Resistant starch in plantain (*Musa AAB*) and Implications for the glycaemic index

Ebun-Oluwa Peace Oladele

Submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds

School of Food Science and Nutrition

July, 2013

The candidate confirms that the work submitted is her own and appropriate credit has been given within the thesis where reference has been made to the work of others.

Dedication

This thesis is dedicated

To the almighty God, the creator of the heavens and the earth, the one who was, who is, and who is to come. The owner of life and the custodian of knowledge. To you be the glory now and forevermore.

And

To my father, Mr Gabriel Oluwadare Ojokan (of blessed memory), it is painful you are not alive to witness this day despite your desires, prayers, advice and struggle to get me to the top in life. I miss you dearly.

Acknowledgements

I would like to express my profound gratitude to my supervisor, Professor Gary Williamson, who saw in me the potential to succeed despite the ordeal I had gone through at the initial stages of this learning process. Your advice, corrections, enthusiasm, dedication and an eye for details were really helpful not only for this thesis but for my career in the academia. I honestly wish I had your professional guide right from the beginning.

I am indebted to the Commonwealth Scholarship Commission in the United Kingdom and my employers, the Federal University of Technology, Akure, Nigeria, for providing me the funding this work. Special thanks go to Prof A. A. Oshodi for his care and fatherly counsel all through the work. I am equally grateful to Late Prof K. O. Ipinmoroti, Prof O. O. Ajayi, Prof I. A. Amao, Prof A.V. Popoola and Dr J. O. Olukunle all of the Federal University of Technology, Akure. A grateful acknowledgement is also made to my first supervisor Dr Santosh Khokhar and to Dr Kale Girish of the Department of Materials Engineering, University of Leeds. I cannot but appreciate Prof Mike Morgan, the Head of the School of Food Science and Nutrition, Ms Angela Morrinson and Mrs Catherine Roberts for their intervention at a critical point in this endeavour. My appreciation also goes to other members of the administrative office, Mrs. Nicola Bhuller and Ms. Debbie who were always willing to help with administrative matters. Sincere thanks goes to all my colleagues, Stephania, Regiane, Kayleigh, Samantha, Joana, Islamiyat, Hanis, Maryam, Nicolai, Wafa, Fatima, Idolo, Hilda, etc for the times we shared together in the laboratory, computer clusters, office and in other areas of this great adventure. Special thanks go to Sarah, my only partner in the

available carbohydrates/glycaemic index journey, to Dr Tristan Dew for trainings in the use of the laboratory equipments and to Dr. Caroline Orfila for her advice on sugar analysis. Special thanks also go to all members of the Food technology laboratory, Dr. Mel Holmes, Mr. Ian Hardy and Jemma Levantiz.

Many thanks to my mum, for her unending prayers, love and support, sweet mother how can I ever forget you; and to my sisters and their families, I thank you for the emotional support, the nickname you all gave me, 'Prof', is gradually coming to reality, all glory to God. I am grateful to all members of the Redeemed Christian Church of God (RCCG) at home and abroad, especially to members of RCCG, everlasting father's assembly and victory centre, Leeds, and my home parishes RCCG Tabernacle of His refuge and heaven's glory. I also acknowledge the prayers and concern of Pastor and Pastor (Mrs) Julius Olalekan, Pastor and Pastor (Mrs) Julius Adetoro, Pastor and Mrs Adewale Aribatise, Deacon Steve Amuda, Mr and Mrs Adewale Samuel, Deacon and Mrs Bolaji Cole and Mrs Nlewedim, to mention a few. You are all appreciated for your sacrifice of love.

Finally, my deepest thanks and appreciation go to my husband, Mr Ademola Oladele, for his love and support throughout the period of this study. You encouraged me to pursue this PhD abroad and to keep moving on and stay afloat even in the deepest oceans and troubled waters. I appreciate and love you today and always. Lastly, my heartfelt appreciation goes to my special gifts from God, my lovely children, Victor, Emmanuel and Justus, for their understanding, love, obedience and the ability to cope in times I needed to focus more on this work than on them. You are all rare gems.

Abstract

Consumption of foods rich in resistant starch and slowly digestible starches has been associated with lower postprandial glycaemic responses. The need to identify and quantify resistant starch in potential resistant starch sources and optimise processing conditions to maximise their benefit is vital in the quest for more healthy diets required for the control and management of diabetes and related conditions.

Significant quantities of three types of resistant starch were identified in plantains: these are physically entrapped starch (RS1), native resistant starch (RS2) and retrograded starch (RS3). However, the relative quantities of each type vary with the conditions/state of processing/storage conditions applied to the food before consumption. The high correlation ($r^2 = 0.8$) obtained between increased total resistant starch content of plantain products and reduced glycaemic index suggests that factors which promote the formation of enzyme resistant starch in plantain can also influence the glycaemic response to the available carbohydrates.

Apart from native resistant starch which has been commonly reported for the *Musa ssp*, our data suggest that some alcohol extractable components of plantain may act as enzyme inhibitors. The presence of these components resulted in an increase in the value of RS2 in flours when compared to the starch isolates. It may be necessary therefore to distinguish between resistant starch type 2 (RS2), which is due to the inherent nature of starch and

resistant starch produced from the interference from other food components such as enzyme inhibitors. This type of resistant starch may not be present in all foods and its properties need to be further investigated.

Contents

List of figures

List of tables

Abbreviations

AUC	Area under curve
AV CHO	Available carbohydrate
BUP	Boiled unripe plantain
BUPC	Boiled unripe plantain crisps
CON A	Cocanavalin A
DSC	Differential scanning calorimetry
DWB	Dry weight basis
G20	Starch digested within 20 min *in vitro*
G120	Starch digested between 20 and 120 min *in vitro*
GAE	Garlic acid equivalent
GI	Glycaemic index
GOPOD	Glucose oxidase/peroxidase
IAUC	Incremental area under curve
IDSD	*In vitro* degree of starch digestion
Min	Minutes
RDS	Rapidly digestible starch
RPF	Ripe plantain flour
RPS	Ripe plantain starch
RRP	Ripe raw plantain
RS	Resistant starch
RS1	Physically trapped resistant starch
RS2	Native resistant starch

RS3	Retrograded resistant starch
SDS	Slowly digestible starch
SI	Satiety index
TEMP	Temperature
TG	Total glucose
TS	Total starch
URPF	Unripe plantain flour
URPS	Unripe plantain starch
USP	United States pharmacopeia
XRD	X-ray diffraction

Chapter 1 Literature review

1.1 Plantain and banana

Plantains and bananas belong to the *Musa* genus of the plant kingdom and are amongst the world's major fruit crops with a worldwide production of 103 million tonnes in 2004, consisting of 71 million tonnes of dessert bananas and 32 million tonnes of plantains (Aurore *et al*., 2009). They are also classified as fruit-bearing herbs and are grown abundantly in the tropical humid regions of the world where they also serve as an important part of the diet. In the developing countries, plantain and banana are the fourth most important food crops after maize, wheat and rice and are amongst the cheapest food crops to produce.

From the two wild ancestors, *Musa acuminata* COLLA and *Musa balbisiana* COLLA, descended the wide spread fruit banana, *Musa sapientum* and the starch banana, *Musa paradisiaca*, also called plantain. Plantains are characterised by a higher starch concentration than bananas and the cooking banana (the cooking banana which descended from *Musa acuminata* is botanically different from the plantain). While cooking bananas and plantains are a major staple food in Africa, the cooking bananas are mainly produced in Uganda, East Africa (Simmonds, 1987). Most of the existing cultivars are hybrids and polypoids based on two wild diploid species *Musa acuminata* and *Musa balbisiana*. The main genome groups are AA, AB, AAA, AAB and ABB (Robinson and Sauco, 2010).

Plantain or plantain banana as it is sometimes called is not as popular as dessert banana, especially in the western world. However in Nigeria, other parts of Africa, in the Caribbean and in some parts of Asia, plantain serves as a major staple food. It is particularly desired for the variability in the stages of ripeness and in cooking methods. Plantains can be consumed in the unripe, fairly ripe, ripe and overripe stages. They are usually eaten raw by most farmers on the farm but many households prefer to have them as boiled, grilled or fried (Figure 1.1 d, e and f). Sometimes steaming is done without removal of the plantain peels. Ripening stage or cooking method used is always a function of individual preference but sometimes as a result of the fact that plantains, just like bananas, are very perishable and need to be consumed in good time if wastage is to be avoided. It is quite unfortunate that despite its importance in the diet of many tropical populations, only a small quantity of plantains produced annually has been considered for industrial processing. The more unfortunate aspect is the fact that not less than 40% of the annual production is wasted due to spoilage. Existing processing on a domestic, artisanal and regional scale may be sources of inspiration for development of industrial productions. The processing of plantain and banana into chips, flour, dried pulps, jam, spirits, etc are growing very slowly in plantain/banana producer countries (Emaga *et al*., 2007).

Starch is the major component of green plantains with a total starch content of 61.3 - 76.8 g/100 g (DWB), (Faisant *et al*. (1995), Juarez-Garcia *et al*. (2006), Rodriguez-Ambriz *et al*. (2008)). Green plantain contains a total

dietary fibre content of 14.5% (DWB), (Juarez-Garcia *et al*., 2006). Plantain is low in protein but relatively high in minerals and vitamins (Best *et al*., 1984).

1.1.1 Some differences between plantain and banana

A major difference between plantain and dessert bananas is in the total sugar and starch contents. Dessert bananas convert almost all of their starch into soluble sugar during ripening, while ripe plantains still have a high starch content (from 10% to 15% fresh pulp), which usually affects their taste. The difference in starch degradation efficiency between plantain and bananas may, among other factors, be due to starch content, more efficient starch degradation enzymatic apparatus in dessert bananas, and/or the structural differences in the granules. Susceptibility of starch to degradation during ripening also depends on structural features, such as the degree of crystallinity, the amylopectin branch chain length distribution, the amylose content, and the shape and size of the granules (Cordenunsi and Lajolo (1995), Soares *et al*. (2011)).

Bananas have lower amylose content (15 - 19%) than plantains (21 - 24%) (Soares *et al*., 2011). Ripe plantains contain 66% starch and 17% sugars while ripe bananas contain 5% starch and 80% sugars (Ketiku, 1973). The quantity of most nutrients in banana and plantain are similar except that plantain contains more starch as earlier mentioned. They both contain significantly high quantity of potassium (4 mg/g pulp) but only trace amounts of sodium and iron (0.01 mg/g pulp), (Simmonds, 1987). They also have similar levels of B vitamins, thiamine, niacin and riboflavin; however, plantains

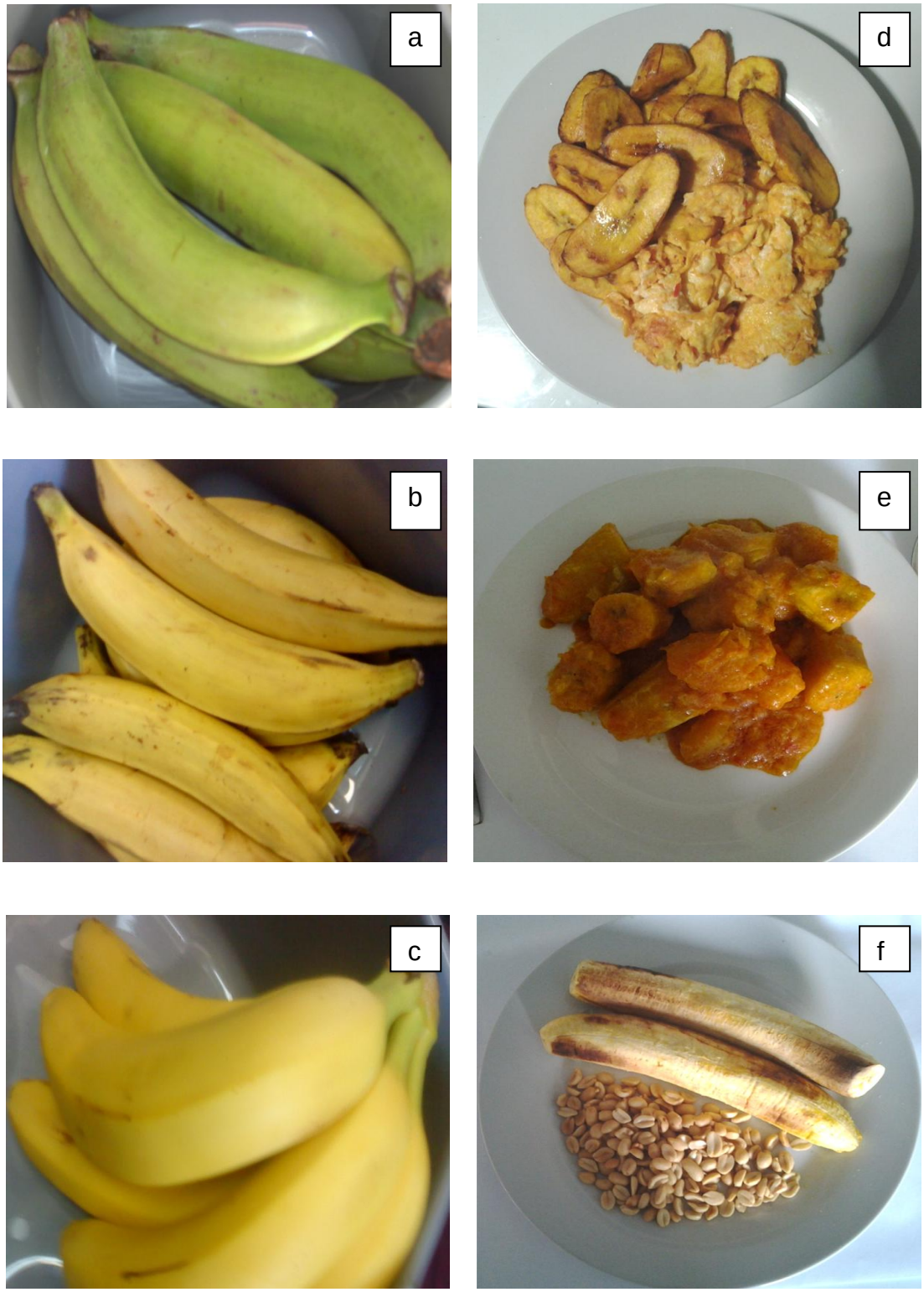


Figure 1.1: Pictures of *Musa* cultivars and processed plantain foods
a- unripe plantain fingers, b- ripe plantain fingers, c- bunch of ripe banana, d- fried ripe plantain with omelette meal, e- unripe plantain cooked with red oil, spices and sauce, f - grilled unripe plantain with roasted groundnuts.

contain twice the amount of vitamin C (20 mg/100 g pulp) in banana (Chandler, 1995). The total phenolics in banana is lower (ripe - 8.1 ± 0.1 and unripe - 9.0 ± 0.2) than in plantains (ripe – 9.8 ± 0.2 and unripe – 18.9 ± 1.4), (values are mean ± SEM mg of GAE equivalents/g DWB (Bennett *et al*., 2010). Unripe plantain has a bitter taste when eaten raw (Best *et al*., 1984).

Dessert bananas are usually consumed in the raw uncooked form when they are ripe, while plantains are consumed at different stages of maturity and are usually cooked before consumption. This is because of the higher starch content in plantains. On the basis of the literature and personal assessment, differences in banana and plantain (apart from genomic differences) occur in size, colour and shape (Figure 1a, b, c), as well as taste and texture.

1.1.2 Therapeutic properties of plantain

Numerous preparations of dried unripe plantains have been found to be effective both as a prophylactic treatment and in healing aspirin-induced ulcers in rats, whereas ripe fruits preparations of plantains were inactive. Furthermore some other conventional substances with known anti-ulcerogenic activity were used to compare the results obtained. Aluminium hydroxide, cimetidine, prostaglandin E2, N6, 02-dibutyryl adenosine 3',5' cyclic monophosphate were also tested and found to be anti-ulcerogenic when used prophylactically in rats but were not effective in healing ulcers that were already formed by aspirin, and in addition these substances did not stimulate the growth of gastric mucosa. It was therefore concluded that anti-

ulcerogenic action of the plantain preparations must have been due to their ability to stimulate the growth of gastric mucosa. The active ingredients (~ 3.2 mg/g DWB) were discovered to be water soluble and were successfully and completely extracted from the plantain flours using 95% ethanol at room temperature, however, they could not be extracted with anhydrous ethanol or other organic solvents while extractions with aqueous ethanol at elevated temperatures destroyed the anti-ulcerogenic compounds (Best *et al*., 1984). The anti-ulcerogenic agent was later identified as the natural flavonoid leucocyanidin (Lewis *et al*., 1999).

Green plantain was also discovered to have a beneficial role in the dietary management of persistent diarrhoea in hospitalized children in relation to diarrheal duration, weight gain and costs, but the prophylactic effects are also lost when plantains are cooked (Best *et al*. (1984), Lewis *et al*. (1999), Alvarez-Acosta *et al*. (2009)).

1.1.3 Plantain in Nigeria

In Nigeria, plantain serves a major staple as well as a snack. It is usually consumed cooked and in various forms and dishes, examples are given on Figure 1.1 d, e, f. The ripe ones are usually consumed fried and this is the favourite form of consuming ripe plantain in the western part of Nigeria. In western Nigeria, ripe plantain is sometimes cooked with beans and some condiments to increase sweetness and palatability. Grilled plantains are a favourite snack usually prepared from semi-ripe plantain and normally consumed with either roasted groundnuts or palm oil. Boiled green plantain is

the favourite form of consuming plantain in eastern Nigeria. It is the form that attracts the greatest attention in this work because in Nigeria and other parts of Africa there are claims (which are generally locally accepted) that it helps to cure diabetes, but this has not been scientifically tested. Moreover, it is not clear what component of the food could be responsible for this claim.

The only scientifically related information to this assertion is the low glycaemic index (GI) of green unripe plantain as reported in the literature. Furthermore, there is overwhelming evidence of the fact that green plantain is one of the few sources of granular resistant starch. Due to the fact that resistant starch has been linked to low GI (this is discussed in more detail in section 1.7) and low GI foods have been regarded as beneficial for the control and management of diabetes (this is discussed in more detail in section 1.6), there may be a possible link between resistant starch content and the low GI of plantain, and this therefore is the focus of this work.

1.2 Starch

Starch is widely distributed in nature and is the major component of cereals, tubers, legumes, and some fruits. The functional properties of starches vary with botanical source for e.g. normal maize starch produces an opaque paste and a firm gel whereas waxy maize and potato starches produce clear pastes that do not set to a gel (Lilia and Harold, 2003). Starch has therefore been isolated for use as a raw material in diverse food products to produce or improve specific functionalities and properties.

Starch is a polysaccharide composed of two polymers of glucose, amylose and amylopectin. Amylose (Figure 1.2) is basically a linear polymer of glucose (molecular weight $5x10^5$ to $1x10^6$ and degree of polymerisation (DP) of 10^3 - 10^4) composed of (1, 4) - linked glucan residues with about one α (1-6) branch per 300-1000 glucan residues (Neil *et al*. (2005), Sajilata *et al*. (2006)). The side-chains on the molecules that are branched are few and these molecules are so long that they act similarly to unbranched molecules. The linear chemical structure of amylose enables it to change its conformation and the presence of many hydroxyl groups produces a high hydrogen bonding capability, with strong internal forces that allow these conformational changes. Amylose usually constitutes 10–30% of starch and acts as the structural stabiliser of the starch granule and therefore starches with a greater amount of amylose are more resistant to digestion Lilia and Harold (2003), Sajilata *et al*. (2006), Neil *et al*. (2005)).

Amylopectin (Figure 1.3) is a branched molecule with a molecular weight of tens of millions formed by α (1, 4)-linked anhydroglucose units with α (1, 6)-linked branches and a DP of $5x10^4$ to $5x10^5$. The average chain length between branches in amylopectin is about 20 residues. Amylopectin is less prone to gelation and retrogradation because of the branched structure. Crystallinity is produced when clustered branches are packed together while the branch points are considered to be in the amorphous regions. The crystalline regions are more resistant to enzymatic and chemical action and to

penetration by water than are amorphous regions in starch granules (Lilia and Harold, 2003).

There are two crystalline structures in starch: type A is found mainly in cereal, tapioca and mango starches, whereas B type starches are found in tubers and potato. A third type, C, is a mixture of A and B allomorphs and can be found in banana and some legumes (Topping and Clifton, 2001). Amylopectin chain lengths of the A type structure is between 23 to 29 glucose units and the hydrogen bonding between the hydroxyl groups of the amylopectin molecule chains lead to the formation of outer double helical structure. amylopectin chain lengths in the B type starches is between 30 to 44 glucose molecules with water inter-spread. For the C type starches amylopectin chain lengths is between 26 to 29 glucose molecules (Sajilata *et al*., 2006).

1.3 Resistant starch

Resistant starch was initially discovered and defined as starch that was remaining undigested together with non-starch polysaccharides in *in vitro* dietary fibre analysis despite rigorous digestion treatments with amylases to remove starch completely (Englyst *et al*., 1982). Thereafter, Englyst and Cummings (1985), (1986b), (1987) carried out studies on ileostomists that provided more evidence that not all starch ingested is completely digested and absorbed in the human small intestine. Some of the starch in some foods, e.g. cereals, potato and banana, were recovered in effluents collected from ileostomists. Furthermore, the amount of undigested starch recovered *in vivo*

was usually more than that obtained *in vitro* from the dietary fibre analysis. Partial hydrolysis products namely, maltose, maltotriose and dextrins, accounted for up to 69 ± 9 % of ingested starch in some of the effluents. On the basis of these observations, a definition for resistant starch was therefore redefined so as to include all the starch that escaped digestion in the human small intestine (Englyst and Cummings, 1990). This definition was later adopted and modified by the European Resistant Starch Research Project (EURESTA), (1992) as 'the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals'. Various attempts were then made to develop *in vitro* models that would mimic the human digestive system in order to save the huge cost of carrying out *in vivo* studies. The studies that gave resistant starch portions that matched the mean values of starch recovered in ileostomy studies were those reported by (Englyst *et al*. (1996a), Silvester *et al*. (1995)).

Resistant starch does not add to the caloric content of foods since it is not digestible and it is characterised by physiological effects that make it comparable to dietary fibre. Resistant starch has many physiological benefits, which includes improved bowel functions due to the production of short chain fatty acids, reduction in colonic pH, enhancement of faecal weight and output, alteration of intestinal bacterial activity and reduction of secondary bile acid excretion. It has also been found to be useful in energy metabolism and weight management as well as improved glycaemic and insulinaemic

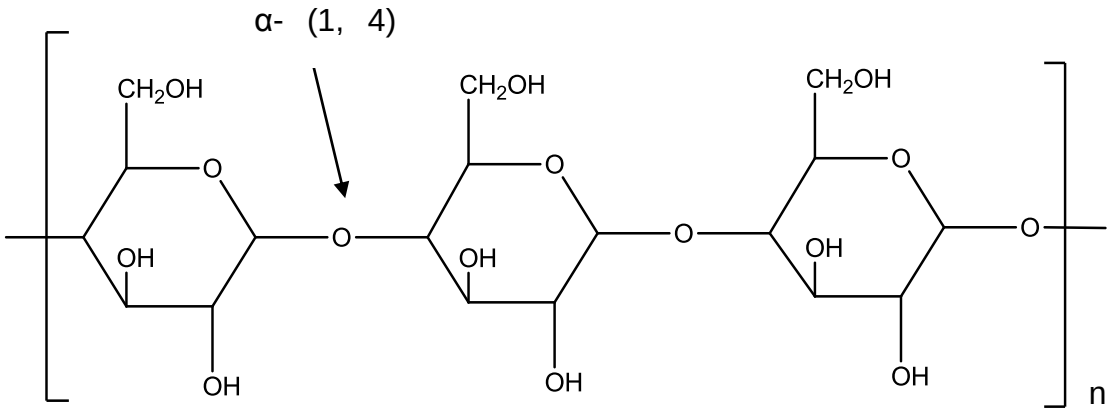


Figure 1.2: Starch amylose structure: α-D-glucopyranose residues are linked by 1, 4 bonds

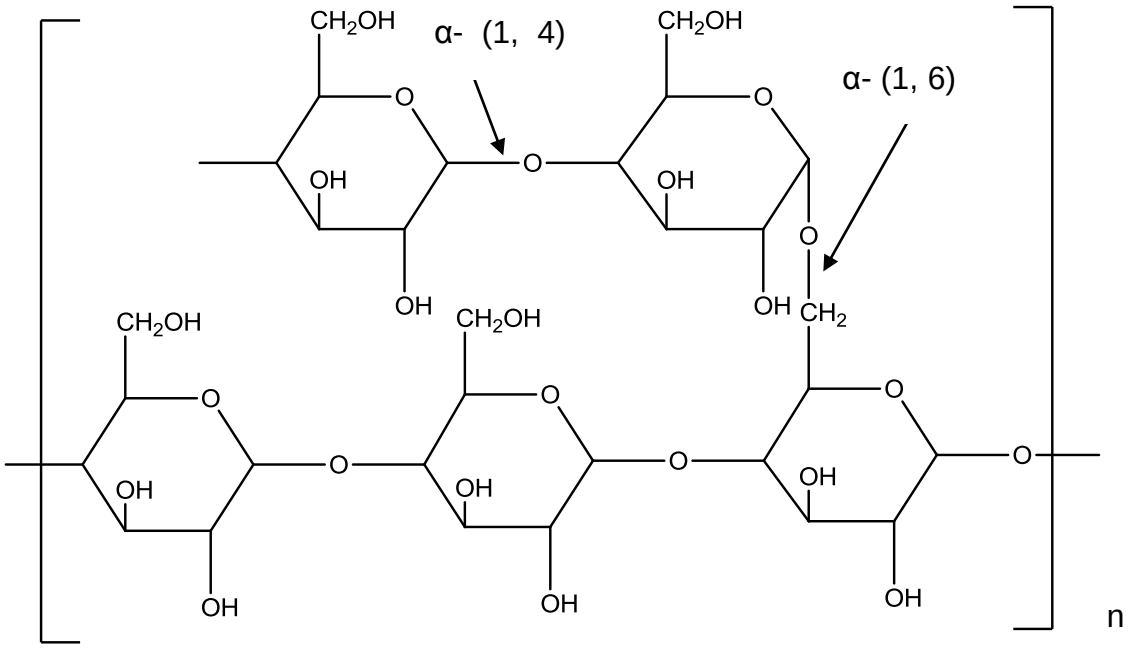


Figure 1.3: Starch amylopectin structure: α-D-glucopyranose residues are linked linear by 1, 4 bonds and branched 1, 6 bonds

response (Asp (1997), Cummings *et al*. (1996), Champ (2004), Nugent (2005)).

Resistant starch was categorised into three types to reflect the reasons why starch may escape digestion and absorption in the human small intestine by Englyst and Kingman (1990). These are physically inaccessible starch – RS1, resistant starch granules – RS2 and retrograded starch – RS3. A fourth category, which is resistant starch produced by chemical modification – RS4, was later included by Brown *et al* (1995).

1.3.1 Resistant starch type 1

Resistant starch type 1 is produced when starch is physically trapped within the food matrix. Physical inaccessibility of digestive enzymes to starch results when starch is impounded in food fragments that have been incompletely broken down (Neil *et al*., 2005). An important factor in enzyme digestibility is accessibility to the substrate. Inaccessibility of enzymes to some portion of starch may therefore make it indigestible. This type of resistant starch is found in whole grain foods and partly milled grains and seeds and in some very dense types of starchy foods, hence milling or grinding can release the starch to make it digestible (Sajilata *et al*., 2006).

1.3.2 Resistant starch type 2

Resistant starch type 2 occurs because of the nature of the starch granules. Resistance occurs as a result of the structure and conformation of the starch granules (Lilia and Harold, 2003). Dietary sources of native resistant starch

are some raw foods e.g. banana and partly gelatinised foods e.g. cooked high-amylose corn starch. It is not all raw or partly gelatinised foods that can be sources of RS2 because it is an inherent property of the nature of the starch and source. This type of resistant starch has only been detected in B and C type starches such as potato, banana, some legumes and high amylose corn starch (Neil *et al*., 2005).

1.3.3 Resistant starch type 3

Resistant starch type 3 is retrograded starch which occurs as a result of cooling gelatinised starch when starch changes from an initial amorphous state to a more ordered crystalline state (Magnus and Eliasson, 2006). It is found in most moist-heated foods that are not absolutely fresh. It has been detected in cornflakes, cooled potatoes, stale bread and many other cooked and cooled foods.

1.3.4 Resistant starch type 4

Resistant starch type 4 is produced by introducing chemical bonds that can inhibit the action of digestive amylases to a starch polymer. The interference usually depends on the type and the degree of bonding. These types of bonds can include dextrinization, etherification, esterification and oxidation. They are usually referred to as cross-linked starches; examples include starch ethers and phosphate esters.

1.3.5 Resistant starch in plantain

The discovery of enzyme resistant starch in *Musa ssp* started with the work of Englyst (1986b), when up to 90% of ingested starch content of banana was recovered in effluents from ileostomists. An enzyme resistant starch value of 66.7%, 10.5% and 3.9% of total starch was also reported for freeze-dried samples of raw, freshly cooked and cooked and re-heated green plantain respectively *in vitro* (Englyst and Cummings, 1986a). Thereafter, other studies on the digestibility of banana starch were carried out while that of plantain is scarcely reported (Faisant *et al*. (1995), Lehmann *et al*. (2002)). This may be due to the fact that most of the research was performed in Western countries where plantain is not well known. A study of the starch granule structural characteristics and starch degradation patterns of plantain and banana reveals that plantain starch is more resistant to digestive enzymes than banana starch (Soares *et al*., 2011). Apart from two recent studies (Pelissari *et al*. (2012) and Ovando-Martinez *et al*. (2009)) which reported a resistant starch (RS2) value of 42.5 ± 0.4 to 50.3 ± 1.0 g/100 g sample (DWB) for raw green plantain flour and starch, no papers were found on the quantification of resistant types and starch digestibility in ripe and unripe plantain and plantain products.

1.4 Starch digestion

Starch digestion is a multifaceted process, which includes different phases: the diffusion of the enzyme towards the substrate, the adsorption of the

enzyme to the starchy material, and the eventual multiple step hydrolysis of starch to glucose (Colonna *et al*., 1992).

The diffusion of α-amylase unto the substrate surface is considered an important step in starch hydrolysis and can be prevented by starch interactions with fibre, protein and other food components. The hydrolysis of starch was initially considered as starting from the granule surface, however, it was later discovered that some native cereal starches such as corn and sorghum contain peripheral pores and channels, which enable α-amylase penetration, thus resulting in an inside-out hydrolysis mechanism (Fannon *et al*., 1992). On the other hand B type starches such as potato and banana starches are digested by ex-corrosion i.e. hydrolysis starts from the surface of the starch (Gallant *et al*., 1997). This difference in hydrolysis mechanism appears to explain the reason why cereal starches have higher digestibility than tuber starches such as potatoes.

1.4.1 Starch digestion in the human digestive system

Salivary amylase in the mouth is the first enzyme to act on starch during digestion; this takes place in a relatively short time before the bolus of food is transferred into the stomach by the oesophageal peristalsis. The gastric juice in the stomach which is composed of hydrochloric acid retards the action of salivary α-amylase which remains active in the stomach only as long as it is protected from stomach acid. This implies that starch digestion by salivary amylases can continue in the stomach if trapped within a large bolus of food

until the bolus is broken up and exposed to stomach acid (Goodman, 2010). The food then proceeds to the duodenum where it encounters the pancreatic fluid. The acidity of the fluid arriving from the stomach is neutralized to a pH of ~8 by sodium hydrogen carbonate; the pancreatic fluid, which contains α-amylase, continues the hydrolysis of starch into maltose, maltotriose and oligosaccharides. Further enzymatic digestion by glucosidases such as glucoamylase and isomaltase are required to break these starch digestion products to glucose. Glucose is subsequently absorbed by active transport mediated by the sodium-glucose co-transporter (SGLT-1), across the apical membrane of intestinal absorptive cells and then exits the gastrointestinal tract across the basolateral membrane via the facilitative glucose transporter GLUT2, into the blood stream (Dona *et al*. (2010), Wachters-Hagedoorn *et al*. (2004)). Products of starch digestion not converted to glucose e.g. resistant starch and maltodextrins, are transported to the large intestine where they are metabolised by colonic bacteria through fermentation into short-chain fatty acids (Wong and Jenkins, 2007).

1.4.2 Food properties affecting the rate of starch digestion

Foods are complex structures, in which starch may be present in different forms and quantities and therefore starch digestion will be expected to be influenced by a variety of factors, some of which are highlighted below.

1.4.2.1 Amylose content

The extent of starch digestion generally decreases as the amylose content increases; however amylose content alone is not the sole predictor of digestibility. Amylose that is bound to lipids is less accessible to digestive enzymes than free amylose (Vesterinen *et al*. (2002), Nebesny *et al*. (2004)).

1.4.2.2 Gelatinisation and retrogradation

Despite the large molecular weight of starch, it has been demonstrated that gelatinised starch is rapidly digested and absorbed in the small intestine of man (Inger, 2006). Under limited water concentration, gelatinisation of starch granules is not complete and happens at higher temperatures. This is because under these conditions, starch chains become mobile and the microstructure of the granule is modified but not its entire integrity; this results in granules that are more slowly digestible than gelatinised ones (Wang *et al*. (1991), Shin *et al*. (2005)). Gelatinised starch granules are more susceptible to α-amylase degradation than are native starch granules. Gelatinised starch molecules, whose granular architecture have been destroyed, can undergo inter and intra-molecular association into and ordered structure by the process of retrogradation. Starch retrogradation also results in reduced enzyme susceptibility.

1.4.2.3 Food Matrix

The structure of the food matrix whether a liquid or solid matrix has an important effect on accessibility of digestive enzymes to starch. The physical texture of the food may affect the starch digestion and also the absorption of

hydrolysis products. Mastication of food in the mouth and peristaltic effects in the stomach reduce the particle size of solid foods, increasing the area/volume ratio and improving the digestion process in the small intestine. The rate of gastric emptying varies from one food to another, and it can influence the starch digestion because of its effect on the mass balance in the small intestine. The faster food particles enter the small intestine the more rapid they will be available to digestive enzymes (Parada and Aguilera, 2011).

Usually, solid food particles empty from the stomach only when they have been broken down (e.g. to sizes of < 2 mm). The food matrix will determine the rate of food particle size reduction and viscosity of the food bolus which will subsequently affect the gastric emptying rate (Holt *et al*., 1982).

Insoluble fibre in food tissue retards the rate of starch hydrolysis, especially in whole-grain products and legumes. Destruction of the food matrix during milling or grinding increases the rate of starch digestion. The presence of viscous fibre (soluble fibre) e.g. guar gum, increases the viscosity of the food matrix even at relatively low concentrations. This can then lead to reduced susceptibility of starch to digestive enzymes and lower absorption of glucose after digestion (Brennan *et al*., 1996). An inhibitory action on α-amylase was detected when a low concentration (0.5%) of galactomannan (a legume based viscous fibre) was added to a gelatinised starch slurry (Slaughter *et al*., 2002). It is sometimes difficult to determine the exact mechanism by which starch digestion is slowed down in the presence of viscous fibre, whether it is

by inhibition of enzyme access to substrate or by increase in viscosity of gastrointestinal contents based on mass transfer or both. This may also depend on the source of the fibre.

1.4.2.4 Other food components

Food components that may affect starch digestion apart from dietary fibre include tannin and phytic acid in legumes, maltose and maltotriose (from incomplete hydrolysis of starch) in high concentrations (Colonna *et al*. (1992), Tormo *et al*. (2004)). Granule surface proteins and lipids can reduce starch surface accessibility by blocking the adsorption sites needed for enzymes to bind to the substrates (Oates, 1997). Some food components may not only inhibit starch digestion but also play a role in glucose uptake. Some polyphenols e.g. diacetylated anthocyanin have been shown to inhibit α-glucosidase activity, also green tea polyphenols inhibit the glucose transport. Inhibition of starch digestive enzymes or glucose transporters would reduce the rate of glucose release and/or absorption in the small intestine.

1.4.2.5 Starch particle size and characteristics

Regardless of botanical origin, a smaller granule size shows lower enzymatic susceptibility than a larger one (Franco and Ciacco, 1992). The rate of hydrolysis of raw starch granules is proportional to the surface area as earlier reviewed by Parada and Aguilera (2011), therefore a smaller particle size results in a reduced surface area and consequently a reduced adsorption of enzyme unto the granule surface. The kinetics of enzymatic starch digestion also depends largely on molecular architecture and physicochemical

characteristics of the starch granule (Dona *et al*., 2010). This is particularly true for raw starches in food.

1.4.2.6 Chemical modification

There are conflicting reports regarding the impact of chemical modifications on starch digestibility. Substitution of starch hydroxyl groups with acetyl (acetylation) or hydroxypropyl (hydroxypropylation) substituent was found to reduce starch digestibility by α-amylase (Wolf *et al*., 1999). Also a considerable reduction in digestibility of starch cross-linked by phosphate bridges was observed in a study whereas no reduction (or only slight) reductions were found in another study (Xie and Liu (2004), Han and BeMiller (2007)). It appears that the disparity in the observations made with different types of cross-linked starches is linked with the inherent property of the starch used and the type of modifications made (Juansang *et al*., 2012).

1.4.3 Nutritional starch fractions

Starch is nutritionally classified into three types based on the rate of starch digestion *in vitro*. This classification was developed by Engyst *et al* (2005) from a series *of in vivo* and *in vitro* studies. It should be noted that the *in vitro* measurements proposed are based on the average of measurements obtained *in vivo* because of differences observed in both the rate and extent of starch digestion that is seen between individuals in human studies. These are rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS). RDS is the portion of starch that is digested in the first 20 minutes of starch digestion. It causes a rapid increase in blood glucose

concentration after consumption of starchy foods. The total content of free sugar and rapidly digested starch in a meal constitute the rapidly available glucose (RAG) content (Englyst and Englyst, 2005) and have some implications for predicting the glycaemic index of foods.

SDS is the portion of starch that is digested after the RDS but in no longer than 120 min i.e. between 20 and 120 min of starch digestion *in vitro*. It is completely but slowly digested in the human small intestine. SDS offers a stabilising and sustaining effect of the blood glucose level and subsequently lowered glycaemic index (GI) (Lehmann and Robin, 2007). SDS in cooked and cooled foods may consist of retrograded amylopectin since retrograded amylopectin is slowly but completely digested (Eerlingen *et al*., 1994) and/or amylose that is not fully re-associated.

Resistant starch is the portion of starch that is not digested after 120 minutes of enzyme incubation *in vitro* and represents the starch that is not digested in the human small intestine. It is discussed in detail in section in section 1.3).

1.5 The glycaemic index

The glycaemic index (GI) theory was developed over thirty years ago by Jenkins and co-workers (1981) as a tool to classify foods based on their effects on the blood glucose level with an initial aim of aiding diabetic patients in controlling their postprandial hyperglycaemia. The GI ranks individual foods according to their postprandial rate of carbohydrate digestion and glucose absorption. It is defined as the incremental area under the postprandial blood

glucose curve after the consumption of 50 g of (digestible) carbohydrates from a test food, divided by the area under the corresponding curve after a meal containing a similar amount of the reference food, normally white bread or glucose (Wolever *et al*., 1991). The reference food is given a value of 100. Although a quantity of 50 g available carbohydrate portion is generally accepted for GI testing, the relative responses of foods at different levels of available carbohydrate intake (at least between 25 - 100 g) are the same (Wolever, 2006). Within the limits determined by expected GI difference and by day-to-day variation of glycaemic responses, the GI forecasts the ranking of the glycaemic potential of different meals in individual subjects.

The postprandial blood glucose concentration is the sum of the endogenous glucose (circulating in the body) and the exogenous glucose (provided by the food). In the postprandial period, the exogenous glucose and the total glucose first increase and then decrease as a result of the transport of glucose from the intestinal lumen into the blood (Parada and Aguilera, 2011). The effect of starchy foods on blood glucose and insulin were originally considered only in diabetics but today with the upsurge of conditions such as obesity, etc, the promotion of healthy diets containing low GI foods is becoming popular. This is because low GI foods reduce insulinemia which may provide greater access to fatty acids as a source of fuel, promoting greater fat oxidation.

Over the years, there have been many variations in the methodology employed for determining the GI of foods. This led to a discussion on methodology for measuring GI by an expert panel, as part of a global discussion on the role of dietary carbohydrate in human nutrition (Food and Agriculture Organization, 1998). This panel agreed on a reference methodology and provided guidelines for measurement in future GI testing. The outcome of this was an increase in the number of foods reported in the international table of GI published by Foster-Powell *et al* (2002). Nevertheless, debates still took place thereafter with regards to some aspects of measuring GI, such as the accurate measurement of the type of carbohydrates present in food and food portion sizes as summarised by Monro (2003) and Pi-Sunyer (2002). This was then followed by a review by Brouns *et al* (2005) based on available scientific studies on GI. A summary of the agreed conclusions and recommendations for the accurate measuring of the glycaemic index of foods is presented below:

- At least ten test subjects should be tested to obtain a sufficient statistical power
- The inclusion of both genders in a study is acceptable
- The food portion should be based on available carbohydrate
- A test dose of 50 g available carbohydrates is recommended;
- For foods with a low carbohydrate content it is justified to lower the test dose to 25 g available carbohydrate
- The reference food should be measured at least twice

- The use of healthy human volunteers is recommended
- If the test food is a fluid, ingestion (250 ml) should take place within 5-10 min
- If the test food is a solid or semi-solid, ingestion should take place within 10-15 min
- Glucose or white bread are recommended as reference foods
- The evening before a test, each subject should consume a meal of choice and repeat that meal before each subsequent test. Unusual vigorous physical activity should be avoided
- Test foods should be randomised in blocks of a maximum of six
- The total duration of the tests should not exceed 4 months
- When testing multiple foods, a reference test should be done at the beginning and a repetition should take place after every 6 – 8 weeks;
- Glucose measurement alone (without the measurement of insulin response) is appropriate
- For more mechanistic and/or metabolic studies, measurement of both glucose and insulin is recommended;
- Blood sampling times should be at 0 min (baseline sample), followed by 15, 30, 45, 60, 90 and 120 min after starting to eat the test meal;
- AUC calculation should be based on incremental AUC, ignoring area under the baseline;
- It is also recommended to calculate GI as the mean of the individual ratios.

1.6 Low glycaemic index foods and diabetes

The consumption of low GI foods has been linked with positive impacts on a wide variety of chronic diseases, such as diabetes, obesity and coronary heart disease as earlier reviewed (Chiu *et al*. (2011), Brand-Miller *et al*. (2009a)). This is because lower blood glucose and insulin levels are also associated with an improved metabolic profile of high-density lipoprotein cholesterol, glycosylated proteins, oxidative status, haemostatic variables and endothelial function.

When glucose is present in the blood at elevated levels, it causes a metabolic stress. Foods that produce a moderate rate of change in blood glucose are therefore perceived as nutritionally better than those that generate a rapid change because postprandial hyperglycemia plays a direct pathogenic role in type 2 diabetes and some cardiovascular diseases. Therefore an important diet modification considered for the treatment and management of diabetes is the consumption of low GI foods (Food and Agriculture Organization (1998), Brand-Miller *et al*. (2003)).

The rate of glucose transport and bioavailability depends on the impact of the food on postprandial glucose levels (Figure 1.4) as well as the health status of the individual. Some foods e.g. high-amylose rice and different types of rye breads have been shown to decrease insulin responses of healthy subjects with no effect on glucose responses while some foods such as barley pasta enriched with β-glucan led to a reduction in insulin response when compared

with the consumption of wheat pasta, although the plasma glucose in both meals did not differ significantly (Bourdon *et al*. (1999), Leinonen *et al*. (1999)). This implies that different foods may need to be studied with regard to their impact on glucose and insulin responses.

Today, evidence supporting the therapeutic potential of low-glycaemic index diets in diabetes, hyperlipidaemia cardiovascular and diseases is over-whelming (Jenkins and Wolever (1981), Jenkins *et al*. (1987), Augustin *et al*. (2002)). The results of clinical studies indicate that a low GI diet is linked with a reduced risk of diabetes and cardiovascular disease (Jenkins *et al*., 2002). The clinical utility of the GI has been supported by many studies. In long-term trials, low-GI meals resulted in modest improvements in overall blood glucose control in patients with insulin-dependent and non-insulin-dependent diabetes. Low-GI diets also reduced insulin secretion and lowered blood lipid concentrations in patients with hypertriglyceridemia (Wolever *et al*., 1991).

Excess consumption of high glycaemic index foods may lead to hyperinsulinaemia, insulin resistance, weight gain, and possibly obesity, resulting in insulin-resistant syndrome. Even though wide fluctuations occur in glucose inflow into circulation (e.g. during feeding) and outflow of circulation (e.g. during fasting and exercise), the plasma glucose concentration is normally maintained within a relatively narrow range, between approximately 3.3 and 8.3 mmol/l. The peak postprandial plasma glucose, which occurs normally within the first hour after the start of the meal, seldom exceeds 8.3 mmol/l (in healthy people) and the postprandial increase in glucose rarely

lasts beyond 120 min. In response to this rise in blood glucose concentration, the pancreas increases its secretion of insulin and suppresses the release of glucagon, thereby limiting hepatic glucose production and promoting the uptake of glucose by the muscle and fat tissues. Increased insulin levels effectively deposit a large proportion of glucose into these tissues if receptor sensitivity to insulin is normal. Defects in insulin secretion and/or insulin action results in elevated blood glucose levels and consequently diabetes mellitus. In type 1 diabetes, there is an absolute insulin deficiency while in type 2 diabetes insulin levels are normal or, more commonly elevated but the responses of tissues to insulin are inadequate resulting in a relative insulin deficiency. Chronic hyperglycemia causes damage and dysfunction of various cells, tissues and organs (Wachters-Hagedoorn *et al*., 2004).

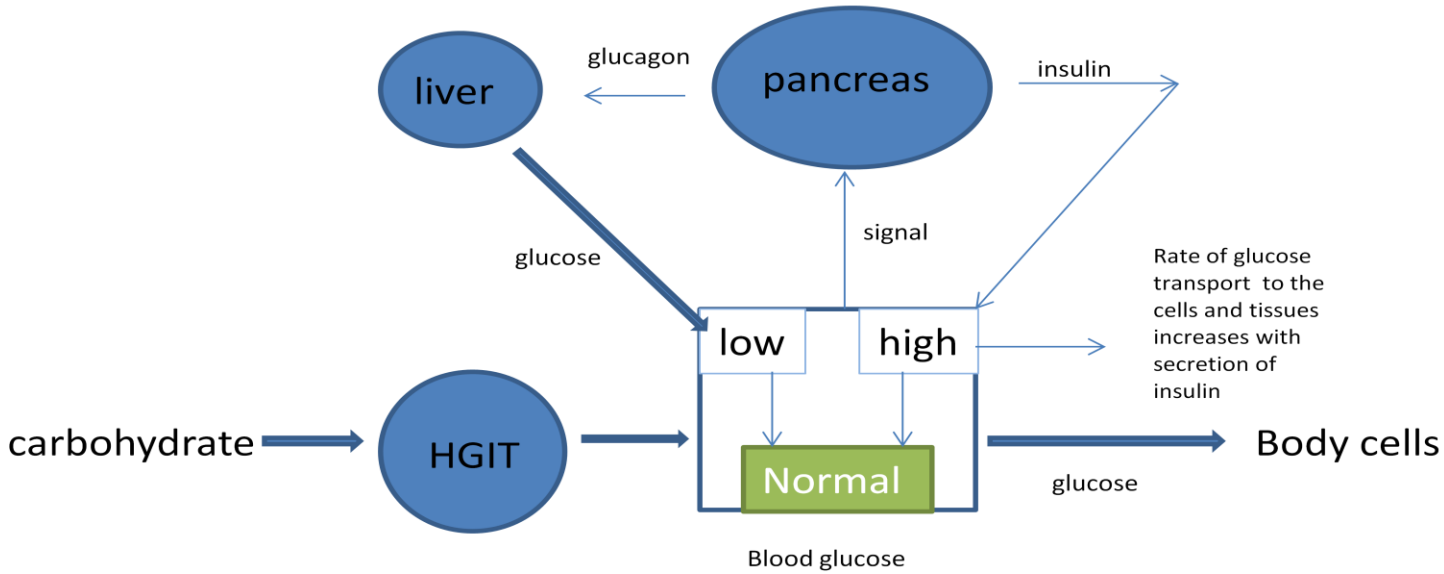


Figure 1.4: Glucose transport from digestion of carbohydrate food
HGIT – human gastro intestinal tract. In the fasting state, plasma glucose concentrations are relatively stable (NORMAL). Following glucose ingestion, the increase in plasma glucose concentration triggers insulin release, which stimulates peripheral glucose uptake and suppresses endogenous glucose production. If blood glucose levels fall below normal (LOW), glucagon is released by the pancreas which acts on the liver to increase hepatic glucose production within minutes and the plasma concentration returns to normal. If blood glucose levels rise above normal (HIGH), more insulin is secreted to increase the rate of glucose transport to the body cells so as to restore the plasma glucose level to normal.

1.7 Impact of resistant starch on glycaemic index

There is increasing interest in consuming resistant starch (RS) containing foods because of their potential health benefits, especially in terms of hypoglycaemic impact. For over two decades consumption of food products rich in RS has been associated with low postprandial glucose and insulin response, however, due to the lack of consensus by researchers on this hypothesis more evidence is needed as to the hypoglycaemic effect of RS. This also may be dependent on food type and starch source. As earlier reviewed by Nugent (2005), fifteen studies have reported a reduction in postprandial glucose and insulin response with the consumption of RS-rich test-meal, while ten studies have showed no significant effect. Although it was concluded from a summary of these studies that RS must contribute at least 14% of total starch intake in order to bestow any benefits on glycaemic or insulinaemic responses, it is not clear if the studies that reported no effect of RS on glycaemic response used quantities lower than 14% RS.

Some authors (Wolever (2006), Raben *et al*. (1994), Jenkins and Kendall (2000)) at a time had suggested that the claim that RS reduced postprandial glucose responses and GI may be true only when it replaces part of the available carbohydrates in a food. They further added that it was a case of reducing the amount of starch available for digestion rather than having any specific impart on starch digestion and/or glucose metabolism. However, it has been revealed in other studies that RS content of a food influences not

only the amount of available starch but also the overall digestibility of starch and absorption of digestion products (Granfeldt *et al*. (1993), Granfeldt *et al*. (1995a), Kendall *et al*. (2009), Hallstrom *et al*. (2011)). One of the mechanisms by which this is achieved is by increasing the overall viscosity of the food bolus as well as the creation of products of incomplete digestion of starch such as maltose and maltotriose which may inhibit α-amylase activity as well as increase viscosity of the food bolus in the gastrointestinal tract (Warren *et al*., 2012).

Despite the lack of consensus regarding the precise effects of RS on insulin and blood glucose responses it is noteworthy that there are yet no reports of RS worsening insulin and glucose response. Other studies also revealed the positive impact of RS on glycaemic and insulin responses when ingested with soluble fibre (Behall *et al*., 2006). Although there appears to be no general correlation between GI and content of resistant starch (RS) in foods *per se*, RS content of foods is a nutritional variable that may be linked to low GI property of a food (Truswell, 1992). In this regard the nutritional/nutraceutical potential of unripe banana has been linked to its high resistant starch content (Pacheco-Delahaye *et al*. (2004), da Mota *et al*. (2000)).

Since resistant starch has a low GI because it is not digested in the human small intestine, it is logical to believe that adding it to a food will result in an overall lowering of the GI of the food (or in the worst scenario maintain the initial GI of the food) than when a soluble starch is added. It appears that the

lack of consensus on the impact of RS on GI is due to the fact that in most studies, the types of enzyme resistant starch present in the foods had not been clearly characterised. Jenkins (2000) in his review had indicated the need to distinguish between RS that produce low glycaemic response because they are not digested and RS that in addition also behave like *lente* carbohydrates which produce low glycaemic response by slow release of sugars from food (Kendall *et al*., 2004).

1.8 The glycaemic index of plantain

A summary of the work reported so far on the glycaemic index of plantain is highlighted in Table 1.1 below and inferences from each study are also discussed.

1.8.1 Comments/observations on each study reported in Table 1.1

1.8.1.1 Study 1 (Menezes *et al*., 2010)

The objective of the work was to determine the extent of *in vitro* colonic fermentation of unavailable carbohydrates of unripe plantain flour and starch and to evaluate their glycaemic response in healthy volunteers. In the manuscript, the GI values were not given but were calculated from reported values of the area under the curve (AUC) of standard and samples. The unripe banana mass, which was made from cooked homogenised green plantain, contained 6.5 g RS per portion served, however the food form was destroyed by homogenising the sample. The quantity of sugar in the sample was ignored in the calculation of the available carbohydrate because it was

less than 2g/100g sample and the method used for estimation of blood glucose was accu-check glucometer using finger prick capillary blood meter. A 50 g total starch basis used for the food portion sizes was wrong, especially for the raw starch sample.

1.8.1.2 Study 2 (Bahado-Singh *et al*., 2006)

The aim of the study was to add some commonly eaten indigenous Caribbean foods to the over 400 foods on the GI data base. The content of unavailable/resistant starch in the test foods was not reported and the method of glucose determination was glucose oxidase/peroxidase by spectrophotometry using finger prick capillary blood. There is no reason to fault the result based on the experimental protocol provided (Table 1.1).

1.8.1.3 Study 3 (Alegbejo and Ameh, 2012)

A 50 g portion size of test food and 50 g of control (glucose) was used. The results obtained are therefore misleading because portion size of the test food is not based on 50 g available carbohydrates (like the control); moreover plantain was served with condiments (meat and stew) as for study 4 below, as a mixed meal and the condiments were part of the 50 g portion size.

1.8.1.4 Study 4 (Godwin, 2010)

The aim of the study was to check the impact of traditional processing methods on the GI of green plantain based on the belief that Nigerian diabetic patients usually consume green plantain to reduce postprandial glucose levels. The boiled plantain and plantain flour (cooked) were served with a

bowl of soup made up of tomato sauce (containing fresh pepper, tomato, onions grounded to a smooth paste and mixed with 100 ml of hot red palm oil) and 30 g of boiled beef meat as eaten in the Nigerian culture. There were however major errors in the study e.g. different subjects were used for the reference and samples and tests were conducted after overnight fast > 12 hours. The results obtained are therefore misleading.

1.8.1.5 Study 5 (Pacheco-Delahaye *et al*., 2004)

The aim of the study was to compare the impact of fibre on GI of rice bran, papaya and plantain drinks. Plantain which had the highest content of total dietary fibre (16.1g/100g) caused the least increase in glycaemic response. The low GI of the raw green plantain drink was attributed to presence of fibre, however, this may not be entirely true because raw plantain is known to also contain some significant levels of resistant starch. There is no reason to fault the result based on the experimental protocol provided (Table 1.1).

1.8.1.6 Study 6 (Perry *et al*. (2000), Atkinson *et al*. (2008))

The GI of plantain was determined alongside those of 28 other New Zealand foods. The GI value reported was based on results from both healthy and diabetic subjects, however, venous blood samples rather than finger-prick blood samples were used. There is no reason to fault the result based on the experimental protocol provided (Table 1.1).

Table 1.1: Reported studies on the glycaemic index of plantain

study	Products used	Food portion size (g)	Size basis	Subjects	Ref food	GI
1.	a. Unripe banana mass- cooked pulp of unripe banana b. Unripe banana starch- starch isolate (un-gelatinised)	a. 81 b. 59	50 g TS	9	White bread	a. 43.2 b. 6.4
2.	a. Boiled green plantain b. Boiled ripe plantain c. Fried green plantain d. Fried ripe plantain	a. 259.2 b. 308.6 c. 175.9 d. 211.6	50 g AV CHO	10	Glucose	a.39±4 b.66±2 c.40±3 d.90±6 NB: SE indicated
3.	Boiled green plantain with stew and meat	50	No basis	D- 9 H– 7	Glucose	a.68 b.69
4	a. Boiled plantain with soup and meat b. Roasted plantain c. Fried plantain d. Boiled and pounded plantain e. Cooked plantain flour meal	a.124.4 b.224.4 c.62.9 d.138.2 e.121	50 g AV CHO	50 for samples, another 10 for reference	Glucose	a.64.9±10 b.56.9±9 c.64.9±9 d.66.6±3 e.65.1±1
5	Green plantain powder in 125 ml water (meringue)	Not given	50 g AV CHO	10	Glucose*	39
6	Boiled green plantain	120	21 g AV CHO	8	Glucose	38±10

Information provided was extracted from published manuscripts. Study 3: D-diabetic, H – Healthy, Study 5: glucose* - commercial solution

1.8.2 Inferences from the studies above

1. Green plantain (in different forms) was the most common sample used, only in one study was ripe (processed) plantain used.

2. The impact of resistant starch on the glycaemic index of plantain has not been studied.

3. Glycaemic index of raw ripe plantain has not been reported.

4. Glycaemic index of plantain crisps/chips has not been reported

Amongst other reasons, the scarcity of literature on *in vitro* digestion of ripe plantain may explain why there are very few studies on the GI of ripe plantain.

1.9 Aim

The goal of this research is to try to establish a link between resistant starch content of plantain and its glycaemic index. The experiments were tailored towards detection and quantification of three types of resistant starch in plantain products and finally checking the GI of plantain in the absence of resistant starch.

The specific objectives of the thesis were:

1.) To estimate the gelatinisation temperatures and the extent of gelatinisation in cooked plantain foods (chapter 3). This was required in order to estimate the cooking time/point that will remove any residual

resistant starch due to incomplete gelatinisation that usually occurs in some processed foods.

2.) To determine the differences between nutritional starch fractions in plantain flours and starches and the impact of gelatinisation on these fractions (chapter 4) as well as the implication of gelatinisation on sucrose hydrolysis in ripe and unripe plantains (chapter 5). This was done to check if the other components of plantain flour had any influence on the quantity of resistant starch. Also accurate estimation of the quantity and types of free sugars (fructose, glucose and sucrose) as part of the available carbohydrates in plantain was key to measuring GI.

3.) To quantify type 1 and type 3 RS in cooked plantain (chapter 6). This was done to check the differences in the quantities of RS1 and RS3 between freshly cooked plantain and stored plantain products

4.) To determine the glycaemic index of plantain product devoid of all forms of resistant starch in order to check if plantain GI will be low in the absence of resistant starch (chapter 7).

Chapter 2 Method optimisation and methodological challenges

2.1 Materials

2.1.1 Equipment

Incubation for starch digestion was carried out in a Grant OLS200 combined orbital/linear shaking water bath (Grant instruments, UK). Heat treatment at various temperatures was performed using a Grant aqua 12 plus linear shaking water bath (Grant instrument UK) and boiling was done using a Grant SBB 14 aqua plus unstirred water bath (Grant instrument UK). Absorbance measurements were performed using a Cecil CE7200 spectrophotometer (Cecil Instruments Ltd. Cambridge, UK). Centrifuge used was an eppendorf 5810R while the microfuge used was an eppendorf centrifuge 5415C and freeze drier was Scanvac cool safe 55-9 (Vitaris Switzerland).

2.1.2 Enzymes

Amyloglucosidase from Aspergillus *niger* (EC 3.2.1.3, Megazyme E-AMGDF, specific activity = 3260 U/ml on soluble starch; 1U = 1 µmol/min) was purchased from Megazyme international, Ireland. Pancreatin used was from porcine pancreas (EC 232-468-9, Sigma Cat. no. P-7545), with an activity of 8 x USP; (amylase activity ≥ 200 USP units/mg, lipase activity ≥ 16 USP units/mg and protease activity ≥ 200 USP units/mg). Invertase, EC 3.2.1.26 from yeast (stabilized with glycerol) with an enzyme activity of ≥11020≤ 13340 U/ml, was purchased from Fisher scientific, UK.

2.1.3 Standards

Soluble starch from potato (product number: S2004, CAS-number : 9005-25-8), amylose from potato starch (product number: A05121-1G, CAS-number 9005-82-7, containing ≤10% water and ≤2% butanol as impurities) and amylopectin from potato starch (product number: 10118-SG-F, CAS-number 9037-22-3, containing ≤10% water as impurity) were purchased from Sigma Aldrich, UK.

2.1.4 Assay kits

Resistant starch assay (K-RSTAR), amylose/amylopectin assay (K-A-AMYL) and D-glucose oxidase/peroxidise assay (K-GLUC) kits were purchased from Megazyme International, Ireland.

2.1.5 Statistical analysis

Analysis of variance (ANOVA) was used to assess statistical differences between samples and data are expressed as mean plus standard deviation.

2.2 Methods

2.2.1 Sample preparation

Cultivars of ripe and unripe plantains from Columbia were purchased from Leeds city market. Unripe plantains selected were full green (stage 2) while ripe plantains used were in the fully ripe stage (yellow) in colour (stage 6) on the colour index scale (Aurore *et al*., 2009). The samples were cut into thin

slices of about 2 mm thickness, freeze dried, blended into a fine flour and stored in clean plastic containers at ambient temperature.

2.2.2 Measurement of resistant starch

Two methods were explored for the measurement of resistant starch; the methods by Megazyme International and Englyst *et al* (1992).

2.2.3 Determination of resistant starch, non-resistant starch and total starch by Megazyme method

The megazyme protocol was provided as an assay kit and is based on the methods of the Association of Analytical Chemist (AOAC) method 2002.02 (2000) and McCleary *et al* (2002).

2.2.3.1 Hydrolysis and solubilisation of non-resistant starch

A 100 mg sample was accurately weighed into three of each screw cap corning culture tubes. 4.0 ml of an enzyme solution containing pancreatic α-amylase and amyloglucosidase was added to each tube. The tubes were tightly capped, mixed on a vortex mixer and attached horizontally in a shaking water bath, aligned in the direction of motion. The tubes were incubated at 37^{o}C with continuous shaking (200 strokes/min) for exactly 16 hours. The tubes were removed from the water bath and excess surface water was removed with a paper towel. The tube caps were removed and the contents treated with 4.0 ml of ethanol (99 % v/v). The tubes were centrifuged at 1,500*g* (approx. 3,000 rpm) for 10 min (non-capped). The supernatants were

carefully decanted and pellets were re-suspended in 2 ml of 50% ethanol with vigorous stirring on a vortex mixer and a further 6 ml of 50% ethanol was added, the tubes were mixed and centrifuged again at 1500*g* for 10 min. The supernatants were decanted and this suspension and centrifugation step was repeated once more. The supernatants were carefully decanted and the tubes inverted on absorbent paper to drain excess liquid.

2.2.3.2 Measurement of resistant starch

A magnetic stirrer bar (5 x 15 mm) and 2 ml of 2 M potassium hydroxide (KOH) was added to each tube and the pellets re-suspended (to dissolve the RS) by stirring for approximately 20 min in an ice/water bath over a magnetic stirrer. An aliquot of 8 ml of 1.2 M sodium acetate buffer (pH 3.8) was added to each tube with stirring on the magnetic stirrer and 0.1 ml of amyloglucosidase was immediately added; tubes were mixed well and incubated in a water bath at 50°C for 30 min with intermittent mixing on a vortex mixer.

After the incubation the contents of the tubes were quantitatively transferred to a 100 ml volumetric flask (using a water wash bottle). Volume was adjusted to 100 ml with distilled water and mixed well. An aliquot of the solution was centrifuged at 1,500*g* for 10 min. 0.1 ml aliquots (in triplicate) of the supernatants were transferred into glass test tubes, 3.0 ml of glucose oxidase/peroxidise (GOPOD) reagent was added and tubes were incubated

at 40°C for 20 min before measuring the glucose concentration at 510 nm against a reagent blank.

2.2.3.3 Measurement of non-resistant (solubilised) starch

The combined supernatant solutions obtained on centrifugation of the initial incubation steps were transferred to 100 ml volumetric flasks and adjusted to volume with 100 mM sodium acetate buffer (pH 4.5) and mixed well**.** A 0.1 ml aliquot of this solution (in triplicate) was incubated with 10 µl of dilute AMG solution (300 U/ml) in 100 mM sodium maleate buffer (pH 6.0) for 20 min at 40^{o}C. 3.0 ml of glucose oxidase peroxidise GOPOD reagent was added and the tubes incubated for a further 20 min at 40°C. Absorbance was measured at 510 nm against a reagent blank. The principle of the glucose oxidase/peroxidise method involves the oxidation of D-glucose by glucose oxidase to produce hydrogen peroxide and subsequent reaction of the hydrogen peroxide with a dye in the presence of peroxidase enzyme to produce a coloured product (Figure 2.2). The concentration of the final product which is measured at 510 nm with a spectrophotometer is equivalent to the glucose concentration. Contents of resistant starch and of non-resistant (solubilised) starch were calculated using a glucose standard curve (Figure 2.1). Total starch content was calculated as the sum of resistant starch and non resistant starch.

2.2.3.4 Amendments to the megazyme protocol

The method by megazyme is good for the determination of RS in starches and flours, with good reproducibility (Table 2.3). The reason for this is the removal of soluble starch and alcohol soluble substances before solubilisation of starch with KOH. However, the protocol may not be appropriate for measuring non-resistant starch and total starch content of flours and samples containing significant quantities of soluble sugars (glucose and sucrose). This is because the step which involves the use of absolute ethanol and 50% ethanol to remove the soluble starch will also remove soluble sugars and will therefore lead to an overestimation of non-resistant starch content and subsequently an overestimation of the total starch content. Moreover, the low pH (4.5 - 6) used in the protocol may lead to hydrolysis of some of the sucrose in solution in samples susceptible to hydrolysis e.g. plantains (this is discussed in detail in chapter 5). Due to this reason, an additional step that involves removal of soluble sugars for flours was incorporated before the digestion protocol outlined above. This involved treating the samples with 10 ml of 80% ethanol for 10 min in a water bath at 37^{o}C, centrifugation at 1500*g* for 10 min after which the supernatant was discarded. This was done three times followed by a final rinse with absolute ethanol, centrifugation and decanting of supernatants. The residues were subsequently air-dried in a fume cupboard overnight.

2.2.4 Englyst's method

2.2.4.1 Measurement of G20 and G120

For 10 analysis tubes, each of 500 mg sample, 9 g of pancreatin was mixed with 60 ml deionised water and stirred on a magnetic stirrer for 10 min, the resulting suspension was subsequently centrifuged at 1500*g* for 10 min at 20^{o}C; 45 ml of the supernatant was taken and mixed with 4 ml amyloglucosidase and 3 ml invertase. A 5 ml aliquot of the enzyme mixture and 4 glass balls (~15 mm diameter) was used for the digestion. Incubation of sample with digestive enzymes was done at pH 5 and 37°C in capped tubes immersed horizontally in a shaking water bath. At all times a sample blank was prepared in duplicate. A value for rapidly available glucose (RAG) was measured as the glucose released from the food at 20 min (G20), of enzyme incubation and G120 as glucose released at 120 min of enzyme incubation. Glucose was determined in all the samples using a glucose oxidase/peroxidise analysis. This initial protocol was however, modified to give targeted reference (control) values as explained in the next section and as outlined on Table 2.1.

2.2.4.2 Determination of total glucose (TG)

2.2.4.2.1 Dispersion of resistant starch

After removal of the G120 aliquots from the tubes, the tubes were removed from the shaking water bath, vortex-mixed vigorously and the rack of tubes

was placed in a boiling water bath for 30 min. The tubes were then removed from the bath, vortex-mixed and placed in ice-water until thoroughly chilled. A 10 ml aliquot of 7 M KOH was added into each tube, the tube was capped, and the contents were mixed by inversion. The tubes were immersed horizontally in a shaking water bath containing ice for 30 min.

2.2.4.2.2 Hydrolysis of starch to glucose

The tubes were removed singly from the ice-water and 0.2 ml of the contents taken into a tube containing 1 ml of 1 M acetic acid. To these tubes 40 µl of amyloglucosidase solution was added. Tubes were mixed and placed into a 70^{o}C water bath for 30 min followed by 10 min in a boiling water bath. The tubes were cooled to room temperature before addition of 8 ml of ethanol; this was the total glucose (TG) portion and was measured by glucose reaction with GOPOD reagent.

2.2.4.3 Measurement of free-sugar glucose

A sample of 0.5 g of sample was weighed into a 50 ml tube**,** 20 ml of water was added before capping the tube. The tube was then heated in a boiling water bath for 30 min, the tube was vortex-mixed, cooled to 37 ^{o}C, before the addition of 0.2 ml of invertase. The tubes were capped and immersed horizontally in the shaking water bath at 37 ^{o}C for 30 min. It was then vortex-mixed vigorously before removal of 0.2 ml into a test-tube containing 4 ml of methanol.

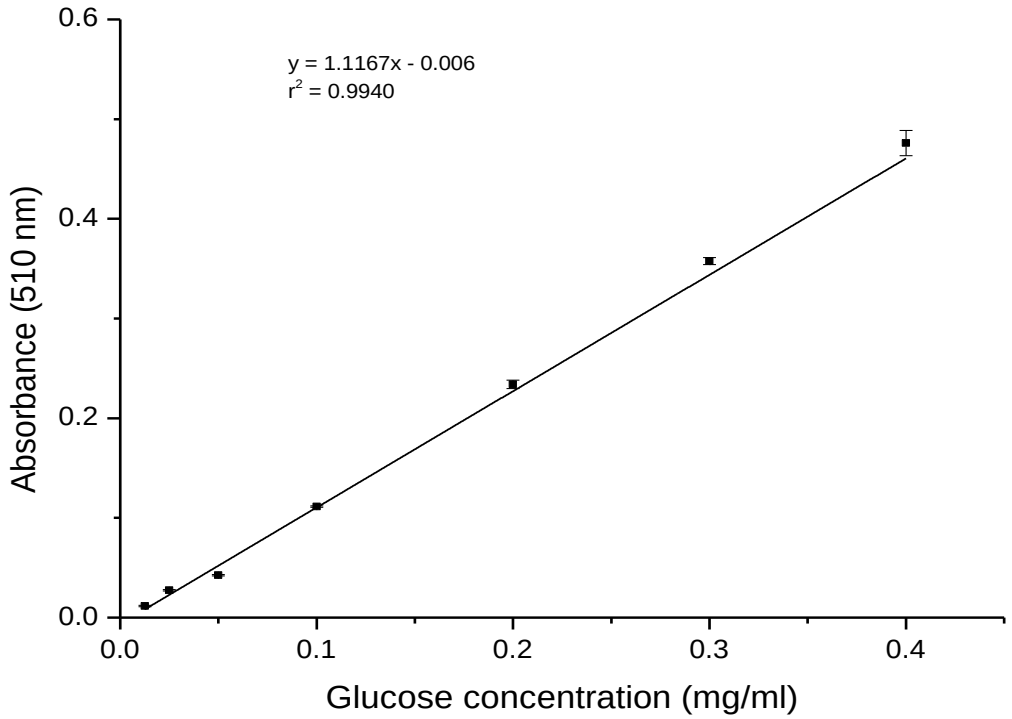


Figure 2.1: Glucose standard calibration curve
Glucose stock solution (1 mg/ml) was prepared in deionised water and further dilutions were made by taking aliquots for the preparation of standards in the range of 0.0125 – 0.4 mg/ml. 100µl vol plus 3 ml glucose/oxidase was incubated for 20 min at 40Oc before taking measurements against a reagent blank at 510 nm using a spectrophotometer

The free-sugar glucose (FSG) portion was measured by glucose reaction with GOPOD reagent and estimated from the glucose standard curve.

2.2.4.4 Estimation of resistant starch

Resistant starch and total starch were estimated from the values of TG, G120 and FSG using the equations below.

TS = (TG - FSG) x 0.9

RS = (TG - G120) x 0.9

(Englyst *et al*., 1992)

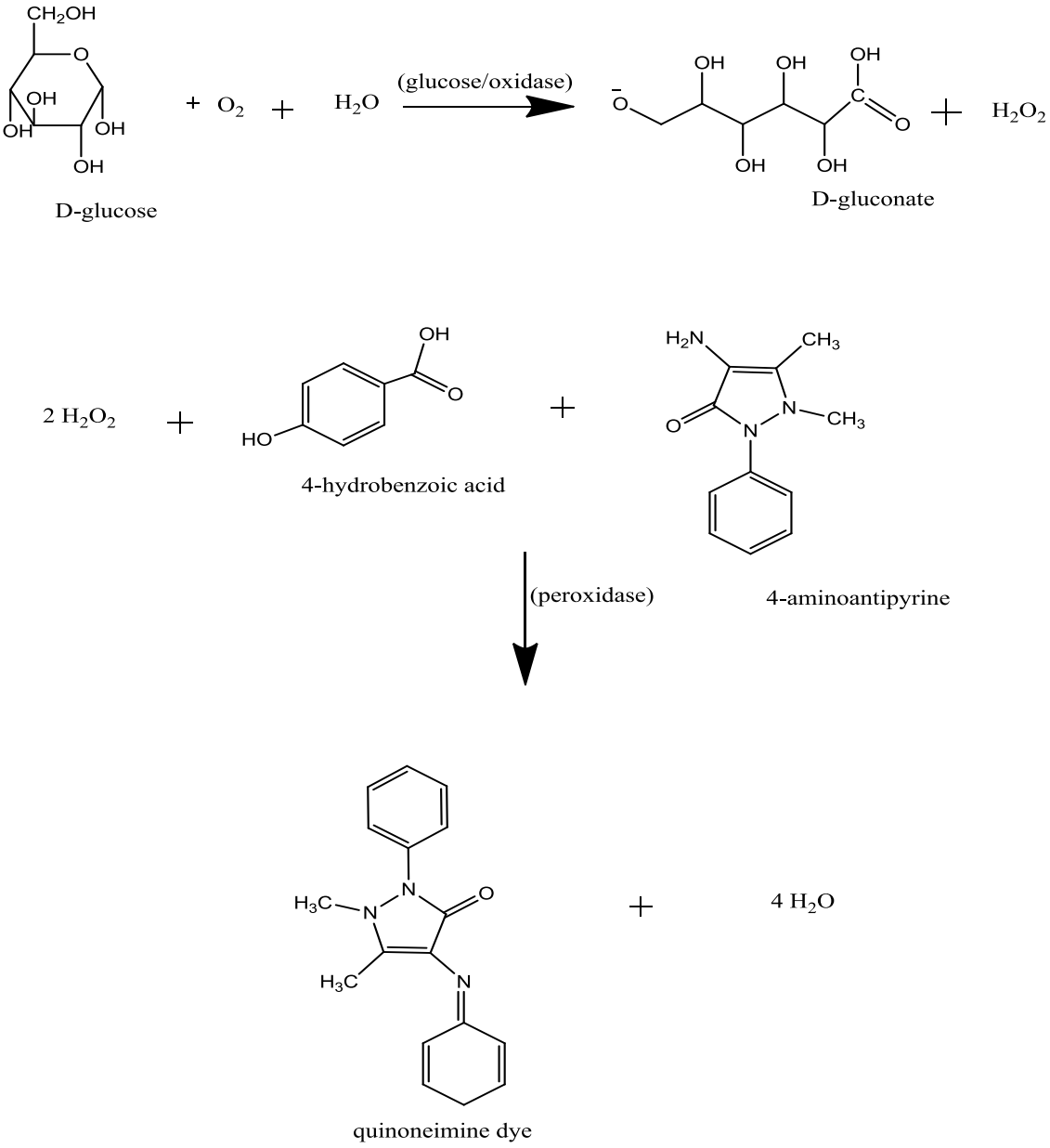


Figure 2.2: Reaction mechanism of glucose oxidase/peroxidise with D-glucose
The reagent was prepared by mixing glucose oxidase plus peroxidase and 4-aminoantipyrine (all 3 supplied as a freeze-dried powder) in p-hydroxybenzoic acid buffer (pH 7.4). The principle involves the oxidation of D-glucose by glucose oxidase enzyme to produce hydrogen peroxide and subsequent reaction of the hydrogen peroxide with a dye (4-aminoantipyrine) in the presence of peroxidase enzyme to produce a coloured product (quinoneimine dye). The concentration of the final product which is measured spectrophotometrically at 510nm is equivalent to the glucose concentration. The sweet pink colour formed with glucose was stable at room temperature for at least two hours after development.

2.2.4.5 Optimising enzyme activity in Englyst's method

Enzyme activity optimised for the protocol is presented in Table 2.1 below. The values for protocol 'G' were the ones that were not significantly different from the control values. Enzyme concentrations were subsequently optimised for different sample weights as shown in Table 2.2, however, the water bath speed had to be optimised as well, in order to attain the desired control values.

2.2.4.5.1 The role of reference materials in the *in vitro* digestion process

Wheat flour

Wheat flour has high SDS content and was used to check the efficiency of starch hydrolysis during the amylolytic incubation. Values for G120 that were too low (when compared with the control values (Table 2.1), indicated that the activity of the amylolytic enzymes was insufficient.

Cornflakes

Cornflakes have high RDS content and were used to check the efficiency of starch and maltose hydrolysis during the first stage of amylolytic incubation. Values for G20 that were too low (Table 2.1), indicated that the amyloglucosidase activity was insufficient.

Potato starch

Glucose release from potato starch is limited by the rate at which the surface of the starch granule becomes available to the amylolytic enzymes, which is determined by the extent of mechanical disruption, i.e., by glass balls and the

effect of the speed of the shaking water-bath. Potato starch was used to establish the optimum speed of the shaking water bath during the amylolytic incubation. If the G120 value for the potato starch was too low (Table 2.1), speed was increased and vice versa.

2.2.4.6 Modifications

2.2.4.6.1 The problem with invertase

Invertase was removed from the enzyme mixture because it interfered with the digestion process. From data on Table 2.1, G20 values (in the presence of invertase) suggest that amyloglucosidase activity was inhibited by ~12% while G120 values indicate a reduction in the overall activity of the amylolytic enzymes by ~ 6.5% in the presence of invertase.

2.2.4.6.2 Free glucose

Free glucose (FG) was determined instead of free sugar glucose since invertase had been excluded from the enzyme mixture. This was done by treating the sample with acetate buffer solution (pH 8.9) and placing the tube in a water bath at 100°C for 30 min. It was then vortex-mixed vigorously before removal of 0.2 ml into a test-tube containing 4 ml of methanol. The free glucose (FG) portion was determined from the glucose standard curve.

2.2.4.6.3 Determination of total glucose

Results obtained were not reproducible especially in samples that were not pure starches. The reason for this is not clear, however, this may be due to

hydrolysis of other polysaccharides by KOH and interference from other substances.

A value for total glucose (TG) was obtained by gelatinising samples at 100^{o}C before digestion and measuring glucose with GOPOD reagent.

2.2.4.7 Method chosen for determination of resistant starch

Values for RS (after optimisation and modifications) were not significantly different ($p \leq 0.05$) between two the methods, however, Englyst's method was chosen to be used in the thesis because it can be used to measure the different types of RS, as well as the RDS and SDS which serve as a means of measuring the rate of starch digestion. These additional parameters are critical to the determination of GI. In addition, the Megazyme method is designed for use in the analysis of dry powders whereas the Englyst's method allows for analysis of foods in the form in which they are usually eaten (i.e. without drying and grinding).

Although Englyst's method has been described as complicated and laborious (Finocchiaro *et al*., 2009), a major advantage of this method is that it is relatively fast, when compared with Megazyme assay that requires 16 hours digestion. Moreover, a proper understanding of the principles involved in the Englyst's method enables modifications to suit desired purposes, for example unnecessary steps can be eliminated to make the protocol less complicated

Tabl e 2.1: Optimisation of enzyme activity for starch digestion *in vitro*

	A	B	C	D	E	F	G	H	Control	Control*
pH	**5**	**5**	**5**	**5**	**5**	**5**	**7**	**7**		
Amyglucosidase	4ml	2ml	4ml	5ml	5ml	5ml	5ml	5ml		
Invertase	3ml	3ml	6ml	4ml	4ml	0ml	0ml	4ml		
Pancreatin	9g	9g	10g	9g	10g	10g	10g	10g		
Parameter	**G20**	**G20**	**G20**	**G20**	**G20**	**G20**	**G20**	**G20**	**G20**	
Wheat flour	23.3 ± 1.1^{b}	20.6 ± 2.4^{a}	27.4 ± 3.0^{c}	30.5 ± 1.3^{de}	32.6 ± 0.9^{ef}	38.1 ± 0.9^{h}	36.1 ± 1.4^{gh}	28.9 ± 1.3^{cd}	35 ± 1^{fg}	
Cornflakes	63.2 ± 3.0^{b}	57.3 ± 1.1^{a}	69.4 ± 0.2^{c}	69.8 ± 1.5^{c}	70.5 ± 1.3^{c}	76.6 ± 1.0^{d}	75.6 ± 0.6^{d}	70.9 ± 1.2^{c}	79 ± 1^{e}	75 ± 1^{d}
Raw potato starch	4.0 ± 0.6^{b}	1.3 ± 0.5^{a}	4.3 ± 0.4^{b}	4.7 ± 0.5^{b}	3.8 ± 0.7^{b}	4.6 ± 0.4^{b}	4.2 ± 0.7^{b}	4.0 ± 0.5^{b}	4 ± 0.5^{b}	
Unripe pl. flour	4.3±0.5	1.8±0.6	4.4±0.7	4.6±0.2	4.5±1.1	4.4±0.6	3.8±0.5	4.4±0.6	NA	
Ripe pl. flour	14.5±2.0	14.8±0.5	15.1±0.8	15.9±1.2	19.2±1.1	19.7±1.6	7.3±1.1	16.2±1.3	NA	
Parameter	**G120**	**G120**	**G120**	**G120**	**G120**	**G120**	**G120**	**G120**	**G120**	
Wheat flour	57.3 ± 1.2^{b}	49.2 ± 1.9^{a}	60.3 ± 1.7^{c}	70.4 ± 1.0^{d}	71.5 ± 0.9^{d}	72.2 ± 1.2^{d}	77.0 ± 0.3^{e}	70.3 ± 0.5^{d}	77 ± 1^{e}	
Cornflakes	72.7 ± 1.1^{b}	66.8 ± 1.5^{a}	72.4 ± 0.9^{b}	72.2 ± 0.8^{b}	73.3 ± 0.7^{b}	76.3 ± 0.9^{c}	77.6 ± 1.2^{c}	71.6 ± 0.8^{b}	81 ± 1^{d}	77 ± 1^{c}
Raw potato starch	24.9 ± 1.1^{bcd}	9.3 ± 1.1^{a}	23.1 ± 1.0^{b}	26.4 ± 1.9^{cde}	24.5 ± 1.0^{bc}	28.2 ± 0.6^{e}	26.7 ± 1.2^{de}	25.5 ± 0.5^{cd}	26 ± 1^{cd}	
Unripe pl. flour	14.9±0.7	9.9±1.5	16.5±1.1	15.3±0.9	14.7±1.6	16.4±1.2	13.7±0.9	16.8±1.6	NA	
Ripe pl. flour	20.0±2.2	15.5±1.9	19.5±1.6	22.5±1.2	21.1±1.3	20.5±1.2	8.4±1.0	16.8±1.3	NA	

Englyst's recommended enzyme concentration is 'A' but was adjusted using reference samples and target (control) values supplied in the protocol. Control* represents the control values minus free sugar glucose content. Enzyme concentrations are for 10 tube of 0.5 g sample per tube. G20 and G120 values are in g/100 g DWB. Only enzyme mixture 'G' has values that are not significantly different (n=3, $p \leq 0.05$) (gray highlight) from the target vales in all instances. Values with the same superscript in the same row are not significantly different from each other at 95% confidence level.

Table 2.2: Enzyme concentration and speed optimized for different sample weights

Sample weight (mg)	Pancreatin (g)	AMG (ml)	Water bath speed (rev/min)	Vol of water in pancreatin (ml)	supernatant taken (ml)	Total enzyme volume (ml)	Added water to make up to 50ml	Vol of enzyme for digestion (ml)
500	10	5	160	60	45	50	0	5
400	8	4	140	60	45	49	1	5
300	6	3	120	60	45	48	2	5
200	4	2	100	60	45	47	3	5
100	2	1	80	60	45	46	4	5

Enzyme concentration (G) from Table 2.1 was used to calculate the concentrations needed for some sample weights. Enzyme concentrations were based on 2 g/g pancreatin/sample and 1ml/g amyloglucosidase/sample. The speed of the water bath was optimised with potato starch to achieve target values outlined in Table 2.1. Sample weights are on DWB and if wet samples are to be used, the moisture contents are first determined in order to estimate the dry weight content of the sample. Values are for ten sample tubes. AMG = amyloglucosidase

In this thesis, the Englyst's protocol has been modified to suit the samples used for each chapter and the determination of each type of resistant starch.

It should be noted that glucose release and absorption in the small intestine does not occur as discrete increments, but is rather a continuous process. Likewise, glucose release during *in vitro* amylolytic hydrolysis for the determination of the G20 (RAG) and G120 (SAG) fractions is a continuous process, even though measurements are taken at those points. Therefore, the RAG and SAG terms should be viewed as measures of the rate at which glucose is `likely' to become available for absorption in the small intestine (Englyst *et al*., 2004).

2.2.5 Determination total starch content

The method of Englyst without the KOH hydrolysis step was adopted for the determination of total starch in samples in this work. This involved heating starch suspension in excess moisture (100 - 500 mg in 15 ml H_2O) for 30 min and subsequent hydrolysis of the soluble starch to glucose with pancreatin and amyloglucosidase as earlier described in section 2.2.3.

The KOH hydrolysis step is required for the hydrolysis of RS3, which is not solubilised by heating at 100^{o}C. In cases where this is required, extraction of sugars and soluble starch before solubilisation of enzyme resistant starch pellets (as in the case of Megazyme method described earlier) is recommended.

2.2.6 Determination of amylose content

2.2.6.1 Amylose-Iodine complex formation method

The method of Hoover and Ratnayake (2001) with slight modification was used. 20 mg of sample was weighed in a round-bottomed tube, an 8 ml aliquot of 90% dimethylsulphoxide (DMSO) was added and the tube was heated in a water bath at 85^{o}C for 15 min with intermittent mixing. The tube was allowed to cool to room temp (~ 45 min) and checked for the presence of clear gels (or else the procedure was repeated with a fresh sample). A 50 µl aliquot of the dissolved sample (dispersed starch solution) plus 2.5 ml water was taken in a glass tube and 500 µl Iodine/Potassium iodide solution was added. This was mixed vigorously and allowed to stand for 30 min before measurement of the absorbance at 600 nm against a reagent blank (50 µl 90% DMSO + 2.5 ml water + 500 µl Iodine/Potassium iodide solution).

The total amylose content for each sample was calculated from the standard curve (Figure 2.3) which was prepared over the range of 0 - 100% amylose using mixtures of pure amylose and amylopectin standards.

2.2.6.2 Determination of amylose by the megazyme method

The megazyme assay for amylose/amylopectin is sold as a kit and the procedure is a modification (involves using an ethanol pre-treatment step to remove lipids prior to analysis) of a concanavalin A (Con A) method developed by Yun and Matheson (1990). The principle involves complete

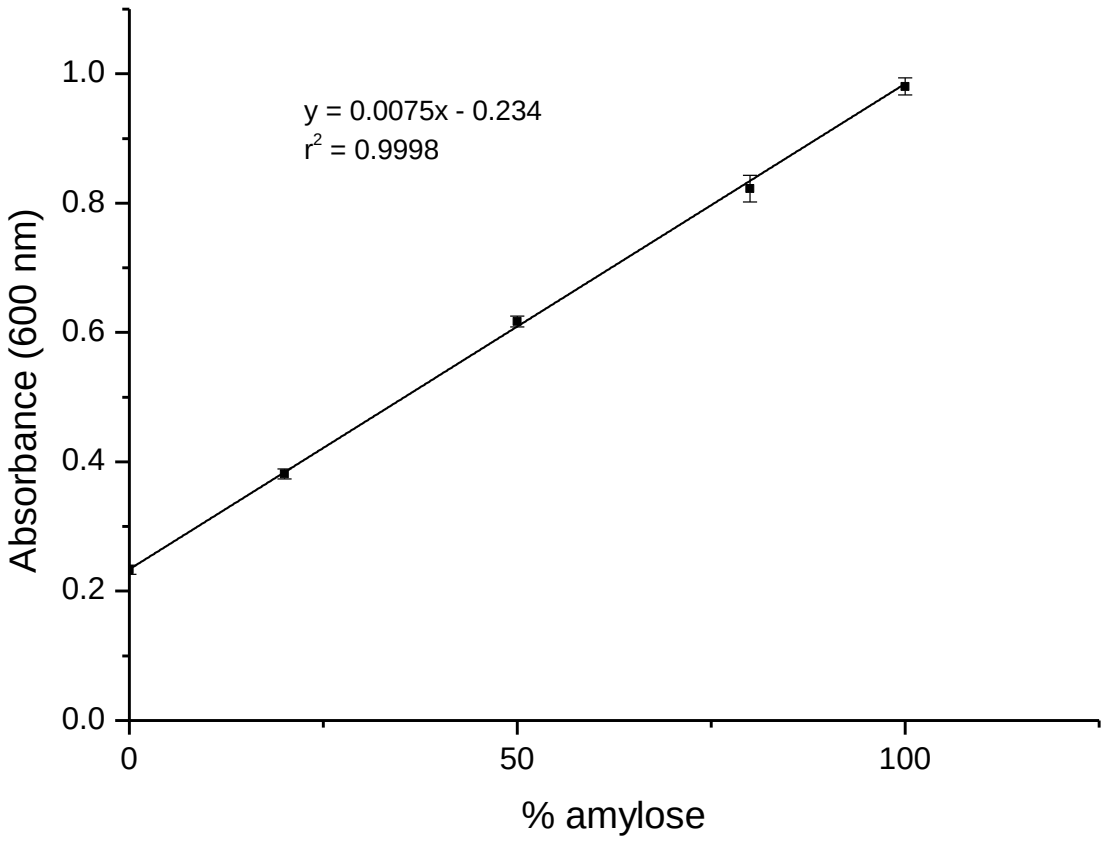


Figure 2.3: Amylose standard calibration curve
Mixtures of 20+0, 16+4, 10+10, 4+16 and 0+20 mg of amylose+amylopectin were used to prepare 100, 80, 50, 20 and 0% amylose standards respectively. 50 µl of the dissolved standard plus 2.5 ml water was mixed with 500 µl Iodine/Potassium iodide solution and the absorbance read at 600 nm against a reagent blank. The absorbance of 0% amylose is due to the I_2 affinity of the long outer branches of amylopectin.

dispersion of starch by heating in dimethyl sulphoxide (DMSO), and precipitation of starch with ethanol. After dissolution of the precipitated sample in an acetate/salt solution, amylopectin is specifically precipitated by the addition of Con A and removed by centrifugation. The amylose, in an aliquot of the supernatant, is hydrolysed to D-glucose with amyloglucosidase,

which is analysed using glucose oxidase/peroxidase reagent. The total starch, in a separate aliquot of the acetate/salt solution, is similarly hydrolysed to D-glucose and measured colorimetrically by glucose oxidase/peroxidase. The concentration of amylose in the starch sample is estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample, to that of the total starch content.

2.2.6.3 Experimental procedure for the megazyme method

2.2.6.3.1 Starch pre-treatment

A 20 mg of sample was weighed into a 10 ml screw capped tube. 1 ml of DMSO was added to the tube while gently stirring it at low speed on a vortex mixer. The tube was capped and heated in a boiling water bath until the sample was completely dispersed (approx. 1 min). It was ensured that no gelatinous lumps of starch were remaining. The content of the sealed tube was vigorously mixed at high speed on a vortex mixer and the tube was heated in a water bath for 15 min, with intermittent high-speed stirring on a vortex mixer. The tube was then stored at room temperature for ~ 5 min and 2 ml of 95% (v/v) ethanol was added with continuous stirring on a vortex mixer. A further 4 ml of ethanol was added; the tube was capped and inverted to mix. A starch precipitate was formed, and the tube was allowed to stand for 15 min. The tube was centrifuged at 2,000*g* for 5 min. The supernatant was discarded and the tube was properly drained on tissue paper for 10 min. The pellet was used in the subsequent amylose and starch determinations. A reference sample (with specified amylose content, supplied with the kit) was included in

each batch. 2 ml of DMSO (with gentle vortex mixing) was added to the starch pellet. The tube was placed in a boiling water bath for 15 min and mixed occasionally to ensure that there were no gelatinous lumps. On removing the tubes from the boiling water bath, 4 ml of Con A solvent (600 mM sodium acetate buffer pH 6) was immediately added to the tube, mixed thoroughly and the content of the tube was quantitatively transferred (by repeated washing with Con A solvent) to a 25 ml volumetric flask. This was further diluted to volume with Con A solvent (this resulting solution is called solution A and was required to be analysed within 2 hours if retrogradation and precipitation of amylose is to be avoided).

2.2.6.3.2 Con A precipitation of amylopectin and determination of amylose

A 1 ml of solution A was transferred to a 2.0 ml Eppendorf microfuge tube and 0.50 ml of Con A solution was added, the tube was capped and gently mixed by repeated inversion while avoiding frothing of the sample. The tube was allowed to stand for 1 hour at room temperature and centrifuged at 14,000g for 10 min at room temperature. 1 ml of the supernatant was separated into a 15 ml centrifuge tube; 3 ml of 100 mM sodium acetate buffer, pH 4.5 was added (to reduce the pH to ~ 5). The contents were mixed, lightly capped (with a marble) and heated in a boiling water bath for 5 min to denature the Con A. The tube was placed in a water bath at 40°C and allowed to equilibrate for 5 min. 0.1 ml of amyloglucosidase/α-amylase enzyme mixture was added and incubated at 40°C for 30 min, after which it was centrifuged at

2,000g for 5 min. To 1.0 ml aliquots of the supernatant 4 ml of GOPOD reagent was added, incubated for 20 min at 40°C. (The reagent blank and the D-glucose controls were incubated concurrently). The absorbance of each sample and the D-glucose controls was read at 510 nm against a reagent blank.

2.2.6.3.3 Determination of total starch

0.5 ml of solution A was mixed with 4 ml of 100 mM sodium acetate buffer, pH 4.5. 0.1 ml of amyloglucosidase/α-amylase solution was added and the mixture was incubated at 40°C for 10 min. Glucose was determined concurrently with that of section 2.2.6.3.2 above.

Calculation of amylose content (%):

$$\text{Amylose, \% (w/w)} = \frac{\mathbf{A510\ nm\ (Con\ A\ supernatant)}}{\mathbf{A510\ nm\ (Total\ starch\ aliquot)}} \times \frac{\mathbf{6.15}}{\mathbf{9.21}} \times 100$$

$$= \frac{\mathbf{A510\ nm\ (Con\ A\ supernatant)}}{\mathbf{A510\ nm\ (Total\ starch\ aliquot)}} \times 66.8$$

Where 6.15 and 9.2 are dilution factors for the Con A and total starch extracts respectively.

2.2.6.4 Megazyme versus iodine method

The starch iodine method gave an amylose content of 22.7 ± 1.8 (n = 5) for plantain starch. However, when amylose content was determined on flours *in situ* results obtained was 8.6 ± 1.4 and 16.7 ± 0.9 %, n = 5, for ripe and unripe

plantain flours respectively. These values were then converted to the relative starch contents of the samples and total amylose content was 24 ± 2.1 and 23.2 ± 1.6 %, n = 5, for ripe and unripe plantain, hence, the determination of amylose content of flours needs to take into account the relative amount of starch in the sample. A conversion factor from flour to total starch content needs to be used to accurately estimate the amylose content. This observation may the reason for conflicting data and the wide variation in amylose content (8.6 - 38.8%) previously reported for plantain (Soares *et al*., 2011).

Precipitation of amylopectin appears to be a better alternative for the determination of amylose because under defined conditions of pH, temperature and ionic strength, Con A specifically complexes branched polysaccharides based on α-D-glucopyranosyl or α-D-mannopyranosyl units at multiple non-reducing end-groups with the formation of a precipitate. Thus, Con A effectively complexes the amylopectin component of starch but not the primarily linear amylose component. However, the experimental protocol by Megazyme is very long and quite cumbersome and reproducibility of results requires extra measures (especially in terms of timing) which are not usually feasible in a multiple-user laboratory.

Other problems that may arise from the use of this protocol as experienced during the analyses is that in this method, extraction with 95% ethanol at room temperature, precipitates starch and removes lipids but may not

completely remove soluble sugars from all types of samples (e.g. plantains, as discovered in this work). The procedure was therefore modified for adaptation for use for flours so as to incorporate the removal of sugars in order to avoid overestimation of starch and amylose contents**.**

Based on the issues above, it is recommended therefore that determination of total amylose content should not be performed on flours *in situ.*

2.3 Sample collection

Plantain samples were bought from three different shops in Leeds city market in order to establish sample homogeneity during the work. Resistant starch contents of the samples collected are presented in Table 2.3. For ripe samples obtained from shop A at different times, RS values were significantly different ($p \leq 0.05$) from each other but mean RS values for ripe samples were not significantly different for the three shops. In the case of unripe samples, RS values were not significantly different from each other for samples obtained from the three shops. Samples required for analysis in this thesis were subsequently obtained from shops B and C.

Table 2.3: Resistant starch content of ripe and unripe plantain (g/100g, DWB)

	Sampling time	Shop A	Shop B	Shop C
Ripe	**1**	29.8±1.6^{a}	33.9±0.9^{a}	33.0±1.3^{ab}
	2	31.0±1.2^{ab}	33.9±0.9^{a}	31.0±1.6^{ab}
	3	33.5±1.4^{bc}	33.2±1.0^{a}	30.4±1.2^{a}
	4	29.8±1.0^{a}	33.6±1.4^{a}	34.2±1.5^{b}
	5	35.4±1.1^{c}	33.4±0.4^{a}	31.6±0.1^{ab}
	Mean	**31.9±2.5^{x}**	**33.6±0.8^{x}**	**32.0±1.7^{x}**
Unripe	**1**	63.4±0.7^{a}	61.4±1.6^{a}	59.5±1.6^{a}
	2	61.9±1.5^{a}	60.9±1.3^{a}	59.9±1.8^{a}
	3	61.2±0.9^{a}	61.6±1.3^{a}	62.0±0.8^{a}
	4	61.8±1.2^{a}	61.5±0.6^{a}	61.8±1.4^{a}
	5	62.1±0.7^{a}	61.7±1.1^{a}	61.6±0.9^{a}
	Mean	**62.1±1.1^{x}**	**61.4±1.0^{x}**	**60.9±1.5^{x}**

Plantain samples were randomly collected five times from each of the three shops and at each collection time resistant starch analysis was carried out in duplicate. The mean RS value for each shop represents an average and standard deviation of 10 replicates. RS values with the same superscript in the same column are not significantly different from each other at 95% confidence level, while mean RS values with the same superscript in the same row are not significantly different ($p \leq 0.05$).

Chapter 3 Determination of gelatinisation temperature of starch in plantain products *in situ*

3.1 Hypothesis

Cooking processes employed for the consumption of plantain foods may not allow starch to be completely gelatinised and these foods will therefore contain some levels of native resistant starch (Type 2 RS).

3.2 Abstract

Gelatinisation temperature in excess moisture was determined on plantain starches and flours and an attempt was made to deduce the degree of starch gelatinisation in whole cooked foods. The gelatinisation temperature of plantain starch was determined using an enzymatic method (with confirmation from amylose leaching and microscopic methods). Starch gelatinisation temperature for plantain starch was observed at 75 ± 2^{o}C while it was 80 ± 2^{o}C for starch in the flour. The enzymatic digestion method was then applied for the determination of the *in vitro* degree of starch digestion in ripe and unripe flours of plantain (*in situ*) at different treatment temperatures, and an attempt was made to deduce the equivalent processing temperatures of cooked foods from the flours using three equations. One-way anova statistical analysis was used to determine the equation that gave the best fit and this was applied to the values obtained for the degree of starch gelatinisation obtained from enzyme incubation in some cooked plantain foods. Processing temperatures extrapolated from the equation of best fit ranged between 73.2 ± 0.3 - 79.5 ± 0.2^{o}C and 67.8 ± 0.5 - 80.1 ± 1.3^{o}C for ripe and unripe plantain foods respectively. Processing temperatures obtained also revealed that some of the samples were cooked either at the gelatinisation temperature or at temperatures very close to the gelatinisation temperature of plantain starch. Under normal domestic cooking conditions applied here, with the use of salt, it is required that plantain products be cooked for at least 20 min before starch can be completely gelatinised. Since starch is completely gelatinised at

the gelatinisation temperature and no resistant starch type 2 remains, processing conditions become very important in preserving the nutritional quality of starch with regards to ungelatinised/native starch in plantain foods.

3.3 Introduction

Ripe and unripe plantain in their raw/uncooked forms contain about 32 g/100g and 61 g/100g native resistant starch respectively (refer to chapter 2). Native resistant starches suffer poor enzyme digestibility but this quality is lost once they are gelatinised and they become easily and rapidly digested (Englyst *et al*., 1992). When starch granules are heated in the presence of water, an order-to-disorder phase transition, referred to as gelatinisation, occurs. The determination of gelatinisation temperature and extent of starch gelatinisation becomes very important because most foods are usually processed before consumption. In the past, gelatinisation has been considered as affecting product quality through changes in physicochemical properties such as texture, viscosity, etc.; however, with the increasing incidence of diet-related health conditions such as diabetes and obesity, it has become important to consider the health implications of processing starch. The nutritional properties of starch are, to a large extent, related to its availability for digestion and/absorption in the gastrointestinal tract, hence starch bioavailability is also very much dependent on processing conditions (Qiang, 2005).

Much work has been done on starch gelatinisation temperature (Shyam, 2009) but it is not certain if this can be applied to domestic cooking which does not involve the use of pure starches or flours, but whole foods. In some studies, the degree of gelatinisation had been determined but this has not been linked to gelatinisation temperature (Baks *et al*. (2007), Liu and Han (2012).

Domestic cooking temperatures by many individuals are not usually regulated so as to preserve food nutrients, because the need to satisfy hunger is sometimes given priority over the nutritional values that can be derived from the meal. The ability to introduce a system where domestic cooking can be optimised by effective monitoring of food processing temperatures to preserve the nutritional qualities of starch will be of great benefit to all. This is much more relevant in an age where there are a lot of campaigns regarding the consumption of more healthy foods.

Although it is known that most resistant starches do not survive hydrothermal treatment, especially under high moisture and high temperature treatments, it is not certain if some or all domestic cooking processes used for some foods actually retain significant quantities of resistant starch. This is because in domestic cooking it is quite difficult to ascertain the starch concentration of the sample/food. The determination of the degree of starch gelatinisation will help to maintain good levels of resistant starch in processed foods. Zhang *et al* (2005), indicated that a lot of questions still remain unanswered regarding cooked banana starch (in that review, cooked banana starch referred to

starches of the *musa* family), especially regarding its digestion properties. In this chapter, we are concerned with the fate of starch in domestically processed plantain, especially green plantains due to their significantly high content of native resistant starch (RS2).

Numerous approaches based on starch properties have been used to follow the gelatinisation process. These include granule swelling, water holding capacity, starch–iodine complex formation/amylose leaching, solubility, paste viscosity, birefringence, nuclear magnetic resonance (NMR), X-ray diffraction patterns, differential scanning calorimetry (DSC), electrical conductivity and enzyme susceptibility, (Miao *et al*. (2010), Baks *et al*. (2007), Chaiwanichsiri *et al*. (2001)). Previously, loss of birefringence was the most broadly used technique but it is lengthy and usually subject to sampling errors. This is because starch granules are generally difficult to count in heterogeneous mixtures and starch cannot be easily separated from other components in the cooked material. At the moment, DSC and viscosity are the most commonly used methods to assess starch gelatinization; however DSC requires an equipment that is not easily accessible in food industry laboratories, and viscosity measurement (amylographic method) is not so reliable to monitor the gelatinization process (Di Paola *et al*., 2003). The fact that gelatinised starch is susceptible to enzyme hydrolysis has been a basis for the study of the gelatinisation process and proves to be one of the most sensitive methods

for the measurement of this parameter, (Tester and Sommerville (2001), Shyam (2009)).

In the past, starch gelatinisation had been checked mainly for textural qualities of starch but recently, a major concern is the nutritional quality of starch. This has led to many studies of which glycaemic index stands out as a major tool. Although it is true that many food products are formulated from starches and flours, a large group of foods are also consumed as whole foods. Differential scanning calorimetry has been applied for the measurement of starch gelatinisation *in situ* in a few studies (Pelissari *et al*., 2012) but it is not certain if this can be applied to all concentrations of food materials as accuracy of DSC measurements is somewhat limited to some starch/water concentrations. Moreover, the presence of other more crystalline particles in the sample may interfere with the melting process which is the basis for DSC measurements (Abboud and Hoseney (1984), Eliasson (1992)).

It is true that gelatinisation temperature is a function of starch-in-water concentration, and this has caused some forms of limitation to the determination of gelatinisation temperatures of some concentrations using certain methods (Garcia *et al*. (1996), Garcia-Alonso *et al*. (1999), Eliasson (1992)). The strategy adopted in this chapter involved the determination of the gelatinisation temperature in excess water, determination of the degree of digestion and deduction of equations to relate the two together.

In this work, we have determined the gelatinisation temperature of plantain starch using an enzymatic method (with confirmation from amylose leaching and microscopic methods). This was then applied to the determination of starch gelatinisation temperature in ripe and unripe flours of plantain *in situ* and an attempt was made to deduce the equivalent treatment temperatures of cooked foods from the flours and subsequently the degree of gelatinisation. An advantage of this method is that it does not require costly instrumentation or special devices such as DSC and biosensor methods which have also been applied to measurement of starch gelatinisation *in situ*, even though these were not applied to whole foods (Marconi *et al*., 2004).

3.4 Materials and Methods

3.4.1 Materials

Materials used are as described in the previous chapter.

3.4.2 Sample preparation

Samples were prepared as described in the previous chapter.

3.4.3 Starch isolation

Flour from unripe plantain was used for starch extraction because of higher starch content. The first step was to eliminate pigments and sugars by extraction and centrifugation with 50% ethanol/water mixture until the colour and sugars were removed (complete removal of sugars was ascertained by testing the supernatant for sugars using phenol-sulphuric acid method).

Aqueous starch slurry (about 5% starch) was prepared from the residue and filtered through a 100 µm aperture sieve to remove fibre and other non-starch particles. The starch obtained was rinsed several times with water and subsequent sedimentation to obtain a pure starch product that was free from proteins and lipids (with proteins and lipids below detection limit), (Regmi *et al*. (2011), Garcia-Alonso *et al*. (1999)). This was subsequently freeze-dried to less than 1% moisture content and stored in plastic containers at room temperature for further analysis. The purity and yield of the starch was 90 ± 1.1% and 50 ± 3.2%, n = 3, respectively.

3.4.4 Domestically Prepared Foods

3.4.4.1 Boiling

One finger of plantain (ripe or unripe) was cut into five portions and cooked in 750 ml boiling water (ordinary/pre-salted by addition of 3 g of table salt). One finger each was removed from the cooking pot at 3, 6, 10, 15 and 20 min, cut into thin slices on petri-dishes and quenched in liquid nitrogen. Samples were then stored in the -80^{o}C freezer and freeze-dried, blended and sieved to obtain fine powder and stored in plastic containers for the digestion experiment. For digestion, 500 mg of cooked and dried samples in 15ml water were subjected to enzymatic hydrolysis.

3.4.4.2 Grilling

150 g of sample was grilled at high/medium heat in a grill for 30 min.

For each cooking experiment, 3 fingers were used and the experiment was repeated three times.

3.4.5 Heat treatment

500 mg starch/flour was treated with 15 ml de-ionised water and vortex-mixed for 5 min to produce a starch suspension. This was subsequently incubated in a shaking water bath for 30 min at temperatures ranging from 40^{o}C to 100^{o}C. After incubation, the sample tube was immediately transferred to another water bath maintained at 37^{o}C to prevent retrogradation from occurring. The tubes were allowed to equilibrate for about 15 min before commencement of the digestion process.

3.4.6 Digestion

The procedure of Englyst *et al*., (1992) with slight modification was used. For 10 analysis tubes, 10 g of pancreatin was mixed with 60 ml deionised water and stirred on a magnetic stirrer for 10 min, the resulting suspension was subsequently centrifuged at 1500*g* for 10 min at 20^{o}C; 45 ml of the supernatant was taken and mixed with 5 ml amyloglucosidase. A 5 ml aliquot of the mixture was used for the digestion. Invertase was omitted because it interfered with the digestion (see chapter 2). Incubation of sample with pancreatic α-amylase and amyloglucosidase was done at pH 7.0 and temperature 37°C in capped tubes immersed horizontally in a shaking water-bath. At all times, a sample blank was prepared in duplicate. A value for rapidly available glucose (RAG) was measured as the glucose released

from the food at 20 min (G_{20}), of enzyme incubation. For flours, an aliquot was taken before digestion as the free glucose (FG). For cooked samples, tubes were incubated at 37^{o}C, for 30 min, an aliquot was then taken for FG, before the commencement of enzyme digestion. Glucose was determined using the glucose oxidase peroxidise (GOPOD) analysis kit as described in chapter 2.

3.4.7 Determination of leached amylose

Total amylose was determined by the method of Hoover and Ratnayake (2001). Leached amylose was determined on heated treated samples at each temperature considered. A 0.2 ml aliquot of heat-treated sample suspension was taken in 2.67 ml water and centrifuged. Then 500 µl of I_2/KI reagent (0.5395 g KI plus 0.1575 g Iodine in 500 ml of water) was added to 250 µl aliquot of the supernatant in 2.5 ml water. Tubes were allowed to stand for 30 min in the dark and the absorbance read against a blank at 600 nm. The percentage amylose leached was calculated from the total amylose content determined for each starch.

3.4.8 Microscopy

Field Emission Gun Environmental Scanning Electron Microscope (FEGESEM) Quanta 200F model, made by FEI was used for the microscopic observations. Samples were run in the ESEM mode. Briefly, a small sample of starch slurry was placed on an aluminium stub, which was then placed on a

black blocky stage called a "Peltier Cooling Stage" for the resulting pictures to be taken.

3.4.9 Statistical analysis

All analyses carried out were performed on 3 sets of five plantain fingers, each analysed in triplicate. Data obtained (except for microscopy) was analysed using analysis of variance (ANOVA), and expressed as mean ± standard deviations.

3.5 Results and discussion

Extrapolation of gelatinisation temperature from *in vitro* rate of starch digestion was carried out on dry powdered samples using parameters described by Englyst i.e. G20 (glucose released in the first 20 min of enzyme incubation) and G120 (glucose released between 20 and 120 min of enzyme incubation). Gelatinisation temperature was determined on plantain starch using pure potato starch as a reference/control. This is because the gelatinisation temperature of potato starch has been well studied for decades and data for validation is more readily available. Many methods have been used for the determination and validation of the gelatinisation temperature of potato starch; some of these are indicated in Table 3.1 on page 78.

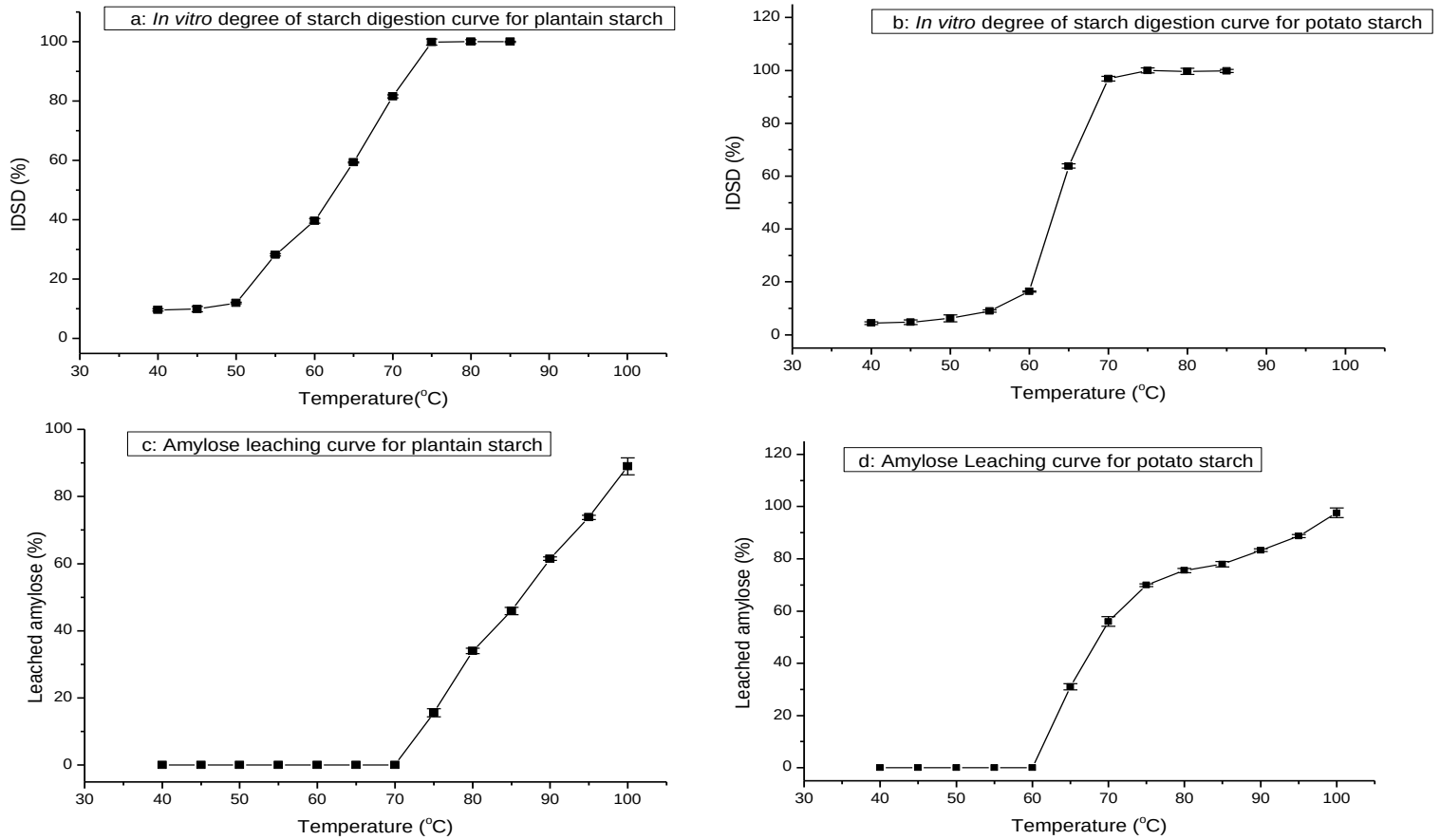


Figure 3.1: Controlled gelatinisation temperature curves for plantain and potato starches
c and d are leached amylose curves for the determination of gelatinisation temperature; potato shows a point of inflexion at 70°C while plantain shows no inflexion; a and b show similar curve patterns for monitoring gelatinisation in both potato and plantain starches. 3 replicates were performed; some standard deviation bars are hidden behind data points. IDSD is defined in section 3.5.2.

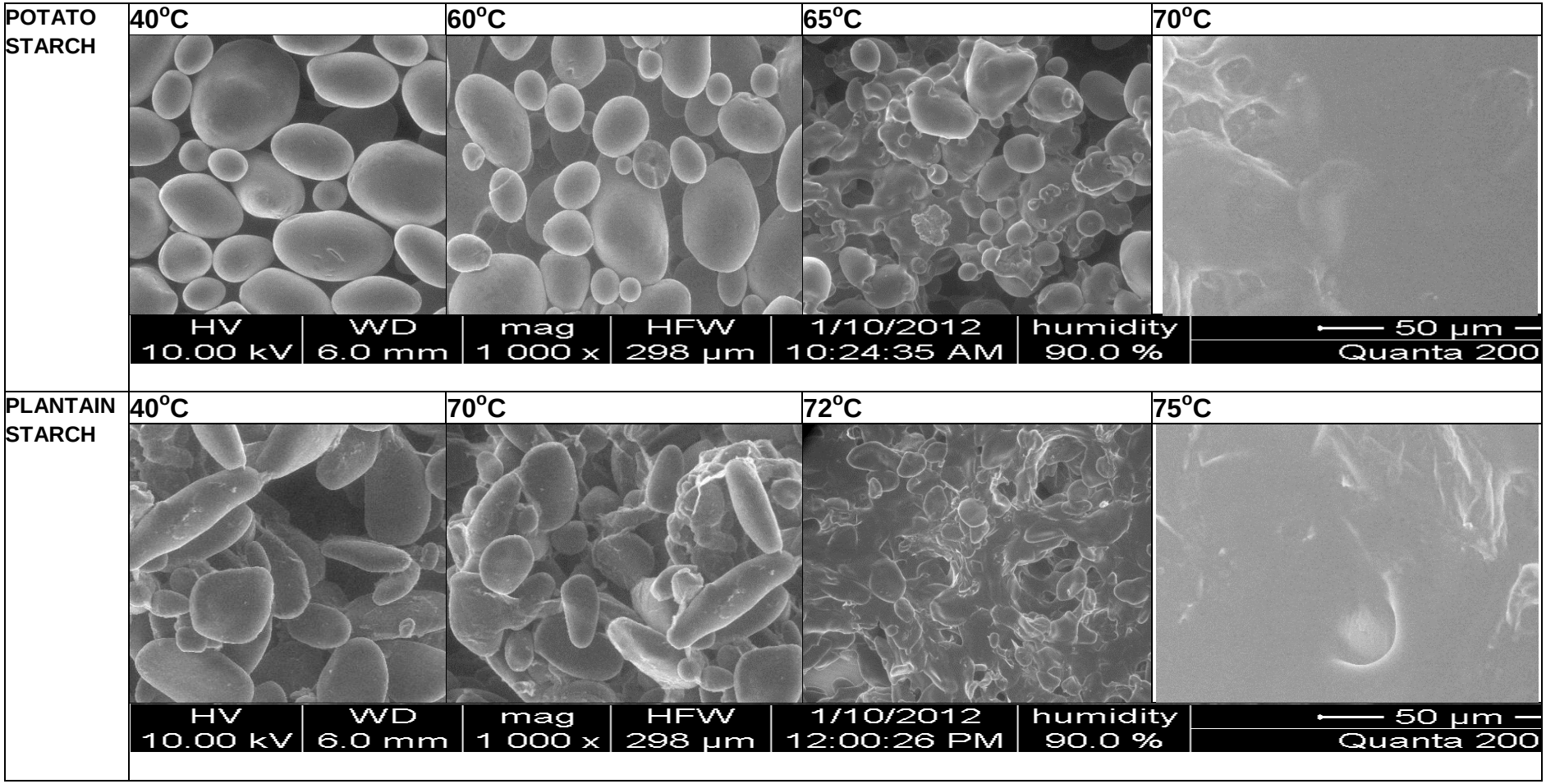


Figure 3.2: Environmental Scanning Electron Microscopy observations of potato and plantain starch at selected temperatures
500 mg/15 ml potato/plantain starch slurries after heat treatment at temperatures indicated were used to monitor progress in starch gelatinisation by disappearance of starch granule morphology. At gelatinisation temperature (70^{o}C for potato and 75^{o}C for plantain), starch granules had melted.

3.5.1 Graphical method for the determination of gelatinisation temperature

The *in vitro* degree of starch digestion (IDSD) (IDSD is defined in section 3.5.2) was plotted against temperature of the hydrothermal treatment of starch (Figures 3.1a and b). In Figure 3.1a, IDSD was constant between 40 - 45^{o}C; however there was an increase in IDSD from 50^{o}C up to 75^{o}C when IDSD became constant. A change in IDSD is an indication of the susceptibility of starch to enzyme hydrolysis. The fact that IDSD becomes constant with further increases in temperature at 75^{o}C, indicates there are no more starch granules to be hydrolysed and this point can be considered as the gelatinisation point/temperature for plantain. A confirmation of this behaviour was done using pure potato starch which gave a similar curve style to that observed with plantain starch (Figure 3.1b) but with gelatinisation point/temperature at 70^{o}C. Amylose leaching curves (Figures 3.1c and d) were also used to deduce the gelatinisation temperature for both plantain and potato starches. In the amylose leaching method, the gelatinisation curves obtained for potato and plantain were not similar as in the case of the enzymatic method (Figures 3.1a and b). It is interesting to note however that the leached amylose versus temperature curve obtained for potato (Figure 3.1d), has a similar outline/pattern to that for the leaking of potato earlier reported by Magnus and Elliasson (2006). The graph of % leached amylose versus temperature for potato starch indicates that amylose began to be leached after 60^{o}C, there was a linear curve observed between 60 - 70^{o}C (a similar trend to the % IDSD versus temperature curve for potato (Figure 3.1b)

and at 70^{o}C, a deviation from linearity was observed. This was further confirmed by checking the granule morphology with an environmental scanning electron microscope (ESEM), at various temperatures, namely 40, 60, 65 and 70^{o}C (Figure 3.2) for potato starch. ESEM observation reveals that at 70^{o}C no starch granules remained. The value obtained is in tandem with previous results obtained from determination in excess water of potato gelatinisation temperature (Table 3.1).

Table 3.1: Gelatinisation temperature of potato determined by various authors

Method	Gel	Reference
DSC	71.2	Donavan and Mapes (1980)
DSC	71.0	Eberston *et al*. (1980)
Microscopic	68	Eberston *et al*. (1980)
Plastograph	70	Goto (1969)
Microscopic	68	Schoch and Maywald (1956)

Adapted from (Shyam, 2009)

The % amylose leaching versus temperature curve for plantain starch showed a different pattern to that observed for potato. This may be due the fact that amylose leaching (which is very much dependent on swelling is always a function of botanical origin (Tester and Sommerville, 2001). Figure 3.1c indicates that amylose began to leach from plantain starch after 70^{o}C and continues at a steady linear rate, and even at 100^{o}C, amylose was not completely leached. There was no other change observed on the amylose leaching curve up to 100^{o}C, so we had to rely on the digestibility and

microscopic results (Figure 3.2) to confirm the gelatinisation temperature. The observations in this work reveal that the use of amylose leaching may be a good indicator of the onset temperature of gelatinisation but not the final/gelatinisation temperature and therefore susceptible to some pitfalls and errors for some types of starches. This fact is supported by some other authors (Baks *et al*., 2007).

Having established that the enzymatic method was good at predicting accurately the gelatinisation temperature at low starch concentrations, this was then applied to ripe and unripe plantain flours and the gelatinisation curves obtained are as shown in Figures 3.3a and b. The point at which further increases in temperature resulted in no further increase in IDSD i.e. the gelatinisation temperature was observed at 80^{o}C. It is interesting to note that both ripe and unripe plantain flours have the same starch gelatinisation temperatures; however the curve patterns indicate that the gelatinisation rate in the ripe flours is much slower especially below 75^{o}C, this may be unconnected with the fact that ripe plantains contain more sugar than the unripe. The presence of sugars has been associated with an increase in gelatinisation onset temperature (Magnus and Eliasson, 2006)**.** Gelatinisation temperature determined here using the enzymatic method is similar to the work of Pelissari *et al* (2012), where values of 78.3 ± 0.2^{o}C and 80.5 ± 0.3^{o}C were obtained for unripe plantain starch and flour, respectively using DSC.

The use of an enzymatic method provides an advantage over other methods because it also gives a clue to the digestibility of starch as a function of

temperature. Starch digestibility is a subject that is attracting more interest today, due to its importance in nutrition because of its connection to blood glucose response and glycaemic index of foods (Wachters-Hagedoorn *et al*., 2004). Rapidly digestible starch will therefore be a useful tool for the assessment nutritional the quality of starch in whole foods prepared from plantains.

3.5.2 Mathematical deduction of gelatinisation temperature

For purpose of clarity we define the following terms;

RDS = G20 X 0.9 (for pure starch)

RDS = (G20 – FG) X 0.9 (for samples containing sugars)

NB: RDS = rapidly digestible starch, IDSD = *in vitro* degree of starch digestion, G20= glucose released after 20 minutes of enzyme incubation, FG = free glucose in sample.

(Englyst *et al*., 1992)

For the purpose of this work we redefine RDS for a particular temperature as $G20_t(S)$ and RDS at complete gelatinisation as $G20_g(S)$.

The first step was to determine RDS at complete gelatinisation ($G20_g$ (S)) and this was based on the principle of total starch determination (without consideration of retrograded starch).

Observations

Below the gelatinisation temperature $G20_t(S) < G20_g(S)$

At gelatinisation temperature and beyond $G20_t(S) = G20_g(S)$

We therefore define a term for the extent of gelatinisation based on the rapidly digestible starch as:

$$\boldsymbol{IDSD} = \frac{\mathbf{G20t}(\boldsymbol{S})}{\mathbf{G20g}\,(\boldsymbol{S})} \times 100,$$ where IDSD = *In vitro* degree digestion

When IDSD = 100%, there is complete gelatinisation.

It is possible to define the gelatinisation temperature as the point when IDSD becomes 100%, subject to a rational standard deviation. This was used for plantain and potato starch above, and the corresponding temperature were 75^{o}C and 70^{o}C respectively. It therefore implies that RDS values can be used without plotting them as a function of temperature, but by converting them to their corresponding IDSD values for mathematical inferences rather than plotting them as absolute values. However IDSD values can also be used for the graphical inference of gelatinisation temperature as described in the previous section.

3.5.3 Determination processing temperatures of whole plantain foods

A graph of IDSD as a function of temperature was plotted for ripe and unripe plantain flours (Figures 3.3a and b). The graphical appearance of the curves

led to the need to develop appropriate equations for the purpose of relating the two functions.

Equation 1

A logistic fit nonlinear equation curve fitting on Origin software (Figure 3.4) gave the following equation for ripe and unripe flours with the corresponding parameters in Table 3.2.

IDSD = $\frac{A1-A2}{1+\left(\frac{T}{T0}\right)^{p}} + A2$ 1

Where T= pre-treatment temperature, A_1, A_2, p and T_0 are equation constants whose values are given in Table 3.2.

Equation 2

Points AB and BC were plotted separately (Figures 3.3b1 and b2) and the resulting equations were solved simultaneously to give rise to a linear equation (2).

IDSD = 4.8T - 246.6 2

(where T is treatment temperature)

This was only applicable to unripe sample as two straight lines could not be extrapolated from the ripe sample gelatinisation curve.

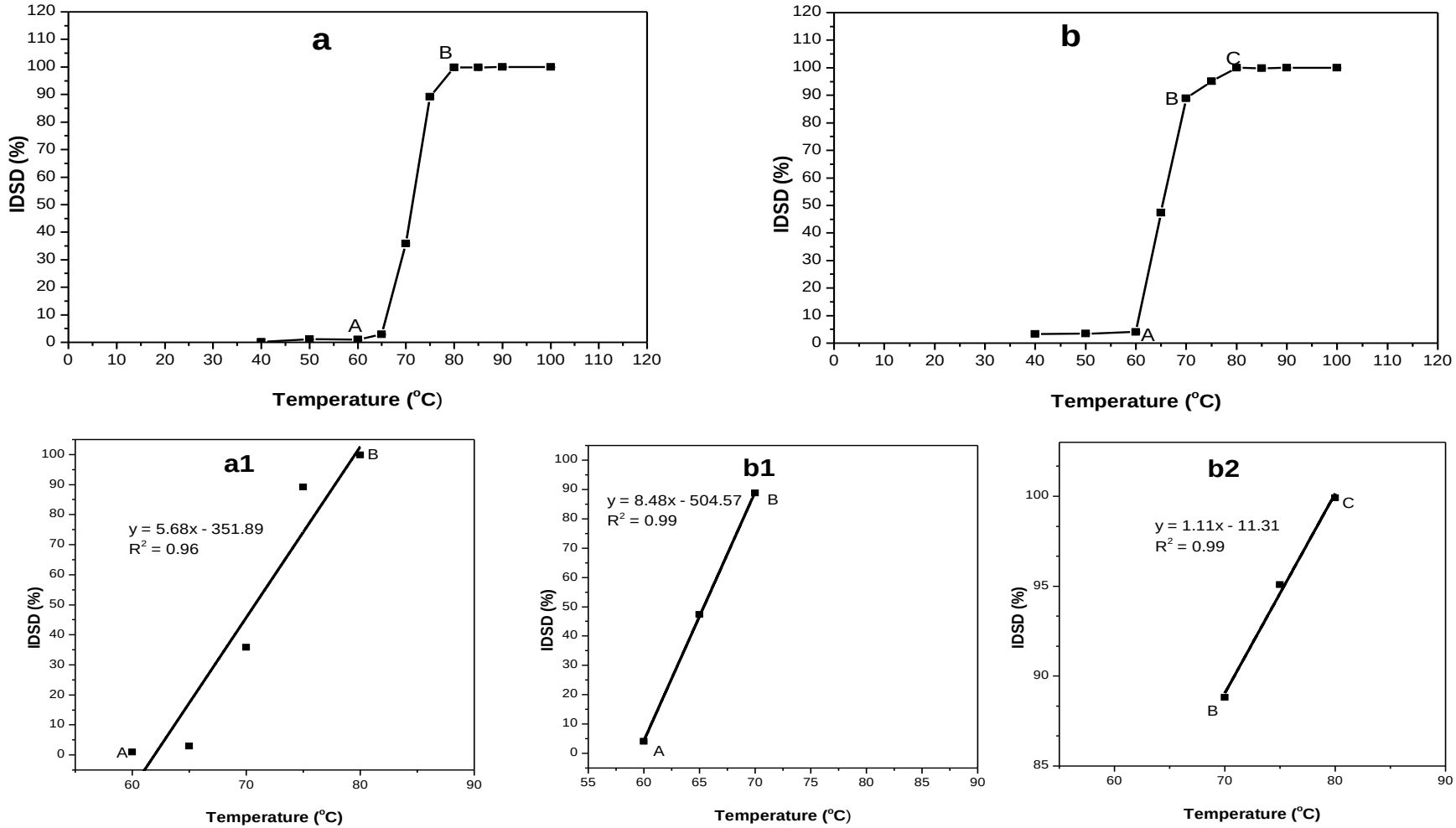


Figure 3.3: Controlled gelatinisation curves for ripe and unripe plantain flours.
Plots 3a and 3b are the plots of the value of IDSD Vs. temperature at increasing temperatures for controlled gelatinisation for ripe and unripe plantain flours respectively. Plot 3a1 was extracted from portion AB of 3a while plots b1 and b2 were extracted from portion AB and BC of plot b respectively. Equations generated through these plots were used in mathematical deduction of equivalent processing temperature and the degree of gelatinisation for cooked plantain foods, as described in section 3.5.3.

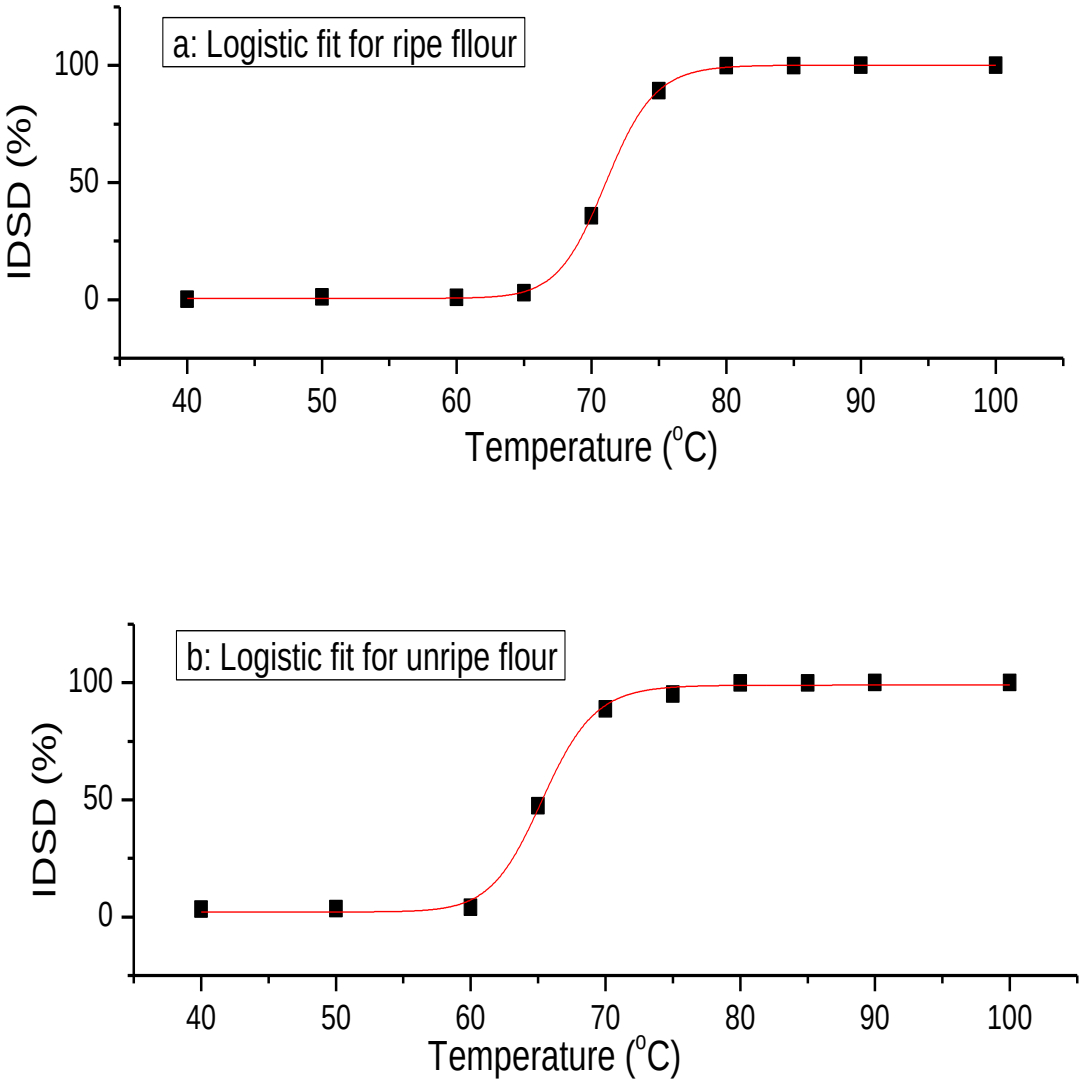


Figure 3.4: Nonlinear curve for the plot of IDSD vs. Temperature for ripe and unripe plantain flour
Points were plotted to obtain an equation for the relationship between *in vitro* digestion rate and temperature using a nonlinear curve. (Eqn 1 above was generated from these graphs)

Table 3.2: Parameters for nonlinear curve fitting for equation 1 and figure 3.4

	Unripe plantain		**Ripe Plantain**	
ADJ R^2	**0.99**		**0.99**	
Parameter	**Value**	**Std error**	**Value**	**Std error**
A1	2.137	1.473	0.554	0.269
A2	98.984	1040	100.149	0.265
T^0	65.3525	0.194	71.089	0.043
P	33.808	3.670	39.392	0.864

Values were generated on origin software curve fit

Equation 3

Equation of plots for points AB for ripe sample (Figure 3.3a) and equations for plots of points AB and BC (treated separately) for unripe sample were used.

IDSD = 5.7T – 351.8.....3 (ripe)

IDSD = 8.4T – 504.6.....3a (unripe)

IDSD = 1.1T – 11.3.....3b (unripe)

For all the equations, some selected values of IRSD in the range of the curves were used to determine corresponding temperature and then compared to real values using a one-way analysis of variance at 95% confidence level (Table 3.3). Equation 3 was chosen for both ripe and unripe samples because it has the highest number of values that were not significantly different from the real values. This was then applied to the determination of the degree of gelatinisation in some domestically prepared plantain foods (Table 3.4). The degree of gelatinisation for domestically cooked plantain foods were obtained from the deduced equivalent treatment temperatures using the equation below.

Degree of gelatinisation (DG) = $\left(\frac{Treatment\ temp\ (sample)}{gelatinisation\ temperature}\right)$ x 100

Recommendation for the application of this principle to other starches: Due to the fact that some starches behave differently (Eliasson, 1992), which may result in different types of curves, it is logical to start from the curve of IDSD as a function of temperature and then to graphically determine the best fit plot and equation to be used. Values obtained for corresponding treatment temperatures and the degree of gelatinisation of domestically prepared plantain (Table 3.4) indicate that temperature values ranged between 73.2 ± 0.3 – 79.5 ± 0.2^{o}C for ripe samples and between 67.5 ± 0.4 and 80.1 ± 1.3^{o}C for unripe plantain samples. Plantain products (ripe and unripe) cooked at 10 minutes and above are completely gelatinised but those cooked with salt are not completely gelatinised until after 20 min of cooking. There was no data available for comparing these values. Although boiling of plantains is usually done for about 10 - 15 min before consumption, we have included 3 and 6 minutes to see progression in the rate of gelatinisation. Data obtained for IDSD indicate that samples cooked with salt have slower rates of gelatinisation in the first few minutes of cooking, and this observation is more pronounced with the unripe plantain products. This is not unconnected with impact of salts on gelatinisation (Wootton and Bamunuarachchi (1980), Lii and Lee (1993)). Although most of the studies done were on pure starches it is interesting to observe a similar effect even in the presence of other food components.

Table 3.3: Comparison of equations for determination of best fit

IDSD (%)	EQN 1 (^oC)	EQN 2 (^oC)	EQN 3 (^oC)	IDEAL FIG (^oC)
RIPE				
2.9±0.6	64.9±0.2^b	N/A	62.5±0.6^a	65^b
35.8±1.2	70.2±0.8^a	N/A	68.3±1.4^a	70^a
89.1±0.5	75.2±0.3^a	N/A	77.6±0.6^b	75^a
99.9±0.1	84.8±0.2^b	N/A	79.5±0.5^a	80^a
100±0.2	90.1±0.3^b	N/A	79.6±0.6^a	80^a
UNRIPE				
4.0±1.1	70.0±0.5^c	52.3±0.6^a	60.0±0.0^b	60^b
47.4±1.3	70.2±0.9^c	61.3±1.0^a	65.1±0.2^b	65^b
95.1±0.8	70.5±0.5^a	71.3±0.9^a	75.4±0.1^b	75^b
99.9±0.2	70.8±0.7^a	72.3±0.1^b	79.8±0.3^c	80^c
100±0.2	71.3±0.7^a	72.3±0.3^a	79.9±0.9^b	80^b

n=3, values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level). N/A = not applicable

Table 3.4: Deduced treatment temperatures and degree of gelatinisation for domestically cooked plantain foods

	Ripe			unripe		
Sample	**IRD (%)**	**Eqv treatment**	**DG (%)**	**IRD (%)**	**Eqv treatment**	**DG (%)**
BWS						
3 min	64.1±1.6^{a}	73.2±0.3^{a}	91.5±0.4^{a}	68.4±0.9^{a}	67.6±0.1^{a}	84.4±0.1^{a}
6 min	81.6±1.9^{c}	76.3±0.3^{c}	95.4±0.4^{c}	82.3±1.0^{b}	69.2±0.1^{ab}	86.5±0.1^{ab}
10 min	94.0±1.5^{d}	78.5±0.3^{d}	98.1±0.3^{d}	90.4±1.0^{c}	71.0±0.9^{b}	88.7±1.15^{b}
15 min	97.4±1.1^{d}	79.1±0.2^{d}	98.9±0.2d^{e}	97.8±1.7^{de}	77.9±1.6^{d}	97.4±2.0d^{e}
20 min	98.4±0.8^{e}	79.3±0.2^{e}	99.1±0.2^{e}	99.3±1.2^{e}	79.2±1.1^{de}	99.1±1.4^{de}
CWOS						
3 min	67.4±3.3^{ab}	73.8±0.6^{ab}	92.3±0.7^{ab}	88.4±1.8^{c}	69.9±0.2^{b}	87.4±0.3^{b}
6 min	81.8±4.7^{c}	76.4±0.8^{c}	95.4±1.0^{c}	95.6±1.4^{d}	75.9±1.3^{c}	94.9±1.6^{c}
10 min	98.2±1.2^{e}	79.2±0.2^{e}	99.0±0.3^{e}	99.4±1.6^{e}	79.3±1.4^{de}	99.1±1.7^{de}
15 min	98.7±1.9^{e}	79.3±0.3^{e}	99.2±0.4^{e}	100.3±1.5^{e}	80.1±1.3^{e}	100.1±1.7^{e}
20 min	99.8±1.0^{e}	79.5±0.2^{e}	99.4±0.2^{e}	100.0±1.0^{e}	79.8±0.9^{e}	99.8±1.1^{e}
Grilled	70.4±2.0^{b}	74.3±0.4^{b}	92.9±0.4^{b}	67.8±3.3^{a}	67.5±0.4^{a}	84.4±0.5^{a}

BWS= boiled with salt, CWOS= boiled without salt, n=3. Values with the different superscripts in the same column are significantly different at 95% confidence level.

3.6 Conclusion

The enzymatic method of measuring starch gelatinisation developed in this chapter is suitable for the determination of gelatinisation temperatures in starches, flours and degree of gelatinisation and processing temperatures in whole foods when milled. It is useful for both retrograded and unretrograded food samples because it does not take into account the retrograded portion of the food. It is generally agreed by many authors that only gelatinised portion of starch can retrograde (Garcia-Alonso *et al*. (1999), Gonzalez-Soto *et al*. (2006), Tian *et al*. (2012)), therefore if there is any ungelatinised starch

granules in the food it is not affected by the inference made. Since $G20_g(S)$ is determined based on the available carbohydrates in the food when heated in excess moisture at 100^oC, it implies that RS1, RS2 and RS3 have been eliminated. RS1 was eliminated by grinding the food samples, RS2 was eliminated by heating samples in excess water to 100^oC and RS3 was not considered because solubilisation in potassium hydroxide or dimethyl sulphoxide was not done (Murphy *et al*. (2008), Englyst *et al*. (2004)). The use of KOH is time consuming and has the additional drawback of likelihood of solubilising non-starch polysaccharides. DMSO on the other hand does inhibit digestive enzymes and results in underestimation of starch.

This enzymatic method will allow the comparison of degree of gelatinisation and pre-treatment temperatures of different concentrations of the same type of food. Due to the fact that IDSD is determined on dry powder, whatever the starting concentration of gelatinised or ungelatinised material, it will be possible to compare IDSD and therefore the degree of gelatinisation and/or treatment temperature.

Chapter 4 Comparing the effect of controlled gelatinisation on nutritional starches and *in vitro* starch digestion in plantain starch and flour

4.1 Hypothesis

Plantain starch is rich in resistant starch; however, the presence of other components in the flour will possibly increase enzyme resistance of plantain starch. Nevertheless, this observation will be dependent on treatment temperature.

4.2 Abstract

The impact of controlled gelatinisation on digestion rates of ripe and unripe plantain flours and their starch isolates as well as changes which occur in nutritional starch fractions namely RDS, SDS and RS were examined. There was virtually no RDS observed in ripe plantain flour at 40 - 45^{o}C and only ~ 1 ± 0.6% (n = 3), of RDS between 50 - 65^{o}C. Marked increases in RDS were observed between 65 and 70^{o}C for all the samples except in ripe plantain flour where this increase was spread over a broader range of temperature i.e. between 65 - 75^{o}C. RS values were significantly different for all samples between 40^{o}C and 55^{o}C with flours showing higher values of RS than the starch isolates. At 60^{o}C and $p \leq 0.05$, starches have RS values that are not significantly different from each other (22.3 ± 1.8% and 21.3 ± 1.4%) while the flours have values not significantly different from each other (89.1 ± 0.4% and 89.5 ± 0.4%). High values of SDS were observed on enzyme digestion of starches, while flours had higher values of RS and RDS than starches. *In vitro* rate of digestion curve patterns indicate that plantain flours offer lower rates of starch digestion at temperatures 40 - 60^{o}C and this is an indication that the use of flours rather than starches of plantain will offer slower digestibility when incorporated into food products. The rate of starch digestion in plantain flours is constant after 120 min, whereas starch digestion continues to increase gradually even beyond 120 min for starches. The results confirm the probable presence of digestion inhibiting substances in plantain flours which may be responsible for their lower starch digestibility at temperatures below

gelatinisation temperature; nevertheless, it seems that these substances are deactivated at temperatures above the gelatinisation temperature.

4.3 Introduction

Plantain and banana starches are amongst starch sources that still remain either unexploited or underutilized. New starches from unconventional sources are beginning to gain interest not just due to their functional characteristics, especially for new food product formulations, but because of their observed nutritional qualities. Plantain is one such starch source that has not been fully assessed.

The impact of banana starch and flour on starch digestibility when added to other foods have been tested (Pacheco de Delahaye (2001), Pacheco-Delahaye *et al*. (2004), Osorio-Diaz *et al*. (2008), Rendon-Villalobos *et al*. (2008)). However, it is not clear from these results if improved nutritional qualities are better from foods supplemented with starches than with flours, because the difference between the influence of non-digestible starch components in flours and starches have not been tested. The quantities of resistant starch, slowly digestible starch and rapidly digestible starches and how these change before and after the gelatinisation temperature have also not been tested. Also information on starch digestibility in ripe plantain is not available. Ripe plantain, processed in many ways, forms an important part of the diet of many tropical populations. It therefore becomes very important that

its digestion properties as well as the impact of heat-moisture treatment on its nutritional properties are studied in order to maximise its benefits.

The nutritional quality of starch, however, strongly depends on its structure and the conditions of its processing (Lehmann and Robin, 2007). When starch is heat-treated in the presence of water, diverse changes are produced; these changes are referred to as gelatinisation, and involve events such as swelling of the granule, leaching of amylose, loss of birefringence, and disorganization of the crystalline order (Henry and Alistair (2006); Biliaderis (2009)). Gelatinisation increases starch digestion and may be a tool to modify the nutritional benefits of starch. The ability to manipulate the hydrolysis of starch granules has nutritional implications. For example the controlled granule hydrolysis of starch products may help to optimise blood glucose and insulin concentrations (Guzar *et al*., 2012).

The increasing incidence of diabetes and obesity in today's world (Narayan *et al*., 2006) has stimulated the search for healthier foods and for better processing methods for foods intended for human consumption. The fact that starchy foods vary in their glycaemic response has been a subject of much interest, especially following the discovery that slowly digested and absorbed carbohydrates are beneficial in the management of hyperglycaemia (Lehmann and Robin, 2007). The dietary quality of starch-based foods is attributed to the relative amounts and proportions of RDS, SDS and RS. A low-GI starch ingredient should contain lower amounts of RDS and higher proportions of SDS and RS (Zhang *et al*., 2008). Information on the digestion

properties of cooked plantain starch as well as the influence of cooking on the rate of digestion is scarce (Zhang *et al*., 2005). In this work, we have attempted to study the differences in starch digestibility and nutritional starches of ripe and unripe plantain starches/flours, and how they change under controlled gelatinisation conditions as a gateway to understanding how the inherent benefits of some of its nutraceutical components can be maximised.

4.4 Materials and method

4.4.1 Materials

Materials used are as described in chapter 2.

4.4.2 Sample preparation

Samples were prepared as described in chapter 2.

4.4.3 Starch isolation

Starch was isolated as described in chapter 3.

4.4.4 Preparation of residues from ethanol extraction

Residues from ethanol extraction of flours were obtained by extracting pigments and sugars with 80% ethanol/water mixture followed by several rinses with water until the colour and sugars were removed (complete removal of sugars was ascertained by testing the supernatant for sugars using the phenol-sulphuric acid method). The residue obtained was then

freeze-dried and stored in clean plastic containers at ambient temperature for further analysis.

4.4.5 Heat Treatment

A 500 mg sample was treated with 15 ml de-ionised water and vortex-mixed for 5 min to produce a starch suspension. This was subsequently incubated in a shaking water bath for 30 min at temperatures ranging from 40^{o}C to 85^{o}C. After incubation, the sample tube was immediately transferred to another water bath maintained at 37^{o}C to prevent the occurrence of starch retrogradation.

4.4.6 Digestion

The procedure of Englyst *et al.,* (1992) with slight modification was used (modifications have been described earlier in chapter 2). For 10 analysis tubes, 10 g of pancreatin was mixed with 60 ml deionised water and stirred on a magnetic stirrer for 10 min, the resulting suspension was subsequently centrifuged at 1500*g* for 10 min at 20^{o}C; 45 ml of the supernatant was taken and mixed with 5 ml amyloglucosidase. Then 5 ml of the mixture was used for the digestion. Invertase was omitted because it interfered with the digestion process (refer to chapter 2). Incubation of sample with pancreatic a-amylase and amyloglucosidase was done at ~ pH 7 and 37°C in capped tubes immersed horizontally in a shaking water bath. At all times a sample blank was prepared in duplicate. A value for rapidly available glucose (RAG) was measured as the glucose released from the food at 20 min (G_{20}), of enzyme

incubation and G_{120} as glucose released at 120 min of enzyme incubation. For flours, an aliquot was taken before digestion as the free glucose (FG). Glucose was determined using the glucose oxidase analysis kit. A value for total glucose (TG) was obtained by gelatinising sample at 100^{o}C before digestion.

4.4.7 Calculations

TS = TG x 0.9

TS = (TG-FG) x 0.9

RDS = G_{20} x 0.9

RDS = (G20 – FG) x 0.9 (for flours)

SDS = (G_{120}-G_{20}) x 0.9

RS = (TG – G_{120}) x 0.9

(Englyst *et al*., 1992)

4.4.8 Statistical analysis

All analyses carried out were performed on 3 sets of samples. Each set was composed of five fingers of plantain and each set was analysed in triplicate. The mean of each set was taken. Data obtained was analysed using analysis of variance (ANOVA), and expressed as mean values.

4.5 Results and discussions

4.5.1 Dietary starches

In this chapter, an *in vitro* procedure developed by Englyst (1992), which involves starch hydrolysis by enzyme susceptibility to pancreatin and amyloglucosidase, has been used to determine nutritionally-important starch fractions (resistant starch, slowly digestible starch and rapidly available starch) in flours and starch isolates from plantain, and to study the changes which occur to these fractions with increasing temperature. Values of starch fractions have been expressed as percentages of total starch content to enable easier comparison of data.

4.5.1.1 Rapidly digestible starch

Results in Table 4.1 reveal that rapidly digestible starch values (RDS) for starches were higher while those for flours were lower than those recorded for native potato starch (< 6% of total starch content), (Lu *et al*., 2012). RDS showed a steady and progressive increase with temperature and demonstrates the progress in gelatinisation of starch as well as the damage/loss of native resistant starch (RS2).

It is interesting to note that there is virtually no rapidly digestible starch in RPF at 40-45^{o}C and ~ 1 - 3% of RDS is present between 50 - 65^{o}C. Marked increases in RDS were also observed between 65 and 75^{o}C for all the samples, however, RPF had the highest increase in RDS between 70 and 75^{o}C. The unique behaviour of ripe plantain flour is not unconnected with the presence of sugars in this sample (Table 4.4). It has been reported that the

presence of sugars (glucose, fructose, fructose, sucrose, maltose and lactose) increases the gelatinisation onset temperature as summarised by Magnus and Eliasson (2006). Sugars reduce the plasticising effect of water on starch by binding to water molecules and subsequent reduction of water available to starch. This effect is more pronounced with disaccharides than with monosaccharides and greatest with sucrose amongst the disaccharides. Also the presence of more than 10% sucrose is said to decrease the swelling volume of starch (Magnus and Eliasson, 2006). Rapidly digestible starch is very important because it has been found to be correlated with glucose response and glycaemic index (Englyst *et al*., 2003a). The lower value of RDS in flours before gelatinisation temperatures (75^{o}C for starch and 80^{o}C for flours, chapter 4) indicate that though plantain starch has low RDS, this is further reduced in the presence of other components of the whole flour. At and after gelatinisation, no significant differences are observed for all samples. This gives an indication that the reduction of RDS in flours may have been due to the presence of some amylase and amyloglucosidase inhibitors or substances which reduce digestion rates in the flours, and which must have

Table 4.1: Rapidly digestible starch content of plantain flour and starch isolates at various temperatures

	RDS (%Total starch)				RDS (g/100g sample)			
TEMP(^{o}C)	**URPS**	**RPS**	**URPF**	**RPF**	**URPS**	**RPS**	**URPF**	**RPF**
40	9.7±0.2^{c}	11.1±0.4^{d}	3.4±0.0^{b}	0.1±0.2^{a}	8.7±0.2	7.8±0.3	2.4±0.0	0.0±0.1
45	9.9±0.1^{c}	11.0±0.2^{d}	3.4±0.2^{b}	0.2±0.2^{a}	8.9±0.1	7.8±0.2	2.4±0.2	0.1±0.1
50	12.0±0.2^{d}	11.4±0.3^{c}	3.5±0.1^{b}	1.0±0.3^{a}	10.8±0.2	8.0±0.2	2.5±0.1	0.4±0.1
55	28.2±0.7^{d}	26.7±1.0^{c}	3.8±0.2^{b}	1.0±0.4^{a}	25.4±0.7	18.8±0.7	2.7±0.2	0.4±0.2
60	39.6±1.5^{d}	34.8±1.1^{c}	4.0±0.3^{b}	1.0±0.6^{a}	35.7±1.4	24.5±0.8	2.8±0.2	0.4±0.2
65	59.3±0.8^{c}	58.5±1.0^{c}	47.0±1.8^{b}	3.0±0.8^{a}	53.4±0.7	41.2±0.7	33.6±1.3	1.1±0.3
70	81.5±0.8^{b}	88.5±1.9^{c}	88.2±0.3^{c}	36.5±2.2^{a}	73.5±0.8	62.3±1.3	63.0±0.2	13.6±0.8
75	99.8±0.2^{c}	99.7±0.5^{c}	94.4±0.7^{b}	88.4±1.8^{a}	89.9±0.2	70.2±0.4	67.4±0.5	33.1±0.7
80	99.9±0.3^{a}	99.6±0.6^{a}	99.3±0.1^{a}	99.3±0.8^{a}	90.0±0.3	70.1±0.4	70.9±0.1	37.1±0.3
85	99.9±0.6^{a}	99.50.3^{a}	99.7±1.6^{a}	99.3±0.2^{a}	90.0±0.5	70.0±0.2	71.2±1.1	37.1±0.1

Values are means ± standard deviations of triplicate determinations. Flour/starch suspension in excess moisture was subjected to heat- treatment at various temperatures. RDS was determined by taking an aliquot of the digesta in the first 20 min of starch digestion after heat treatment at the indicated temperature. Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level). URPS – unripe plantain starch, RPS – ripe plantain starch, URPF – unripe plantain flour, RPF – ripe plantain flour.

Table 4.2: Slowly digestible starch content of plantain flour and starch isolates at various temperatures

	SDS (%Total starch)				SDS (g/100g sample)			
TEMP(oC)	**URPS**	**RPS**	**URPF**	**RPF**	**URPS**	**RPS**	**URPF**	**RPF**
40	28.9±0.04^{c}	24.6±0.4^{c}	10.4±0.4^{b}	8.8±0.0^{a}	26.0±0.4	17.3±0.3	7.4±0.3	3.3±0.0
45	29.4±0.3^{d}	24.8±0.2^{c}	9.9±0.2^{b}	8.6±0.4^{a}	26.5±0.3	17.5±0.2	7.0±0.2	3.2±0.0
50	31.0±0.2^{c}	25.0±0.8^{b}	9.4±0.1^{a}	8.9±0.4^{a}	27.9±0.2	17.6±0.6	6.7±0.1	3.3±0.0
55	32.80±0.9^{c}	28.7±0.9^{b}	8.2±0.5^{a}	8.8±0.3^{a}	29.5±0.8	20.2±0.6	5.8±0.4	3.3±0.0
60	38.1±1.1^{c}	43.2±1.0^{d}	7.0±0.3^{b}	8.8±0.0^{a}	34.3±1.0	30.4±0.7	5.0±0.2	3.3±0.0
65	21.6±0.5^{b}	27.0±0.7^{c}	25.4±1.8^{c}	9.1±0.5^{a}	19.4±0.5	19.0±0.5	18.2±1.3	3.4±0.0
70	11.2±0.4^{c}	7.3±0.3^{b}	5.1±0.8^{a}	19.3±0.8^{d}	10.1±0.4	5.1±0.2	3.6±0.6	7.2±0.0
75	0.0±0.0^{a}	0.0±0.1^{a}	3.8±0.5^{b}	11.9±0.6^{b}	0.0±0.0	0.0±0.1	2.7±0.4	4.4±0.0
80	0.0±0.0^{a}	0.1±0.0^{b}	0.8±0.1^{c}	0.3±0.0^{d}	0.0±0.0	0.1±0.0	0.6±0.1	0.1±0.0
85	0.0±0.0^{a}	0.1±0.1^{a}	0.2±0.2^{a}	0.0±0.0^{a}	0.0±0.0	0.1±0.1	0.2±0.2	0.0±0.0

Values are means ± standard deviations of triplicate determinations. Flour/starch suspension in excess moisture was subjected to heat- treatment at various temperatures. SDS was determined by finding the difference in glucose content between 20 min at 120 min of starch digestion after heat treatment at the indicated temperature. Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level). URPS – unripe plantain starch, RPS – ripe plantain starch, URPF – unripe plantain flour, RPF – ripe plantain flour.

Table 4.3: Resistant starch content of plantain flour and starch isolates at various temperatures

	RS (%Total starch)				RS (g/100g sample)			
TEMP(^{o}C)	**URPS**	**RPS**	**URPF**	**RPF**	**URPS**	**RPS**	**URPF**	**RPF**
40	61.4±0.2^{a}	63.7±0.2^{b}	86.4±0.3^{c}	90.3±1.2^{d}	55.3±0.2	44.9±0.2	61.7±0.2	33.8±0.5
45	60.3±0.5^{a}	63.8±0.1^{b}	86.3±0.1^{c}	90.3±0.6^{d}	54.3±0.4	44.9±0.1	61.6±0.1	33.8±0.2
50	56.9±1.2^{a}	63.4±0.4^{b}	87.2±1.0^{c}	89.7±1.2^{d}	51.3±1.1	44.6±0.3	62.3±0.7	33.5±0.5
55	38.3±0.7^{a}	44.3±2.1^{b}	87.6±1.9^{c}	89.6±0.3^{c}	34.5±0.7	31.2±1.5	62.5±1.4	33.5±0.1
60	22.3±1.8^{a}	21.3±1.4^{a}	89.1±0.4^{b}	89.5±0.4^{b}	20.1±1.6	15.0±1.0	63.6±0.3	33.5±0.2
65	19.4±0.7^{b}	14.3±0.7^{a}	27.5±1.9^{c}	87.5±1.5^{d}	17.5±0.7	10.0±0.5	19.6±1.3	32.7±0.6
70	7.3±0.4^{b}	3.1±0.4^{a}	6.8±0.6^{b}	44.6±1.5^{c}	6.6±0.4	2.2±0.3	4.9±0.4	16.7±0.6
75	0.2±0.2^{a}	0.0±0.0^{a}	1.8±0.1^{b}	0.3±0.3^{a}	0.2±0.2	0.0±0.0	1.3±0.1	0.1±0.1
80	0.1±0.2^{a}	0.0±0.0^{a}	0.1±0.1^{a}	0.0±0.0^{a}	0.1±0.2	0.0±0.0	0.1±0.1	0.0±0.0
85	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Values are means ± standard deviations of triplicate determinations. Flour/starch suspension in excess moisture was subjected to heat- treatment at various temperatures. RS was determined by taking subtracting the glucose content at 120 min of starch digestion after heat treatment at the indicated temperature from the total starch content. Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level). URPS – unripe plantain starch, RPS – ripe plantain starch, URPF – unripe plantain flour, RPF – ripe plantain flour.

been inactivated at high temperatures (Bennett *et al*., 2010). It is therefore reasonable to conclude that plantain starches/flours, like many other starches will have a better effect in reducing glucose response when they are either ungelatinised or partially gelatinised. RDS values at 70^{o}C are not significantly different for RPS and URPF and this may result from the fact that both have similar starch content (Table 4.4), and probably because at this temperature, differences arising from other components may have been inactivated by high temperature treatments.

4.5.1.2 Slowly digestible starch

The SDS portion is digested gradually but completely in the human intestine. The possibility of a starch to generate slowly digestible starch depends to a large extent on the botanical source of the starch and treatment conditions used (Lehmann and Robin, 2007). Treatment conditions tested in this work give very interesting results for SDS (Table 4.2). Unlike the case of RS and RDS, the starches produced higher values of SDS than the flours, especially between 40^{o}C and 60^{o}C. This implies that starches from plantain may be better than flours for use in products requiring more SDS. At 65^{o}C, URPF and RPS are not significantly different from each other and this again may be related to the same content of starch present in both samples. A slower rate of gelatinisation in RPF seems to be responsible for the low quantity of SDS when compared to other samples at temperatures below 70^{o}C, however at this temperature and beyond, ripe plantain flour elicits highest values of SDS.

When starch is completely gelatinised, no SDS values are observed for all samples.

The structural properties of SDS are not well understood and there are no commercial SDS products yet existing, although novel slowly-digestible carbohydrates (SDCs) such as isomaltulose (Palatinose, Palatinit) have been marketed (Lehmann and Robin, 2007). A few studies have investigated the postprandial physiological responses to the ingestion of RDS and SDS in healthy subjects and type 2 diabetics and showed that SDS had more positive impact on GI than did RDS (Ells *et al*. (2005), Harbis *et al*. (2004), Seal *et al*. (2003)).

4.5.1.3 Resistant starch

RS2, a native resistant starch present in some raw foods such as potatoes, green bananas and high amylase corn, is barely digestible. However, in the presence of heat and excess moisture, starch may be gelatinised and become available for digestion. The importance of thermal properties on the digestibility of starches has, therefore, led to the need to optimise processing conditions to maximise their potential benefits. This is especially important for heat processed foods. From Table 4.3, native resistant starch values are significantly different for all samples between 40^{o}C and 55^{o}C. At 60^{o}C starches have RS values that are not significantly different from each other while the flours have values not significantly different from each other.

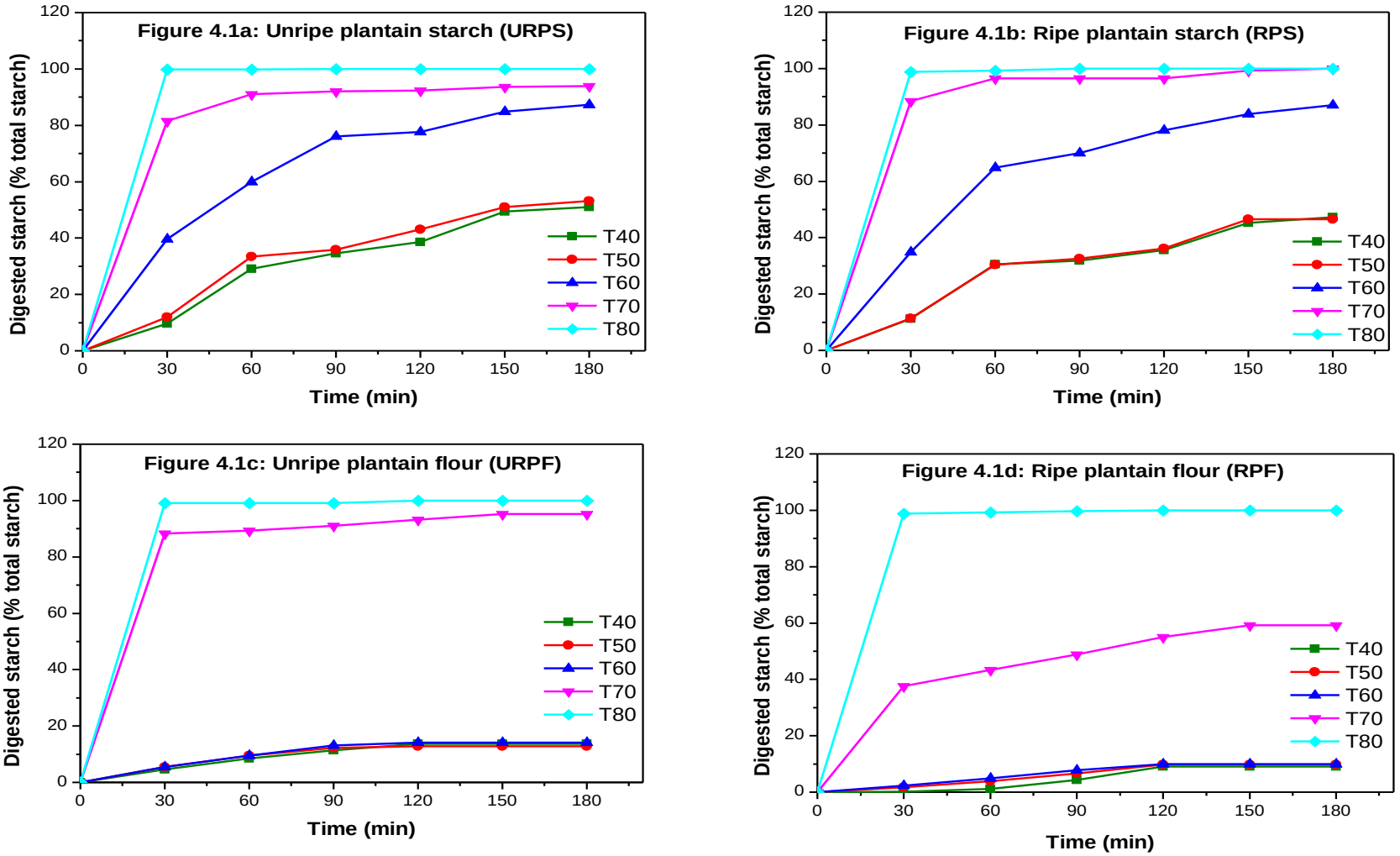


Figure 4.1: *In vitro* starch digestion curves for plantain flours and starch isolates

The quantity of starch digested was monitored at 0, 30, 60, 90, 120, 150 and 180 min of starch digestion of starch suspension at five temperatures (^{0}C) T40, T50, T60, T70, T80 for each sample. Some curves are overlapping and therefore not clearly visible. Values plotted are means ± standard deviations of triplicate determinations and are on DWB

Table 4.4: Total Starch and total sugar composition for plantain flours and starch isolates (g/100g) DWB

	URPS	RPS	URPF	RPF
STARCH	90.1±0.3	70.4±1.1	71.4±0.5	37.4±0.4
SUGARS	NIL	NIL	5±0.8	32±2.1

n = 3, total starch content of plantain flour and starch samples were determined by the modified method of Englyst *et al* (as described in chapter 2) and total sugars was quantified by the phenol sulphuric acid method

Table 4.5: Nutritional starch fractions for residues of unripe plantain flour (% total starch)

	RDS	SDS	RS
URPS	8.1±1.1[a]	29.8±0.5[a]	62.1±1.5[a]
URPR	9.7±0.9[a]	29.0±2.3[a]	61.3±1.3[a]

Values are means ± standard deviations of triplicate determinations. Residues were obtained from 80% ethanol extractions on unripe plantain flour and RDS, SDS and RS quantities were determined on rinsed and freeze-dried residues as earlier described. Values with the same superscript in the same column are not significantly different – $p \leq 0.05$, n = 3. URPS = unripe plantain starch, URPR = unripe plantain residue

Table 4.6: Nutritional starch fractions for residues of ripe plantain flour (% total starch)

	RDS	SDS	RS
RPS	11.1±0.4[a]	24.6±0.4[a]	63.7±0.2[a]
RPR	9.7±0.9[a]	29.0±2.3[a]	61.3±1.3[a]

Values are means ± standard deviations of triplicate determinations. Residues were obtained from 80% ethanol extractions on ripe plantain flour and RDS, SDS and RS quantities were determined on rinsed and freeze-dried residues as earlier described. Values with the same superscript in the same column are not significantly different – $p \leq 0.05$, n = 3. RPS = ripe plantain starch, RPR = ripe plantain residue

Gelatinisation is described as a swelling-driven process (Donald, 2001) and at a certain point during the swelling process the crystalline regions of starch are broken and gelatinisation is initiated (Svihus *et al*., 2005). At excess water content as we have in this study, this onset of gelatinisation is said to occur at temperatures between 50 - 70^{o}C. The onset gelatinisation temperature appears to have occurred between 55^{o}C and 60^{o}C for starches, while it occurred between 60^{o}C and 65^{o}C and between 65^{o}C and 70^{o}C for URPF and RPF respectively. These values are quite close to onset gelatinisation temperatures of between 62.3 ± 0.4 - 72.0 ± 0.05^{o}C earlier reported for some *Musa* flours (da Mota *et al*., 2000) using differential scanning calorimetry. Onset of starch gelatinisation in the context of this work is evidenced by a large change/drop in RS values of 16, 23, 62 and 42% respectively for URPS, RPS, URPF and RPF respectively. Differences in RS values between ripe and unripe flours can be explained by the differences in components, especially starch and sugar content. RS is however depleted for all samples upon gelatinisation. It is evident; therefore, that native resistant starch in plantains, though non-degradable by digestive enzymes, does not survive heat-moisture treatment at and above the gelatinisation temperature, even in the presence of other food components.

4.5.2 *In vitro* rate of starch digestion

Starch digestion *in vivo* is a complex process that is affected by many factors such as the botanical source of the starch, its physical and chemical properties, processing method and the presence of other food materials such

as lipids, enzyme inhibitors and proteins as discussed earlier in chapter 1. Englyst (1992) was able to establish an *in vitro* method that does not use human digestive enzymes, but compares favourably with *in vivo* digestion in humans. We have exploited this method in examining the rate at which plantain starch digests when present with other food components in comparison to the starch alone**.**

The *in vitro* rate curves covering digestion over a period of three hours are shown in Figure 4.1. All the samples show unique curve patterns, with little similarities in some cases. More similarities were however observed between starches than between flours. Digestion curve patterns indicate that treatment of starch at 80^{o}C yield the same curve pattern, with a very sharp rise in the first 30 min and then no further change in starch digestion, obviously because of complete gelatinisation of starch at this temperature. Pure starches, i.e. RPS and URPS exhibit the same curve pattern at all temperatures but with the RPS (Figure 4.1b) having its 50^{o}C digestion rate curve overlapping with the 40^{o}C curve whereas there is a slight separation between 40^{o}C and 50^{o}C for URPS (Figure 4.1a). This may not be unconnected with the purity of the starch. The presence of up to 30% non-starch components in the ripe sample (Table 4.4) might have accounted for a slower rate with a higher temperature treatment between 40 - 50^{o}C. It is very clear from the curve patterns that flours offer lower rates of starch digestion at temperatures 40 - 60^{o}C and this is an indication that the use of flours rather than starches of plantain will offer slower digestibility when incorporated into products. This is similar to the

observation for barley grain when digestion of starch in the meal versus digestion of starch in pure starch of barley grain were compared (Asare *et al*., 2011). Another interesting phenomenon observed, is that the quantity of starch digested reaches a plateau for flours at 120 min, whereas starch digestion continues for starches after 120 min (Figure 4.1 on page 104). This observation supports the fact that there may be some digestion retardants such as enzyme inhibitors (such as procyanidin, proanthocyanithin, and narigenin (Williamson, 2013)) in the plantain flours. It is also obvious from Figure 4.1 that RPF offers a distinct curve at 70^{o}C. This curve, unlike what is observed with the other samples, stands out clearly from the 80^{o}C digestion rate curve and has the lowest values of digested starch at all time intervals studied. Again, this may be due to the presence of high levels of sugars. Many studies relating to the digestibility of plantains has focused mainly on green/unripe plantain. The reason for this is that plantain starch has shown significantly high levels of resistant starch which obviously appears to be more abundant in unripe than in ripe samples due to a higher starch content in the unripe plantain samples. We have, however, been able to deduce from this study that starch digestion is slower in ripe plantain than in unripe plantain, particularly when it is not gelatinised. Although ripe plantain flour contains high level of simple sugars (Table 4.4), it also contains a reasonably high level of resistant starch 33.8 g/100g). The benefit of this quantity of RS present in ripe plantain can be maximised when it is eaten in the raw, unprocessed form. The fact that the 60^{o}C temperature curve stands out

distinctly from those of the 40 and 50^{o}C with higher values of digested starch at the various times studied for the starches but not for flours (Figure 4.1), further points out a slower rate of starch digestion for flours in comparison with their starch counterparts.

In vitro digestion curves observed at 70^{o}C for the starches have similar patterns to those obtained for cooked pasta supplemented with banana (Ovando-Martinez *et al*., 2009), while digestion curve patterns obtained for all samples at 80^{o}C are similar to the curve patterns obtained for cookies produced by substitution of wheat flour with unripe banana flour (Agama-Acevedo *et al*., 2012). Due to the observations we have made in this work, we can infer that there was a lower degree of gelatinisation in the spaghetti than the cookies, suggesting a higher *in vitro* digestibility in the cookie when compared to the spaghetti.

The marked variation in RS, SDS and RDS values for plantain starch and flours was not observed in potato where similar or negligible differences were observed between starch and flours (Lu *et al*., 2011). Unripe plantain flour data in this study when compared with data for unripe banana flour (Englyst *et al*., 1992) had higher RS (86.4% vs. 76%), lower SDS (10% vs. 20%) and lower RDS (3.4% vs. 4%). This further justifies the importance of the need to study the differences in nutritional starches of different starch sources in order to obtain maximum benefits from the food crop.

Changes in the levels of RS, RDS and SDS observed are quite difficult to correlate with one another, which further suggest that the mechanism of starch digestion is complex and needs further study (Lehmann and Robin, 2007). The data do suggest, however, that temperature optima for best yields of nutritional starches (RDS, SDS and RS) in plantains using high water-starch heat-moisture treatments may be between 50 - 60^{o}C.

4.6 Tentative conclusions

a. Substances present in foods which may interfere with the digestion process include tannins, phytic acid, enzyme inhibitors, lipids, proteins and fibre. Due to the fact that flours and starches of the same particle size were used, factors due to the physical form of the food were eliminated. An attempt to check the impact of food components not included in the ethanol extract was made. *In vitro* starch digestion was performed on residues from 80% ethanol and water extracts (Tables 4.5 and 4.6). There are no significant differences observed between the RDS, SDS and RS fractions of ripe and unripe plantain ethanol residues when compared to their starch counterparts.

It can be deduced from this observation that the differences in nutritional starch fractions and starch digestibility in plantain flours are largely derived from some components which are water/ethanol extractable. There is scarcity of information on what the likely components of these extract may be. Two

studies have however confirmed the presence of some polyphenols in some *Musa spp* (Bennett *et al*. (2010), Ovando-Martinez *et al*. (2009)). The effect of polyphenols on starch digestion by α and β amylases is also known to a certain extent as recently reviewed by Williamson (2013).

b. Granular integrity has been explained to be the main factor responsible for the indigestibility of native banana starch (RS2) (which shares some similar properties with plantain) (Zhang *et al*., 2005). The loss of RS2 on heating in all samples is explained by the loss of granular integrity of starch. However, the difference of 25% - 26.6% in RS2 between plantain starches and flours, resulting from interference from other components present in the flours is also lost. It therefore becomes important to distinguish and also separate native resistant starch (RS2) which is due to the inherent nature of starch and starch properties from resistant starch produced from the interference from other food components.

4.7 Conclusion

Plantain starch has significantly high levels of enzyme resistant starch because of its inherent granular properties; however resistance of plantain starch to digestive enzymes is increased by the presence of other components in the flours, mainly ethanol extractable substances. Both native resistant starch and resistance starch produced by the presence of alcohol extractable substances are lost when starch is gelatinised.

Chapter 5 Sucrose inversion and implications of gelatinisation on quantification of simple sugars in plantains

5.1 Hypothesis

Sucrose in plantain is hydrolysed by sucrase before gelatinisation. Sucrose hydrolysis may lead to an over estimation of glucose and fructose in such mixtures. This phenomenon may also lead to errors in starch content determinations in solutions where the pH is not controlled.

5.2 Abstract

Sucrose inversion and its effect on extraction of glucose, fructose and sucrose in ripe and unripe plantain flours were examined at different temperatures using water as solvent**.** There was complete sucrose hydrolysis in ripe samples whereas it was less pronounced in unripe samples. Sucrose hydrolysis occurred below but not the above the gelatinisation temperature of plantain. Also sucrose hydrolysis did not occur in cooked plantain products. Sucrose hydrolysis was not a function of dehydration as both freeze-dried and wet samples were hydrolysed in water. Sucrose hydrolysis was at a maximum between 20 - 40^{o}C in both ripe and unripe samples. Complete hydrolysis occurred in ripe samples while only ~ 50% of sucrose in the unripe samples was hydrolysed.

Extraction efficiency of water for mono and disaccharides of plantain was assessed by performing similar extractions using the same sample to solvent ratio in two other commonly used and previously reported solvents for the extraction of simple sugars, namely, 50% ethanol and 80% ethanol (Ebell (1969), AOAC (1980), Hall (2003), Englyst *et al*. (2004)). Results of sugar extractions with water were affected by sucrose hydrolysis. The highest values for sucrose content in ripe samples were recorded in hot aqueous ethanol extracts; sucrose values obtained from extractions performed in 80% ethanol at 60^{o}C and 100^{o}C and in 50% ethanol at 80^{o}C were not significantly different from each other at 95% confidence level (21.2 ± 0.7 g/100g, 20.6 ± 0.1 g/100g, 20.8 ± 0.7 g/100g respectively). Extractions of sugars in unripe

samples had significantly lower values ($p \leq 0.05$) using 80% ethanol at all temperatures, while 50% ethanol at 80^{o}C appears to be best extraction condition for extracting glucose, fructose and sucrose from an unripe plantain matrix.

5.3 Introduction

All mono- and disaccharides are soluble in water, but solubility differs between various sugars. As expected, solubility of sugars also increases with increased temperature (Kirsi, 2006). The presence of other sugars in a solution also reduces the solubility of individual sugars. For example, the solubility of lactose decreased with an increasing content of sucrose in solutions containing both lactose and sucrose (Kirsi, 2006).

The solubility of simple sugars in water and mixtures of ethanol/methanol and water has been employed in their extractions from many food and non food substances (Rodriguez-Sevilla *et al*. (1999), Basha (1992)). In most cases, extraction in hot aqueous ethanol (mainly 50% or 80% has been employed (Davis *et al*., 2007). For many plant products, extraction procedures have had to be optimised with regards to solvent, time, temperature and sample to solvent concentrations and for some plant products, there have been some discrepancies in the quantities of individual as well as total sugars measured (Johansen *et al*. (1996), Knudsen and Li (1991), Giannoccaro *et al*. (2006)). Despite the disparities observed in quantification of some mono- and di-saccharides extracted in different solvents, little or no recent data exists to

explain if these variations are due to the possible effects of sucrose hydrolysis. Moreover, nowadays, with the emergence of more sophisticated measuring techniques for sugars, over-emphasis is placed on quantification of sugars rather than their extraction. This is evidenced by the large volume of literature on quantification techniques when compared to the very small number of papers on extractions. Both are important, since both extraction and quantification errors will produce misleading results. Due to the fact that the sucrose content is usually higher than glucose and fructose in most fruits e.g. ripe banana or plantain (Torija *et al*. (1998), Kanellis *et al*. (1989)), the phenomenon of sucrose hydrolysis cannot be overlooked when considering these fruits for nutritional, rheological or chemical analysis purposes.

Sucrose has a unique carbonyl-to-carbonyl linkage (the glycosidic bond is formed between the reducing ends of both glucose and fructose, and not between the reducing end of one and the non-reducing end of the other), which makes it very labile in acidic medium. Acid hydrolysis of sucrose occurs more rapidly than other oligosaccharides (Constantina *et al*., 2012). Sucrose undergoes hydrolysis to yield equimolar amounts of D-(+)-glucose and D-(-)-fructose (Figure 5.1).

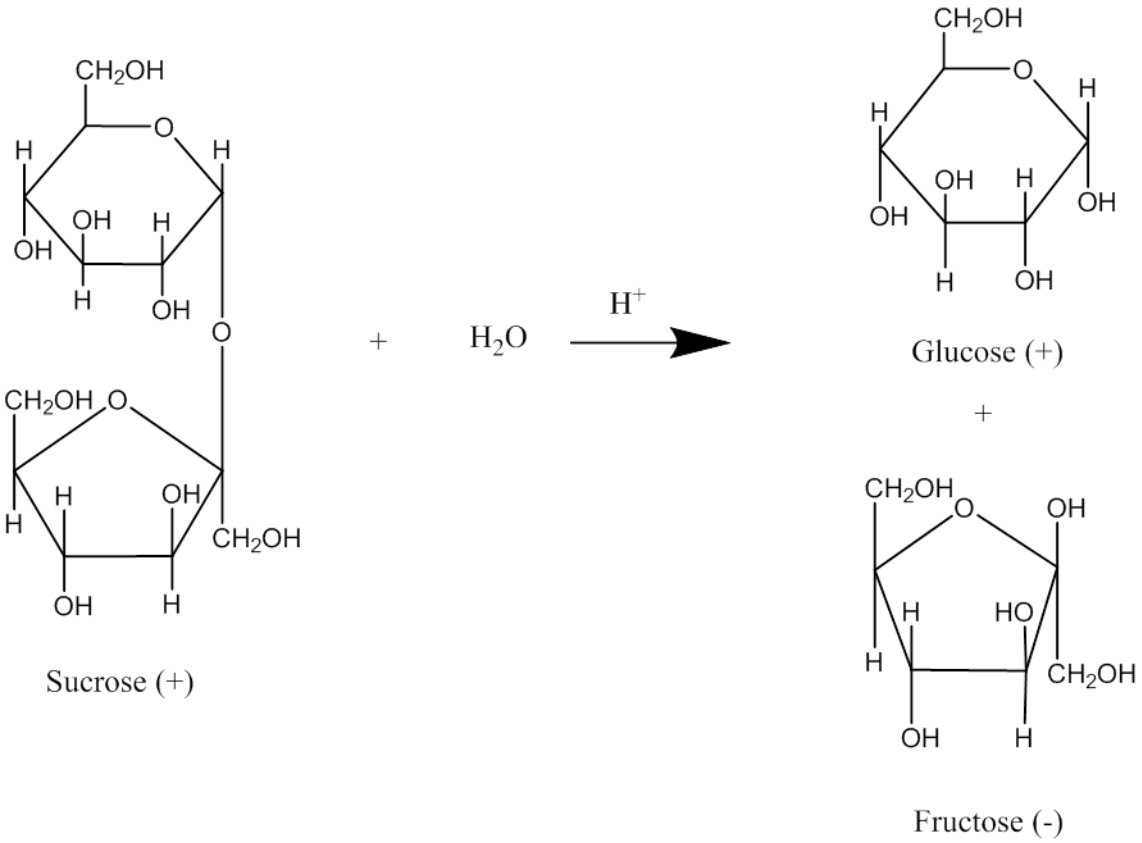


Figure 5.1: Sucrose hydrolysis in acidic medium
H_2O splits into its hydrogen and hydroxyl components and they break the oxygen bond between the monosaccharide units in the sucrose molecule. The hydrogen combines with the oxygen to give the glucose its regular monosaccharide configuration, while the OH radical combines with the appropriate fructose carbon atom, which after the splitting of the sucrose molecule has one of its four valence bonds free.

Sucrose is optically active with a positive specific optical rotation of $(\propto)_D^{20}$ = +66.5^o; it rotates the polarized light plane to the right (dextrorotatory). The mixture of glucose and fructose resulting from the hydrolysis of sucrose turns polarized light to the left (levorotatory). This is because fructose has a higher optical rotation (-92.9^o) than glucose (+52.7^o). Hence the process is called

inversion and the mixture is called invert sugar. Sucrose can be enzymatic or acid hydrolysed (Constantina *et al*., 2012).

The investigation of sucrose hydrolysis is also important in starch content determination (which is an important part of this work) when samples are analysed *in situ*. In the past, sucrose hydrolysis was not a problem in starch analysis because the procedures used always involved the isolation of starch before measuring its properties or quantity. Today, with the emergence of the glycaemic index and resistant starch concepts and the need to determine resistant starch and available carbohydrates on samples *in situ*, it becomes very important to optimise experimental protocols so as to avoid sucrose hydrolysis which may lead to overestimation or under estimation of some of the parameters measured, e.g. total starch and rapidly available glucose. This is because analytical methods for the estimation of polysaccharides usually involve breaking them down into their constituent monosaccharide(s) (Folkes and Jordan, 2006).

Information exists for the impact of sucrose on gelatinisation but not vice versa (Magnus and Eliasson, 2006). The impact of gelatinisation on sucrose/sucrose hydrolysis is important because many starch containing foods e.g. plantains, are usually cooked before consumption; the fate of sucrose before and after processing stages/processes needs to be investigated.

In this study three commonly used extraction solvents for non structural carbohydrates namely: water, 50% ethanol and 80% ethanol were employed at different temperatures to check the extent of sucrose hydrolysis, and how it affected the quantification of glucose, fructose and sucrose in ripe and unripe plantain flours. Possible reasons for observed hydrolysis in some samples and not in others were also tested as a means of preventing sucrose hydrolysis when it is not desired either in food products or in experimental protocols.

5.4 Materials and method

5.4.1 Sugars

Glucose**,** EC Number 200-075-1, molecular weight 180.16 of ≥99.5% purity, fructose**,** EC Number 200-333-3, molecular weight 180.16 of ≥99 % purity, sucrose**,** EC Number 200-334-9, molecular weight 342.3 of ≥99.5 % purity, and fucose**,** EC Number 200-792-9, molecular weight 164.16 of ≥99.5% purity were purchased from Sigma Aldrich**.**

5.4.2 Solvents

Absolute ethanol analytical grade and 99.8% purity was purchased from Fisher scientific, UK, while acetonitrile, HPLC grade was purchased from Sigma Aldrich, UK.

5.4.3 HPLC materials

Prevail carbohydrate ES HPLC column 5 μm (250×4.6 mm), Alltech All-Guard Cartridge system pre-column (7.5×4.6 mm cartridge) and guard cartridges

were purchased from Grace Davison Discovery Sciences, UK. A Shimadzu HPLC UFLC instrument (Shimadzu Scientific, Japan) was used for the analysis. A Shimadzu low temperature evaporative light scattering detector (ELSD-LT II) was used for analyte detection. The ELSD-LT II detector was operated at 40°C, 350 kPa and air was used as the nebulising gas.

5.4.4 Sample preparation

Plantain flours used were prepared as described in chapter 2.

5.4.5 Samples prepared to check effect of processing on sucrose hydrolysis

One finger of ripe plantain was cut into five portions one portion each was either boiled, fried, grilled, freeze-dried or left unprocessed (i.e. raw). Two fingers of plantain were used for each assay and the experiment was repeated three times. The average of each experiment was taken.

5.4.5.1 Boiling

One portion of the cut plantain finger (about 50 g) was cooked in 750 ml boiling water for 15 min.

5.4.5.2 Grilling:

One portion of the cut plantain finger (about 50 g) was grilled at high/medium heat in a grill for 30 min

5.4.5.3 Frying

One portion of the cut plantain finger was further cut into slices of 2 mm in diameter and deep fried in pre-heated vegetable cooking oil for approximately 2.5 min.

5.4.6 Extractions

A 100 mg sample was extracted in 5 ml solvent in a capped tube immersed in a water bath for 30 min at temperatures ranging from 20 - 100^{o}C with intermittent shaking on a vortex mixer, after which tubes were centrifuged at 2000*g* for 10 min at 25^{o}C. This process was repeated 3 times resulting in a pulled extract of 15 ml per sample and each sample was prepared in triplicate. For samples analysed 'as is' i.e. wet, weight of samples taken were based on 100 mg dry matter content in each sample and all results are presented on dry weight basis.

5.4.7 Quantification of Soluble Sugars

Sugar standards were prepared in the range 1- 4 mM. Standard solutions were filtered through a 0.25 μm filter, 0.9 ml of standard plus 0.1 ml 0.01 M fucose (to give 1 mM internal standard) were transferred into vials, capped, and loaded on to the Shimadzu SIL-10AXL auto sampler. Extracts of plantain samples were treated in the same way as standards after appropriate dilutions to fit into the standard curve range. The mobile phase used was 75% acetonitrile and the equipment was run in isocratic mode at a flow rate of 1 ml/min. Pump pressure was 115 - 120 bars (11.5 - 12 MPa), oven

temperature was at 20^{o}C, the sample injection volume was 5 μl and the run time was 30 min.

5.5 Statistical analysis

All analyses carried out were performed on 3 sets of samples. Each set was composed of five fingers of plantain (except for the samples used to check the effect of processing on sucrose hydrolysis, where each set of sample was composed of three plantain fingers) and each set was analysed in triplicate. The mean of each set was taken. Data obtained was analysed using analysis of variance (ANOVA), and expressed as means ± standard deviations.

5.6 Results and discussion

Peaks of sugars analysed were well resolved in both the standards (Figure 5. 2) and samples (Figure 5.3) with stable baselines. Detection limit was 250 µM sugar solution, however a good baseline was not achieved in solutions below 1 mM concentration, hence the standard curve was prepared in the range of 1 to 4 mM. A linear curve was also obtained in the concentration range used.

5.6.1 Sucrose hydrolysis in plantain

Simple sugars extraction for ripe plantain samples (Figures 5.6 - 5.10) reveal that sucrose was detected in 50% and 80% ethanol extractions at all temperatures with sucrose values ranging from 17.1±0.6 to 21.2±0.7 g/100 g. In the case of extractions made in water, no sucrose was detected in extracts at 20, 40 and 60^{o}C (Figures 5.4 and 5.6 - 5.8).

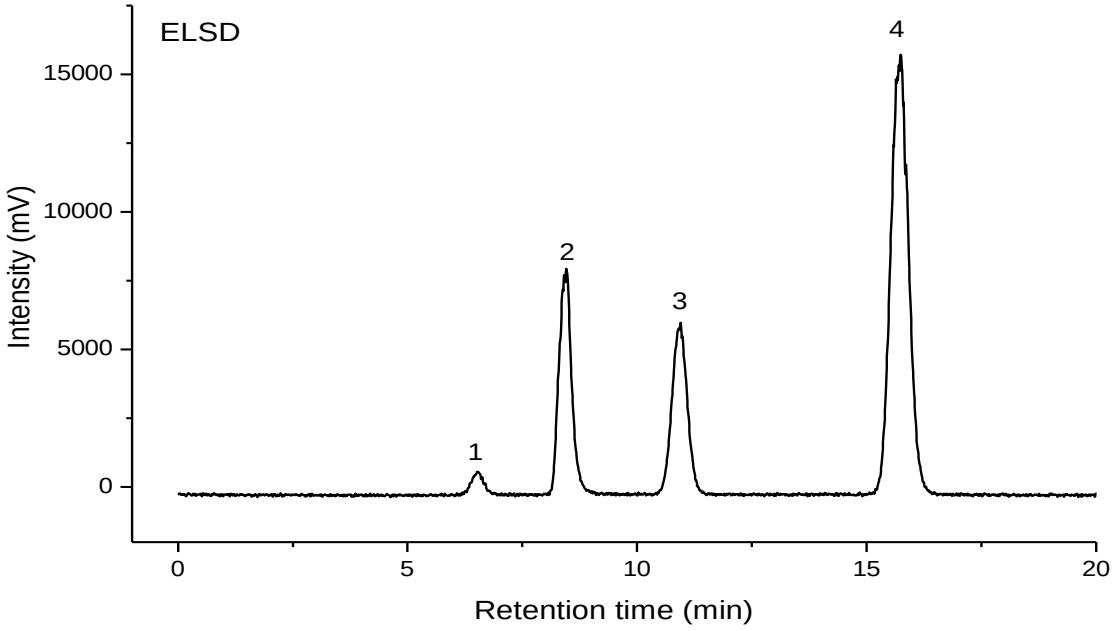


Figure 5.2: Chromatogram of a mixture of three sugars plus fucose analysed isocratically.
Sugar mixture of fructose, glucose and sucrose was 4 mM and fucose (1 mM) was added as internal standard. Peak 1= fucose, peak 2= fructose, peak 3 = glucose and peak 4 = sucrose

Sucrose was however seen in extractions made at 80^{o}C and 100^{o}C (Figures 5.5, 5.9 and 5.10). The relatively higher values of fructose and glucose with water extractions at temperatures 20 - 60^{o}C when compared with those of the aqueous ethanol extracts is an indication of sucrose hydrolysis. If this result is to be viewed in terms of the starch gelatinisation process, it would be reasonable to conclude that sucrose was hydrolysed in samples treated at temperatures below the gelatinisation temperature (80 ± 2^{o}C, from chapter 3) but not hydrolysed in those samples treated at greater 80^{o}C.

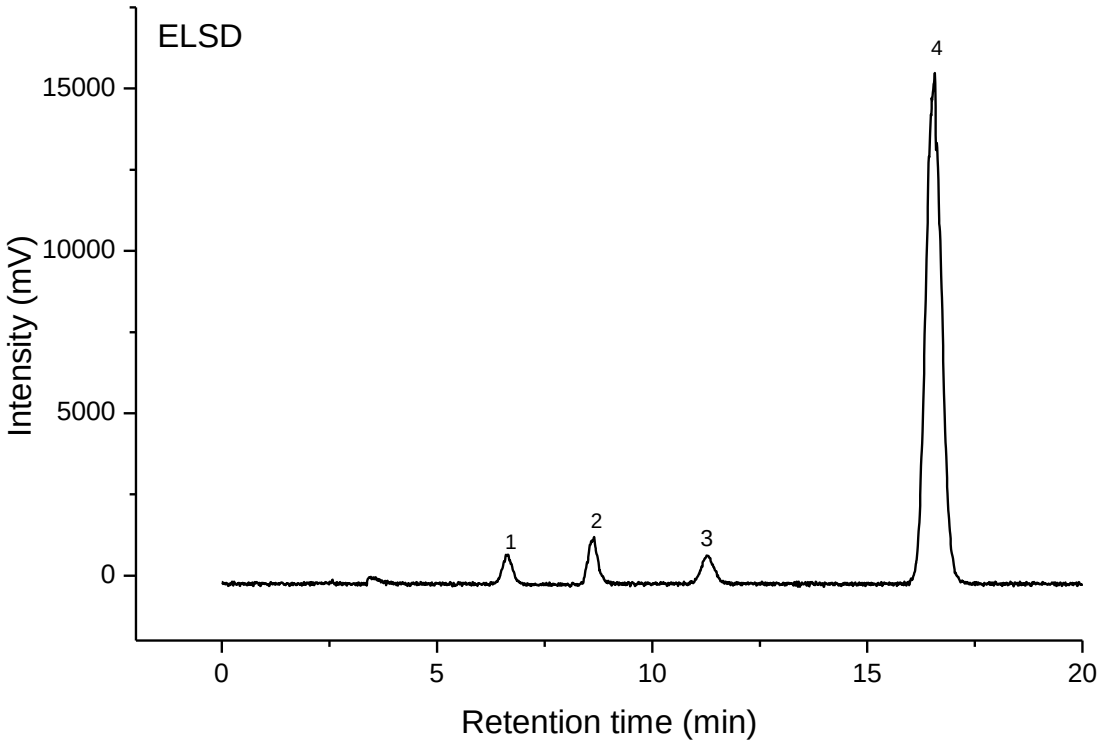


Figure 5.3: Chromatogram of ripe plantain sugars in an extract of 80% ethanol at 100°C

1 mM fucose was added as internal standard, chromatogram shows the mono and disaccharides detected in plantain, peak 1= fucose, peak 2= fructose, peak 3 = glucose and peak 4 = sucrose

It appears that both the pH of the water/ethanol/plantain mixture, which was ~ 7 (Table 5.2) and the presence of ethanol were responsible for the prevention of sucrose hydrolysis in those mixtures. In addition these conditions will not be favourable for starch gelatinisation to occur because for water-alcohol moistures, acid-alcohol treatments are more favourable for the production of soluble starch (Chang *et al*. (2004), Ma and Robyt (1987), Robyt and Ma (1987), Lin *et al*. (2003).

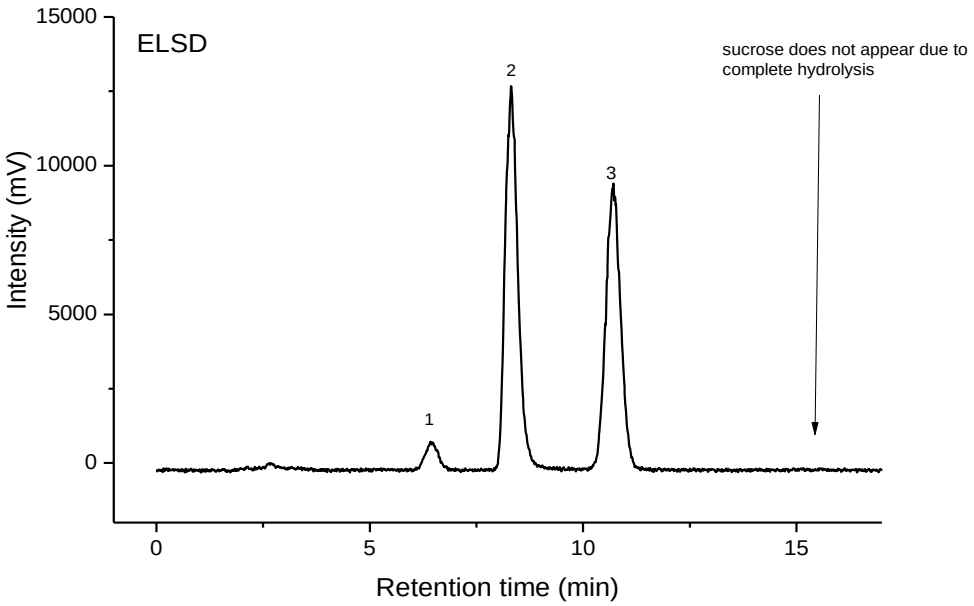


Figure 5.4: Chromatogram of ripe plantain sugars extracted with de-ionised water at 60°C

1mM fucose was added as internal standard, chromatogram shows that no sucrose was detected due to sucrose inversion in this mixture, peak 1= fucose, peak 2= fructose, peak 3 = glucose.

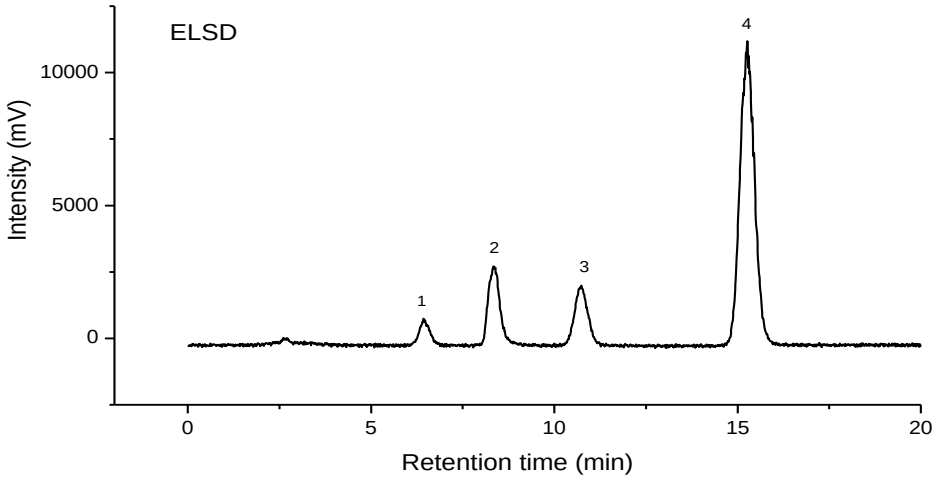


Figure 5.5: Chromatogram of ripe plantain sugars extracted with deionised water at 100°C

1mM fucose was added as internal standard, chromatogram shows that sucrose was detected because sucrose inversion did not occur in this mixture, peak 1= fucose, peak 2= fructose, peak 3 = glucose, peak 4: sucrose.

The extent of sucrose hydrolysis in the unripe plantain samples shows a different pattern to what was observed for the ripe samples. In unripe samples water extracts, unlike the ripe flour extracts, sucrose was detected in water extracts at all temperatures, however the quantity of sucrose increased at an almost steady rate with increasing temperature (from 2.1 ± 0.4 to 5.6 ± 0.1 g/100 g). The quantities of sucrose extracted at 20 and 40^{o}C are about 50% of the values obtained for sucrose at 80^{o}C and 100^{o}C. This implies that though sucrose hydrolysis took place in water extractions of unripe samples, the degree of sucrose hydrolysis in unripe plantain samples was not as high as it occurred in ripe samples where no sucrose was salvaged at all.

The observations above may not be unconnected with the presence of a sucrose enzyme in plantain which is higher in ripe than in unripe plantains. The activity of acid invertase (EC 3.2.1.26) of the *Musa ssp* during ripening was reported to be close to zero in harvested green banana and increases during postharvest ripening (Fils-Lycaon *et al*. (2011), Iyare and Ekwukoma (1992)).

High temperature treatments results in starch gelatinisation and also appears to inactivate plantain invertase. The fact that sucrose is totally hydrolysed at 60^{o}C in ripe plantain samples but only ~ 20% hydrolysed (i.e. a reduction in enzyme activity to hydrolyse sucrose rather than increase) in unripe plantain samples at the same temperature, suggests that starch gelatinisation reduces the activity of invertase possible by increased viscosity of the mixture

produced by gelatinised starch (It will be recalled that higher content of sugars delayed the unset of gelatinisation in ripe plantain flours - chapter 3).

Enzymes are organic catalysts which thrive more in some conditions than others and usually have their optimum operating conditions. Invertase activity is optimum at pH 4.5 and 55^{o}C (Sturm, 1999). The pH of extraction mixtures of water and plantain at 20 -100^{o}C is given in Table 5.1 on page 133. The pH range observed indicates an average of pH 5 for ripe plantain and pH 6 for unripe plantain mixtures. Plantain water mixtures observed at all temperatures here are therefore acidic suspensions. It appears that the lower pH of ripe plantain suspensions is responsible for complete hydrolysis while the higher pH for unripe is responsible for reduced extent of sucrose hydrolysis. The fact that the pH values of plantain-water mixtures are not significantly different from each other at all temperatures examined however suggests that pH may not be important when temperature is involved.

Various mixtures were further tested for the activity of sucrose hydrolysing enzymes in ripe plantain water mixtures. Sucrose hydrolysis was checked in a plantain water mixture at pH 7 and sucrose hydrolysis was not observed (Table 5.2 on page 133). Hydrolysis of pure sucrose was tested in water at pH 5 and no hydrolysis occurred. The fact that plantain sucrose (and not pure sucrose) was hydrolysed at pH 5 suggests that the hydrolysis must have been aided by other factors apart from acidity of the mixture. The optimum condition for non enzymatic sucrose hydrolysis had also been tested and

reported to be between a pH of 0.8 - 2.5 (Pinheiro Torres and Oliveira, 1999). Furthermore, when plantain/water mixture at 40^{o}C was spiked with pure sucrose and the percentage hydrolysis calculated based on the initial content of sucrose in the plantain flour, a value of 119 ± 1.1% hydrolysed sucrose was obtained (Table 5.2), indicating that some of the added sucrose was also hydrolysed. If sucrose was not hydrolysed in water at pH 5 but hydrolysed in plantain solution/suspension at the same pH, it simply means there must have been some sucrose hydrolysing enzymes in the plantain samples. Therefore, we can reasonably conclude that sucrose hydrolysis in plantain occurs due to the presence of acid active sucrose hydrolysing enzymes, which are deactivated at high temperatures (above 60^{o}C) however, enzyme activity and the extent of hydrolysis are still a function of enzyme quantity, pH, starch gelatinisation and probably other factors.

Freeze-drying/dehydration has been reported to be a possible cause of sucrose hydrolysis in some food products (Karel and Labuza (1968), Chen *et al*. (2002)). In order to check and possibly eliminate the fact that freeze-drying could be the cause of sucrose hydrolysis plantain, fresh (un dehydrated) samples were analysed in selected solvents, 50% ethanol, 80% ethanol and water at boiling and water at 40^{o}C (Figure 5.13).

These next set of samples were analysed “as eaten” i.e. they were not dried and grounded before analysis. The raw freeze-dried sample was used to compare the values of the raw “as eaten” samples, especially regarding sucrose hydrolysis. The results obtained in Figure 5.13 indicate that both the raw and freeze-dried ripe plantain samples showed comparable values of sucrose, fructose and glucose with the different solvents used. Again at 40^{o}C, (a temperature below gelatinisation), sucrose was conspicuously absent in these two samples, while the values of glucose and fructose increased. We were therefore able to confirm the occurrence of sucrose hydrolysis both in wet and dry plantain samples.

5.6.2 Sucrose hydrolysis and extraction efficiency of simples sugars in plantain

From Figures 5.6 - 5.10, extraction capacity of the solvents used, varied with temperature, however the quantities of glucose and fructose observed in water extracts are affected by sucrose hydrolysis of samples both in the ripe and unripe plantain samples. An 80% ethanol solution has always been the recommended extraction solvent for sugars because it deactivates any enzyme present in the sample (Steve and Yolanda, 2006). Results of sugar extractions made with 50% ethanol and 80% ethanol revealed that sucrose hydrolysis did not occur in these solvents. The highest values for sucrose content in ripe samples were recorded in hot aqueous ethanol; sucrose values obtained from extractions in 80% ethanol at 60 and 80^{o}C and in 50%

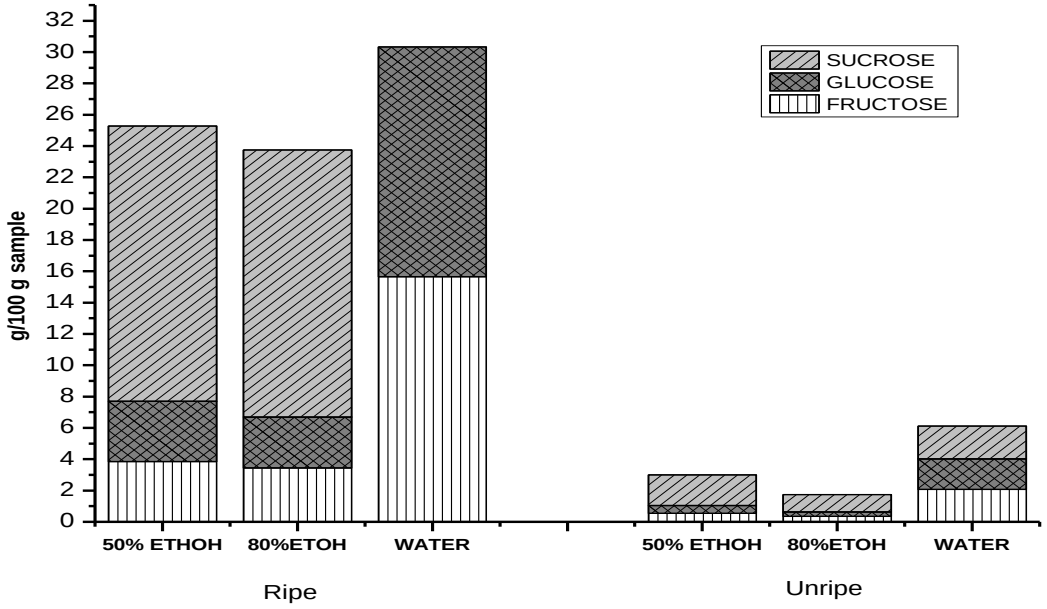


Figure 5.6: : Extractions of fructose, glucose and sucrose from raw plantain flours using 50% ethanol, 80% ethanol and water at 20°C.
Sucrose is not present in water extract of ripe plantain due to complete hydrolysis of sucrose**.**

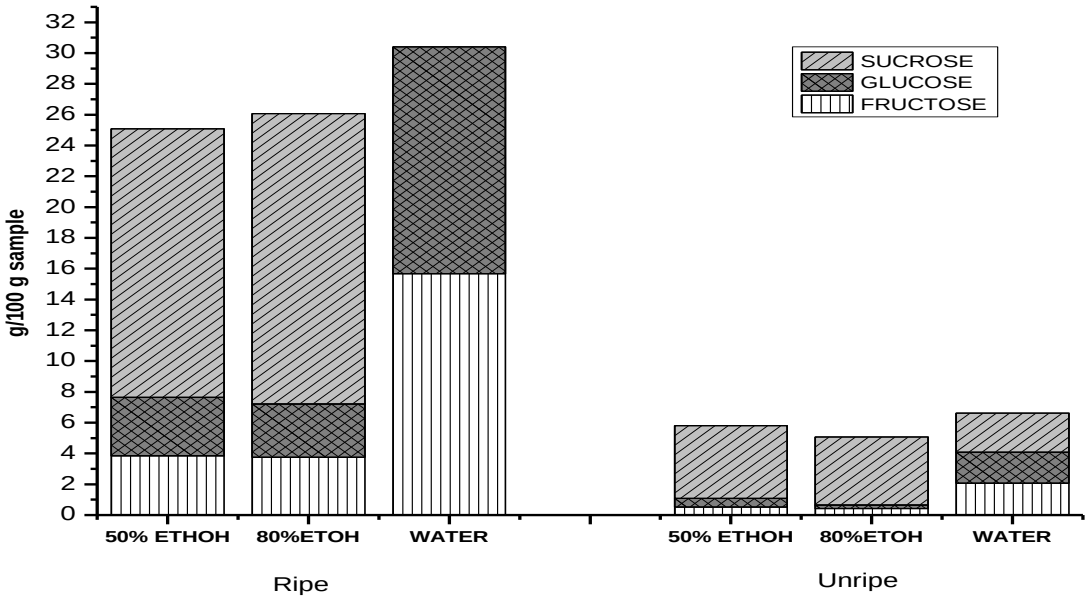


Figure 5.7: Extractions of fructose, glucose and sucrose from raw plantain flours using 50% ethanol, 80% ethanol and water at 40oC
Sucrose is not present in water extract of ripe plantain due to complete hydrolysis of sucrose. Water has lowest quantity of sucrose in unripe plantain due to partial hydrolysis of sucrose

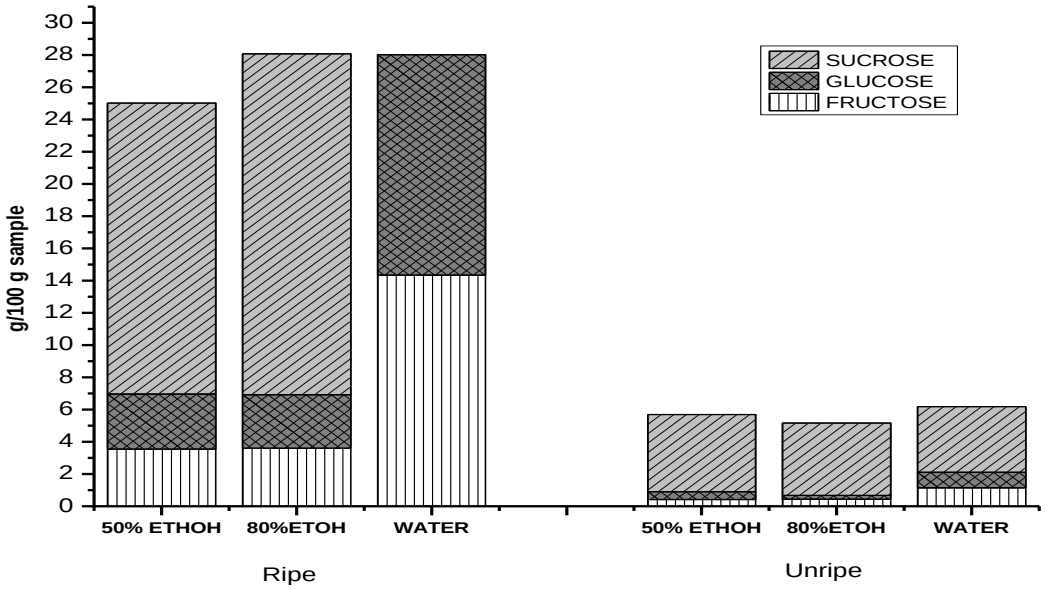


Figure 5.8: Extractions of fructose, glucose and sucrose from raw plantain flours using 50% ethanol, 80% ethanol and water at 60^{o}C

Sucrose is not present in water extract of ripe plantain due to complete hydrolysis of sucrose. In unripe plantain, water has higher quantity of sucrose when compared with extractions at 20 and 40^{o}C (figures 6 and 7) due to reduced inversion of sucrose.

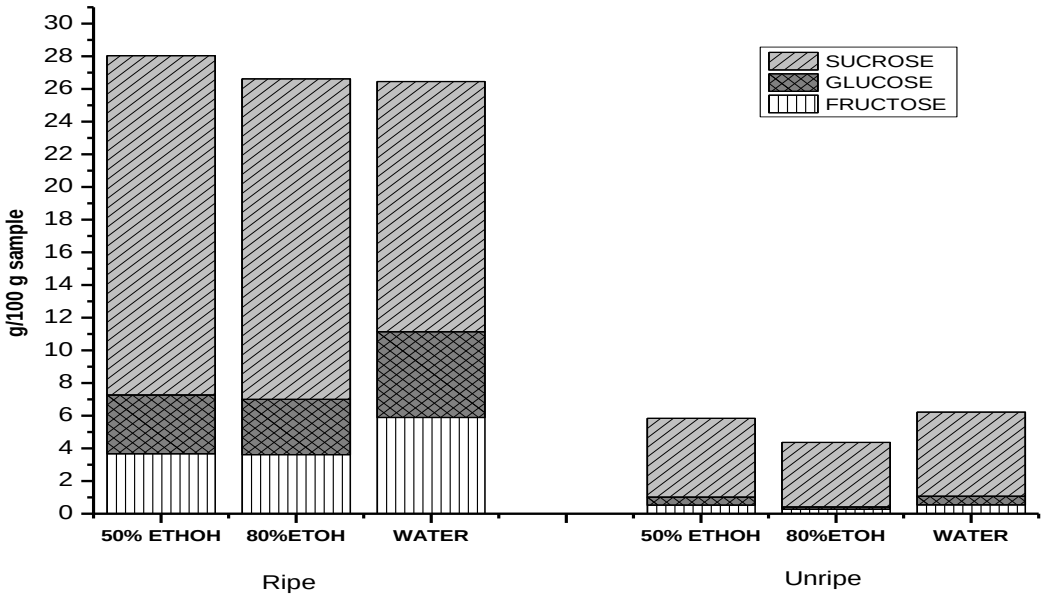


Figure 5.9: Extractions of fructose, glucose and sucrose from raw plantain flours using 50% ethanol, 80% ethanol and water at 80^{o}C

Sucrose inversion did not occur in water extracts of both ripe and unripe plantain. In ripe plantain, reduced quantity of sucrose vs. increased quantity of fructose and glucose observed when compared with other solvents must have been due to slight hydrolysis during mixing.

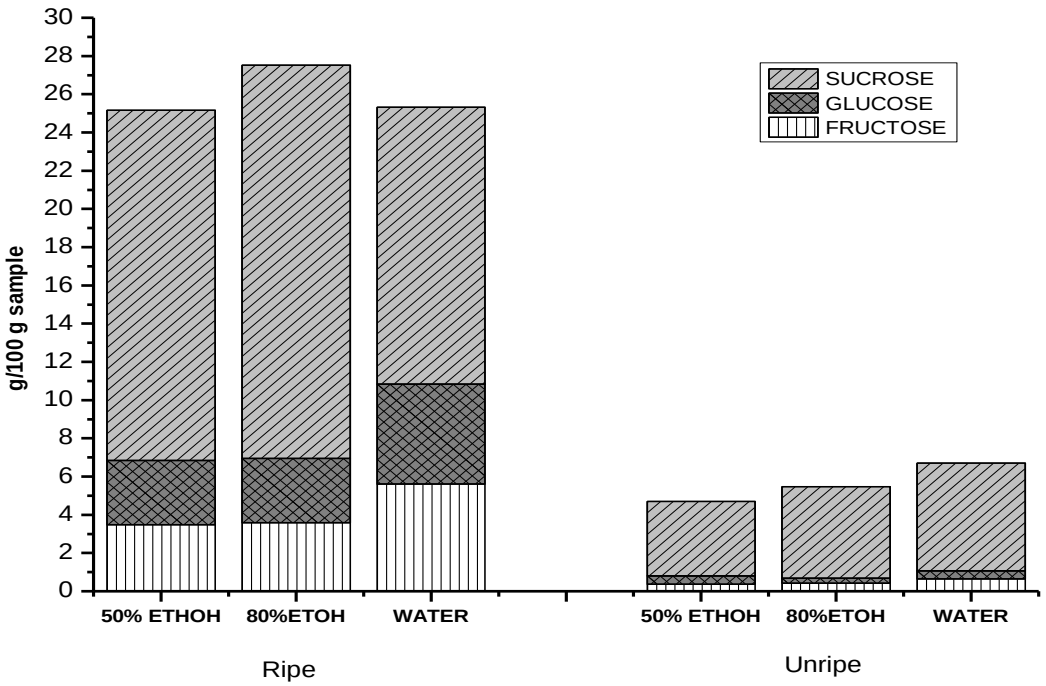


Figure 5.10: Extractions of fructose, glucose and sucrose from raw plantain flours using 50% ethanol, 80% ethanol and water at 100^{o}C

Sucrose inversion did not occur in water extracts of both ripe and unripe plantain. Reduced quantity of sucrose vs. increased quantity of fructose and glucose observed in water extracts of ripe plantain, when compared with other solvents must have been due to slight hydrolysis during mixing.

ethanol at 80^{o}C were not significantly different from each other at 95% confidence level (21.2 ± 0.7 g/100 g, 20.6 ± 0.1 g/100 g, 20.8 ± 0.7 g/100 g respectively). This observation is similar to that of Giannoccaro and co-workers (2006) in the extraction of defatted soy bean using various mixtures of solvents. Sucrose extraction efficiency was slightly different for the unripe samples as significantly high values ($p \leq 0.05$) were recorded in 50% ethanol extracts at 40 - 100^{o}C and water at 80 and 100^{o}C (sucrose values at these extraction conditions were not significantly different from each other at 95%

confidence level). The results of a number of studies show that high temperature extractions were more effective for 80% ethanol extractions and less effective for water extractions, hence the use of hot 80% ethanol is more commonly recommended for sugar extraction (Johansen *et al* (1996), Giannoccaro *et al*. (2006), Hall (2003)). Unfortunately this recommendation was not favourable for extractions of sugars in unripe samples as values for fructose, glucose and sucrose and total sugars obtained using 80% ethanol were significantly ($p \leq 0.05$) low at all temperatures. The reason for this is not clear but may have to do with both the lower quantity of sugars in unripe samples coupled with a denser food matrix in the unripe samples than in the ripe plantain. Extraction efficiency is usually dependent on the relative affinity of the substance for the solvent compared to its existing matrix and is affected by physical, ionic, covalent, hydrophilic and hydrophobic properties (Hall, 2003). This may also be the reason for the conspicuous poorer extractability of 80% and 50% ethanol at 20^{o}C, especially in unripe plantain samples. The 50% ethanol solution at 80^{o}C appears to be best extraction condition for extracting glucose, fructose and sucrose from an unripe plantain matrix.

Values of total sugars obtained from water extracts at 20 and 40^{o}C (30.3 ± 0.2 g/100 g and 30.4 ± 1.3 g/100 g, respectively) in ripe samples were significantly higher than other water extractions at 60 - 100^{o}C.

Table 5.1: pH values for plantain flour mixtures in water at different temperatures

	RIPE	UNRIPE
TEMPERATURE (^oC)	**pH**	**pH**
20	5.16±0.5[a]	6.07±0.07[a]
40	5.06±0.10[a]	6.11±0.03[a]
60	5.05±0.20[a]	6.04±0.14[a]
80	5.00±0.13[a]	5.95±0.11[a]
100	4.92±0.20[a]	5.88±0.28[a]

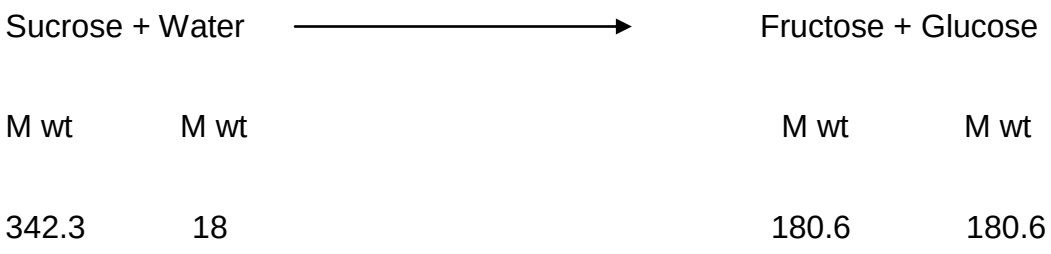
Values are means of three determinations. Each mixture is 100 mg/5 ml plantain in water. pH of mixtures were determined with a pH meter. Values with the same superscript in the same column are not significantly different.

Table 5.2: Test of sucrose hydrolysis in ripe plantain with various solvent-solute mixtures

Mixture	pH of mixture	%sucrose hydrolysed
Flour + water at 100^{o}C	4.96±0.05	3.2 ± 0.9
Flour + water at 40^{o}C	5.1±0.1	98.6 ± 1.3
sucrose + water at 40^{o}C	7.1±0.03	0.0 ± 0.0
sucrose + water of (adjusted to pH 5 with buffer)	5.2±0.06	0.0 ± 0.0
Flour + sucrose + water (spiking)	5.1±0.03	119.3 ± 1.1
Flour + water (adjusted to pH 9 with buffer)	7.15±0.03	0.0 ± 0.0
Flour + 50% ethanol 20^{o}C	6.98±0.2	0.0 ± 0.0
Flour + 80% ethanol 20^{o}C	7.11±0.3	0.0 ± 0.0

Values are means of triplicate determinations. Volume of solvent taken was 15 ml in each case, quantity of flour was 100 mg and sucrose was 50 mg.

It appears that sucrose hydrolysis in these samples might have resulted in significantly higher values of total sugars than may actually occur in the samples. This can be explained by the Brigs concept (Maurice, 2004) as indicated by the sucrose inversion equation below.



The implication of the equation is that sucrose hydrolysis will always result in an increase in total dissolved solids (Maurice, 2004), and this is reflected in the higher quantity of total sugars in water extractions where sucrose was completely hydrolysed (Figures 5.6 - 5.10) . The increase factor can be calculated as 360.32/342.3 = 1.053, based on the molecular weights of species in solution. This phenomenon was however not detected in water extracts of unripe samples; and this can be attributed to incomplete sucrose hydrolysis observed in these samples.

5.6.3 Sucrose hydrolysis and non-enzymatic browning in plantain

Sucrose is a non-reducing disaccharide and does not undergo non-enymatic browning (Chen *et al*., 2002). Sucrose hydrolysis, which results in the production of fructose and glucose may induce browning in many products such as banana. It has been reported that reducing sugars in banana fruits

were involved in browning during processing and storage (Torija *et al*., 1998). Torija and co-workers (1998) were able to avoid browning by using the pre-treatments of blanching and microwaving. These were high temperature treatments which must have inactivated the sucrose hydrolysing enzymes. On the other hand, plantain has been reported to be less prone to undergo browning than banana because of deactivation of polyphenol oxidase by high temperature and the use of copper complexing agents and reducing sugars (Ngalani *et al*., 1993). If non-enzymatic browning is aided by reducing agents and enzymatic browning is countered by reducing agents which deactivate the polypehol oxidase enzymes, it then becomes quite unclear which form of browning takes precedence over the other in food systems. Nevertheless, the more important type of browning here is that which occurs during heat processing, i.e. the non-enzymatic browning; this has however, not been reported for plantain. Also, the evidence that plantains can be cooked without manifesting the Maillard reaction and still maintain their orange/yellow colour (Aurore *et al*., 2009) supports the fact that heat inhibits sucrose hydrolysis and therefore prevents browning (Figures 5.10 and 5.11). The implication of this is that the undesirable change in colour of some processed foods may not occur if plantain flours are used in these applications.

5.6.4 Implication of sucrose inversion on glycaemic index of plantain

Glucose and fructose are monosaccharides and do not need to be digested before they are absorbed in the human digestive system. Sucrose on the other hand is a disaccharide that needs to be digested by intestinal brush

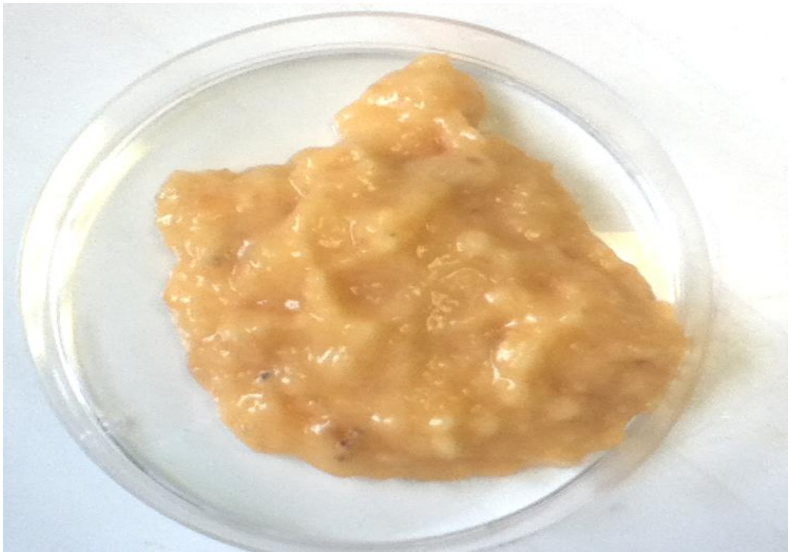


Figure 5.11: Raw ripe plantain

Plantain finger was peeled, sliced and minced with a hand mincer

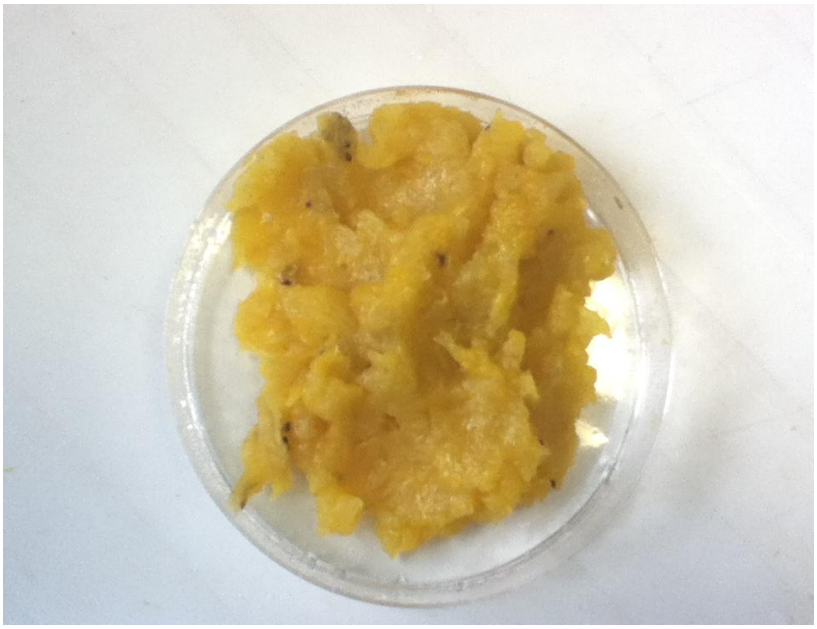


Figure 5.12: Ripe boiled plantain

Plantain finger was cut into chucks, cooked in boiling water for 10 min, it was cooled to room temperature and then minced with a hang mincer. No browning was observed because sucrose was not hydrolysed.

border sucrase (E.C. 3.2.1.48) into its components; glucose and fructose before absorption (Robayo–Torres *et al*., 2006). When glucose is consumed, it causes an elevation of the blood glucose concentration, dietary fructose however is only partially converted to glucose mainly in the liver and therefore produces less pronounced blood glucose elevation (David and Gregory, 2001). The level and duration of the blood glucose rise after a meal depends on the rate of absorption, tissue uptake, insulin levels, etc. which in turn depend upon factors such as gastric emptying as well as the rate of hydrolysis and diffusion of hydrolysis products in the small intestine. On the GI scale (based on 50 g of sugar) with glucose as 100, fructose has a lower GI (23) than sucrose (60) and sucrose has a lower GI than glucose (Wolever and Miller (1995), David and Gregory (2001)). Sucrose hydrolysis in plantain will make the glucose ready for absorption and may lead to an increase in GI. If sucrose hydrolysis is prevented, a slow rate of sucrose digestion may lead to slower rate of glucose being released into circulation. In essence the pre-hydrolysis of sucrose before consumption may be a disadvantage. On the other hand, the hydrolysis mixture of glucose and fructose (invert sugar) may actually behave like honey which has a similar GI with sucrose in some studies (Wolever and Miller, 1995) while it had either higher or lower GI than sucrose in others (Abdulrhman *et al*., 2013): These variations have however been attributed to differing ratios of fructose and glucose in those samples (Wolever and Miller, 1995). Excluding other factors, it is logical that an equimolar concentration of fructose and glucose (invert sugar) should elicit a

GI of (glucose + fructose)/2 = (100+23)/2= 61.5. If this is the case, then sucrose hydrolysis which occurs in plantain-water mixtures at low temperatures may actually not be a disadvantage.

5.6.5 Impact of sucrose hydrolysis on sweetness of plantain

The sweetness ranking is in the order fructose > sucrose> glucose. It is quite interesting to note that this order is in the reverse when compared to the GI values of the sugars as indicated in the previous section. Inversion of sugar will result in a mixture that is sweeter due to the presence of more fructose molecules (Maurice, 2004). Therefore sucrose hydrolysis in plantain products may be desired for the purpose of enhancing their sweetness.

5.6.6 Sucrose hydrolysis and enzymatic analysis of total starch and related parameters

The phenomenon of sucrose hydrolysis in plantains needs to be monitored in order to avoid errors in the estimation of free glucose, rapidly available glucose (RAG), total starch, and other measurements directly related to the measurement of glucose in the food matrix. If experimental pH measurements are not properly monitored, glucose from sucrose will be measured as part of free glucose or even as part of glucose from starch after digestion. This is because these measurements involve the determination of glucose, and subsequent calculation of the appropriate values. For example the Englyst protocol, requires the measurement of rapidly available glucose and rapidly digestible starch (RAG and RDS) using a pH of 5 and temperature of 37^{o}C (Englyst *et al*., 1999). This experimental protocol may be suitable for pure

starches and gelatinised foods because these do not involve the occurrence of sucrose hydrolysis but will not be suitable for the determination of RDS in ripe raw plantain samples. This is because the experimental conditions in this protocol are favourable for sucrose hydrolysis in plantain to take place and may lead to inclusion of glucose from sucrose in the measurement of RDS (especially when invertase is not included in the enzyme mixture used for starch digestion) and subsequently misleading results. Therefore the use of buffer solutions at appropriate pH for the sample being analysed is recommended for carbohydrate analysis. In the case of plantain products *in situ* a working pH of ~ 7 will be appropriate in order to eliminate sucrose hydrolysis.

5.6.7 Sucrose hydrolysis and starch gelatinisation in plantain

Sucrose, glucose and fructose have been shown to increase the gelatinisation onset temperature for many starches in the order sucrose>glucose>fructose (Zhang *et al*. (2013), Hoover and Senanayake (1996), Perry and Donald (2002)). Since sucrose hydrolysis has been shown to occur in plantains, it implies that the presence of glucose/fructose in solution and not sucrose is the likely cause of the observed increase in gelatinisation temperature of flours when compared to their pure starches (chapter 3). It further implies that if sucrose hydrolysis had been avoided in the samples, the onset temperature of gelatinisation and subsequently gelatinisation temperature in flours might have been further increased (especially in ripe plantain samples where the level of sucrose is above the 10%

sucrose per sample required to decrease the swelling volume of starch (Magnus and Eliasson, 2006)). This will be particularly useful in products where a low degree of gelatinisation is desired during heat-treatment in order to reduce the depletion of native resistant starch.

Can sucrose hydrolysis then be used as an indicator of gelatinisation in plantain? This should be a question for further research. The study of starch gelatinisation at controlled pH also remains an issue for further investigation. In this work we have been more concerned about domestically processed samples and their impact on GI. For this reason, these samples are required to be fed to human subjects; which implies that we are not able to use buffers to adjust pH during cooking to monitor gelatinisation for health and safety reasons.

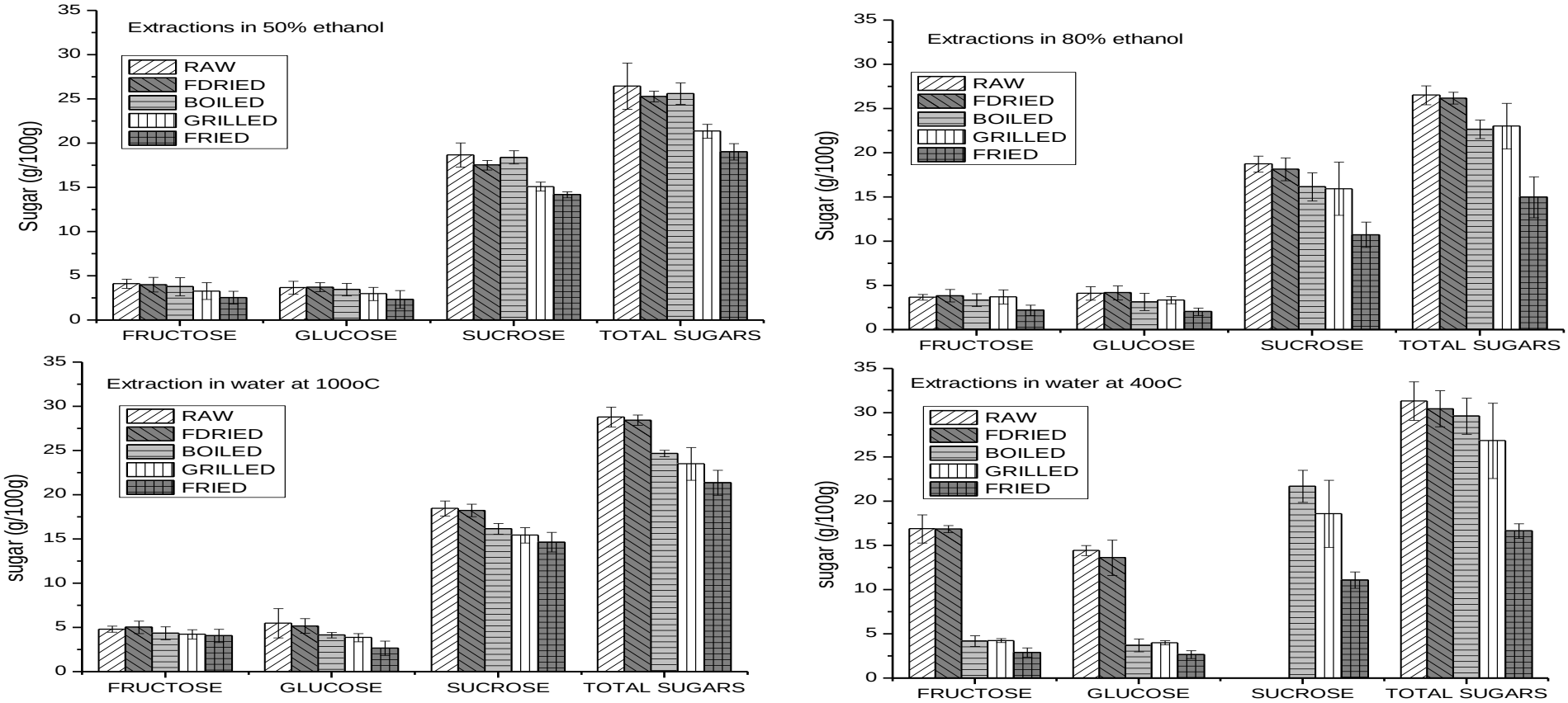


Figure 5.13: Fructose, glucose, sucrose and total sugars extracted from raw and processed ripe plantain

Three plantain fingers were each cut into five portions for raw, fried, boiled, grilled and f dried (freeze-dried). Extractions (in three replicates for each solvent) were done in water, 50% ethanol and 80% ethanol at 100^{o}C and in water at 40^{o}C. Freeze-dried sample was used to check effect of dehydration on sucrose hydrolysis when compared to wet samples. Results indicate the following (i) sucrose hydrolysis did not occur in processed samples as shown by extractions in water at 40^{o}C. In addition, sucrose was conspicuously absent in this extract for raw samples (both fresh and dried, which implies dehydration is not a condition for sucrose inversion (ii) sucrose in raw, wet and freeze-dried plantain was not hydrolysed in water

5.7 Conclusion

Reports on sucrose hydrolysis in food systems had always been associated with dehydrated products (Acevedo *et al*. (2008), Cristina Acevedo *et al*. (2012), Schebor *et al*. (2010)). In this work, it is concluded that dehydration of plantain is not necessarily a factor for sucrose hydrolysis to take place because inversion was found to occur even in fresh (non-dehydrated) plantain. However, starch gelatinisation was detected as an important determinant of the extent of sucrose hydrolysis. High temperatures required for starch gelatinisation processes also lead to deactivation of sucrose hydrolysing enzymes.

Sucrose hydrolysis in plantain has both advantages and disadvantages. It may lead to experimental errors in carbohydrate analysis if appropriate conditions of pH and temperature are not properly monitored. It also affects extraction of simple sugars when using water as the extraction solvent. It may however be a useful tool in increasing sweetness in plantain products that do not require heat treatments because in these products, sucrose hydrolysis increases fructose content and subsequently product sweetness.

The phenomenon of sucrose hydrolysis is important in glycaemic index studies because sucrose, glucose and fructose have different glycaemic indices and the relative quantities of each type of monosaccharide/disaccharide in a food will influence the overall glycaemic index elicited by the food.

Chapter 6 Retrogradation, retrograded resistant starch (RS3) and physically trapped resistant starch (RS1) in plantain

6.1 Hypothesis

Resistant starch type 2 which is abundant in plantain (chapters 2, 3 and 4), is lost when starch undergoes gelatinisation during cooking. However cooked and cooled plantain foods may contain high levels of retrograded starch (resistant starch type 3) and physically trapped starch (resistant starch type 1) which may contribute to the low glycaemic index of plantain.

6.2 Abstract

Starch retrogradation in plantain starch gels was studied by an amylose leaching method (starch-iodine reaction), an enzymatic method and X-ray diffraction analysis. Amylose leaching method was not suitable for the measurement of heat stable retrograded starch while the enzymatic method and X-ray diffraction gave comparable results. Retrograded starch (RS3) and physically trapped starch (RS1) contents of plantain foods were subsequently determined by the enzymatic method.

A thermally stable RS3 content 5.0 ± 0.9 to 13.2 ± 3.0 g/100 g dry weight basis was obtained for unripe samples while a value ranging from 0.9 ± 0.4 to 2.5 ± 0.9 g/100 g was obtained for ripe samples. Retrogradation increased slowly digestible starch (SDS) and physically inaccessible starch (RS1) portions in some of the samples but not in others. Retrogradation resulted in an increase in the quantity of slowly digestible starch (SDS) for ripe plantain samples. Samples cooked without salt produced an increase from ~ 0 at 0 hour to 2.8 ± 1.1 g/100 g at 48 hours and for samples cooked with salt the increase was from ~0 to 5.3 ± 1.3 g/100 g at 48 hours. There was a significant increase in the value of RS1 from 4.6 ± 3.6 to 7.7 ± 2.0 g/100 g after 48 hours of cold storage ($p \leq 0.05$) for unripe plantain samples cooked without salt but no significant difference for samples cooked with salt. Plantain RS3 isolate exhibited an X-ray diffraction pattern similar to that of retrograded starches but had a significantly ($p \leq 0.05$) lower crystallinity (25.5 ± 2.3%) when

compared to those of plantain granular starch (34.1 ± 1.1%) and native resistant starch, RS2 (31.2 ± 1.6%).

6.3 Introduction

Starch retrogradation is the term used to describe changes in physical and chemical behaviour following the gelatinisation of starch. During retrogradation, starch molecules re-associate to from an ordered structure. This usually takes place in two stages: in the first stage, two chains may associate and in the second stage and under favourable conditions a crystalline order and a physical phase separation occurs (Qiang, 2005). The extent of retrogradation depends on storage temperature. Storage of starch gels with 45 to 50% water content at low temperatures but above –5.0°C increases the retrogradation compared to storage at room temperature, especially during the first days of storage. Storage at freezing temperatures below –5.0°C virtually inhibits recrystallization while higher temperatures (above 32°C) effectively reduce retrogradation (Magnus and Eliasson, 2006).

Due to the fact that re-crystallisation and increase in firmness are both referred to as retrogradation, different techniques are used to measure the retrogradation process (Magnus and Eliasson, 2006). This, coupled with the fact that the molecular mechanism behind the phenomenon is still incompletely known, makes the evaluation of retrogradation more complex because different techniques may not be essentially measuring the same

process. This leaves us with a phenomenon that has been widely studied but still not completely understood.

Various techniques based on different properties of starch have been used to monitor starch retrogradation. The most commonly used methods include differential scanning calorimetry (DSC) (Abd Karim *et al*., 2000), X-ray diffraction analysis (XRD), enzyme digestion, nuclear magnetic resonance (NMR) (Abd Karim *et al*., 2000), and starch iodine methods (Jankowski, 1992).

Karim and co-workers (2000) in the review of methods used to measure starch retrogradation had indicated that following changes in a single parameter with time may not provide an adequate description of retrogradation. Moreover, retrogradation kinetics determined using different methods may also not be in agreement (Roulet *et al*., 1990). This therefore implies that correct interpretation of any results obtained using a particular method would depend on the accurate understanding of the physical and/or chemical basis on which the determination is made, as well as an appreciation of the limitations of the method.

Despite the volume of evidence surrounding the physiological benefits of retrograded starch, retrogradation does affect the textural qualities of food by increasing their hardness and rigidity and subsequently decreased acceptability in terms of sensory qualities (Gudmundsson, 1994).

Interest in resistant starch type 3 (RS3) has arisen because it is the most common type of resistant starch present in processed foods. In the past, It

was measured as part of dietary fibre because it shares similar properties with fibre, especially in being undigested (Bjorck *et al*., 1987). RS3 is formed from gelatinised starch and consists mainly of retrograded amylose and re-crystallized amylose fragments released during starch degradation (Qiang, 2005). RS3 is has been attributed to amylose retrogradation, however, RS3 can include retrograded amylopectin (Eerlingen *et al*., 1994) as well as re-crystallizations caused by the association of amylose and amylopectin upon cooling. Only RS3 occurring from retrograded amylose can survive heating at 100^{o}C (Eerlingen *et al*. (1994), Donald (2006)). This needs to be considered when choosing/designing experimental protocols and methods to be used for the determination of RS3.

Although it is proposed that the proportion of RS3 usually produced from starches with normal levels of amylose (< 30%) is currently too low to be useful in the production of RS - ingredients, there are reports supporting the fact that these low levels when present in foods may have physiological benefits (Qiang, 2005).

Investigations into the amylopectin chain length of banana starch indicate that it will be a good source for the generation of RS3 (Lehmann *et al*., 2002). On the other hand, Eggleston and co-workers (1992) reported that plantain starches (except for one diploid hybrid labelled 566/32) showed little retrogradation upon cooling of gelatinised starches after 24 hours of storage. The observed higher retrogradation property of the diploid hybrid was

attributed to its higher amylose content (12.04%) and larger granule size when compared to other hybrids studied.

It is always assumed that RS3 is the most common type of resistant starch in processed foods and little attention is given to RS1. Literature contains information regarding the decline of RS1 quantity by size-reduction treatments such as grinding and milling, but little information is available on how to boost type 1 RS by processing or other treatments. The enzyme resistance of RS1 is based on the inaccessibility of the enzyme to the starch, and it does not depend on the intrinsic chemical resistance of the starch itself. RS1 is therefore measured chemically as the difference between the glucose released from enzyme digestion of starch in a homogenised portion of a food sample and that released from a non-homogenised portion of the same food (Sajilata *et al*. (2006), Englyst and Englyst (2005)). This further indicates that the form of a food as well as the nature of other non-starch components of the food will play an important role in determining the quantity of RS1 that can be obtained from it.

Information on retrogradation properties of plantain starch is very scarce, probably because of loss of interest due to the reported low levels in banana starches as earlier reviewed by Zhang and co-workers (2005). Earlier research did not look at retrogradation in the whole fruit to see if there were any improvements. The large majority of the work done on resistant starch in the *Musa ssp* were carried out on dessert banana and as their name implies they are usually eaten raw rather than cooked. It was therefore easy to have

overlooked the importance of retrogradation of the whole fruit which is the major focus of this chapter.

Up to now there have been no studies to quantify RS1 and RS3 in cooked and cooled plantain starch or foods.

We have adopted an enzymatic method similar to that used in previous chapters to quantify RS1 and to monitor the extent of formation of type 3 resistant starch in cooked and cooled plantain foods. Retrogradation results in resistance of gelatinised starch to acid or amylolytic enzymes (Berry (1986), Bjorck *et al*. (1987)) and it has also been established that the extent of crystallinity is a major factor affecting starch digestibility (Cairns *et al*. (1990), Sievert and Pomeranz (1990), Sievert *et al*. (1991)). Therefore, an enzymatic method is likely to measure resistance to amylolytic enzymes as a result of substances which cannot be accessed by these enzymes due to their crystalline structures. The enzymatic method was also chosen because of the need to quantify RS3 for nutritional purpose and not just to check retrogradation as in other methods such has XRD and DSC. However, we have used XRD to validate retrogradation of starches and to measure the relative crystallinity of resistant starch types in plantain. An additional benefit of using an enzymatic method is that it can be applied to the measurement of RS3 in whole foods and on 'as eaten basis' i.e. without drying and milling. RS3 was measured as heat stable retrograded resistant starch that is not susceptible to enzyme hydrolysis after 2 hours. We are more interested in heat stable resistant starch because cooked foods, which have been stored in

the refrigerator for 1 - 2 days, are usually re-heated in a microwave oven or similar equipment before consumption. When such foods are re-heated at temperatures above 60^{o}C, retrograded amylopectin is lost.

6.4 Materials and method

6.4.1 Materials

Enzymes used are as described in chapter 2.

6.4.2 Gelatinisation of samples

Samples were completely gelatinised (please refer to chapter 3) before subjecting them to retrogradation storage as described in section 6.4.5.

6.4.3 Starch

5% (0.1 g in 2 ml) and 10 % (0.2 g in 2 ml) starch solutions were prepared in 50 ml Eppendorf tubes and subsequently heated in a boiling bath for 15 min. Three tubes were used for each analysis.

6.4.4 Whole plantain

For each retrogradation experiment, one finger of plantain was cut into 6 equal portions and cooked in 750 ml of water for 15 min. Three fingers were used for each measurement. Freshly cooked samples were analysed in duplicate within 30 min of cooking which included the time for mincing and weighing.

6.4.5 Retrogradation storage

Gelatinised starch/ food were allowed to cool for 10 min at ambient temperature before transferring them to the refrigerator (at 5^{o}C) or water bath (at 20^{o}C). For starch, samples were stored for 3, 6, 12, 24 and 48 hours. Whole food samples were only stored for 24 and 48 hours.

6.4.6 Determination of retrogradation in plantain starch

6.4.6.1 Amylose leaching method

A 13 ml volume of water was added and mixed with the retrograded starch suspension, vortex-mixed and then centrifuged for 15 min at 3000*g* and 20^{o}C. Quantity of leached amylose was determined using the same method as described in chapter 3. Measurement for completely gelatinised starch was taken at time zero, i.e., without subjecting samples to retrogradation storage.

6.4.6.2 Enzymatic method

A 13 ml volume of water was added to each tube, tubes were reheated in a boiling water bath for 30 min and starch digestion and glucose measurement was carried out as earlier described in chapter 3. The quantity of RS3 was calculated using the equation below.

$$RS3 = [G120_{t0} - G120_{tx}] \times 0.9 \quad \ldots\ldots 1$$

Where $G120_{t0}$ is the digestible starch for freshly gelatinised starch and $G120_{tx}$ is the digestible starch at retrogradation storage time x (x = 3, 6, 12, 24 and 48 hours).

6.4.6.3 X-ray diffraction assay

6.4.6.3.1 Preparation of samples

Starch gels (retrograded and unretrograded) were freeze-dried and then homogenised before measurements. For 5% starch samples, it was initially difficult to get XRD measurements because they were very light and full of air so the samples had to be flattened by storing them for a week under a heavy weight to achieve a flat material of not more than 0.5 mm thickness**.**

6.4.6.3.2 Isolation of native resistant starch (RS2)

Starch digestion as earlier described was carried out on starch suspensions. After digestion, tubes were centrifuged at 3000*g* for 15 min at ambient temperature. The supernatants were discarded while undigested matter in the tubes were pulled and rinsed with acetone and centrifuged again. This was done three times before rinsing twice with ethanol. Samples were then air-dried at room temperature and used for the XRD measurements.

6.4.6.3.3 Isolation of retrograded starch (RS3)

Retrograded starch gels were heated in a boiling water bath for 15 min and starch digestion carried out as earlier described. After digestion, tubes were centrifuged at 3000*g* for 15 min at ambient temperature. The supernatants were discarded while the RS3 residues in the tubes were pulled and rinsed with acetone and centrifuged. This was done three times before rinsing twice with ethanol. Samples were then air-dried at room temperature and used for the XRD measurements.

6.4.6.4 X-ray diffraction measurements

Crystallinity of starches was determined from the measured X-ray diffraction patterns obtained. To determine the crystalline diffraction patterns of samples, a small sample holder of about 5 mm diameter was filled with sample, diffractograms were recorded using an X-ray diffractometer (P analytical X'Pert MPD). The scattered X-ray radiation was recorded using programmable divergence slits with an irradiated area of 15x15 mm over an angular range of 4 - 60° (2θ) in steps of 0.1° and scan time of one hour. Each sample was run twice. 560 data points were obtained for each run and origin software was used to make XRD plots after deduction of the intensities of the blank pan. The degree of starch crystallinity was determined using equation 3 on page 168.

6.4.7 Determination of retrograded starch (RS3) in boiled plantain

After 24/48 hours storage of cooked plantain, a portion of about 30 g was cut and minced using a hand operated kitchen mincer and then homogenised with a laboratory mortar and pestle to disrupt the food matrix. 0.5 g of homogenised sample was mixed with 15 ml water in an Eppendorf tube, heated in a boiling bath for 15 min, and then equilibrated in a water bath at 40^{o}C. Starch digestion was carried out as described in chapter 3. Starch digestion was also carried out on a minced and homogenised portion of freshly cooked plantain. The quantity of RS3 in samples after retrogradation was calculated using equation 1 above. RDS (rapidly digestible starch) and

SDS (slowly digestible starch) were determined as described earlier in chapter 4.

6.4.8 Determination of physically trapped starch (RS1) in plantain foods

Two portions of about 30 g each was cut from a cooked plantain finger. One was minced using a hand operated kitchen mincer while the other was minced and homogenised. 0.5 g of minced/homogenised portion was mixed with 15 ml water and then equilibrated in a water bath at 40^{o}C (for samples analysed hot, cooked minced samples were maintained at 100^{o}C in a water bath before equilibrating in at 40^{o}C). Starch digestion was carried out as described in chapter two. Starch digestion was also carried out on minced/homogenised portions of freshly cooked plantain.

For each sample at time 0, 24 or 48 hour, the value of RS1 was calculated using equation 2 below.

$$RS1 = [G120_{(homogenised)} - G120_{(minced)}] \times 0.9 \quad \ldots\ldots 2$$

6.4.9 Statistical analysis

All analyses were performed in triplicate, and the mean and standard deviations of each set was taken. Data obtained was analysed using analysis of variance (ANOVA) on SPSS statistics software and values are expressed as mean ± standard deviation.

6.5 Results and discussion

6.5.1 Detection and measurement of retrogradation in plantain starch

Completely gelatinised starches were used to study the formation of retrograded starch. Starches used had ~ 98.5 ± 1.1% degree of gelatinisation (refer to chapter 3).

The results from leached amylose determined on cooked and cooled samples are shown in Figures 6.1 - 6.3. The quantity of leached amylose in completely gelatinised starch 5% suspension was monitored at room temperature (20^{o}C) and at refrigerator temperature (5^{o}C) to determine the degree of retrogradation. Quantity of leached amylose was determined with two types of sample treatments (i) with re-heating before measurements and (ii) without re-heating before measurements. From Figure 6.1, there was marked reduction in the quantity of amylose leached between 3 hours and 48 hours, when compared to leached amylose at time 0, in both room and fridge temperatures. This gave an indication that retrogradation had taken place.

However, samples subjected to re-heating (Figure 6.2), revealed that some of the amylose not leached initially was actually trapped in the starch matrix rather than re-associated (retrograded). The rate of reduction in quantity of leached amylose was consistent between 20^{o}C and 5^{o}C, but the 5^{o}C samples had lower values of leached amylose at all times studied (Figures 6.1 and 6.2). Variation in each storage time between values of leached amylose for the re-heated and non-re-heated samples are shown in Figure 6.3.

In order to confirm that observations made with the leached amylose method were an indication of starch retrogradation; samples were subjected to enzyme hydrolysis. Unfortunately, no retrograded starch was detected (Table 6.1). A 10% starch solution was then prepared and checked by an enzymatic method and a small quantity of RS3 (0.8 ± 0.2 g/100 g) (n = 3) was detected after a 48-hour storage (Table 6.1).

Retrogradation was further confirmed by a non-chemical method using X-ray diffraction (XRD) which detects crystalline regions in samples.

X-ray diffraction patterns obtained for gelatinised starches (labelled Gel) and for starch gels stored for 48 hours (labelled Ret) are shown in Figures 6.4 and 6.5. Diffraction patterns obtained for gelatinised starches are large amorphous areas with no crystalline peaks typical for an amorphous material (Zobel *et al*., 1988). Starch is a semi-crystalline material but its crystallinity is lost on gelatinisation (Magnus and Eliasson, 2006). X-ray diffraction pattern obtained for samples stored for 48 hours for both the 5% and 10% starch samples indicate that the areas of the amorphous regions were reduced in the two samples (Figures 6.4 and 6.5). A further deduction was the observation of a very small crystalline peak (CP) in the X-ray diffraction pattern for the 10% starch solution. The absence of this peak in the amorphous control is an indication of the presence of RS3 produced by the crystallisation of amylose in the stored 10% starch gels.

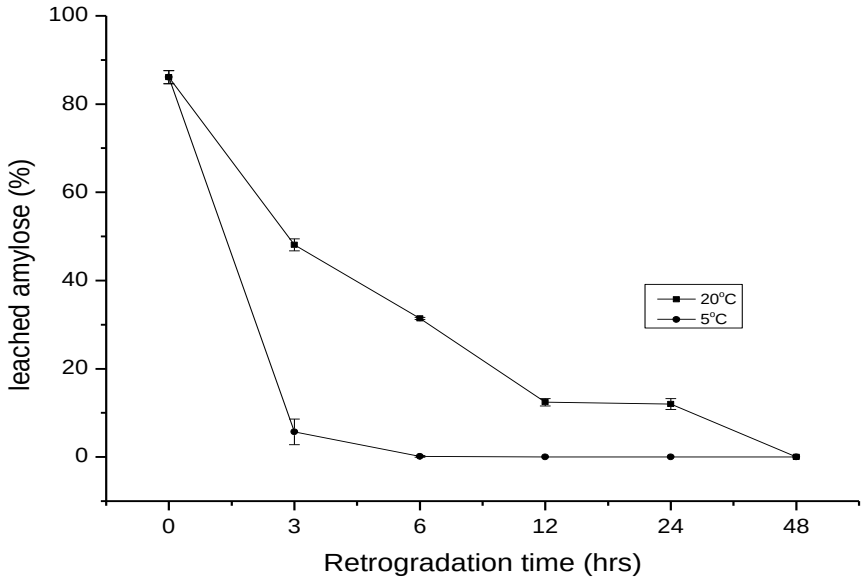


Figure 6.1: Leached amylose curves at 5°C and 20°C without re-heating
Quantity of leached amylose was measured in starch gels (5% conc.) at time 0 and after storage for 3, 6, 12, 24 and 48 hours

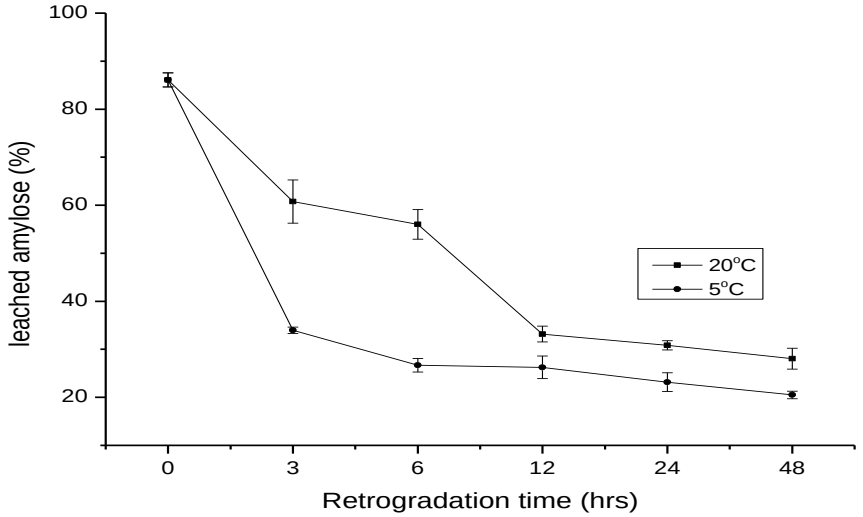


Figure 6.2: Leached amylose curves at 5°C and 20°C with re-heating;
Quantity of leached amylose was measured in starch gels (5% conc.) at time 0 and after storage for 3, 6, 12, 24 and 48 hours. Gels were re-heated at 100°C for 10 minutes before measurements.

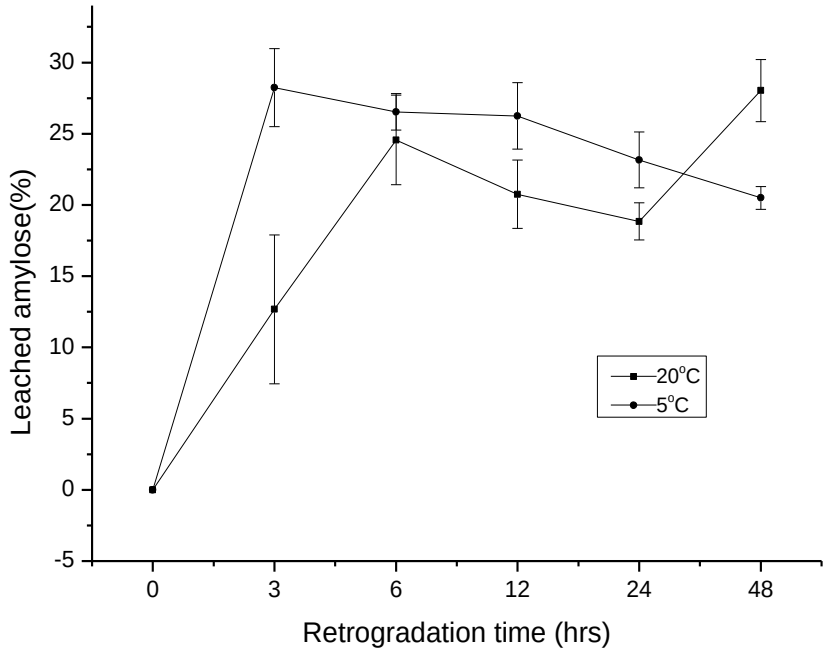


Figure 6.3: Leached amylose curves at 5°C and 20°C for difference between re-heating and no re-heating
Values of leached amylose used in this figure represent subtractions made on values for figures 6.1 and 6.2 at the experimental times shown.

Table 6.1: Quantity of retrograded resistant starch in plantain starch (g/100g dry weight basis)

Starch conc.	0 hour	24 hours	48 hours
5 %	ND	ND	ND
10 %	ND	ND	0.8±0.2

ND= not detected, n=3, detection limit was 10% starch suspension after 48 hours storage

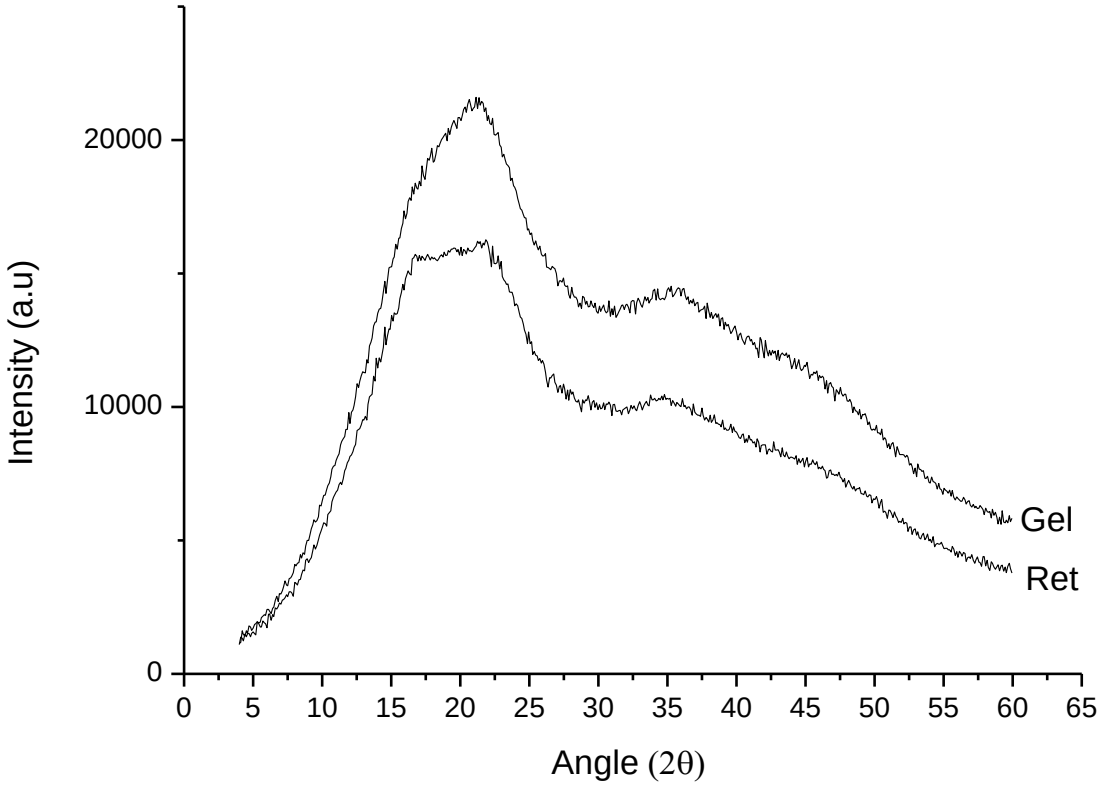


Figure 6.4: X-ray diffraction patterns for 5% starch gels
Gel = armorphous control, Ret = retrograded starch after 48 hours storage at 5^{o}C. A reduction in armorphous area is observed with the retrograded starch gel

This was a confirmation of the results obtained by using an enzymatic method. The XRD study of the development of crystallinity in amylose and starch gels had earlier been carried out and the observation made was that the initial development of crystallinity occurred at the same rate, however the crystallization of amylose reached a limit after 48 hours, whereas that of starch gel continued (Miles *et al*., 1985). Other studies later confirmed that the further crystallization observed in starch gels and not in amylose gels are due

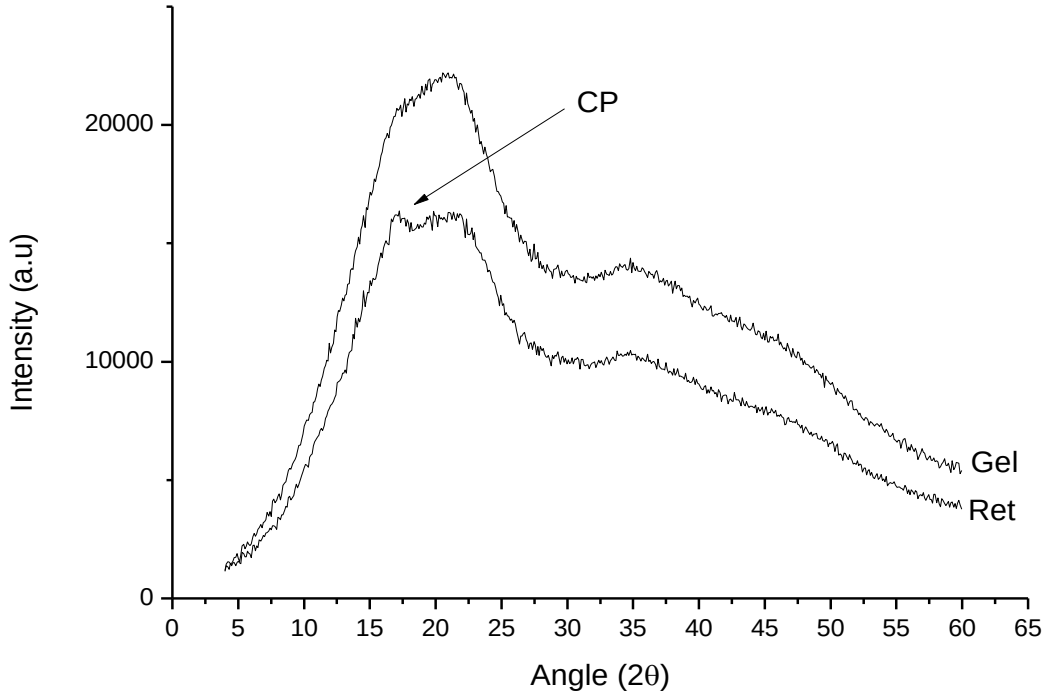


Figure 6.5: X-ray diffraction patterns for 10 % starch gels
Gel = armorphous control, Ret = retrograded starch after 48 hours storage at 4^{o}C. A reduction in armorphous area is observed with the retrograded starch gel as well a small crystalline peak - CP

to amylopectin crystallisation which occurs more slowly than that of amylose (Ring *et al*. (1987), Ring *et al*. (1988)).

The reduction in the amorphous area (Figures 6.4 and 6.5) in the stored starches, is an evidence of the re-association of starch molecules and may be interpreted as a reduction in amorphous strength of the starch gel which may literally imply an increase in the crystallinity of the starch gel. Although this is not conclusive, especially for the stored 5% starch gels where no crystals

were detected, it seems to explain the decrease in the quantity of leached amylose observed in the amylose leaching method above (Figures 6.1 and 6.2). Furthermore, because no RS3 was detected and no crystals were observed, it can be deduced that the re-association of amylose occurring in these gels were not strong enough to produce RS3, which may likely be due to the low starch concentration as well as the nature of the starch granules (Magnus and Eliasson, 2006). The reduction in the quantity of leached amylose gives a good picture of the probable re-association of starch granules as part of the initial steps in retrogradation but not of the final formation of RS3. Again, as we observed in the case of determination of gelatinisation temperature (chapter 3), the leached amylose method may not be appropriate for the determination of retrogradation of starch, especially in terms of RS3 quantification because it does not give a true picture of amylose retrogradation. However, it may be a suitable method for the determination of amylopectin retrogradation and this needs to be further evaluated.

Our observation of detection of RS3 in 10% starch but not in the 5% solution is in tandem with an earlier report for banana, indicating that starch suspensions of Taiwan dessert banana underwent retrogradation during cooling only at concentrations greater than 8% (Lii *et al*., 1982).

Though RS3 was not detected in 5% solution it was easier to monitor other changes observed with a lower concentration. The use of the amylose leaching method was difficult to apply to plantain starch at concentrations higher than 5% concentration whereas we were able to go up to 10%

concentration with potato starch (unreported). This is due to a higher viscosity - it was earlier reported that banana starch has a viscosity four times that of a corn starch paste of the same starch concentration of 6% (Ling *et al*., 1982).

6.5.2 Starch retrogradation in whole foods

The results for the digestion of samples on 'as eaten' basis, to quantify retrograded starch (RS3), physically trapped starch (RS1), slowly digestible starch (SDS), and rapidly digestible (RDS) are shown in Tables 6.2 and 6.3. Values on dry weight basis (DWB) are conversions based on the moisture content of the samples.

Results reveal that RS3 values after 24 hours and 48 hours storage were not significantly different ($p \leq 0.05$) for both ripe and unripe samples that were cooked without salt. In addition, for these samples, the extent of retrogradation after 48 hours storage in the ripe was higher than in the unripe samples (55.6 ± 1.8 vs. 40.0 ± 1.1%). This may be due to the higher quantity of sugars present in the ripe samples (refer to chapters 4 and 5). It was earlier reported that retrogradation of starch gels increased in the presence of sugars in the order glucose >fructose> sucrose (Hoover and Senanayake, 1996).

Table 6.2: Resistant starch fractions in boiled unripe plantain

DWB (g/100 g sample)

		RDS	SDS	RS1	RS3	RS TOTAL
NS	**0HOUR**	54.0±2.4^{c}	13.6±4.4^{a}	4.6±3.6^{a}	0.0±0.0^{a}	4.6±2.1^{a}
	24HOUR	49.8±2.8^{bc}	13.2±0.6^{a}	4.6±1.5^{a}	5.0±0.9^{b}	9.6 ±2.1^{b}
	48HOUR	44.0 ±4.9^{b}	14.2 ± 2.8^{a}	7.7 ± 2.0^{b}	7.0 ± 0.8^{b}	14.7±2.8^{c}
WS	**0HOUR**	33.4±6.6^{a}	25.3±4.5^{b}	15.0±3.2^{c}	0.0±0.0^{a}	15.0±3.2^{c}
	24HOUR	31.7±3.9^{a}	23.6±4.2^{b}	12.9±5.3^{bc}	6.2±2.7^{b}	19.1±2.6^{c}
	48HOUR	28.9±2.3^{a}	15.0±1.6^{a}	18.0±3.4^{c}	13.2±3.0^{c}	31.2±3.2^{d}

AS EATEN (g/100 g sample)

		RDS	SDS	RS1	RS3	RS TOTAL
NS	**0HOUR**	18.6±0.8	4.7±1.5	1.6±0.7	0.0±0.0	1.6±0.7
	24HOUR	17.1±1.0	4.6±0.5	1.6±0.7	1.7±0.3	3.3±0.7
	48HOUR	15.1±1.7	4.9±1.0	2.7±0.7	2.4±0.3	5.0±1.0
WS	**0HOUR**	10.3±2.0	7.8±1.4	4.6±1.0	0.0±0.0	4.6±1.0
	24HOUR	9.7±1.2	7.3±1.3	4.0±1.6	1.9±0.8	5.9±0.8
	48HOUR	8.9±0.7	4.6±0.5	5.5±1.1	4.1±0.9	9.6±1.0

Analyses were made on fresh, 'as eaten'. DWB = dry weight basis, and were calculated from fresh weight values based on moisture content of samples i.e. 65.8 ± 3.2% for NS (samples cooked with no salt) and 69.2 ± 1.8% for WS (samples cooked with addition of salt). n=3, values with the same superscript in the same column are not significantly different at 95% confidence level.

Table 6.3: Resistant starch fractions in boiled ripe plantain

DWB (g/100 g sample)

		RDS	SDS	RS1	RS3	RS TOTAL
NS	**0HOUR**	38.2±1.2^{d}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
	24HOUR	35.2±1.3^{c}	1.4±0.8^{ab}	0.7±0.4^{ab}	0.9±0.4^{ab}	1.7±0.2^{c}
	48HOUR	32.8±1.5^{b}	2.8±1.1^{bc}	1.1±0.5^{b}	1.4±0.6^{b}	2.5±0.6^{d}
WS	**0HOUR**	35.7±0.8^{c}	0.1±0.2^{a}	0.8±0.4^{ab}	0.0±0.0^{a}	0.8±0.4^{b}
	24HOUR	30.8±1.2^{b}	3.2±1.1^{c}	1.2±0.5^{b}	1.5±0.5^{b}	2.7±0.5^{d}
	48HOUR	25.5±1.0^{a}	5.3±1.2^{d}	3.5±0.7^{c}	2.5±0.9^{c}	6.0±0.3^{e}

AS EATEN (g/100 g sample)

		RDS	SDS	RS1	RS3	RS TOTAL
NS	**0HOUR**	12.1±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	24HOUR	11.2±0.4	0.4±0.2	0.2±0.1	0.3±0.1	0.5±0.1
	48HOUR	10.4±0.5	0.9±0.3	0.4±0.2	0.4±0.2	0.8±0.2
WS	**0HOUR**	10.3±0.2	0.0±0.0	0.2±0.1	0.0±0.0	0.2±0.1
	24HOUR	8.9±0.3	0.9±0.3	0.3±0.1	0.4±0.1	0.8±0.1
	48HOUR	7.4±0.3	1.5±0.4	1.0±0.2	0.7±0.2	1.7±0.1

Analyses were made on fresh, 'as eaten'. DWB = dry weight basis, and were calculated from fresh weight values based on moisture content of samples i.e. 68.3 ± 1.5 % for NS (samples cooked with no salt) and 71.2 ± 1.4 % for WS (samples cooked with addition of salt). n = 3, values with the same superscript in the same column are not significantly different at 95% confidence level.

For samples cooked with salt, the quantity of RS3 increased by about 113% between 24 hours to 48 hours for the unripe samples (i.e. from 6.2 ± 2.7 g/100 g to 13.2 ± 3.0 g/100 g) while for ripe samples the increase observed was about 66.7% (1.5 ± 0.5 to 2.5 ± 0.9 g/100 g). It appears that cooking with salt is favourable for the formation of RS3 contrary to earlier studies which reported a decrease in retrogradation at 4^{o}C with sodium chloride (Beck *et al*. (2011), Baker and Rayas-Duarte (1998)). It is also not clear why the extent of retrogradation in ripe samples was lower in the presence of both salt and sugars because the expectation would be that a combination of two factors that lead to an increase in retrogradation should produce a higher value rather than a lower one. The impact of the interactions of sodium chloride with sugars (fructose, glucose and sucrose) on starch retrogradation is scarcely reported in literature and needs to be investigated. In addition, the fact that the reorganisation of amylose and amylopectin chains during cooling is a function of the starch granular structure which varies for different starches implies that these interaction may vary for different starches (Belloperez and Paredeslopez, 1995).

Reported values for quantification of RS3 in whole foods are scarce because most of the studies on retrogradation were performed on starch samples and in the majority of these studies, retrogradation was studied by other methods such as XRD and DSC which can be used to determine crystallinity levels (Gidley *et al*., 1995) but cannot be used to quantify RS3 in terms of weight per food quantity as required in nutritional studies. Quantification of RS3 by an

enzymatic assay was however reported in one study by Yadav *et al* (2010) for the following foods after 24 hours storage at 4^{o}C on percent dry weight basis. Wheat - 2.5 ± 0.1, rice – 1.6 ± 0.1, barley 3.5 ± 0.2, chickpea – 7.1 ± 0.0, pea – 5.9 ± 0.1, lentil 6.5 ± 0.2, kidney bean 5.3 ± 0.1. RS3 values obtained after 24 hours storage for unripe plantain (5.0 ± 0.9 – 6.2 ± 2.7 g/100 g) is lower than that of chickpea, which was the highest value reported in that study. Quantification of RS3 in banana was reported in only one study, where RS3 value for banana starch extrudate (30% moisture content) after 24 hours was found to be 5.25 ± 0.06% of sample (Bello-Perez *et al*., 2005b) . This is similar to values we have obtained for cooked unripe plantain (after 24 hours) in this study (5.0 ± 0.9%). This value was however not the maximum in our study as an increase to 7.0 ± 0.8 g/100 g was observed after 48 hours of storage as opposed to a maximum of 5.04 ±0.06% after a 12-hour storage reported for banana, by Bello-Perez and co-workers (2005a).

Retrogradation resulted in an increase in the quantity of slowly digestible starch (SDS) in ripe plantain samples (Tables 6.2 and 6.3). Samples cooked without salt showed an increase from ~0 at 0hour to 2.8 ± 1.1 g/100 g at 48 hours and from ~0 to 5.3 ± 1.3 g/100 g at 48 hours for samples cooked with salt. Hence it can be concluded that retrogradation in ripe plantain products is favourable for the production of slowly digestible starches. SDS formation has been attributed to factors such as the presence of longer amylose chains in starch e.g. non-waxy rice starch which prevents aggregation of amylose chains and results in the formation of a cross-linked network rather than the

formation of a more ordered crystalline structure (RS3) due to non-alignment of double helices. Therefore SDS is structurally considered to consist of less perfect crystals and amorphous components which are digestible (Lehmann and Robin, 2007). The mechanism for the production of SDS during starch retrogradation as it affects various starch sources/structures needs to be further investigated. In the case of unripe plantain, there were no significant changes ($p \leq 0.05$) in SDS levels for samples cooked without salt, however, for samples that were cooked with salt the significant drop in SDS levels appeared to have resulted in corresponding significant increases in RS3 levels from 0 hour to 48 hours. SDS values obtained for freshly cooked unripe plantain (13.6 ± 4.4 g/100 g) was higher than for freshly cooked potatoes (3 ± 4.5 g/100 g), whereas values for cold storage potatoes were higher (23 ± 10 g/100 g) than for unripe plantain (14.2 ± 2.8 g/100 g) (Monro *et al*., 2009). However, it appears the higher values on storage was due to the fact that RS1 was measured along with the RS3 (as is the case with many studies) as samples used in the study by Monro *et al* (2009) were minced but not homogenised. Retrograded starch and physically trapped starch have different physiological benefits and correct determinations will help to maximise these benefits.

6.5.3 How crystalline is RS3 from plantain starch

The crystallinity of retrograded starch (RS3) isolated from starch was determined by XRD and compared with those of the plantain starch and native resistant starch (RS2) isolate (Figure 6.6). The crystallinities of the

starches were determined from the X-ray diffraction patterns obtained, against an amorphous control which is gelatinised plantain starch using equation 3 below.

$$\% \text{ Crystallinity} = \frac{\text{peak area of } sample - \text{peak area of amorphous control}}{\text{total peak area of sample}} \times 100 \quad \text{..... (3)}$$

RS3 isolate had a significantly lower crystallinity value when compared with its whole starch and RS2 counterparts (Table 6.4). Crystallinity is important because it usually affects starch digestibility. Native starches are semi-crystalline substances and highly crystalline starches have lower susceptibility to digestive enzymes (Carlos-Amaya *et al*. (2011), Zhang *et al*. (2006)).

When starch is gelatinised it loses its crystallinity, however, during the retrogradation process, some of its molecules associate to form crystals. These crystals are usually not in the same form as the initial native starch (Ek *et al*., 2012).

The absence of the first peak at 15^{o} 2θ in the RS3 curve appears to further confirm a reduction in the number crystalline regions in the retrograded starch when compared with the native starches. Smaller peaks observed after angle 25^{o} 2θ appear to be more conspicuous in the order RS3 > RS2 > starch.

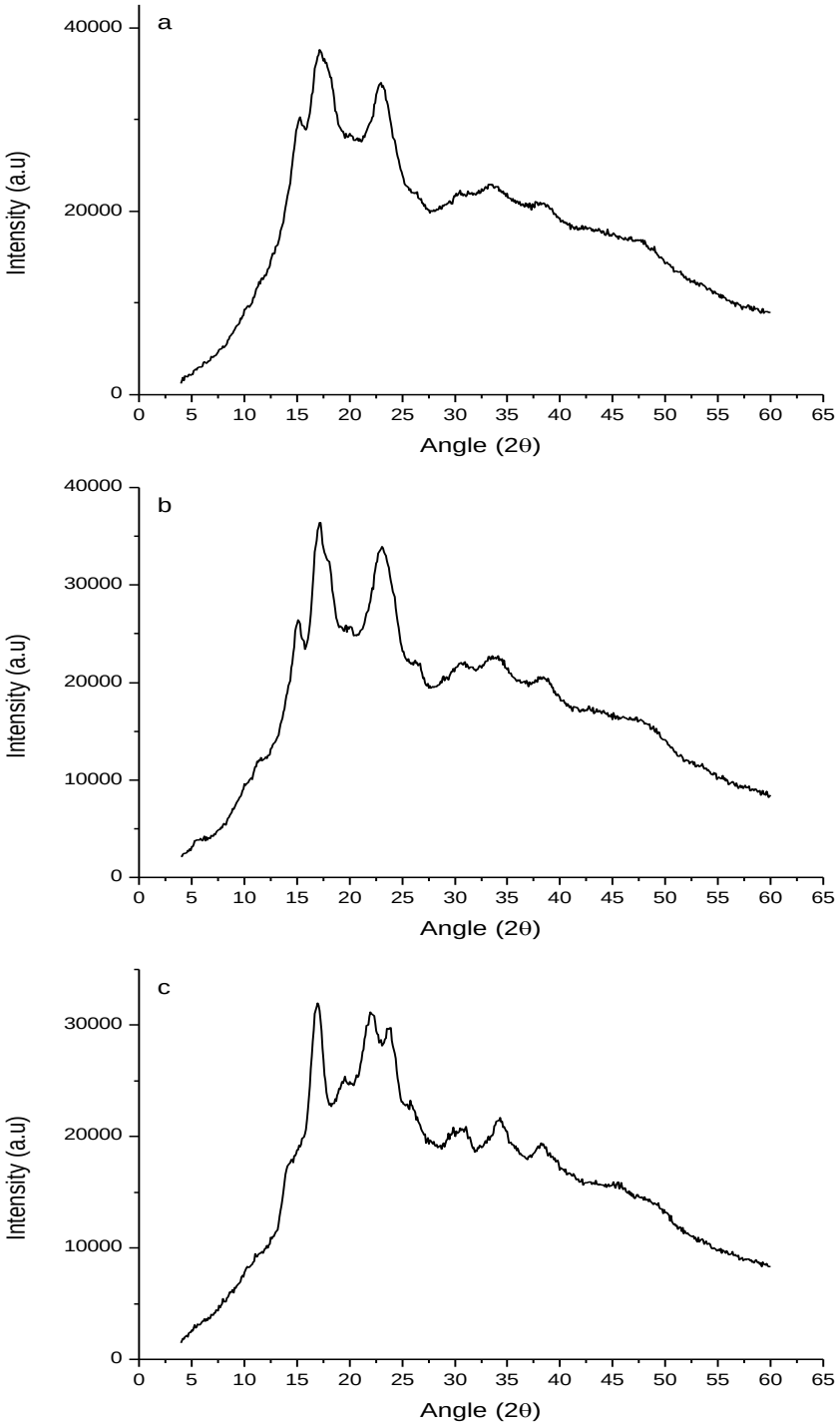


Figure 6.6: X-ray diffraction patterns for (a) plantain granular starch (b) native resistant starch isolate (RS2) and (c) retrograded resistant starch isolate (RS3)

a and b peaks and patterns are very close in similarity except that peaks after angle 25^{o} 2θ are more conspicous for b while the crystallinity pattern for c is different from those of a and b, also in c, the peak at 15^{o} 2θ is absent and peaks after angle 25^{o} 2θ are more conspicous and sharper.

The reason for these observations needs to be further investigated in structural studies relating to the differences in these starches, as there were no literature reports available to clarify this.

It is important to note however that granular banana starch X-ray diffraction pattern reported in literature (Faisant *et al*. (1995) Bello-Perez *et al*. (2005a)), have a peak at angle 5^{o}, this was not observed for plantain starch where the first crystalline peak occurred at angle 15^{o} 2θ.

The crystallinity value obtained for RS3 isolate in this work (25.5 ± 2.3%) is comparable with a value of 26.0% obtained by Zhang and Hamaker (2012), which was the only study that has reported the crystallinity value of retrograded *Musa* starch (banana starch was used in this case) as determined by XRD. However, in the study by Zhang and Hamaker (2012) the retrograded starch isolate was not used.

The crystalline peaks observed in the X-ray diffraction pattern of plantain RS3 isolate at 17^{o} 2θ and at 22^{o} and 24^{o} 2θ are typical of retrograded starch isolates from other starch sources as earlier reported by Gidley *et al* (1995) and Eerlingen *et al* (1993a, 1993b, 1995). However, the crystallinity level for the plantain starch RS3 isolate (25.5 ± 2.3 %) falls in the lower range of values for RS3 isolates, which is usually between 25 ± 3 – 30 ± 3 % (Gidley *et al*. (1995), Eerlingen *et al*. (1993a), (Eerlingen *et al*., 1993b), (Eerlingen and Delcour, 1995)). We can infer from this result that RS3 from plantain does not display any exceptional quality in terms of crystallinity when

compared with RS3 isolates from other sources. Also the crystallinity of amylomaize VII starch was lower (17 ± 2%) than that of its retrograded counterpart (30 ± 3%) while plantain starch had a lower crystallinity on retrogradation.

Table 6.4: Crystallinity of different starches from plantain

Sample	Starch	RS2	RS3
Crystallinity (%)	34.1 ± 1.1[a]	31.2 ± 1.6[a]	25.5 ± 2.3[b]

% crystallinity was calculated from XRD patterns in figures 6.6 using equation 3 on page 168, values are means of and standard deviations of 2 determinations. RS3 has a significantly lower crystallinity at 95% confidence level.

6.5.4 Physically trapped starch (RS1) in plantain

Results in Tables 6.2 and 6.3 reveal that in the unripe plantain samples that were cooked without salt, RS1 values were not significantly different ($p \leq 0.05$) after 24 hours of storage whereas there was a significant increase in RS1 quantity from 4.6 ± 3.6 to 7.7 ± 2.0 g/100 g after 48 hours of cold storage. Cooking with salt resulted in a significantly higher level of RS1 when compared with the samples that were cooked without salt at each time examined, however, RS1 values at 0, 24 and 48 hours for samples cooked with salt were not significantly different from each other at 95% confidence

level. The reason for higher RS1 values when samples were cooked with salt may be attributed to higher bonding and re-association of food particles due to the presence of salt. In addition, stabilisation of the plantain cell walls upon cooling may be a contributing factor to this observation as earlier reported by Micklander *et al* (2008). This is because the stabilisation of plant cell walls will decrease accessibility of enzymes to starch that is enclosed within the cellwall.

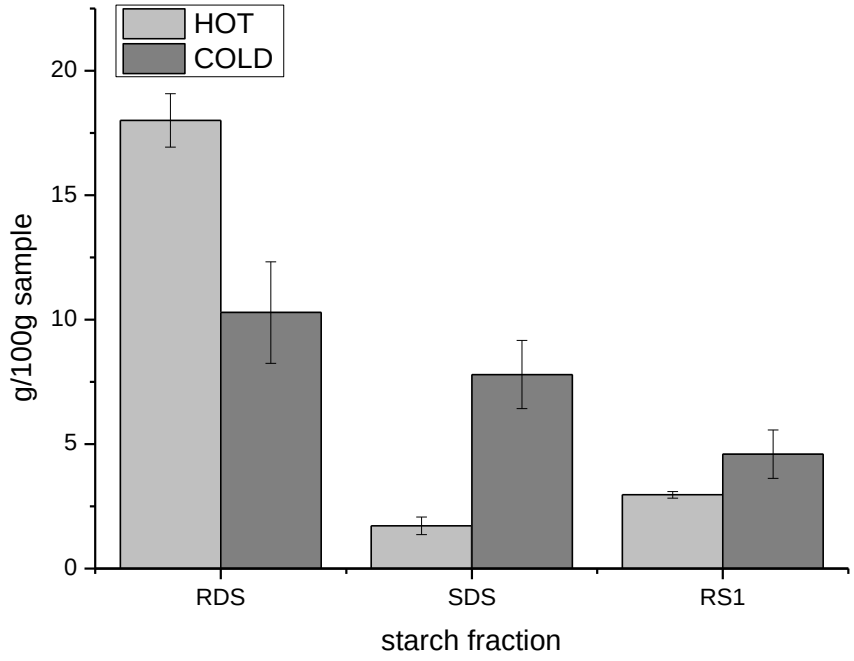


Figure 6.7: Starch fractions in hot and cold unripe boiled plantain
in vitro starch digestion was carried out on samples with two treatments before digestion (i) hot – heating at 100^{o}C (ii) cold – 20^{o}C, values plotted are means ± standard deviations of triplicate determinations

For ripe plantain, the rates of increase in RS1 after 24 hours of storage were approximately the same in both samples cooked with salt and those cooked without salt because the values obtained for RS1 after 24 hours were not significantly different from those at 0 hour. Contrary to the observation for unripe samples, a significant increase in RS1 was observed after 48 hours for samples cooked with salt (in the case of unripe plantain a significant increase in RS1 at 48 hours was observed for samples cooked without salt). At this moment we are unable to give reasons for these observations because ripe and unripe plantains are different in terms of components and their relative quantities and the interactions of these components during retrogradation and in the presence of salts need to be further investigated.

6.5.5 Impact of cooling on physically trapped starch (RS1)

The impact of cooling on the starch fractions of freshly cooked plantain was determined by carrying out *in vitro* digestion analyses on the same samples as hot (100^{o}C) and cold (20^{o}C) according to Englyst (1992). Results obtained (Figure 6.7) reveal that samples analysed cold had higher quantities of slowly digestible starch (SDS) and physically trapped starch (RS1) and lower quantities of rapidly digestible starch (RDS) than those analysed hot.

A recent study disclosed that the glycaemic index (GI) of cooked potato (which has been classified has a high GI food) could be reduced by serving it cold (Ek *et al*., 2012). The reason given for this observation was ascribed to the probable conversion of potato RDS into SDS.

Our results however indicate that the cooling of boiled plantain foods not only resulted in an increase in the quantity of SDS but a significant increase of about 50% of the initial RS1 quantity. The combination of a higher quantity of SDS and higher RS1 value may lead to significantly lower glycaemic index (GI) values for plantain foods when consumed cold rather than hot. The fact that the presence of SDS in foods results in lower GI values has been reviewed elsewhere (Lehmann and Robin, 2007), while Jenkins *et al* (2000) gave some insights into the positive impact of RS1 in lowering postprandial glucose levels. The minimum quantity/dose of SDS/RS1 required to have a positive impact on glycaemic index reduction has however not been investigated. It is also not certain if the structure of RS1/SDS (as implied in the case of dietary fibre in grains (Juntunen *et al*., 2002)) is more important to regulate glucose metabolism than its quantity.

Zhang *et al* (2012) studied banana starch retrogradation by differential scanning calorimetry (which is known to be more specific for the measurement of amylopectin retrogradation as earlier reviewed by Karim *et al* (2000)) and discovered that banana starch showed faster retrogradation kinetics than corn and potato starches. This was attributed to a higher proportion of long chains of amylopectin in banana starch. It is not certain if this is true for all *Musa* starches, however, it seems to provide a good explanation for the differences in starch fractions (Figure 6.7) observed between the hot and cold plantain samples. This opinion of a fast rate of

amylopectin retrogradation is further supported by the results earlier reported by the amylose leaching method in section 6.5.1 of this chapter.

Despite its nutritional and physiological importance, it appears that RS1 is the neglected resistant starch type as it seems to be receiving less emphasis today. No current literature reports are available relating the impact of RS1 to glycaemic index (earlier reports having been cited in chapter 1). This is not surprising because the focus of the study and measurement of resistant starch type 1 in many studies had always been on cereal grains and pulses when they are not milled. Other commonly consumed foods in the western world, such as potatoes and rice have not been reported to contain significant amounts of RS1. Low quantities of RS1 detected in rice and potato seems to be related to the low fibre content of these food types. Though high fibre in foods have been advocated to reduce digestion and flatten glycaemic response, it is not clear if higher fibre content is correlated with an increase in the quantity of RS1. Cooling increases food rigidity and hardness and may help to improve RS1 levels in foods. The increase in RS1 in some cooked foods may be due to stickiness caused by the leaching of amylose from starch granules during gelatinisation (Magnus and Eliasson, 2006). The good level of RS1 obtained for plantain in this study arouses a fresh interest into the importance of RS1 in foods and this needs to be further investigated, quantified and maximized.

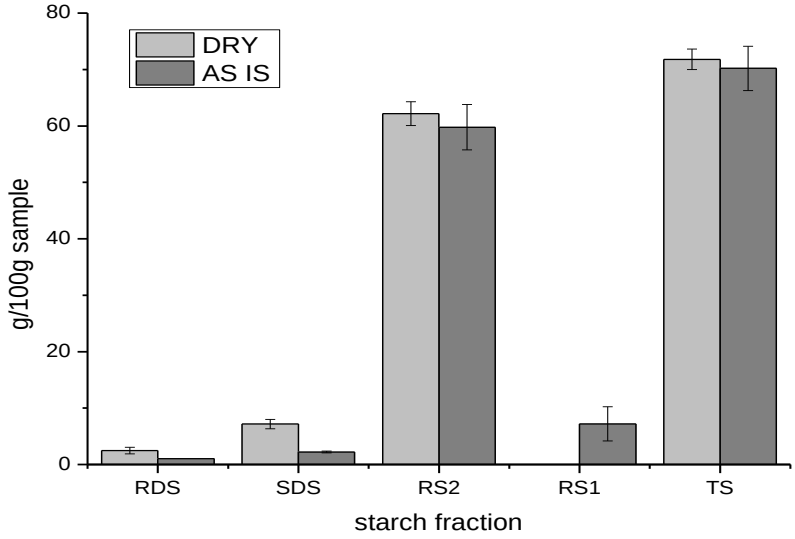


Figure 6.8: Starch fractions in raw unripe plantain
in vitro starch digestion was carried out on two types of samples (i) DRY- freeze-dried and blended (ii) AS IS – wet and minced. Results are reported on dry weight basis for comparison purposes, and values plotted are means ± standard deviations of triplicate determinations. RS1 is not measured when samples are analysed as dry powders

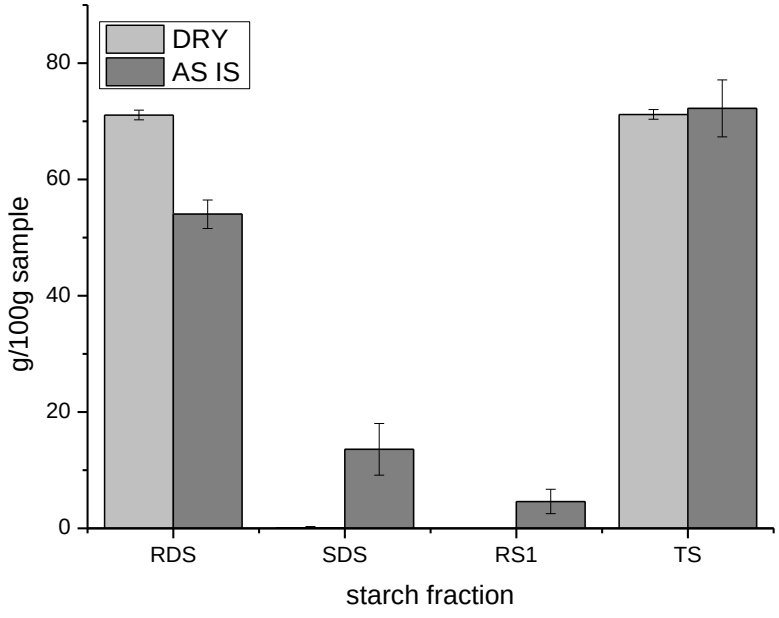


Figure 6.9: Starch fractions in boiled unripe plantain
in vitro starch digestion was carried out on two types of samples (i) DRY- freeze-dried and blended (ii) AS IS – wet and minced. Results are reported on dry weight basis for comparison purposes, values plotted are means ± standard deviations of triplicate determinations. RS1 is not measured when samples are analysed as dry powders

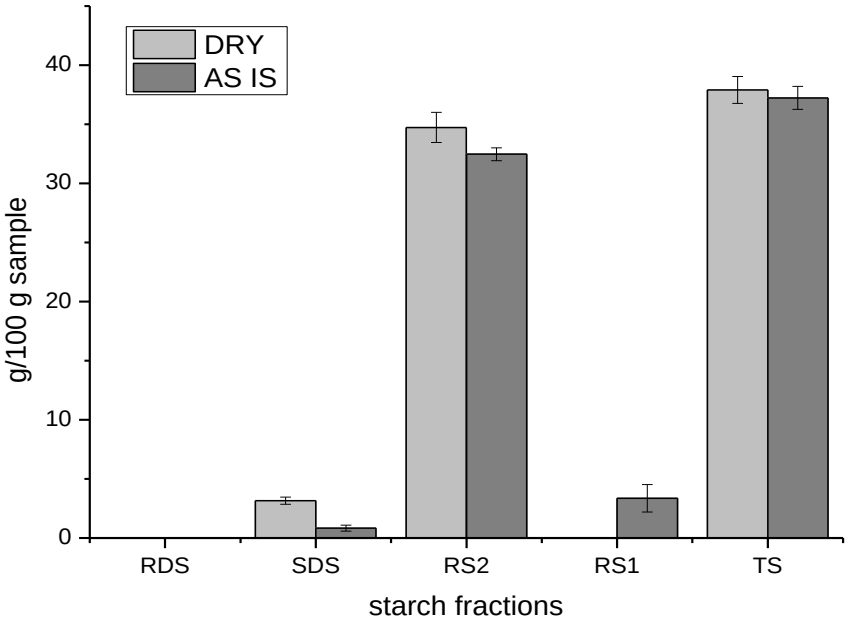


Figure 6.10: Starch fractions in raw ripe plantain
in vitro starch digestion was carried out on two types of samples (i) DRY- freeze-dried and blended (ii) AS IS – wet and minced, Results are reported on dry weight basis for comparison purposes, values plotted are means ± standard deviations of

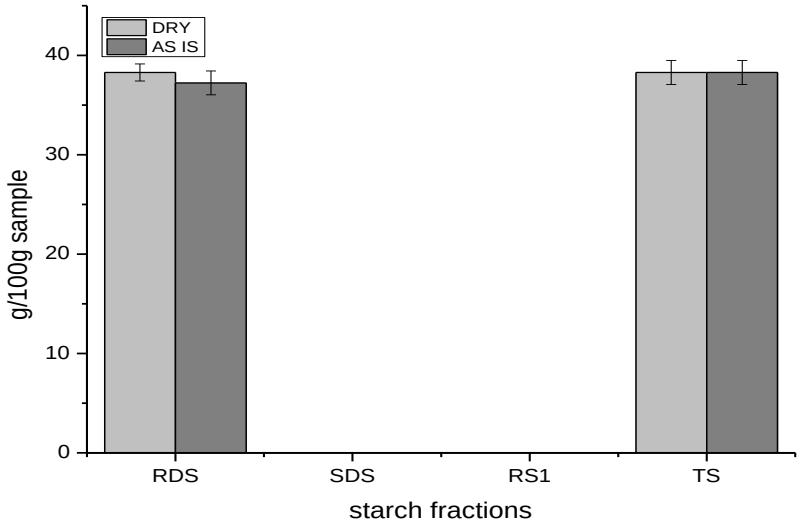


Figure 6.11: Starch fractions in boiled ripe plantain
in vitro starch digestion was carried out on two types of samples (i) DRY- freeze-dried and blended (ii) AS IS – wet and minced. Results are reported on dry weight basis for comparison purposes, values plotted are means ± standard deviations of triplicate determinations. Though RS1 is not measured when samples are analysed as dry powders, RS1 was also not detected in boiled ripe plantain analysed on an as is basis

6.5.6 Implication of experimental protocols on the measurement of RS1

Samples that are analysed in dry powdered forms may not allow the accurate estimation of resistant starch, slowly digestible starch and rapidly digestible starch. In Figures 6.8 - 6.11, RS1 was not detected in samples analysed as dried powder. This is of course expected as RS1 is by definition physically trapped starch, a food matrix is usually disrupted by grinding, or milling and this destroys the presence of RS1. The consequence however is an increase in rapidly digestible starch. This increase in RDS was observed for all the samples studied (Figures 6.8 – 6.11) except in the raw ripe samples, where no RDS was observed in both milled and un-milled samples. In this sample, however, even though no increase in RDS was observed, there was a marked increase in SDS. In the case of boiled ripe plantain, neither RS1 nor SDS was detected in these samples, even when analysed in the wet 'as eaten' basis, and therefore the quantity of RDS detected using both dry and wet samples were quite similar. It may therefore be logical to conclude that analyses involving boiled ripe plantain can be carried out either in the wet or dry sample basis. It is always more convenient to use dry samples due to better sample homogeneity and storage stability. Analysing samples on an 'as eaten' basis seems to be more significant with unripe samples, as both raw and boiled samples showed wide variations in the parameters measured when analysed wet and dry. Dry powder analysis of boiled unripe samples resulted in loss of RS1, SDS and a corresponding increase in RDS quantity. The implication of this is that available carbohydrates will be overestimated

using this method. For the raw unripe sample, RS1 was lost while SDS and RDS increased.

The idea of analysing samples on an 'as eaten' basis arose about a decade ago and is gaining more acceptance because of the need to accurately measure available carbohydrates, resistant starch and other nutritionally important carbohydrate fractions in foods. Accurate determinations of these parameters are required for better prediction of *in vivo* outcomes from *in vitro* measurements. Furthermore, determinations carried out on food on an 'as eaten' basis will allow more accurate measurements of available carbohydrates in food and consequently accurate food portion sizes. This is because the quantity of food needed for the measurement of GI is usually based on the available carbohydrate quantity in the food. The overall effect is that more reliable glycaemic index values of foods will be obtained.

6.6 Conclusion

Plantain foods have potential for the generation of retrograded resistant starch (RS3). A review on the production of RS3 by Thompson (2000) reveals that many methods have been employed for the generation of RS3 from starch using various strategies such as partial depolymerisation, re-heating cycles, enzyme de-branching etc. whereas only very few studies have quantified RS3 in whole foods. Our study has revealed that RS3 production could be aided by the food-form as well as other components in the food. As mentioned earlier *Musa* starches were thought to have poor retrogradation

properties, however we are able to confirm that this may not be the case with plantain, especially when starch retrogradation is studied *in situ* (i.e. within the food matrix). An understanding of what components may be involved and in what concentrations they will be useful, is a subject for further investigation.

RS3 may not be the most common type of resistant starch in foods. Freshly cooked foods in most cases do not usually contain RS3 but may contain reasonably high quantities of RS1, depending on their food matrix. Moreover, most studies have been based on measuring resistant starch in starches and flours and the consequences are that intact food matrices that would have aided the formation of RS1 in the cooked foods would have been disrupted by milling. It is quite ironic that a lot of effort has been put into the study of trying to produce more RS3 while a more/equally important resistant starch type, RS1, which is potentially available by the nature of the food matrix, is being destroyed in the same process.

Chapter 7 Resistant starch in plantain and glycaemic index

7.1 Hypothesis

A completely gelatinised plantain food has high quantity of rapidly digestible starch (RDS) and will consequently have high glycaemic index (GI).

7.2 Abstract

Three plantain products differing in their available carbohydrate types and resistant starch contents were tested. Boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP) (25 g available carbohydrate portion) and 25 g available carbohydrate portion white bread as reference were given to 10 pre-screened healthy individuals. Postprandial glycaemic response, satiety scores and the glycaemic indices (GI) were measured. Peak blood glucose response times for BUP and BUPC occurred at 45 min post-meal time while that of RRP occurred at 30 min postprandial time and the peak blood glucose response values for BUP, BUPC and RRP (1.8 ± 0.8, 2.3 ± 0.8, 1.9 ± 0.7, n = 10 respectively) reflected the *in vitro* quantities of rapidly available glucose (RAG) in the samples. GI values obtained for the test products were BUP = 44.9 ± 6.7, BUPC = 55.0 ± 7.9, RRP = 38 ± 6.9, n = 10 and were inversely correlated with the total quantity of resistant starch in the test products.

Satiety scores were highly and inversely correlated with blood glucose responses for unripe plantain products (r^2 = 0.99, r^2 = 0.91 for BUP and BUPC respectively) but exhibited very low positive correlation for RRP (r^2 = 0.43). Peak satiety scores for white bread and all tests products were also not significantly different at 95% confidence level.

7.3 Introduction

In vitro tests to determine resistant starch and other dietary starch fractions have been carried out and used to estimate the glycaemic index (GI) of foods because of the high costs of conducting *in vivo* GI tests. However, *in vitro* starch digestion data have not been reliable in the accurate prediction of GI values of food (Dona *et al*., 2010).

GI is food specific because it compares equal weights of available carbohydrate and therefore it is a unique measure of biological quality or property of the available carbohydrates in the food rather than the property of the food in itself (Monro (2002), Wolever (2006)). GI is based on glycaemic response which measures the net result of a sequence of chemical and biological processes between food ingestion and release of glucose into the peripheral blood stream (Monro, 1999). Foods are generally classified as low GI (≤55), medium GI (56 - 69) and high GI (≥70) (Brand-Miller *et al*., 2009b).

The basis for the determination of GI has been to compare the same quantity of available carbohydrates in a food to the reference food (usually glucose or white bread). This is because the same quantity of available carbohydrates in different foods can produce very different measure of glycaemic response (Monro, 1999). The argument by Monro *et al* (2003) that GI is a function beyond just the quantity of the available carbohydrates is logical because the type of available carbohydrates is also very important. This is supported by an earlier report by Englyst *et al* (1996b) that GI is correlated with the quantity of

rapidly available glucose (RAG), which precludes slowly digestible starch (SDS). The implication of this is that the GI of a food with higher quantity of slowly available glucose (SAG) in its available carbohydrates is likely to be lower than the one with a higher quantity of rapidly available glucose (RAG).

Digestible starch in food usually produces lower blood glucose response than an equivalent carbohydrate load of glucose, however major differences in blood glucose response are observed with starch in different foods leading to wide variation in GI values between foods and food types ((Inger, 2006) and references within). It is, however, still unclear why starch in some foods e.g. boiled potatoes causes a glycaemic response that is similar to that of glucose while starch in white bread with the same type of available carbohydrate (rapidly digestible starch) produces a lower glycaemic response than glucose. Many reasons have been suggested, this includes the presence of fibre, resistant starch, enzyme inhibitors, lipids, etc (Wolever, 2006). It is easier to study the impact of some of these substances when they are added to foods, but when they are present within the food matrix it may be the difficult to measure their impact if the foods are not properly characterised. Even in the case of well characterised products, when there are many interfering components, it may be difficult to measure the relative impact of each component as well as how component interactions affect the overall blood glucose response.

Starch fractions in foods have different impacts on blood glucose response and subsequently GI. Rapidly digestible starch is known to induce a rapid

increase of blood glucose and insulin levels after food ingestion, whereas slowly digestible starch prolongs the release of glucose, consequently helping to lower GI. On the other hand, resistant starch reduces starch availability for digestion thus lowering blood glucose response (Juansang *et al*. (2012), Lehmann and Robin (2007)).

It appears that a major challenge to the GI concept at the moment is the fact that a high percentage of foods whose GI values have been determined and reported in the international table of glycaemic index (Foster-Powell *et al*. (2002), Atkinson *et al*. (2008)) have not been characterised/well-characterised in terms of food components e.g. resistant starch, that could be responsible for reported GI values. Apart from the fact that this will aid better understanding of published GI values, there is the potential benefit that it will help in the development of more low GI foods, when appropriate conditions e.g. processing are optimised.

The satiety index was proposed along with the GI as the two indices of carbohydrate foods based on their physiological functions (Mann *et al*., 2007). The satiety index (SI) measures the satiety value of equal energy portions of foods relative to a standard, which is white bread (Holt *et al*., 1996).

The rate of gastric emptying and glucose absorption as well as the presence of dietary fibre have been identified as factors which are likely to affect postprandial satiety (Inger, 2006). Even though these factors have also been related to GI, the correlation of GI and satiety is still a subject of much debate.

A significant inverse relationship was obtained between GI and peak satiety scores for 6 different starchy foods (Holt *et al*., 1996). The low GI products caused a significantly higher rating of stomach fullness which may suggest that a slower rate of gastric emptying was induced. The mechanism by which low GI foods support high satiety has however not been reported. Reports available are not clear as to whether it is satiety which induces a low glycaemic response and consequently results in low GI or that it is low GI foods that induce stomach fullness which subsequently increases postprandial satiety.

It was however suggested that the potential effects of resistant starch on satiety needs to be evaluated because many products reported as having low satiety indices were suspected to contain high amounts of resistant starch (Inger (2006), Kendall *et al*. (2009)). This brings us back to the reason why evaluation of GI and/or satiety should be carried out on well characterised products in order to enhance a better understanding of the dietary benefits and implications

Based on the fact that GI is a value that is not dependent on food portion size but an index of the extent to which the available carbohydrates in a high carbohydrate food raises the blood glucose levels (Monro (2002), Wolever (2006)) we have examined three different types of available carbohydrates in plantain foods and how the GI varies with respect to resistant starch components within the food matrix. Satiety based on equi-carbohydrate

content was also investigated. The products tested are ripe raw plantain, boiled unripe plantain and boiled unripe plantain crisps.

The impact of the extent of gelatinisation on glycaemic index of foods has been reported by many authors. The evidence that gelatinisation affects GI values is overwhelming, because partially gelatinised foods have been reported to have lower GI values than fully gelatinised ones (Jung *et al*. (2009), Inger (2006)). The possible reason is that partially gelatinised starches may contain some residual quantities of native resistant starch (RS2) as well as a good quantity of slowly digestible starch (chapter 4), which will help to lower GI. Also the presence of retrograded resistant starch (RS3) will contribute to lower GI values (Englyst and Cummings, 1987).

In order to eliminate RS3 and RS2 we have used freshly cooked and completely gelatinized plantain (refer to chapter 3) so as to determine the GI of green unripe plantain in the absence of resistant starch.

GI data for well characterised plantain products are scarce. The aim of the work described in this chapter is therefore to determine the GI of three characterised plantain products (which are well defined in terms resistant starch and nutritional starch contents) and their impact on postprandial satiety in healthy subjects. We are therefore reporting for the first time the GI of completely gelatinised green plantain, boiled plantain crisps and ripe raw plantain.

7.4 Materials and methods

7.4.1 Test products, preparation and basis for choice

7.4.1.1 Unripe boiled plantain

One finger of unripe plantain was cut into five portions of ~ 300 g each and cooked in slightly salted and pre-boiled water for 20 min to ensure complete gelatinisation (see chapter 3). The cooked plantain portions were allowed to cool down to room temperature (for approximately 10 min) before weighing and serving. The boiled unripe plantains were freshly cooked before each test to avoid starch retrogradation. The presence of retrograded resistant starch (RS3) was checked in freshly cooked plantain foods as outlined below. This is the most common form of consuming unripe plantain in Nigeria. It is also the form usually recommended as having therapeutic properties (see chapter 1). The only difference to its usual form of consumption is that we have not added condiments so as to avoid any interference in measurements from other substances.

7.4.1.2 Unripe boiled plantain crisps

Unripe plantain crisps were prepared by cooking unripe plantain as described in section 7.4.1.1 above. After cooling to room temperature, they were cut into thin slices of about 2 mm thickness with a kitchen knife and subsequently freeze-dried.

The basis for using this test product was to check the GI of plantain in the absence of all types of resistant starch in plantain i.e. RS1, RS2, and RS3.

This product was prepared as a follow up to test product 1 which still contains some quantity of RS1 (see Table 7.3). No form of resistant starch was detected in the unripe plantain crisps we prepared for the study.

7.4.1.3 Raw ripe plantain

Consumption of ripe plantain is not as common as the consumption of the cooked form or the consumption of bananas. This is probably the reason why the GI of ripe plantain has been scarcely reported. This product was therefore included in order to report for the first time, the GI.

7.4.1.4 Reference food: white wheat bread

White bread used for the study was purchased from Morrisons supermarket, Leeds city centre, on the morning of each test day. White bread was chosen over glucose because it is a common food and would be generally more acceptable by subjects. Moreover, consumption of concentrated glucose drink after an overnight fast can induce nausea in some subjects. The GI values obtained were however calibrated against glucose by dividing by a value of 1.4, (Wolever (2006), Brouns *et al*. (2005)).

7.4.2 Chemical analysis and characterisation of test meals

7.4.2.1 Determination of available carbohydrates

Available carbohydrate was determined and calculated as available starch (RDS + SDS) + total free sugars (glucose +fructose + sucrose), (please refer to chapters 4 and 5 for detailed methodology). Fructose, glucose and sucrose in the ripe and unripe samples were determined by extraction of sample in 80%

ethanol at boiling and 50% ethanol at 80^{o}C, respectively, and subsequent estimation using ELSD HPLC as earlier described in chapter 5.

7.4.2.2 Determination retrograded starch (RS3)

Retrograded starch was determined in cooked unripe plantain at 10, 20 and 30 min using the method described in the previous chapter. In addition, RS3 was determined in the dried products to further rule out the formation of retrograded starch in the first 30 min. One plantain finger was cut into four portions, three portions were cooked. After cooling for 10 min, one portion was cut into thin slices, quenched in liquid nitrogen and subsequently freeze-dried. The same treatment was carried out on portions 3 and 4 after 20 and 30 min of cooling respectively. Portion one was cut into thin slices and freeze-dried without cooking. Total starch content in the four portions was determined using the modified method of Englyst *et al* (2005) as described earlier in Chapter 2.

7.4.3 Subjects

Twelve pre-screened healthy subjects (using a pre-screening questionnaire and anthropometry measurements) were selected for the study. Inclusion criteria were: healthy male or female of any ethnicity, 20 to 40 years of age, body mass index (BMI) < 30 kg/m2, not on medication, not diabetic, not pregnant or lactating mother, not allergic to any of the test products and/or their constituents.

The ethics committee of the Faculty of Mathematics and Physical sciences, University of Leeds gave the approval for the study (MEEC 11-048) and all volunteers gave their written consents.

7.4.4 Study design

Subjects were allowed to eat their normal diet the day before participating in the test but were to avoid unusual and/or strenuous exercise, drinking alcohol and smoking (Brouns *et al*., 2005). They arrived at the School of Food Science & Nutrition, University of Leeds between 8am-8.45am on each test day. All meals were served as breakfast in random order after an overnight fast of 10-14 hours with a two-day washout period between tests (Brouns *et al*., 2005).

All subjects consumed all three test foods once and the reference food twice (25 g available carbohydrate portions). The meals were served with water (200 ml) and were consumed over a 10 min period (Wolever, 2006).

Subjects remained seated throughout the experimental session. Capillary blood samples were collected during the fasting state and at 15, 30, 45, 60, 90 and 120 min after consumption of the test meal, for analysis of glucose. Satiety was measured using a subjective rating scale described in detail in the next section.

7.4.5 Sampling and analysis

Capillary blood samples were taken using accu-chek fastclix finger pricker in the fasting state and at 15, 30, 45, 60, 90, and 120 min after each meal. One drop of blood was placed on a test strip for immediate analysis of glucose with aviva nano meter for glucose measurement (Goni and Valentin-Gamazo (2003), Nilsson *et al*. (2008), Leeman *et al*. (2008)). The incremental areas under the blood glucose curves were calculated geometrically using an excel software. The GI was estimated by expressing the glycaemic response area for the test foods as a percentage of the mean response area of the reference food taken by the same subjects (Wolever *et al*., 1991) .

The coefficient of variation (CV) of analytical variation for various portable glucose meters ranges from 1.5% to 8.0% (Solnica *et al*., 2003). However, the mean GI values obtained with portable glucose meters are not significantly different ($p = 0.11$) from GI values obtained using glucose oxidase and hexokinase methods values (Wolever (2006), Wolever *et al*. (2008)) .

Information from the Accu-check aviva manual for blood glucose measurement used in our study reveals that the meter, which measures the glucose concentration in whole blood using a test strip containing glucose dehydrogenase, was calibrated using hexokinase method and the detection limit is 0.6 mmol/L and system measurement range is 0.6 - 33.3 mmol/L. A CV of 3.0% was obtained for repeatability (within series) while a CV of 1.8% was obtained for reproducibility (day-to-day).

Assessments of feelings of hunger/satiety were performed immediately after blood sampling at 30, 60, 90 and 120 min using a rating scale of 1-10 with 1 as very hungry and 10 as very full. The test subjects filled out the satiety questionnaires themselves. At the end of the study the mean and standard deviation of satiety scores at each time and for each test food and control from ten subject was determined and the significant differences in satiety scores for the various foods was measured using analysis of variance on SPSS software at $p = 0.05$.

7.5 Results and discussion

Twelve participants in all were recruited for the study; however, only data for ten participants were used for the computation of the results. The data of the first two participants were omitted from the study because they were initially fed with 50 g available carbohydrate portions of bread but could not consume 50 g available carbohydrate portions of cooked unripe plantain foods within 15 min. This was due the texture and taste of boiled unripe plantain. Unripe plantain has a coarse dry texture and does not have sweet taste due to very low sugar content (Table 7.1), it is therefore usually consumed with condiments to enhance palatability, but because our interest was in the GI of the characterised product, it was presented without condiments. All the remaining ten participants were therefore fed with 25 g available carbohydrate portions of the test foods and reference food (Table 7.1). The lesson here is that pilot studies are very important in order to determine the feasibility of an

experiment, especially with human studies since they are usually expensive and time consuming.

7.5.1 The presence of resistant starch, the type of available carbohydrate and glycaemic response

The blood glucose response curves for white bread (WB), boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP) are shown in Figure 7.1. The sharp rise in blood glucose response, the fast peak (which occurred at 45 min post meal period) and fast decline of BUPC appear to reflect a rapid breakdown of starch into glucose and fast absorption of the glucose released. Although BUPC has a postprandial peak at the same time as WB (45 min), its blood glucose response is lower than that of white bread at all times after 45 min and lower than BUP at 90 min post meal stage. BUPC does not contain any type of resistant starch (Table 7.3), and is the only sample whose blood glucose curve peaks with bread and at exactly the same time as bread (Figure 7.1). This observation leads us to think that the presence or absence of resistant starch may be related to the peak of the blood glucose curves; more so because BUP also peaks at the same time but with lower glucose response (Figure 7.1). Although the available carbohydrates in white bread, BUP and BURC are composed mainly of digestible starch (Table 7.2), the lower blood glucose response of BUP is not unconnected with the fact that ~ 50% of its digestible starch is slowly digestible (as determined *in vitro*), (Table 7.2).

.

Table 7.1: Starch, sugar, moisture and available carbohydrate contents of plantain products and white bread

	BUP (g/100 g WWB)	**BUPC** (g/100 g DWB)	**RRP** (g/100 g WWB)	**WB** (g/100 g WWB)
Total starch	21.7±1.4	72.4±1.7	12.1±1.2	47.5±1.3
Total sugars	1.7±0.2	5.5±0.5	14.9±1.5	2.6±0.6
Glucose	0.1±0.0	0.3±0.1	2.0±0.1	ND
Fructose	0.1±0.2	0.4±0.1	1.9±0.1	ND
Sucrose	1.4±0.2	4.8±0.5	10.8±0.8	2.6±0.6
Total RS	1.6±0.8	ND	11.4±1.9	ND
Total CHO	23.4±1.2	78.2±1.3	27.0±1.3	49.8±1.6
RS1	1.6±0.8	0.0±0.2	2.1±0.6	ND
RS2	ND	ND	10.7±1.3	ND
Moisture	69.2±1.8	1.3±0.5	60.3±2.1	50.2±1.8
Available CHO	19.8±0.8	77.9±0.5	15.3±1.1	49.2±2.1
Food portion size (25 g AV CHO)	125±2.1	32 ±1.5	163±2.6	50.3±2.5

BUP = Boiled unripe plantain, BUPC = Boiled unripe plantain crisps, RRP = Ripe raw plantain, WB = white bread, ND = not detected. WWB = wet weight basis, DWB = dry weight basis. Results are expressed as means ± standard deviations, n= 3

Table 7.2: Available carbohydrate components of plantain products and white bread (g/ 25g available carbohydrate food portion size)

	BUP	BUPC	RRP	WB
Fructose	0.1±0.2	0.1±0.0	3.1±0.1	0.0±0.0
Glucose	0.1±0.0	0.1±0.1	3.3±0.1	0.0±0.0
Sucrose	1.9±0.2	1.5±0.2	17.6±1.2	1.2±0.4
RDS	12.9±1.5	23.2±0.5	0.8±0.3	23.9±0.8
SDS	9.8±0.7	0.0±0.1	0.2±0.3	0.1±0.2
Major AV CHO	RDS	RDS	sucrose	RDS
% of major AV CHO	52±3	93±1	71±1	96±2
RAG	15±1.1	24.9±0.2	12.9±0.4	24.5±0.7

Table gives breakdown of types and quantities of available carbohydrates (AV CHO) present in the tested plantain products. RDS= rapidly digestible starch, SDS = slowly digestible starch, RAG= rapidly available glucose (RDS + total sugars). Results represent quantities in 25 g available carbohydrate food portion sizes (Table 7.1) as served and are expressed as means ± standard deviations, n= 3. Fructose was not included in the calculation quantity of RAG.

Slowly digestible starches are expected to produce lower glycaemic responses (Englyst *et al*. (2003a), Lehmann and Robin (2007)), however, this effect is not limited to slowly digestible starches alone but also applicable to products containing inaccessible resistant starch (RS1) (Jenkins and Kendall, 2000). Therefore, the presence of 2.0 ± 0.9 g/25 g RS1 quantity in BUP (Table 7.3) may be a contributing factor to the observed low glucose response when compared with its counterpart, BUPC.

Table 7.3: **Resistant starch content of plantain products (g/ 25g available carbohydrate food portion size)**

	RS1	**RS2**	**RS3**	**TOTAL RS**
BUP	2.0±0.9	ND	ND	2.0±0.9
BUPC	ND	ND	ND	ND
RRP	3.4±1.2	17.4±2.3	0.0	20.8±1.9

Table gives breakdown of types and quantities of resistant starch present in the tested plantain products. ND = not detected, RS1 = inaccessible starch, RS2 = native resistant starch, RS3 = retrograded starch. Results represent quantities in 25 g available carbohydrate food portion sizes (Table 1) as served and are expressed as means ± standard deviations, n = 3

Ripe raw plantain (RRP) elicits a significantly low glucose response between 60 and 120 min post meal time, and the of shape its glucose response curve is similar to that obtained for sucrose (Wolever, 2006). However, a lower glycaemic index (38.5 ± 6.9, n = 10) for RRP when compared to that of sucrose (~ 65) raises some questions. It appears that the presence of

resistant starch type 2 (determined *in vitro*) in high quantity (10.7 ± 1.3 g/100 g WWB) and in an equivalent quantity to sucrose (10.8±0.8 g/100 g WWB) in RRP is responsible for this observation. In addition, results obtained earlier in chapter 3 reveals very poor *in vitro* enzyme digestibility of ripe plantain starch, which may be due the presence of other food components acting as enzyme inhibitors such as phenolics (Bennett *et al*., 2010) and products of incomplete digestion of starch such as maltose and maltotriose (Colonna *et al*. (1992), Warren *et al*. (2012)), which are usually measured as resistant starch (European Resistant Starch Research Project (EURESTA) (1992). Some of these components may also affect the transport of glucose from the food matrix to the blood circulation (Williamson, 2013). More *in vivo* studies are however required to understand the impact of other components of plantain on starch digestion and glucose transport.

At 15 min post-meal stage, RRP has the highest blood glucose response but this was not significantly higher ($p = 0.05$) than those of WB and BUPC. This observation indicates the presence of free sugars in high quantity constituting 97% of available carbohydrates in the sample (Table 7.2).

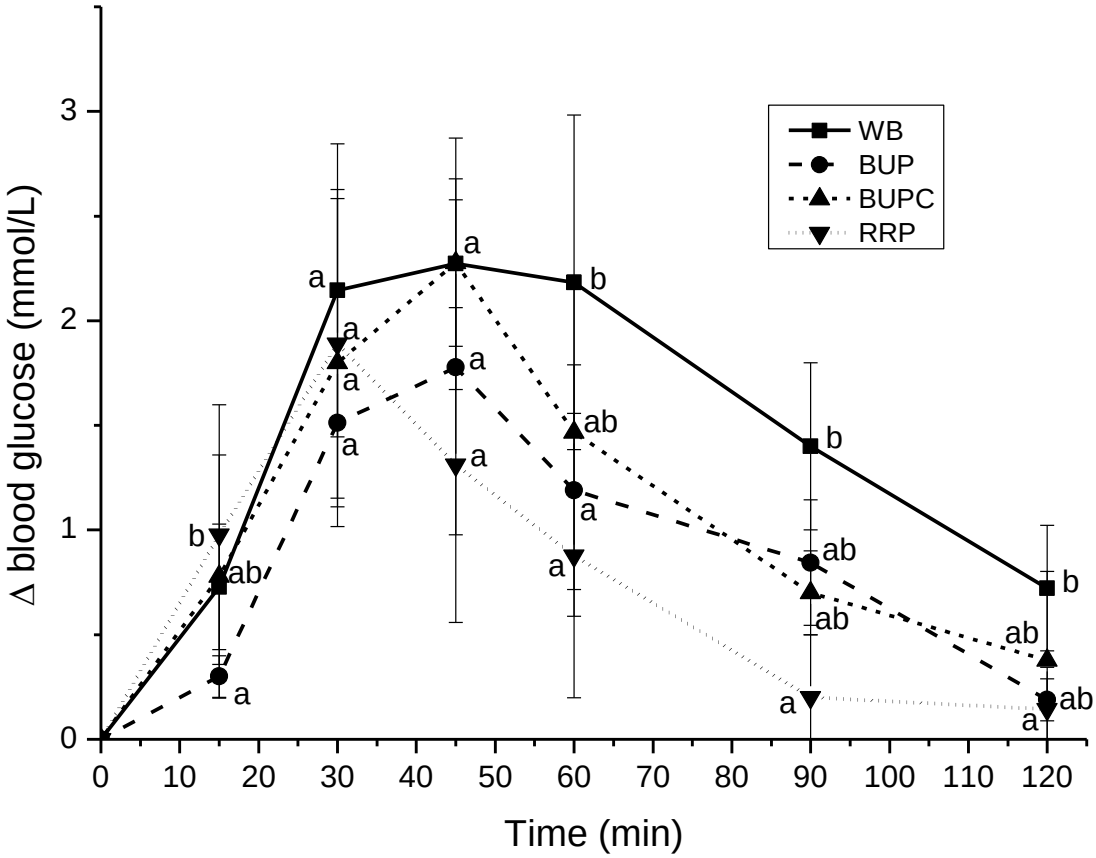


Figure 7.1: Blood glucose increments (responses) after ingestion 25g available carbohydrates portion of white bread (WB), boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP)
Values plotted are means ± standard deviations of 10 subjects at time 0, 15, 30, 45, 60, 90 and 120 mins. Significant differences (at 95% confidence levels) in blood glucose response values between samples for each time are indicated by letter annotations beside the data points

7.5.2 Quality and quantity of rapidly available glucose

Englyst *et al* (2003c) proposed that the quantity of RAG was a good predictor of GI in a study involving twenty-three cereal products. In our study, it appears the peak glucose response values (Table 7.4) are connected with the quantity of RAG.

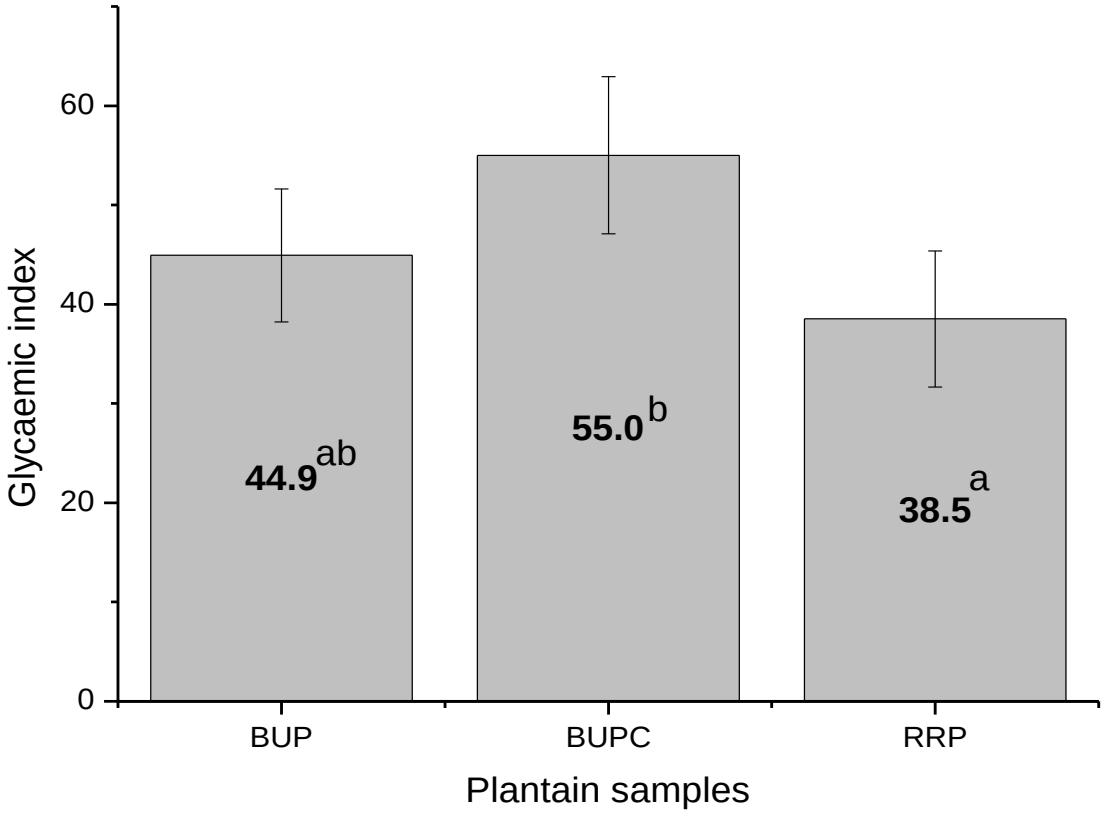


Figure 7.2: GI values of boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP)
GI was calculated from IAUC values at 120 min of food ingestion when compared with bread and converted to glucose by dividing values by 1.4. GI values obtained are means ± standard deviation (indicated with error bars) from 10 subjects. The GI values with the same superscript are not significantly different at 95% confidence level.

A close examination of the peak glucose values of white bread and BUPC (2.3 ± 0.6 and 2.3 ± 0.8 mmol/L, n = 10) and their RAG values (24.5 ± 0.7 and 24.9 ± 0.2 g/food portion, n = 3) suggests that 1 g of RAG will elicit ~ 0.1 mmol/L (literally). It is quite interesting to observe that both bread and BUPC which contain the same type of available carbohydrate (Table 7.2) elicit similar peak glucose responses and the peak responses at the same time of 45 min post meal period (Figure 7.1).

In addition, BUP, which contains starch as its digestible carbohydrate but with some quantity of SDS, has a peak blood glucose response at the same time but with a lower response which may be attributed to a lower quantity of RAG (15 ± 1.1 g/food portion) - Table 7.2. Only RRP has a different type of RAG (mainly sucrose and glucose) and this was reflected in its earlier peak time of 30 min. It can therefore be concluded that the time of attaining the blood glucose response peak is a function of the type of RAG while the peak height is a function of quantity of the RAG.

Table 7.4: Peak blood glucose response, peak time and rapidly available glucose values for reference and test foods

	Peak blood glucose response (mmol/L)	Peak Time (min)	Rapidly available glucose (RAG) g/ portion
White bread (WB)	2.3±0.6[a]	45	24.5±0.7[c]
Boiled Unripe plantain (BUP)	1.8±0.8[a]	45	15±1.1[b]
Boiled plantain crisps (BUPC)	2.3±0.8[a]	45	24.9±0.2[c]
Ripe raw plantain (RRP)	1.9 ±0.7[a]	30	12.9±0.4[a]

Peak blood glucose responses after ingestion 25g available carbohydrate portion of each test meal and reference are means ± standard deviations of 10 subjects at time while RAG values are means ± standard deviations of triplicate *in vitro* determinations for each test food. Values with the same superscript in the same column are not significantly different at 95% confidence level.

Although the number of food samples involved here is not large, this is an interesting observation that should be further investigated with other food types and products. This further emphasizes the need for products to be characterised in terms of their carbohydrate types in GI determinations.

Further deductions can be made from Table 7.4;

(i) If 1 g of RAG elicits a blood glucose response of ~ 0.1 mmol/L, it would be expected that RRP with RAG quantity of 12.9 ± 0.4 g/portion should elicit a response of ~ 1.3 mmol/L; however, it elicited a response of 1.9 mmol/L, which implies an additional 0.6 mmol/L. It may be reasonable to conclude that the extra 0.6 mmol/L glucose (which represents ~ 50% of total fructose in sample – Table 7.2) must have come from fructose metabolism to glucose. The conclusion that fructose malabsorption may be prevented when consumed with glucose appears to be true here (Riby *et al*., 1993). (Fructose metabolism is discussed below).

(ii) If 1 g of RAG elicits a blood glucose response of ~ 0.1 mmol/L, it would be expected that BUP with RAG quantity of 15 ± 1.1g/portion should elicit a response of ~ 1.5 mmol/L, however, it elicited a response of 1.8, which implies an additional 0.3 mmol/L. It may be reasonable to conclude that the extra 0.3 mmol/L glucose must have come from slowly digestible starch which constitutes ~ 37% (9.8 ± 0.7 g/portion- Table 7.2) of its available carbohydrate content. Since the peak glucose response of BUP was attained at 45 min

and slowly digestible starch (SDS) is by definition that portion of starch that is digestible between 20 and 120 min of food ingestion (Englyst *et al*., 1992), it is expected that only a little quantity of it will be reflected in the blood glucose at 45 min postprandial period just as we have observed.

The observations made from the impact of available carbohydrate type and content on blood glucose response represent a clue to predicting *in vivo* results from *in vitro* determinations and should be further investigated.

7.5.3 Glycaemic index of plantain products and resistant starch

7.5.3.1 Glycaemic index of boiled unripe plantain (BUP)

A majority of the studies on physical and chemical properties of banana and plantain starches reported in the literature have utilised green (unripe) fingers as their substrate for starch extraction. This is quite logical because unripe plantains have higher starch content than their ripe counterparts. In most cases the reason why green unripe plantain was chosen was the presence of a high quantity of enzyme resistant starch *in vivo* in bananas as initially reported by Englyst and Cummings (1986a) and Faisant *et al* (1995).

As earlier indicated in the introductory chapter, green boiled plantains when eaten whole (not in powder or paste form) are believed by the Nigerian populace and in other parts of Africa to be good in the control and management of diabetes. Incidentally, it is not clear which type of diabetes is inferred and whether this is a myth or a scientific fact still remains to be

explored. The only scientific basis available at the moment to give credibility to these beliefs is the low GI (37 ± 4, mean ± SE) (Bahado-Singh *et al*., 2006), that has been reported for boiled green plantain. Although the low GI of boiled green plantain has been attributed to the presence of resistant starch (Bahado-Singh *et al*., 2006), it is still unclear why this is so and what other reasons, if any, may be responsible for its low GI according to GI classification of foods (Brand-Miller *et al*., 2009b).

An increase in GI when unripe plantain is gelatinised/cooked has been reported (Menezes *et al*. (2010), Bahado-Singh *et al*. (2006)). It is quite logical to accept that this is true because gelatinisation increases the rate of digestion and may lead to a higher GI. Other authors have also confirmed this with other food products (Ek *et al*. (2012), Granfeldt *et al*. (1995b), Jung *et al*. (2009)). The GI of boiled green plantain obtained in our study (44.9 ± 6.7, n = 10) is higher than earlier reported (37 ± 4). It is possible that samples used in those studies were not completely gelatinised and would therefore have contained some quantity of native resistant starch (chapters 3 and 4), leading to a lower GI. What is however unclear is that freshly prepared BUP, which is completely gelatinised and un-retrograded, elicited a low GI. On the contrary, other starchy foods which were boiled, completely gelatinised and freshly cooked have high GI, e.g. potatoes, 93 - 141 (Leeman *et al*. (2008), Soh and Brand-Miller (1999)), rice, 64 - 93 (Miller *et al*., 1992). A possible reason for this observation is the presence of RS1 (2.0 ±0.9 g/portion) and SDS (9.8 ±

0.7 g/portion) - Tables 7.2 and 7.3, in BUP. We investigate this in the next section.

7.5.3.2 Glycaemic index of boiled unripe plantain crisps (BUPC)

Plantain crisps which are usually prepared by frying thin slices of unripe plantain in vegetable oil are a popular snack in the African continent. Information on GI of plantain crisps is scarce. The plantain crisps we have produced and tested in this study were made by freeze-drying of boiled unripe plantain and are therefore virtually fat-free. Frying in oil was not used so as to avoid the inhibitory effect of fat/lipids to starch digestion. Moreover, as a follow up from the previous section, plantain crisps used in this study did not contain any form resistant starch and SDS (Table 7.2 and 7.3). A GI value of 55.0 ± 7.9, n = 10, (Figure 7.2) obtained for boiled unripe plantain chips (BUPC), is ~ 10 points higher than obtained for BUP (44.9 ± 6.7), it is therefore reasonable to conclude at this point that the presence of RS1 along with the SDS content in boiled unripe plantain (BUP) was responsible for the observed lower GI for boiled plantain than that of dried plantain crisps.

The next question that then needs to be answered is why the GI of the dried plantain is lower than the GI of white wheat bread (71.4) if they both contain starch that is rapidly digested (Table 7.2) and they both reached the peak value of blood glucose response at the same time (Figure 7.1). Blood glucose response may also be affected by other physiological and nutritional factors apart from the digestibility of the starch and related features of the food as discussed earlier in chapter 1.

Studies on the efficiency of the digestive system in adults reveal that pure soluble starch irrespective of its viscosity produces the same glucose response as that of starch dextrin or glucose. This is because in contrast to fibre, the viscosity of starch decreases when passing through the gastrointestinal tract. Starch viscosity, however appears to be important in intact food forms and may need to be investigated in relation to GI of plantain foods (Inger, 2006).

One possible factor which is readily suspected to be responsible for low GI of plantains in the absence of any resistant starch is the presence of dietary fibre (soluble fibre 2.1-3.1%, insoluble fibre 4.1-12.6%) (da Mota *et al*., 2000). Although the quantity of dietary fibre in foods *per se* is of little or no value in controlling plasma glucose in diabetics as earlier reviewed by Nuttall (1993) and in lowering GI as discussed by (Inger, 2006), when considering dietary fibre as a fibre network within a botanical tissue, encapsulation of starch in the fibre network may significantly reduce the amylolytic digestion of starch due to reduced access of enzymes to the substrate (Wolever, 1990). It appears as though the presence of an intact botanical tissue is more important than the presence of the dietary fibre itself. This view is supported by results obtained in several studies e.g. the milling of rice significantly increased the postprandial glycaemic response, and furthermore fibre did not have any noticeable effect because both brown and white rice elicited the same glucose response (O'dea *et al*., 1980). Bread products made with intact kernels produced a lower glucose response than bread products based on

milled flours e.g. whole meal milled wheat bread has a GI of 96 ± 5 whereas wholemeal wheat kernels bread has a GI of 63 ± 6, wholemeal milled rye bread has a GI of 78 ± 3 while whole meal rye kernels bread has a GI of 48 ± 5 (Jenkins *et al*., 1986). Porridges made from milled barley flours had higher GI (55 - 65) than those made from intact barley kernels (28 - 37) (Granfeldt *et al*., 1994). These observations are synonymous with our results as the GI of BUP (with the intact food form) is lower than that of boiled plantain crisps. However, since the GI values of BUPC and BUP are not significantly different ($p = 0.05$) (Figure 7.2), the impact of other food components in lowering postprandial GI of plantain cannot be ruled out and therefore needs to be further investigated.

7.5.3.3 Glycaemic index of ripe raw plantain (RRP)

The GI of ripe raw plantain has been scarcely reported while that of banana is common because plantain is preferentially eaten cooked (please refer to introductory chapter). The GI of ripe plantain obtained here (38.5 ± 6.9, n = 10) is lower than an average GI of 52 ± 4 obtained from ten studies on banana (Foster-Powell *et al*., 2002). A higher GI value obtained for banana may be due to higher quantity of sugar in banana than plantain.

The attainment of an earlier peak (at 30 min), for ripe plantain is similar to the observation made for most fruits and for sugars (Wolever *et al*., 1993). Sugars with a low/medium GI (e.g. sucrose) are therefore believed to have a lower GI than starch because they contain a smaller amount of glucose than starch rather than because of slow absorption (Lee and Wolever, 1998).

Moreover, the presence of fructose as part of sucrose or in a food product contributes to low GI due to slow and incomplete absorption of fructose (Riby *et al*., 1993). It therefore becomes necessary to re-consider the inclusion of fructose as a glycaemic carbohydrate since it does not raise blood glucose levels appreciably because it is converted to glucose in the liver and only a small proportion of this glucose is released into circulation.

On the other hand, fructose that is consumed with glucose or starch is said to be more rapidly absorbed because these carbohydrates aid more rapid and complete transport of fructose out of the intestine. Riby *et al* (1993) reported that simultaneous intake of fructose and glucose can prevent fructose malabsorption, which implies that the pair of monosaccharide may be absorbed by the disaccharidase-related transport system as if they were the product of the enzymatic hydrolysis of sucrose. This may be due to the translocation of GLUT2 to the brush border membrane by glucose. This absorptive mechanism may, however, not be able to transport fructose when ingested without glucose. Furthermore, recent evidence indicating that fructose increases visceral adipose deposition, lipogenesis and insulin insensitivity has not been confirmed with the consumption of fructose in the form of sucrose or high fructose corn syrup (HFCS) as reviewed by Stanhope (2012).

The inclusion of the fructose content of a food/product/meal in the calculation of available carbohydrates may lead to underestimation of the total available

carbohydrate, if all the fructose is not absorbed, especially within the two-hour postprandial period.

Wolever (1993) predicted the glycaemic index of nine fruits from their sugar (fructose, glucose and sucrose) contents using equation 1 below and discovered a correlation value of r = 0.7 (n = 9, $p < 0.05$) between the predicted values and GI values determined *in vivo*.

$$\text{Predicted GI} = \frac{Pf\ x\ GIf + Pg\ x\ GIg + Ps\ x\ GIs}{100} \quad \text{.....1}$$

Where P_f, P_g, P_s are percentage contents of fructose, glucose and sucrose in the fruits and GI_f, GI_g and GI_s are the glycaemic indices of fructose, glucose and sucrose, respectively.

We adopted the same equation for the prediction of the glycaemic index of ripe raw plantain (RRP) from its sugar content (Table 7.2) and the published GI of fructose (23), glucose (100) and sucrose (65) (Foster-Powell *et al*., 2002) and obtained a value of 63.4 ± 2.1, n = 3. It is obvious that the wide variation between the predicted value and the determined GI of 38.5 ± 6.9, n = 10, must have been accounted for by other intrinsic and extrinsic factors related to both food digestion and absorption of glucose.

It is reasonable to think that the sharp decline and lower glucose response between 30 - 120 min post meal period observed for RRP could be due to high insulin responses induce by the sharp peak at 15 min post meal period,

but earlier investigations revealed that sugars do not produce inappropriately high postprandial insulin responses, i.e., the insulin responses elicited by sucrose and fructose are not different from what would be expected from a starchy carbohydrate food with the same glycaemic index (Lee and Wolever, 1998). Conclusions made from several studies also reveal that a resistant starch content (in most cases RS2 or RS3) of not less than 14% of total starch ingested usually confers some benefits on glycaemic or insulinaemic response (Nugent, 2005). Therefore, high level of resistant starch in the RRP (20.8 ± 1.9, n = 3 - Table 7.3) appears to be a possible reason for its the observed low GI. Nevertheless, this is not conclusive because of the presence of other food components in the food matrix such as fibre (David and Gregory, 2001), polyphenols (Williamson, 2013) and cell wall and/or components (Bennett *et al*., 2010). Further investigations on the possible reason for the observed low GI of ripe raw plantain are therefore required.

7.5.3.4 Correlation between glycaemic index (GI) and total resistant starch (RS)

A negative correlation (r^2 = 0.8 – Figure 7.4) was obtained between GI and total RS content of the plantain samples. Although this is in tandem with earlier conclusions made, investigations as to which RS type significantly affects GI needs to be carried out. Resistant starch like other unavailable carbohydrates can cause a reduced digestion and/absorption of available carbohydrates either by substrate encapsulation or by increasing food

viscosity (Menezes *et al*., 2010). The relationship between RS and GI is far from being established. For high amylose foods, a high negative correlation has been established, however for some other type of starches e.g. wheat starch, no correlation was obtained (Inger, 2006). This appears to be related to the types of resistant starch in various products, for example RS2 is found in raw potatoes and raw bananas but not in raw wheat. It is therefore necessary to try to establish the relationship, if any, between GI and each type of resistant starch (i.e. RS1, RS2, RS3 and RS4) rather than between GI and total RS.

The mechanism of enzyme resistance of each type of resistant starch is important in considering how glycaemic index and glucose metabolism are affected. The presence of RS1 which is a consequence of an intact food form (as discussed earlier in chapter 6) is known to slow down the availability of starch to digestive enzymes, leading to a slow increase in postprandial blood glucose response just like SDS and may be more important in lowering the GI of a food than RS2 and RS3 (Jenkins and Kendall (2000), Lehmann and Robin (2007)). Nevertheless since resistant starches in general undergo fermentation by colonic bacteria, short chain fatty acids produced during the process may enhance glucose tolerance at subsequent meals or in the long term (Inger, 2006).

Overall, amongst the samples studied, boiled unripe plantain (BUP) appears to be the best in terms of glycaemic control because it exhibits a slow and gradual rise in blood glucose response as well as a gradual decline (Figure

7.1). This observation appears to be the reason for the claim of its ability to be used in the management of diabetes in Nigeria (please see introductory chapter).

7.5.4 Glycaemic index and satiety

The impact of postprandial glycaemic response on satiety was determined by comparing the incremental area under the blood glucose response curve (IAUC) at 30, 60, 90 and 120 min with satiety values at those times (Figure 7.5). High negative correlations were obtained for BUP and BUPC (r^2 = 0.99 and 0.91 respectively) while WB showed a high positive correlation (r^2 = 0.88) and ripe plantain had a low positive correlation (r^2 = 0.43). Overall, GI versus peak satiety scores (Figure 7.6) showed a good positive correlation (r^2 = 0.71).

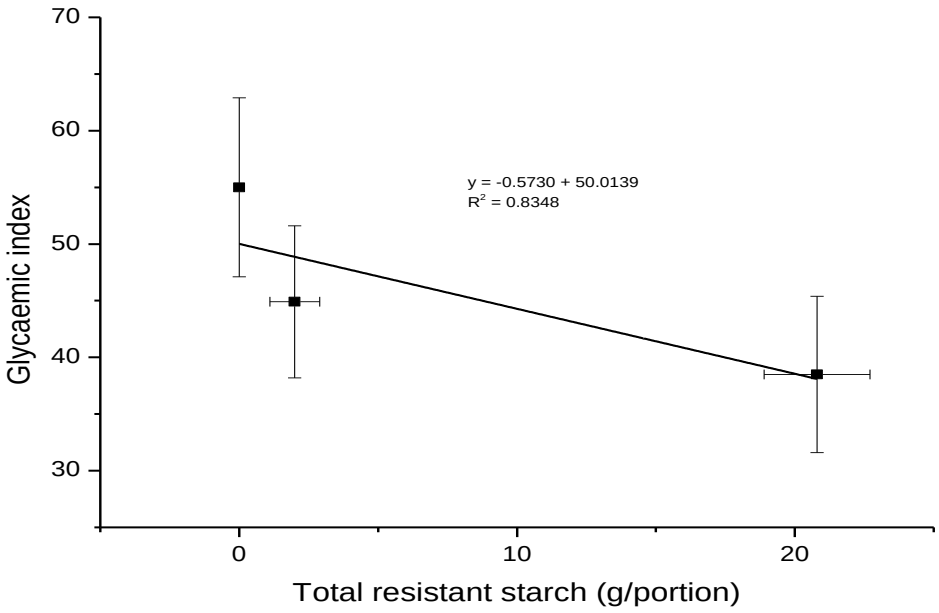


Figure 7.3: Glycaemic index values for boiled plantain unripe, boiled unripe plantain crisps and raw ripe plantain vs. total resistant starch correlation graph
Data points were obtained from mean glycaemic indices ± standard deviation of 10 subjects for each test product and total resistant starch contents determined *in vitro* for each test product, n=3

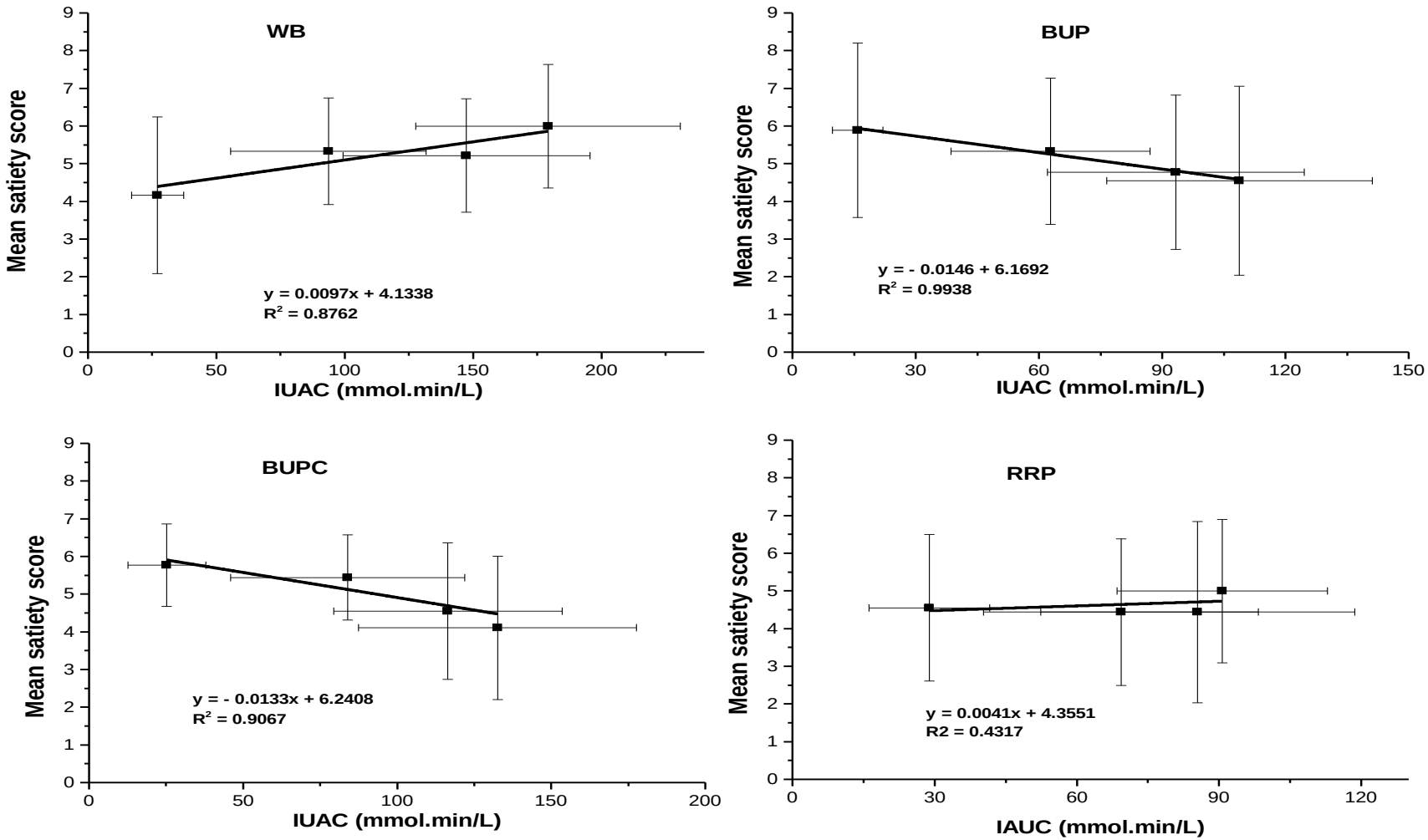


Figure 7.4: Mean satiety score vs. incremental area under the blood glucose response curve (IAUC) graphs for white bread (WB), boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP)

Data points were obtained from mean satiety scores ± standard deviations of 10 subjects at 30, 60, 90 and 120 min after food ingestion and mean IAUC values ± standard deviations of 10 subjects at 0-30, 0-60, 0-90 and 0-120 min.

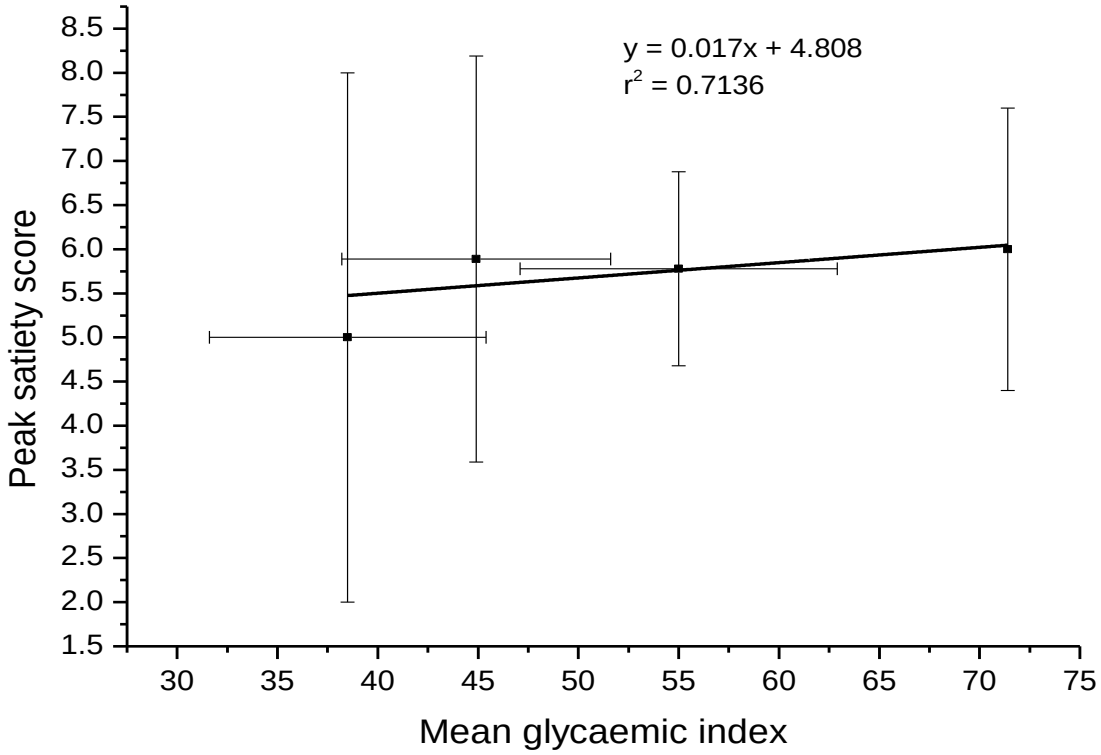


Figure 7.5: Peak Satiety scores vs. glycaemic indices correlation graph.
Data points were obtained from peak satiety scores of 10 subjects for each test product and reference and their glycaemic indices. X and y standard deviations indicate between subject variations.

Table 7.5: Peak satiety score for plantain products and reference food

	WB	BUP	BUPC	RRP
Peak satiety	6.0±1.6[a]	5.9±2.3[a]	5.8±1.1[a]	5.0±3.0[a]

Peak satiety scores after ingestion 25 g available carbohydrates portion of white bread (WB), boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP). Values plotted are means ± standard deviations of 10 subjects. Values with same superscript are not significantly different from each other at 95% confidence level.

These variations in trend amongst samples indicate that it is actually difficult to correlate blood glucose response with satiety, and because GI is calculated from blood glucose response over a period of time (2 hours), satiety may not be a basis for GI. Nevertheless, the observation with unripe plantain samples only appears to negate this assertion, and suggests that for some food products, postprandial blood glucose responses at between 0 - 120 min may be correlated with satiety. Leeman *et al* (2008), however, found no correlation between blood glucose response and satiety in the study of three potato products (French fries, boiled potatoes and boiled potatoes + oil) and white wheat bread. Non-correlation of GI with satiety cannot therefore be generalised as much as its correlation with GI cannot be overruled.

It was initially claimed that low GI foods have an overall health benefit because they promote satiety and would therefore be useful in abetting weight control (Brand-Miller *et al*., 2002). This view was later contradicted in some studies that revealed that there was no correlation between GI and satiety. For example, in a study to compare potatoes and pasta on an equi-carbohydrate basis (49 g), potatoes which had higher GI also exhibited a higher satiety than pasta with a lower GI (Anderson *et al*., 2002). A review later published by Anderson and Woodend (2003) revealed that high GI carbohydrates suppress short-term food intake (1 hour), while low GI carbohydrates appeared to be more effective over longer periods (6 hours).

We have measured satiety in products with equi-available carbohydrate content and peak satiety scores obtained are not significantly different from

each other (p = 0.05), (Table 7.5). The same observation was made by Leeman *et al* (2008) in the study of potato products with equi-carbohydrate quantities, however in that same study, differences in satiety indices were observed when equi-energy portions were used. This suggests that available carbohydrate quantity, regardless of food portion size is a major determinant of satiety. However, because we have considered only high starch foods with virtually no fat or protein, this proposition may not be applicable to all food groups.

It is more logical to state that the glycaemic effects exhibited by the plantain foods examined portray their available carbohydrate content and do not seem to generally reflect the impact of satiety (Anderson and Woodend, 2003).

7.6 Conclusion

The presence of resistant starch and slowly digestible starch reduces plantain glycaemic index but the low values of plantain glycaemic index cannot be attributed solely to the contributions from resistant and/or slowly digestible starches. Equivalent quantity of available carbohydrates from the same source (as we observed for three different plantain products) may not elicit the same glycaemic index and confirms the fact that glycaemic index is a property of the quality of available carbohydrates in the food. However, this may not be entirely true because glycaemic index is also dependent on the ability of the food to release the available carbohydrates within its matrix into circulation as glucose and this is can be affected by other food components.

The contribution of other food components to the low glycaemic index of plantain therefore needs to be further investigated.

An important observation in this work was that peak glycaemic response values were predictable from both the quantities and types of rapidly and slowly available glucose (*in vitro*) present in samples while peak glycaemic response attainment times were a function of the type of available carbohydrates in the products tested.

Satiety may be a good predictor of blood glucose responses for unripe plantain products but not for ripe plantain products. The reason for this needs to be further elucidated.

Chapter 8 General conclusions and future perspectives

8.1 General conclusions and future perspectives

Starch is quantitatively the most important source of available carbohydrate as well as a prominent source of indigestible carbohydrates in food. Resistant starch and glycaemic index are two phenomena that are well-known with regards to the dietary management of cardiovascular diseases and diabetes in almost every part of the world today. The rate of the search for healthier foods does not however seem to match the rate at which complications derived from eating unhealthy foods is gaining prominence. Moreover, the identification of resistant starch types in various foods, their specific impacts on GI and how to optimise them in dietary management is still a challenge to food scientists. This is because the mechanism by which a potential source of resistant starch elicits resistance is somewhat specific to the food, its source, chemical composition, physical and rheological properties, processing and manner of consumption amongst other factors. It appears that factors which produce an increased resistance of starch to digestion also reduce overall starch digestion and lead to the production of more quantities of slowly digestible starch (SDS). This implies that formation of resistant starch can influence the rate of digestion of the available starch fraction.

Although gelatinisation of plantain starch destroys RS2; this actually starts off another process that appears to be more beneficial nutritionally. It leads to the formation of RS3 and more importantly, slowly digestible starch (SDS). The quantity of SDS is significantly increased in both gelatinised and retrograded plantain foods by about 100% in unripe plantain samples and by about 200%

in unripe plantain foods cooked with salt (chapters 4 & 6). With regards to the rate at which starch is digested, SDS resulted in reduced glycaemic response in boiled unripe plantain and the impact of SDS on glycaemic index appears to be more pronounced and easier to monitor than that of resistant starch. SDS is present in uncooked, partially gelatinised as well as freshly cooked plantain and retrograded products. It is therefore recommended the relationship that between SDS and GI, as well as the minimum quantity required to significantly reduce glycaemic response in plantains should be addressed.

Ironically, results obtained in chapter 4 reveal that plantain starch also has the potential to generate more SDS than flours, especially at temperatures below the gelatinisation temperature. The mechanism for which SDS is generated therefore needs to be studied further in plantain and other potential sources of SDS. In this study, the observation is that formation of SDS is promoted when starch digestion rate is increased, for example, starch digestion rate is increased in starches as opposed to flours and more SDS is formed. Also heat treatment/gelatinisation increases starch digestion and also increases SDS (chapter 4). In the reverse process (retrogradation), the trend is also reversed: when starch is completely gelatinised, the rate of digestion is at its maximum *as it were* and SDS becomes zero. Retrogradation of gelatinised starch results in reduction of the rate of starch digestion and leads to the production of more SDS (chapter 6).

The mechanism leading to the production of SDS is not fully understood. In gelatinised starch, SDS formation has been attributed to factors such as the presence of longer amylose chains such as occurs in plantain starch (Lehmann and Robin, 2007). This prevents aggregation of amylose chains and results in the formation of a cross-linked network rather than the formation of a more ordered crystalline structure (RS3) due to non-alignment of double helices. Encapsulation of gelatinised starch between layers of RS3 has been proposed as another possible mechanism behind the increased content of a slowly digestible starch fraction (Zhang *et al*., 2006). An a A-type crystalline structure, including the distribution and perfection of crystalline regions (both crystalline and amorphous lamellae), determines the slow digestion property of native cereal starches (Zhang *et al*., 2006). The structural bases for significantly high levels of SDS in plantain starch (gelatinised and ungelatinised) which has both A-type and B-type crystalline forms need to be investigated. The results obtained on the quantification of SDS in plantain under various processing conditions in this thesis (chapters 4, 6 and 7) and the fact that by definition, SDS is the starch fraction that is slowly but eventually digested in the human small intestine, suggests that SDS components could be products of incomplete digestion of starch and could also contain products of incomplete conversion of gelatinised starch to RS3 in gelatinised starch. This may be a gateway to understanding the factors which produce a slower digestion of starch within a food matrix and

may also be related the ability of digestive enzymes to act on the required substrate.

For plantain products, our observation is that RS1, like RS2, is an inherent property of the plantain food but unlike RS2, it does not change significantly when cooked, except when plantain is cooked with the addition of salt. The reason for this observation also needs to be further investigated. RS1 has been scarcely reported in food except in grains and pulses and the quantification of RS1 in plantain products is reported for the first time in this thesis (chapters 6 & 7). It is not certain if some other sources of starch can be trapped in foods as to make them inaccessible similar to when they are present in a food matrix. However if this is true, then efforts can be geared toward food formulations that will enhance the trapping of starch to induce the generation of RS1 which has similar physiological impact on GI like SDS.

Although resistant starch formation is influenced by the rate of starch digestion, and the rate of starch digestion may be useful in predicting GI, data and information on the types and quantities of RS and other food components which promote starch resistance to digestive enzymes will help to optimise processing conditions required to promote the benefits of slow digestion of starch. Kinetic models have been used for the study of starch digestion rate which has been shown to follow a first order reaction (Butterworth *et al*., 2012), however, it is important not only to study the digestion kinetics of starch but food factors that may be responsible for the observed rate of reaction. Knowledge of how the relative quantities of food substances within

the food matrix impacts the starch digestion kinetics will be beneficial in the formulation of functional foods required for the control and management of hyperglycaemia.

The Englyst model used in our study has proved useful in determining impact of different types of available carbohydrates on peak time and peak glycaemic response of foods. We have also reported for the first time that peak glycaemic response values were predictable from the quantity and type of available carbohydrate (*in vitro*) in samples tested. If this is investigated for other foods, a possible relationship with GI may help to reduce the cost of conducting *in vivo* studies for the determination of GI. Nevertheless, the application of proper and accurate experimental protocols is a key factor *in vitro* studies. This includes the choice of enzymes and enzyme concentrations as well as buffers for accurate pH measurements, etc and these may vary for different plant sources due to varied food components as we have observed in this study (chapters 2 and 5).

Research for over two decades has helped to establish resistant starch as a valuable ingredient for functional food development but this has not helped to ameliorate or reduce the surge in diet-related diseases. It appears as though this development has led to a reduction in actually identifying potential resistant starch sources and more attention is focused on incorporating resistant starch into existing food products. Just like the case of dietary fibre (Juntunen *et al*., 2002), the presence of resistant starch in the food itself may actually be more beneficial in the control of hyperglycaemia than when it is

added as an ingredient. The identification of potential resistant starch sources and their useful application in diet may therefore help to reduce the high incidence of obesity, hyperglycaemia and diabetes. Furthermore, traditional and conventional processing methods are usually better than industrial processes such as extrusion and autoclaving which usually produce more soluble starch, greater digestibility of starch and destruction of the food matrix (Alonso *et al*., 2000). The domestic cooking conditions employed in the consumption of green plantains in Nigeria, which usually involves the addition of condiments may lead to a reduction in the extent of starch gelatinisation, a reduction in starch digestion and consequently result in lower GI. It is therefore recommended that the GI of boiled plantain in a mixed meal should be properly and carefully investigated

8.2 Further recommendations

1. Despite its low glycaemic index, long term studies are still required to investigate if plantain is beneficial in the management of diabetes.
2. A systematic study of the relationship between GI and different RS types in plantain using a large number of food samples is recommended.
3. The impact of having hot vs. cold plantain foods on GI has not been investigated and needs to be studied.
4. Because the same quantity of available carbohydrates from the same source may not elicit the same glycaemic index, there is need to

characterise and determine the GI of other plantain products especially grilled plantain which are usually processed below the gelatinisation temperature (chapter 3).

5. Because the low glycaemic index of plantain cannot be attributed only to nutritional starches and resistant starch as we have observed in this study (chapter 7), the contribution of other food components to the low glycaemic index of plantain therefore needs to be further investigated.
6. Insulin response to plantain products has not been reported and needs to be investigated.
7. The impact of low-GI foods on glucose and insulin balance has been shown to extend even to the next meal, so that foods eaten during dinner might influence the glycaemic response at breakfast, therefore second meal effect of consuming plantain foods need to be examined.
8. The enzyme inhibitors in the alcohol extractable fraction of plantain also need to be investigated.
9. Plantains have potential uses as flours, starches, whole foods and may serve as a major dietary component in this generation. The long term consequences of a food that lowers postprandial glucose responses may have implications far wider than the treatment of diabetes. This is because any factor that slows down the digestion and absorption of carbohydrate will lower insulin secretions and will consequently be of potential benefit in the treatment of conditions such as diabetes and cardiovascular diseases. Moreover, due to the fact that plantains have

been implicated in the treatment of other types of ailments, a detailed study of its nutraceutical components may help to proffer solutions to many health-related problems.

Chapter 9 References

9.1 Alphabetical list of all references used in the thesis

1980. Official Methods of Analysis of. *Analytical Chemistry.* American Chemical Society.

1992. Resistant Starch. Proceedings for the 2nd plenary meeting of EURESTA: European FLAIR Concerted Action No. 11 on physiological implications of the consumption of resistant starch in man. Crete, 29 May-2 June 1991. *European Journal of Clinical Nutrition,* 46 Suppl 2**,** S1-148.

Abboud, A. M. & Hoseney, R. C. 1984. Differential scanning calorimetry of sugar cookies and cookie doughs. *Cereal Chemistry,* 61**,** 34-37.

Abd Karim, A., Norziah, M. H. & Seow, C. C. 2000. Methods for the study of starch retrogradation. *Food Chemistry,* 71**,** 9-36.

Abdulrhman, M., El Hefnawy, M., Ali, R., Abdel Hamid, I., Abou El-Goud, A. & Refai, D. 2013. Effects of honey, sucrose and glucose on blood glucose and C-peptide in patients with type 1 diabetes mellitus. *Complementary therapies in clinical practice,* 19**,** 15-9.

Acevedo, N. C., Schebor, C. & Buera, M. D. P. 2008. Sorption Properties of Dehydrated Model Systems and Their Relationship to the Rate of Non-Enzymatic Browning. *Food Engineering: Integrated approaches,* 295-300

Agama-Acevedo, E., Islas-Hernandez, J. J., Pacheco-Vargas, G., Osorio-Diaz, P. & Bello-Perez, L. A. 2012. Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. *Lwt-Food Science and Technology,* 46**,** 177-182.

Alegbejo, J. O. & Ameh, D. A. 2012. Glycaemic Index of Market Plantain in Healthy and Diabetic Subjects. *Journal of Pure and Applied Microbiology,* 6**,** 677-682.

Alonso, R., Aguirre, A. & Marzo, F. 2000. Effects of extrusion and traditional processing methods on antinutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. *Food Chemistry,* 68**,** 159-165.

Alvarez-Acosta, T., Leon, C., Acosta-Gonzalez, S., Parra-Soto, H., Cluet-Rodriguez, I., Rossell, M. R. & Colina-Chourio, J. A. 2009. Beneficial Role of Green Plantain Musa paradisiaca in the Management of Persistent Diarrhea: A Prospective Randomized Trial. *Journal of the American College of Nutrition,* 28**,** 169-176.

Analysis, O. M. O. 2000. AOAC International.

Anderson, G. H., Catherine, N. L. A., Woodend, D. M. & Wolever, T. M. S. 2002. Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. *American Journal of Clinical Nutrition,* 76**,** 1023-1030.

Anderson, G. H. & Woodend, D. 2003. Effect of glycemic carbohydrates on short-term satiety and food intake. *Nutrition Reviews,* 61**,** S17-S26.

Asare, E. K., Jaiswal, S., Maley, J., Baga, M., Sammynaiken, R., Rossnagel, B. G. & Chibbar, R. N. 2011. Barley Grain Constituents, Starch Composition, and Structure Affect Starch *in vitro* Enzymatic Hydrolysis. *Journal of Agricultural and Food Chemistry,* 59**,** 4743-4754.

Asp, N. G. 1997. Resistant starch - An update on its physiological effects. *In:* Kritchevsky, D. & Bonfield, C. (eds.) *Dietary Fiber in Health and Disease.* New York: Plenum Press Div Plenum Publishing Corp.

Atkinson, F. S., Foster-Powell, K. & Brand-Miller, J. C. 2008. International Tables of Glycemic Index and Glycemic Load Values: 2008. *Diabetes Care,* 31**,** 2281-2283.

Augustin, L. S., Franceschi, S., Jenkins, D. J. A., Kendall, C. W. C. & La Vecchia, C. 2002. Glycemic index in chronic disease: a review. *European Journal of Clinical Nutrition,* 56**,** 1049-1071.

Aurore, G., Parfait, B. & Fahrasmane, L. 2009. Bananas, raw materials for making processed food products. *Trends in Food Science & Technology,* 20**,** 78-91.

Bahado-Singh, P. S., Wheatley, A. O., Ahmad, M. H., Morrison, E. & Asemota, H. N. 2006. Food processing methods influence the glycaemic indices of some commonly eaten West Indian carbohydrate-rich foods. *British Journal of Nutrition,* 96**,** 476-481.

Baker, L. A. & Rayas-Duarte, P. 1998. Retrogradation of amaranth starch at different storage temperatures and the effects of salt and sugars. *Cereal Chemistry,* 75**,** 308-314.

Baks, T., Ngene, I. S., Van Soest, J. J. G., Janssen, A. E. M. & Boom, R. M. 2007. Comparison of methods to determine the degree of gelatinisation for both high and low starch concentrations. *Carbohydrate Polymers,* 67**,** 481-490.

Basha, S. M. 1992. Soluble sugar composition of peanut seed. *Journal of Agricultural and Food Chemistry,* 40**,** 780-783.

Beck, M., Jekle, M. & Becker, T. 2011. Starch re-crystallization kinetics as a function of various cations. *Starch-Starke,* 63**,** 792-800.

Behall, K. M., Scholfield, D. J., Hallfrisch, J. G. & Liljeberg-Elmståhl, H. G. M. 2006. Consumption of Both Resistant Starch and β-Glucan Improves Postprandial Plasma Glucose and Insulin in Women. *Diabetes Care,* 29**,** 976-981.

Bello-Perez, L. A., De Francisco, A., Agama-Acevedo, E., Gutierrez-Meraz, F. & Garcia-Suarez, F. J. L. 2005a. Morphological and molecular studies of banana starch. *Food Science and Technology International,* 11**,** 367-372.

Bello-Perez, L. A., Ottenhof, M. A., Agama-Acevedo, E. & Farhat, I. A. 2005b. Effect of storage time on the retrogradation of banana starch extrudate. *Journal of Agricultural and Food Chemistry,* 53**,** 1081-1086.

Belloperez, L. A. & Paredeslopez, O. 1995. Effects of solutes on retrogradation of stored starches and amylopectins - a calorimetric study. *Starch-Starke,* 47**,** 83-86.

Bennett, R. N., Shiga, T. M., Hassimotto, N. M. A., Rosa, E. a. S., Lajolo, F. M. & Cordenunsi, B. R. 2010. Phenolics and Antioxidant Properties of Fruit Pulp and Cell Wall Fractions of Postharvest Banana (Musa acuminata Juss.) Cultivars. *Journal of Agricultural and Food Chemistry,* 58**,** 7991-8003.

Berry, C. S. 1986. Resistant starch - formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. *Journal of Cereal Science,* 4**,** 301-314.

Best, R., Lewis, D. A. & Nasser, N. 1984. The anti-ulcerogenic activity of the unripe plantain banana (musa species). *British Journal of Pharmacology,* 82**,** 107-116.

Biliaderis, C. G. 2009. *Structural Transitions and Related Physical Properties of Starch*.

Bjorck, I., Nyman, M., Pedersen, B., Siljestrom, M., Asp, N. G. & Eggum, B. O. 1987. Formation of enzyme resistant starch during autoclaving of wheat-starch - studies invitro and invivo. *Journal of Cereal Science,* 6**,** 159-172.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B. & Schneeman, B. O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. *American Journal of Clinical Nutrition,* 69**,** 55-63.

Brand-Miller, J., Hayne, S., Petocz, P. & Colagiuri, S. 2003. Low-glycemic index diets in the management of diabetes. *Diabetes Care,* 26**,** 2261-2267.

Brand-Miller, J., Mcmillan-Price, J., Steinbeck, K. & Caterson, I. 2009a. Dietary glycemic index: health implications. *American Journal of Clinical Nutrition,* 28 Suppl**,** 446S-449S.

Brand-Miller, J. C., Holt, S. H. A., Pawlak, D. B. & Mcmillan, J. 2002. Glycemic index and obesity. *American Journal of Clinical Nutrition,* 76**,** 281S-285S.

Brand-Miller, J. C., Stockmann, K., Atkinson, F., Petocz, P. & Denyer, G. 2009b. Glycemic index, postprandial glycemia, and the shape of the curve in healthy subjects: analysis of a database of more than 1000 foods. *American Journal of Clinical Nutrition,* 89**,** 97-105.

Brennan, C. S., Blake, D. E., Ellis, P. R. & Schofield, J. D. 1996. Effects of guar galactomannan on wheat bread microstructure and on the *in vitro* and *in vivo* digestibility of starch in bread. *Journal of Cereal Science,* 24**,** 151-160.

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G. & Wolever, T. M. S. 2005. Glycaemic index methodology. *Nutrition Research Reviews,* 18**,** 145-171.

Brown, I. L., Mcnaught, K. J. & Moloney, E. 1995. Hi-maize(tm) - new directions in starch technology and nutrition. *Food Australia,* 47**,** 272-275.

Butterworth, P. J., Warren, F. J., Grassby, T., Patel, H. & Ellis, P. R. 2012. Analysis of starch amylolysis using plots for first-order kinetics. *Carbohydrate Polymers,* 87**,** 2189-2197.

Cairns, P., Leloup, V., Miles, M. J., Ring, S. G. & Morris, V. J. 1990. Resistant starch - an x-ray-diffraction study into the effect of enzymatic-hydrolysis on amylose gels invitro. *Journal of Cereal Science,* 12**,** 203-206.

Carlos-Amaya, F., Osorio-Diaz, P., Agama-Acevedo, E., Yee-Madeira, H. & Bello-Perez, L. A. 2011. Physicochemical and Digestibility Properties of Double-Modified Banana (Musa paradisiaca L.) Starches. *Journal of Agricultural and Food Chemistry,* 59**,** 1376-1382.

Chaiwanichsiri, S., Ohnishi, S., Suzuki, T., Takai, R. & Miyawaki, O. 2001. Measurement of electrical conductivity, differential scanning calorimetry and viscosity of starch and flour suspensions during gelatinisation process. *Journal of the Science of Food and Agriculture,* 81**,** 1586-1591.

Champ, M. M. J. 2004. Physiological aspects of resistant starch and *in vivo* measurements. *Journal of Aoac International,* 87**,** 749-755.

Chandler, S. 1995. The nutritional value of bananas. *World Crop Series: Bananas and plantains*, 468 -480.

Chang, Y. H., Lin, J. H. & Lii, C. Y. 2004. Effect of ethanol concentration on the physicochemical properties of waxy corn starch treated by hydrochloric acid. *Carbohydrate Polymers,* 57**,** 89-96.

Chen, W. S., Liu, D. C. & Chen, M. T. 2002. Effects of high level of sucrose on the moisture content, water activity, protein denaturation and sensory properties in Chinese-style pork jerky. *Asian-Australasian Journal of Animal Sciences,* 15**,** 585-590.

Chiu, C. J., Liu, S. M., Willett, W. C., Wolever, T. M. S., Brand-Miller, J. C., Barclay, A. W. & Taylor, A. 2011. Informing food choices and health outcomes by use of the dietary glycemic index. *Nutrition Reviews,* 69**,** 231-242.

Colonna, P., Leloup, V. & Buleon, A. 1992. Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition,* 46**,** S17-S32.

Constantina, T., Virginia, G., Dimitra, L. & Charikleia, C. 2012. Chemistry and Functional Properties of Carbohydrates and Sugars (Monosaccharides, Disaccharides, and Polysaccharides). *Sweeteners.* CRC Press.

Cordenunsi, B. R. & Lajolo, F. M. 1995. Starch breakdown during banana ripening - sucrose synthase and sucrose-phosphate synthase. *Journal of Agricultural and Food Chemistry,* 43**,** 347-351.

Cristina Acevedo, N., Schebor, C. & Buera, P. 2012. Impact of Starch Gelatinization on the Kinetics of Maillard Reaction in Freeze-Dried Potato Systems. *Food and Bioprocess Technology,* 5**,** 2428-2434.

Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A. & Englyst, H. N. 1996. Digestion and physiological properties of resistant starch in the human large bowel. *British Journal of Nutrition,* 75**,** 733-47.

Da Mota, R. V., Lajolo, F. M., Ciacco, C. & Cordenunsi, B. R. 2000. Composition and functional properties of banana flour from different varieties. *Starch-Starke,* 52**,** 63-68.

David, C.-S. & Gregory, C. 2001. Dietary Fiber and Glucose Metabolism and Diabetes. *Handbook of Dietary Fiber.* CRC Press.

Davis, F., Terry, L. A., Chope, G. A. & Faul, C. F. J. 2007. Effect of extraction procedure on measured sugar concentrations in onion (Allium cepa L.) bulbs. *Journal of Agricultural and Food Chemistry,* 55**,** 4299-4306.

Di Paola, R. D., Asis, R. & Aldao, M. a. J. 2003. Evaluation of the Degree of Starch Gelatinization by a New Enzymatic Method. *Starch - Stärke,* 55**,** 403-409.

Dona, A. C., Pages, G., Gilbert, R. G. & Kuchel, P. W. 2010. Digestion of starch: *In vivo* and *in vitro* kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers,* 80**,** 599-617.

Donald, A. M. 2001. Plasticization and self assembly in the starch granule. *Cereal Chemistry,* 78**,** 307-314.

Donald, T. 2006. Resistant Starch. *Functional Food Carbohydrates.* CRC Press.

Ebell, L. F. 1969. Specific total starch determinations in conifer tissues with glucose oxidase. *Phytochemistry,* 8**,** 25-&.

Eerlingen, R. C., Crombez, M. & Delcour, J. A. 1993a. Enzyme-resistant starch .1. Quantitative and qualitative influence of incubation-time and temperature of autoclaved starch on resistant starch formation. *Cereal Chemistry,* 70**,** 339-344.

Eerlingen, R. C., Deceuninck, M. & Delcour, J. A. 1993b. Enzyme-resistant starch .2. Influence of amylose chain-length on resistant starch formation. *Cereal Chemistry,* 70**,** 345-350.

Eerlingen, R. C. & Delcour, J. A. 1995. Formation, analysis, structure and properties of type-iii enzyme resistant starch. *Journal of Cereal Science,* 22**,** 129-138.

Eerlingen, R. C., Jacobs, H. & Delcour, J. A. 1994. Enzyme-resistant starch .5. Effect of retrogradation of waxy maize starch on enzyme susceptibility. *Cereal Chemistry,* 71**,** 351-355.

Eggleston, G., Swennen, R. & Akoni, S. 1992. Physicochemical studies on starches isolated from plantain cultivars, plantain hybrids and cooking bananas. *Starch-Starke,* 44**,** 121-128.

Ek, K. L., Brand-Miller, J. & Copeland, L. 2012. Glycemic effect of potatoes. *Food Chemistry,* 133**,** 1230-1240.

Eliasson, A. C. 1992. A calorimetric investigation of the influence of sucrose on the gelatinization of starch. *Carbohydrate Polymers,* 18**,** 131-138.

Ells, L. J., Seal, C. J., Kettlitz, B., Bal, W. & Mathers, J. C. 2005. Postprandial glycaemic, lipaemic and haemostatic responses to ingestion of rapidly and slowly digested starches in healthy young women. *British Journal of Nutrition,* 94**,** 948-955.

Emaga, T. H., Andrianaivo, R. H., Wathelet, B., Tchango, J. T. & Paquot, M. 2007. Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. *Food Chemistry,* 103**,** 590-600.

Englyst, H., Wiggins, H. S. & Cummings, J. H. 1982. Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst,* 107**,** 307-18.

Englyst, H. N. & Cummings, J. H. 1985. Digestion of the polysaccharides of some cereal foods in the human small intestine. *American Journal of Clinical Nutrition,* 42**,** 778-87.

Englyst, H. N. & Cummings, J. H. 1986a. Digestion of the Carbohydrates of Banana (Musa-Paradisiaca-Sapientum) in the Human Small-Intestine. *American Journal of Clinical Nutrition,* 44**,** 42-50.

Englyst, H. N. & Cummings, J. H. 1986b. Digestion of the carbohydrates of banana (Musa paradisiaca sapientum) in the human small intestine. *American Journal of Clinical Nutrition,* 44**,** 42-50.

Englyst, H. N. & Cummings, J. H. 1987. Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition,* 45**,** 423-31.

Englyst, H. N. & Cummings, J. H. 1990. Non-starch polysaccharides (dietary fiber) and resistant starch. *Advances in Experimental Medical Biology,* 270**,** 205-25.

Englyst, H. N. & Kingman, S. M. 1990. Dietary Fiber and Resistant Starch - a Nutritional Classification of Plant Polysaccharides. *Dietary Fiber***,** 49-65.

Englyst, H. N., Kingman, S. M. & Cummings, J. H. 1992. Classification and Measurement of Nutritionally Important Starch Fractions. *European Journal of Clinical Nutrition,* 46**,** S33-S50.

Englyst, H. N., Kingman, S. M., Hudson, G. J. & Cummings, J. H. 1996a. Measurement of resistant starch *in vitro* and *in vivo*. *British Journal of Nutrition,* 75**,** 749-55.

Englyst, H. N., Veenstra, J. & Hudson, G. J. 1996b. Measurement of rapidly available glucose (RAG) in plant foods: a potential *in vitro* predictor of the glycaemic response. *British Journal of Nutrition,* 75**,** 327-37.

Englyst, K., Englyst, H. & Eliasson, A. 2004. Detecting nutritional starch fractions. *Starch in food: structure, function and applications***,** 541-559.

Englyst, K. N. & Englyst, H. N. 2005. Carbohydrate bioavailability. *British Journal of Nutrition,* 94**,** 1-11.

Englyst, K. N., Englyst, H. N., Hudson, G. J., Cole, T. J. & Cummings, J. H. 1999. Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. *American Journal of Clinical Nutrition,* 69**,** 448-54.

Englyst, K. N., Vinoy, S., Englyst, H. N. & Lang, V. 2003a. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition,* 89**,** 329-40.

Englyst, K. N., Vinoy, S., Englyst, H. N. & Lang, V. 2003b. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition,* 89**,** 329-339.

Englyst, N. A., Taube, J. M., Aitman, T. J., Baglin, T. P. & Byrne, C. D. 2003c. A novel role for CD36 in VLDL-enhanced platelet activation. *Diabetes,* 52**,** 1248-55.

Faisant, N., Buleon, A., Colonna, P., Molis, C., Lartigue, S., Galmiche, J. P. & Champ, M. 1995. Digestion of raw banana starch in the small-intestine of healthy humans - structural features of resistant starch. *British Journal of Nutrition,* 73**,** 111-123.

Fannon, J. E., Hauber, R. J. & Bemiller, J. N. 1992. Surface pores of starch granules. *Cereal Chemistry,* 69**,** 284-288.

Fils-Lycaon, B., Julianus, P., Chillet, M., Galas, C., Hubert, O., Rinaldo, D. & Mbéguié-a-Mbéguié, D. 2011. Acid invertase as a serious candidate to control the balance sucrose versus (glucose + fructose) of banana fruit during ripening. *Scientia Horticulturae,* 129**,** 197-206.

Finocchiaro, E., Anne, B. & Monika, O. 2009. Resistant Starch (RS). *Fiber Ingredients.* CRC Press.

Folkes, D. & Jordan, M. 2006. Mono- and Disaccharides. *Carbohydrates in Food, Second Edition.* CRC Press.

Food and Agriculture Organization, F. 1998. Food and Nutrition Paper no. 66. Report of a Joint FAO/WHO Expert Consultation, Rome, 14–18 April 1997. 1998 ed. Rome: FAO.

Foster-Powell, K., Holt, S. H. A. & Brand-Miller, J. C. 2002. International table of glycemic index and glycemic load values: 2002. *American Journal of Clinical Nutrition,* 76**,** 5-56.

Franco, C. M. L. & Ciacco, C. F. 1992. Factors that affect the enzymatic degradation of natural starch granules - effect of the size of the granules. *Starch-Starke,* 44**,** 422-426.

Gallant, D. J., Bouchet, B. & Baldwin, P. M. 1997. Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers,* 32**,** 177-191.

Garcia-Alonso, A., Jimenez-Escrig, A., Martin-Carron, N., Bravo, L. & Saura-Calixto, F. 1999. Assessment of some parameters involved in the gelatinization and retrogration of starch. *Food Chemistry,* 66**,** 181-187.

Garcia, V., Colonna, P., Lourdin, D., Buleon, A., Bizot, H. & Ollivon, M. 1996. Thermal transitions of cassava starch at intermediate water contents. *Journal of Thermal Analysis,* 47**,** 1213-1228.

Giannoccaro, E., Wang, Y. J. & Chen, P. Y. 2006. Effects of solvent, temperature, time, solvent-to-sample ratio, sample size, and defatting on the extraction of soluble sugars in soybean. *Journal of Food Science,* 71**,** C59-C64.

Gidley, M. J., Cooke, D., Darke, A. H., Hoffmann, R. A., Russell, A. L. & Greenwell, P. 1995. Molecular order and structure in enzyme-resistant retrograded starch. *Carbohydrate Polymers,* 28**,** 23-31.

Godwin, O. H. a. a. E. V. 2010. Glycemic indices of processed unripe plantain (Musa paradisiaca) meals. *African Journal of Food Science,* 4**,** 514-521.

Goni, I. & Valentin-Gamazo, C. 2003. Chickpea flour ingredient slows glycaemic response to pasta in healthy volunteers. *Food Chemistry,* 81**,** 511-515.

Gonzalez-Soto, R. A., Sanchez-Hernandez, L., Solorza-Feria, J., Nunez-Santiago, C., Flores-Huicochea, E. & Bello-Perez, L. A. 2006. Resistant starch production from non-conventional starch sources by extrusion. *Food Science and Technology International,* 12**,** 5-11.

Goodman, B. E. 2010. Insights into digestion and absorption of major nutrients in humans. *Advances in Physiology Education,* 34**,** 44-53.

Granfeldt, Y., Drews, A. & Bjorck, I. 1995a. Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. *Journal of Nutrition,* 125**,** 459-465.

Granfeldt, Y., Hagander, B. & Bjorck, I. 1995b. Metabolic responses to starch in oat and wheat products - on the importance of food structure, incomplete gelatinization or presence of viscous dietary fiber. *European Journal of Clinical Nutrition,* 49**,** 189-199.

Granfeldt, Y., Liljeberg, H., Drews, A., Newman, R. & Bjorck, I. 1994. Glucose and insulin responses to barley products - influence of food structure and amylose-amylopectin ratio. *American Journal of Clinical Nutrition,* 59**,** 1075-1082.

Granfeldt, Y. E., Drews, A. W. & Bjorck, I. M. E. 1993. Starch bioavailability in arepas made from ordinary or high amylose corn - concentration and

gastrointestinal fate of resistant starch in rats. *Journal of Nutrition,* 123**,** 1676-1684.

Gudmundsson, M. 1994. Retrogradation of starch and the role of its components. *Thermochimica Acta,* 246**,** 329-341.

Guzar, I., Ragaee, S. & Seetharaman, K. 2012. Mechanism of Hydrolysis of Native and Cooked Starches from Different Botanical Sources in the Presence of Tea Extracts. *Journal of Food Science,* 77**,** C1192-C1196.

Hall, M. B. 2003. Challenges with nonfiber carbohydrate methods. *Journal of Animal Science,* 81**,** 3226-32.

Hallstrom, E., Sestili, F., Lafiandra, D., Bjorck, I. & Ostman, E. 2011. A novel wheat variety with elevated content of amylose increases resistant starch formation and may beneficially influence glycaemia in healthy subjects. *Food & nutrition research,* 55.

Han, J.-A. & Bemiller, J. N. 2007. Preparation and physical characteristics of slowly digesting modified food starches. *Carbohydrate Polymers,* 67**,** 366-374.

Harbis, A., Perdreau, S., Vincent-Baudry, S., Charbonnier, M., Bernard, M. C., Raccah, D., Senft, M., Lorec, A. M., Defoort, C., Portugal, H., Vinoy, S., Lang, V. & Lairon, D. 2004. Glycemic and insulinemic meal responses modulate postprandial hepatic and intestinal lipoprotein accumulation in obese, insulin-resistant subjects. *American Journal of Clinical Nutrition,* 80**,** 896-902.

Henry, Z. & Alistair, S. 2006. Starch. *Food Polysaccharides and Their Applications.* CRC Press.

Holt, S., Reid, J., Taylor, T. V., Tothill, P. & Heading, R. C. 1982. Gastric-emptying of solids in man. *Gut,* 23**,** 292-296.

Holt, S. H. A., Miller, J. C. B. & Petocz, P. 1996. Relationships between satiety and plasma glucose and insulin responses to foods. *Proceedings of the Nutrition Society of Australia, Vol 20,* 20**,** 177-177.

Hoover, R. & Ratnayake, W. S. 2001. Determination of Total Amylose Content of Starch. *Current Protocols in Food Analytical Chemistry.* John Wiley & Sons, Inc.

Hoover, R. & Senanayake, N. 1996. Effect of sugars on the thermal and retrogradation properties of oat starches. *Journal of Food Biochemistry,* 20**,** 65-83.

Inger, B. 2006. Starch. *Carbohydrates in Food, Second Edition.* CRC Press.

Iyare, O. A. & Ekwukoma, B. O. 1992. Changes in the activities of carbohydrate-degrading enzymes with ripening in musa-paradisiaca. *Journal of the Science of Food and Agriculture,* 58**,** 173-176.

Jankowski, T. 1992. Influence of starch retrogradation on the texture of cooked potato-tuber. *International Journal of Food Science and Technology,* 27**,** 637-642.

Jenkins, D. J., Jenkins, A. L., Wolever, T. M., Collier, G. R., Rao, A. V. & Thompson, L. U. 1987. Starchy foods and fiber: reduced rate of digestion and improved carbohydrate metabolism. *Scandinavian journal of gastroenterology. Supplement,* 129**,** 132-41.

Jenkins, D. J. A. & Kendall, C. W. C. 2000. Resistant starches. *Current Opinion in Gastroenterology,* 16**,** 178-183.

Jenkins, D. J. A., Kendall, C. W. C., Augustin, L. S. A. & Vuksan, V. 2002. High-complex carbohydrate or lente carbohydrate foods? *American Journal of Medicine,* 113**,** 30S-37S.

Jenkins, D. J. A. & Wolever, T. M. S. 1981. Slow release carbohydrate and the treatment of diabetes. *Proceedings of the Nutrition Society,* 40**,** 227-235.

Jenkins, D. J. A., Wolever, T. M. S., Jenkins, A. L., Giordano, C., Giudici, S., Thompson, L. U., Kalmusky, J., Josse, R. G. & Wong, G. S. 1986. Low glycemic response to traditionally processed wheat and rye products - bulgur and pumpernickel bread. *American Journal of Clinical Nutrition,* 43**,** 516-520.

Jenkins, D. J. A., Wolever, T. M. S., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., Bowling, A. C., Newman, H. C., Jenkins, A. L. & Goff, D. V. 1981. Glycemic index of foods - a physiological-basis for carbohydrate exchange. *American Journal of Clinical Nutrition,* 34**,** 362-366.

Johansen, H. N., Glitso, V. & Knudsen, K. E. B. 1996. Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry,* 44**,** 1470-1474.

Juansang, J., Puttanlek, C., Rungsardthong, V., Puncha-Arnon, S. & Uttapap, D. 2012. Effect of gelatinisation on slowly digestible starch and resistant starch of heat-moisture treated and chemically modified canna starches. *Food Chemistry,* 131**,** 500-507.

Juarez-Garcia, E., Agama-Acevedo, E., Sayago-Ayerdi, S. G., Rodriguez-Ambriz, S. L. & Bello-Perez, L. A. 2006. Composition, digestibility and application in breadmaking of banana flour. *Plant Foods for Human Nutrition,* 61**,** 131-137.

Jung, E. Y., Suh, H. J., Hong, W. S., Kim, D. G., Hong, Y. H., Hong, I. S. & Chang, U. J. 2009. Uncooked rice of relatively low gelatinization degree resulted in lower metabolic glucose and insulin responses compared with cooked rice in female college students. *Nutrition Research,* 29**,** 457-461.

Juntunen, K. S., Niskanen, L. K., Liukkonen, K. H., Poittanen, K. S., Holst, J. J. & Mykkanen, H. M. 2002. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *American Journal of Clinical Nutrition,* 75**,** 254-262.

Kanellis, A. K., Solomos, T. & Mattoo, A. K. 1989. Changes in sugars, enzymic activities and acid-phosphatase isoenzyme profiles of bananas ripened in air or stored in 2.5 percent O2 with and without ethylene. *Plant Physiology,* 90**,** 251-258.

Karel, M. & Labuza, T. P. 1968. Nonenzymatic browning in model systems containing sucrose. *Journal of Agricultural and Food Chemistry,* 16**,** 717-&.

Kendall, C., Esfahani, A., Sanders, L., Potter, S. & Jenkins, D. 2009. Resistant Starch reduces postprandial glycemic and insulinemic response and increases satiety in humans. *Faseb Journal,* 23.

Kendall, C. W. C., Emam, A., Augustin, L. S. A. & Jenkins, D. J. A. 2004. Resistant starches and health. *Journal of AOAC International,* 87**,** 769-774.

Ketiku, A. O. 1973. Chemical composition of unripe (green) and ripe plantain (musa-paradisiaca). *Journal of the Science of Food and Agriculture,* 24**,** 703-707.

Kirsi, J. 2006. Mono- and Disaccharides. *Carbohydrates in Food, Second Edition.* CRC Press.

Knudsen, K. E. B. & Li, B. W. 1991. Determination of oligosaccharides in protein-rich feedstuffs by gas-liquid-chromatography and high-performance liquid-chromatography. *Journal of Agricultural and Food Chemistry,* 39**,** 689-694.

Lee, B. M. & Wolever, T. M. S. 1998. Effect of glucose, sucrose and fructose on plasma glucose and insulin responses in normal humans: comparison with white bread. *European Journal of Clinical Nutrition,* 52**,** 924-928.

Leeman, M., Ostman, E. & Bjorck, I. 2008. Glycaemic and satiating properties of potato products. *European Journal of Clinical Nutrition,* 62**,** 87-95.

Lehmann, U., Jacobasch, G. & Schmiedl, D. 2002. Characterization of Resistant Starch Type III from Banana (Musa acuminata). *Journal of Agricultural and Food Chemistry,* 50**,** 5236-5240.

Lehmann, U. & Robin, F. 2007. Slowly digestible starch - its structure and health implications: a review. *Trends in Food Science & Technology,* 18**,** 346-355.

Leinonen, K., Liukkonen, K., Poutanen, K., Uusitupa, M. & Mykkanen, H. 1999. Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. *European Journal of Clinical Nutrition,* 53**,** 262-267.

Lewis, D. A., Fields, W. N. & Shaw, G. P. 1999. A natural flavonoid present in unripe plantain banana pulp (Musa sapientum L. var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. *Journal of Ethnopharmacology,* 65**,** 283-288.

Lii, C. Y., Chang, S. M. & Young, Y. L. 1982. Investigation of the physical and chemical-properties of banana starches. *Journal of Food Science,* 47**,** 1493-1497.

Lii, C. Y. & Lee, B. L. 1993. Heating a-type, b-type, and c-type starches in aqueous sodium-chloride - effects of sodium-chloride concentration and moisture-content on differential scanning calorimetry thermograms. *Cereal Chemistry,* 70**,** 188-192.

Lilia, C. & Harold, C. 2003. Starch Properties and Functionalities. *Characterization of Cereals and Flours.* CRC Press.

Lin, J. H., Lee, S. Y. & Chang, Y. H. 2003. Effect of acid-alcohol treatment on the molecular structure and physicochemical properties of maize and potato starches. *Carbohydrate Polymers,* 53**,** 475-482.

Ling, L. H., Fernandes, J. B., Reilly, P. J. & Osman, E. M. 1982. Physical-properties of starch from cavendish banana fruit. *Starke,* 34**,** 184-188.

Liu, K. S. & Han, J. C. 2012. Enzymatic method for measuring starch gelatinization in dry products in situ. *Journal of Agricultural and Food Chemistry,* 60**,** 4212-4221.

Lu, Z.-H., Donner, E., Yada, R. Y. & Liu, Q. 2012. The synergistic effects of amylose and phosphorus on rheological, thermal and nutritional properties of potato starch and gel. *Food Chemistry,* 133**,** 1214-1221.

Lu, Z.-H., Yada, R. Y., Liu, Q., Bizimungu, B., Murphy, A., De Koeyer, D., Li, X.-Q. & Pinhero, R. G. 2011. Correlation of physicochemical and nutritional properties of dry matter and starch in potatoes grown in different locations. *Food Chemistry,* 126**,** 1246-1253.

Ma, W. P. & Robyt, J. F. 1987. Preparation and characterization of soluble starches having different molecular sizes and composition, by acid-hydrolysis in different alcohols. *Carbohydrate Research,* 166**,** 283-297.

Magnus, G. & Eliasson, A.-C. 2006. Starch. *Carbohydrates in Food, Second Edition.* CRC Press.

Mann, J., Cummings, J. H., Englyst, H. N., Key, T., Liu, S., Riccardi, G., Summerbell, C., Uauy, R., Van Dam, R. M., Venn, B., Vorster, H. H. & Wiseman, M. 2007. FAO/WHO scientific update on carbohydrates in human nutrition: conclusions. *European Journal of Clinical Nutrition,* 61 Suppl 1**,** S132-7.

Marconi, E., Messia, M. C., Palleschi, G. & Cubadda, R. 2004. A maltose biosensor for determining gelatinized starch in processed cereal foods. *Cereal Chemistry,* 81**,** 6-9.

Maurice, S. 2004. Brix-Sugar Inversion. *The Soft Drinks Companion.* CRC Press.

Mccleary, B. V., Mcnally, M., Rossiter, P., Aman, P., Amrein, T., Arnouts, S., Arrigoni, E., Bauer, L., Bavor, C., Brunt, K., Bryant, R., Bureau, S., Camire, M. E., Champ, M., Chen, Q., Chin, M. L., Colilla, W., Coppin, J., Costa, D., Crosby, G., Dean, J., Berrios, J. D., De Valck, J., Doerfer, J., Dougherty, M., Eybye, K., Fahey, G., Femenia, A., Forssell, P., Gelroth, J., Geske, J., Hidaka, H., Isaksen, M. F., Kettlitz, B., Kozlowski, F., Laerke, H. N., Li, B., Lincoln, Y., Liu, Q., Martensson, A.

C., Mattys, S., Meuser, F., Monro, J., Niba, L., Niemann, C., Panozzo, J., Rossiter, P., Roturier, J. M., Sampson, C., Sanders, P., Suter, D., Tas, A. A., Themeier, H., Tudorica, C., Watson, R., Weber, L., Weinstein, M., Wilkinson, M., Yen, J. & Zheng, T. 2002. Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: Collaborative study. *Journal of AOAC International,* 85**,** 1103-1111.

Menezes, E. W., Dan, M. C. T., Cardenette, G. H. L., Goni, I., Arturo Bello-Perez, L. & Lajolo, F. M. 2010. *In vitro* Colonic Fermentation and Glycemic Response of Different Kinds of Unripe Banana Flour. *Plant Foods for Human Nutrition,* 65**,** 379-385.

Miao, M., Zhang, T., Mu, W. M. & Jiang, B. 2010. Effect of controlled gelatinization in excess water on digestibility of waxy maize starch. *Food Chemistry,* 119**,** 41-48.

Micklander, E., Thybo, A. K. & Van Den Berg, F. 2008. Changes occurring in potatoes during cooking and reheating as affected by salting and cool or frozen storage - a LF-NMR study. *Lwt-Food Science and Technology,* 41**,** 1710-1719.

Miles, M. J., Morris, V. J., Orford, P. D. & Ring, S. G. 1985. The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research,* 135**,** 271-281.

Miller, J. B., Pang, E. & Bramall, L. 1992. RICE - A high or low glycemic index food. *American Journal of Clinical Nutrition,* 56**,** 1034-1036.

Monro, J. 2003. Redefining the glycemic index for dietary management of postprandial glycemia. *Journal of Nutrition,* 133**,** 4256-4258.

Monro, J., Mishra, S., Blandford, E., Anderson, J. & Genet, R. 2009. Potato genotype differences in nutritionally distinct starch fractions after cooking, and cooking plus storing cool. *Journal of Food Composition and Analysis,* 22**,** 539-545.

Monro, J. A. 1999. Available carbohydrate and glycemic index combined in new data sets for managing glycemia and diabetes. *Journal of Food Composition and Analysis,* 12**,** 71-82.

Monro, J. A. 2002. Glycaemic glucose equivalent: combining carbohydrate content, quantity and glycaemic index of foods for precision in glycaemia management. *Asia Pacific Journal of Clinical Nutrition,* 11**,** 217-225.

Murphy, M. M., Douglass, J. S. & Birkett, A. 2008. Resistant starch intakes in the United States. *Journal of the American Dietetic Association,* 108**,** 67-78.

Narayan, K. M. V., Saaddine, J. B., Boyle, J. P., Thompson, T. J. & Geiss, L. S. 2006. Impact of recent increase in incidence on future diabetes burden - US, 2005-2050. *Diabetes Care,* 29**,** 2114-2116.

Nebesny, E., Rosicka, J. & Tkaczyk, M. 2004. Influence of conditions of maize starch enzymatic hydrolysis on physicochemical properties of glucose syrups. *Starch-Starke,* 56**,** 132-137.

Neil, R., Peter, R., Ellis. & Peter, J., Butterworth. 2005. Starch molecular and nutritional properties: A review. *Advances in Molecular Medicine,* 1**,** 5-14.

Ngalani, J. A., Signoret, A. & Crouzet, J. 1993. Partial-purification and properties of plantain polyphenol oxidase. *Food Chemistry,* 48**,** 341-347.

Nilsson, A. C., Ostman, E. M., Granfeldt, Y. & Bjorck, I. M. E. 2008. Effect of cereal test breakfasts differing in glycemic index and content of indigestible carbohydrates on daylong glucose tolerance in healthy subjects. *American Journal of Clinical Nutrition,* 87**,** 645-654.

Nugent, A. P. 2005. Health properties of resistant starch. *Nutrition Bulletin,* 30**,** 27-54.

Nuttall, F. Q. 1993. Dietary fiber in the management of diabetes. *Diabetes,* 42**,** 503-508.

O'dea, K., Nestel, P. J. & Antonoff, L. 1980. Physical factors influencing postprandial glucose and insulin responses to starch. *American Journal of Clinical Nutrition,* 33**,** 760-765.

Oates, C. G. 1997. Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science & Technology,* 8**,** 375-382.

Osorio-Diaz, P., Aguilar-Sandoval, A., Agama-Acevedo, E., Rendon-Villalobos, R., Tovar, J. & Bello-Perez, L. A. 2008. Composite durum wheat flour/plantain starch white salted noodles: Proximal composition, starch digestibility, and indigestible fraction content. *Cereal Chemistry,* 85**,** 339-343.

Ovando-Martinez, M., Sayago-Ayerdi, S., Agama-Acevedo, E., Goni, I. & Bello-Perez, L. A. 2009. Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chemistry,* 113**,** 121-126.

Pacheco-Delahaye, E., Perez, R. & Schnell, M. 2004. Nutritional and sensory evaluation of powder drinks based on papaya, green plantain and rice bran. Glycemic index. *Interciencia,* 29**,** 46-51.

Pacheco De Delahaye, E. 2001. Nutritional evaluation of green plantain flour and vegetable dehydrated soups. Study of starch *in vitro* digestibility. *Acta Cientifica Venezolana,* 52**,** 278-282.

Parada, J. & Aguilera, J. M. 2011. Review: Starch Matrices and the Glycemic Response. *Food Science and Technology International,* 17**,** 187-204.

Pelissari, F. M., Andrade-Mahecha, M. M., Sobral, P. J. D. & Menegalli, F. C. 2012. Isolation and characterization of the flour and starch of plantain bananas (Musa paradisiaca). *Starch-Starke,* 64**,** 382-391.

Perry, P. A. & Donald, A. M. 2002. The effect of sugars on the gelatinisation of starch. *Carbohydrate Polymers,* 49**,** 155-165.

Perry, T., Mann, J., Mehalski, K., Gayya, C., Wilson, J. & Thompson, C. 2000. Glycaemic index of New Zealand foods. *New Zealand Medical Journal,* 113**,** 140-142.

Pi-Sunyer, F. X. 2002. Glycemic index and disease. *American Journal of Clinical Nutrition,* 76**,** 290S-298S.

Pinheiro Torres, A. & Oliveira, F. a. R. 1999. Application of the acid hydrolysis of sucrose as a temperature indicator in continuous thermal processes. *Journal of Food Engineering,* 40**,** 181-188.

Qiang, L. 2005. Understanding Starches and Their Role in Foods. *Food Carbohydrates.* CRC Press.

Raben, A., Tagliabue, A., Christensen, N. J., Madsen, J., Holst, J. J. & Astrup, A. 1994. Resistant starch - the effect on postprandial glycemia, hormonal response, and satiety. *American Journal of Clinical Nutrition,* 60**,** 544-551.

Regmi, P. R., Metzler-Zebeli, B. U., Ganzle, M. G., Van Kempen, T. & Zijlstra, R. T. 2011. Starch with High Amylose Content and Low *In vitro* Digestibility Increases Intestinal Nutrient Flow and Microbial Fermentation and Selectively Promotes Bifidobacteria in Pigs. *Journal of Nutrition,* 141**,** 1273-1280.

Rendon-Villalobos, R., Osorio-Diaz, P., Agama-Acevedo, E., Tovar, J. & Bello-Perez, L. A. 2008. Composite wheat-plantain starch salted noodles. Preparation, proximal composition and *in vitro* starch digestibility. *Interciencia,* 33**,** 658-662.

Riby, J. E., Fujisawa, T. & Kretchmer, N. 1993. Fructose absorption. *American Journal of Clinical Nutrition,* 58**,** S748-S753.

Ring, S. G., Colonna, P., Ianson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J. & Orford, P. D. 1987. The gelation and crystallization of amylopectin. *Carbohydrate Research,* 162**,** 277-293.

Ring, S. G., Gee, J. M., Whittam, M., Orford, P. & Johnson, I. T. 1988. Resistant starch: Its chemical form in foodstuffs and effect on digestibility *in vitro*. *Food Chemistry,* 28**,** 97-109.

Robayo–Torres, C. C., Quezada–Calvillo, R. & Nichols, B. L. 2006. Disaccharide Digestion: Clinical and Molecular Aspects. *Clinical Gastroenterology and Hepatology,* 4**,** 276-287.

Robinson, J. C. & Sauco, V. G. 2010. *Bananas and Plantains, 2nd Edition*, Cabi Publishing-C a B Int, Cabi Publishing, Wallingford 0x10 8de, Oxon, Uk.

Robyt, J. F. & Ma, W. P. 1987. A new method of preparing soluble starches of different size and composition by acid-hydrolysis in different alcohols. *Abstracts of Papers of the American Chemical Society,* 194**,** 16-CARB.

Rodriguez-Ambriz, S. L., Islas-Hernandez, J. J., Agama-Acevedo, E., Tovar, J. & Bello-Perez, L. A. 2008. Characterization of a fibre-rich powder prepared by liquefaction of unripe banana flour. *Food Chemistry,* 107**,** 1515-1521.

Rodriguez-Sevilla, M. D., Villanueva-Suarez, M. J. & Redondo-Cuenca, A. 1999. Effects of processing conditions on soluble sugars content of carrot, beetroot and turnip. *Food Chemistry,* 66**,** 81-85.

Roulet, P., Macinnes, W. M., Gumy, D. & Wursch, P. 1990. Retrogradation kinetics of 8 starches. *Starch-Starke,* 42**,** 99-101.

Sajilata, M. G., Singhal, R. S. & Kulkarni, P. R. 2006. Resistant starch - A review. *Comprehensive Reviews in Food Science and Food Safety,* 5**,** 1-17.

Schebor, C., Florencia Mazzobre, M. & Del Pilar Buera, M. 2010. Glass transition and time-dependent crystallization behavior of dehydration bioprotectant sugars. *Carbohydrate Research,* 345**,** 303-308.

Seal, C. J., Daly, M. E., Thomas, L. C., Bal, W., Birkett, A. M., Jeffcoat, R. & Mathers, J. C. 2003. Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and

rapid hydrolysis rates determined *in vitro*. *British Journal of Nutrition,* 90**,** 853-864.

Shin, S. I., Kim, H. J., Ha, H. J., Lee, S. H. & Moon, T. W. 2005. Effect of hydrothermal treatment on formation and structural characteristics of slowly digestible non-pasted granular sweet potato starch. *Starch-Starke,* 57**,** 421-430.

Shyam, S. 2009. Gelatinization of Starch. *Food Properties Handbook, Second Edition.* CRC Press.

Sievert, D., Czuchajowska, Z. & Pomeranz, Y. 1991. Enzyme-resistant starch .3. X-ray-diffraction of autoclaved amylomaize vii starch and enzyme-resistant starch residues. *Cereal Chemistry,* 68**,** 86-91.

Sievert, D. & Pomeranz, Y. 1990. Enzyme-resistant starch .2. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chemistry,* 67**,** 217-221.

Silvester, K. R., Englyst, H. N. & Cummings, J. H. 1995. Ileal recovery of starch from whole diets containing resistant starch measured *in vitro* and fermentation of ileal effluent. *American Journal of Clinical Nutrition,* 62**,** 403-11.

Simmonds, R. H. S. a. N. W. 1987. *Bananas,* London, Longman.

Slaughter, S. L., Ellis, P. R., Jackson, E. C. & Butterworth, P. J. 2002. The effect of guar galactomannan and water availability during hydrothermal processing on the hydrolysis of starch catalysed by pancreatic alpha-amylase. *Biochimica Et Biophysica Acta-General Subjects,* 1571**,** 55-63.

Soares, C. A., Peroni-Okita, F. H. G., Cardoso, M. B., Shitakubo, R., Lajolo, F. M. & Cordenunsi, B. R. 2011. Plantain and Banana Starches: Granule Structural Characteristics Explain the Differences in Their Starch Degradation Patterns. *Journal of Agricultural and Food Chemistry,* 59**,** 6672-6681.

Soh, N. L. & Brand-Miller, J. 1999. The glycaemic index of potatoes: the effect of variety, cooking method and maturity. *European Journal of Clinical Nutrition,* 53**,** 249-254.

Solnica, B., Naskalski, J. W. & Sieradzki, J. 2003. Analytical performance of glucometers used for routine glucose self-monitoring of diabetic patients. *Clinica Chimica Acta,* 331**,** 29-35.

Stanhope, K. L. 2012. Role of Fructose-Containing Sugars in the Epidemics of Obesity and Metabolic Syndrome. *In:* Caskey, C. T., Austin, C. P. & Hoxie, J. A. (eds.) *Annual Review of Medicine, Vol 63.*

Steve, C. & Yolanda, B. 2006. Detection and Determination of Polysaccharides in Foods. *Food Polysaccharides and Their Applications.* CRC Press.

Sturm, A. 1999. Invertases. Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant Physiology,* 121**,** 1-7.

Svihus, B., Uhlen, A. K. & Harstad, O. M. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science and Technology,* 122**,** 303-320.

Tester, R. F. & Sommerville, M. D. 2001. Swelling and enzymatic hydrolysis of starch in low water systems. *Journal of Cereal Science,* 33**,** 193-203.

Thompson, D. B. 2000. Strategies for the manufacture of resistant starch. *Trends in Food Science & Technology,* 11**,** 245-253.

Tian, Y., Zhang, L., Xu, X., Xie, Z., Zhao, J. & Jin, Z. 2012. Effect of temperature-cycled retrogradation on slow digestibility of waxy rice starch. *International journal of biological macromolecules,* 51**,** 1024-7.

Topping, D. L. & Clifton, P. M. 2001. Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews,* 81**,** 1031-1064.

Torija, E., Diez, C., Matallana, C., Camara, M., Camacho, E. & Mazario, P. 1998. Influence of freezing process on free sugars content of papaya and banana fruits. *Journal of the Science of Food and Agriculture,* 76**,** 315-319.

Tormo, M. A., Gil-Exojo, I., De Tejada, A. R. & Campillo, J. E. 2004. Hypoglycaemic and anorexigenic activities of an alpha-amylase inhibitor from white kidney beans (Phaseolus vulgaris) in Wistar rats. *British Journal of Nutrition,* 92**,** 785-790.

Truswell, A. S. 1992. Glycemic index of foods. *European Journal of Clinical Nutrition,* 46**,** S91-S101.

Vesterinen, E., Myllarinen, P., Forssell, P., Soderling, E. & Autio, K. 2002. Structural properties in relation to oral enzymatic digestibility of starch gels based on pure starch components and high, amylose content. *Food Hydrocolloids,* 16**,** 161-167.

Wachters-Hagedoorn, R., Priebe, M., Vonk, R. & Eliasson, A. 2004. Analysing starch digestion. *Starch in food: structure, function and applications***,** 575-589.

Wang, S. S., Chiang, W. C., Zhao, B., Zheng, X. G. & Kim, I. H. 1991. Experimental-analysis and computer-simulation of starch-water interactions during phase-transition. *Journal of Food Science,* 56**,** 121-124.

Warren, F. J., Butterworth, P. J. & Ellis, P. R. 2012. Studies of the effect of maltose on the direct binding of porcine pancreatic alpha-amylase to maize starch. *Carbohydrate Research,* 358**,** 67-71.

Williamson, G. 2013. Possible effects of dietary polyphenols on sugar absorption and digestion. *Molecular Nutrition & Food Research,* 57**,** 48-57.

Wolever, T. M. S. 1990. Relationship between dietary fiber content and composition in foods and the glycemic index. *American Journal of Clinical Nutrition,* 51**,** 72-75.

Wolever, T. M. S. 2006. *Glycaemic Index: A Physiological Classification of Dietary Carbohydrate*.

Wolever, T. M. S., Brand-Miller, J. C., Abernethy, J., Astrup, A., Atkinson, F., Axelsen, M., Bjorck, I., Brighenti, F., Brown, R., Brynes, A., Casiraghi, M. C., Cazaubiel, M., Dahlqvist, L., Delport, E., Denyer, G. S., Erba, D., Frost, G., Granfeldt, Y., Hampton, S., Hart, V. A., Hatonen, K. A., Henry, C. J., Hertzler, S., Hull, S., Jerling, J., Johnston, K. L., Lightowler, H., Mann, N., Morgan, L., Panlasigui, L. N., Pelkman, C., Perry, T., Pfeiffer, A. F. H., Pieters, M., Ramdath, D. D., Ramsingh, R. T., Robert, S. D., Robinson, C., Sarkkinen, E., Scazzina, F., Sison, D. C. D., Sloth, B., Staniforth, J., Tapola, N., Valsta, L. M., Verkooijen, I., Weickert, M. O., Weseler, A. R., Wilkie, P. & Zhang, J. 2008.

Measuring the glycemic index of foods: interlaboratory study. *American Journal of Clinical Nutrition,* 87**,** 247S-257S.

Wolever, T. M. S., Jenkins, D. J. A., Jenkins, A. L. & Josse, R. G. 1991. The glycemic index - methodology and clinical implications. *American Journal of Clinical Nutrition,* 54**,** 846-854.

Wolever, T. M. S. & Miller, J. B. 1995. Sugars and blood-glucose control. *American Journal of Clinical Nutrition,* 62**,** S212-S227.

Wolever, T. M. S., Vuksan, V., Relle, L. K., Jenkins, A. L., Josse, R. G., Wong, G. S. & Jenkins, D. J. A. 1993. Glycaemic index of fruits and fruit products in patients with diabetes. *International Journal of Food Sciences and Nutrition,* 43**,** 205-212.

Wolf, B. W., Bauer, L. L. & Fahey, G. C. 1999. Effects of chemical modification on *in vitro* rate and extent of food starch digestion: An attempt to discover a slowly digested starch. *Journal of Agricultural and Food Chemistry,* 47**,** 4178-4183.

Wong, J. M. W. & Jenkins, D. J. A. 2007. Carbohydrate digestibility and metabolic effects. *Journal of Nutrition,* 137**,** 2539S-2546S.

Wootton, M. & Bamunuarachchi, A. 1980. Application of differential scanning calorimetry to starch gelatinization .3. Effect of sucrose and sodium-chloride. *Starke,* 32**,** 126-129.

Xie, X. J. & Liu, Q. 2004. Development and physicochemical characterization of new resistant citrate starch from different corn starches. *Starch-Starke,* 56**,** 364-370.

Yadav, B. S., Sharma, A. & Yadav, R. B. 2010. Effect of storage on resistant starch content and *in vitro* starch digestibility of some pressure-cooked cereals and legumes commonly used in India. *International Journal of Food Science and Technology,* 45**,** 2449-2455.

Yun, S. H. & Matheson, N. K. 1990. Estimation of amylose content of starches after precipitation of amylopectin by concanavalin-A. *Starch-Starke,* 42**,** 302-305.

Zhang, G. Y., Sofyan, M. & Hamaker, B. R. 2008. Slowly digestible state of starch: Mechanism of slow digestion property of gelatinized maize starch. *Journal of Agricultural and Food Chemistry,* 56**,** 4695-4702.

Zhang, G. Y., Venkatachalam, M. & Hamaker, B. R. 2006. Structural basis for the slow digestion property of native cereal starches. *Biomacromolecules,* 7**,** 3259-3266.

Zhang, P. & Hamaker, B. R. 2012. Banana starch structure and digestibility. *Carbohydrate Polymers,* 87**,** 1552-1558.

Zhang, P. Y., Whistler, R. L., Bemiller, J. N. & Hamaker, B. R. 2005. Banana starch: production, physicochernical properties, and digestibility - a review. *Carbohydrate Polymers,* 59**,** 443-458.

Zhang, X. Y., Tong, Q. Y., Zhu, W. X. & Ren, F. 2013. Pasting, rheological properties and gelatinization kinetics of tapioca starch with sucrose or glucose. *Journal of Food Engineering,* 114**,** 255-261.

Zobel, H. F., Young, S. N. & Rocca, L. A. 1988. Starch gelatinization - an x-ray-diffraction study. *Cereal Chemistry,* 65**,** 443-446.