooked or raw, either as green, half ripe or ripe fruit. African and Western cultures generally eat the inside raw and throw away the skin. Bananas and plantains together constitute the fourth most important global food commodity after rice, wheat and maize in terms of gross production and consumption (VIB, 2016). Banana flour is an important raw material in the confectionery industry and complementary infant food formulation in various parts of the globe (Adeniji & Empere, 2001). Ripe banana fruit is utilized in a number of ways in the diet, from simply being peeled and eaten out of hand to being sliced and served in fruit drinks, and salads, sandwiches, custards, etc. Banana fruits are also smashed and incorporated into ice cream, bread and cream pies. The fruit is used in making jam, sauce or jelly. Banana puree is an important component of most infant food. Matured unripe banana fruits are boiled or baked and eaten with soups or stews; or the fruit is thinly sliced and fried till crisp to make banana chips. The banana fruit is also used as the dietary food against intestinal disorders, including constipation, because of its soft texture and smoothness. A ripe banana fruit when eaten neutralizes acidity and reduces irritation by coating the stomach lining (Morton, 1987). Ripe banana fruits that have been rejected are usually supplemented with proteins, vitamins and mineral salts and used as animal feeds. In Uganda, banana peels particularly from the unripe bananas provide a delicate relish to cattle, goats and sheep. Elsewhere, beef cattle can be fed with green mature banana fruits which have been mixed with urea and molasses to mask the astringent taste (Okaka et al., 2002).

Commercially, banana can be processed into edible starches for instance the green culled bananas and the pith of pseudostem which are removed during fruit selection and processing (Abdul Aziz, Ho, Azahari, Bhat, Cheng, & Ibrahim, 2011; Zhang et al. 2005). Pectin can be produced from discarded banana peels via acid extraction and precipitation by using ethanol or ammonium salts (Emaga et al., 2008). Carboxy methyl cellulose can be manufactured from banana pseudostem though the quality is needs to be improved for food grade applications (Adinugraha, Marceno, & Haryadi, 2005). The abundance of anthocyanins reported in the banana inflorescence bracts (*Musa acuminata* and *Musa acuminata x balbisiana*), ranging from 14–32 mg anthocyanin/100 g bracts, mainly comprising of cyanidin-3-rutinoside compound,

are potential sources of cheap natural food colorant that can be exploited (Jenshi, Saravanakumar, Aravinthan, & Suganya, 2011). The content of anthocyanins is slightly higher than the commercially available anthocyanins from red cabbage and due to the abundance of bracts produced by bananas, bananas may provide sufficient and sustainable market outlook as a source of food colorant (Jenshi et al. 2011; Pazmino, Giusti, Wrolstad, & Gloria, 2001). Bananas can be used as a sustainable target for biogeneration of flavors that can be used in the food industry. (Kuo, Hwang, Yeh, Pan, Tsai, & Pan, 2006) reported that banana leaves (*Musa* cv. Cavendish) contain a membrane-bound enzyme of 9-LOX, which is able to produce oolong tea-like, melon-like, and fruity cucumber-like flavor upon pickling or when treated with soybean oil, linoleic acid and linolenic acid. The kinetic properties of the enzyme were comparable to LOX obtained from canola seed and English pea (Kuo et al., 2006).

A few studies have been done on the by-products of banana and plantain in order to evaluate its nutrient content as a potential source of dietary food components such as carbohydrate, proteins, dietary fibers, and minerals for human consumption (Emaga et al. 2007; Mohapatra, Mishra, & Sutar, 2010). Banana pith from the pseudostem has long been eaten as a vegetable in some parts of the world such as India, Sri Lanka, and Malaysia (Kennedy, 2009). It contains considerable amount of starch, sugars, and minerals (Mohapatra et al. 2010). In most of the Southeast Asian countries, banana inflorescence has been consumed as vegetables and salad for a very long time. Emaga et al. (2007) reported that banana peels from three different genetic makeups namely AAA, AAB, ABB, and AAAB were rich in total dietary fibre (40–50 %), protein, and amino acids (8–11 %), lipids and fatty acids (2.2 % to 10.9 %). The dietary fibre content was slightly higher compared to wheat, barley, oats and rice brans (Sudha, Vetrimani, & Leelavathi, 2007). The peels were reported to contain significant amount of potassium. An incorporation of banana peels at a ratio of 10 % into biscuits did not show significant differences in the overall color, aroma, and taste, which makes it suitable for the production of low calorie food products with high dietary fiber content (Joshi, 2007).

Banana peel extracts could be used as a preservative. Antibacterial compounds such as ß-sitosterol, 12-hydroxystrearic acid and malic acid isolated from banana peels (*Musa paradisiaca*) have shown to be a good suppressor of foodborne pathogens including *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis* and *Escherichia coli* (Mokbel & Hashinaga, 2005) and could potentially be applied into food systems in the future.

Devatkal, Kumboj, & Paul (2014) also added that the preservative capability of banana peel water extract from similar banana varieties in reducing lipid oxidation process in raw meat was comparable to synthetic antioxidants such as butylated hydroxy toluene (BHT). The existence of known antioxidative substances in banana peels has been reported by Mokbel and Hashinaga (2005).

2.9 Nutritive components

Bananas are considered to be one of the most important sources of energy and starchy staple food for the people of tropical humid regions (Onwuka & Onwuka, 2005). Bananas are nutritionally low in protein but relatively high in carbohydrates, vitamins and minerals (Offem & Njoku, 1993). They are particularly rich in nutrients for instance starch, sugar and vitamins A & C, minerals such as potassium, calcium, sodium, magnesium, iron, Zinc, phosphorus and selenium. (Doymaz, 2010; Wall, 2006).

Ripe bananas are the ideal food source to provide vitality. They have three natural sugars: sucrose, fructose and glucose, as well as fiber. The glycemic index of ripe bananas is high raising the energy levels immediately once consumed (Pius, 1994; Kyamuhangire et al., 2002). A banana of 120 g has a glycemic index of 52 compared to an apple with a glycemic index of 38 (Kaye, Susanna, Holt, & Brand, 2002) implying that if one is to choose between a banana and an apple from the supermarket shelf for revitalizing the body, a banana would be a better choice. The crude protein content of bananas is relatively low ranging from 1.67 to 2.66% depending on the banana part (pulp or peel) (Maina et al., 2012); the fat content can be as low as 0.1% (Maina et al., 2012) or sometimes undetectable. Bananas are good sources of moisture for the human body. Different bananas have varying moisture contents depending on the cultivars, environmental and growth conditions. In general, moisture varies between 68 and 80% (Swaminathan, 2002; Maina et al., 2012). The presence of high amounts of carbohydrates and minerals such as potassium and magnesium can stimulate the intellectual coefficient of humans hence serving as an excellent ingredient for children and teenagers in their school years. Bananas are a valuable source of vitamin B_6, vitamin C, and potassium (Nelson et al., 2006). Bananas contain vitamins B_1, C, D and E as well as β-carotene and vitamin A, which are ideal for rejuvenation of the skin in men and women of over 40 years. Eight Fe'i banana cultivars were found to contain riboflavin in the range of 0.10 to 2.72 mg/100 g, some having substantial concentrations above this range (Englberger, Lyons, Foley, Daniells, Aalbersberg, & Dolodolotawake et al., 2010). A banana cultivar Karat with 200 g edible flesh would provide three times the estimated requirement of niacin (14 mg/day) and almost one-half of the estimated requirements of α-tocopherol (7.5 mg/day) for a non-

pregnant non-lactating woman (Englberger *et al*., 2010). Pro-vitamin A carotenoids (including beta-carotene) are important in protecting against vitamin A deficiency and anemia as vitamin A is involved in iron metabolism (Chittaranjan, 2011). Studies on bananas grown in Africa and South America have shown that bananas with yellow-to-orange flesh coloration have a higher carotenoid content (Amorim, Vilarinhos, Cohen, Amorim, Santos-Serejo, Silva et al, 2009; Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2008). Table 3 below provides the average nutritional composition of fresh bananas.

Table 3: Nutritional composition of fresh bananas

Constituents	**Unit**	**Bananas (*Musa* spp.)**
Energy	Kcal	89 - 122
Water	g	65 - 74
Protein	g	1.1 – 2.66
Total lipid	g	0.3 – 0.4
Carbohydrate	g	21.8 - 32
Fibre	g	2 – 3.4
Sodium	mg	1 - 4
Potassium	mg	385 - 500
Calcium	mg	3 - 8
Magnesium	mg	30 - 35
Iron	mg	0.4 – 0.6
Phosphorus	mg	22 – 30
Vitamin C (Ascorbic acid)	mg	11.7 – 20
Equivalent β. carotene	µg	68 - 1035
Thiamin (Vitamin B1)	µg	40 – 80
Riboflavin (Vitamin B2)	µg	40 - 70
Niacin	µg	600 - 610
Pantothenic acid (Vitamin B5)	µg	280
Pyridoxine (Vitamin B6)	µg	470
Folic acid	µg	23

***Source*:** Data adopted from Aurore et al. (2009) and Maina et al. (2012)

2.10 Consumer preferred banana characteristics

Key sensory characteristics for banana include hardness (softness), taste (sweetness), and color (light yellow) (Dzomeku, Darkey, Bam, & Ankomah, 2007). Depending on the preparation method, the amount of ripeness is also an important selection factor. Dzomeku et al. (2007) stated that "Because of the varying methods of cooking and uses of plantains, the texture, particularly, the softness of the cooked plantain is very important in determining a good cooking plantain cultivar." Another study conducted at an urban market in Kwara State of Nigeria found similar sensory attributes among *Musa* varieties. The results of a consumer preference panel indicated that consumers generally prefer "fingers" (banana fruit) of medium or big size, hands containing 9-12 fingers, pulp with light yellow color, absence of black spots in the peel, firm texture, aroma and flavor of medium intensity, and medium-sweet fruits with a shelf-life of seven to nine days (Ayinde, Adiwume & Folorunsho, 2007). Some new banana cultivars have not been well accepted for cooking by consumers for instance Nowakunda et al. (2000) found that, among 14 *Musa* cultivars introduced in Uganda, consumers rated new varieties as unacceptable for cooking purposes due to the high tannin contents, hard texture, and poor taste compared with traditional cultivars. Ssali et al (2010) found that sensory properties for 18 hybrids of matooke (East African Highland cooking bananas) in Nakaseke district, central Uganda, were more important to consumer acceptability than yield or disease resistance. Some hybrids were not liked by consumers because of their pronounced sticky placentas and being less soft relative to the indigenous cultivars such as '*Mbwazirume*' (Ssali et al., 2010). Diverse sensory qualities have been identified in various dessert banana varieties (Bugaud, Daribo, Rosalie et al., 2010).

2.11 Food texture

"Texture is defined as that group of physical characteristics that arise from the structural elements of the food, sensed primarily by the feeling of touch, related to the deformation, disintegration and flow of the food under a force, and measured objectively by functions of mass, time, and length" (Bourne, 2002). The perception of texture is a dynamic process composed of feedback from several senses (Wilkinson et al., 2001). There are also temporal elements to textural perception, from first bite to swallow. Classification of textural characteristics and their relationship to popular nomenclature is shown in Table 4.

The textural quality of fruits and vegetables is strongly dependent on cell wall polymers (Sila et al., 2005). Cell wall components consist mainly of insoluble cellulose fibers meshed into a matrix of hemicellulose and pectin (Lodish, Berk, Zipursky, Matsudaira, Baltimore, & Darnell, 2000). Cellulose and hemicellulose exhibit a distinctive microfibril network via hydrogen bonds, which enhances cell wall rigidity and resistance to tearing. Pectin and hemicellulose confer plasticity and the ability to stretch. In the middle lamella, pectin plays a primary role in intercellular adhesion (Padayachee, Day, Howell, & Gidley, 2017; Lodish et al., 2000; van Buren, 1979). The unique mixture of pectin, hemicellulose and cellulose in cell walls, and the changes that occur during ripening and storage are highly implicated in the textural changes observed in plant foods (Daniel et al., 2005; Abbott, 1999).

Table 4: Classification of textural characteristics and their relationship to common nomenclature

Characteristics	Primary parameter	Secondary parameter	Popular terms
Mechanical	Hardness		Soft, firm, hard
	Cohesiveness	Brittleness	Crumbly-crunchy, brittle
		Chewiness	Tender-chewy-tough
		Gumminess	Short-mealy-pasty gummy
	Viscosity		Thin-thick
	Springiness		Plastic-elastic
	Adhesiveness		Sticky-tacky-gooey
Geometrical	Particle size/ shape		Gritty, grain & course etc.
	Shape & orientation		Fibrous, cellular, crystalline etc.
	Moisture content		Dry, moist, wet, juicy
Others	Fat content	Oiliness	Oily
		Greasiness	Greasy

Adapted from Szczesniak (1963).

In the living tissues, changes in the composition of cell wall polymers results from the action of hydrolytic enzymes, mostly pectinases, and the activities of these enzymes often increase during ripening of fruits (Fischer & Bannett, 1991). There are majorly three pectolytic enzymes i.e. pectin methyl esterase (PME), polygalacturonase (PG) and pectin lyase (PL) that hydrolyze pectin during ripening and subsequently lead to softening (Verma, Mani Tiwari, & Mishra, 2017). PME mostly influences texture (Roy et al., 2001; Smout et al., 2004).

Foods undergo changes in texture during thermal processing. The most important changes in texture during thermal processing are related to the structure of both protopectin and pectin (Van Buren, 1986; Mc Cann & Roberts, 1991; Carpita & Gibeaut, 1993). Pectin has also been shown to influence the textural properties of carrots. For instance, cell separation occurs during cooking of carrots (Ng & Waldron, 1997), which is related to solubilization of pectic components. The β-elimination reaction, which is dependent on the degree of methylation (DM) of pectic polymers, is associated with the heat induced softening of carrots (van Buren 1986; Sajjanantakul et al., 1989; Waldron et al., 1997). Besides influence of the pectin structure, turgor pressure contributes to textural properties during thermal processing, for instance in carrots (Martens, 1986; Greve et al., 1994; Verlinden, 1996).

2.12 Factors influencing texture of foods

Owing to the great diversity in textural attributes, there are many genetic, physicochemical, environmental, and processing-related drivers of food texture. Measurable food product characteristics which can explain food texture include:- Biochemical characteristics such as lipid content, cell wall content and composition, moisture content, amylose content etc; Cellular organelles; Chemical composition; Gelatinization properties; Granule morphology; Macro-structure of the food such as arrangement of starch granules in cells; Microstructural organization of cells or the arrangement of tissue in the food product; Physicochemical properties, morphology and molecular structure of starch (amylose and amylopectin content); Genetic drivers (e.g. polymorphism); Starch digestibility; Swelling capacity; and Mechanical factors such as particle size and shape.

Texture of foods is affected by factors such as turgor pressure, content of calcium, starch, pectin, dry matter (Terefe & Verseeg, 2016) as well as pH and temperature depending on the stage of maturity and condition of the food material. For instance, calcium is associated with

increased hardness in carrots which is attributed to the protective role of calcium on membrane integrity and enhanced intramolecular and intermolecular cross-linking between pectic polymers and calcium ions (Marchiner, 1986). The degree of methylation of pectin negatively correlates with tissue hardness particularly after thermal treatment (Daniel et al., 2005). The higher the methyl ester content, the lower the resistance toward texture degradation during cooking. The level of pectin de-methylation in carrots was said to be the primary factor influencing the thermal softening mechanism (Sila et al, 2005).

Heat and pressure also affect the cell wall structure, leading to reduction in cell wall adhesion and cell separation which starts at approximately 50°C (Aguilera & Stanley, 1990; De Belie et al., 2002). Higher temperatures lead to tissue weakening and hence less hardness. Microscopic studies on carrots reveal thickening of the cell wall coupled with disruption of the plasma membrane at about 60°C (Ramana et al., 1992), which results in tissue weakening. However, these temperatures do not affect cell wall pectins (Greve et al., 1994). Tissue disruptions also lead to reduced hardness in bananas just like in other foods. Cooking is often associated with the degradation of pectic polymers via β-elimination, which is usually related to the DM of pectins (Waldron et al., 1997). De-polymerization through β-elimination reaction solubilizes esterified pectins at a pH above 4.5 and is enhanced by heating. Solubilization of pectic compounds in the middle lamella leads to intercellular weakening and cell separation causing texture degradation (Daniel et al., 2005).

The effect of calcium on hardness in living tissues can be explained by calcium binding to the free carboxylic groups of pectin leading to formation of calcium pectate that is insoluble (Banjongsinsiri, 2003). However, upon heating of the tissues, this behavior changes depending on the degree of methylation of pectin. According to Daniel et al. (2005), a weak positive correlation (0.29 to 0.48) was found between the calcium content of pretreated carrot samples and hardness values after thermal processing (115°C, 120°C, and 125°C).

High dry matter content also increases hardness in certain foods (Kaur et al., 2002; Higley et al., 2003). The fracturability, hardness and cohesiveness of fresh raw potatoes were positively correlated with their dry matter content ($r = 0.699$, 0.648 and 0.874 respectively) (Kaur et al., 2007). However, only the correlation of cohesiveness and dry matter was statistically significant ($P < 0.05$). Gibert et al. (2010) found a strong correlation between initial dry matter

content and firmness, as well as differences in cooking behavior among banana genotypes. According to Tesfaye, Shermarl, Thunya, & Oranuch (2012), a very strong correlation exists between dry matter and starch content.

Chen & Ramaswamy (2002) found decreasing hardness of bananas over time of storage although with slower rates of decrease at higher storage temperatures and attributed this decrease in hardness to degradation of starch with time during storage. The study used kinetic methods to assess color and texture of bananas stored at different temperatures.

Interest in manipulating the texture and other properties of processed fruits and vegetables has spurred research regarding the cell wall, and in particular the role of pectin and its degradation (reviewed in Van Buggenhout, Sila, Duvetter, Lowey, & Hendrickx, 2009). In a complementary publication, Sila et al. (2009) reviewed evidence on pectin structure-function relationships and methodological approaches toward understanding them, including such applications to texture as the role of pectin in gel formation in plant-based foods.

2.13 Textural attributes of bananas

Texture of bananas is a common criterion in determining the degree of maturity and ripeness (softness of texture) (Kudachikar et al., 2011; Mahajan et al., 2011; Kulkarni et al., 2011). Texture is also one of the most important attributes that consumers use to determine the quality of cooked banana or plantain (Qi et al., 2000). Hardness affects cooking time where soft bananas take a short cooking time to reach acceptable softness while hard ones are a disadvantage as they consume more fuel before reaching an acceptable level of softness. Sometimes, the acceptable softness may not be reached due to the general hardness of a particular banana cultivar. Hardness of cooked bananas also affects their taste and enjoyment in the mouth. Gibert et al. (2010) characterized banana texture after boiling using a mechanical approach, with attention to variation in the cooked texture with genotype. These authors found a strong correlation between initial dry matter content and firmness, as well as differences in cooking behavior among genotypes. They also found bananas to have lower starch content than plantains, 81.9% and 86.5%, respectively (Gibert et al., 2010).

Among cooking bananas, hardness varies depending on the cultivar, the climate, soil conditions and stage of maturity. Banana is a climacteric fruit and exhibits a respiratory peak during

natural ripening at 20^{o}C after harvest. If bananas are to be consumed ripe, then most of the banana bunches are harvested at complete maturity while they are green and unripe and subsequently allowed to ripen at ambient conditions (Kudachikar, Kulkarni, & Keshava, 2011).

The texture of bananas is dynamic due to the degradation of starch during fruit ripening. Such dramatic changes in banana texture due to post-harvest ripening are important considerations in the interpretation of measured textural traits. Peroni-Okita et al. (2010) found a pattern of starch degradation and conversion to soluble sugar in the days after fruit harvest, as well as differences in the size and shape of starch granules depending on maturity of the bananas (*Musa acuminata* AAA cv. Nanicão). Peroni-Okita et al. (2010) found that while green banana starch granules have oval and rounded shapes with smooth surfaces, ripe banana have elongated granules with circular depressions and layered striations due to postharvest enzymatic degradation. On the other hand, skin color can be used as a predictor of shelf-life for retail distribution and texture is an important part of eating quality. External skin color changes during ripening often reflect changes in flesh color (Wainwright & Hughes, 1990). As the yellowing of the skin color intensifies, the fresh color changes from the typical opaque white of a product with high starch content to a very soft yellow. Loss of firmness or softening during ripening has been linked to the breakdown of cell walls or reduction in cohesion of the middle lamella due to solubilization of pectic substances (Smith, Tucker, & Jeger, 1990) and finally migration of water from the skin to the flesh as a result of osmosis. Besides skin color, texture is also an important factor in the eating quality of bananas. Flesh texture depends on a number of factors such as variety, growing practices and the ripening procedure (Charles & Tung, 1973). In case of cooked bananas, due to the high starch content, retrogradation takes place during cooling and this leads to hardening of the texture. Gibert et al. (2010) characterized banana texture after boiling using a mechanical approach, with attention to variation in the cooked texture with genotype. Gibert et al. (2010) found a strong correlation between initial dry matter content and firmness, as well as differences in cooking behavior among genotypes. Bugaud, et al. (2010) noted important environmental relationships, such as a high correlation between rainfall level and banana fruit firmness (R=0.88) and peel hardness (R=0.80). Bugaud et al. (2010) related some sensory properties to measurable attributes such as acidity, noting that “textural properties of bananas were predictable by titratable acidity and dry matter content

(R2 = 0.62)," but that predictions of mealiness, adhesiveness, and heterogeneity were not efficient (Bugaud et al., 2013).

Kheng, Ding, & Abdul Rahman (2012) found a significant interaction between harvesting weeks and days in pulp firmness of the Malaysian Rastali dessert banana as ripening occurred. During ripening, pulp firmness decreased by 90.91% and 96.53% as the banana ripened from day 0 to 5, respectively, for banana harvested at weeks 11 and 12. Banana pulp harvested at week 12 was significantly softer than banana pulp harvested at week 11 on days 0 and 1 during ripening. Normally, as bananas ripen, softening occurs due to breakdown of cells and the conversion of starch to sugars during hydrolysis, resulting in loss of turgidity.

2.14 Role of starch in influencing food texture

For root tuber crops, starch structures are considered a primary characteristic that affects texture (Charoenkul, Uttapap, Pathipanawat et al., 2011). Approximately 80% of the dry matter in root tuber crops is carbohydrates, which consist primarily of starch, mucilage, and sugars (Huang, Chiang, Chen & Wang, 2007). Different cultivars have different levels of starch for instance, bananas have been found to have lower starch content than plantains, 81.9% and 86.5% respectively (Gibert et al., 2010). Starch itself has two major sub-components: amylose (a spiral polymer made up of D-glucose units) and amylopectin (a soluble polysaccharide and highly branched polymer of glucose found in plants) (Figure 3). Amylose is a linear molecule while amylopectin is a larger branched polymer and the two are arranged in semi-crystalline granules (Burrell, 2003; Peroni-Okita, Simão, Cardoso et al., 2010).

The ratio of amylose and amylopectin in starch may explain textural traits of food products. Nearly all key textural traits in starchy foods can be related to functions of amylose and amylopectin ratios, including viscosity, shear resistance, gelatinization, textures, solubility, tackiness, gel stability, cold swelling, and retrogradation (Satin, 1998). A basic physical property of starch that breeders utilize is starch granule size (Table 5). A comparison of the degradation of starch in root tuber crops including green banana, cassava and potato found that banana and potato starches had a higher heterogeneity of particle sizes while cassava starch had more homogenous granule size (Pineda-Gomez, Angel-Gil, Valencia-Munez, Rosales-Rivera, & Rodriguez-Garcia, 2014). The textural traits for sorghum have been framed in terms of grain quality, which can be characterized by amylose content, protein content, lipid content,

hardness, endosperm texture and peak gelatinization temperature (Goddard, Harris, Kelly, Cullen, Reynolds, & Anderson, 2015). Starch content plays a role in determining these qualities, as do grain filling and thousand grain weight (De Alencar Figueiredo, et al., 2010). In an early paper on texture in a grain crop (sorghum), Rami (1998) found close correlations among several quality traits including amylose content, dehulling yield, kernel friability, kernel hardness and kernel flouriness. These correlations pointed to high amylose content grains being more brittle, harder, and less susceptible to abrasion. Amylose content was negatively correlated with protein content but positively correlated with yield factors, providing evidence of amylose content tracking variations in starch content.

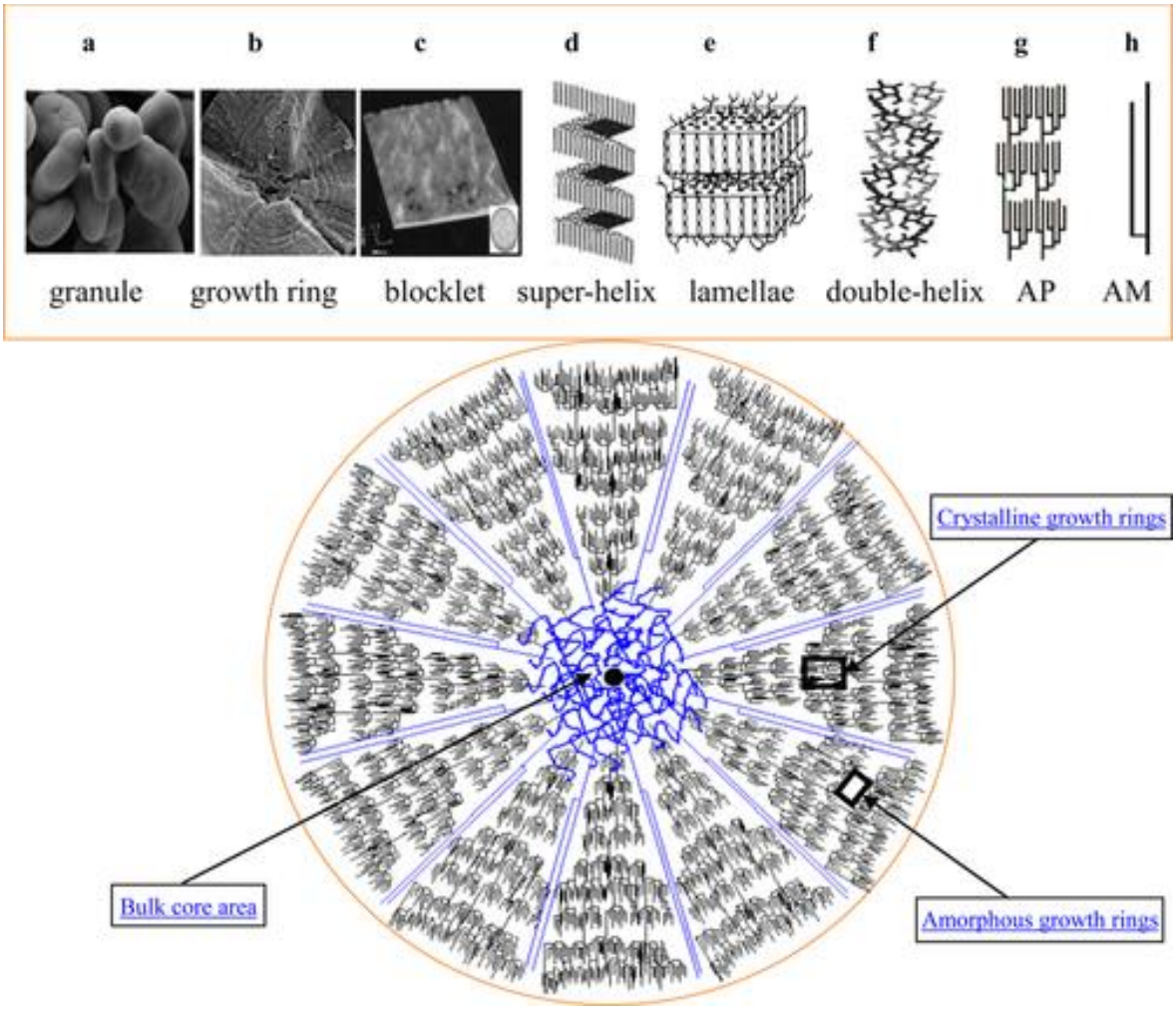


Figure 3: The hierachical structure of starch granules and a stylized model representing the distribution of amylose and amylopectin molecules. (a) Native pea starch granules as viewed by SEM; (b) growth rings as observed by SEM; (c) blocklet structures as revealed

by AFM; (d–h) representations of super helix, lamellar, double helical structures and amylopectin and amylose molecules, respectively. The blue lines represent amylose molecules, and the black lines represent amylopectin molecules. Adapted from Wang et al. (2015) as reproduced from (Pérez & Bertoft, 2010).

Table 5: Granule size of starch from different food sources

Starch species	Granule size range (µm)	Average size
Waxy Rice	2-13	5.5
High Amylose Corn	4 - 22	9.8
Corn	5 - 25	14.3
Cassava	3 - 28	14
Sorghum	3 - 27	16
Wheat	3 - 34	6.5; 19.5
Sweet potato	4 - 40	18.5
Arrowroot	9-40	23
Sago	15 - 50	33
Potato	10 - 70	36

Source: Adapted from Satin (1998)

2.15 Starch gelatinization and retrogradation

The changes that starch undergoes during gelatinization and retrogradation are major determinants of its functional properties for food processing, during digestion, and in industrial applications. These properties determine the quality, acceptability, nutritional value, and shelf-life of the finished foods (Wang & Copeland 2013). Starch is the most abundant carbohydrate reserve in plants and is found in leaves, flowers, fruits, seeds, different types of stems and roots (Alcázar-Alay & Meireles, 2015). When heated in the presence of excess water, starch undergoes a transition phase known as gelatinization, which occurs at a characteristic temperature corresponding to each starch species (Singh, et al., 2003). Gelatinization occurs when water diffuses into the starch granule, which then swells

substantially due to hydration of the amorphous phase causing loss of crystallinity and molecular order (Jiménez et al., 2012) (Figure 4).

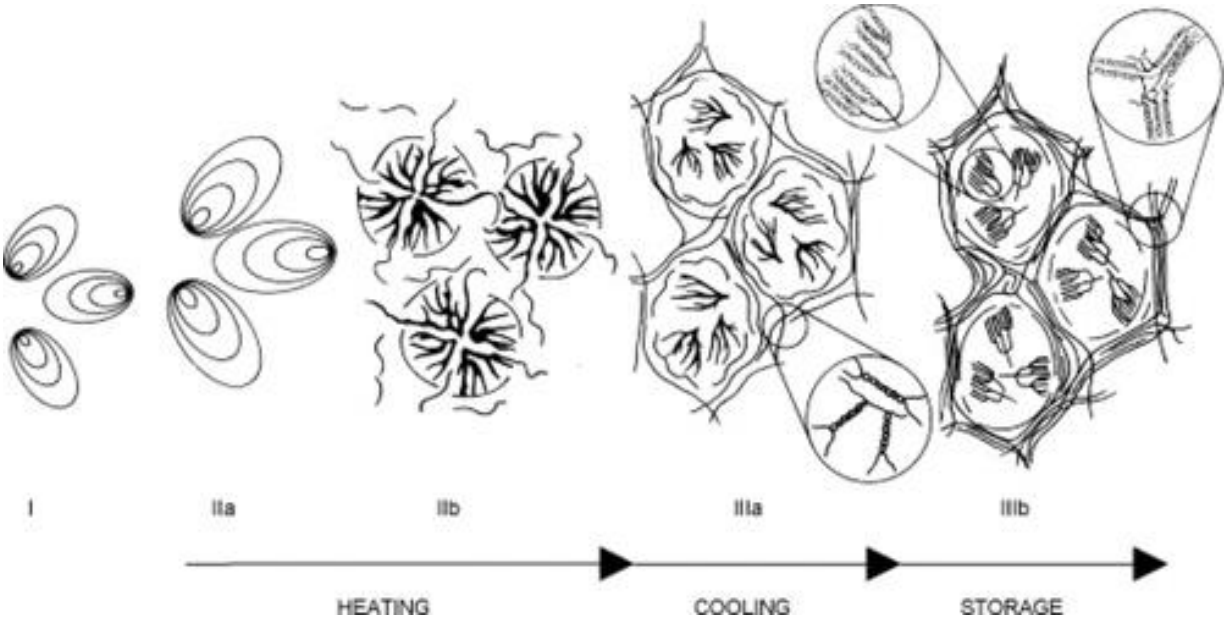


Figure 4: Schematic representation of changes that occur in a starch-water mixture during heating, cooling and storage. (I) Native starch granules; (II) gelatinization, associated with swelling [a] and amylose leaching and partial granule disruption [b], resulting in the formation of a starch paste; (III) retrogradation: formation of an amylose network (gelation/amylose retrogradation) during cooling of the starch paste [a] and formation of ordered or crystalline amylopectin molecules (amylopectin retrogradation) during storage [b]. Adapted from Wang et al. (2015).

Gelatinization occurs initially in the amorphous region, favored by the weak hydrogen bonds present in this area. The process then extends to the crystalline region. The presence of amylose reduces the fusion point in the crystalline region and the amount of energy necessary to initiate gelatinization (Sasaki et al., 2000). The gelatinization process is represented by transition temperatures and gelatinization enthalpies in the paste, and these measures are characteristic for each starch species (Alcázar-Alay & Meireles, 2015). High transition temperatures correspond to a high degree of crystallinity, high stability and resistance of the granule structure to gelatinization (Tester et al., 2004). Gelatinization of starch granules is associated with a loss of birefringence and crystalline order due to the breaking of the double helix in the crystalline region and the leaching of amylose (Evans & Haisman, 1982). As a result, starch transitions from a semi-crystalline form (relatively indigestible) to an amorphous form that is easily

digestible (Tester & Debon, 2000). The principal consideration during gelatinization is the ability of starch molecules to form hydrogen bonds with the molecules in the solvent, in this case water. Gelatinization affects the rheological properties and viscosity of the paste, making the starch granule more accessible to enzymatic action. When starch granules swell and its components are in solution, the medium properties change from a simple starch granules suspension to a starch paste. This starch behavior also affects the texture of solid foods by making them soft. The process of gelatinization is affected by the physicochemical properties of the starch, the presence of other ingredients, the availability of water and favorable conditions such as temperature, time and mechanical energy (Schirmer et al., 2015).

On cooling, starch undergoes a process of retrogradation. According to Wang et al., (2015), starch retrogradation is a process in which disaggregated amylose and amylopectin chains in a gelatinized starch paste re-associate to form more ordered structures. Retrogradation of starch is normally caused by the loss of moisture from the gelatinized starch structure leading to re-crystallization of amylose and amylopectin. For instance, during syneresis, a decrease in temperature causes a reduction in the kinetic energy that facilitates the amylose molecules to associate and form a three-dimensional network. As a consequence, water is squeezed out of the gel, while intermolecular interaction between amylose molecules becomes stronger and gel shrinks (Figure 5).

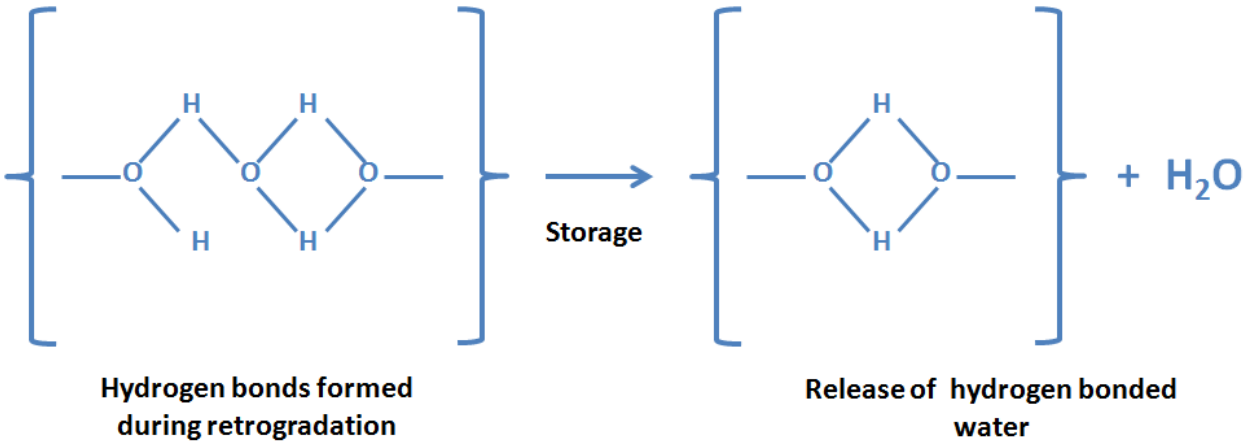


Figure 5: Syneresis of starch gel showing release of water from the amylose gel

The changes that starch undergoes during gelatinization and retrogradation are major determinants of its functional properties for food processing and during digestion (Wang &

Copeland, 2013). According to Wang and Copeland (2013), these properties determine the quality, acceptability, nutritional value, and shelf-life of the finished foods. In many cases, starch retrogradation is considered to have undesirable effects due to its major contribution to the staling of bread and other starch-rich foods, which can cause reduced shelf-life and consumer acceptance and lead to significant waste, and thereby posing significant challenges to food processors (Collar & Rosell, 2013). In terms of cooked bananas, this leads to hardening of the texture. On the other hand, starch retrogradation may be considered desirable in some applications, such as in the production of breakfast cereals, parboiled rice, dehydrated mashed potatoes, and Chinese rice vermicelli, due to modification of the structural, mechanical, and sensory properties (Karim et al., 2000).

The properties of banana starch such as its digestibility, solubility, swelling power etc. are of particular interest as they affect the functional properties of the starch during industrial applications (Ssonko and Muranga, 2017). These properties depend on the starch source such as bananas, potatoes, cassava etc. Starch is mainly made up of two polymers of D-glucose: the lightly branched amylose with a small number of long glucan chains, and the highly branched amylopectin, which contains many clusters of short chains (Manners, 1989). The amount of amylose depends on the plant cultivar and probably growth conditions (Patron, et al., 2002). Native starches contain amylose varying from 20 to 30% (Hoover et al., 2010; Wang et al., 2011) and is normally digested slowly by enzymes due to the highly ordered molecular structure in intact granules. Processing or cooking disrupts the ordered structure of granular starch, resulting in its increased susceptibility to enzymatic digestion (Wang & Copeland, 2013). The digestibility of retrograded starch is largely dependent on time and temperature of storage. Subsequent cooling and storage leads to retrogradation, in which starch regains an ordered structure that is more resistant to enzymatic digestion (Eerlingen et al., 1994; Chung et al., 2006; Zhou & Lim, 2012). Retrogradation of starch makes foods harder than when starch is still gelatinized. A good example is when bananas are cooked and allowed to cool; the texture hardens and makes the cooked bananas undesirable.

2.16 Prevention of retrogradation of starch

Retrogradation negatively affects the functional and sensory properties of foods (Sikora *et al.*, 2003). For semi-solids foods where syneresis is a major challenge, retrogradation can be

minimized or controlled by chemical modifications of starch. Chemically cross-linked starch, in which phosphate or adipate is commonly used as a bifunctional reagent, is often found in fillings, puddings, soups, and sauces due to increased resistance to mechanical shearing, thus providing short and non-sticky texture or 'mouthfeel' (Wurzburg, 2006). Also, esterified starch (so-called stabilized starch), in which acetate or propylene oxide is commonly used as a monofunctional reagent, is often found in frozen and retorted foods due to increased stability in viscosity enhancement, thus preventing hardening and syneresis during storage (Wurzburg, 2006). Alternatively, blending starch with milk or polysaccharide hydrocolloids can function as an alternative to the expensive chemical modification (Appelqvist & Debet, 1997). Most polysaccharides used by the food industry as bio-thickeners which are derived from plants for instance pectin, guar and seaweed gums (carrageenan, alginate) have a high Water Holding Capacity (WHC). Starch/hydrocolloid mixtures are widely used to modify and control the texture of foodstuffs. The addition of a hydrocolloid strongly influences the gelatinization and retro-gradation of starch (Sikora *et al.*, 2003). Specifically, food hydrocolloids are used to thicken gel, control syneresis, stabilize an emulsion or suspension, function as a coating and bind water (Fu, Chen, Luo, Liu, & Liu, 2015). It has been demonstrated that the structure of the hydrocolloid, including the type and number of monosaccharide backbone as well as the type, number and distribution of side units, determines its characteristics and behavior in solutions. Usually, polymers exhibit significant influence in their flow behavior and hence are used at low concentrations in food preparations in the range of 0.1 to 1 % (Sikora, Juszczak, Sady, & Krawontka, 2003) to retard retrogradation. Additives such as fat, glucose (Muira, Nishimura, & Katsuta, 1992), sodium nitrate and emulsifiers (Yang, Han, Wu, Zhang, Zhang, & Iqbal, 2017) can reduce retrogradation of starch.

Reduction of the retrogradation process can be enhanced by the use of plasticizers such as water, glycerol and sugars which enable the de-structuring of starch during processing. Plasticizers lower the glass transition temperature of starch, which may cause the material to be above the glass transition at room temperature (Smits, Kruiskamp, van Soest, & Vliegentharta, 2003). Knowledge of influence of plasticizers on the glass transition of foods helps food scientists to control storage stability and quality of foods (Levine & Slade, 1991). Reduction in re-crystallization increases with increasing plasticiser size. For instance, Smits et al. (2003) found the largest reduction in recrystallization with threitol (4 OH) and xylitol (5 OH).

According to Smits et al. (2003), the use of xylitol instead of glucose in bakery or other food industry, would probably be too expensive for a small improvement in reducing retrogradation. These plasticizers can also be used in non-food products such as those based on tuber starches (with B-type crystallinity) where using threitol instead of glycerol can be interesting for reducing retrogradation (Smits et al., 2003). Most of these substances can be added to bananas in sliced form and then steamed and mashed for proper distribution in the banana matrix.

Delay or prevention of retrogradation in cooked bananas may have challenges. Most importantly, how to incorporate either of the above different elements into bananas (matooke) in order to ensure proper contact with starch molecules or better still to maintaining a significant amount of free water in the banana matrix. In general terms, though energy consuming, the easiest way would be to maintain the temperature of the bananas at or above the gelatinization temperature of the inherent starch. This is based on the natural principle that at such temperatures, the starch remains gelatinized. However, this is only applicable for as long food can be held at that temperature, otherwise it may not be possible for most processing applications since food has to be processed, packed and distributed.

2.17 Starch isolation and extraction

Starch is a glucose polymer that has tremendous economic, nutritional and technological applications. Starch from different plant sources exhibits different physicochemical properties. The products in which starch is used are determined by the properties of that particular starch including the amylose/amylopectin ratio and the structure of the starch (Kenji, Komae, Kohyama, Kato, Tamiya, & Komaki, 2002). Starch can be extracted from various sources using various techniques. Besides corn, cassava and sweet potatoes, bananas are also important sources of starch for research and industrial applications. The utility of starch can be increased by developing appropriate processing techniques to prepare the starch with desirable properties (Jangchud et al., 2003).

Isolation method of starch with different agents influences starch properties and this requires special attention when selecting the most appropriate method for isolation of starch. Utilization of starch in food industries is chiefly determined by the physical, functional and pasting characteristics (Adebowale & Lawal 2002).

In general, starch is isolated from roots and tubers through rasping, sieving and decanting or centrifugation (Daiuto et al., 2005). In bananas, starch isolation may involve grinding the raw material, sieving in water and decanting. Starch isolation employs a number of techniques involving use of sodium metabisulphate, sodium chloride and distilled water.

Isolation of starch using sodium metabisulphate, according to Vasanthan (2001), is done by blending of the raw material for instance sweet potato with water at a ratio of 1:10 until a smooth slurry is formed. Sodium metabisulphate of 0.01% (w/v) is added during slurrying. After slurrying, the filtration is done with double-layered cheesecloth then filtered through a series of polypropylene screens (250, 175, 125, and/or 75 µm) and centrifuged for 20 min at 5000 × g at 20°C. Starch settled at the bottom of centrifuge tube is washed with toluene, oven dried at 30° to 40°C and the dried starch is ground with a mortar and pestle into a fine powder.
Isolation of starch using distilled water, according to Wickramasinghe et al. (2009), is done (with slight modifications) by getting the edible portion of the raw material, in this case banana, and is cut into small pieces and homogenized with distilled water for 1-2 min. The slurry is then passed through a double-layered cheesecloth and the filtrate is allowed to settle for a minimum of 3h at room temperature. The precipitated starch is washed three times with distilled water, dried at room temperature for two days and then the dried starch is kept in an oven at 50^{o}C for three hours and then ground with a mortar and pestle into a fine powder. An experiment conducted by Babu & Parimalavalli (2014) to isolate starch from sweet potatoes using three techniques involving the use of sodium metabisulphate, water and sodium chloride, found that starch isolation with only distilled water yielded the greatest amount of starch (10.20%) followed by Sodium chloride (8.72%) and Sodium metabisulphate (6.96%).

2.18 Pectin and its properties

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry matter of higher plants. The highest concentration of pectin is found in the middle lamella of cell walls, with a gradual decrease as one passes through the primary wall toward the plasma membrane (Sundar Raj et al., 2012). Pectins provide consistence and mechanical resistance to vegetal tissues (Canteri-Schemin et al., 2005). Pectin is often associated with other cell wall components such as cellulose, hemicellulose and lignin (Harholt et al., 2010).

Pectins are mainly composed of polymers rich in galacturonic acid (Figure 6), with significant amounts of rhamnose, arabinose, galactose and other sugars and are characterized by three major chains i.e. homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Fissore et al., 2009). The main chain of pectin may or may not be esterified with methyl-ester groups in the carboxylic acid units. The extent of esterification gives it the degree of esterification (DE) which may vary from low to high. Pectins are commonly classified according to their DE as high methoxyl (HM) or low methoxyl (LM) pectins (Luzio , 2013), with a DE >50 % and <50 %, respectively. HM pectins produce a gel under acidic conditions with high sugar concentrations (Evageliou et al., 2000); whereas LM pectins form gels by the interaction of divalent cations, especially Ca^{2+} with free carboxyl groups (Cardoso et al., 2003).

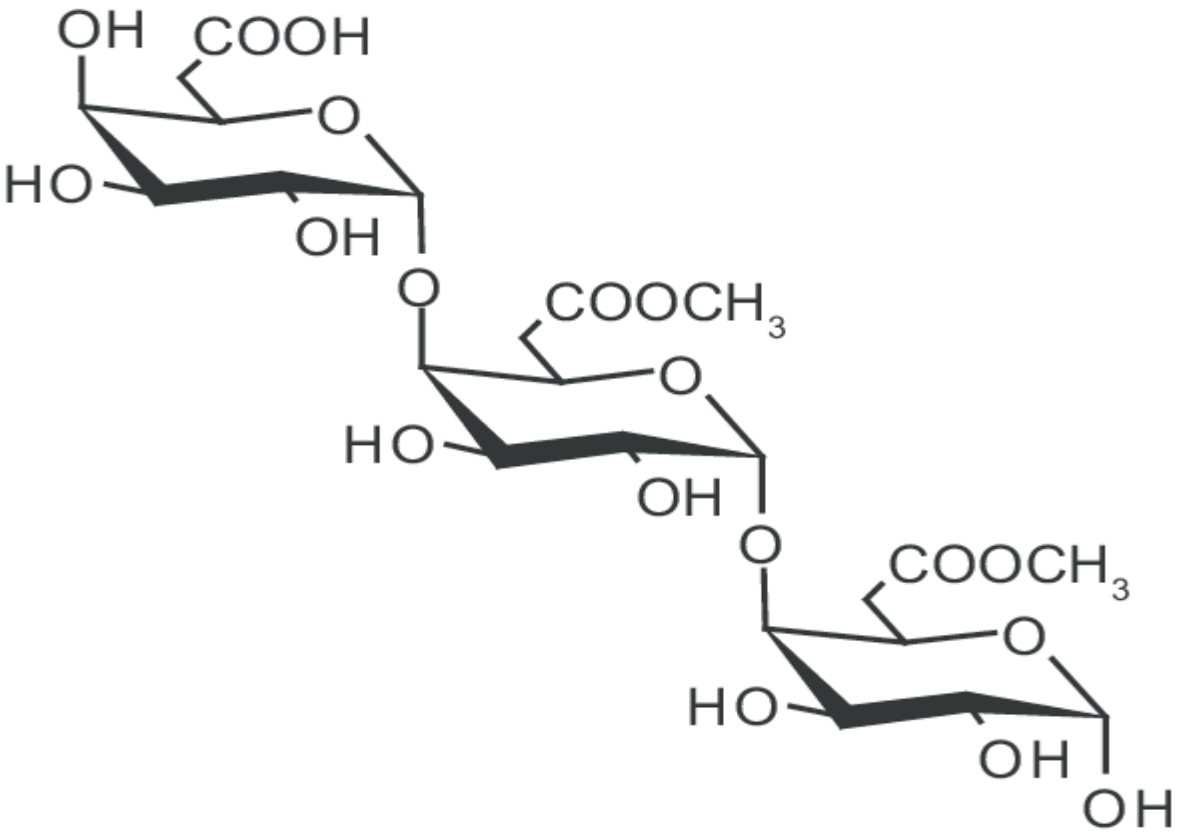


Figure 6: Structure of pectin (Ali et al., 2015)

Pectins are able to form gels depending on their molecular size, and DE which also vary depending on the source. Hence pectin from bananas does not form gels the same way as that

from apples or oranges due to variations in molecular size and DE. Pectin contains from a few hundred to about 1000 saccharide units in a chain-like configuration; this corresponds to average molecular weights from about 50,000 to 150,000 daltons (Sundar et al., 2012). According to these authors, large differences may exist between samples and between molecules within a sample and estimates may differ between methods of measurement. The amount and nature of pectin are critical for texture in fruits and vegetables during their growth, maturation, storage and processing (Crombie et al., 2003).

2.19 Role of pectin in preventing retrogradation

Generally, pectic substances are physiologically active structural polysaccharides of the plant cell wall, which play an important role in the middle lamella (Ognyanov, Georgiev, Petkova, Ivanov, Vasileva, & Kratchanova, 2018). Pectin is a hygroscopic molecule whose functional properties are based on the magnitude of its water solubility, water absorption and water holding capacity. Pectin exhibits a high water holding capacity particularly that with a high degree of esterification (DE) and has been reported at values of 37.84 g water/g (Boulos, Greenfield, & Wills, 2000) and 57 g water/g in case of sun flower pectin (Miyamoto & Chang, 1992). However, the water holding capacity of some other isolated pectins with low DEs have been found to range from 8 - 10.8 g water/g (Ognyanov et al., 2018; Lundberg, Pan, White, Chau, & Hotchkiss, 2014). The high water binding and holding capacity of pectin implies that pectin can reduce syneresis and modify the texture of foods (Gannasin, Ramakrishnan, Adzahan, & Muhammad, 2012) through increased water availability to starch in cooked bananas hence keeping them soft. The amount of pectin in the bananas is very important since it determines how much water can be retained in the banana matrix to retard retrogradation and hence hardening. On the other hand, pectins with a high DE would also imply high cohesiveness which would increase firmness for instance in case of gels (Gannasin et al., 2012). The major factors affecting water holding capacity of pectin are particle size, pH, and ionic strength (Takeshi, Yasufumi, Yumiko, Takaaki, & Toshiyuki, 1996) depending on the source.

Recovery of pectin is a crucial unit operation in the food industry in order to provide adequate supply for the growing demand (Cristina et al., 2012; Vasco-correa & Zapata, 2017). Pectin is extracted at high temperature by hydrolyzing protopectin into pectin at commercial level, but

there are novel perspectives in pectin production (Tripodo, 2015; Wang et al., 2014). The conventional method is comprised of two main steps, hydrolysis of proto-pectin into pectin using acids and subsequently precipitated by ethanol (Djilas, 2009; Ibarz et al., 2001).

Studies have been performed to investigate the effects of process variables on extraction of pectin from bananas, banana peels and other materials (Qiu et al., 2010). Acids are the strongest extracting agents of pectin as they facilitate extraction of insoluble pectin that is tightly bound to the cell matrix of the plant material and result in higher yields (Assoi et al., 2014; Yapo, 2007). Emaga et al. (2008b) studied the difference in pH, temperature and time on pectin extraction from banana peels using sulphuric acid. Oliveira, et al. (2015) evaluated pectin extraction from banana peels using organic acids. The use of mineral acids has been found to be more effective than organic acids (Chan & Choo, 2013; Pinheiro, et al., 2008). However, due to their low dissociation constant, organic acids have a lower hydrolyzing capacity than mineral acids implying they are less likely to cause proton-catalyzed depolymerization of pectins (Kermani, et al., 2014).

Some studies have found pectin yields from extraction of apple pomace, cocoa husks and passion fruit peels with citric acid to be similar to those obtained with hydrochloric acid (Canteri-Schemin et al., 2005; Chan & Choo, 2013). Pectin extraction yields vary with the method of extraction. Oliveira et al (2015) obtained yields of 5.2 to 12.2% w/w dry weight from banana peels within range reported by Emaga et al. (2008b) which was from 2.4 to 21.7%. The yield was increased by increasing temperatures and lowering pH. Conditions used by Emaga et al. (2008b) involved use of 90^{o}C at pH 1.5 for 240 min. However, the harsher conditions reduced the quality of pectin by lowering the DE to 49%. These authors have reported time to have a positive effect on yield which was highly significant. Other studies by Garna et al. (2007) and Masmoudi et al. (2008) have reported harsh conditions to favor pectin extraction yields from apple pomace and lemon by-products.

Depending on the extraction conditions, the quality of pectin will also vary. Emaga et al. (2008b) have reported the Degree of Methyl Esterification (DE) values from 43.5 to 79% when extracting pectin from banana peels using sulphuric acid. Oliveira et al (2015) found optimum conditions of extraction using citric acid to be at 87^{o}C at pH 2.0 for 160 min. Emaga et al. (2012) and Garna et al. (2007) reported increased Galacturonic acid (GA) with increasing

extraction time and temperature. Chan and Choo, (2013) also found that increasing extraction time resulted in higher uronic acid. A very low pH (1.5) was demonstrated by Garna et al. (2007) to result in higher extraction of low molecular weight compounds (non-pectic substances or degraded fractions of pectins) from apple pomace when compared to pH 2.0.

Microwave Assisted Extraction (MAE) has been used to extract pectin from plant materials (Sandarani, 2017). It involves dielectric heating of plant molecules through the exposure of microwaves. Dipolar rotation of water takes place due to the absorbance of microwave energy, which leads to generation of heat inside the plant tissues. MAE has been recently investigated by many researchers and found that it can lead to a considerable increase in the yield and quality of extracted pectin (Kratchanova et al., 1994; Mohapatra & Mishra, 2011). For example, when orange peels are subjected to microwave radiation, there is inactivation of pectin esterase enzyme and destruction of orange skin cells due to rapid heat generation in microwave environment (Zhongdong et al., 2006). According to these authors, since pectin esterase interacts with the pectic substances in the orange peels and reduces their solubility, its inactivation improves the pectin extraction. Moreover, due to the disintegration of parenchyma cells, there is also increase in specific surface area, which facilitates the water absorption capacity of the plant cell. MAE has been used to reduce extraction time and energy (Kratchanova et al., 2004; Fishman et al., 2006).

Extraction of pectin using enzymes has also been applied. In this case, cell wall degrading enzymes with minimum pectinolytic activity are used to hydrolyze non pectin plant cell wall components in enzymatic extraction of pectin (Puri et al., 2012; Fissore et al., 2009). Enzymatic extraction of pectin is environmentally safe and more effective in terms of pectin yield. Different enzymes such as polygalacturonase, hemicellulose, protease and microbial mixed enzymes, cellulose, α-amylase, celluclast, alcalase and α-amylase and neutrase, Xylase, cellulose, b-glucosidase, endopolygalacturonase and pectinesterase are used in pectin extraction as enzymes have the ability to degrade pectin and modify the physicochemical properties of the pectin (Yuliarti et al., 2011; Yu & Sun, 2013; Cui & Chang, 2014).

2.20 Gaps and Challenges in Banana Processing

The major limitation to the utilization of bananas in Uganda is the limited shelf life and their rapid susceptibility to postharvest losses, particularly when fully matured, which necessitate the

need for processing to more stable intermediate or ready- to- consume forms. On the other hand, the hardening phenomenon which bananas undergo after being cooked could be hindering processing of cooking bananas into shelf-stable cooked banana products. In Uganda, bananas processing currently includes cooked banana meals (possibly over 95% in case of cooking cultivars), banana juice/beer, dried or fried banana crisps & chips. Newer products include banana flour and fresh vacuum packed bananas which are yet to gain market on a significant scale. Banana flour is currently being applied in cakes, cookies and dense breads. Processing of mature green bananas is also affected by challenges such as enzymatic browning during processing (Luo & Tao, 2003) and storage which has a negative effect on their appearance, sensory properties such as taste, odor, & texture and nutritional value (Jiang, 2004; Komthong et al., 2006). Enzymatic browning in bananas is affected by a number of factors which include concentration of polyphenol oxidase (PPO) and presence of phenolic compounds, lower pH, favorable temperature including availability of oxygen (Yoruk & Marshall, 2003). However, this can be mitigated using a number of techniques such as chemical treatments (that include the use of antioxidants, acidification, chelating agents) and physical methods (such as blanching, freezing, chilling, and atmospheric modification) (Ioannou & Ghoul, 2013). Drying is one of the traditional methods used in the tropics for processing bananas into shelf stable forms and it is associated with a substantial reduction in weight and volume thereby minimizing packaging, storage, and transportation costs and enables storability of the product under ambient temperature, especially in developing countries (Senadeera et al., 2005). Dried bananas are easy to handle and can be easily incorporated during food formulation and preparation. Hot air drying of food materials also helps to control product quality, enhance hygienic handling and reduce product losses (Corzo et al., 2008). This range of processed banana products is very narrow for such an economically and agriculturally important crop.

In Uganda, cooking bananas are only used for meals where they are cooked by either steaming combined with mashing or boiling to bring about the desired softness and taste. During cooking, bananas undergo softening, the rate of which normally follows first order kinetics. This behavior is observed in the first 10 min of heating and thereafter, there is little change in firmness (Qi et al, 2000). According to Qi et al. (2000), there is an inverse negative relationship

between the force needed to penetrate into the pulp tissue of the cooked bananas and the cooking time in the first 10 min. In general, plantains soften at a slower rate than bananas demonstrating that hardness of banana varies with the cultivar. Generally, cooking time has a greater influence on the cooked banana texture than other factors (Qi et al., 2000) and this could affect the rate and degree of starch retrogradation in the cooked bananas during cooling. According to Huang & Bourne (1983), softening in fruit tissues is due to changes in pectic substances which are also responsible for 95 – 97% firmness in the raw vegetable tissues. There are several challenges, for instance why do the different banana cultivars such as cooking and juice differ in hardness at all levels of processing particularly when still in intact solid form. Is it due to compositional differences of the major components? What role is played by each of the major components in these bananas? How do the major components differ between the different banana cultivars?

CHAPTER 3

CHEMICAL COMPOSITION OF SELECTED BANANA CULTIVARS FROM UGANDA AND PHYSICO-CHEMICAL CHARACTERIZATION OF THEIR STARCH AND PECTIN

3.1 PROXIMATE AND MINERAL COMPOSITION OF SELECTED INDIGENOUS COOKING AND JUICE BANANA CULTIVARS GROWN IN UGANDA

Abstract

Uganda has over 80 banana cultivars which include cooking, juice/beer and desert types. Information on chemical composition is important in understanding differences in physical properties (such as hardness) of these bananas. However, information about their composition is still scanty. Hence, levels of moisture, dry matter, ash, crude fat, protein, fibre and minerals in selected cooking and juice banana cultivars were examined using standard AOAC methods.

Juice bananas had significantly higher dry matter content (30 to 33%) than cooking bananas (19 to 25.6%) ($P<0.05$). The compositional ranges for ash, crude fibre, and crude fat content were 1.97 to 4.24 g/100 g, 0.251 to 0.478 g/100 g and 0.15 to 0.58 g/100 g respectively on dry basis and there were no significant differences between cooking and juice banana cultivars ($P>0.05$). In terms of mineral content, the ranges for calcium, sodium and potassium were 4.76 to 10.84 mg/g; 0.62 to 1.84 mg/g; and 307 to 358 mg/g of banana flour respectively and did not significantly differ between cooking and juice banana cultivars ($P>0.05$). On dry basis, carbohydrate content ranged between 90 and 96% which did not significantly differ between cooking and juice banana cultivars. However, on wet basis, levels of crude carbohydrates (17.6 – 31.8%) were significantly higher in juice bananas (28.26 – 31.75%) than in cooking bananas (17.61 – 23.24%) ($P<0.05$) while the other proximate components did not significantly differ between cooking and juice banana cultivars ($P>0.05$).

Key words: Dry matter, crude ash, fat, fibre, protein and carbohydrate content.

3.1.1 Introduction

Cooking bananas (matooke) are usually harvested at green maturity when they exhibit maximum composition at which stage they are used for preparation of meals. The stage of

maturity greatly affects the concentration of nutrients in bananas just like in other plants (Izonfuo & Omuaru, 1988), thus for cooking bananas (matooke), it is important to harvest them at optimum green maturity (Yu et al., 2004). The stage of maturation at which fruits are harvested not only influences their composition but also the fruit's green-life or its ability to be stored for long periods (Ogbonna et al., 2016). Bananas make significant contribution to human nutrition and health through their composition in terms of energy, minerals, vitamins and fibre. Harvesting bananas prematurely could affect composition and taste when cooked. The integral composition of bananas is important in influencing the textural properties of bananas as living tissues and when cooked. Hence, knowledge of composition of bananas in terms of their ash, fat, fibre, protein, moisture as well as dry matter content not only plays a critical role in understanding the health and, product development properties of bananas but also could serve as basis for correlation of the textural properties. In general, high dry matter content would imply greater hardness (Goddard, Harris, Kelly, Cullen, Reynolds, & Anderson, 2015); more crude fat and protein content would mean formation of more amylose-fat/protein complexes which are insoluble and could further imply greater hardness in bananas (Thachil, Chouksey, & Gudipati, 2014).

In Uganda, cooking bananas are harvested at the green mature stage (95 – 120 days) for preparation of *Matooke (a* local term for bananas) meals. At green maturity, the banana fruits exhibit optimum maturity and hence food reserves beyond which the fruits are ready to ripen. Bananas are generally rich in minerals and ascorbic acid (Ayo-Omogie et al., 2010). Besides texture, knowledge of composition of bananas could give a fair estimate of their potential as health foods as well as for use in developing health and nutritious new products. Calcium has been associated with firmness of tissues especially those containing pectin through formation of calcium pectate whereas sodium has been associated with tenderness through enhancing starch gelatinization (Kittemann, Neuwald, & Streif, 2010). Therefore, information on calcium and sodium composition may help anticipate the hardness of bananas when cooked.

Currently, there is scant information regarding crude ash, protein, fibre, fat and mineral content of Uganda's endemic cooking and juice banana cultivars. The main objective of this part of the study was to determine composition of selected Ugandan cooking and juice banana cultivars.

Major differences in composition between cooking and juice banana cultivars could provide information on why these banana cultivars exhibit differences in hardness.

3.1.2 Materials and methods

3.1.2.1 Raw materials

Fourteen (14) unripe mature green East African Highland Banana cultivars (*Musa* AAA-EA) endemic to Uganda were used in this study. Eleven cooking banana cultivars were selected from four clone sets i.e. *Nfuuka* clone set (*Namande* {NAMD)}, *Nakawere* {NAKW} & *Namweezi* {NAMZ}); *Nakitembe* clone set (*Nakitembe* {NAKT}, *Nakyetengu* {NAKY} & *Kibuzi* {KIB}); *Musakala* clone set (*Musakala* {MUS}, *Mpologoma* {MPO} & *Kisansa* {KIS}); and the *Nakabululu* clone set (*Nakabululu* {NAKB}, & *Kazirakwe* {KAZ}); all were purchased from Kawanda Agricultural Research Institute (KARI). Three juice banana cultivars were also selected i.e. apple banana or *Ndiizi* (NDI) and *Kisubi* (KISB) both from the Ney Poovan AB clone set and *Kayinja* (KAY) from Bluggoes ABB clone set (Karamura, 1998). *Ndiizi* was bought from KARI while KISB and KAY were obtained from a local farm in Wobulenzi, Luweero District, Uganda. All bananas were harvested at green mature stage (90 to 120 days). Maturity was determined by monitoring the dry matter content and pulp-to-peel ratio until they were relatively constant, a sign of optimum maturity. All bananas were free of physical injuries and diseases. Bananas were harvested and immediately transported to the laboratory for sample preparation and analysis.

3.1.2.2 Determination of moisture content

Moisture content was determined according to the AOAC method (1999). Bananas from each of the fourteen cultivars were peeled and carefully sliced to about 1 – 2 mm. Then about 100 g of sliced bananas were weighed into dry pre-weighed petri-dishes and immediately dried in an air drying oven (Yamato, DG400C, Japan) at 105^{o}C for 5 h. The dried bananas in the petri dishes were cooled in a dessicator to room temperature before being weighed again. The dried bananas were carefully pulverized in a grinder (Geepas, GSB5362, Japan) to a fine powder. The fine powder was further dried at 105^{o}C for 5 h and then cooled in a desiccator to room temperature before being weighed again. Moisture content was determined using the formula below:

$$\%\ moisture\ content =$$

$$\frac{Weight\ of\ sliced\ bananas\ before\ drying - weight\ of\ dried\ banana\ flour}{weight\ of\ sliced\ bananas\ before\ drying} x\ 100$$

3.1.2.3 Preparation of banana flour

Fresh bananas were peeled, sliced to about 1 – 2 mm and approx. 100 g weighed into pre-weighed petri dishes. The bananas were immediately dried in an air drying oven (Yamato, DG400C, Japan) at 55^{o}C for 20 h. The dried bananas were cooled in a desiccator and the samples pulverized in a grinder (Geepas, GSB5362, Japan) to a fine powder. The banana flour was carefully sieved through a 150 µm sieve and the coarse particles were further ground using a mortar and pestle and all the flour carefully collected and sieved again. The sieved banana flour was dried further at 105^{o}C for 3 h before being packed in airtight falcon tubes. The banana flour was stored in a cool dry place for further use in determination of proximate composition.

3.1.2.4 Determination of crude protein content

Crude protein was determined using the semi-micro Kjeldahl's method number 920.152 (AOAC, 1995) for protein determination. A measured weight of the test sample 2g was mixed with 10ml of conc. H_2SO_4 in a Kjedahl digestion stand in addition to a tablet of selenium catalyst and heated strongly under a fume cupboard in a heat block (Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA) at 420 ◦C for 2 h. A reagent blank was digested as well but without any sample. All digest were carefully diluted with distilled water and transferred quantitatively to a 100ml volume flask and made up to mark with distilled water. An aliquot 10ml of the digest was mixed with equal volume 10ml of 45% NaOH solution in a machine distillation apparatus. The mixture was distilled and the distillate connected into 10ml of 4% boric acid solution containing three drops of mixed indicator solution (methyl red and bromocressol green) and a total of 50ml of distillate was collected and titrated against 0.1N HCl solution. The end point was marked by a colour change from green to deep red colour. Both the sample and the reagent blank digest were distilled and titrated. The formula below was used to calculate the nitrogen and protein content.

The protein content was calculated using the following formula:

$$\%\ Nitrogen = \frac{(v_1 - v_0)}{sample\ mass\ (g)} x100$$

Percentage protein was then calculated by multiplying % Nitrogen with a factor of 6.25

Where:

% protein = total Nitrogen x K

$v_1 = volume\ of\ acid\ used\ during\ titration$

$v_0 = volume\ of\ the\ blank\ titration$

$N = Normality\ of\ HCl\ used = 0.1N$

$W = weigh\ or\ volume\ of\ Sample\ used$

$K = correction\ factor\ for\ specific\ type\ of\ food\ which\ is\ 6.25$

3.1.2.5 Determination of crude fat

Crude fat content was determined using the Soxhlet apparatus according to method number 920.39 (AOAC, 1990). Continuous Solvent Extraction Gravimetric Method using Soxhlet Apparatus as described by (Correia et al., 2004) was used to determine the fat content in the banana flour. About 5.0g of each sample was wrapped in a porous paper (Whatman NO 45 Filter paper). The wrapped sample was put in a soxhlet flask containing 200ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the flask through electro-thermal heater, the solvent vaporized and condensed into the flux flask such that the wrapped sample was completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned over thus carrying oil extract from the sample down to the boiling flask. Extraction was performed for 60 min. The defatted sample was removed and reserved for crude fibre analysis. The solvent was recovered and the extraction flask with its oil content was dried in the oven at 60^{o}C for 3 min so as to remove any residual solvent. After cooling in a desiccator, the flask was reweighed. By difference, the weight of fat (oil) extracted was determined and expressed as a percentage of the sample weight.

Percentage fat content was determined using the as below:

$$\%\ crude\ fat = \frac{W3-W2}{W1}\ x\ 100$$

$Where;\quad W3 = weight\ of\ extraction\ cup\ (g) + weight\ of\ residue\ (g)$

$W2 = weight\ of\ extraction\ cup\ (g)$

$W1 = Initial\ weight\ of\ the\ samples\ (g)$

3.1.2.6 Determination of crude fibre

Crude fibre was determined according to the AOAC method 978.18 (AOAC, 2006). A measured weight of the defatted sample (4 g) from fat analysis was boiled in 20 ml of 1.25% H_2SO_4 solution for 30 min under reflux. After that, the samples were washed with several portions of hot boiling water using a two-fold nylon cloth to trap the particles. The washed samples were carefully transferred quantitatively back to the flask and 20 ml of 1.25% sodium hydroxide (NaOH) solution was added to it. Again, the samples were transferred to a weighed porcelain crucible and dried in an oven at 105^{o}C for 3hours. After cooling in a desiccator, they were reweighed (W2) and then put in a muffle furnace and incinerated at 550^{o}C for 2 h until they turned into ash. Again they were cooled in a desiccator and weighed. The crude fibre content was calculated gravimetrically as:

$$\%\ crude\ fibre = \frac{w_1 - w_3}{w2} x \frac{100}{1}$$

Where W1 = weight of sample analyzed W2= weight of crucible and sample after boiling and drying W3 = weighed of crucible and sample after ashing.

3.1.2.7 Determination of ash

Ash was determined by the furnace incineration gravimetric method (AOAC, 1984). Two grams (2 g) of banana flour was weighed into a dry crucible and heated in a muffle furnace at 550^{o}C until the sample was completely white ash with no black spots. The crucible and its contents were removed and allowed to cool in a desiccator to about 25^{o}C. The ash content was determined by weighing the cooled crucible and calculated using the following:

$$\%\ ash\ content = \frac{G2-G1}{W} x\ 100$$

$Where:\ G2 = Weight\ of\ crucible\ and\ sample\ after\ ashing;$

$G1 = weight\ of\ the\ crucible\ ;$

$W = original\ weight\ of\ the\ sample$

3.1.2.8 Determination of mineral content

: Mineral content of bananas was determined by atomic absorption spectrometry, according to the method of AOAC (2005).

3.1.2.8.1 Ashing (dry digestion)

A cleaned crucible was dried in the oven at 110^{o}C for 15 min and cooled in a desiccator. Approx. Then 1 g of fine banana flour was weighed into a pre-weighed crucible in triplicate. Then the sample was placed in a muffle furnace at 550^{o}C and heated for 6 h (AOAC, 2005). The crucible was then removed and cooled to room temperature in a desiccator.

3.1.2.8.2 Preparation of the ash solution

To the crucible containing the ash (as prepared in 3.1.2.8), 5 ml of conc. HNO_3 was added. The same acid was used to wash the ash from the sides of the crucible. The acid was then evaporated by heating the crucible on top of a hot plate while not allowing the solution to boil. The remaining residue was dissolved by adding 2 ml of conc. HCl and heated for 3 – 4 min while taking care not to spill the solution. The crucible was then cooled to room temperature and the solution transferred into 25 ml volumetric flask. The crucible was washed with distilled water and the wash added to the volumetric flask. The volumetric flask was brought to volume mark using deionized water.

Five standards of each metal ion were prepared using known concentrations which were read in the Atomic Absorption Spectroscopy (PinAcle 900H, Perkin Elmer) using their specific standard metal lamps to obtain a calibration curve. Then, 10 ml of the sample solution prepared above was pippeted into 250 ml volumetric flask. Then, 1 ml of hydroxyl amine hydrochloride solution was added, mixed well and allowed to stand for 5 min. Ten (10) ml of each of the prepared standard solutions was separately pippeted into the 25 ml volumetric flask, and the same procedure was followed as it was for the samples. Then, 5 ml acetate buffer and 4 drops of phenolphthalein indicator were added to each flask and mixed well for color development. The solution was left to stand for 30 min and then made up to volume using deionized water. The absorbance of the solutions were read using a spectrophotometer at each metal ion's wavelength i.e. Na (589.0 nm), K (766.5 nm), Ca (422.7 nm), Mg (285.2 nm), Zn (213.9 nm) and Fe (248.3nm) using specific metal lamps. The calibration curve was used to obtain the concentration of the mineral elements in the samples.

3.1.2.9 Determination of carbohydrate content

The carbohydrate content was determined by calculating the difference between 100 and the sum total of the other proximate components (AOAC, 2005). Hence it was calculated using the formula below: % CHO= 100-% (Protein + Fat + Fibre + Ash + Moisture content).

3.1.2.10 Data analysis

Data were analysed using SPSS (IBM) package version 23. Means were separated by group and were analyzed using one-way Analysis of Variance (ANOVA) using Fisher's Least Significant Difference (LSD) and means were tested for homogeneity of variance. Significant differences were determined at $P < 0.05$.

3.1.3 Results and discussion

3.1.3.1 Moisture content

Moisture content of bananas ranged between 66.9 and 81.0%. Juice bananas had significantly lower moisture content ($P<0.05$) ranging from 66.9 to 69.2% than cooking bananas whose moisture content ranged from 74.4% to 81.0%. Among cooking bananas, *Namande* (NAMD) and *Musakala* (MUS) had the lowest moisture content (74.4 and 75.0%, respectively) while *Nakitembe* (NAKT) and *Mpologoma* (MPO) had the highest (80.4 and 81.0%, respectively) moisture content. Among juice bananas, *Ndiizi* (NDI) had the lowest (66.9%) relative to *Kisubi* (KISB) and *Kayinja* (KAY) which contained 68.3 and 69.2% moisture, respectively.

The most abundant component of fruits and vegetable is moisture, which may account for up to 90% of the total mass and the maximum moisture content varies between individual fruits and vegetables, because of structural differences (Vicente et al., 2009). For bananas, moisture content depends on cultivar, variety, season of growth as well as the moisture content of soils (Ogbonna et al., 2016; Adeyemi & Oladiji, 2009 and Onwuka & Onwuka, 2005). According to Makanjuola et al. (2013), bananas and plantains contain mainly water and carbohydrates. Ogbonna et al. (2016) reported moisture content of Nigerian bananas, plantain and Saba bananas to be 73.66%, 62.63% and 74.57%, respectively at green mature stage while it varied from 60.44 and 81.68% at the various stages of maturity being highest at ripening stage. This is also in agreement with results of Adeyemi & Oladiji (2009) who reported moisture in bananas grown in Nigeria to range from 73.47 to 79.22% in the unripe to overripe bananas. Onwuka & Onwuka (2005) reported moisture in plantains to vary from 61.62 (unripe bananas) to 62.50 in the ripe bananas. The current study focused on bananas at green mature stage which means

there was potential for increased moisture content towards ripening. Moisture content of bananas influences their characteristics such as hardness of the living tissue (El-Ramady et al., 2015) and when cooked but also juice yield for ripe bananas. The high moisture content found in cooking bananas examined in this study could possibly contribute to their soft texture particularly when cooked relative to juice bananas. This could indeed be an advantage when it comes to saving fuel costs as it takes a lesser time cooking soft banana than hard ones. High moisture content could also be an indication of high perishability of cooking bananas.

3.1.3.2 Dry matter content

Dry matter content generally varied from 19.0 to 33.1% with juice bananas having significantly higher dry matter than cooking bananas ($P<0.05$; Table 6). Dry matter content for cooking bananas ranged from 19.0 to 25.6% while that of juice bananas ranged from 30.8 to 33.1%. Among cooking bananas, MPO, NAKW and NAKT had significantly lower dry matter than NAMD, NAKW and MUS while the rest of cooking bananas had intermediate dry matter content. Just like moisture content, dry matter is a potential indicator of hardness where bananas having higher dry matter content are expected to be harder than those with lower dry matter. Dry matter content of cooking bananas observed in this study was in agreement with dry matter content reported by Ogbonna et al. (2016) for three Nigerian *Musa* species i.e. bananas, plantains and Saba bananas which ranged from 18.32 to 29.69%. Dry matter content of juice bananas investigated in this study was higher than the findings of Ogbonna et al. (2016). Belayneh et al. (2014) also found similar results for Colombian cooking bananas (*Musa* spp.). Ferris et al. (1999) established a positive relationship between textural traits and dry matter content. A significant linear relationship was found between initial firmness and dry matter content of different raw banana varieties (Gibert et al., 2010). The major component of dry matter in bananas and plantains is starch which has been reported to average 81.9 and 86.5 % dry basis, respectively (Gibert et al., 2009). According to Gibert et al. (2009) starch is the main ingredient of dessert and cooking bananas and was noted to contribute to the initial firmness of different raw banana varieties.

According to Belayneh et al. (2014), the high dry matter content of bananas and plantain fruits is an indicator of better cooking quality and storability which is also supported by Forester et al. (2003) and Muchui et al. (2010). However, this is in contrast with results from this work since juice bananas which possessed higher dry matter content are actually not good for

cooking due to their astringent taste (Kyamuhangire et al., 2006) and potentially hard texture (Chapter 4 of this report). This contrast could possibly be due to genetic variability affecting the tannin content as well as the texture. Dry matter content is also a good determinant of the biological value of crops, which shows the fraction of the harvested crop that is available for processing into food products (Baiyeri et al., 1999). On the basis of dry matter, juice bananas would have a higher commercial value and hence processing potential. However, in terms of taste and desirability, experience shows cooking bananas are of higher commercial and culinary value.

3.1.3.3 Ash and mineral content

Ash content in all bananas ranged from 1.97 to 4.24 g/100g banana flour (Table 6). The ash content of juice bananas ranged from 2.30 to 2.81g/100g while that of cooking bananas ranged widely from 1.97 to 4.2 g/100g. The ash content of both juice and cooking bananas did not significantly differ ($P>0.05$). The ash content of NAMD, NAKY, KIB , MUS and MPO was generally significantly higher than that of all other banana cultivars. This could imply that they contain more banana seeds.

The ash content of bananas observed in this study was higher than that given by Forster et al. (2003) who reported ash content of pulp portions from eight different banana cultivars from the North Islands of Tenerife to vary from 0.86 to 1.28%. Forster et al. (2003) also found ash and protein content to be higher in the central part of the banana finger and argued that the higher contents of ash and proteins in the central part of bananas could be due to the presence of seeds which in general have high contents of minerals and proteins. All bananas used in this study had tiny vestigial seeds which could have contributed to the higher ash content values relative to that obtained by Forster et al. (2003). The variation in ash content could also have been affected by differences in soil conditions where these bananas were grown since certain soils in certain locations tend to have more minerals than others (Forster et al., 2003). A study by Ogbonna et al. (2016) found ash content in bananas, plantains and saba bananas at green mature stage to range from 0.71 to 1.29% which is on the lower side of the values obtained in this study.

The profile of mineral content (dry weight) for the selected banana cultivars is shown in Table 7.

Table 6: Crude ash, fibre, protein, fat and carbohydrate content (dry weight) of selected cooking and juice bananas from Uganda

Banana cultivar	Acronyms	Dry matter (%)	Ash (%)	Crude fibre (%)	Crude protein (%)	Crude fat (%)	Crude carbohydrate (%)	Crude carbohydrate (% wetbasis)
Cooking bananas								
Namande	NAMD	25.6^{b}	3.83^{g}	0.341^{ab}	4.63^{bc}	0.41^{cde}	90.8^{abc}	23.24^{c}
Nakawere	NAKW	20.2^{a}	2.03^{a}	0.37^{ab}	5.12^{bc}	0.42^{cde}	92.1^{abcde}	18.60^{ab}
Namweezi	NAMZ	22.2^{ab}	2.52^{cd}	0.468^{b}	5.64^{c}	0.27^{abc}	91.1^{abcd}	20.22^{abc}
Nakitembe	NAKT	19.6^{a}	2.06^{a}	0.404^{ab}	2.82^{abc}	0.58^{e}	94.1^{cdef}	18.44^{ab}
Nakyetengu	NAKY	22.3^{ab}	4.24^{h}	0.322^{ab}	4.72^{bc}	0.42^{cde}	90.3^{a}	20.14^{abc}
Kibuzi	KIB	23.6^{ab}	3.24^{e}	0.354^{ab}	5.57^{c}	0.37^{bcd}	90.5^{ab}	21.36^{bc}
Musakala	MUS	25.0^{b}	3.65^{fg}	0.253^{a}	4.41^{bc}	0.43^{cde}	91.3^{abcd}	22.83^{c}
Mpologoma	MPO	19.0^{a}	3.41^{ef}	0.394^{ab}	3.03^{abc}	0.51^{de}	92.7^{abcdef}	17.61^{a}
Kisansa	KIS	22.6^{ab}	1.97^{a}	0.478^{b}	3.36^{abc}	0.21^{ab}	94^{bcdef}	21.24^{bc}
Kazirakwe	KAZ	23.1^{ab}	2.15^{a}	0.37^{ab}	2.45^{ab}	0.39^{bcde}	94.6^{def}	21.85^{bc}
Nakabululu	NAKB	22.9^{ab}	2.00^{ab}	0.33^{ab}	2.09^{ab}	0.35^{bcd}	94.6^{ef}	21.66^{bc}
Juice bananas								
Kisubi	KISB	31.7^{c}	2.81^{d}	0.317^{ab}	2.80^{abc}	0.15^{a}	94.0^{bcdef}	29.80^{d}
Ndiizi	NDI	33.1^{cd}	2.47^{bcd}	0.375^{ab}	1.23^{a}	0.42^{cde}	96.0^{f}	31.78^{d}
Kayinja	KAY	30.8^{c}	2.30^{abc}	0.251^{a}	2.49^{ab}	0.41^{cde}	95.0^{ef}	29.26^{d}

*Values with same superscripts in the same column are not significantly different (P≤0.05). Values are means of three independent replicates ± standard errors of the means.

Calcium content varied from 5.0 to 10.8 mg/100 g; sodium varied from 0.6 to 1.8 mg/100 g; potassium varied from 307 to 359 mg/100 g; iron ranged from 0.42 to 1.27 mg/100 g while magnesium varied from 24 to 27 mg/100g of banana flour. In general, the content of calcium, sodium, potassium, iron and magnesium did not significantly differ between cooking and juice banana cultivars (P>0.05). However, a detailed analysis shows that there were significant differences among individual banana cultivars. For instance, NAMD, NAMZ and KIS had

significantly higher calcium content than that of NAKT and MUS all of which are cooking banana cultivars. Other cultivars had intermediate calcium content.

Table 7: Mineral content (mg/100g) of banana flour from selected Cooking and juice banana cultivars

Banana cultivar	Calcium	Sodium	Potassium	Iron	Magnesium
Cooking bananas					
NAMD	10.84^{e}	0.89^{bcde}	358.34^{a}	1.20^{f}	24.56^{a}
NAKW	7.32^{abcd}	0.86^{bcd}	343.08^{a}	1.27^{f}	26.61^{cd}
NAMZ	10.01^{de}	0.78^{abc}	308.29^{a}	1.19^{f}	26.24^{bcd}
NAKT	4.76^{a}	0.92^{cde}	308.26^{a}	0.42^{a}	25.05^{ab}
NAKY	8.24^{bcde}	1.84^{g}	307.55^{a}	0.84^{cde}	26.48^{bcd}
KIB	7.99^{bcde}	0.87^{bcd}	308.04^{a}	0.80^{bcd}	27.41^{d}
MUS	5.04^{a}	0.89^{bcde}	359.27^{a}	0.79^{bcd}	26.69^{cd}
MPO	6.25^{abc}	0.72^{ab}	308.66^{a}	1.02^{def}	24.41^{a}
KIS	10.54^{e}	1.03^{def}	307.98^{a}	0.67^{abc}	27.63^{d}
KAZ	5.70^{ab}	1.16^{f}	308.29^{a}	0.76^{bcd}	26.61^{cd}
NAKB	6.93^{abc}	0.62^{a}	308.78^{a}	1.10^{ef}	24.64^{a}
Juice bananas					
KISB	6.51^{abc}	1.00^{def}	308.21^{a}	0.54^{ab}	25.49^{abc}
NDI	8.94^{cde}	1.00^{def}	308.50^{a}	0.67^{abc}	26.63^{cd}
KAY	6.28^{abc}	1.07^{ef}	320.50^{a}	0.61^{abc}	25.63^{abc}

Values with same superscripts in the same column are not significantly different ($P \leq 0.05$). Values are means of three independent determinations ± standard errors of the means.

Results obtained in this study are in agreement with those reported by other authors. Variations in mineral content are influenced by soil composition and conditions as well as seed content (Forster et al., 2003). Forster et al. (2003) reported potassium to range from 433 to 479 mg/100 g; sodium from 0.218 to 0.36 mg/100 g; calcium from 3.31 to 5.2 mg/100 g; magnesium from 36.4 to 41 mg/100 g. The calcium content of bananas in this study was generally higher than what was reported by Forster et al. (2003) in the Tenerife Island bananas. Ozioma et al. (2013) found bananas to contain approx. calicum (7.2 mg/100 g), Mg (45.7 mg/100 g) and Potassium

(380 mg/100 g) which are comparable with mineral content of bananas in this study. However, magnesium and potassium levels were lower than what Forster et al. (2003) reported which also were higher than levels stated in Food Composition charts (Souci et al., 1989; Mataix et al., 1995). However, sodium and iron contents reported in this study were higher than those of Forster et al. (2003).

3.1.3.4 Crude fibre content

Crude fibre content of the selected banana cultivars varied from 0.251 in KAY to 0.478 in KIS (Table 6). The crude fiber content of juice and cooking bananas was not significantly different ($P>0.05$). However, NAMZ and KIS had significantly higher crude fibre while KAY and MUS had significantly lower fibre content ($P<0.05$) than the rest of the banana cultivars which had intermediate crude fibre content. These values were close to 0.51% crude fibre content obtained by Untalan et al. (2015) in bananas from Batangas. However, the values in this study were way too low compared to fibre content obtained by Asif-U-Alam et al. (2014) of 4.2% in hot air dried bananas from Bangladesh and Juarez-Garcia et al. (2006) of 6.3-15.5 g/100 g (dry weight). The low crude fibre content in bananas used in this study could be due to differences in the stage of maturity, since fibre matter develops as the fruit matures (Egbebi and Bademosi, 2012). Egbedi and Bademosi (2012) considered both ripe and unripe bananas and they found that ripe bananas had more fibre than the unripe ones. The low fiber level in the bananas in this study implies that they could be used in developing baby weaning foods. Fibres are known to aid digestion, absorb water and make stools larger and softer so as to prevent constipation (Ayoola & Adeyeye, 2009). However, too much fibre in baby foods is not desirable as it may be objectionable during consumption. The high level of fiber in bananas and plantains suggests that it is capable of promoting digestion as fibers are known to aid and speed up the excretion of wastes and toxins from the body, and also prevent colon cancer as it prevents waste or toxins staying in the intestine for too long (Eromosele & Eromosele, 1993). Oko et al (2015) reported crude fibre content of various species of bananas to vary from 0.19 to 0.51% which agrees well with current results. This imply that Ugandan bananas have a low fibre content and hence good to be used in infant food formulations. However, these results do not show signifcant difference between cooking and juice banana cultivars. Nutritive value of Indian foods provides crude fibre content of bananas as 0.4% (Gopalan et al., 2000) which is close to the crude fibre obtained in this study, while the Food Composition and Nutrition Tables (FCNT, Germany,

2000) and Ramulu & Rao (2003) provide a value of 1.8% which is higher than the valuesobtained in this work. Egbebi and Bademosi (2012) reported crude fiber content in unripe and ripe plantain to vary from 0.7 – 1.11% which increases significantly with progress of maturity. These authors attributed the increase in fiber content at matured stage over tender stage to increase in soluble and insoluble dietary fractions. Bananas used in this study had not reached ripening stage where possibly an increase in soluble matter would affect the fibre content levels.

3.1.3.5 Crude protein content

Crude protein content ranged between 1.23% in NDI and 5.64% in NAMZ (Table 6). Crude protein content in juice bananas ranged between 1.23% and 2.80% with NDI having the lowest and KISB having the highest. In cooking bananas, crude protein ranged between 2.09 in NAKB and 5.64% in NAMZ. The crude protein content in KIB and NAMZ was significantly higher than that in NDI, KAZ, NAKB and KAY ($P<0.05$). The crude protein values obtained in this work were higher than those given in the USDA Nutrient database of 1.09%. However, these results are in agreement with Ogbonna et al. (2016) who reported protein content of green mature plantains to be 3.54% while in Saba bananas it was 1.06% at the green mature stage. Oko et al. (2015) have reported protein content of various species of bananas in the range from 1.22 to 7.22% dry basis which is in agreement with results of this study. Khawas et al. (2014) reported a gradual decrease in protein content as plants mature, decreasing from stage I at 20 days (10.56 g/100 g) to stage V 80 days (2.01 g/100 g) after emergence of the inflorescence. The bananas used in this study had passed stage V (80 days) of maturity implying that possibly their protein content had decreased hence these results could be correct basing on the development stages of bananas. Despite this, there is no information relating protein content in bananas or other foods with hardness.

3.1.3.6 Crude fat content

Crude fat content varied between 0.15 in KISB and 0.58% in NAKT (Table 6). The crude fat content of juice and cooking banana cultivars was not significantly different ($P>0.05$).However, detailed analysis indicates that crude fat content of NAKT was significantly higher than NAMZ, KIB, KIS, NAKB and KISB ($P<0.05$). . On the other hand, the crude fat content of NAMD, NAKW, NAKT, MUS, MPO, KAZ, NDI and KAY was not significantly different. Auta & Kumurya (2015) reported crude fat contents of plantains and bananas to be

0.53% and 1.05%, respectively. Their crude fat value for plantains is close to results obtained in this work. Generally, bananas have been reported to have little fat content relative to the other components and this is an indicator that their products are not seriously affected by rancidity of fats which would contribute to extended shelf-life if other factors are controlled. Khawas et al. (2014) reported crude fat content of bananas to vary from 1.5% to 0.58% from stage I to stage III of maturity in decreasing tendency implying that fat content decreases with the stage of development. Goswami & Borthakur (1996) reported fat content to vary from 0.8 to 1.2% in culinary bananas which was higher during the early stage of development but decreased with maturity. Bananas used in this study were at unripe green mature stage, which means that fat content could have been at low levels. Variation in crude fat content observed between bananas used in this study and bananas used by other authors could also be attributed to cultivar and environmental differences. Vestigial seeds content could also affect the level of crude fat in bananas, an area that needs to be investigated for each of the banana cultivars assessed in this study.

Fats or lipids can affect the functional properties of starch by forming insoluble complexes with amylose (Nimz, Gessler, Usón, Sheldrick, & Saenger, 2004; Luallen & Eliasson, 2004) which tends to increase hardness in foods. In many cases, lipids exist together with starch and as a result, the lipid content in native starches usually correlates well with the amylose content (Copeland, Blazek, Salman, & Tang, 2009). The lipid content of starch is dependent on various factors, such as source, polysaccharide composition, physical structure of the plant source and the amylose content (Lindeboom, Chang, & Tyler, 2004; Copeland et al., 2009). Jane (2009) stated that the presence of lipids in starches increases the gelatinisation temperature, thus retarding granule swelling and preventing amylose from leaching out.

3.1.3.7 Crude carbohydrate

Crude carbohydrate composition (dry weight) was generally high across all banana cultivars and ranged between 90.3 and 96% (Table 6). In general, there was no significant difference in carbohydrate content between juice and cooking banana cultivars ($P>0.05$) on dry weight basis. However, on wet basis, the crude carbohydrate content varied significantly between cooking and juice banana cultivars ($P<0.05$). On wet basis, carbohydrate content generally varied between 17.6 – 31.78%. Carbohydrate content (wet basis) in cooking banana cultivars ranged

from 17.6 to 23.24% while that of juice bananas ranged from 29.3 to 31.78%. NAMD, MUS, KIB, NAKY, NAMZ, KIS, KAZ and NAKB had generally higher levels of carbohydrate but were not significantly different (P>0.05) while MPO, NAKT, NAKW and NAMZ generally had lower carbohydrate content and were also not significantly different (P>0.05). Consideration of crude carbohydrate on wet basis is based on the fact that bananas are cooked and consumed fresh with their inherent moisture which therefore affects the texture (hardness) thus making it prudent to consider carbohydrate content of the bananas on wet basis. Carbohydrate content (dry basis) above 80% has been reported to be a major component in unripe plantains (Odenigbo et al., 2013). Oko et al. (2015) found carbohydrate content (dry basis) to vary from 69.69 to 81.18% in eight unripe Nigerian plantain cultivars. The carbohydrate content determined by proximate reflects majorly two carbohydrates i.e. starch and pectic substances since cellulose and hemicellulose are part of the crude fibre content. Carbohydrate composition of bananas is largely altered during ripening as starch reserves are hydrolyzed to soluble sugars (Cordenunsi & Lajolo, 1995). Starch forms 20 to 25% of the fresh weight (wet basis) of the unripe banana pulp while sugars are present in the green fruit only at levels ranging from 1 to 2% of the fresh pulp (Aquino et al., 2016). The sugar content rises up to 15 to 20% at ripeness while starch can reduce significantly in bananas from 15.7 g/100 g to 3.40 g/100 g during ripening (Adao and Gloria 2005). Carbohydrate content in bananas affects texture (hardness) which is why breakdown of carbohydrates during ripening leads to significant softening (Ammawath, Che Man, Yusof, & Rahman, 2001).

The stage of maturity affects the carbohydrate composition of bananas. For instance, a large proportion of starch in unripe bananas is in the form of resistant starch which could affect the degree of starch gelatinization during cooking. The high carbohydrate content in the bananas used in this study could be a reflection of the high starch content and indicates that these bananas were at the green maturity stage when starch content is highest.

3.1.4 Conclusions and recommendations

According to results, bananas contain more moisture than dry matter on wet basis. The greatest component of dry matter in bananas is carbohydrates. Juice bananas contain significantly higher dry matter than cooking bananas. On wet basis, juice banana cultivars contain significantly higher carbohydrate content than cooking bananas. However, levels of other

components i.e. crude protein, fat, ash and fibre are not significantly different between cooking and juice banana cultivars. Differences in crude carbohydrate content imply that starch and pectic substance content in cooking and juice banana cultivars may be different and this could influence differences in hardness between the two banana cultivars. In general, bananas can serve as good sources of minerals and important sources of carbohydrates which are required by the human body.

Therefore, a study to determine yield and physico-characteristics of starch and pectic substances (the main carbohydrate components) from the selected cooking and juice banana cultivars was conducted and is covered in chapter 3.2.

3.2 PHYSICO-CHEMICAL CHARACTERIZATION OF STARCH AND PECTIN EXTRACTED FROM SELECTED COOKING AND JUICE BANANA CULTIVARS

Abstract

Starch is the most abundant food ingredient and provides energy, stability, bulking and influences textural properties of solid and semi-solid foods. Bananas contain significant levels of starch and pectic substances depending on cultivar. Information on levels and physico-chemical properties of starch and pectin from indigenous banana cultivars grown in Uganda is scanty. Hence, starch and pectin from 11 cooking and 3 juice bananas were extracted and characterized.

Generally, starch yield in all bananas ranged from 4 to 25.7%. Juice bananas (19 to 25.7%) had significantly higher starch yield than cooking bananas (4.0 to 15.2%) ($P<0.05$), with the exception of *Musakala* at 21%. The maximum swelling power and solubility of starch at 98.6^{o}C ranged from 10.14 to 16.58 g H_2O/ g and 5.4 to 13.5 mg/ml respectively. Both swelling power and solubility of banana starch did not significantly differ ($P>0.05$) between cooking and juice banana cultivars. Gelatinization temperature for all banana starch ranged between 78.8 and 80.7^{o}C. Amylose and resistant starch content ranged from 21.6 to 36.4% and 36.2 to 68.6% respectively and both did not significantly differ between cooking and juice banana cultivars. Final viscosities of banana starch ranged from 3040 to 3633 cP and in general, the pasting properties did not differ significantly between cooking and juice banana cultivars ($P>0.05$).

The yield of pectic substances ranged between 7.6 and 31%. Juice bananas had significantly higher crude yield (29.7 to 30.5%) than cooking bananas (7.6 to 21%) ($P<0.05$). Equivalent weight was generally high and ranged between 8156 and 16444. The anhydrouronic acid content was generally low and less than 65% while methoxyl content ranged between 5.8 and 10.6%. The degree of esterification (DE) varied from 88 and 96%. Generally, all the chemical properties of pectic substances did not significantly differ between cooking and juice bananas ($P>0.05$).

Results imply that juice bananas contain more starch and pectic substances than cooking bananas which could affect textural attributes of bananas. The physico-chemical properties of

starch and pectin from both cooking and juice bananas do not differ significantly. The high DE of pectic substances implies that the green mature bananas contain predominantly protopectin.

Key words: Cooking and juice banana cultivars; pectin; physico-chemical properties; Starch

3.2.1 Introduction

Uganda's indigenous bananas belong to the East African Highland Bananas (AAA-EA) which are endemic to the East African Highlands (Robertshaw, 2006). Bananas are a major starchy staple food with high economic value in many parts of the world particularly in Africa. They are rich in carbohydrates (Adeniji et al., 2007). Studies have shown that in countries such as Uganda, Rwanda, and Burundi, bananas contribute close to 30% of daily calorie intake, reaching as much as 60% in certain areas (IPBO, 2016).

Starch is the main carbohydrate and component of bananas. Mature green unripe bananas contain over 80% starch on dry weight basis which makes them important raw materials for various products. Starch has wide applications in the food and non-food industry (Bello-Pérez & Paredes-López, 2009). It is widely used as a thickener, stabilizer and gelling agent (Marriot et al., 1981). Being a major component of bananas, starch contributes greatly to their structural or textural properties in the same way it does for many other foods. Due to retrogradation, starch undergoes structural changes that potentially affect the texture of cooked bananas. This makes starch composition a major factor in influencing hardness of processed bananas. The texture of bananas also varies depending on the cultivar with cooking bananas being normally softer than the non-cooking cultivars such as juice/beer bananas. Resistant starch and amylose composition which are positively correlated (Sang et al., 2008; Benmoussa et al., 2007; Evans & Thompson, 2004) affect functional properties of starch (Mir et al., 2013; Fuentes-Zaragoza et al., 2010). These and other starch properties could affect textural properties of bananas during processing.

The changes that starch undergoes during gelatinization and retrogradation are major determinants of its functional properties during processing/industrial applications and digestion (Wang et al., 2015). These properties determine the quality, acceptability, nutritional value, and shelf-life of the finished foods (Wang and Copeland, 2013). Typically, starch exhibiting a higher tendency for retrogradation may promote hardness (Zaidul et al., 2007) of bananas depending on the cultivar. The chemical composition, structural morphology, amylose-

amylopectin ratio, granule structural arrangement of starch are determined by the botanical sources of starch (Ssonko and Muranga, 2017).

Banana starch characterization has been carried out extensively ((Waliszewski, Aparicio, Bello, & Monroy, 2003; Nwokocha & Williams, 2009; Dufour, et al., 2009; Zhang & Hamaker, 2012; Adewole et al., 2012; Oviri, 2014; Iwe and Agiriga, 2015). However, little has been towards characterization of starch from banana cultivars endemic to Uganda (Muranga, 1998; Ssonko and Muranga, 2017).

On the other hand, pectin is an important structural polymer found in the middle lamella of tissue cells where it functions primarily as an intercellular adhesive providing mechanical support to plant tissues (Bouton, et al., 2002). Plant pectins can be water-soluble or insoluble (protopectins) and they are primarily made up of D-galacturonic acid joined by α - (1- 4) glycosidic linkages (Van Buren, 1991; Mukhiddinov, 2000) and 1, 2-D-rhamnose with D-galactose and D-arabinose side chains (Voragen et al., 2001; Huisman et al.,2001). Pectin is a complex mixture of blocks of homogalacturonic acid called 'smooth regions' mixed neutral sugars rhamnose, galactose, arabinose and glucose in the 'hairy regions' (International Pectin Producers Association (IPPA), 2001). A certain percentage of the galacturonic acid residues is generally esterified with methanol (Lara-Espinoza et al., 2018). Pectins are classified according to their degree of esterification i.e. high methoxyl (HM) pectins with values above 50% esterified carboxylic groups and low methoxyl pectins (LM) with less than 50% esterified carboxylic groups. HM pectins form gels under acid conditions (2.0 <pH < 3.5) with high presence of co-solute concentrations (55-75 %) such as sucrose. LM pectins can form gels in the presence of divalent cations such as calcium ions at a higher pH range between 2.0 and 6.0 (Yapo et al, 2007).

Differences in pectin composition and its properties from different banana cultivars could influence textural attributes during processing. Pectin affects the mechanical and functional properties of plants. In the living tissue, pectin causes firmness whereas during cooking it causes a soft texture (Parre & Geitmann, 2005) as it solubilizes. The physico-chemical properties of pectin depend on the raw material and conditions of isolation/purification (Chan & Chao, 2013), and the maturity stage. Pectic substances can be extracted by chemical or

enzymatic methods, involving a series of physical and chemical stages, in which hydrolysis, extraction and solubilization of pectins are influenced by temperature, pH, acid type and extraction time (Pagan et al., 2001). For example, protopectins are brought into solution by hot dilute acids. The methods of extraction vary with the makeup of plant material which depends on the stage of maturity of the plant (Mukhiddinov, 2000).

Despite all the above, little is known about the chemical properties of pectic substances in bananas at green mature stage, let alone, any differences that exist between pectic substances in cooking and juice banana cultivars.

Therefore, in this study, starch and pectic substances from selected cooking and juice bananas at green maturity were extracted and characterized. Information on levels and physico-chemical properties of starch and pectic substances from these bananas would guide in evaluating differences in hardness as well as aiding in developing innovatively textured banana products.

3.2.2 Materials and methods

3.2.2.1 Raw materials

Fourteen (14) unripe mature green East African Highland Banana cultivars (*Musa* AAA-EA) endemic to Uganda (section 3.1.2.1) were used in this study. Healthy bananas were harvested at green mature stage (90 to 120 days) and were immediately used for extraction of starch and pectin.

Reagents and enzymes

Analytical grade reagents included NaOH, Conc. HCl, Conc. sulphuric acid, 95 – 99% ethanol, KOH, potassium iodide, iodine, phenolpthalene indicator, 96% ethanol, standard amylose, standard glucose monohydrate, and Tris maleate buffer. Enzymes included amyloglucosidase, α-amylase, pepsin and pectinase (*Megazyme*).

3.2.2.2 Extraction and isolation of starch

Starch extraction and isolation was performed according to the method of Zhang et al. (2005) with minor modifications. About 500 g of peeled bananas were sliced to about 1 – 2 mm, mixed with 2 L of 0.05N NaOH and macerated in a high speed coffee grinder (Geepas, GCG289, Japan) for about 2 min. The homogenate was mixed with 5.5 litres of distilled water and screened through a fine nylon cloth and the filtrate collected and allowed to stand for about

2 h. The supernatant was decanted. The sediment was mixed with another 8 litres of distilled water and again screened through a fine nylon cloth to remove residual colored materials and allowed an additional 2 h to settle. The supernatant was decanted again. The extraction was repeated by adding 8 L of distilled water to the sediment, stirred thoroughly well and left to stand overnight. The supernatant was decanted again and the sediment mixed again with 8 litres of distilled water and left to stand for 2 h. The washing steps were repeated until the supernatant was clear and the starch free of discoloration. The supernatant was decanted and the starch sediment was mixed with 3 litres of distilled water and centrifuged at 4000 g at 4^{o}C for 10 min. The supernatant was decanted and the sediment placed and spread on a tray and dried in an air drying oven (Yamato, DG400C, Japan) at 55^{o}C for 16 h. The starch yield was calculated according to the following formula:

$$\%\ starch\ yield = \frac{weight\ of\ starch\ extracted}{weight\ of\ original\ banana\ sample} x100$$

3.2.2.3 Determination of amylose and amylopectin content

Amylose content was determined using the iodine colorimetric method reported by Zakpaa et al. (2010). Approx. 100 mg of banana starch was introduced into 100 ml volumetric flask, wetted with 1 ml of ethanol and 10 ml of distilled water was added, followed by 2 ml of 10% NaOH solution and heated at 90^{o}C in a water bath (WNE 45, India) until a clear solution was formed. The flask with its contents was cooled and diluted to the mark with distilled water. A 5 ml portion of the alkaline solution was introduced into a 500 ml volumetric flask and 100 ml of distilled water added and acidified slightly with 3 drops of 6 M HCl. The contents were well mixed by shaking the flask and 5 ml of iodine solution added and diluted to the mark with distilled water. The absorbance of the solution was read against a blank with a UV spectrophotometer (LKB Biochrom Ultrospec II, model 4050, Cambridge, England) at 640 nm. A standard curve for determination of amylose concentration (Figure 22, Appendix 2) was prepared using standard amylose and the resulting regression equation used to determine amylose concentration (Zakpaa et al., 2010). Amylopectin was determined by difference between 100% and the concentration of amylose.

3.2.2.4 Pasting properties

The pasting properties of the banana starch were determined using a Rapid Viscosity Analyzer (model 3D, New Port Scientific, Sydney, Australia) according to the protocol of Iwe & Agiriga (2015) with modifications. A dry canister was prepared and 2.5 g of banana starch was weighed into it. Then, 25 ml of distilled water was added. The canister was well fitted into the RVA and the starch dispersed and mixed well in the canister by stirring it in the RVA container initially at 960 rpm for about 10 s and finally at 160 rpm for the remaining test time. The slurry was heated from 50^{o}C to 95^{o}C with a holding time of 2 min followed by cooling to 50^{o}C with 2 min holding time. The rate of heating and cooling was constant at 11.25^{o}C/min. All measurements were performed in duplicate. Peak, trough, breakdown, final and setback viscosities including peak time and pasting temperature were read from the pasting profile with the aid of a thermocline for windows software connected to a computer.

3.2.2.5 Swelling power and solubility of banana starch

The swelling power of banana starch was determined according to the method suggested by Lauzon et al. (1995) with modifications. Approx. 0.2 g of banana starch was weighed into a pre-weighed oven dried falcon tube of 15 ml. Then, 10 ml of distilled water was added and the tube capped securely before being shaken to disperse the starch. This was done in triplicate. The starch dispersion in the tube was heated in a water bath (WNE 45, India) at different temperatures from 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95 to 98.6^{o}C for 30 min with continuous shaking. After 30 min of heating, the starch was cooled immediately to room temperature and centrifuged at 4000 g at 4^{o}C for 10 min. The supernatant was decanted and kept safely for determination of starch solubility while the tubes containing the starch gel sediment were weighed and the swelling power determined according to Kusumayanti et al. (2015):

$$Swelling\ power = \frac{weight\ of\ sedimental\ paste\ (g)}{weight\ of\ the\ sample\ (dry\ basis)(g)}$$

Solubility was determined according to Lauzon et al. (1995). Accordingly, 0.5 ml of the supernatant from swelling power determination was taken into an empty glass test tube and 0.5 ml of 5% phenol was added for color development. Then, 1 ml of distilled water was added to

the sample followed by 1 ml of conc. H_2SO_4 (98%). The mixture was placed in a dark corner for 10 min to allow for color development. The absorbance of the samples was read at 490 nm using a UV spectrophotometer (Biochrom Ultrospec II). A standard curve (Figure 24 appendix 2) was prepared using glucose monohydrate. The starch concentration was estimated by taking the glucose concentration x 0.9 (a conversion factor). Solubility of starch was expressed in g/g of starch.

3.2.2.6 Determination of digestible and resistant starch content

3.2.2.6.1 Digestible starch

Digestible starch was determined according to the AOAC method 2002.02 and AACC method 32-40.01 with modifications. Approx. 100 mg of banana flour was weighed into a 15 ml Falcon tube. The flour was mixed with 10 ml of 1 M NaCl/0.5 M HCl containing pepsin at 10 mg/ml concentration and incubated at room temperature (25^{o}C) for 40 min to digest the protein. Tris maleate buffer (pH 6.9) was prepared using 6.05 g in 250 ml of Tris (Hydroxy methyl) amino methane (99.9%) to make a stock solution of 0.2 M Tris maleate and 0.2 M NaOH solution as stock. Then 100 ml of Tris maleate stock and 93 ml of NaOH stock solution were mixed in one beaker to make a stock solution. The volume was raised to 400 ml using distilled water and the pH standardized to 6.9 using 5 M HCl. Then, 0.5 g of α- amylase (3000 U/ml) was mixed with 250 ml of Tris maleate buffer. The pepsin incubated solution was centrifuged at 4000 g for 10 min at 4^{o}C. The supernatant was discarded and the resulting sediment was again treated with 1 ml of pectinase (1000 U/ml) (CAS: 9033-35-6) in Phosphate buffer (containing activity for polygalacturonase, pectin lyase, pectin methylesterase, cellulase and hemicellulase) to digest the pectin and cellulose/hemicellulose (Beldman, Rombouts, Voragen, & Pilnik, 1984). The mixture was incubated at 37^{o}C for 60 min. The pectinase incubated solution was again centrifuged at 6000 rpm for 10 min at 4^{o}C. The supernatant was discarded and the sediment mixed with 5 ml of Tris maleate buffer containing α – amylase and incubated at room temperature for 16 h. It was then incubated at 37^{o}C for 1 h and the mixture centrifuged at 4000 g for 10 min at 4^{o}C to remove the insoluble residues. Using the supernatant, 0.5 ml was drawn and mixed with 0.5 ml of 5% phenol and 1 ml distilled water was added. Then, 1 ml of conc. H_2SO_4 (98%) was added and the solution allowed to stand in the dark for about 10 min to allow for color development before the absorbances were read using a spectrophotometer at 490 nm.

3.2.2.6.2 Resistant starch

To the sediment from determination of digestible starch (AOAC method 2002.02 and AACC method 32-40.01), 3 ml of Tris – maleate buffer (pH 4.75) was added and the sediment solubilized. It was then heated in a water bath (WNE 45, India) at 90^{o}C for 30 min to gelatinize the starch. The gel formed was homogenized using a sample homogenizer before adding amyloglucosidase. Then, 0.3 g of amyloglucosidase (3000 units/ml) was mixed with 6 ml of Tris mealate buffer at pH 4.75 and properly mixed on a vortex. The starch gel formed was homogenized using a sample homogenizer and 120 µl of amyloglucosidase solution was added and mixed thoroughly on a vortex. The mixture was then incubated at 60^{o}C for 45 min at pH 4.75. It was then centrifuged at 4000 g for 10 min at 4^{o}C and the resulting supernatant used to analyze for resistant starch. Using the supernatant, 0.5 ml was taken into a test tube and 0.5 ml phenol was added. Then, 1 ml of distilled water and 1 ml conc. H_2SO_4 were added and allowed 10 min in the dark for color development. The absorbance was read at 490nm. A standard curve (Figure 23 appendix 2) for determination of glucose concentration was prepared using glucose monohydrate. Starch concentration was determined by taking glucose concentration x 0.9.

3.2.2.7 Extraction of pectic substances

Pectic substances were extracted according to the method of McCready (1970) and Normah & Ku Hasnah (2000) with some modifications. Pectic substances were extracted using hot dilute HCL in order to maximize extraction of protopectins predominant in bananas at green maturity. About 50 g of peeled bananas from each sample were weighed and crushed in a coffee grinder (Geepas, GCG289, Japan) to obtain a homogenous pulp. The pulp was mixed with 2.5 l of boiling water acidified with 0.5 ml of 11.91 M HCl to give a pH of 2.2 ±0.1, followed by addition of 20 g washed nylon threads as filter aid. The same water was used to wash out the banana pulp residue on the side of the grinder and added to the mixture. The mixture was heated at 95 – 100^{o}C for 30 min with constant stirring. Then the mixture was filtered and the residue was washed with 1 litre of boiling water. The filtrate was cooled before adding 2 l of 96% ethanol containing 0.2 ml/litre HCl. The mixture was slowly stirred and left to stand for 40 min to precipitate the pectic substances. It was then filtered through a fine nylon cloth. The precipitate was collected on a pre- weighed Petri dish. The dish was weighed again and the precipitate dried in the air drying oven (Yamato, DG400C, Japan) at 50^{o}C for over 16 h. The

dried pectin extract was pulverized in a grinder (Geepas, GCG289, Japan) to a fine powder and stored at room temperature for further use.

3.2.2.8 Determination of equivalent weight

The equivalent weight (Eq. wt) of pectic extracts was determined according to the protocol of Rangana (1986). Approx. 0.1 g of pectic extract was weighed into a 250 ml conical flask and 1 ml ethanol was added. Approx. 0.5 g of sodium chloride and 60 ml of distilled were added. Finally, 2 drops of phenol red were added and the solution titrated against 0.05 N NaOH. The end of the titration was indicated by development of a purple color and the titre volume recorded. Equivalent weight was calculated according to the following formula:

$$Equivalent\ weight = \frac{weight\ of\ sample\ x\ 1000}{ml\ of\ alkali\ x\ normality\ of\ alkali}$$

3.2.2.9 Determination of methoxyl content (MeO)

Methoxyl content was determined according to the method adopted from Rangana (1986) and Castill-Israel et al. (2015). The neutral solution containing 0.5 g pectic substances from the equivalent weight determination was used. To the neutral solution, 25 ml of 0.25 N sodiumhydroxide was added and mixed properly. The mixed solution was stirred thoroughly and kept at room temperature for 30 min in a flask with a stopper. Then, 25 ml of 0.25 N HCl was added and titrated with 0.1 N NaOH until a purple color was observed. The methoxyl content was calculated using the following formula:

$$Methoxyl\ content\ (\%) = \frac{ml\ of\ alkali\ x\ N\ alkali\ x\ 3.1}{weight\ of\ sample\ (g)}$$

3.2.2.10 Determination of anhydrouronic acid (AUA)

Estimation of AUA was performed by making use of the titration volumes in equivalent weight and methoxyl content determinations. Hence, total AUA was determined using the following formula adopted from Castillo-Israel et al. (2015).

$$Percentage\ (\%)AUA = \frac{176 * 0.1z * 100}{w * 1000} + \frac{176 * 0.1y * 100}{w * 1000}$$

Where the molecular weight unit of AUA = 176 g; z = ml of NaOH from the equivalent weight determination; y = ml of NaOH from Methoxyl content determination and w = weight of the sample.

3.2.2.11 Determination of degree of esterification (DE)

The DE of pectic substances was determined on the basis of methoxyl and AUA content (Owen et al., 1952; Castill-Israel et al., 2015) and calculated using the following formula:

$$\% DE = \frac{176 * Meo}{31 * \%AUA} * 100$$

3.2.2.12 Data analysis

Data were analyzed using SPSS (IBM) package, version 23 as in section 3.1.2.9. Simple correlational analysis was also done for the relationship between amylose content and resistant starch as well as amylose content and final viscosity.

3.2.3 Results and discussion

3.2.3.1 Banana starch

3.2.3.1.1 Starch yield

The yield of starch (wet basis) from the 14 banana cultivars varied from 4 to 25.7% (Table 8). Yield of starch from juice bananas (19% to 25.7%) was significantly higher than in cooking bananas (4% to 15%) ($P<0.05$) except for MUS which yielded 21%. A detailed analysis indicates that starch contents of KISB, KAY, MUS and NDI were not significantly different ($P>0.05$). Further analysis showed that starch content of KIS, NAKB, NAMZ, KAZ, NAKY and NAKT were also not significantly different ($P>0.05$). NAMD had significantly lower starch yield than all other bananas ($P<0.05$), followed by KIB, MPO and NAKW.

Starch yield in MUS which is a cooking banana cultivar was significantly higher than in rest of the cooking banana cultivars. It is possible that MUS accumulates more starch reserves relative to water than other cooking cultivars during the dry season, a period during which the study was conducted. Experience with the MUS cultivar is such that during the dry season, its fingers tend to be smaller, longer and also harder upon cooking. Starch yield could also vary according to the banana cultivar (Mota et al., 1997; Fontes et al, 2017). These differences in starch yield

among cooking as well as juice banana cultivars could significantly impact on hardness of these bananas when cooked.

In their studies on Thai bananas, Vatanasuchart et al. (2012) found starch yield to vary depending on the cultivar i.e. 7.5% in Kluai Lebmuenang, 9.6% in Kluai Hom, 13.4% in Kluai Khai and 13.5% in Kluai Namwa. Their results are within the range of starch content for most cooking banana cultivars used in this study. However, starch yield by Vatanasuchart et al. (2012) is way below that of juice bananas investigated in this study. Probably, the Thai bananas studied by Vatanasuchart et al. (2012) are cooking bananas. Nimsung et al. (2007) extracted starch from two Thai banana cultivars, namely Kluai Hom Tong (AAA) and Kluai Namwa (ABB) and they obtained starch yields (wet basis) of 29.15% and 31.12%, respectively. These values are higher than the starch yield of the three juice bananas examined in this study. The higher starch yield in juice bananas compared to cooking bananas could partly explain why juice bananas are normally used for juice production. NAMD had very low starch content which could probably be due to biochemical degradation of starch due to possible advanced maturity (Cordenunsi & Lajolo, 1995). Normally, the starch content drops to less than 1% at the end of the climacteric period while sugars accumulate to more than 10% of the fresh weight of the fruit (Zang et al., 2005).

Starch has been associated with firmness of foods particularly when it undergoes retrogradation. Almost all cooking bananas used in this study had low starch content which may possibly explain why they are normally soft upon cooking. Juice bananas are normally very hard both when raw and when cooked, which could partly be due to their high starch content.

Table 8: Yield of starch from selected cooking and juice banana cultivars

Banana cultivar	% Yield of starch (wet basis)
Cooking bananas	
NAMD	4.0[a]
NAKW	10.1[b]
NAMZ	12.5[b]
NAKT	11.4[b]
NAKY	11.8[b]
KIB	8.8[b]
MUS	21.0[c]
MPO	8.0[b]
KIS	15.2[b]
KAZ	11.9[b]
NAKB	14.3[b]
Juice bananas	
KISB	25.7[c]
NDI	19.0[c]
KAY	25.3[c]

*Values with same superscripts in the same column are not significantly different (P>0.05).

Values are means of two independent determination± SE of the means

3.2.3.1.2 Total, digestible and resistant starch content

The total starch content of banana flour ranged from 48 to 92% on dry basis (Table 9). Total starch content in cooking bananas ranged from 48.4% to 88.3% with NAKW having the lowest total starch while NAKB had the highest starch content. Juice bananas had the highest total starch content ranging from 85% to 92.8% being highest in KISB and lowest in KAY. Resistant starch varied from 36 to 68.7% (Table 9). Resistant starch content did not differ significantly between cooking and juice banana cultivars (P>0.05). In general, all banana cultivars had high resistant starch. RS for cooking bananas ranged from 36.2 to 68.6% while that of juice bananas ranged from 52% to 65%. NAKW and NAMZ had significantly lower RS than NAMD, NAKY, KIS, KIB, MPO, MUS, NAKB, KISB, NDI and KAY.

Table 9: Amylose and resistant starch content (mg/100mg) of banana starch from selected cooking and juice banana cultivars

Banana variety	Digestible starch (mg/100mg)	Resistant starch (mg/100mg)	Total starch (mg/100mg)	Amylose (% content)	Amylopectin (% content)
Cooking bananas					
NAMD	13.4^{a}	53.4^{bcde}	66.8^{bcd}	33.33^{bc}	66.67^{ab}
NAKW	12.2^{a}	36.2^{a}	48.4^{a}	28.76^{abc}	71.24^{abc}
NAMZ	14.4^{a}	37.8^{a}	52.2ab	28.32^{abc}	71.68^{abc}
NAKT	12.5^{a}	46.9^{ab}	59.4^{abc}	35.08^{c}	64.92^{a}
NAKY	12.3^{a}	57.1^{bcdef}	69.4^{bcde}	30.94^{abc}	69.06^{abc}
KIB	17.1^{ab}	61.3^{cdef}	78.4^{def}	34.64^{c}	65.36^{a}
MUS	21.6^{bc}	63.6^{def}	85.2^{ef}	25.05^{ab}	74.95^{bc}
MPO	27.0^{cdef}	68.6^{f}	84.8^{def}	27.02^{abc}	72.98^{abc}
KIS	24.6^{cde}	50.7^{bc}	75.3^{cdef}	25.05^{ab}	74.95^{bc}
KAZ	23.1^{bcd}	54.9^{bcde}	78^{def}	21.57^{a}	78.43^{c}
NAKB	27.3^{cdef}	61^{cdef}	88.3^{f}	22.44^{a}	77.56^{c}
Juice bananas					
KISB	29.4^{def}	65.0^{ef}	92.8^{f}	28.32^{abc}	71.68^{abc}
NDI	30.3^{ef}	57.1^{bcdef}	87.4^{ef}	36.38^{c}	63.62^{a}
KAY	33.1^{f}	52.0^{bcd}	85.1^{ef}	35.51^{c}	64.49^{a}

Values in columns with the same letter are not significantly different (P>0.05). Values are means of three independent determinations.± SE of the means

These results are in agreement with findings on Thai bananas whose resistant starch content (dry basis) was found to range between 52.2 and 68.1% (Vatanasuchart et al., 2012; Vatanasuchart et al., 2009). Green mature bananas are remarkably rich in resistant starch (Ovando-Martinez, Sáyago-Ayerdi, & Goni, 2009). However, RS can be influenced by cultivar, variety and environment differences. The high resistant starch content in the bananas could have implications on hardness of these bananas when cooked. This starch which cannot be digested in the small intestine (Englyst, Trowell, Southgate, & Cummings, 1987) is normally characterized by dense molecular configuration or compact structure which restricts accessibility of water hence hindering gelatinization and thus accessibility by digestive enzymes (Haralampu, 2000). Limited starch gelatinization as a result of restricted penetration of the starch structure by water during cooking could imply a potentially harder texture of the

bananas studied. In general, all types of RS have lower water holding capacity WHC) (Sanz, Salvador, & Fiszman, 2008) which implies that such starch would very easily lose moisture and crystalize hence resulting in increased hardness upon retrogradation. The lower RS values in NAKW, NAMZ and NAKT could imply a softer texture when cooked bananas since their starch can easily be accessed by water thus causing gelatinization. On the other hand, MPO and MUS whose RS values were high could imply a harder texture when cooked. It is important to note that banana starch used in this study was derived from banana flour that was structurally disintegrated which potentially reduces the amount of RS. The implication is that bananas whose structure is still intact could have higher amounts of RS. The high resistance to hydrolysis by enzymes of the native banana starches has been reported (Cummings & Englyst, 1991; Eggleston et al., 1992; Englyst et al., 1996). RS content has been found to positively correlate with amylose content in cereal crops (BenMoussa et al., 2007; Sang et al., 2008; Mir et al., 2013). In cases where bananas are to be used for flour production, it is important to assess the proper stage of banana fruit maturity to produce flour with the right RS content (Englyst & Cummings, 1986, Zhang et al., 2005).

From a medical point of view, RS is well known to have health benefits similar to dietary fiber being almost totally fermented in the colon to short-chain fatty acids that help to prevent colorectal cancer, lower the risk of heart disease, and influence metabolic and inflammatory bowel diseases such as diabetes and diverticulitis (Hung, Cham, & Truc, 2013).

Digestible starch ranged between 12.3 to 33.1%. The content of digestible starch did not significantly differ between cooking and juice banana cultivars ($P>0.05$). Among cooking bananas, NAMD, NAMZ, NAKW, NAKT and NAKY had significantly lower digestible starch content than MPO, KIS and NAKB which had significantly higher digestible starch content ($P<0.05$). Digestible starch content of KISB, NDI and KAY (all juice banana cultivars) was not significantly different from that of MPO and NAKB (cooking banana cultivars). High amounts of digestible starch contribute to a boost in blood glucose levels with the resultant effect of hyperglycemia (Stover & Simmonds, 2003).

3.2.3.1.3 Amylose and amylopectin content

Amylose content of the different banana starches starch varied from 21.7% to 36.4% (Table 9).The amylose content of cooking banana starch ranged between 21.57 and 35.08% being highest in NAKT and KIB and lowest in KAZ and NAKB while Amylose content of juice banana starch ranged from 28.32% to 36.38%. Amylose content of starch from both cooking and juice banana cultivars did not vary significantly (P>0.05). Amylose content of starch from NDI, KAY, KISB, NAMD, NAKW, NAMZ, NAKT, NAKY, KIB and MPO was not significantly different (P>0.05). Amylose content of starch from KAZ, NAKB, MUS and KIS was significantly lower than amylose content of starch from KAY, NDI, KIB and NAKT. In general, amylose content of most starch from the bananas used in this study was very high but also varied widely. These results are in agreement with Rav & Mustaffa (2013) who reported amylose content of plantain and cooking bananas to range from 24.41 to 36.87% and Vatanasuchart et al. (2012) reported amylose content of six Kluai banana cultivars to range between 38.6% – 43.8%. However, other researchers have reported lower amylose content in other banana starches, for instance, Zhang & Hamaker (2012) reported amylose content of 11.2%; Ssonko & Muranga (2017) reported 11.96–12.83% for some East African Highland bananas grown in Uganda. Although amylose comprises a smaller fraction in most starches, the molecule has a large influence on starch properties due to its structural contribution to the amorphous component of the starch granules. For instance, the physicochemical and functional properties of banana starch are dependent on variety, regional climatic conditions, and harvesting periods (Bello-Perez et al., 1999). The botanical source of starch is also a determinant of the chemical composition, granule morphology, amylose/amylopectin ratio and arrangement of the macro molecules, which in turn determine the physicochemical and functional properties of the starch (Ssonko & Muranga, 2017). Some researchers have reported amylose content to be positively correlated with resistant starch content both of which may affect the physico and structural properties of bananas particularly hardness or firmness (Sang et al., 2008; Benmoussa et al., 2007; Evans & Thompson, 2004). Simple correlational analysis between amylose and resistant starch content of the bananas used indicated a correlation coefficient of 15% and a coefficient of determination of 2.3% which is too low to support the above argument. Some authors have reported a positive correlation between hardness and amylose content. For instance, Rolando et al. (2004) found a positive linear correlation

between amylose content and hardness of rice. The wide differences observed in amylose content of the different starches used in this study may be due to cultivar differences and the varying micro-environmental conditions in which the bananas grew.

3.2.3.1.4 Swelling power and solubility

Swelling power of starch at lower temperatures (45 – 65^{o}C) was generally low and ranged between 63 and 143% (0.63 and 1.43 g H_2O/g starch) (Figure 7 and Table 15 in appendix 1). Swelling power was noted to exponentially increase between 70 and 80^{o}C. The amount of water absorbed by starch between 70 and 80^{o}C was more than three to five times the amount of water absorbed between 45 and 70^{o}C. For instance, the lowest water absorbed at 70^{o}C (147%) was observed in KAY starch; this rapidly increased 5-folds to 745% at 80^{o}C. This was the same trend for starch from all banana cultivars examined. Further increase in temperature led to increase in water absorption through 90^{o}C and maximum swelling power was observed at 98.6^{o}C which ranged between 10.14 to 16.58 g H_2O/g starch (1014 to 1658%) exhibited by starch from NAKT and KAZ respectively. Some of the starches (KIS, KISB, NDI and KAY) appeared to saturate earlier at 90^{o}Cwhile others such as KAZ, MUS and KIB exhibited increased swelling power above 90^{o}C. The rapid increase in swelling power between 70 and 80^{o}C could be due to gelatinization of starch for which gelatinization temperature has been observed to range from 78 to 80.7^{o}C according to results of pasting properties.

Swelling power is the ability of starch granules to hydrate and increase in size under excess water conditions. Ratnayake, Hoover, & & Warkentin, (2002) described starch solubility and swelling power as being directly related to the relative proportion of amylose and amylopectin. The low swelling power values at temperatures lower than 70^{o}C could be indicative of high stability of starch granules at temperatures lower than the gelatinization temperature range (Ssonko and Muranga, 2017). The rapid increase in swelling power between 70 and 80^{o}C observed in this study is in agreement with findings by Fontes et al. (2017) who studied swelling power of banana starch from the green variety of Mysore.

This rapid increase in swelling power at 70 and 80^{o}C could be due to the fact that towards gelatinization temperature, the energy is high enough to cause the unwinding of the starch helical structures or breaking up of the intermolecular hydrogen bonds in the amorphous

areas to allow more water to penetrate and sink deep into it hence causing swelling and pasting of the starch (Wang et al., 2015; Somboonchan, 2015). Between 78 and 80^{o}C, starch is able to attain gelatinization. However, water absorption appears to continue beyond gelatinization temperature and start of hydro-saturation is observed beyond 98.6^{o}C. These results are also in agreement with De la Torre-Gutiérrez et al. (2008) who at close to pasting temperature of 70 to 80oC observed high increase in water binding capacity (WBC) in starch from square bananas. The high swelling power which also represents Water Absorption Capacity (WAC) suggests weak association of amylose-amylopectin complex (Otegbayo et al., 2010) which allows permeability of water into the granule structure (Olatunde et al., 2017). High swelling power could also be due to the high amylopectin content (Kusumayanti et al., 2015) of the starch that causes good water absorption and hence swelling. Juice bananas i.e. KISB, NDI and KAY whose starch was among those that appeared to saturate early are normally associated with a hard texture meaning that their starch has limited capacity to gelatinize. This could be important when starch is still embedded in the native structure of the intact bananas. Swelling power of banana starch was reported to be lower than that of cassava starch *(*Kayisu et al. 1981). Higher swelling power implies higher degree of gelatinization and hence potential to produce a more tender product. In general, the individual banana starches showed good swelling power, which shows the potential for their use in products that require water retention, such as meat products and jellies (Fontes et al., 2017). Swelling power is affected by amylose content, molecular weight distribution, degree of branching, length of branches and conformation of the starch molecules (Ratnayake *et al*., 2002).

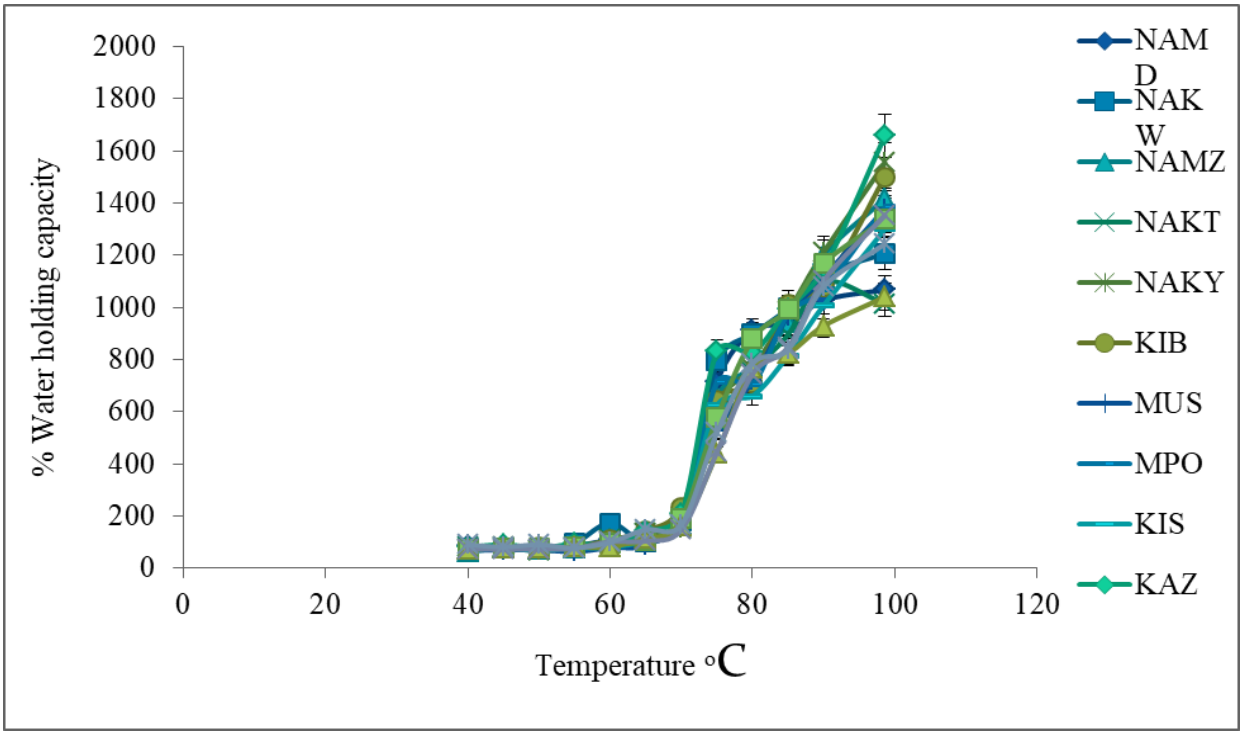


Figure 7: Swelling power (%) of starch from selected cooking and juice banana cultivars

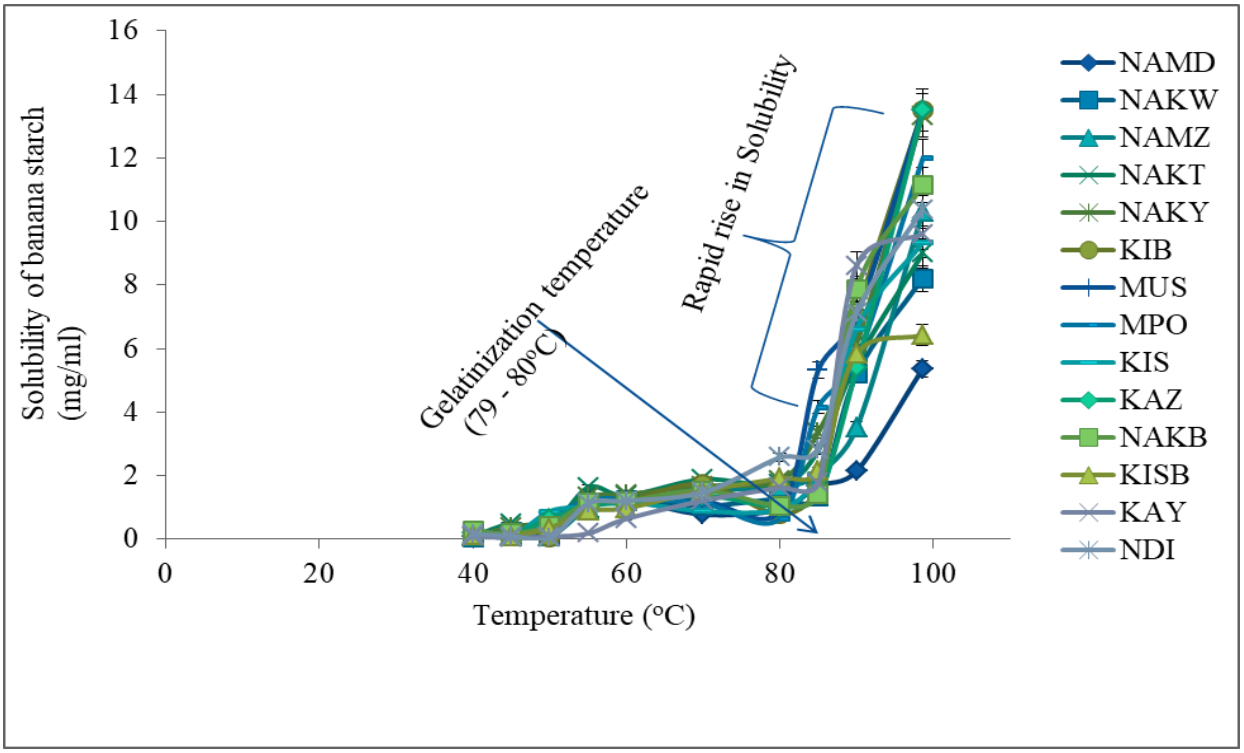


Figure 8: Solubility of banana starch from selected cooking and juice banana cultivars

The solubilities of starch followed a similar trend like that of swelling power. Starch solubility was low at low temperatures (40 – 55^{o}C) (Figure 8 and Table 16 in appendix 1). There was little increase in solubility between 55 and 80^{o}C, however, solubility rapidly increased between 80 and 90^{o}C for all the different banana starch. Solubilities of cooking and juice banana starch were not significantly different (P>0.05). Solubility of the different starch increased with temperature and increased rapidly between 80 and 98.6^{o}C unlike swelling power which rapidly increased between 70 and 80^{o}C. The solubility of starch from NAMD and KISB was significantly lower than the rest while that of KAZ, MUS, KIB, NAKY, MPO and NAKB starches was significantly higher (P<0.05). Starch solubility represents the percentage of starch that leaches out into the solution during determination of swelling power. Solubility and swelling power represent evidence of interaction between the amorphous and crystalline regions of the starch grain (Takizawa et al., 2004).

The rapid increase in solubility of starch after 80^{o}C implies that on gelatinization, more amylose molecules diffuse out of the starch grain structure as water penetrates deeper. Solubility appears to increase even beyond 98.6^{o}C. This may mean that banana starch has limitations for industrial application particularly where good crispness, low water and fat retention are required (Sajilata et al., 2006). According to Fontes et al. (2017), increased solubility may probably be due to deprotonation of the hydroxyl groups of starch molecules, leading to expulsion thereof and allowing greater water penetration into the starch granule leading to increased swelling and solubility. The high starch solubility observed could be due to high amylose content in the banana starch which is the main molecule that leaches out of the starch granule on heating in the presence of water (Jay-lin, 2009).

In general, high swelling power could imply tender bananas on cooking. According to Kumoro et al. (2012) greater swelling power is harmonious with higher solubility which has been implied by the two parameters following a similar trend.

3.2.3.1.5 Pasting properties of the banana starch

Results of pasting properties of banana starch are shown in Table 10.

3.2.3.1.5.1 Peak viscosity

Peak viscosity is a measure of the ability of starch to form a paste on cooking (Adewole et al., 2012). It represents the maximum viscosity developed during or soon after the heating process.

Peak viscosity of banana starch ranged between 2347 and 4046 cP (196 and 337 RVUs). The peak viscosities of cooking and juice banana starch were not significantly different. Starch from MUS had significantly lower peak viscosity ($P<0.05$) while NAMD had significantly higher peak viscosity ($P<0.05$). Peak viscosity of starch NDI, KAY, KIS, NAKY, NAKY and NAKT was not significantly different. Peak viscosity indicates the strength of the starch paste formed during gelatinization and also reflects the extent of granule swelling (Liang & King, 2003). The peak viscosities obtained for banana starch used in this study were lower than those obtained by Ssonko and Muranga (2017) for similar Ugandan bananas. However, these peak viscosities are higher than those obtained by Oviri (2014) for Nigerian banana cultivars of French horn, Cadaba and Agbagba which ranged between 133 and 163 RVU. The peak viscosities obtained in this study were relatively high and accordingly have a potential to form strong gels during gelatinization. The higher peak viscosity displayed by NAMD could be attributed to its high amylose content as observed above. However, the same could not be said of juice bananas whose amylose content was higher than that of cooking bananas. Possibly, this and other bananas displaying high peak viscosities have large starch granules hence greater swelling power (Ssonko & Muranga, 2017). Heat resistance during cooking has been suggested by Dufour et al. (2009) to be a suitable criterion to differentiate between dessert and cooking bananas. The same reasoning could be put forward for juice and cooking bananas used in this study.

3.2.3.1.5.2 Trough viscosity

Trough viscosity ranged between 1880 and 2850 cP (157 and 238 RVUs). Starch from MUS, KIS, NAKB, KISB, KAY and NAKT had lower trough viscosities but were not significantly ($P>0.05$) while NAMD, NAKW and NAMZ had high trough viscosities which were also not significantly different. Trough viscosities of cooking and juice banana starch were not significantly different.

Trough viscosity represents the holding strength which is the ability of granules to remain undisrupted when the starch is subjected to a period of constant high temperature and mechanical shear stress (Oviris, 2014). This hold period is often accompanied by a breakdown in viscosity. The ability of starch to withstand shear thinning or breakdown in viscosity manifested by high breakdown value is of high industrial significance (I.I.T.A, 2011). This

means that all the banana starches analyzed in this study are useful in industrial food applications. The trough viscosity recorded was relatively higher than tht reported by Oviris (2014) for three different plantain cultivars in Nigeria, but lower than what was reported by Ssonko and Muranga (2017) (215 - 285 RVUs) for four East African Highland banana cultivars endemic to Uganda. Adewole et al. (2012) reported trough viscosity values for Nigerian banana cultivars to be in the range of 112 to 141 RVUs. These differences could partly be the result of environmental differences between the cultivars used in this study and those used by Ssonko and Muranga (2017). According to these authors, differences in starch concentrations could also influence trough and peak viscosities.

Table 10: Pasting properties of starch from selected cooking and juice banana cultivars

Banana cultivar	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown Viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)	Pasting temp (^{o}C)
NAMD	4047^{g}	2752^{fg}	1295^{f}	3304^{abcde}	552^{a}	6.1±0.0	78.9
NAKW	3706^{f}	2610^{efg}	1097^{ef}	3259^{abcd}	618^{ab}	5.6±0.8	79.2
NAMZ	3312de	2500^{def}	812^{cd}	3351^{bcdef}	827^{cde}	6.2±0.0	79.3
NAKT	2646^{bc}	2141^{ab}	506^{ab}	3355^{bcdef}	1193^{h}	6.3±0.0	80.0
NAKY	2720^{bc}	2282^{bcd}	438^{ab}	3410^{cdef}	1107^{gh}	6.5±0.2	80.2
KIB	3290de	2304^{bcde}	986de	3339^{abcdef}	1056^{fg}	6.2±0.0	79.2
MUS	2348^{a}	1881^{a}	467^{ab}	3112^{abc}	1207^{h}	6.5±0.0	80.3
MPO	3366^{e}	2850^{g}	516^{ab}	3609^{ef}	763^{bc}	6.7±0.0	79.6
KIS	2624^{bc}	2128^{ab}	496^{ab}	3301^{abcde}	1206^{h}	6.3±0.0	79.6
KAZ	3222de	2419^{bcde}	803^{cd}	3195^{abc}	802^{bcd}	6.3±0.0	78.8
NAKB	2506^{ab}	2117^{ab}	389^{a}	3074^{ab}	990^{ef}	6.5±0.0	79.5
KISB	2803^{c}	2134^{ab}	670^{bc}	3040^{a}	884^{def}	6.2±0.0	80.4
NDI	3112^{d}	2477^{cdef}	636^{ab}	3633^{f}	1188^{gh}	6.3±0.0	79.9
KAY	2784^{c}	2163^{ab}	621^{ab}	3534^{def}	1385^{h}	6.3±0.0	80.7

Values with same superscripts along the column are not significantly different (P>0.05). 1cP = 0.083 RVU

3.2.3.1.5.3 Breakdown viscosity

Breakdown viscosity ranged from 389 to 1294 cP (32 to 108 RVUs). NAKB starch had significantly lower breakdown viscosity ($P<0.05$) followed by NAKY, MUS, KIS, NAKT, MPO, KAY and NDI which were not significantly different. NAMD and NAKW had significantly higher breakdown viscosity ($P<0.05$). The breakdown viscosity of starch from NAMZ, KIB, and KAZ was not significantly different. In general, breakdown viscosities for both cooking and juice banana starch was not significantly different ($P>0.05$). These breakdown viscosities are lower than 235 to 311 RVUs reported by Ssonko and Muranga (2017). However, these values are higher than 28 to 48 RVUs reported by Oviri (2014) and 24 to 29 RVUs reported by Adewole et al (2012) for Nigerian bananas. The differences could be due to cultivar differences, growth conditions as well as extraction and treatment conditions. The relatively high values of breakdown imply that the banana starch investigated in this work has the ability to withstand shear thinning and hence good for industrial application particularly in production of sauces and other products that require high consistency or viscosity.

3.2.3.1.5.4 Set back viscosity

Set back viscosity was between 552 to 1385 cP (46 to 115 RVUs). The starch from NAMD, NAKW and MPO had lower setback viscosities and were not significantly different while starch from KAY, NDI, KIS, MUS, NAKY and NAKT had higher setback viscosities and were also not significantly different ($P>0.05$). Others were intermediate. In general the setback viscosities of starch from both cooking and juice banana cultivars were not significantly different ($P>0.05$). Setback viscosities observed were within the range of 61 to 104 RVUs reported by Ssonko and Muranga (2017) for similar EAHB cultivars and 60 to 124 RVUs reported by Oviri (2014) for Agbagba and Cadaba banana cultivars in Nigeria. High set back viscosity is associated with a cohesive paste, while a low setback viscosity is indicative of a non-cohesive paste (IITA, 2011). This means that the starch from these bananas may be good for industrial application particularly in tablet coating where high cohesiveness is a desired attribute. This may also have implications on the resulting hardness when these bananas are cooked.

Setback viscosity provides information about the tendency of starch to undergo retrogradation (Wang et al., 2015). Typically, starch presenting higher setback values has a greater tendency for retrogradation (Zaidul et al., 2007). In this respect, starch from KAY, MUS, KIS, NAKT,

NDI, NAKY and KIB had the highest setback viscosities implying high tendency to undergo retrogradation. KAY and NDI are juice bananas which are generally harder than cooking bananas. MUS is a cooking banana cultivar which during the dry season is generally known to be hard especially hence the behavior of its starch. On the other hand, KIB and NAKT are usually softer cultivars when cooked, however, the high setback viscosity of their starch could not be explained.

3.2.3.1.5.5 Final viscosity

The final viscosities ranged between 3112 and 3633 cP (259 and 303 RVUs). There was no significant difference in final viscosities of starch from both cooking and juice banana cultivars. These values were slightly lower than 308 to 363 RVUs reported by Ssonko and Muranga (2017) for similar EAHB cultivars also endemic to Uganda. Adewole et al. (2012) reported values in the range 165 to 298 RVU for Nigerian bananas and Plantains, respectively. Final viscosity is an important parameter in predicting and defining the final and textural quality of foods (Kramer & Twigg, 1970). Starch with high final viscosity values can be used as tablet binders in pharmaceutical companies (Bihaderis et al., 1993) implying the banana starch used in this study is suitable for pharmaceutical and other industrial applications. High final viscosity is a reflection of the size of the native starch granules in bananas since large size granules are likely to absorb more water than small size granules which increases the swelling volume and hence final viscosity. The high final viscosities obtained for all the starch in this study imply that the banana starch granules could be large..

Simple correlational analysis performed between amylose content and final viscosity of all the starch used in this study indicated a 36% correlation, which implies that other factors such as starch and water content of the sample could have affected the final viscosities.

3.2.3.1.5.6 Pasting temperature and peak time

Pasting temperature for all the starches ranged between 78.8 and 80.7^{o}C. The peak time taken to reach pasting temperature of all the banana starches ranged between 5.6 and 6.7 min. Ssonko and Muranga (1998) reported relatively lower values of 70.2 to 74.5^{o}C and 4.44 to 4.67 min for pasting temperature and time, respectively for similar EAHB cultivars. The pasting temperature of these starches was relatively lower than that of corn starch (>80°C) (Bello-Pérez et al., 2006). This is a good property for products that require high viscosity at lower processing

temperatures, which could be an advantage during development of new products with heat sensitive ingredients (Ssonko & Muranga, 2017). A lower pasting time also implies quick processability and savings on fuel costs.

3.2.3.2 Banana pectic substances

3.2.3.2.1 Yield

The yield of crude pectic substances ranged from 7.55 to 30.55% (Table 11). The yield of pectic substances in juice bananas ranged from 29.67 to 30.55% and was significantly higher than the yield from cooking bananas (range 7.55 to 21.1%) ($P<0.05$). The yield of pectic substances among juice banana cultivars was not significantly different ($P>0.05$). Among cooking banana cultivars, the yield of pectic substances varied widely. Yields from KAZ, KIB, NAKW, NAKB, NAKT, NAKY, KIS and NAMD did not differ significantly ($P>0.05$) and were significantly higher than yields from NAMZ, MPO and MUS which were significantly lower ($P<0.05$).

The differences in yield of pectic substances between cooking and juice banana cultivars could have implications with respect to hardness of the living tissues since pectin is involved in cementing of the tissues together. In general, the yields were high which could be attributed to the high level of impurities particularly cellulose, hemicellulose, lignin including starch andothers which form part of protopectin (Conrad, 1930). The use of HCl (pH 2.2) which maximizes pectin extraction may also have maximized extraction of the impurities, hence the high yields. The high ionic strength of HCl has the capability to precipitate pectin and other negatively charged ionic molecules such as cellulose and hemi-cellulose which are part of the pectin molecule before the ripening stage. High extraction temperatures have also been reported to increase extraction yields (Gama et al., 2015). Chan & Chao (2013), using waste from cocoa industry, were able to extract between 3.38 and 7.62% of pectin at temperatures from 50 to 95°C for 1.5 to 3 h at a solids concentration of 1:10 to 1:25 (w/v) using citric acid in pH range of 2.5 to 4.0. At 95ºC, it was possible to extract more pectin, characterized as low methoxyl grade, indicating that the temperature has a positive factor in solubilizing these substances as well as the highest concentration of acid. Uzma et al. (2015), extracted pectin from papaya peels and obtained the highest percentage of pectin (16%) on extraction with

hydrochloric acid at pH 2.0, temperature of 80°C and with 60 min extraction time, similar to the conditions used in this study. Yadav et al. (2015) obtained yields of pectin of up to 36% from orange peels using HCl at pH 2 and 85^{o}C extraction conditions.

Table 11: Chemical properties of crude pectic substances extracted from selected cooking and juice banana cultivars at green maturity

Banana cultivar	Acronym	% yield crude pectic substances	EQ. Wt.	% MeO	% AUA	DE
Cooking bananas						
Namande	NAMD	21.1^{b}	8156^{a}	5.8^{a}	37.5^{a}	88^{a}
Nakawere	NAKW	17.4^{b}	12911^{ab}	9.4^{cd}	56.3^{bc}	95^{b}
Namweezi	NAMZ	7.55^{a}	14889^{b}	9.2^{c}	54.4^{b}	96^{b}
Nakitembe	NAKT	19.10^{b}	10800^{ab}	9.7^{cd}	58.5^{bc}	94^{b}
Nakyetengu	NAKY	19.15^{b}	14444^{b}	9.6^{cd}	57.1^{bc}	96^{b}
Kibuzi	KIB	17.03^{b}	14222^{b}	9.1^{c}	54.4^{b}	95^{b}
Musakala	MUS	10.6^{a}	1435^{6}	9.4^{cd}	55.8^{bc}	96^{b}
Mpologoma	MPO	9.38^{a}	13689^{ab}	10.6^{cd}	62.7^{bc}	96^{b}
Kisansa	KIS	20.41^{b}	13467^{ab}	10.3^{cd}	61.0^{bc}	96^{b}
Kazirakwe	KAZ	16.86^{b}	13822^{ab}	6.9^{ab}	41.7^{a}	94^{b}
Nakabululu	NAKB	17.92^{b}	16289^{b}	7.2^{b}	43.2^{a}	95^{b}
Juice bananas						
Kisubi	KISB	29.96^{c}	13956^{ab}	6.8^{ab}	40.9^{a}	94^{b}
Ndiizi	NDI	29.67^{c}	16444^{b}	6.9^{ab}	41.1^{a}	95^{b}
Kayinja	KAY	30.55^{c}	16333^{b}	6.4^{ab}	38.6^{a}	95^{b}

*Values with same superscripts in the same column are not significantly different (P≤0.05). EQ. Wt = Equivalent weight; MeO = Methoxyl content; AUA = Anhydrouronic acid; DE = Degree of esterification

In raw green mature bananas, pectin exists as protopectin which is pectin combined with other substances and is broken down to pectin as bananas ripen. Protopectin is "a group of water-insoluble high-molecular-weight pectin complex (pectic substances) comprised of pectin together with cellulose and hemicelluloses which form the backbone of a cell wall and, when treated with diluted acids, gives soluble pectin extracted usually from plant material" (Ovodov, 2009; Endress, 1991). Conrad (1930) reported that during ripening of fruits, pectin increases at the expense of protopectin. The interconversion of pectic substances is presumed to be involved in the characteristic softening which occurs during fruit ripening. According to Castillo-Israel et al. (2015), the maturity stage of bananas affects pectin yield as the amounts of pectins, hemicellulose, cellulose and lignin vary as the fruit matures. These authors found higher pectin yields of about 16.5% from Saba banana peels at the unripe stage compared to 11.87% from the same bananas at the ripe stage. Castillo-Israel et al. (2015) argued that the ripeness stage of Saba banana peels could affect pectin yield due to amounts of pectins, hemicellulose, celluloses and lignin which vary as the banana fruit matures. Uzma et al. (2015) found the yield of pectin in papaya peels to vary from 2.8% - 16%.

With unripe mature bananas where other impurities as part of protopectin are expected, the high yields observed in this study could be possible. Pectin content of bananas has been reported to range between 0.5 to 1.28% as reported by Baker (1997). Kawabata & Sawayama (1974) examined bananas from three countries (Philippines, Ecuador and Taiwan), and found levels of calcium pectate to range from 0.55–0.68%, with an average of 0.63% which are rather too low compared to yields obtained in the current study. However, the low levels of calcium pectate reported in these bananas could have been due to ripening compared to the bananas used in this study. Over ripening of bananas results in decreased pectin yields due to breakdown of pectin by pectolytic enzymes including polygalacturonase (PG), pectin methyl esterase (PME) and pectin lyase (PL) (Emaga et al., 2007).

The high yield of pectic substances observed in this study could have implications on the texture of bananas particularly during and after cooking. Pectin is the major constituent of all plants and makes up approximately two-thirds of the dry mass of plant primary cell walls and provides structural integrity, strength, and flexibility to the cell wall (Talbott & Ray, 1992). Pectin forms a matrix with celluloses and hemicelluloses which contributes to the cell structure.

This implies that these pectic substances could be contributing to hardness of raw banana tissues and therefore, contributing to the possible differences in hardness between cooking and juice banana cultivars in raw form. The amount and nature of pectic substances are also critical for texture in fruits and vegetables particularly during growth, maturation, storage and processing (Crombie, Scott, & Reid, 2003). However, the argument above may not stand when bananas are cooked since during heating, pectin absorbs water, melts and solubilizes. This process combined with the high water holding capacity of pectin could potentially lead to a soft texture of bananas upon cooking.

3.2.3.2.2 Equivalent weight

The equivalent weight (eq. wt) of the pectic substances ranged between 8156 and 16444 (Table 11). The eq. wt of pectic substances from juice banana cultivars ranged from 13956 to 16444 while that of cooking banana cultivars ranged between 8156 and 16289. Eq. wt of pectic substances from NAMD was significantly lower than that from NAMZ, NAKY, KIB, MUS, NAKB, NDI and KAY ($P<0.05$) while eq. wt of pectic substances from NAKW, NAKT, MPO, KIS, KAZ and KISB was intermediate and not significantly different ($P>0.05$). In general, there was no significant difference in eq. wt of all pectic substances from juice and cooking banana cultivars ($P>0.05$). Pectin produced at lower pH normally has a higher equivalent weight due to the fact that low pH can cause polymerization of the pectin molecules into longer chains (Rouse, 1977; Uzma et al., 2015). This in turn decreases the free acid content of the pectin which implies that the higher the equivalent weight, the longer the pectin polymers.

Equivalent weight represents the quantity of pectin that is reactive which can undergo cross-linking reactions through polyol functional groups and is indicative of a high degree of esterification which is in turn associated with a higher gelling power (Alan, 1984). The Equivalent weight of pectin is also the total content of free galacturonic acid (not esterified) in the molecular chains of pectin (Rangana, 1986). Equivalent weight is also an indicator of PECTIN'S gel-forming ability with higher molecular weight pectins having better gel-forming ability (Vaclavik & Christian, 2008). The high equivalent weight of pectic substances observed in this study could imply increased gel-forming ability (Yadav et al., 2015). The high eq. wt could imply strong associations between the pectin and cellulose/hemicellulose including other

molecules. This could have implications on the texture of bananas where it may lead to a harder texture in both raw and cooked forms. The stage of maturity also affects the equivalent weight of pectin. Relatively high equivalent weights from unripe banana peels were reported by Castillo-Israel et al. (2015). The high equivalent weight found in the banana samples in this study could thereforebe justified by the stage of maturity at which the bananas were harvested.

3.2.3.2.3 Methoxyl content

Methoxyl content was determined by saponification of the pectic extract and titration of the liberated carboxyl groups with NaOH. The methoxyl content varied from 5.8 to 10.6% (Table 11). The methoxyl content of pectic substances from NAMD was significantly lower than the methoxyl content of the pectic substances from the rest of the cooking bananas with the exception of KAZ. The methoxyl content of pectic substances from all juice banana cultivars was not significantly different from that of NAMD and KAZ. In general, the methoxyl content of pectic substances from cooking and juice banana cultivars did not differ significantly ($P>0.05$).

The methoxyl content could be affected by the level of maturity of the bananas. Castillo-Israel et al. (2015) while studying characteristics of pectin from Saba banana peels obtained methoxyl content of 6.4% and 5.25% in ripe and unripe bananas, respectively. According to Aina et al. (2012), the methoxyl content of extracted pectins varies between 0.2 and 12% depending on the source and mode of extraction. Methoxyl content of commercial pectins varies from 8-11% (Castillo-Israel et al., 2015). The methoxyl content of pectic substances extracted from bananas used in the current study was within the ranges reported above. However, the methoxyl content of most pectic substances was above 6.4% implying that they were high methoxyl pectins (Castillo-Israel et al., 2015; Beda & Kouassi, 2014). This property was confirmed by the degree of esterification which was found to be very high ranging from 88 to 96%. This means that the banana pectins in this study could form strong gels with sugar especially those above 7% methoxyl content (Genovese et al., 2010). Pectins with methoxyl content less than 7% can form gels with lower concentrations of sugar. High methoxyl content may also imply strong adhesive and cohesive forces which could imply increased firmness particularly of the gels. It is not clear however, to what extent the high methoxyl pectins can absorb water. Otherwise, high water absorpption and holding capacity would imply maintaining the cooked banana

matrix moist and hence keeping starch gelatimized longer after bananas have been cooked. This would mean that the texture of cooked bananas remains considerably soft upon cooling.

Methoxyl content is important in controlling setting time of pectin, sensitivity to polyvalent metal cations and also determines the functional properties of the pectin-gel texture (Constenla & Lozano, 2003). It also affects the dispersability of pectin in water where pectin with high methoxyl content is readily dispersible in water than that with less than 7.0% methoxyl content (Rouse et al., 1962). Based on these attributes, presence of high methoxyl pectin in banana samples investigated implies their suitability for industrial use particularly in jam and jelly production.

3.2.3.2.4 Anhydrouronic acid (AUA) content

AUA is important in determining the degree of purity of pectin, the degree of esterification and the physical properties of pectin. AUA ranged between 37.5 and 62.7% (Table 11). Pectic substances from NAMD, KAY, KISB, NDI and NAKB had significantly lower AUA than the rest. The AUA of pectic substances from bananas used in this study are within range of values obtained by Castill-Israel et al. (2015) of 57.3% and 39.68% for ripe and unripe banana peels.

Normally, pectin which is a partly esterified polygalactuonide contains about 10% or more organic materials composed of arabinose, galactose, and other sugars. According to the Food Chemical Codex (1996), AUA content above 65% indicates high purity of extracted pectin while AUA content of less than 65% indicates low purity which could imply presence of proteins, starch, and sugars in the precipitated pectin (Norazelina & Nazarrudin, 2011). The generally low AUA content implies that the extracted pectin from the fourteen banana cultivars had low purity hence requiring further purification if the pectin was to be applied commercially. According to Castill-Israel et al. (2015), longer extraction times could be adopted for higher values of AUA. However, this was not required for this study.

3.2.3.2.5 Degree of Esterification

The DE of pectic substances from the fourteen banana cultivars was generally very high and ranged between 88 and 96% (Table 11) implying high methoxyl pectin. The DE of pectic substances from NAMD was significantly lower than the DE of pectic substances from the rest of the banana cultivars. The DE for pectic substances from both cooking and juice banana cultivars was not significantly different ($P>0.05$). DE is important in determining the gelling

and adhesive power of pectins and in case of plant tissue pectins, it has been given in the range of 60 – 90% (Shaha et al., 2013). However, with the exception of NAMD, the DE for all the other pectic substances (94 and 96%) obtained in this study was above the range of 60 – 90%. The high DE of pectic substances from the fourteen banana cultivars studied implies presence of high levels of protopectins (Gee et al., 2006) which are normally present in unripe bananas and other fruits. Protopectin is a precursor for pectin and comprises a combination of cellulose, hemicellulose, lignin and pectin. This means that green mature bananas cooked for meals in Uganda contain protopectin which is insoluble and this may have significant influence on hardness of cooked bananas. The degree of esterification can be different depending on the level of ripeness or maturity, part of the fruit, botanical source and method of isolation (Bonrood et al., 2005). During ripening, solubility of pectic substances increases mainly due to pectic enzymes i.e. Polygalacturonase (PG) and pectin methylesterase (PME) (Maduwanthi & Marapana, 2017). Therefore, the high levels of pectic substances observed in this study could potentially contribute to greater hardness of bananas in both raw and cooked forms. Additionally, the high DE could induce increased adhesiveness thereby complementing starch retrogradation in order to cause increased hardness in bananas cooked at green maturity. According to Joslyn (1963), the great strength of green fruits is due to protopectin which is the water insoluble pectic substance that is partially esterified polygalacturonic acid. On the other hand, the high DE observed in this study could imply high water absorption and holding capacity of the pectic substances which can contribute to a decrease in hardness of cooked bananas.

3.2.4 Conclusions and recommendations

3.2.4.1 Conclusions

Results have shown that juice bananas contain significantly higher levels of starch and pectic substances than cooking bananas which is generally in agreement with dry matter and crude carbohydrate results (chapter 3.1). Results of high DE of pectic substances imply that at green maturity, bananas could be containing predominantly protopectins rather than pectin.

However, the physicochemical properties of starch and pectic substances from both cooking and juice banana cultivars do not significantly differ between the two banana types. This means that the chemistry of starch and pectic substances from cooking and juice bananas is generally

the same and may not significantly influence differences in physical behavior of the two molecules within bananas. This also implies that the possible differences exhibited by these banana cultivars could be due to differences in content of starch and pectic substances.

In general, all the starch and pectic substances investigated in this study possessed good physico –chemical properties implying in their pure forms, they can be utilized as additives and ingredients in the food industry. For instance starch can be used in banana based and pharmaceutical products including sauces. The high DE and methoxyl content of pectic substances implies that these pectins can form strong gels with sugar and may confer high water binding and holding capacity hence contributing to softness of cooked bananas.

3.2.4.2 Recommendations

Basing on information gathered above, it was important to evaluate the hardness profile of both cooking and juice banana cultivars. The major questions were "Do juice and cooking banana cultivars significantly differ in hardness?" and if they do, what component(s) is(are) responsible for these differences in hardness. Therefore, it was important to first profile the hardness of the two banana cultivars (Chapter 4.1) in order to generate objectively verifiable data. Basing on the results in Chapter 3 (3.1 & 3.2), there was need to examine the relationships between hardness and the different principle component molecules and substances in the bananas using Principle Component Analysis (PCA). Key components that have a major correlation with banana hardness would then be investigated for their influence on hardness of cooked bananas (Chapter 4.2). This would then be used to explain the differences in hardness that may exist between cooking and juice banana cultivars and therefore between different banana cultivars and varieties in general.

In order to confirm influence of major macro-molecules on hardness, selected molecules would have to be eliminated from the banana structural matrix by enzymatic hydrolysis and examine its influence on hardness of cooked bananas (Chapter 4.2.).

CHAPTER 4

HARDNESS OF SELECTED UGANDAN INDIGENOUS BANANA CULTIVARS

4.1 HARDNESS OF SELECTED UGANDAN BANANA CULTIVARS UNDER DIFFERENT PROCESSING TREATMENTS

Abstract

Hardness affects cooking time, fuel consumption and the quality of cooked bananas. Hardness of Uganda's indigenous banana cultivars has not been profiled. In this study, hardness of selected Ugandan cooking and juice banana cultivars at green maturity was determined using a Texture Analyser in raw form (control) and at 30, 50, 70, 90, 100 and 130 min of boiling, steaming and "steaming with mashing". Raw juice bananas (JB) (36.17 N to 42.43 N) were significantly harder than raw cooking bananas (CB) (22.37 N to 26.72 N) ($P<0.05$). Upon cooking, JB were harder than CB irrespective of cooking treatment and time. Hardness of all bananas decreased rapidly in the first 30 min under all cooking treatments and gradually thereafter. Boiling produced the softest bananas followed by "steaming with mashing" while "steaming without mashing" produced the hardest bananas. Among JB, *Kayinja* was significantly harder than *Ndiizi* and *Kisubi* under all cooking treatments irrespective of cooking time ($P<0.05$). Among cooking bananas, *Kibuzi* was generally softer while *Kazirakwe* & *Nakabululu* were harder than other CB cultivars. In general, hardness of cooking banana cultivars was not significantly different under each cooking treatment. Overall, hardness of all banana cultivars significantly decreased with cooking time regardless of cooking treatment. Cooling significantly increased hardness of all banana cultivars ($P<0.05$) under all treatments, with JB being significantly harder than CB in all cases. Upon cooling, mashed bananas were harder than the unmashed or boiled ones. During cooling, cooked bananas underwent rapid hardening following first order kinetics, with almost over 80% of hardening happening in the first one hour. Therefore, cooked bananas should be served and consumed while still hot to avoid hardening. Bananas should be boiled for a softer texture while juice bananas should not be cooked due to the hard texture established in this study.

Keywords: Boiling, cooking bananas (CB), juice bananas (JB), mashing, steaming, hardness of raw, cooked & cooled bananas.

4.1.1 Background

Bananas belong to the family *Musaceae* (genus *Musa*) and are a highly perishable crop produced in the tropical and subtropical countries of the world where they are mostly grown for home consumption and local markets. Bananas are subdivided into cooking cultivars (*Musa* spp AAB and ABB) and non-cooking cultivars (*Musa* spp AA and AAA) (Perrier et al., 2011). They are consumed as desert fruit, prepared for consumption as food by cooking or as a snack by drying, or extracted for juice and fermented for the production of alcoholic beverages such as beer, wine, and gin. Bananas are hence categorized into desert, cooking, juice/beer and roasting bananas depending on their use. Based on production quantities, bananas are the most popular fresh fruits worldwide followed by apples and grapes and are second in terms of production volume after watermelons (The Portal for Statistics (Statista), 2018).Banana production has steadily increased over the years growing from 142 MT in 2010 to 154 MT in 2015. By 2010, Uganda was the world's largest producer and consumer of cooking bananas with an estimated per capita consumption of 250 – 480 kg (Kabahenda & Kapiriri, 2010). Bananas occupy the largest cultivated area among staple food crops in Uganda and are primarily grown on small subsistence farm plots of average 0.5 ha (Nalunga et al., 2015). They are a source of income and are fast becoming a cash crop for Ugandans. Most banana varieties grown in Uganda are endemic to the East African highlands, a region recognized as a secondary center of banana diversity.

Despite the high production volumes, banana processing remains largely limited to cooking for domestic meals, juice/beer, dried chips and fried crisps. The rest are consumed as dessert. In Uganda, processing of bananas into solid-state shelf-stable products is rare. The reasons for this trend are not well known but hardening of cooked bananas could be a major challenge. Globally, texture remains the main sensorial criterion when evaluating the taste and acceptability of bananas (Qi et al., 2000). When cooking bananas are cooked, they soften, but harden immediately on cooling leading to loss of the desirable soft texture and taste. Hardness or softness is a key sensory characteristic for bananas which is important in determining a good cooking plantain cultivar depending on the method of cooking (Dzomeku et al., 2007). Hardness affects cooking time and the amount of fuel used. A short cooking time to reach acceptable softness is an advantage (Almazan, 1990). Bananas are cooked in different ways (Dury et al.,, 2002). In Uganda, bananas are cooked by boiling, steaming combined with

mashing or roasting.

Understanding texture characteristics of bananas allows for control of processing operations such as heating (boiling, steaming, mashing, frying, drying etc.) and processing time to attain the desired quality attributes of the end product (Chen & Opara, 2013a). Data on hardness of bananas is needed to develop solutions to the hardening phenomenon that occurs in cooked bananas and inform breeders, processors, traders and consumers about banana cultivars that produce tender meals. This information could also be applicable to product development models in case of banana products that are desired globally. However, there is scant information on hardness of Uganda's cooking and juice banana cultivars. This part of the study aimed at determining the i) hardness of selected Ugandan indigenous cooking and juice banana cultivars when raw, cooked and cooled ii) influence of different processing treatments (i.e. boiling, steaming, steaming combined with mashing and cooling) on the hardness of the bananas ii) influence of cooking time on the hardness of the cooked bananas.

4.1.2 Materials and methods

4.1.2.1 Materials

Unripe mature green East African Highland Bananas (*Musa* AAA-EA) (section 3.1.2.1) endemic to Uganda were used in this study.

4.1.2.2 Pre-harvest evaluation of sample maturity

The study used two groups of banana cultivars i.e. juice and cooking banana cultivars. Eleven cooking banana cultivars were selected from four clone sets and three juice bananas drawn from two clone sets (Karamura, 1998) depending on availability. Cooking banana samples were subjected to dry matter and pulp-to-peel ratio evaluation which were monitored until their values were relatively constant, an indication of optimum maturity. At this stage, bananas are manufacturing little or no additional food reserves. All bananas had expressed signs of maturity i.e. fruit angles were filled in completely, the top leaves had dried, the colour of banana finger had changed from deep green to light green (Amin, Hossain, Rahim, & Uddin, 2015) and the flesh of the pulp was orange-yellow for all cooking bananas. The banana fingers had reached relatively good size in comparison with the normal size of the particular banana cultivar basing on information from the on-farm guide at Kawanda Agricultural Research Institute (KARI). The edges of the banana fingers were fully round without being sharp (Amin et al., 2015).

None of the bananas had reached the ripening stage. The stage of maturation at which the fruit is harvested influences the storage life, eating quality of bananas and could also affect texture. Bananas which are not fully mature have less appealing pulp and normally harder than mature ones when cooked. Every fruit attains full characteristics such as flavour, taste and colour during storage if it was harvested at optimum maturity (Amin et al., 2015). Bunches of bananas are harvested when the fruits are fully developed, that is, 75% matured, the angles are becoming less prominent and the fruits on the top are changing to light green; and the flower remnants (styles) are easily rubbed off the tips. All these factors were considered during selection of banana samples examined in this study.

4.1.2.3 Sample harvesting

Before harvesting, all cooking banana samples had attained constant levels of dry matter content and "pulp to peel ratio" which had been monitored for three weeks. The flesh of all banana samples had attained pale to lightyellow-color determined visually as a sign of maturity. Samples were harvested between 8.00 and 10.00 am and immediately transported to the Department of Food Science & Nutrition, Makerere University for texture analysis. Analysis of hardness was performed within three hours after harvest to avoid influence of respiration on banana texture.

4.1.2.4 Sampling and preparation of "raw banana samples"

For determination of hardness of raw bananas, samples were taken by plucking two banana fingers randomly from the bottom, middle and top of the bunch. The fingers were manually washed using potable water and immediately thinly peeled. Peeling was performed by making a longitudinal slit along each banana finger and using the knife to carefully open and strip off the skin without removing the surface pulp. Samples were placed on a piece of aluminium foil and transferred to the texture analyzer (*TA.XT plus stable micro-systems*, Surrey, UK) for measurement of hardness.

4.1.2.5 Preparation of "boiled banana samples"

Boiling of banana samples was conducted according to the method of Paciulli et al. (2016) with modifications. A mixture of banana fingers from the top, middle and bottom of the bunch were randomly picked, washed and peeled. Peeled banana fingers were gently added to boiling water in a stainless steel pot at a temperature of about 98^{o}C and completely covered in a ratio of 1:2

bananas to water. The water level was marked and the cooking pot covered with the lid and the bananas boiled on gas flame using an Automatic Ignition Gas system (stainless steel, FL423GC/Gas stove – *Flamingo*, India). Boiling was continued for 30, 50, 70, 90, 110 and 130 min at 97 - 98^{o}C for each of the samples. The water level in the cooking pot was maintained by topping up using boiled water prepared at the side. At the end of each time point, two banana fingers were picked and placed on a piece of aluminium foil and immediately transferred to the texture analyzer platform for analysis. Three measurements (1 to 1.5 inches apart) were performed and recorded for each sample.

4.1.2.6 Preparation of "steamed banana samples"

A random mixture of banana fingers as in section 4.1.2.3 were picked, washed and peeled. Steamed bananas were prepared by placing the peeled fingers into the middle chamber of a 3 Tier Food Steamer (HS6000, Black & Decker, China). Samples were steamed for 30, 50, 70, 90, 110 and 130 min at 98 - 99^{o}C and at each sampling time, two banana fingers were removed, placed on a piece of aluminium foil and hardness measured as in section 4.1.2.8.

4.1.2.7 Preparation of "Mashed Banana Samples"

A random sample of banana fingers as in section 4.1.2.5 were picked and treated accordingly. Bananas for mashing were prepared by taking peeled bananas and steaming them in the middle chamber of a 3 Tier Food Steamer (*Black & Decker*) for 30 min before being mashed. Mashing was conducted by taking steamed bananas and wrapping them into two heat-softened banana leaves. They were wrapped further in another layer of a 30 micron plastic bag to enhance pressing and mashing. Banana samples were then hand-mashed by squeezing and pressing 30 times to produce the mashed bananas. The plastic bag was removed and the mashed bananas left wrapped in banana leaves, returned to the steamer and steamed for a further 20 min before taking the first sample. Samples of bananas steamed with mashing were placed in a 4 x 4.5 cm cup formed using aluminium foil and taken for hardness analysis as in section 4.1.2.8.

4.1.2.8 Preparation of cooled bananas

In order to determine the effect of cooling on hardness of cooked bananas, banana samples were taken at the end of each cooking treatment, placed on a table and left in the open at room temperature (~25^{o}C) to cool for 1 and 4 h after which hardness was measured, respectively.

4.1.2.9 Determination of hardness

Analysis of hardness was performed using a Texture Analyzer (*TA.XT plus stable micro-systems, Surrey, UK*) according to the methods of Setiady, et al (2009) with modifications. The measurement was performed using a penetration probe or rig of 6 mm diameter. The Texture Analyzer was set in *Return-to-start* mode with the following test settings:- penetration distance into the sample - 20 mm, pre-test speed – 1.0 mm/s, test speed into sample – 2.0 mm/s, post-test speed – 10 mm/s, a trigger force of 0.049 N and calibrated using a 2 kg load cell. The banana sample was positioned in the middle of the Texture Analyzer platform and the equipment was commanded to start. The measurements were performed in duplicate using two independent samples. The profile of the force in form of texture curves was monitored on a Personal Computer (PC) interfaced to the Texture Analyzer. The force needed to fracture or penetrate the banana sample was recorded as the first peak under the force-time curves and was taken as the hardness (N) (Jha et al., 2010a; Bagaud, Chillet, Beaute, & Dubois, 2006).

4.1.2.10 Statistical analysis

Data were analyzed using IBM (SPSS) package version 23. Means were separated by group and were analyzed using one-way Analysis of Variance (ANOVA) using Fisher's Least Significant Difference (LSD) and means were tested for homogeneity of variance. Other statistical procedures used include comparing means using the Paired Samples T-Test to determine significant differences in texture of a pair of cooked and cooled banana samples depending on the treatment. Significant differences were determined at $P < 0.05$.

4.1.3 Results and Discussions

4.1.3.1 Hardness of the raw bananas

Hardness of raw green unripe juice bananas ranged between 36.1 N and 42.43 N and was significantly higher ($P<0.05$) than that of raw green unripe cooking bananas which ranged from 22.30 N to 26.72 N (Table 12). For juice bananas, *Ndiizi* was significantly harder than both *Kayinja* and *Kisubi* ($P<0.05$). The hardness of *Kisubi* and *Ndiizi* both of which belong to the Ney Poovan AB clone set varied significantly ($P<0.05$). There was no significant difference between *Kisubi* of Ney Poovan AB and *Kayinja* of the Bluggoes ABB clone sets ($P>0.05$). Among cooking bananas, *Kibuzi* was significantly softer than *Nakabululu* and *Kisansa* which were significantly harder ($P<0.05$). Hardness of the rest of the cooking banana cultivars was not significantly different from that of *Kibuzi* ($P>0.05$). Hardness of *Namande, Nakawere* and

Namweezi of the *Nfuuka* clone set was not significantly different (P>0.05). In the *Nakitembe* clone set, *Nakitembe* cultivar was harder than *Nakyetengu* and *Kibuzi*. Cultivars under *Musakala* and *Nakabululu* clone sets were the hardest and there was no significant difference in hardness between cultivars in these two clone sets.

Table 12: Hardness of selected raw cooking and juice banana cultivars at green maturity

Local name of cultivar	**Synonym	Clone set	Hardness (N)
Cooking banana cultivars			
Namande	NAMD	*Nfuuka*	$23.81^{abc} \pm 3.19$
Nakawere	NAKW	*Nfuuka*	$22.81^{ab} \pm 1.98$
Namweezi	NAMZ	*Nfuuka*	$22.80^{ab} \pm 1.97$
Nakitembe	NAKT	*Nakitembe*	$25.40^{abc} \pm 1.49$
Nakyetengu	NAKY	*Nakitembe*	$22.53^{ab} \pm 2.79$
Kibuzi	KIB	*Nakitembe*	$22.37^{a} \pm 1.59$
Musakala	MUS	*Musakala*	$25.10^{abc} \pm 1.00$
Mpologoma	MPO	*Musakala*	$23.65^{abc} \pm 1.50$
Kisansa	KIS	*Musakala*	$26.00^{bc} \pm 2.03$
Nakabululu	NAKB	*Nakabululu*	$26.36^{bc} \pm 1.98$
Kazirakwe	KAZ	*Nakabululu*	$25.14^{abc} \pm 1.15$
Juice banana cultivars			
Kisubi	KISB	Ney Poovan AB	$36.17^{d} \pm 1.45$
Ndiizi (apple banana)	NDI	Ney Poovan AB	$42.43^{e} \pm 3.73$
Kayinja	KAY	Bluggoes ABB	$36.86^{d} \pm 2.16$

*Values with the same superscript letters in the same column are not significantly different (P>0.05).

*N = Newtons, the penetration force as measured by the Texture analyzer (TA).

*Names and "clone set classification" adopted from Karamura (1998).

***Note*: The synonyms for banana varieties in this Table are used in all sections hereafter.

Values are means of three independent determinations ± standard errors of the means

Hardness of bananas is a reflection of their eating quality. According to current results, raw juice banana cultivars were significantly harder by an average of 59% than cooking banana cultivars (Table12). In their experiment on prediction of textural attributes using color values of banana (*Musa sapientum*) during ripening, Jaiswal et al. (2014) reported that the initial pulp firmness of banana fruits (at 0 days) was 38.04 N which decreased to 14.00 N on the 10^{th} day as the banana ripened. The initial firmness value of 38.04 N is close to the hardness of juice bananas examined in this study. Jaiswal et al. (2014) possibly used desert or juice bananas which fall within the range of firmness recorded in this study. Baoxiu et al. (2000) working on desert banana (green mature Cavendish) obtained hardness of about 22 N for the raw bananas which is close to the values (22.37 - 26.36 N) obtained for cooking bananas in this study. Cavendish is a non-cooking banana whose hardness is expected to be higher than that of cooking bananas. Probably the bananas used by Qi et al. (2000) were obtained from commercial sources when they were beginning to ripen, a process that would have reduced their firmness. In the previous work, the same juice bananas used in this study were found to have higher starch, crude pectic extracts and dry matter content but lower moisture than cooking bananas which may partly explain why juice bananas are harder as observed in this study. NDI also called “apple banana” is both a juice and a desert banana cultivar and was found to be harder in raw form than all banana cultivars investigated in this study. In the day to day experiences, green or unripe juice and desert bananas are generally harder than cooking bananas in raw and cooked form which supports the observations from this study. Hardness is influenced by, banana cultivar and variety, maturity stage, growth season, soils, etc. (Amin, et al., 2015). Current results indicated that hardness of KIB, NAMZ, NAKW, NAKY, NAMD, NAKT, MPO, MUS and KAZ was not significantly different ($P>0.05$); whereas KIS and NAKB were significantly harder than KIB ($P<0.05$). In practice these banana cultivars differ in their hardness when cooked, which means that small variations in measurement may have significant textural implications when these bananas are cooked. Basing on personal experience, Ugandan consumers tend to prefer banana cultivars such as KIB, NAKT and NAKY for softer texture. This study was conducted during the wet season of April and May 2017 which could have influenced hardness particularly in terms of cell turgor pressure.

As results indicate, there were a few noticeable differences in hardness between cultivars in different clone sets as grouped by Karamura (1998). The differences based on clone sets may

not be conclusive because banana classification by Karamura (1998) was largely based on growth and physical characteristics of the different banana cultivars, and not eating quality profiles. However, results of this study showed that hardness of the selected banana cultivars in each clone set was close which may be related to differences in their genetic composition.

4.1.3.2 Influence of boiling, steaming and mashing on hardness

All banana cultivars (both cooking and juice) were subjected to boiling, "steaming with mashing" and "steaming without mashing" treatments and sampled for hardness tests at 30, 50, 70, 90, 110 and 130 min. The boiling temperature ranged between 97 to 98^{o}C while that of steaming ranged between 98 to 99^{o}C. In general, boiling caused rapid decrease in hardness for all the banana samples (Figure 9A). Juice bananas were significantly harder than cooking bananas ($P<0.05$). For juice bananas, boiling for 30 min caused a rapid decrease in hardness by an average of 81% and by 130 min, hardness had decreased by 92%.KAY was significantly harder than KISB and NDI ($P<0.05$) and their hardness decreased significantly with cooking time ($P<0.05$). For instance, hardness of KAY, KISB and NDI decreased from 8.89 N, 6.58 N, and 6.29 N at 30 min to 4.90 N, 2.67 N and 1.38 N respectively at 130 min (Table 17, appendix 1).

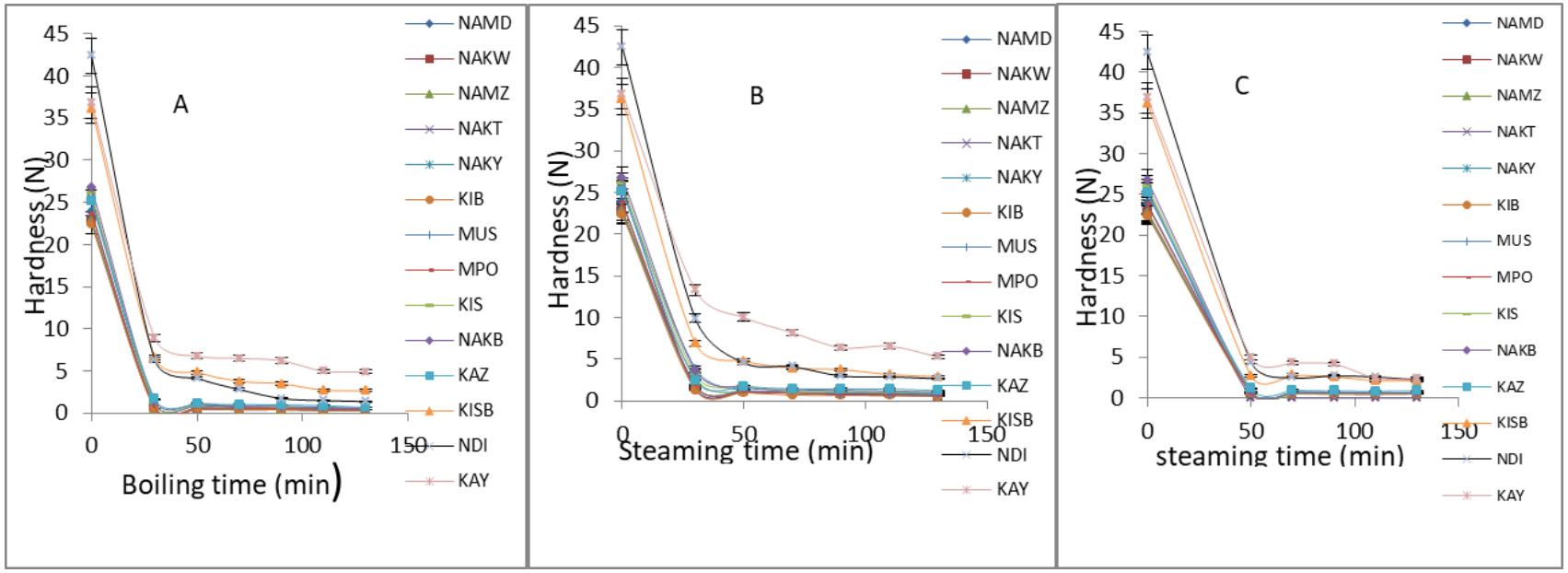


Figure 9: Changes in hardness of cooking and juice bananas during (A) Boiling, (B) Steaming and (C) "Mashing with steaming"

For cooking bananas, boiling for 30 min also caused a rapid decrease in hardness by an average 96.2% and by 130 min, hardness had decreased by an average 98%. Between 30 and 130 min of boiling, the decrease in hardness for most cooking banana cultivars was not significant ($P>0.05$; (Figure 9 A and appendix 1, Table 17). However, NAKB, KAZ and NAKT experienced a significant decrease in hardness with increasing cooking time ($P<0.05$) and were harder than other cultivars (Table 17, appendix 1). On the other hand, KIB, NAMZ and NAKW maintained the lowest hardness profile with KIB being the softest.

Similarly, steaming caused rapid decrease in hardness for both cooking and juice bananas within the first 30 min followed by a slow gradual decrease until 130 min (Figure 9 B). Juice bananas were generally harder than cooking bananas. In the first 30 min of steaming, hardness of juice bananas decreased rapidly by 73.9% and by 130 min, it had decreased by 90.6%. Hardness of all the three juice banana cultivars significantly decreased between 30 and 70 min ($P<0.05$) but further decrease was not significant ($P>0.05$) except for KAY (Figure 9 B, Table 18, appendix 1). Among juice banana, KAY was significantly harder than KISB and NDI ($P<0.05$). Hardness of cooking bananas also decreased rapidly by 90.3% within 30 min of steaming and by 130 min, it had decreased by 96.7%. Hardness of almost all banana cultivars significantly decreased between 30 and 70 min of steaming ($P<0.05$) but further decrease was not significant ($P>0.05$; Figure 9 B). Among the cooking banana cultivars, hardness of KIB was the lowest while that of NAKB and KAZ was the highest.

Hardness of mashed bananas decreased between 50 and 130 min of steaming although the decrease was not significant ($P>0.05$) except for NDI (Figure 9 C and Table 19 appendix 1). Again mashed juice bananas were significantly harder than mashed cooking bananas throughout cooking ($P<0.05$). For juice bananas, hardness decreased by 92.50% by 130 min of steaming. KAY was significantly harder than both KISB and NDI ($P<.05$). For cooking bananas, hardness decreased by 97.62% by 130 min of steaming. Once again, KIB had the lowest level of hardness while KAZ had the highest level of hardness. These results indicate that hardness of mashed bananas was intermediate between that of boiled and steamed bananas upon cooking.

Current results indicated that boiling and steaming caused rapid decrease in hardness in the first 30 min and a little thereafter. On the other hand, steaming in combination with mashing led to

significant reduction in hardness relative to steaming alone. These results imply that the rate of softening of bananas during heating follows first-order kinetics (Harada & Paulus, 1987). This is also in agreement with observations made by Qi et al. (2000) who studied the effect of cooking on banana and plantain texture. Qi et al. (2000) reported firmness of desert banana pulp to decrease sharply during the first 10 min of cooking, losing 75% of its original firmness. The observed decrease in hardness of bananas during cooking (boiling and steaming) implies absorption of water by banana starch which on heating hydrates and swells (Wang et al., 2015). Wang et al. (2015) state that during cooking, the starch granule structure collapses due to melting of the crystallites, unwinding of the double helices, breaking up of hydrogen bonds and hence gelatinization of starch. Cooking also causes alteration of organelles in the cytoplasm and swelling of the cell walls (Xu et al., 2015; Prestamo et al., 1998) leading to a gradual breakdown of the protoplasmic structure. This breakdown causes subsequent loss of turgor pressure and a softening effect which is related to changes in the pectic polymers of the cell wall and the middle lamella (Xu et al., 2015). According to Waldron et al. (2003) the high-temperature exposure during industrial thermal processing and/or home cooking of vegetables causes cell separation, which is related to the solubilization of pectic components, often accompanied by the swelling of cell walls. Starch has a significant influence on the textural properties of foods. When heated in water, starch granules become hydrated, swell, and are transformed into a paste. These structural changes are accompanied by loss of the characteristic birefringence of intact starch granules (Wang, Li, Copeland, Niu, & Wang, 2015).

In general, boiling produced the softest texture followed by “steaming combined with mashing” and lastly “steaming without mashing” (Figures 9 A, B, C and Tables 17, 18, 19 appendix 1). This is probably because boiling provided bananas with adequate access to water relative to steaming. Boiling bananas in plenty of water probably increases their water content due to osmotic exchange (Dadzie & Orchard, 1997). Gouado et al. (2011) reported that after boiling, the water content of unripe and ripe bananas increased significantly ($P<0.05$). Excess water, at prolonged heating enhances further swelling and gelatinization of starch and further enhances breakdown and solubilization of other intercellular materials such as pectin leading to further structural separation (Qi et al., 2000). Depending on water availability, time of exposure and temperature, higher degrees of swelling and gelatinization of starch and pectin can be achieved, hence further lowering hardness (Hunag & Bourne, 1983; Qi et al., 2000). “Steaming combined

with mashing" resulted in intermediate hardness while steaming alone produced the hardest texture in hot cooked form. Mashing causes structural breakdown of the native bonds within the banana structural matrix which exposes starch and pectin to more moisture hence increased uptake of water and gelatinization. Access to moisture during cooking is in ascending order – boiling > steaming with mashing > steaming alone. Steaming restricts the amount of moisture in contact with the bananas which in turn limits starch gelatinization. Boiled cooking bananas were observed to disintegrate in the boiling water compared to their steamed counterparts, which remained firm and intact during the whole process of cooking. This re-affirms observations made by Ambigaipalan et al. (2013) showing that the amount of water available influences the degree of starch gelatinization.

Current results also showed that juice bananas were harder than cooking bananas under all the three cooking treatments. This means that the tendency for banana starch to gelatinize and cause structural breakdown during cooking is more pronounced in cooking bananas than in juice bananas. It is not well established why juice bananas are generally harder than cooking bananas. This may probably be related to the native water, starch, pectin and dry matter content, and degree of molecular compactness which may restrict the extent and rate of water penetration into the banana structural matrix in order to cause swelling and tissue separation (Sato, 2016). In chapter three of this report, juice bananas were found to have significantly more starch, pectic substances and dry matter content but lower moisture than cooking bananas. These differences in firmness loss could also be due to inherent differences in cell size and structure, as well as differences in chemical composition (Dadzie, 1998).

4.1.3.3 Influence of cooking time on hardness

All banana samples were subjected to a cooking time ranging from 30 to 130 min in order to determine if extended cooking time leads to significant reduction in hardness of bananas. Bananas cooked for a shorter time were significantly harder than those cooked for a longer time. Hardness of all the banana cultivars decreased with cooking time from 30 to 130 min depending on the cooking treatment. During boiling, hardness of cooking bananas at 30 min ranged between 0.5 N to 1.67 N and was not significantly different from hardness at 130 min which ranged between 0.34 N and 0.68 N ($P>0.05$) (Table 17 appendix 1). However, hardness of juice bananas at 30 min of boiling was significantly higher than hardness at 130 min of boiling ($P<0.05$). Hardness of all bananas steamed for 30 min was higher than their

corresponding hardness at 130 min of steaming (P<0.05) (Figure 10). In general, cooking beyond 70 minutes for all the three cooking treatments resulted in little decrease in hardness implying that for soft texture, bananas should be cooked for at least 70 minutes regardless of cooking treatment applied.

When boiled bananas were cooled and evaluated for hardness, all bananas boiled for 30 min were significantly harder than those bananas boiled for 130 min (Figure 11) (P<0.05). For instance, the hardness of cooking bananas boiled for 30 min and allowed to cool for 4 h ranged between 2.33 N (KIB) and 5.11 N (KAZ) while that of juice bananas ranged between 13.02 N (KISB) to 18.41 N (KAY). However, when the same bananas were boiled for 130 min and cooled for 4 h, hardness of cooking bananas ranged between 1.21 N (KIB) and 4.02 N (NAKB) while that of juice bananas ranged between 9.36 N (KISB) and 12.65 N (KAY) (Table 18 appendix 1). This effect was more pronounced in steamed bananas (mashed and unmashed) than in boiled bananas.

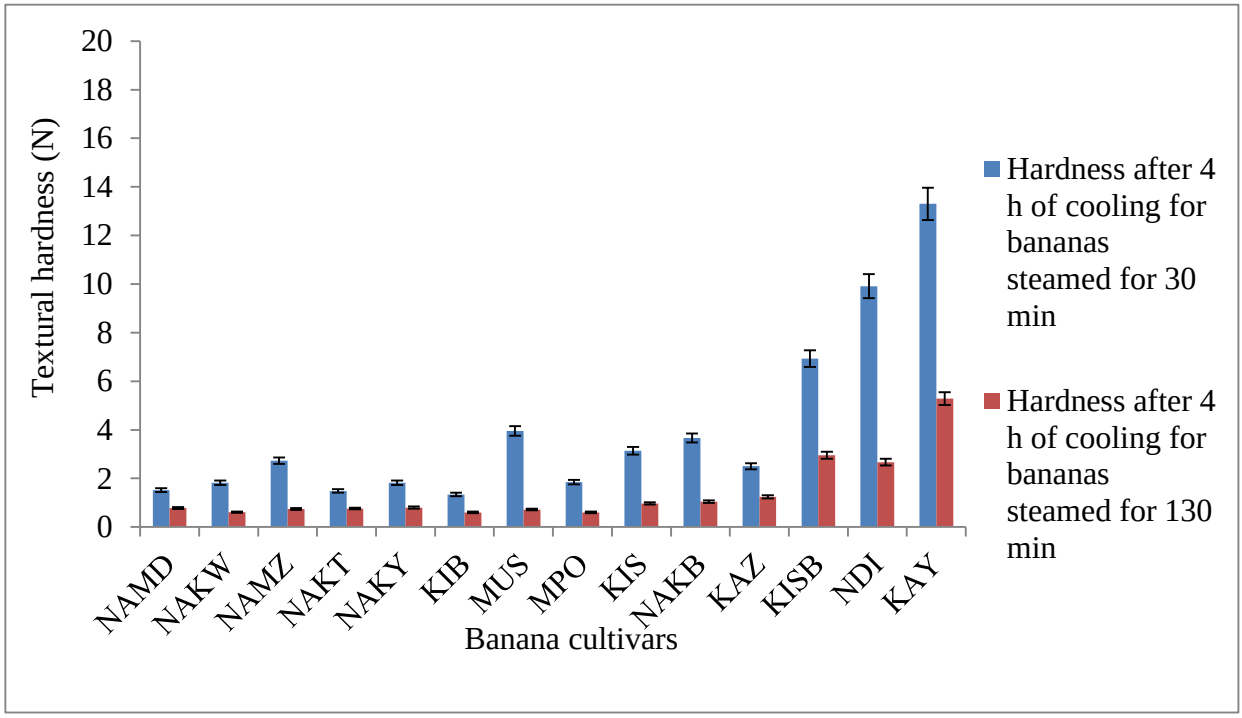


Figure 10: Effect of cooking time on hardness of steamed bananas. The shorter bars (red) represent hardness after 4 h of cooling for bananas steamed for 130 min while longer bars (blue) represent hardness after 4 h of cooling for bananas steamed for 30 min.

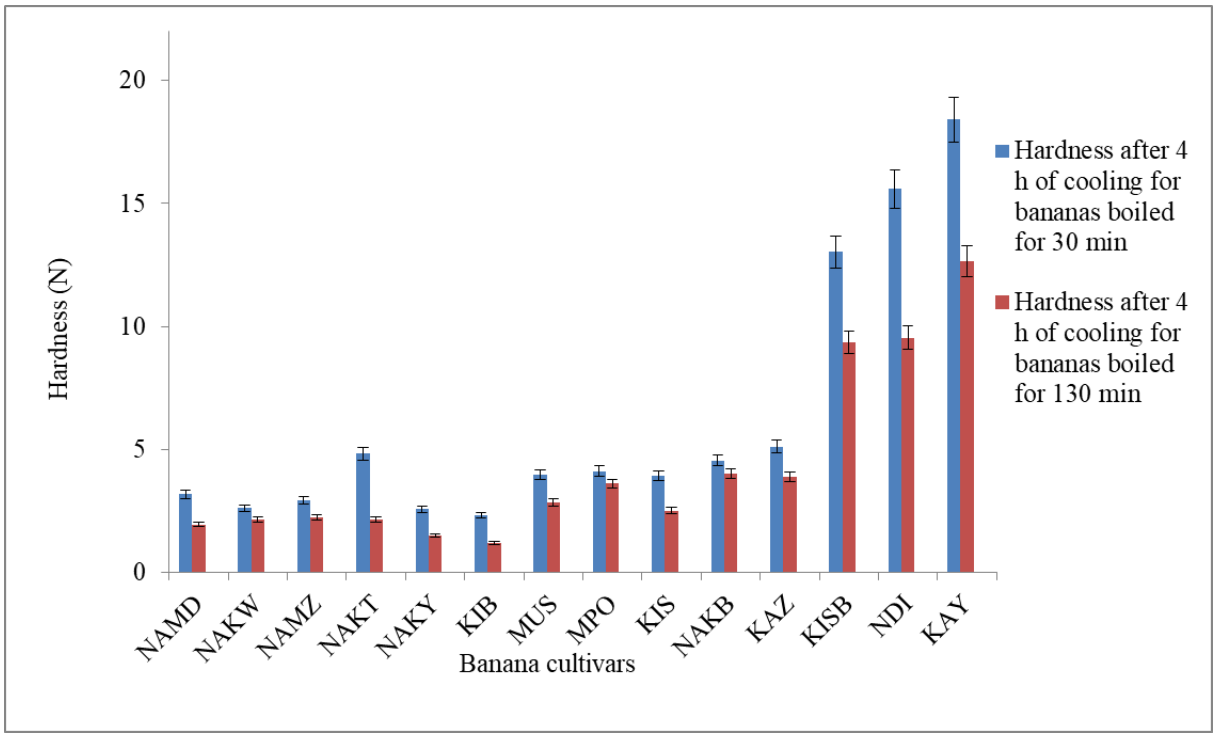


Figure 11: Effect of cooking time on hardness of boiled bananas. The shorter bars (red) represent hardness after 4 h of cooling for bananas boiled for 130 min while longer bars (blue) represent hardness after 4 h of cooling for bananas boiled for 30 min

Hardness was more pronounced when bananas were allowed to cool for 4 h at room temp implying that longer cooking time allows bananas to absorb more water thus allowing greater gelatinization of starch and pectin. This enhances extensive structural breakdown, enabling tissues to gain and retain high moisture levels, and reduces their propensity to lose it during cooling. This in turn could keep banana starch gelatinized longer and hence softer texture.

4.1.3.4 Influence of cooling on hardness

All cooked banana samples subjected to cooling became significantly harder ($P<0.05$) (Figures 12, 13 & 14 and Tables 20, 21 & 22 appendix 1). Juice bananas were significantly harder than cooking bananas in all cases ($P<0.05$). In cooled form, hardness of boiled cooking bananas ranged from 1.04 N to 4.02 N while that of boiled juice bananas ranged from 9.54 N to 12.65 N. Hardness of steamed cooking bananas ranged between 2.97 N and 5.39 N while that of steamed juice bananas ranged between 15.85 N and 18.33 N upon cooling. Hardness of mashed cooking bananas ranged between 3.84 N to 6.89 N while that of mashed juice bananas ranged from 14.41 N to 20.44 N upon cooling.

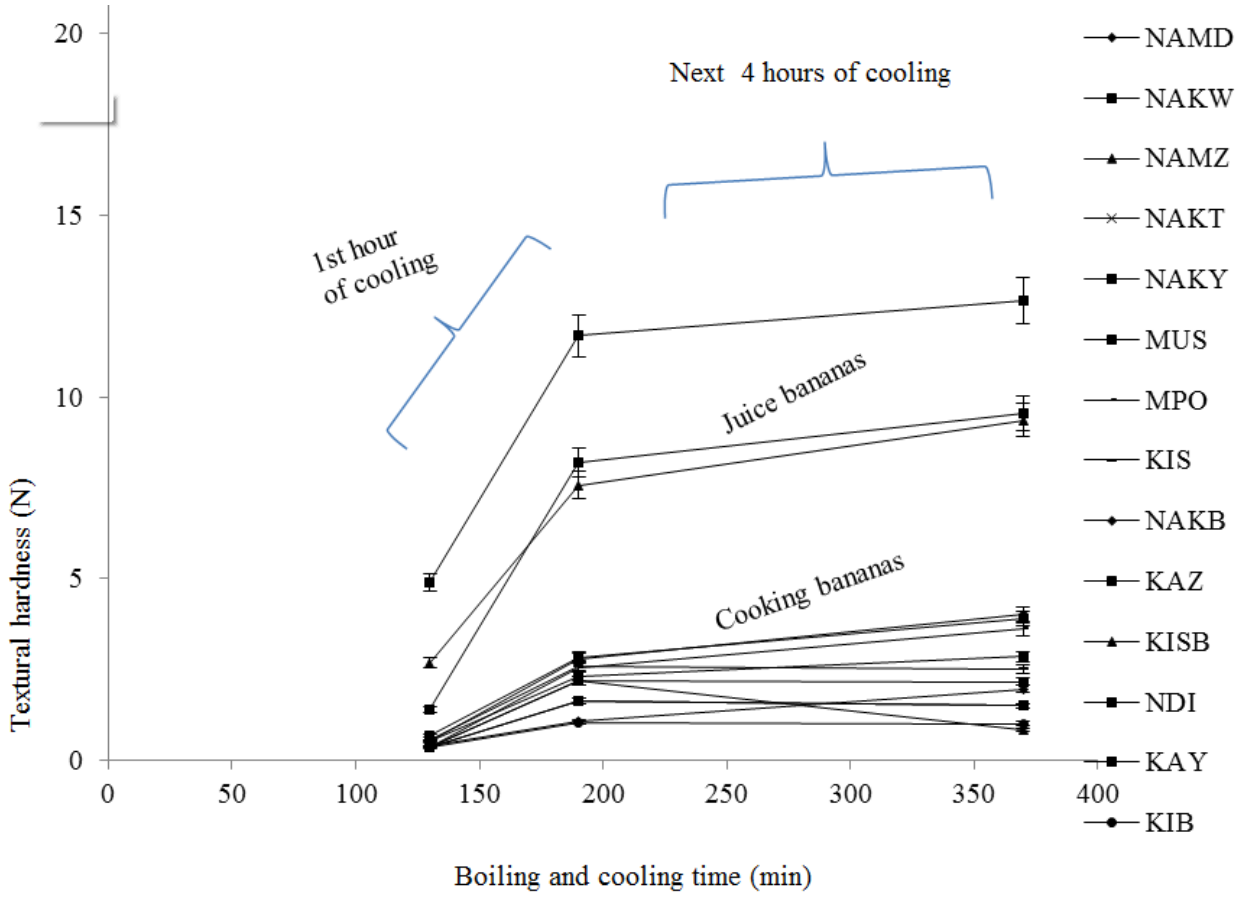


Figure 12: Effect of cooling on hardness of boiled bananas - Rapid hardening of bananas boiled for 130 min during cooling at room temperature.

Overall upon cooling, KAZ and NAKB were the hardest cooking bananas while KIB was the softest under all cooking treatments. Among juice bananas, KAY was the hardest and KISB was the softest in cooled form irrespective of the cooking treatment employed.

Rate of hardening: During cooling, all banana samples underwent rapid hardening in the first 1 h, followed by a slow gradual increase in hardness over the next 4 h of cooling (Figures 12, 13 & 14). The magnitude of hardening was influenced by the cooking treatment and time of cooking. For example, when MUS was boiled for 30 min and cooled for 1 h, hardness increased rapidly from 1.10 N to 3.36 N (305%). A further 4 h of cooling increased hardness to only 3.98 N (by a factor of 18.5%). Whereas hardness of MUS boiled for 130 min and cooled, experienced rapid hardening to 2.31 N (444%) in the first 1 h and further cooling for an extra 4 h only increased hardness to 2.85 N (104%). Basing on the cooking treatment, steamed bananas

experienced a higher rate of hardening in the first hour of cooling compared to mashed and boiled bananas. Results indicated that upon cooling, mashed bananas were generally harder than bananas subjected to steaming alone or boiling (Table 21 appendix 1).

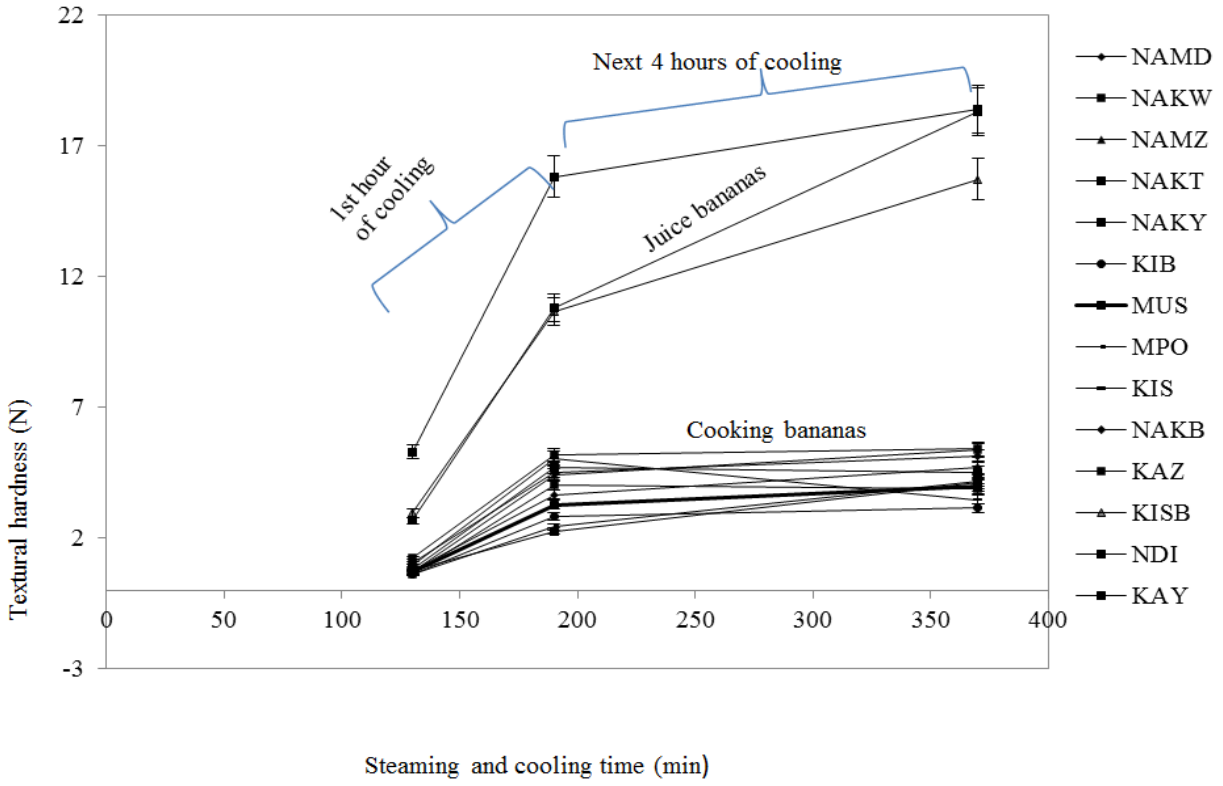


Figure 13: Effect of cooling on hardness of steamed bananas - Rapid hardening of texture for bananas steamed for 130 min during cooling at room temperature

Results have indicated that all banana cultivars exposed to different cooking treatments significantly hardened upon cooling ($P<0.05$). The observed hardening of cooked bananas during cooling could be attributed to retrogradation of starch. Starch in form of disaggregated amylose and amylopectin chains in a gelatinized starch paste normally undergoes re-association to form more ordered crystalline structures (Wang et al., 2015). On cooling, the disaggregated starch chains retrograde gradually into partially ordered structures that differ from those in native starch granules which may have detrimental effects on the quality of starch-rich foods such as bananas. According to Wang & Copeland (2013), the quality and nutritional properties of starch-based foods are largely determined by the changes that starch undergoes during processing and subsequent storage. These changes involve water uptake, granule swelling, formation of a viscoelastic paste during heating, followed by re-association of dispersed starch

chains on cooling and formation of a firm gel (Wang et al., 2015) which is probably responsible for hardening in cooked bananas.

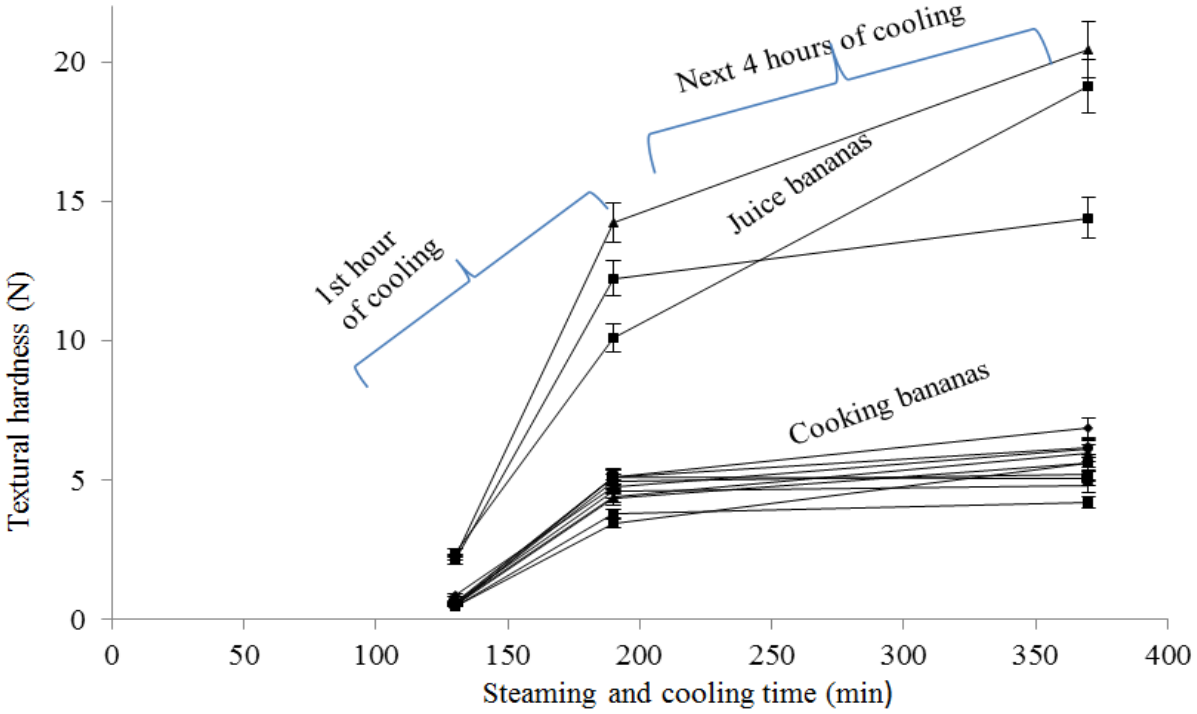


Figure 14: Effect of cooling on hardness of mashed bananas - Rapid hardening of texture of mashed bananas steamed for 130 min during cooling at room temperature

The extent of hardening may be influenced by the degree of retrogradation which is in turn influenced by storage temperature, water content of the gelatinized starchy food, starch source, extent of gelatinization of the starch, amylopectin chain length (Ambigaipalan et al., 2013; Fu, Wang, Li, Zhou, & Adhikari, 2013) and possibly cooking treatment and time. For instance, results have shown that banana samples boiled or steamed for a shorter time of 30 min were harder than those cooked for a longer time of 130 min. Current results have also shown that upon cooling, the magnitude of hardening was affected by the cooking treatment. For instance mashed bananas (range 3.84 - 6.89 N for CB and 14.41 - 20.44 N for JB) were much harder than "unmashed steamed bananas" (range 2.97 – 5.39 N for CB and 15.85 – 18.33 N for JB) which were also harder than boiled bananas (1.04 – 4.02 N for CB and 9.36 – 12.65 N for JB) (Tables 17, 18 & 19 Appendix 1). It is possible that mashing and pressing of bananas led to creation of new bonds upon cooling that are stronger than the native bonds. Pressing may increase adhesive and cohesive forces (Murdia & Wadhwani, 2010), which also excludes air

spaces in the banana structural matrix making the product more compact and harder upon cooling. Mashing could also have increased the surface area leading to increased water loss and hence contributing to hardening.

Results showed that a greater part of hardening (over 80%) occurred in the first hour of cooling, a trend that was observed in all banana cultivars. This implies that hardening of bananas during cooling follows first order kinetics. Wang et al. (2015) reported retrogradation of starch to be rapid initially and slows down thereafter which may explain rapid hardening of cooked bananas in the first hour of cooling followed by slow gradual hardening thereafter.

4.1.4 Conclusions and recommendations

This study has shown that juice banana cultivars are harder than cooking banana cultivars irrespective of treatment and cooking time. Among juice bananas, *Kayinja* is the hardest in cooked form regardless of cooking treatment and time. Among cooking bananas, *Kibuzi* exhibited the softest texture while *Nakabululu*, *Kazirakwe, Kisansa & Musakala* were the hardest both in raw and cooked forms. "Steaming without mashing" results in harder cooked bananas than "mashing with steaming" or boiling. Cooling results in hardening of cooked banana texture which occurs rapidly in the first 1 h following first order kinetics implying that bananas should be served and consumed hot to avoid hardening of texture. Also hardness of bananas decreases with cooking time regardless of banana cultivar and cooking treatment. Lastly, boiling produces the softest cooked bananas followed by "steaming with mashing" while "steaming without mashing" produces the hardest cooked bananas.

Therefore, this study recommends boiling of bananas for softest texture. Alternatively, steamed bananas should be coupled with mashing for a softer texture. Bananas should be cooked longer (at least 70 min) particularly bananas steaming with or without mashing in order to attain the softest texture possible. On the basis of these results, cooked bananas once served should be consumed as soon as possible when texture is still soft.

Lastly, a correlational analysis to establish the key chemical components that are more related with hardness of bananas should be performed. This should be done using yield of starch, pectic substances and proximate composition against hardness of selected bananas in raw form. Resulting from Principle Component Analysis (PCA), the major macro molecules which are more related with hardness of raw bananas should further be investigated for their effect on

hardness of cooked bananas.

4.1.5 Principle Component Analysis

Principle component analysis (PCA) was done to using excelstat to analyse the correlation between proximate composition (crude ash, fibre, protein, fat, carbohydrates and moisture content), yield of starch and pectic substances with hardness of bananas in raw form.

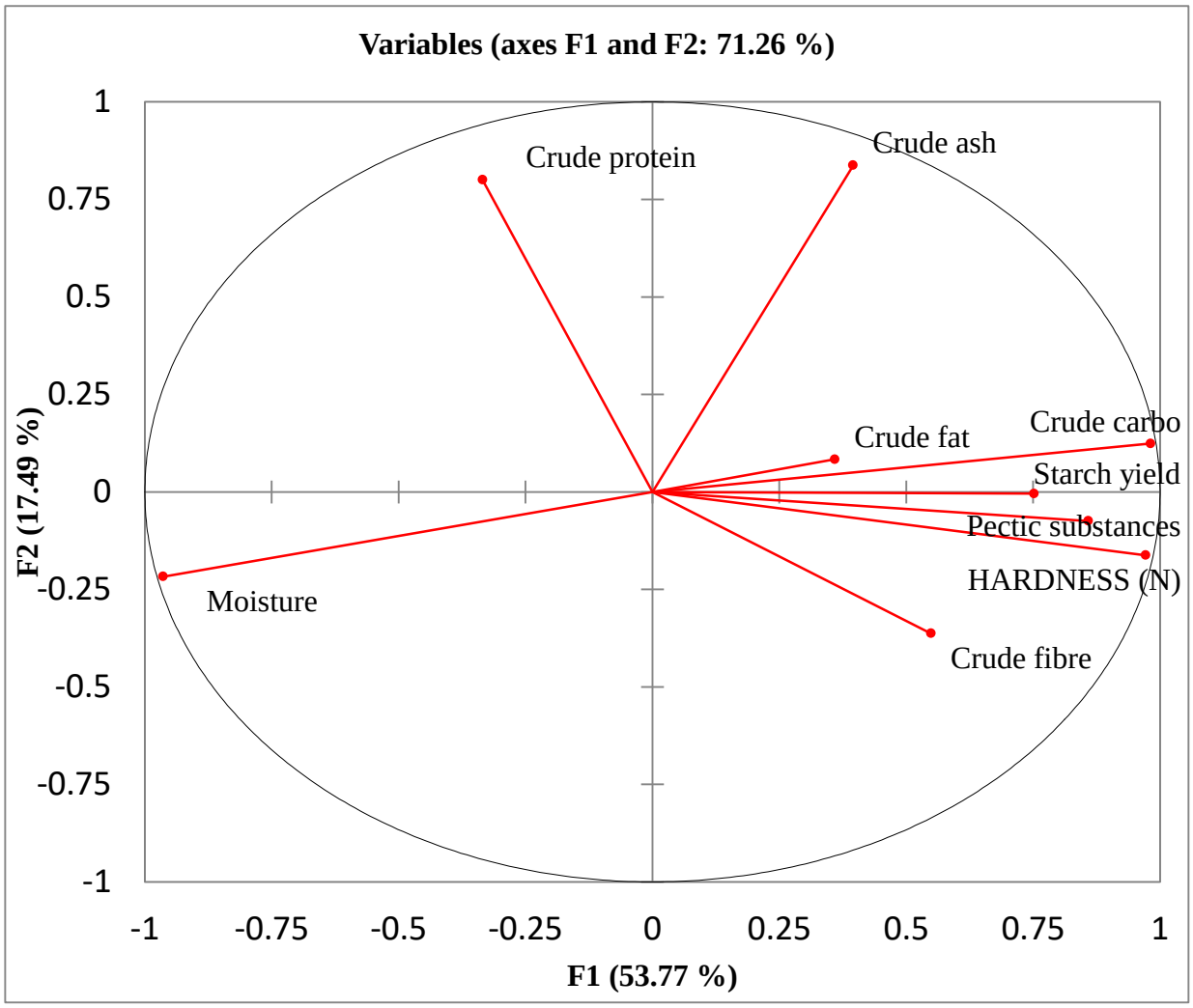


Figure 15: Correlational analysis of proximate composition, starch and pectic substances with hardness of raw bananas.

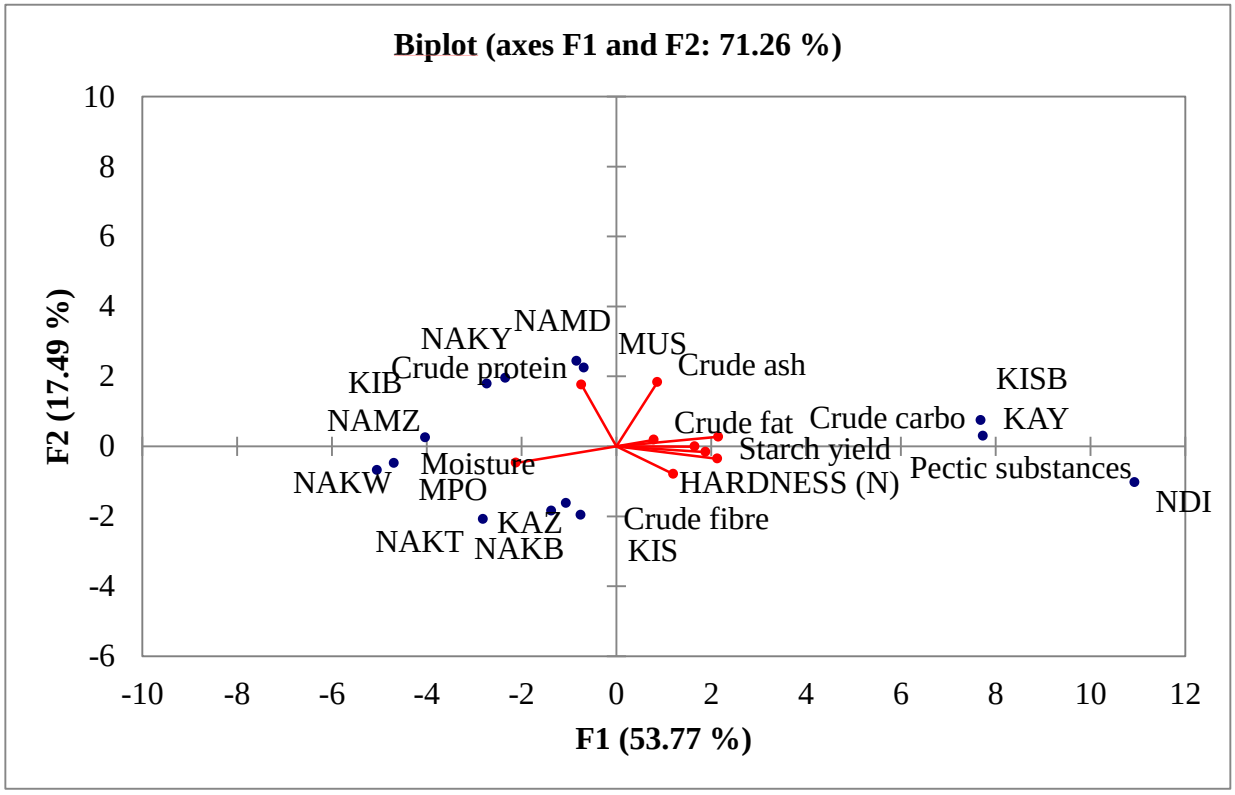


Figure 16: Principle Component Analysis (PCA) cluster diagram showing correlations between composition factors and banana cultivars

Table 13: Correlational matrix for composition with hardness of the raw bananas

Variables	Crude ash	Crude fibre	Crude protein	Crude fat	Crude carbo	Moisture	Starch yield	Pectic sub'nces (%)	HARDNESS (N)
Crude ash	**1**	**-0.05**	**0.41**	**0.26**	**0.47**	**-0.54**	**0.19**	**0.26**	**0.25**
Crude fibre	**-0.05**	**1**	**-0.26**	**-0.17**	**0.53**	**-0.51**	**0.20**	**0.46**	**0.56**
Crude protein	**0.41**	**-0.26**	**1**	**-0.32**	**-0.20**	**0.11**	**-0.22**	**-0.31**	**-0.47**
Crude fat	**0.26**	**-0.17**	**-0.32**	**1**	**0.32**	**-0.30**	**0.09**	**0.25**	**0.40**
Crude carbo	**0.47**	**0.53**	**-0.20**	**0.32**	**1**	**-1.00**	**0.73**	**0.81**	**0.93**
Moisture	**-0.54**	**-0.51**	**0.11**	**-0.30**	**-1.00**	**1**	**-0.72**	**-0.79**	**-0.89**
Starch yield	**0.190**	**0.20**	**-0.22**	**0.09**	**0.73**	**-0.72**	**1**	**0.55**	**0.74**
Pectic substances (%)	**0.26**	**0.46**	**-0.31**	**0.25**	**0.81**	**-0.79**	**0.55**	**1**	**0.81**
HARDNESS (N)	**0.25**	**0.56**	**-0.47**	**0.40**	**0.93**	**-0.89**	**0.74**	**0.81**	**1**

Values in bold are different from 0 with a significance level alpha=0.95

Results of PCA (Figures 15 & 16 and Table 13) show that hardness of bananas in raw form is more correlated with crude carbohydrates (93%), pectic substances (81%) and starch content (74%) implying that carbohydrates, pectic substances and starch have a strong influence on hardness of raw bananas. Crude fibre (56%) had a lower correlation with hardness of raw bananas. Crude fat and ash had very low correlations with hardness of raw bananas implying that fat and ash may not significantly affect hardness of bananas. On the other hand, hardness had a strong negative correlation with moisture content and a weak negative correlation with crude protein. This implies that moisture content of bananas is strongly related with softness of bananas while protein content may have little influence. The cluster diagram (Figure 16) shows that hardness is associated more with juice bananas than cooking bananas.

Therefore, based on these results, the key components in bananas that could strongly influence hardness of bananas are moisture, starch and pectic substances. Therefore, in chapter 4.2, the latter two components i.e. starch and pectin were investigated.

4.2 EFFECT OF STARCH AND PECTIN ON HARDNESS OF COOKED BANANAS

Abstract

Texture is an important quality attribute of fresh and processed foods. In plant foods, texture is closely related with the structural integrity of the primary cell walls and middle lamella that is mainly composed of pectic substances. Previous studies have shown that juice bananas have more starch and pectic substances and have been found to be generally harder when raw, cooked or cooled than cooking bananas. Results of Principle Component Analysis (PCA) have shown that carbohydrates, starch content and pectic substances are highly correlated with texture. However, little information is available on the effect of starch and pectin on hardness of cooked bananas. Therefore, in this work, the effect of added pectin and starch and structural elimination of pectin on hardness of cooked bananas was investigated.

Treatment of bananas with pectin did not significantly increase hardness upon cooking relative to the control ($P>0.05$). However, upon cooling, hardness of cooked bananas with added pectin decreased significantly with increasing pectin concentration ($P<0.05$). Hydrolysis of pectin resulted in significantly harder bananas during cooking and upon cooling ($P<0.05$). Similarly, hardness of starch treated bananas increased significantly with increasing starch concentration upon cooking and cooling relative to the control ($P<0.05$). Hardness of cooked bananas treated with a combination of starch and pectin increased but was not significantly different from the control. Upon cooling, hardness of cooked bananas with added starch-pectin composite decreased with increasing concentration similar to the effect of pectin when added alone.

Current results indicate that starch increases hardness of bananas upon cooking and cooling. However, pectin decreases hardness of cooked bananas implying that pectin contributes to a softer texture of bananas during cooking and cooling. Pectin can therefore be added up to 5% to decrease hardness in cooked bananas.

Key words: Cooked bananas; Hardness; Pectin; Starch; Structural pectin hydrolysis;

4.2.1 Background

Texture is a group of physical characteristics that arise from the structural elements of a food perceived by the sense of touch, which are related to deformation, disintegration and flow of food under force, and measured objectively by functions of mass, time and distance (Bourne, 2002). Texture is a major sensory and quality attribute that plays an important role when assessing the quality of food. The texture of foods is related to the structure formed by micro and macro molecular elements forming the cell wall and other regions (Davison, et al., 2013). The major challenge to cooking and consumption of bananas is their susceptibility to hardening of the texture after cooling. Changes in the texture of bananas affect other attributes such as flavor and aroma (Gowen, 1995) which are lost upon cooling of the cooked bananas. Changes in the texture of bananas during cooking are caused by structural changes in starch, pectin, cellulose and hemicellulose (Chaïb et al., 2007). The basic structure of the primary cell wall consists of a cellulose-hemicellulose network with pectin interwoven within this network forming the basis for structural integrity of the cell. In raw plant tissues, pectin which is mainly found in the middle lamella, cements cell walls together and gives firmness and elasticity to plant tissues (Zamil & Geitmann, 2017).

Cooking affects the structural integrity and biochemical composition of the cells and cell walls leading to loss of turgor pressure and the 'fresh' texture of bananas. Starch which is the most important carbohydrate and a major component in bananas undergoes gelatinization during cooking and retrogradation upon cooling (Wang et al., 2015), which affect the hardness of cooked bananas. With increase in temperature over a temperature range of 50 - 90^{o}C, the water binding capacity of starch increases (Gani, Sul Haq, Masoodi, Broadway, & Gani, 2010) leading to softening as the starch gelatinizes. However, in general, the water binding and holding capacity of starch is low (Gani et al., 2010) thus increasing the tendency of retrogradation. The low water binding and holding capacity could be attributed to the involvement of a larger proportion of the hydroxyl groups in forming hydrogen and covalent bonds between the starch chains than with water (Hoover & Sosulski, 1986). Additionally, gelatinized banana starch has the largest thermal stability because its crystals dissociate at higher temperatures accompanied with a larger enthalpy change (Espinosa-Solis et al., 2009). From experience, its almost impossible to re-soften cool "cooked bananas" by reheating. These properties of starch affect its functional qualities (Wang & Copeland, 2013). As a result,

differences in starch content could have significant impact on hardness of bananas particularly when cooked. It's not clear what would happen to the degree of hardness and retrogradation in bananas if starch levels were increased.

On the other hand, the pectin content of raw green mature bananas has been found to consist majorly of protopectin which is characterized by a high degree of esterification (D.E) ranging between 88 to 96%. Pectin consists of a linear chain of α- (1→4)-linked D-galacturonic acid units in which varying proportions of acid groups are esterified with methyl groups which are responsible for gel formation during heating in presence of sugar and divalent cations such as Ca^{2+} (Padival et al., 1979; Sundar Raj et al., 2012). This property would increase water binding and holding properties of pectin which in turn would decrease hardness of cooked bananas. In order to fully understand the effect of pectin in cooked bananas, it could be removed by enzyme hydrolysis before cooking bananas and determine hardness of the bananas upon cooking and cooling. Structural elimination of pectin can be performed by hydrolysis using pectolytic enzymes such as polygalacturonase (PG) and pectin metylesterase (PME) which are the key enzymes responsible for pectin breakdown (Tapre & Jain, 2014) in plant tissues causing softening of fruits and vegetables as they mature and ripen. During ripening, PME removes methyl ester groups from the cell wall pectic substances, making them accessible to depolymerisation by PG which in turn reduces intercellular adhesiveness and tissue rigidity (Payasi, Mishra, Chaves, & Singh, 2009). Most commercial preparations of pectinases are produced from fungal sources (Sharma, Rathore, & Sharma, 2012) with the commonest source being *Aspergillus niger* (Gummadi & Panda, 2003). Depending on the need, these pectic enzymes can be used as processing aids particularly in the production of fruit juices. Due to the high water holding capacity of pectin within plant tissues, it is possible that if pectin is eliminated from the structural matrix of bananas, it would lead to increased dryness and hence hardness upon cooking and cooling. Thus, structural elimination of pectin would confirm its role in bananas particularly when cooked. Besides foods such as cheese where the effect of added pectin on texture has been studied, there is no information regarding the effect of structural elimination of pectin using pectinases, let alone added pectin on hardness of cooked bananas. Similarly, there is scant information on the effect of added starch on the hardness of cooked bananas. Starch and pectin are major structural components of bananas and both starch and pectic substances have been found to strongly correlate with hardness in raw bananas.

Thus, adding pectin and starch to bananas separately would help in establishing the effect of each molecule on hardness of bananas upon cooking. Pectin and starch addition has been shown to modify the moisture content, texture, and melting properties of foods (Ibáñez et al., 2016; Lobato-Calleros et al., 2006; Delcour, et al., 2009; Li, Dhital, & Wei, 2017). Therefore, this study was aimed at investigating the effect of added starch and pectin and structural elimination of pectin on hardness of cooked bananas.

Understanding these effects would promote innovation of cooked banana products that have more industrial and economic value. One of the challenges to banana processing is how to use recent advances in processing technologies to adjust banana composition, innovation of creative ingredient mixes and processes to improve the texture of cooked bananas. As a result, future banana processors would produce banana products of a more desirable softer texture and flavor.

4.2.2 Materials and methods

4.2.2.1 Materials

Three East African Highland Banana (AAA-EA) cultivars endemic to Uganda were selected for this study. These include *Kayinja* (KAY), a local juice banana cultivar from Central and Western Uganda under the Bluggoes ABB clone set and two cooking banana cultivars namely *Kazirakwe* (KAZ), a local cooking banana cultivar common in Western Uganda under the *Nakabululu* clone set; and *Nakitembe* (NAKT), a local cooking banana cultivar from Central Uganda under the *Nakitembe* clone set (Karamura, 1998). The choice of these banana cultivars was based on the general degree of hardness as determined in Chapter 4.1 and as well as availability at the time of research. In chapter 4.1, KAZ was among the hardest cooking bananas upon cooking. In chapter 4.1, NAKT was found to be moderately soft upon cooking and was also immediately available. Cooking and eating experience has also shown that NAKT is a soft cultivar as long as it's well matured. KAY was also the hardest cultivar among juice bananas upon cooking and cooling. A general assumption taken was that regardless of the banana cultivar, the effects of pectin and starch would be the same. KAZ and NAKT were purchased from Kawanda Agricultural Research Institute (KARI) while KAY was bought from a farm near Kyengera trading centre in Wakiso district, Uganda. All samples were harvested and immediately transported to Makerere University Food Science and Nutrition laboratory for

texture analysis. Pectin (CAS 9000-69-5; 75% D.E) and the enzyme pectinase (CAS: 9032-75-1; activity 1 U/mg) were purchased from Sigma Aldrich (USA) through Kobian Scientific, a local agent. Banana starch was locally extracted from NAKT banana cultivar using the alkaline starch extraction method (Zhang, Whistler, BeMiller, & Hamaker, 2005). Other materials were obtained from local supermarkets.

4.2.2.2 General preparation of bananas for treatments

Banana fingers (21) were picked from green mature banana bunches (7 from each of the top, middle and bottom), then washed, peeled and dipped in portable water to prevent browning. The peeled bananas were sliced to approx. 1 – 2 mm discs and subdivided into three equal portions. Each portion was placed in a glass beaker and covered with aluminium foil before being weighed. Steaming combined with mashing which is commonly used in preparation of local bananas meals was used in this work. Though boiling was found to produce the softest cooked bananas, the method was not suitable for the treatments. This is because for thorough distribution of the added pectin and starch, it required that the bananas be thinly sliced and mashed upon cooking. Boiling mashed bananas is practically not suitable as the bananas would become a watery paste or mass.

4.2.2.3 Preparation of pectinase-treated bananas

Approx. 240 g of sliced banana sample was weighed into a beaker. Then, 1.5 g of pectinase (polygalacturonase) was weighed and mixed well with 410 ml of distilled water. The banana sample was mixed into the enzyme solution and the pH of the banana-enzyme mixture measured to be 5.3. The enzyme-banana mixture as well as the control were incubated at 50^{o}C for 2 h in a shaking water bath (Julabo-Germany) with constant agitation. After incubation, the solutions in the enzyme-treated bananas and the control were drained and the banana slices lightly squeezed in a nylon cloth to remove excess water. Each of the control and the enzyme-treated bananas were placed in a small plastic bag of 30 microns which was perforated to allow normal exchange of air and moisture. The bag containing the banana sample was steamed in a 3 Tier Food Steamer (Black & Decker, China) for 30 min, and thereafter removed and mashed. The mashed bananas were returned to the steamer and steamed for a further 20 min (50 min of cooking overall) before the first sample was taken for texture analysis. The bananas were steamed further for 70, 90, 110 and 130 min and appropriately sampled for texture analysis as in section 4.1.2.6. The remaining banana masses for each replicate of the respective treatments

were rolled together, cooled for 4 h at room temperature (25^{o}C) and used to measure texture of the cool samples. Mean values were tabulated and used to generate a profile of curves used for analysis of changes in hardness of the enzyme treated bananas.

4.2.2.4 Preparation of starch and pectin-treated bananas

For each of starch and pectin treatments, i.e. 0 g (0%), 1 g (1.7%), 3 g (5%), 5 g (8.3%), and 7 g (11.7%) of banana starch extracted from *Nakitembe* banana cultivar and pectin (*Sigma*) were weighed separately. Approx. 60 g of sliced bananas (section 4.2.2.2) were weighed into a plastic bag of 30 microns. The weighed starch or pectin was dispersed onto the banana slices carefully little by little from a plastic sieve by tapping the sieve while agitating the bananas to ensure uniform distribution. The plastic bag for each treatment was perforated at the upper side to prevent buildup of internal pressure and to allow normal exchange of air and moisture during steaming. A control for each of the treatments was prepared and comprised of bananas without added starch or pectin. All banana samples were cooked by steaming in a Food Steamer for 30 min as in section 4.2.2.3 before being mashed. Mashed bananas were placed back into the steamer and steamed for a further 20 min (50 min overall) before sampling for texture analysis. The remaining banana masses were treated as in section 4.2.2.3 before being used to measure texture of the cool samples. Treatments were conducted in three independent replicates.

4.2.2.5 Preparation of starch-pectin composite treated bananas

Sliced bananas (60 g) from section 4.2.2.2 were weighed into a plastic bag as in section 4.2.2.3. Then, a starch-pectin composite was prepared by weighing 0, 1, 3, 5, and 7 g of banana starch and 0, 1, 3, 5, and 7 g of pectin (Sigma) separately. The starch and pectin powders were mixed thoroughly in a 1:1 ratio [i.e. 0 g (0%), 2g (3.33%), 6 g (10%), 10 g (16.7%), and 14 g (23.3%)] to achieve uniform composites of the different levels of treatments. Then, each starch-pectin composite was applied separately onto 60 g of banana slices [i.e. 0 g (0%), 2g (3.33%), 6 g (10%), 10 g (16.7%), and 14 g (23.3%)] and the treated samples processed as in section 4.2.2.3. (Note: both starch and pectin were added at the same levels as previously used to avoid effects of over or under addition in the cooked bananas).

4.2.2.6 Determination of hardness and cohesiveness

Hardness was measured using a Texture Analyzer (TXT Plus Stable micro-systems) as done in section 4.1.2.9 above. Cohesiveness was defined as the ratio of the area under the second

penetration curve to the area under the first penetration and values were generated by the Texture Analyzer according to the manufacturer's instructions (Texture technologies Corp. and Stable Micro Systems, 2018).

4.2.2.7 Data analysis

Data were analyzed using IBM (SPSS) package, version 23. All means were split and separated by group and analyzed using one-way Analysis of Variance (ANOVA) using Fisher's Least Significant Difference (LSD) and Turkey's test procedures. Means were tested for homogeneity of variance and significant differences were determined at $P < 0.05$. For graphed results, simple regression analysis was also done to determine the strength of the correlation between the added molecules and hardness of cooked bananas.

4.2.3 Results and Discussion

4.2.3.1 Effect of pectin-treatment on hardness

Upon cooking, there was a small increase (range 1.04 – 1.24 N) in hardness of pectin treated bananas relative to the control (0.93 N) (Figure 17 A, curve (b) and Table 23 appendix 1). After 5% added pectin, there was a slight decrease in hardness with increasing pectin concentration (Table 23 appendix 1). Hardness increased from 0.93 N (control) through 1.04 to 1.24 N at 1.7 and 5% added pectin, respectively. Further increase in pectin concentration resulted in a decrease in hardness to 1.14 and 1.06 N at 8.3% and 11.7% added pectin respectively. However, in general, hardness of pectin treated bananas was not significantly different from hardness of the control ($P>0.05$). When simple regression analysis was performed on the cooked banana data curve, there was a low regression coefficient (43%) and a coefficient of determination (r^2) of 18.3% implying that added pectin contributed very little to the observed increase in hardness. Upon cooling, there was a general increase in hardness of both the control and pectin treated bananas which was attributed to retrogradation of starch (Figure 17 A, curve (a) and Table 23 appendix 1). However, there was a general decrease in hardness of pectin treated bananas as the concentration of pectin increased decreasing from 4.50 N (1.7%) to 1.87 N (8.3%) followed by a slight increase to 1.92 N at 11.7% added pectin. In general, hardness of pectin treated bananas was significantly lower than hardness of the control except at 1.7% ($P<0.05$). Hardness of bananas at 1.7% added pectin was not significantly different from the control ($P>0.05$). Simple regression analysis performed on the cooled data curve (Figure 17 A,

curve a) indicated a strong negative regression coefficient of 89.6% and a coefficient of determination (r^2) of 80.3% implying that much of the decrease in hardness of cooked bananas was due to added pectin.

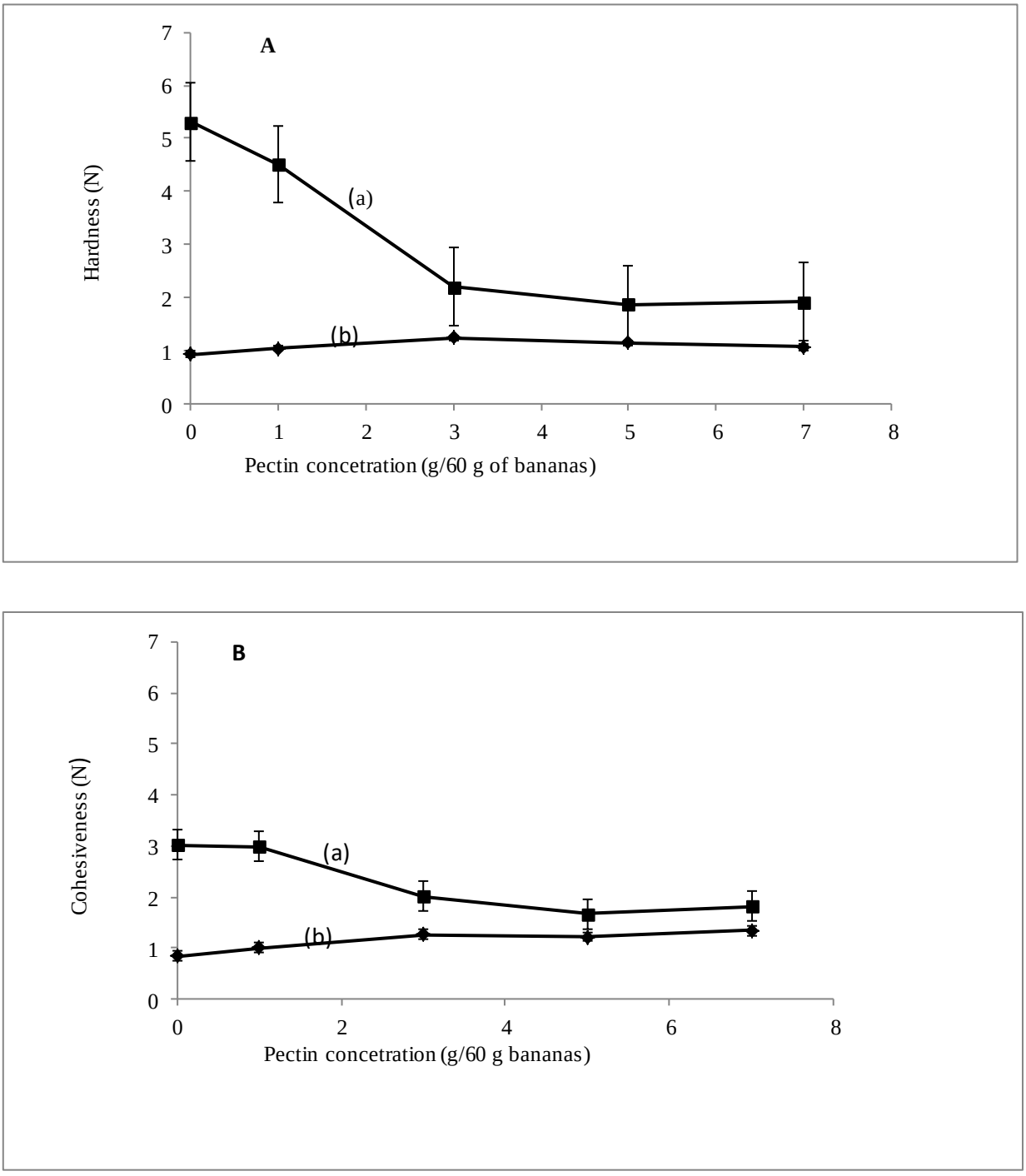


Figure 17: Effect of added pectin on (A) hardness and (B) cohesiveness of bananas (case of cooking bananas i.e. NAKT) upon cooking (curve b) and cooling (curve a)

Cohesiveness of pectin-treated bananas was higher than the control (0.84 N) and increased significantly with increasing pectin concentration from 1.01 to 1.34 N upon cooking ($P<0.05$) (Figure 17 B, curve b and Table 23 appendix 1). Upon cooling, cohesiveness of the control and pectin-treated bananas generally increased probably due to retrogradation of starch. However, cohesiveness of pectin-treated bananas (2.99 to 1.67 N) decreased significantly with increasing added pectin concentration relative to the control (3.02 N) ($P<0.05$) (Figure 17 B, curve a) and Table 23 appendix 1).

In general, these results imply that pectin enhances softness or tenderness of cooked bananas. The observed increase in hardness upon cooking of pectin treated bananas could be attributed to a number of factors: adhesiveness of pectin leading to increased compactness of starch and other molecules; inadequate gelatinization of starch due to the high Water Binding Capacity (WBC) of pectin (Lobato-Calleros et al., 2006) making water unavailable for starch to gelatinize adequately; the increased dry matter content due to added pectin. The low regression coefficient implies that pectin had little effect in increasing hardness of cooked bananas. These results are in agreement with Tan et al. (2007) who observed an increase in stiffness of rennet gels and a reduction in syneresis as the concentration of high methoxyl pectin increased from 0% to 0.1% attributed to a more compact microstructure. According to Kopjar et al. (2009), pectin increased firmness and cohesiveness of strawberry jam which increased with increasing degree of esterification of pectin. These authors attributed the increase in firmness to increased adhesiveness and compactness of molecules. On the other hand, the general increase in hardness of pectin-treated bananas and the control upon cooling was attributed to starch retrogradation (Wang et al., 2015). Retrogradation is accompanied by a series of physical changes such as exudation of water and increased crystallinity with the appearance of β-type crystalline polymorphs (Hoover et al., 2009). Besides, the observed significant decrease in hardness of cooked bananas as pectin concentration increased upon cooling may be attributed to the High Water Retention Capacity (WRC) of pectin. Pectin is highly hygroscopic and absorbs water leading to formation of soft gels. The high water binding and holding capacity of pectin (Armstrong et al., 1993) keeps the cooked banana structural matrix moist, thus maintaining starch in a gelatinized state thus making pectin-treated bananas softer in cooled form. Pectins have high water absorption capacity, for instance sunflower seed pectin can absorb upto 57 g of water per g of pectin (Miyamoto & Chang, 1992). Apple pectin has a high

methoxyl content with DE higher than 50% and a water holding capacity of approx. 6.7 g water/g pectin (Sri Puvanesvari et al., 2012). According to Sri Puvanesvari et al. (2012), apple pectin also has a high emulsifying activity of 90% and above implying that pectin can prevent loss of water from the food matrix hence preventing extensive starch retrogradation. Studies on cheese with added pectin found increased moisture content coupled with reduced hardness (Ibáñez et al., 2016; Lobato-Calleros et al., 2006). Added pectin could also interrupt interactions between starch molecules within the banana matrix hence preventing starch-starch associations and re-crystallization leading to a decrease in hardness.

On the other hand, the increase in cohesiveness of pectin-treated bananas relative to the control could be attributed to the adhesive properties of pectin. Cohesiveness measures the extent to which a food product stays together after deformation or the extent to which a product adheres to itself under some compressive or tensile stress (Texture technologies Corp. and Stable Micro Systems, Ltd, 2018). It is a good indicator of how the banana holds together upon cooking and may be directly affected by adhesiveness of the sample (Sozer et al., 2007). The adhesiveness of pectin tends to keep particles in the cooked banana matrix cohesive. The decrease in cohesiveness of the pectin treated bananas upon cooling could be due to the high moisture levels retained by pectin. This may be true only in the short run as other authors such as Kopjar, et al. (2009) found that the cohesiveness of all pectin treated jam samples had increased over the storage period which may be due to loss of moisture causing structural re-arrangement and increased adhesiveness and hence cohesiveness.

4.2.3.2 Effect of added starch on hardness

Upon cooking, there was a general increase in hardness (1.06 to 1.97 N) of starch-treated bananas which was higher than that of the control (0.86 N) and increased with increasing starch concentration (Figure 18 A, curve b and Table 24 appendix 1). Hardness increased from 1.06 (1.7%) to 1.97 N (11.7% added starch) becoming significantly higher than the control beyond 5% added starch ($P<0.05$). Simple regression analysis performed on the data indicated a strong positive regression coefficient of 99% and a coefficient of determination (r^2) of 98% implying that almost all the observed increase in hardness is due to added starch. Upon cooling, hardness generally increased for both the control (4.04 N) and starch-treated bananas (4.52 to 5.99 N) (Figure 18 A, curve a) and Table 24 appendix 1), which was attributed to starch retrogradation. Hardness of cooled starch-treated bananas was higher than that of the control and increased

significantly with increasing starch concentration from 4.52 N at 1.7% to 5.99 N at 11.7% ($P<0.05$).

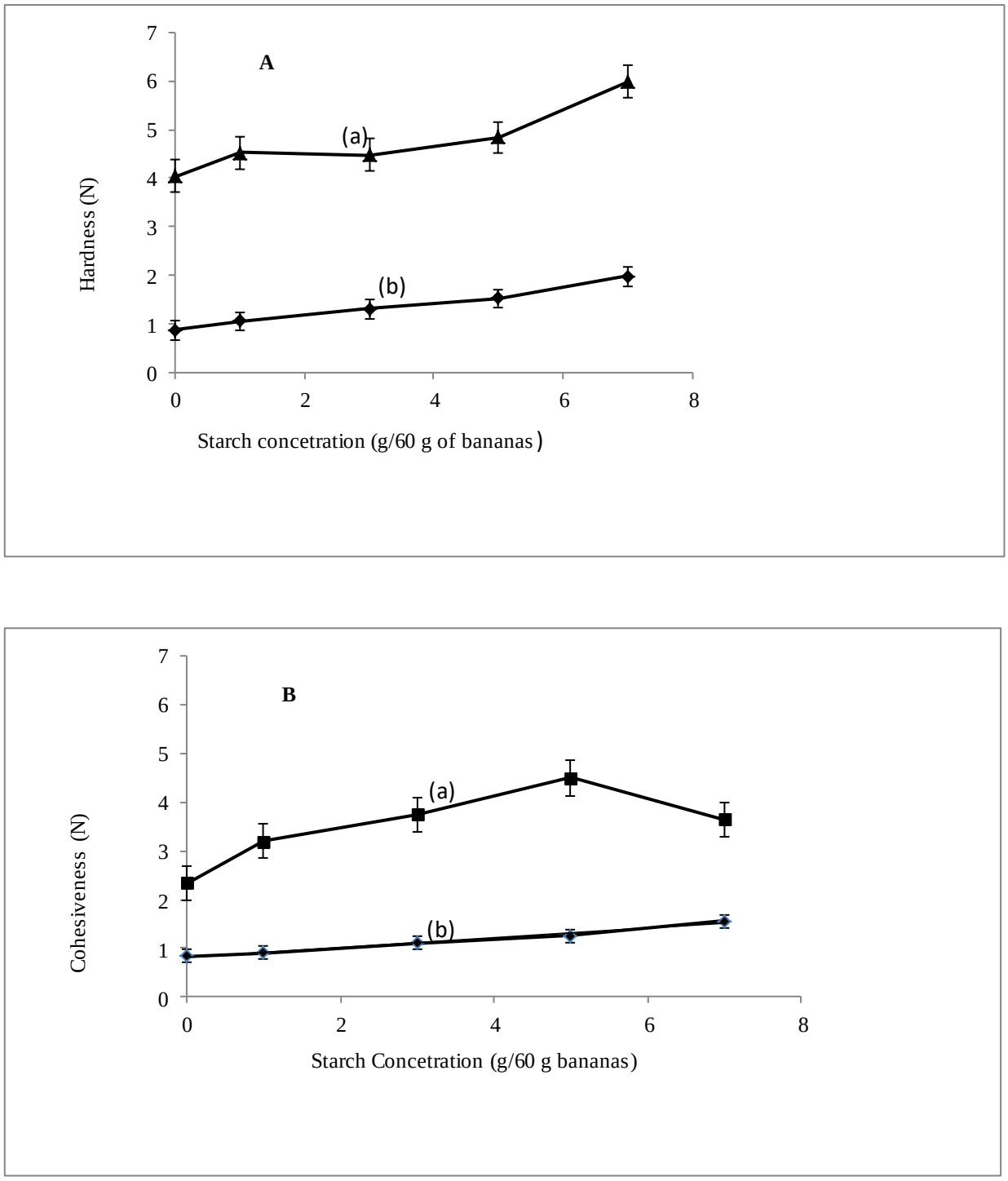


Figure 18: Effect of added starch on (A) hardness and (B) cohesiveness of bananas (case of cooking bananas i.e. NAKT) upon cooking (curve b) and cooling (curve a)

Results of simple regression analysis performed on the cooled data curve indicated a high positive regression coefficient of 91% and a coefficient of determination (r^2) of 83%, implying strong dependence of observed increase in hardness on added starch.

Cohesiveness of starch-treated bananas (0.91 to 1.56 N) was also higher than that of the control (0.84 N) and increased with increasing added-starch concentration from 0.91 N (1.7%) to 1.56 N (11.7% added starch) upon cooking (Figure 18 B, curve b and Table 24 appendix 1). Simple regression analysis indicated a coefficient of determination of 98% implying that most of the observed increase in cohesiveness is due to starch. Upon cooling, cohesiveness of the control and starch-treated bananas generally increased possibly due to starch retrogradation. However, cohesiveness of starch-treated bananas was significantly higher than that of the control (2.34 N) and increased significantly with increasing starch concentration from 3.20 N (1.7%) to 4.49 N (8.3% added starch) ($P<0.05$) before decreasing slightly to 3.65 N at 11.7% added starch with a coefficient of determination of 54% (Figure 18 B, curve a). This implies a small dependence of cohesiveness in cooled bananas on starch.

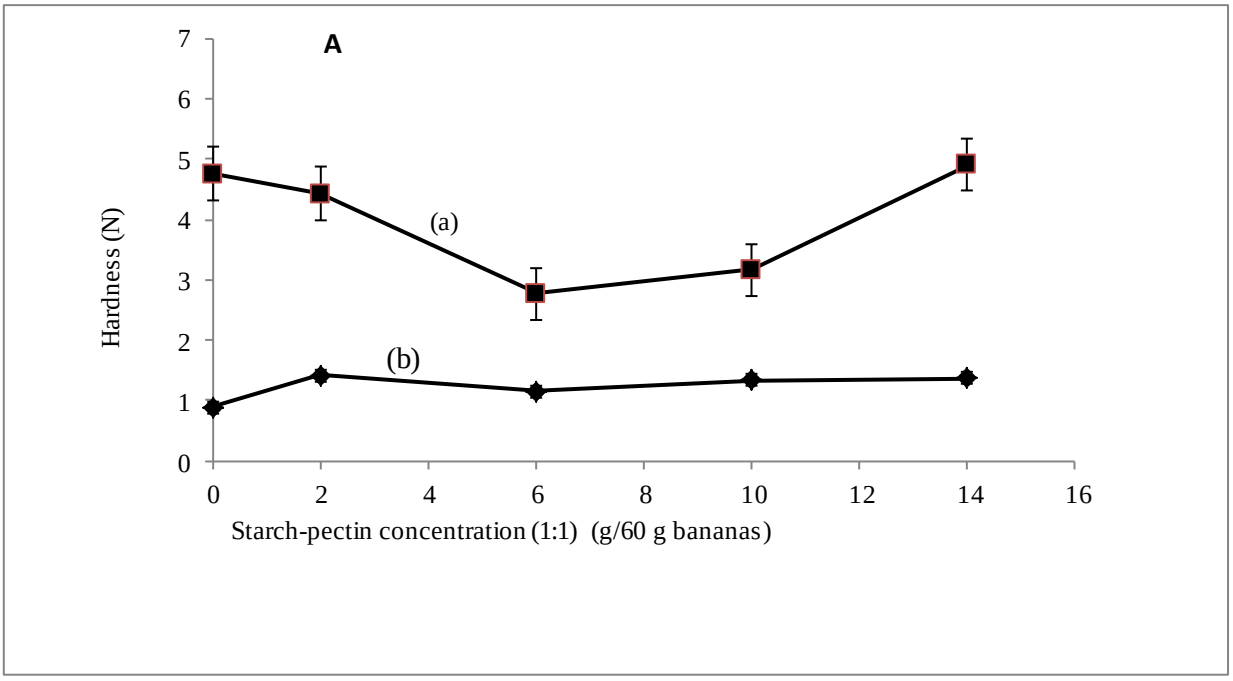
Results have shown that starch-treated bananas were significantly harder than the control ($P<0.05$) upon cooking and cooling and hardness increased with increasing starch concentration. In general, these results imply that starch increases hardness in cooked bananas. Despite the high Water Binding Capacity (WBC) particularly during heating, starch has a low Water Holding Capacity (WHC) which tends to reduce the water activity (aw) of foods (Homayouni et al., 2013). This property coupled with increased dry matter content possibly enhances increased hardness during cooking of bananas. Upon cooling, there was a general increase in hardness of starch-treated bananas and the control which could be due to starch retrogradation (Wang and Copeland, 2013). Retrogradation of starch involves re-association of gelatinized starch (amylose) molecules to form an ordered crystalline structure (Eliasson & Wahlgren, 2004) associated with increased solidity and hence hardness. These results are in agreement with findings by Liu et al.(2013) who found a significant increase in hardness of Surimi gels ($P<0.05$) prepared with added corn starch. Some studies on manufacture of noodles have found that use of starch increases hardness and cohesiveness (Kim et al., 1996; Singh et al., 2002; Toyama et al., 1997).

There was a high correlation between cohesiveness of cooked bananas and starch concentration implying that starch strongly increases cohesiveness. This may be attributed to the adhesiveness of gelatinized starch being higher in samples with more starch (Sozer et al., 2007). Upon cooling, cohesiveness was observed to increase with starch concentration although with a reduced coefficient of determination of 54% implying less adhesiveness of starch as the bananas cooled. Upon cooling, starch loses water (or its gelatinized state) leading to retrogradation and this increases fragility of cooked bananas. Beyond 8.3% added starch, there was a sudden decrease in cohesiveness (Figure 18 B, curve a). These results are in agreement with findings by Kim et al. (1996) and Singh et al. (2002) who found noodles made from starch to be more cohesive.

4.2.3.3 Effect of starch-pectin composite on hardness

Upon cooking, hardness of starch-pectin composite treated bananas (1.42 and 1.15 N) was slightly higher than the control (0.89 N) but not significantly different ($P > 0.05$) (Figure 19 A, curve b and Table 25 appendix 1). Hardness was 1.42 N at 3.33% composite treatment which was significantly higher than the control ($P<0.05$) and decreased to 1.15 N at 10% composite but then increased to 1.33 and 1.37 N at 16.6 and 23.4% composite, respectively. Upon cooling, hardness generally increased for the control and starch-pectin composite treated bananas due to starch retrogradation (Figure 19 A, curve (a) and Table 25 appendix 1). Hardness of the control (4.76 N) was generally higher than that of the composite treated bananas (4.43 to 2.77 N) but not significantly different ($P > 0.05$). Hardness decreased from 4.43 at 3.33% to 2.77 N at 10% composite but then rose to 3.16 N at 16.6% and finally to 4.91 N at 23.4% composite.

Cohesiveness of the starch-pectin composite treated bananas (1.09 to 1.38 N) was higher than that of the control (0.86 N) upon cooking but was not significantly different ($P>0.05$) (Figure 19 B, curve b) and Table 25 appendix 1). Cohesiveness decreased from 1.30 at 3.33% to 1.09 N at 10% composite and then increased to 1.26 and 1.38 N at 16.6 and 23.4% composite, respectively. Upon cooling, cohesiveness of the control (2.83 N) and that of the composite-treated bananas (2.13 to 3.76 N) generally increased due to starch retrogradation (Figure 19 B, curve (a) and Table 25 appendix 1). Cohesiveness at 3.33% composite was 2.80 which decreased to 2.13 N at 10% composite but rose up to 2.47 and 3.76 N at 16.6 and 23.4% composite, respectively.



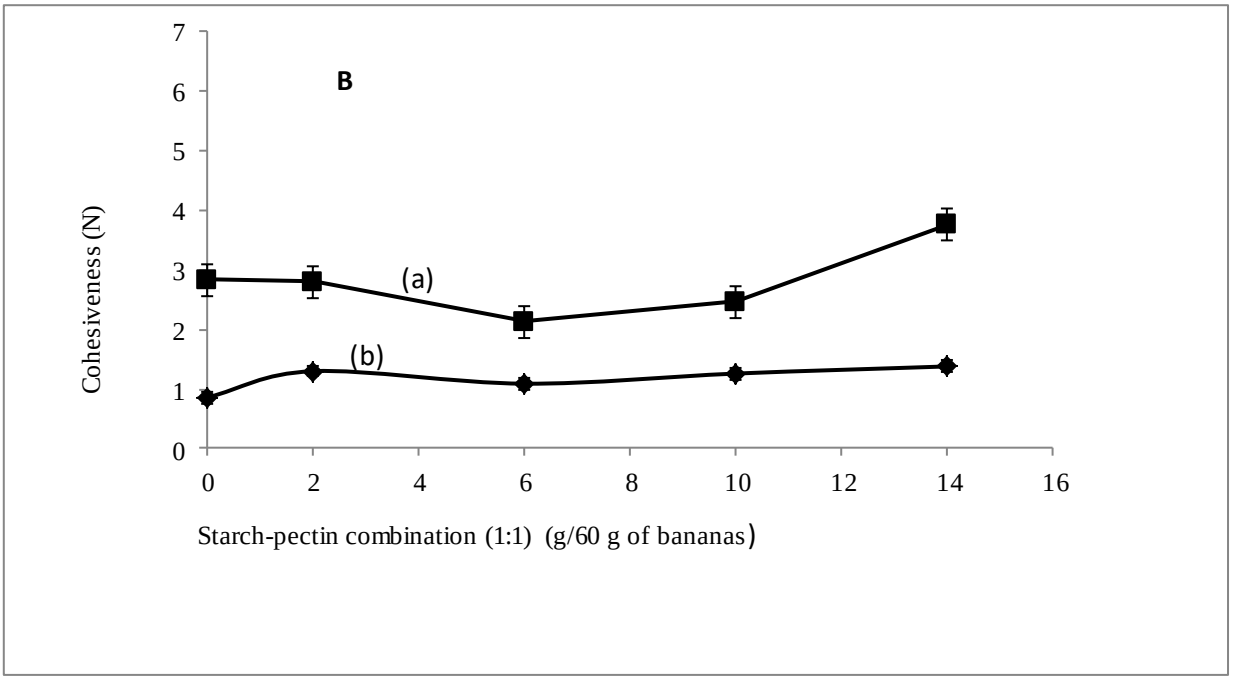


Figure 19: Effect of added starch-pectin composite on (A) hardness and (B) cohesiveness of cooking bananas (case of NAKT) upon cooking (curve b) and cooling (curve a)

The trend of these results was similar to that observed for pectin-treated bananas. The decrease in hardness between 0 and 10% of the composite during cooling could be attributed to the high

water binding and holding capacity of pectin making the bananas softer. The high water holding capacity of pectin ensures that pectin remains gelatinized long enough to cause a considerable proportion of starch to remain gelatinized thus reducing the extent of starch retrogradation and firming. Pectin could also have had a diluting effect on starch by occupying the intermolecular spaces between starch molecules. This keeps starch molecules apart and possibly prevents extensive association of the starch molecules which could cause formation of a crystalline starch structure. However, as the concentration of starch-pectin composite increased beyond 10%, hardness started increasing. This could be due to increased competition for water between starch and pectin where water becomes significantly depleted resulting in pectin forming lumps while starch begins to form a crystalline structure thus increasing hardness.

The increase in hardness upon cooling of the starch-pectin composite treated bananas was also accompanied by an increase in cohesiveness. Generally, cohesiveness of starch-pectin composite treated bananas was higher than the control and decreased initially but then increased with increasing composite concentration upon cooking. The increase in cohesiveness relative to the control could be attributed to increased adhesiveness of starch and pectin upon gelatinization.

4.2.3.4 Effect of pectinase treatment on hardness

The experiment was conducted within recommended optimum temperature and pH range for PG catalytic activity which vary between 45 and 55^{o}C and 4.4 to 7.2, respectively depending on the enzyme source and environment (Duvetter et al., 2009).

Elimination of pectin from the structural matrix of bananas significantly increased hardness ($P<0.05$) during cooking and upon cooling. Results indicated that pectinase-treated cooking bananas (KAZ) were significantly harder than the control ($P<0.05$) during cooking and upon cooling (Figure 20 A and Table 26 appendix 1). For juice bananas (KAY), hardness of pectinase treated bananas was significantly higher than the control ($P<0.05$) at lower cooking time but became gradually indifferent as cooking time increased (Figure 21 A, curve a) and Table 27 appendix 1). In general, during cooking, hardness of pectinase-treated cooking bananas decreased with cooking time from 1.82 to 1.23 N relative to that of the control which fluctuated between 0.56 and 0.61 N. Hardness of juice bananas decreased from 3.49 to 1.80 N

relative to the control which decreased from 1.78 to 1.57 during cooking. Upon cooling, hardness of all pectinase-treated bananas and the controls increased significantly ($P<0.05$) probably due to starch retrogradation (Table 14). Upon cooling, all the pectinase-treated cooking and juice bananas were all significantly harder than their respective controls ($P<0.05$). Hardness of cooled pectinase-treated cooking bananas was 6.62 N while that of its corresponding control was 3.62 N while hardness of cooled pectinase-treated juice bananas was 15.36 N and that of its corresponding control was 12.34 N.

Cohesiveness of pectinase-treated cooking bananas was significantly higher than that of the control and decreased from 1.47 to 1.10 N while that of the control decreased from 0.61 to 0.57 N during cooking (Figure 20 B and Table 26 appendix 1). Cohesiveness of the pectinase-treated juice bananas decreased from 2.47 to 1.26 N while that of the control decreased from 1.47 to 1.23 N (Figure 21 B and Table 27 appendix 1) and was not significantly different ($P>0.05$). Upon cooling, cohesiveness of the pectinase-treated bananas and that of the control generally increased due to starch retrogradation. Cohesiveness of the cooled pectinase-treated cooking bananas was 5.12 N which was significantly higher than 2.63 N of the control (Table 14). Cohesiveness of cooled pectinase-treated juice bananas was 6.83 N which was not significantly different from 6.50 N of the control (Table 14).

Polygalacturonase (PG) eliminates pectin by hydrolysis through cleaving the α-1, 4-D galacturonan linkages in the pectin homogalacturonan chain preferring the non-esterified substrate with decreasing activity as the degree of methoxylation increases (Duvetter et al., 2009). According to these authors, ExoPG attacks the chain from the non-reducing end and removes terminally (1→)-linked GalA residues.

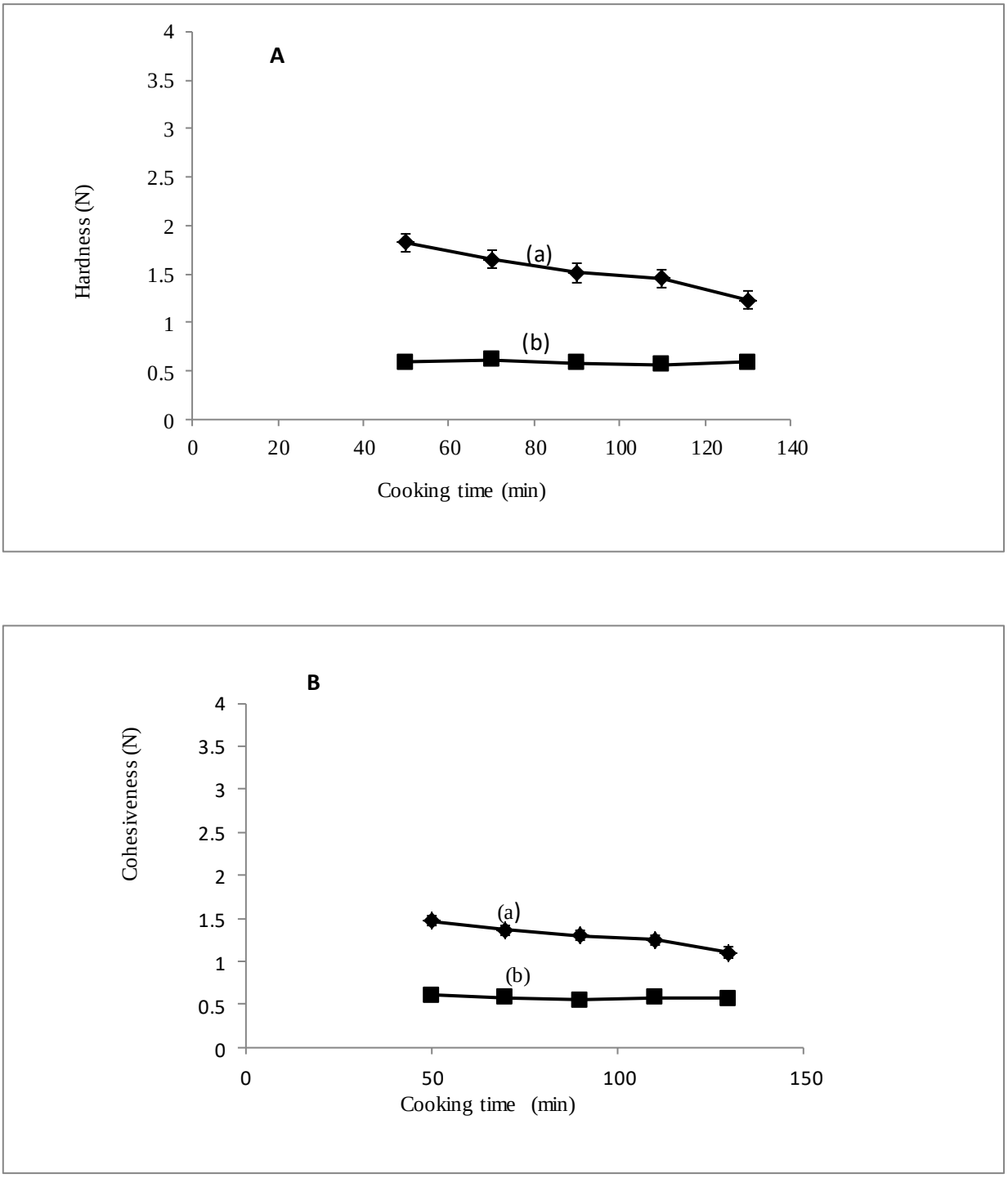
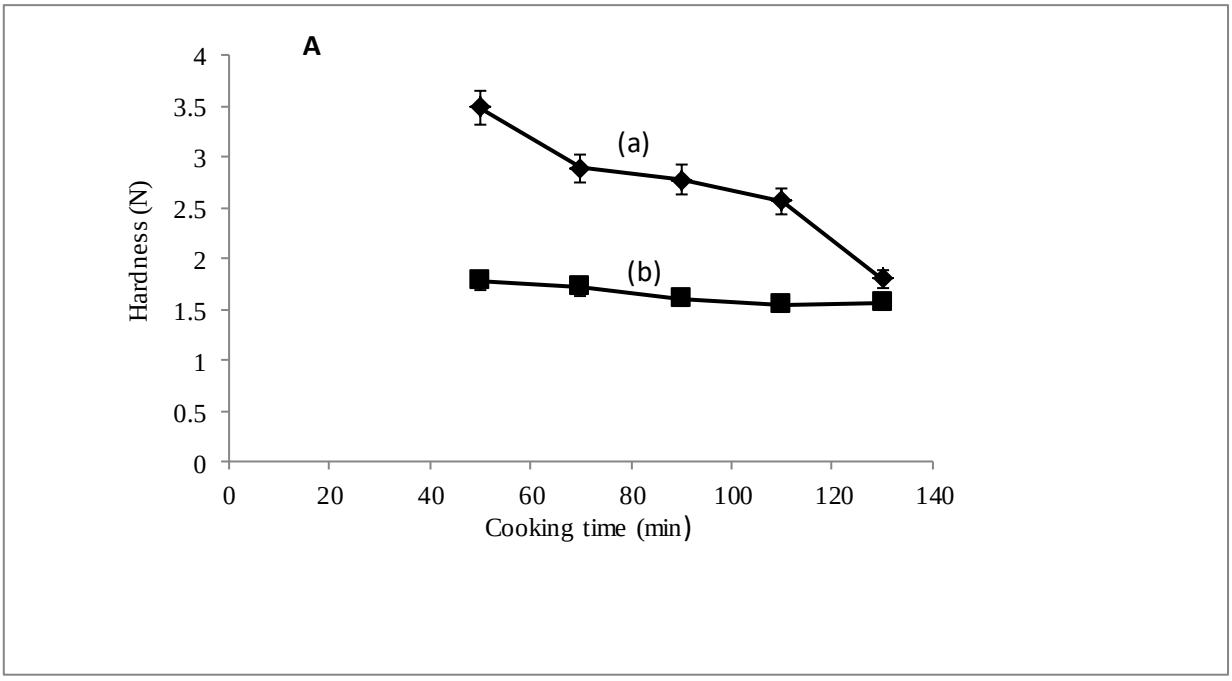


Figure 20: Effect of structural elimination of pectin on (A) hardness and (B) cohesiveness of bananas (case of cooking bananas i.e. KAZ) during cooking. Curves (a) pectinase-treated bananas (b) control



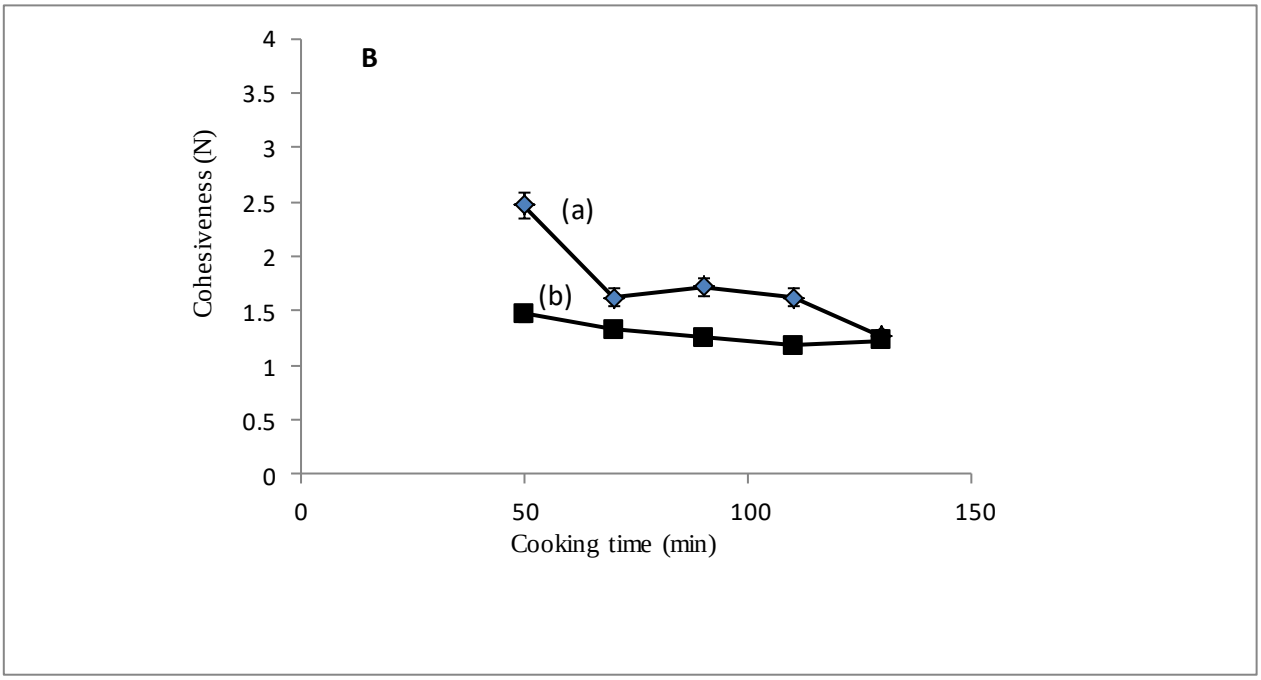


Figure 21: Effect of structural elimination of pectin on (A) hardness and (B) cohesiveness of bananas (case of juice bananas i.e. KAY) during cooking. Curves (a) pectinase-treated bananas and (b) control

Table 14: Effect of structural elimination of pectin on hardness of cooked bananas in cooled form

Treatment	Texture			
	Hardness (N)		Cohesiveness (N)	
	Treated	Control	Treated	Control
Cooking bananas				
Cooked for 130 min	1.23±0.27[a]	0.59±0.07[b]	1.10±0.21[a]	0.57±0.09[b]
After cooling (4 h)	6.63±1.59[a]	3.62 ± 0.59[b]	5.12±0.56[a]	2.63±0.15[b]
Juice bananas				
Cooked for 130 min	1.80±0.21[b]	1.57±0.10[b]	1.26±0.31[d]	1.23±0.11[d]
After cooling (4 h)	15.36±a	12.34±0.88[b]	6.83±1.45[d]	6.50±0.13[d]

Values with same superscript letters along the same row under the same subheading are not significantly different (P>0.05).

The elimination of pectin from the structural matrix of bananas using pectinase (polygalacturonase) provided an opportunity to understand the role of pectin in influencing hardness of cooked bananas. All bananas where pectin was hydrolyzed were harder than the controls upon cooking and cooling implying that hydrolysis of pectin leaves starch and cellulose/hemicellulose as the main structural elements that profoundly affect texture. This reduces the water holding capacity or water activity (Homayouni et al., 2013) of bananas which in turn reduces the extent of starch gelatinization during cooking thus leading to increased hardness.

The reduced water activity in the treated bananas was evident during sample preparation where enzyme-treated bananas appeared dryer than the corresponding controls. Pectinase treated juice bananas were harder than the pectinase treated cooking bananas. This could be because juice bananas contain more starch than cooking bananas as already found out in this work. Despite pectinase treated bananas being harder in all cases, hardness reduced with cooking time. The decrease in hardness with cooking time could be due to the increasing degree of starch gelatinization as more starch takes up moisture during cooking. These findings re-enforce the

results on effect of added pectin on hardness of cooked bananas where it was found to decrease hardness of cooked bananas.

Results indicated a significant increase in cohesiveness of bananas from which pectin was hydrolyzed which decreased with cooking time while that of the control remained more or less constant. This implies that pectin lowers cohesiveness relative to starch. This increase could be attributed to the relatively reduced water activity in the bananas. Increased cohesiveness of bananas would probably improve their processability as the product becomes more tolerant of manufacturing, packaging and delivery stresses. This enhances presentation of the product to the consumers in its expected state (Texture technologies Corp. and Stable Micro Systems, Ltd, 2018). However, the increased hardness found in this study would make the product undesirable to consumers who prefer tender banana products.

4.2.4 Conclusions and recommendations

Results have shown that starch increases hardness of cooked bananas implying that it could be partly responsible for differences in hardness between different banana cultivars. On the other hand, pectin has been found to significantly decrease hardness of cooked bananas particularly upon cooling implying that it is responsible for tenderness in cooked bananas. Structural hydrolysis and elimination of pectin from the banana matrix using pectinase also significantly increased hardness of bananas during cooking and upon cooling confirming that pectin is responsible for tenderness in cooked bananas.

Therefore, based on these results, pectin could be added to sliced bananas at an optimized level (between 1 and 5%) to enhance tenderness of cooked bananas. Furthermore, controlled breakdown of starch molecules in sliced bananas using beta-amylase could decrease molecular size distribution of starch molecules in bananas and hence hardness. Similarly, both starch and pectin have been observed to increase cohesiveness during cooking while upon cooling, pectin appears to decrease cohesiveness and starch increases it. This implies that starch is important in maintaining product structural integrity and hence could promote marketability of banana products.

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CHAPTER 5

GENERAL DISCUSSION

5.1 Introduction

Global production and utilization of bananas is increasing according to FAOSTAT data. International trade in bananas is majorly for the desert type while the cooking types are mainly consumed in the producing countries. Uganda is the leading producer and consumer of the East African Highland cooking bananas (EA-AAA). Cooking bananas are Uganda's important staple food crop eaten by 70% of the population every day (CropLife International, 2018). According to Promusa (2018), more than 75% of all farmers grow bananas. Ugandans eat as much as 1 kg of bananas a day, mainly cooking bananas that have been domesticated in the Great Lakes region for more than 2000 years. According to the 2008/2009 Uganda National census 68% of the banana crop is produced in the western region, followed by 23% in the central region, 8% in the eastern region and 1% in the northern region (Uganda Bureau of Statistics (UBOS), 2010). This demonstrates the importance of bananas based on the regions of Uganda. Botanical remains found in Uganda and dated at more than 2000 years before the Christian era suggest that bananas were introduced in East Africa earlier than previously thought (Lejju et al., 2006; Lejju et al., 2005).

Despite the high production and consumption volumes, processing of cooking bananas into shelf-stable products remains a challenge. Hardening of cooked bananas upon cooling as established in this study could limit the shelf-life and therefore consumption of cooked bananas. Due to the hardening phenomenon, cooked bananas lose taste and aroma upon cooling and hence their desirability among consumers. This leads to discard of banana meals as waste translating into food and monetary losses. This problem is serious among banana consumers who rely on cash flows to access food particularly in cities and major towns of Uganda.

Therefore, understanding the composition of bananas, the physical and chemical properties of starch and pectin as major structural and textural components, profiling hardness of the bananas including studying the role played by both starch and pectin in cooked bananas were the core of this study. This study used Uganda's indigenous banana cultivars as case studies since they

had not been well researched. This was critical in laying a foundation for future studies and therefore solving the hardening phenomenon commonly observed in cooked bananas.

5.1 Composition of bananas

Compositional analysis of bananas revealed that water, starch and pectic substances were the major components of bananas. Examination of cooking and juice banana cultivars revealed that juice bananas had more starch, pectic extracts and dry matter content but lower moisture content than cooking bananas. The starch yield was found to range from 4 to 25.7% wet basis being predominantly higher in juice bananas than in cooking bananas. The presence of starch as a main component of bananas besides water is supported by various authors such as Zhang et al., (2005); Gaurav & Manoj (2014).

This study used two types of bananas i.e. the cooking and the juice banana cultivars in order to compare the composition and texture of the generally harder juice banana cultivars with the generally softer cooking banana cultivars. Comparision was done on all components of bananas analyzed as well as some physical and chemical properties of the two major components (starch and pectin). Since consumers generally consume bananas when they are fresh and unripe, use of mature but unripe bananas was critical in this study. Any major differences in the composition of bananas in the unripe form were used as major factors contributing to the differences in texture and hence important elements in the determination of which components influence hardness of bananas particularly when cooked.

Other components such as crude ash, fibre, protein and fat did not vary significantly between cooking and juice banana cultivars implying that their influence on textural differences between the two banana cultivars could be minor. For instance, crude ash ranged between 1.97 and 4.24%; crude fibre ranged between 0.25 and 0.48%; crude protein ranged between 1.27 and 5.64 while fat ranged between 0.15 and 0.58% with no significant differences between cooking and juice banana cultivars ($P>0.05$). Egbebi and Bademosi (2012) examined unripe plantain flour and obtained the following values i.e. 2.8% crude protein, 3.8% crude ash, 0.2% crude fibre while crude fat was 0.7%. According to these authors, chemical composition of plantains varies in proportion to maturity. The results of this study are within range of Egbebi and Bademosi's values on composition of bananas. According to results of crude protein content, juice banana cultivars were found to be on the lower side relative to cooking bananas. Since crude protein, ash, fibre and fat were not significantly different between cooking and juice

banana cultivars, it was concluded that they may not affect potential differences in hardness between these two banana cultivars.

The presence of more starch, pectic substances and dry matter content in juice bananas than in cooking bananas could be justified by their general differences in texture notable during cooking applications. The generally softer cooking bananas had more moisture which is involved in hydration of starch and pectin thus causing gelatinization during thermal processing. More moiture also means that molecules in that food are more mobile, which can alter food structure and microstructure, crystallisation, rates of diffusion and chemical and biochemical reactions (Slade & Levine, 1991) relative to foods in which there is little moisture. More moisture causes greater hydration and consequent weakening of the structural network (Pereira, Bennett, Hemar, & Campanella, 2001). The revrese is true of foods with more dry matter. This could explain why cooking banana cultivars with more moisture are much softer than juice banana cultivars.

5.2 Physical and chemical characteristics of banana starch and pectin

Investigation into the physico-chemical characteristics of banana starch from both cooking and juice banana cultivars revealed that there were no major differences between starch from the two banana cultivar groups. Amylose generally varied between 21.5 and 36.4% with no significant difference between juice and cooking banana cultivars. In contrast, the amylose content of all juice banana cultivars that were sampled was in the upper range between 28 and 36.4% relative to cooking banana cultivars. Resistant starch which is said to correlate positively with amylose content (Yoon et al., 2013) also varied between 36.2 and 65% with no significant difference between juice and cooking banana cultivars. Despite this, resistant starch content of juice bananas was also in the upper range between 52 and 65%. Simple correlation performed between resistant starch and amylose content of the studied bananas was only 14% implying a very negligible relationship between the two properties. Resistant starch is structurally complex and is resistant to in-vitro hydrolysis by amylase treatment (Englyst et al., 1992). It is mainly composed of the linear part of starch or amylose with α-(1–4) D-glucan units (Otten et al., 2006). Resistance of starch to digestion could also be positively correlated to hardness of cooked bananas. A simple correlation analysis performed between resistant starch content and hardness of raw bananas indicated a positive correlation of 20% which was too low. This could have been attributed to the fact that the samples used for both hardness and composition

analysis were harvested at different times, hence the variation. Resistance to digestion is related to i) the dense molecular configuration which restricts accessibility of digestive enzymes, like amylase (Haralampu, 2000) including heat penetration; and ii) the starch granules themselves are protected by botanical cell wall which inhibits the digestive enzymes to break them down (Nugent, 2005). Whereas RS has health benefits in humans (Garg et al., 2017), the dense molecular configuration of this molecule makes it inaccessible to not only enzymes but also water (particulalry during heating) to cause gelatinization and this may confer added hardness to the bananas particularly in juice bananas where starch occurs most.

Some authors have reported a positive correlation between hardness and amylose content. For instance, Rolando et al. (2004) found a positive linear correlation between amylose content and hardness of rice. It is now well known that rice with high amylose content provides dry and fluffy textures while low amylose rice gives moist, chewy and clingy textures after cooking (Mir et al., 2013). Generally, high-amylose rice varieties give high hardness, high tensile strength, and high consistency (Lu et al., 2009). However, a correlational analysis done between amylose content and hardness of raw bananas indicated correlation coefficient of 37% with a coefficient of determination of 14% implying that the influence of amylose on hardness of bananas is low.

Other characteristics such as pasting properties were not significantly different ($P>0.05$) between cooking and juice banana cultivars. In particular, there were no major differernces in swelling power and solubility of starch between cooking and juice banana cultivars ($P>0.05$). However, it was evident that all the banana starch was very stable at low temperatures ($<70^{o}C$) which reflects the stable nature of this molecule particularly when bananas are still intact in their natural form i.e. in the fresh unripe banana pulp. The gelatinization temperature of 78 to $80^{o}C$ appears to be very high for bananas. This implies that in intact unripe fresh bananas, it is difficult for water to penetrate the intact structure in order to cause starch gelatinization and hence softening. This could mean that little starch attains gelatinization temperature under normal cooking temperatures and time hence leaving a high percentage of ungelatinized starch in the bananas particularly in juice cultivars which have a harder texture. This means that prolonged cooking at boiling temperature can allow more time at high temperatures for water to penetrate deep into the banana structural matrix and cause starch gelatinization. Because of the limited gelatinization, it is possible that starch in cooked bananas could also have a high

propensity to retrograde upon cooling. This phenomenon may be more complex in juice bananas whose pulp structure is normally harder hence limiting penetration of water into the banana structure and hence the starch structure.

Setback viscosity informs about the tendency of starch (amylose) to undergo retrogradation (Wang et al., 2015). In this study, there was no significant difference between setback viscosities of cooking and juice banana starch ($P>0.05$). The high tendency of starch towards retrogradation would imply that starch in bananas would increase firmness upon cooling. However, the results of this work show that the behavior of isolated starch (when it's withdrawn from its natural environment) could be different from that of starch that is still intact within the banana pulp structural matrix.

In general terms, results of this study imply that differences in hardness usually observed between juice and cooking banana cultivars could partly be due to differences in the quantity of starch.

On the other hand, in living tissues, pectic substances serve to cement tissues and cells firmly together. Their breakdown loosens the tissue's coherence leading to softening as experienced in ripe bananas and other fruits (Misoon & Abu-Bakr 2005). In general, the chemical properties of pectic substances extracted from juice and cooking banana cultivars did not differ significantly ($P>0.05$). The pectic substances were all found to have high equivalent weight ranging from 8156 to 16444 implying high reactivity, and hence could undergo cross-linking reactions through polyol functional groups (Alan, 1984). All other chemical properties of the pectic substances from both cooking and juice banana cultivars did not differ significantly ($P>0.05$) i.e. methoxyl content (5.8 to 10.6%); Anhydrouronic acid (37.5 to 62.7%); and degree of esterification (88 to 96%). The lack of significant difference in chemical properties between pectic substances from juice and cooking banana cultivars implies that these substances behave in the same way and have the same effects regardless of the banana cultivar.

In general, all pectic substances extracted from juice and cooking banana cultivars had DE above 94% with the exception of one banana cultivar (NAMD) whose pectic substances had a DE of 88%, an indication of over maturity. Presence of high DE (94 to 96%) in banana pectin implies presence of protopectin in green mature unripe bananas. The presence of protopectin (which is insoluble and also highly reactive due to the high equivalent weight) in green mature

unripe bananas implies that this could affect texture making bananas harder due to possible extensive crosslinking with other molecules such as cellulose and hemicellulose. Based on experience, green mature unripe bananas are generally harder in texture than ripe ones. The relative insolubility of protopectin could be due to its existence as a polygalacturonide in which the hydroxyl groups on carbon atoms C4 and C5 are masked by glycosidic and ring formations, and the carboxyl group on C1 is either free, esterified with methyl alcohol, or esterified with araban, galactan, or some other polysaccharide (Joslyn, 1963). According to this author, the hydroxyl groups on carbon atoms C2 and C3 may be free, esterified with acetyl groups, or linked by ether-like linkage to polysaccharides or lignins. The degree of acetylation is a factor in determining the extractability of pectin and its combination with other cell-wall constituents (Joslyn, 1963). The water insoluble protopectin occurs only in the cell wall, lying closest and fused to the cellulose layer. Any existing free pectin merges into the protopectin layer without a sharp line of division and in a similar manner the protopectin merges into the cellulose layer in the cell wall (Zykwinska, Thibault, & Ralet, 2008). This has a lot of implications for the yield of pectic substances obtained in this report. The yields were very high between 7 and 29% being significantly higher in juice bananas than in cooking bananas ($P<0.05$). This also implies that the extracted pectic substances could have contained a lot other substances particularly cellulose and hemicellulose which are normal constituents of protopectin, hence increasing the extraction yields. It is therefore possible that protopectin being insoluble could increase hardness of cooked bananas upon cooling. This is in sharp contrast to the presence of soluble pectin which encourages a softer texture in cooked bananas as results in Chapter 4.2 have revealed.

5.3 Hardness of selected banana cultivars

Results of hardness revealed that juice banana cultivars were generally harder than cooking banana cultivars in raw, cooked and cooled forms regardless of the cooking treatment applied. In raw form, hardness of juice bananas ranged between 36.17 N and 42.43 N (average 38.5 N) whereas hardness of cooking bananas ranged between 22.37 N and 26.36 N (average 24.17 N). This gives an average difference of 14.33 N in hardness between cooking and juice banana cultivars which is very high. This clearly shows that juice banana cultivars are harder than cooking banana cultivars. Upon cooking, a similar trend was observed where juice bananas were significantly harder than cooking bananas ($P<0.05$). Extreme hardness is one of the

reasons why juice bananas are not cooked for food. Food for human consumption should taste good with a good aroma but most importantly it should have a favorable texture, which is not too soft and not too hard, one that brings enjoyment to the consumer. Texture has been cited as one of the most important attributes used by consumers in selecting bananas for consumption (Qi et al., 2000). Factors like starch, pectin and dry matter content affect texture in foods. Dry matter content affects hardness of food stuffs; the higher the dry matter content, the harder or firmer the food item. Ferrisa et al. (1999) in their study to evaluate the fruit quality of plantains, plantain hybrids and cooking bananas found that plantains which had high dry matter content were associated with higher hardness occurring at some level initially during growth. These authors argued that pulp texture is an important food quality characteristic for plantains and cooking bananas particularly in West Africa where specific fruit textures are preferred for particular dishes. Bananas meant for frying should not be too soft as they may absorb excess oil that may be undesirable to the consumers. Bananas for cooking are desired when they are soft upon cooking and remain relatively soft upon cooling. The texture of the East African Highland cooking bananas becomes soft upon cooking but hardens immediately upon cooling making the texture undesirable to consumers. Ferrisa et al. (1999) suggested that fruit dry matter content may be correlated with fruit quality and storage life. This implies that the high dry matter content exhibited by juice banana cultivars used in this study could be of advantage particularly in flour and juice production.

5.4 Influence of starch and pectin on hardness of bananas

The effect of added starch and pectin on hardness of bananas has been examined in this study as discussed in Chapter 4.2. It has been found that hardness of cooked bananas increased with increasing added starch concentration. Simple regression analysis performed indicated a very strong regression coefficient of 99% between hardness and starch concentration. In general, cooked bananas treated with starch were 1.3 times (130%) harder than pectin treated bananas. Similarly, hardness of cooled bananas increased with starch concentration and also exhibited a strong positive regression coefficient of 91%. Furthermore, hardness of cooled starch-treated bananas was 1.89 times (189%) harder than the cooled pectin-treated bananas implying that starch has a stronger influence on increasing hardness more than pectin. Starch being the main dry matter component of bananas implies that hardness exhibited by juice bananas relative to cooking bananas could be due to presence of high starch content. The means by which starch

increases hardness could be explained in several ways. Starch itself is an insoluble molecule which means it exists in a solid or semi-solid form. This solidity increases hardness. Upon cooking, starch gelatinizes and subsequently causes softening (Pither, 2003). However, as more starch is added, there is increasing hardness among bananas upon cooking which may possibly be due to increased competition for water hence less gelatinization and increasing solidity. In contrast, cooling of bananas causes the constituent starch to undergo retrogradation during which it loses moisture and becomes highly crystalline (Wang, 2015). This change from the amorphous partially gelatinized form to the highly crystalline structure causes hardening in cooled bananas. The more starch added to the banana pulp, the more hardness cooked bananas exhibited. In general, the texture of cooked bananas was found to harden significantly upon cooling and was more serious in the first one hour of cooling. It has been argued that starch has a high water binding capacity upon heating but similarly it has a comparatively high propensity to lose it upon cooling and this may be important in promoting hardness during cooling of cooked bananas.

In contrast, pectin has been found to decrease hardness of cooked bananas particularly during cooling. Although added pectin was found to cause a slight increase in hardness of bananas during cooking, the low regression coefficient of 42% and coefficient of determination of 18.3% mean that only 18% of the observed increase in hardness can be explained by added pectin. This increase in hardness could partly be due to increase in dry matter content as well as adhesiveness or cohesiveness of pectin. Upon cooling, added pectin exhibited a strong negative regression coefficient (89%) with hardness of cooked bananas which means pectin favors tenderness of cooked bananas. These results are in agreement with findings by Alvarez & Canet (2001) who suggested that pectic substances are intimately involved in changes in firmness and play a much more important role in softening of tissues. Alvarez and Canet (2001) suggested that the chemistry of pectin during processing is complex and is responsible for physical changes that are dependent on the degree of pectin depolymerization and de-esterification, which determines whether the tissue becomes softer or firmer. The extent of tissue softening differs widely during processing by different methods.

It is important to note that the method of processing can influence hardness. Steaming (as applied in this study) does not provide adequate moisture to the tissues to cause sufficient

gelatinization of starch which may explain why steamed bananas exhibited a harder texture than boiled bananas. This becomes more evident upon cooling when steamed bananas (not mashed) become relatively much harder.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

First and foremost, on dry basis, bananas are dominated by carbohydrates ranging from 90 to 96% with the highest percentage being starch. On fresh weight (wet) basis, green mature bananas contain more moisture, starch and pectic substances. In general, juice bananas contain more starch, pectic substances and therefore more dry matter than cooking bananas implying that composition of bananas varies depending on cultivar and these compositional differences could influence differences in hardness exhibited between cooking and juice banana cultivars. However, the physico-chemical properties of starch and pectin derived from these two banana cultivars do not differ significantly implying that the properties of banana starch and pectin may not have significant influence on differences in hardness between cooking and juice banana cultivars. This means that differences in hardness between the two banana cultivars could be due to differences in dry matter (starch and pectic substance) content.

Based on current results, juice banana cultivars are significantly harder than cooking banana cultivars either in raw, cooked or cooled forms regardless of cooking method or treatment. However, hardness reduces with cooking time implying that bananas should be cooked a bit longer to enhance tenderness depending on the cultivar and heating source. This study has also proven that when bananas are cooked at green maturity and allowed to cool, they undergo significant hardening of the texture which is rapid in the first one hour of cooling. This implies that cooked bananas should be served and consumed when still hot to avoid the hardening phenomenon that occurs in cooked bananas due to starch retrogradation. Among cooking treatments, boiling produces the softest bananas followed by “steaming with mashing” and lastly “steaming without mashing”.

Further investigations into the role of starch and pectin on hardness of cooked bananas have confirmed that i) starch increases hardness of cooked bananas implying that starch could be the major factor responsible for the observed hardness in cooked bananas and therefore responsible for differences in hardness observed between different banana cultivars; ii) Pectin on the other hand decreases hardness in cooked bananas and is generally responsible for tenderness in cooked bananas as was confirmed by structural hydrolysis.

6.2 Recommendations

According to observations made in this study, consumers should boil bananas for a softer texture. Alternatively, steamed bananas should be mashed in order to achieve a much tender texture as traditionally done in local banana eating communities. Steaming combined with mashing should be done for at least 70 min in order to attain a much softer cooked banana texture. Extended cooking beyond 70 min (as locally done for 2 - 3 h in banana consuming communities) could increase the degree of softness of the banana meal due to increased starch gelatinization. Cooked bananas should be served hot and once served, should be consumed as fast as possible while still hot to avoid the textural hardening phenomenon observed in this study.

Industrial processors of bananas should first of all undertake a study to find out tastes and preferences of banana consumers. Based on results of such a study, pectin could be added and optimized at levels between 1 and 5% in order to reduce hardening of texture that occurs upon cooling of cooked bananas. This will produce a much softer mashed banana meal. Alternatively, banana processors could also undertake a study to perform controlled partial breakdown of starch in bananas using β-amylase in order to reduce the size of the starch molecules and study the textural changes in the cooked bananas with the view to reduce hardness of cooked bananas particularly upon cooling. This should be aimed at optimizing tenderness of cooked bananas.

6.3 Areas for further investigation

Further studies could focus on investigating the molecular size/weight distribution of starch in both cooking and juice banana cultivars. This could provide further information on the causes of differences in hardness between cooking and juice banana cultivars.

Further studies should also examine the role of protopectin on the texture of unripe mature bananas. In the same way, the molecular size distribution of protopectin and pectin molecules in cooking and juice bananas should be investigated in order to reveal their structural profiles and how this could impact on texture. This could be useful in further explaining the differences in hardness observed between different banana cultivars.

Last but not least, a further study should be conducted on the influence of sap (tannins) on the water activity in bananas and its role on the development behavior of banana fruits, their starch and finally its effect on hardness of raw and cooked bananas.

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Appendix 1: Tables of results

Table 15: Swelling power (%) of starch from selected cooking and juice banana cultivars at different temperatures

Banana cultivar	Treatment temperature											
	40°C	**45°C**	**50°C**	**55oC**	**60°C**	**65°C**	**70°C**	**75°C**	**80°C**	**85°C**	**90°C**	**98.6°C**
Cooking bananas												
NAMD	85.8	75	70	84.2	109.2	129.2	168.3	714	912	937	1027	1068^{ab}
NAKW	77.3	75.8	70	95.8	173.3	99.2	180	794	897	996	1127	1206^{abc}
NAMZ	63.3	75.8	71.7	77.5	107.5	127.5	165.8	656	771	943	1200	1418^{bcd}
NAKT	80.8	77.5	66.7	80	107.5	134.2	166.7	558	751	896	1098	1014^{a}
NAKY	70	83.3	66.7	78.3	103.3	135	150.8	628	768	983	1212	1554^{cd}
KIB	70	77.5	79.2	81.7	108.3	143.3	230.8	643	708	1012	1079	1501^{cd}
MUS	69.2	69.2	70	63.3	78.3	87.5	186.7	525	719	967	1116	1378^{abcd}
MPO	81.7	78.3	70.8	82.5	94.2	102.5	196.7	713	706	976	1071	1386^{abcd}
KIS	70	76.7	69.2	77.5	80	105.8	175.8	623	660	816	1008	1300^{abcd}
KAZ	80	90.8	78.3	93.3	91.7	141.7	207.5	832	826	995	1148	1658^{d}
NAKB	72.5	78.3	76.7	82.5	93.3	103.3	191.7	578	878	991	1170	1337^{abcd}
Juice bananas												
KISB	72.5	76.7	78.3	80	81.7	114.2	161.7	441	768	823	929	1040^{ab}
NDI	90	80.8	90	77.5	99.2	148.3	165.8	520	784	838	1080	1243^{abc}
KAY	72.5	71.7	72.5	75.8	101.7	103.3	147.5	445	745	848	1103	1353^{abcd}

Values with the same superscript along the last column are not significantly different (P>0.05).
Values are means of three independent replicates ± standard errors of the means

Table 16: Solubility mg/ml) of starch from selected cooking and juice banana cultivars at different temperatures

Banana cultivar	Treatment temperature									
	40°C	**45°C**	**50°C**	**55°C**	**60°C**	**70°C**	**80°C**	**85°C**	**90°C**	**98.6°C**
Cooking bananas										
NAMD	0.08	0.19	0.32	1.25	1.3	0.77	0.93	1.7	2.1	5.40[a]
NAKW	0.03	0.21	0.12	1.14	1.2	1.1	1.3	1.3	5.2	8.20[abc]
NAMZ	0.08	0.14	0.08	0.97	1.2	1.5	1.6	2.1	3.5	10.3[bcd]
NAKT	0.08	0.48	0.35	1.62	1.4	1.9	1.7	2.8	5.6	9.0 [abcd]
NAKY	0.09	0.39	0.57	1.33	1.4	1.7	1.9	3.4	6.2	13.30[d]
KIB	0.15	0.17	0.05	1.15	1.2	1.7	0.77	1.7	7.0	13.50[d]
MUS	0.25	0.19	0.42	1.17	1.2	1.2	0.87	5.3	7.0	13.50[d]
MPO	0.14	0.16	0.72	1.23	1.3	1.1	0.64	4.2	5.7	12.0[cd]
KIS	0.18	0.09	0.87	1.03	1.2	0.9	0.95	2.1	6.6	9.30[abcd]
KAZ	0.20	0.17	0.63	1.07	1.1	1.4	1.0	1.4	5.4	13.50[d]
NAKB	0.27	0.19	0.42	1.15	1.2	1.5	1.0	1.4	7.9	11.10[cd]
Juice bananas										
KISB	0.12	0.10	0.35	0.89	1.0	1.5	1.9	2.1	5.8	6.40[ab]
NDI	0.16	0.10	0.04	1.12	1.2	1.5	2.6	2.8	7.1	10.40[abcd]
KAY	0.08	0.05	0.08	0.18	0.6	1.2	1.6	1.8	8.6	9.60[bcd]

Values with the same superscript along the last column are not significantly different (P>0.05). Values are means of three independent replicates ± standard errors of the means

Table 17: Changes in hardness selected cooking and juice banana cultivars during boiling

	Cooking treatment						
	Boiling						
Cooking time (min)	0	30	50	70	90	110	130
NAMD	23.81^{abc}	0.64_{f}^{f}	0.62_{f}^{f}	0.36_{f}^{f}	0.43_{f}^{f}	0.35_{f}^{f}	0.38_{g}^{f}
NAKW	22.81^{ab}	0.69_{f}^{f}	0.47_{f}^{f}	0.47_{f}^{f}	0.41_{f}^{f}	0.34_{f}^{f}	0.35_{g}^{f}
NAMZ	22.80^{ab}	0.5_{f}^{f}	0.7_{f}^{f}	0.52_{f}^{f}	0.39_{f}^{f}	0.33_{f}^{f}	0.37_{g}^{f}
NAKT	25.40^{abc}	1.08_{f}^{g}	0.94_{f}^{fg}	0.77_{f}^{fg}	0.65_{f}^{fg}	0.62_{f}^{fg}	0.56_{g}^{f}
NAKY	22.53^{ab}	0.65_{f}^{f}	0.5_{f}^{f}	0.45_{f}^{f}	0.42_{f}^{f}	0.42_{f}^{f}	0.35_{g}^{f}
KIB	22.37^{a}	0.56_{f}^{f}	0.5_{f}^{f}	0.43_{f}^{f}	0.42_{f}^{f}	0.38_{f}^{f}	0.34_{f}^{f}
MUS	25.10^{abc}	1.1_{f}^{g}	0.99_{f}^{fg}	0.64_{f}^{f}	0.55_{f}^{f}	0.54_{f}^{f}	0.52_{g}^{f}
MPO	23.65^{abc}	0.68_{f}^{f}	0.64_{f}^{f}	0.58_{f}^{f}	0.54_{f}^{f}	0.43_{f}^{f}	0.35_{g}^{f}
KIS	26.00^{bc}	1.11_{f}^{f}	1.07_{f}^{f}	0.87_{f}^{f}	0.69_{f}^{f}	0.73_{g}^{f}	0.54_{g}^{f}
NAKB	26.36^{bc}	1.47_{f}^{h}	1.14_{f}^{gh}	0.83_{f}^{fg}	0.82_{g}^{fg}	0.69_{g}^{fg}	0.51_{g}^{f}
KAZ	25.14^{abc}	1.67_{f}^{h}	1.13_{f}^{gh}	1.03_{f}^{gh}	0.98_{g}^{gh}	0.89_{g}^{g}	0.68_{g}^{g}
KISB	36.17^{d}	6.58_{g}^{k}	4.82_{g}^{jk}	3.77_{g}^{ij}	3.49_{h}^{ij}	2.73_{h}^{i}	2.67_{h}^{i}
NDI	42.43^{e}	6.29_{g}^{k}	4.14_{g}^{jk}	2.79_{g}^{ij}	1.74_{g}^{i}	1.49_{g}^{i}	1.38_{g}^{i}
KAY	36.86^{d}	8.89_{h}^{m}	6.79_{h}^{kl}	6.5_{h}^{kl}	6.22_{i}^{kl}	4.97_{i}^{jk}	4.9_{i}^{jk}

Values with different superscripts along rows or subscripts along columns are significantly different.

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Table 18: Changes in hardness of selected cooking and juice banana cultivars during steaming

	Cooking treatment						
	Steaming						
Cooking time (min)	0	30	50	70	90	110	130
Cooking banana cultivars							
NAMD	23.81^{abc}	1.52_{fg}^{f}	1.18_{f}^{fg}	0.99_{f}^{fg}	0.95_{f}^{g}	0.92_{f}^{g}	0.78_{f}^{g}
NAKW	22.81^{ab}	1.82_{fg}^{f}	1.19_{f}^{fg}	0.97_{f}^{g}	0.91_{f}^{g}	0.85_{f}^{g}	0.61_{f}^{g}
NAMZ	22.80^{ab}	2.73_{fg}^{f}	1.1_{f}^{g}	1.05_{f}^{g}	1.04_{f}^{g}	0.92_{f}^{g}	0.74_{f}^{g}
NAKT	25.40^{abc}	1.48_{fg}^{f}	1.18_{f}^{fg}	0.98_{f}^{fg}	0.89_{f}^{g}	0.94_{f}^{fg}	0.76_{f}^{g}
NAKY	22.53^{ab}	1.82_{fg}^{f}	1.26_{f}^{fg}	1.03_{f}^{g}	0.97_{f}^{g}	0.95_{f}^{g}	0.8_{f}^{g}
KIB	22.37^{a}	1.34_{f}^{f}	1.05_{f}^{fg}	0.72_{f}^{gh}	0.70_{f}^{gh}	0.64_{f}^{gh}	0.60_{f}^{g}
MUS	25.10^{abc}	3.95_{g}^{f}	1.29_{f}^{g}	1.13_{f}^{gh}	0.74_{f}^{h}	0.73_{f}^{gh}	0.72_{f}^{g}
MPO	23.65^{abc}	1.85_{fg}^{f}	1.1_{f}^{g}	1.05_{f}^{g}	0.72_{f}^{g}	0.71_{f}^{g}	0.6_{f}^{g}
KIS	26.00^{bc}	3.14_{g}^{f}	1.82_{f}^{g}	1.13_{f}^{g}	1.10_{f}^{g}	1.04_{f}^{g}	0.97_{f}^{g}
NAKB	26.36^{bc}	3.67_{g}^{f}	1.65_{f}^{g}	1.36_{fg}^{g}	1.27_{f}^{g}	1.19_{f}^{g}	1.05_{f}^{g}
KAZ	25.14^{abc}	2.5_{fg}^{f}	1.76_{f}^{fg}	1.47_{fg}^{g}	1.44_{f}^{g}	1.45_{f}^{g}	1.24_{f}^{g}
Juice banana cultivars							
KISB	36.17^{d}	6.93_{h}^{f}	4.8_{f}^{fg}	$3.97_{g}^{g}\pm$	3.77_{g}^{g}	3.18_{g}^{g}	2.95_{g}
NDI	42.43^{e}	9.91_{i}^{f}	4.58_{f}^{g}	$4.13_{g}^{g}\pm$	3.00_{g}^{g}	2.91_{g}^{g}	2.67_{g}^{g}
KAY	36.86^{d}	13.3_{j}^{f}	10.0_{f}^{fg}	8.19_{h}^{gh}	6.40_{h}^{gh}	$6.5_{h}^{gh}8$	5.29_{h}^{i}

Values with different superscripts along rows or subscripts along columns are significantly different.

Table 19: Changes in hardness of selected cooking and juice banana cultivars subjected to "mashing with steaming"

	Cooking treatment						
	"Steaming combined with mashing"						
Cooking time (min)	0	30	50	70	90	110	130
Cooking banana cultivars							
NAMD	23.81^{abc}	---	0.76_{f}^{f}	0.74_{f}^{f}	0.70_{f}^{f}	0.69_{f}^{f}	0.64_{f}^{f}
NAKW	22.81^{ab}	---	0.62_{f}^{f}	0.59_{f}^{f}	0.49_{f}^{f}	0.47_{f}^{f}	0.52_{f}^{f}
NAMZ	22.80^{ab}	---	0.76_{f}^{f}	0.62_{f}^{fg}	0.60_{f}^{g}	0.58_{f}^{g}	0.56_{f}^{g}
NAKT	25.40^{abc}	---	0.98_{fg}^{f}	0.64_{f}^{g}	0.60_{f}^{g}	0.58_{f}^{g}	0.60_{f}^{g}
NAKY	22.53^{ab}	---	0.71_{f}^{f}	0.67_{f}^{f}	0.64_{f}^{f}	0.64_{f}^{f}	0.59_{f}^{f}
KIB	22.37^{a}	---	0.60_{f}^{f}	0.54_{f}^{fg}	0.44_{f}^{fg}	0.45_{f}^{fg}	0.42_{f}^{g}
MUS	25.10^{abc}	---	0.71_{f}^{f}	0.63_{f}^{f}	0.65_{f}^{f}	0.61_{f}^{f}	0.48_{f}^{f}
MPO	23.65^{abc}	---	0.54_{f}^{f}	0.57_{f}^{f}	0.53_{f}^{f}	0.51_{f}^{f}	0.5_{f}^{f}
KIS	26.00^{bc}	---	0.74_{f}^{f}	0.52_{f}^{f}	0.55_{f}^{f}	0.55_{f}^{f}	0.54_{f}^{f}
NAKB	26.36^{bc}	---	0.77_{f}^{f}	0.69_{f}^{f}	0.65_{f}^{f}	0.61_{f}^{f}	0.61_{f}^{f}
KAZ	25.14^{abc}	---	1.17_{fg}^{f}	0.96_{f}^{f}	0.86_{f}^{f}	0.85_{f}^{f}	0.88_{f}^{f}
Juice banana cultivars							
KISB	36.17^{d}	---	2.69_{fg}^{f}	2.82_{g}^{f}	2.61_{g}^{f}	2.14_{g}^{f}	2.12_{g}^{f}
NDI	42.43^{e}	---	4.41_{h}^{f}	2.54_{g}^{g}	2.74_{g}^{g}	2.59_{g}^{g}	2.24_{g}^{g}
KAY	36.86^{d}	---	4.96_{h}^{f}	4.34_{h}^{f}	4.17_{h}^{f}	4.34_{h}^{f}	4.3_{h}^{f}

Values with different superscripts along rows or subscripts along columns are significantly different.

Table 20: Effect of cooling on hardness of boiled bananas

Banana cultivar	BOILED BANANAS	
	Hardness (N) at 130 min boiling	**Hardness (N) when readily cooled (4h)**
Cooking bananas		
NAMD	0.38 ± 0.10^{a}	1.95 ± 1.19^{v}
NAKW	0.35 ± 0.11^{a}	2.16 ± 0.86^{v}
NAMZ	0.37 ± 0.10^{a}	2.19 ± 0.51^{v}
NAKT	0.56 ± 0.30^{a}	2.16 ± 1.52^{v}
NAKY	0.35 ± 0.12^{a}	1.49 ± 0.36^{v}
KIB	0.34 ± 0.20^{a}	1.04 ± 0.17^{v}
MUS	0.52 ± 0.15^{a}	2.85 ± 0.43^{v}
MPO	0.35 ± 0.12^{a}	3.61 ± 0.31^{v}
KIS	0.54 ± 0.22^{a}	2.51 ± 0.50^{v}
NAKB	0.51 ± 0.16^{a}	4.02 ± 1.48^{v}
KAZ	0.68 ± 0.10^{a}	3.88 ± 1.19^{v}
Juice bananas		
KISB	2.67 ± 0.49^{a}	9.36 ± 1.28^{v}
NDI	1.38 ± 0.67^{a}	9.54 ± 1.33^{v}
KAY	4.90 ± 0.83^{a}	12.65 ± 1.60^{v}

Data is compared along rows: Values with different superscripts along the same row are significantly different ($P<0.05$). S.P = Spoiled sample

Table 21: Effect of cooling on hardness of steamed bananas

Banana cultivar	STEAMED BANANAS	
	Hardness (N) at 130min steaming	Hardness (N) after cooling (4h)
Cooking bananas		
NAMD	0.78± 0.17 ^{a}	4.77± 0.95 ^{b}
NAKW	0.61± 0.11 ^{a}	3.82± 0.47 ^{b}
NAMZ	0.74± 0.15^{a}	5.31± 1.07 ^{b}
NAKT	0.76 ± 0.19 ^{a}	4.13± 0.49 ^{b}
NAKY	0.80± 0.09 ^{a}	4.48± 0.62 ^{b}
KIB	0.60± 0.12 ^{a}	2.97± 0.50 ^{b}
MUS	0.72± 0.15 ^{a}	4.05± 0.46 ^{b}
MPO	0.60± 0.06^{a}	4.11± 1.32 ^{b}
KIS	0.97± 0.30^{a}	3.44± 1.02 ^{b}
NAKB	1.05± 0.14^{a}	5.12± 0.63 ^{b}
KAZ	1.24±0.15^{a}	5.39± 1.23 ^{b}
Juice bananas		
KISB	2.95± 0.49 ^{a}	15.85±2.23 ^{b}
NDI	2.67± 0.24 ^{a}	18.22± 3.73 ^{b}
KAY	5.29± 0.56 ^{a}	18.33± 1.66 ^{b}

Data is compared along rows: Values with different superscripts along the same row are significantly different ($P<0.05$). S.P = Spoiled sample

Table 22: Effect of cooling on hardness of bananas "mashed with steaming"

Banana cultivar	MASHED BANANAS	
	Hardness (N) at 130min steaming	**Hardness (N) after cooling (4 h)**
Cooking bananas		
NAMD	0.70± 0.16 ^{a}	6.89± 0.53 ^{b}
NAKW	0.52± 0.03 ^{a}	5.22± 0.39 ^{b}
NAMZ	0.58± 0.09 ^{a}	5.99± 0.34 ^{b}
NAKT	0.60± 0.12 ^{a}	s.p
NAKY	0.59± 0.07 ^{a}	5.09± 0.62 ^{b}
KIB	0.49± 0.05 ^{a}	3.84± 0.36 ^{b}
MUS	0.48± 0.07 ^{a}	6.15± 0.62 ^{b}
MPO	0.50± 0.06 ^{a}	5.62± 1.97 ^{b}
KIS	0.54± 0.12 ^{a}	5.62±0.77 ^{b}
NAKB	0.61± 0.06 ^{a}	4.79± 0.30 ^{b}
KAZ	0.88± 0.22 ^{a}	6.14± 0.63 ^{b}
Juice bananas		
KISB	2.61± 0.34 ^{a}	14.41± 1.24 ^{b}
NDI	2.24± 0.20 ^{a}	20.44± 0.98 ^{b}
KAY	4.30± 0.56 ^{a}	19.15± 1.07 ^{b}

Data is compared along rows: Values with different superscripts along the same row are significantly different ($P<0.05$). S.P = Spoiled sample

Table 23: Effect of added pectin on hardness and cohesiveness of bananas upon cooking and cooling

Treatment	Texture			
Pectin conc. (g/60 g bananas)	**Cooked bananas**		**Cooled bananas**	
	Hardness (N)	**Cohesiveness (N)**	**Hardness (N)**	**Cohesiveness (N)**
0 (0%)	0.93±0.17^{a}	0.84±0.12^{a}	5.31±0.28^{b}	3.02±0.04^{b}
1 (1.7%)	1.04±0.19^{a}	1.01±0.19^{ab}	4.50±0.31^{b}	2.99±0.28^{b}
3 (5%)	1.24±0.10^{a}	1.27±0.07^{bc}	2.20±0.51^{a}	2.02±0.15^{a}
5 (8.3%)	1.14±0.16^{a}	1.22±0.14^{bc}	1.87±0.19^{a}	1.67±0.04^{a}
7 (11.7%)	1.06±0.29^{a}	1.34±0.11^{c}	1.92±0.69^{a}	1.81±0.32^{a}

Values with same superscript letters under the same subheading along the same column are not significantly different (P>0.05).

Table 24: Effect of added starch on hardness and cohesiveness of bananas upon cooking and cooling

Treatment	Texture			
Starch conc. (g/60 g bananas)	**Cooked bananas**		**Cooled bananas**	
	Hardness (N)	**Cohesiveness (N)**	**Hardness (N)**	**Cohesiveness (N)**
0	0.86±0.09^{a}	0.84±0.05^{a}	4.04±0.61^{a}	2.34±0.63^{a}
1 (1.7%)	1.05±0.14^{ab}	0.91±0.12^{a}	4.52±0.48^{a}	3.20±0.19^{ab}
3 (5%)	1.30±0.21^{ab}	1.11±0.14^{ab}	4.48±0.63^{a}	3.75±0.46^{bc}
5 (8.3%)	1.52±0.21^{bc}	1.26±0.14^{bc}	4.84±0.88^{ab}	4.49±0.29^{c}
7 (11.7%)	1.97±0.46^{c}	1.56±0.24^{c}	5.99±0.59^{b}	3.65±1.05^{bc}

Values with same superscript letters under the same subheading along the same column are not significantly different (P>0.05).

Table 25: Effect of starch-pectin composite on hardness of bananas upon cooking and cooling

Treatment		Texture			
Starch-pectin composite (1:1 ratio) (g)	**% composition**	**Cooked**		**Cooled**	
		Hardness (N)	**Cohesiveness (N)**	**Hardness (N)**	**Cohesiveness (N)**
0 + 0	0%	0.89 ± 0.13^{a}	0.86 ± 0.13^{a}	4.76 ± 0.17^{a}	2.83 ± 0.41^{ab}
1.7 + 1.7	3.33%	1.42 ± 0.11^{b}	1.30 ± 0.12^{ab}	4.43 ± 0.17^{a}	2.80 ± 0.46^{ab}
3 + 3	10%	1.15 ± 0.16^{ab}	1.09 ± 0.33^{ab}	2.77 ± 0.11^{a}	2.13 ± 0.33^{a}
5 + 5	16.6%	1.33 ± 0.31^{ab}	1.26 ± 0.16^{ab}	3.16 ± 1.22^{a}	2.47 ± 0.44^{a}
7 + 7	23.4%	1.37 ± 0.31^{ab}	1.38 ± 0.24^{c}	4.91 ± 2.41^{a}	3.76 ± 0.61^{b}

Values with same superscript letters under the same subheading along the same column are not significantly different (P>0.05).

Table 26: Effect of structural elimination of pectin on hardness and cohesiveness of bananas (the case of cooking bananas i.e. KAZ) during cooking and cooling

Treatment	Texture of cooked bananas			
Cooking time (min)	**Hardness (N)**		**Cohesiveness (N)**	
	Treated	**Control**	**Treated**	**Control**
50	1.82 ± 0.10^{a}	0.59 ± 0.07^{b}	1.47 ± 0.18^{a}	0.61 ± 0.09^{b}
70	1.65 ± 0.12^{a}	0.61 ± 0.04^{b}	1.36 ± 0.17^{a}	0.58 ± 0.02^{b}
90	1.51 ± 0.21^{a}	0.58 ± 0.06^{b}	1.30 ± 0.08^{a}	0.55 ± 0.05^{b}
110	1.45 ± 0.26^{a}	0.56 ± 0.04^{b}	1.25 ± 0.10^{a}	0.58 ± 0.06^{b}
130	1.23 ± 0.27^{a}	0.59 ± 0.07^{b}	1.10 ± 0.21^{a}	0.57 ± 0.09^{b}
Cooling time (min)	**Cooled bananas**			
240	6.63 ± 1.59^{a}	3.62 ± 0.59^{b}	5.12 ± 0.56^{a}	2.63 ± 0.15^{b}

Values with same superscript letters under the same subheading (Hardness or Cohesiveness) along the same row are not significantly different (P>0.05).

Table 27: Effect of structural elimination of pectin on hardness and cohesiveness of bananas (the case of juice bananas i.e. KAY) during cooking and upon cooling

Treatment	Texture of cooked bananas			
Cooking time (min)	Hardness (N)		Cohesiveness (N)	
	Treated	Control	Treated	Control
50	3.49±1.14^{a}	1.78±0.27^{b}	2.47±0.62^{c}	1.47±0.44^{d}
70	2.89±0.71^{ab}	1.72±0.41^{b}	1.62±0.21^{d}	1.33±0.25^{d}
90	2.78±0.35^{ab}	1.61±0.82^{b}	1.72±0.27^{cd}	1.25±0.15^{d}
110	2.57±0.55^{ab}	1.55±0.40^{b}	1.62±0.43^{d}	1.18±0.17^{d}
130	1.80±0.21^{b}	1.57±0.10^{b}	1.26±0.31^{d}	1.23±0.11^{d}
Cooling time (min)	**Cooled bananas**			
240	15.36±a	12.34±0.88^{b}	6.83±1.45^{d}	6.50±0.13^{d}

Values with same superscript letters under the same subheading (Hardness or Cohesiveness) along the same row are not significantly different (P>0.05).

Appendix 2: Standard curves

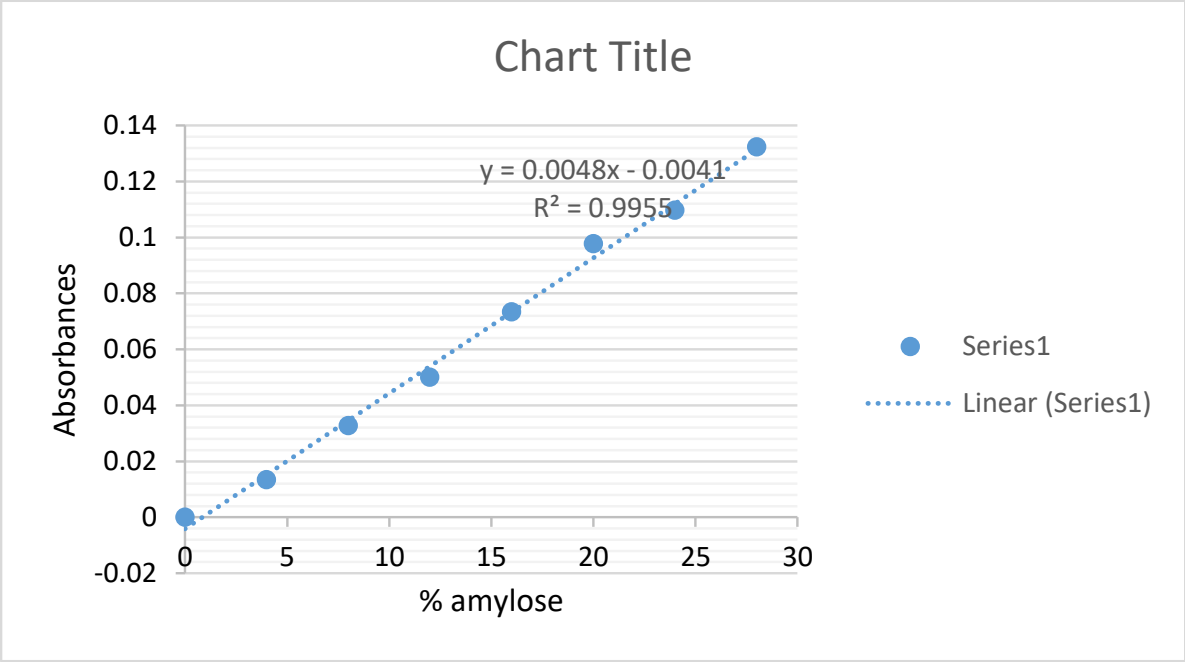

Figure 22: Standard curve for determination of amylose content of starch from selected cooking and juice banana cultivars

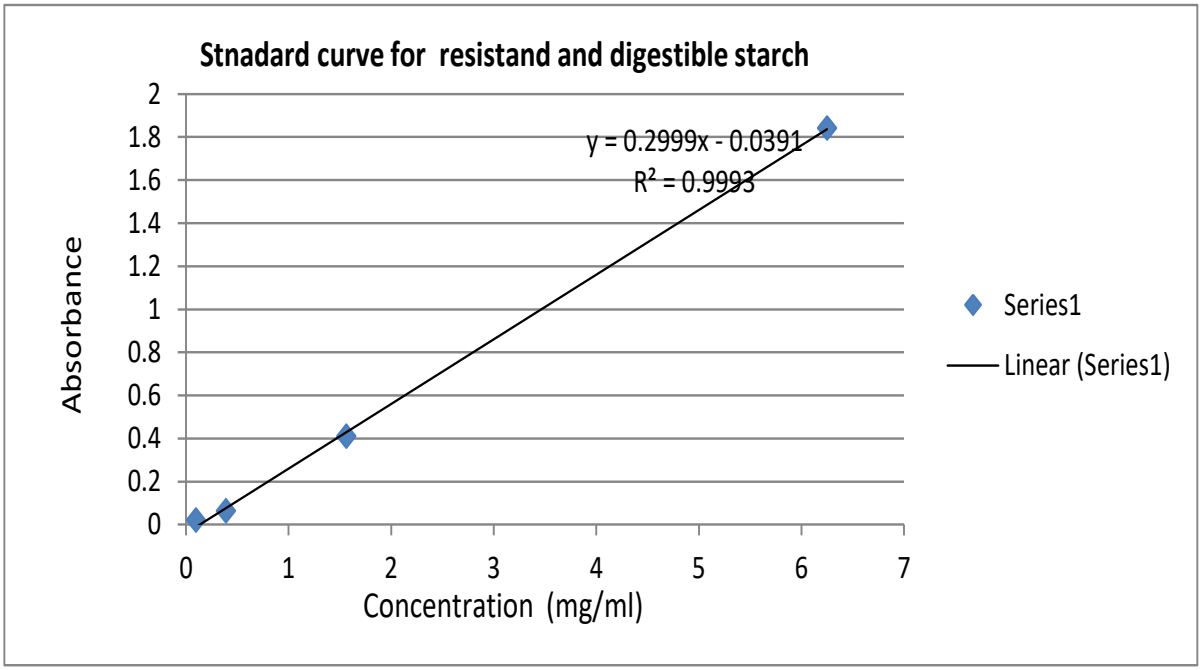

Figure 23: Standard curve for determination of resistant and digestible starch composition of starch from selected cooking and juice banana cultivars

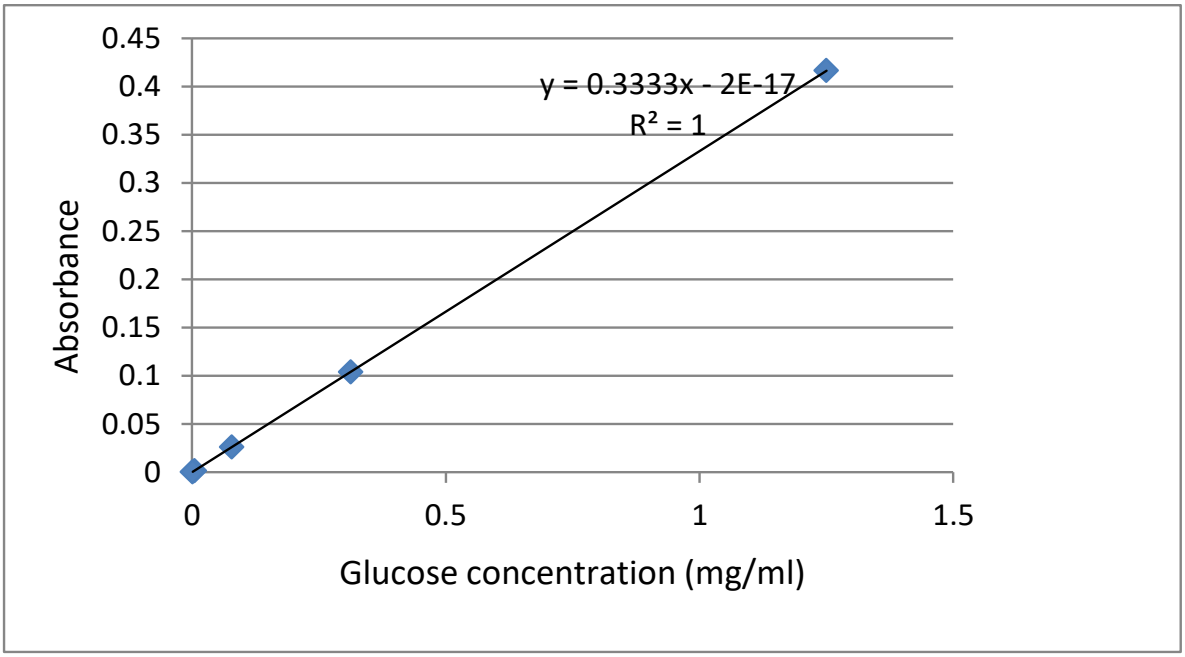

Figure 24: Standard curve for determination of solubility of starch from selected cooking and juice banana cultivars