BANANA PEELS AS AN ALTERNATIVE SOURCE OF SUGAR PRODUCTION

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ABSTRACT

Carbohydrate is the main energy source of humans and animals. It is important for biosynthesis where it acts as precursor for most chemical reaction in our body. Carbohdydrate can be divided into four group which are polysaccharides, oligosaccharides, disaccharides, and monosaccharides. The latter two is commonly known as sugar. There are many foods high in carbohydrate include fruits, sweets, soft drinks, breads, pastas, beans, potatoes, bran, rice, and cereals. Most plants and fruits stored carbohydrate in form of starch. Banana for example contains high percentage of carbohydrate content. Detailed studies on chemical composition of banana peels also have shown high percentage of carbohydrate content. Thus, the large quantities of banana in Malaysia specifically, have the potential of being used industrially for production of sugars. It can alternatively become a new source of food sweetener and therefore reducing Malaysia's need on sugar cane for sugar production and also reduce our dependant on imported sugar. Besides, this study can help to eliminate environmental problems caused by banana waste. Experiment was set up to extract carbohydrate in form of starch from the banana peels by alkaline extraction method using 0.1M NaOH. The confirmation of the extracts was done by microscopy images of starch granules. The microscopy images showed irregular shape of starch granules and when stained with iodine solution, the color changes from brown to deep blue. Further reaction takes place to convert starch into sugar in form of glucose syrup by enzyme hydrolysis using α-amylase and amyloglucosidase. Quantitative confirmation of total sugar syrup was done by Lane Eynon titration method and average result of five sample gives was 655.4 mg/100ml or 0.33 % w/w.

ABSTRAK

Karbohidrat merupakan sumber tenaga utama kepada manusia dan haiwan. Karbohidrat memainkan peranan penting dalam proses biosintesis di mana ia bertindak sebagai pemula tindak balas kimia dalam badan kita. karbohidrat boleh dibahagikan kepada empat kumpulan iaitu polisakarida, oligosakarida, disakarida, dan monosakarida. Disakarida dan monosakarida kebiasaannya dikenali dengan nama komersial gula. Kelas makanan seperti buah-buahan, roti, pasta, kacang, kentang, dedak, beras, bijirin, gula-gula dan juga minuman ringan mempunyai kandungan karbohidrat yang tinggi. Tumbuh-tumbuhan dan buah-buahan menyimpan karbohidrat dalam bentuk kanji. Sebagai contoh, pisang mengandungi peratusan karbohidrat yang tinggi. Menurut kajian yang dilakukan ke atas komposisi kimia kulit pisang, ia juga menunjukkan peratusan kandungan karbohidrat yang tinggi. Maka, jumlah tanaman pisang yang besar di Malaysia khususnya, mempunyai potensi untuk diaplikasikan dalam industri pengeluaran gula. Kulit pisang boleh menjadi sumber alternatif sebagai pemanis makanan dan sekaligus mengurangkan kebergantungan negara terhadap tanaman tebu untuk penghasilan gula. Ini juga boleh membantu mengurangkan jumlah import gula ke dalam negara. Selain itu, kajian ini boleh membantu untuk menghapuskan masalah-masalah alam sekitar yang disebabkan oleh sisa pisang. Kajian melalui proses pengestrakan alkali dengan menggunakan 0.1M NaOH akan dijalankan untuk mendapatkan karbohidrat dalam bentuk kanji. Ujian pengesahan ekstrak akan dilakukan menggunakan teknik mikroskopi granul kanji. Hasil imej mikroskopi menunjukkan bentuk granul kanji yang tidak sekata dan apabila diwarnakan dengan larutan iodin, warna iodin akan berubah dari perang kepada biru gelap. Melalui proses hidrolisis enzim oleh α-amylase dan amyloglucosidase, kanji ditukarkan menjadi pati gula dan ujian pengesahan pati gula cair dijalankan dengan kaedah titratan Lane Eynon. Keputusan purata yang diperolehi hasil titratan 655.4 mg/100ml ataupun 0.33 % w/w

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LIST OF SYMBOLS

°C	degree Celcius
g	gram
M	mol/liter
mm	millimeter
ml	milliliter
U	1/60 micro katal

LIST OF ABBREVIATIONS

DE	Dextrose Equivalent
DSC	Differential Scanning Calorimetry
DSLR	Digitial Single Lens Reflex
ESEM	Environmental Scanning Electron Microscope
HPLC	High Performance Liquid Chromatography
NaOH	Sodium Hydroxide
UK	United Kingdom
USA	United States of America

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Sugar is a term for a class of edible crystalline carbohydrate that can be found in almost every type of fruit and vegetables. There are three main form of sugar family which are sucrose, lactose, and fructose. Sucrose and lactose are disaccharide molecules while fructose is monosaccharide molecule which is the simplest form of sugar. But, in society, sugar is almost exclusively refers to sucrose in food and it is primarily comes from sugar cane and sugar beet, which in its fully refined (or free sugar) form primarily comes from sugar cane and sugar beet, though is present in natural form in many carbohydrates. Other free sugars are used in industrial food preparation, but are usually known by more specific names - glucose, fructose or fruit sugar, high fructose corn syrup, etc.

Banana is known to contain high percentage of sugar. It contains three natural sugars which are sucrose, fructose, and glucose. Thus, banana can give an instant and sustainable boost of energy. Bananas are a staple starch for many tropical populations. Depending upon cultivar and ripeness, the flesh can vary in taste from starchy to sweet, and texture from firm to mushy. Both skin and inner part can be eaten raw or cooked.

Like other plants, banana stores the carbohydrate in form of starch but eventually it will be converted into sugar during ripening stage. Ethylene, a hormone released by the plant in which indirectly affects the flavor of the banana and responsible in stimulating the formation of amylase, an enzyme that breaks down

starch into sugar which contribute the taste of banana. The greener, less ripe bananas contain higher levels of starch and, consequently, have a "starchier" taste. On the other hand, yellow bananas taste sweeter due to higher sugar concentrations.

Plantain also comes from banana family. They belong to the same genus Musa. The difference is that plantain is AAB cultivar while most dessert banana is AAA cultivar. Plantain in contrast is firmer and contain less sugar than banana. Less sugar means they contain more starch. Thus, plantains are used in various stews and curries or cooked, baked or mashed in much the same way as potatoes. Plantain is also dried and ground into flour.

In Malaysia, banana is the second most widely cultivated fruit, covering about 26,000 hectar with a total production of 530,000 metric tones. However, plantain is not widely cultivated in our country. Most of the banana cultivation is monopolied by 'Pisang Berangan' and Cavendish type which accounted about 50% of the banana growing land.

As in banana pulp, the peels also contain high percentage of carbohydrate as revealed in detailed studies on its chemical composition. Anhwange (2009) reported that the content carbohydrate was found to be 59%. Hence, the values indicate that the peel could be a good source of carbohydrates. Thus, the large quantities of banana in Malaysia specifically, have the potential of being used industrially for production of sugars. It can alternatively become a new source of food sweetener and therefore reducing Malaysia's need on sugar cane for sugar production and also reduce our dependant on imported sugar.

1.2 PROBLEM STATEMENT

According to the Food and Agriculture data, sugarcane plantation is surprisingly small with there is only two plantation site. Since in our country the only sugarcane plantation is in Perlis and Kedah, definitely the sugar production from available crop cannot match consumer's needs. Thus, Malaysia is relying on imported sugars from other countries. Compared with sugarcane, banana production

is far greater as it is the second largest cultivars in Malaysia. Lots of products have been produce from banana. However, there are still no studies to utilize the banana peels for commercial value. As stated in the introduction, the banana peels have the potential to further processed producing sugar/sweetener due to high percentage of carbohydrate content.

1.3 OBJECTIVE

The main objectives of this study are to:

- To produce sugar from waste (banana peels)
- To quantitatively determine the amount of total sugar from banana peels

1.4 SCOPE OF STUDY

Based on the objective, the scope of this study is mainly to produce sugar from banana peels. Widely available banana type was chosen as sample in the experiment. Sugar cannot be extracted directly from the peels because they are stored in form of starch. Various extraction methods are available. Hence, the best extraction method should be used to maximize starch yield with high purity. Alkaline extraction method was used with NaOH concentration of 0.1 M and optimum temperature at 40 °C. Starch obtained was hydrolyzed with α – amylase and amyloglucosidase enzyme to yield glucose syrup. The syrup was quantified using Lane-Eynon Titration Methods.

1.5 SIGNIFICANCE OF STUDY

Sugar production is mainly from sugarcane and sugar beet. Corn also has been used to produce industrial sweetener. However, both corn and sugarcane is not the main crop cultivated in our country. Taking to the account that banana is highly produced in Malaysia; the potential of its peels can be recycled to produce sugar/sweetener hence reducing our dependant to imported sugars. If this study yields positive results, then maybe we can have cheap alternative source of producing sugar besides helping towards clean environment by recycling waste.

CHAPTER 2

LITERATURE REVIEW

2.1 STARCH

2.1.1 General View of Starch

Starch is the main storage carbohydrate of plants. A relatively simple polymer, it is composed of glucose molecules that are linked together in two different forms which are amylose and amylopectin. Amylose consists of 20-30 % of normal starch while amylopectin is the major component of starch comprising 70-80 %. Amylose is an essentially linear molecule in which the glucose units are joined end-to-end by α1-4 linkages whereas amylopectin is a much larger branched molecule. Starch is deposited as insoluble, semi-crystalline granules in storage tissues (grains, tubers, roots) and it also occurs to a lesser extent in most vegetative tissues of plants. The moisture content of native starch granules is usually about 10%. Amylose and amylopectin make up 98–99 % of the dry weight of native granules, with the remainder comprising small amounts of lipids, minerals, and phosphorus in the form of phosphates esterified to glucose hydroxyls (Copeland et al., 2009).

According to Lindeboom et al. (2004), the diversity of starch granules and their molecular constituents' influences starch functionality. Starch granules range varies in size of 1 to 100 mm in diameter and the shape are polygonal, spherical and lenticular and can vary greatly with regard to content, structure and organization of the amylose and amylopectin molecules. Lindeboom et al. (2004) also added that

starch granules may occur individually or clustered as compound granules, and in wheat, barley, rye and triticale they occur in bimodal size distributions.

2.1.2 Starch Granules

The composition and structure of starch granules vary considerably between different plants. The functionality of starches is dependent on the proportions of their amylose and amylopectin components (Zhang et al., 2005). As in Table 2.1, the properties and functions of starches are different from each crop. Tuber or root starches have both larger granules and lower protein and lipid contents than cereal starches. Upon processing, tuber and root starches form clear pastes that have a bland taste. This give advantages in many food applications but in its native form, starch has a limited number of uses. Its only usage is mainly as a thickener or binder (Jobling, 2009).

Table 2.1: Comparison of starch granule for different plant

	Maize	**Wheat**	**Potato**	**Cassava**
Type	Cereal	Cereal	Tuber	Root
Granule shape	Round, polygonal	Round, bimodal	Oval, spherical	Oval, truncated
Granule size (mm)	2–30	1–45	5–100	4–35
Phosphate (%, w/w)	0.02	0.06	0.08	0.01
Protein (%, w/w)	0.35	0.4	0.06	0.1
Lipid (%, w/w)	0.7	0.8	0.05	0.1
Size of world market (millions of tons per year)	39.4	4.1	2.6	2.5[a]

[a] includes other minor starch sources, such as sweet potato.

Source: Jobling (2009)

Potato starch is unique among commercial starches. This was mainly due to high level of phosphate groups. The covalent bond of the C6 and C3 positions of the

glucose monomers gives these phosphate groups, coupled with the large size of the granules, a very high swelling power. Most of these starches were produced in the USA in which the maize or corn starch makes up more than 80 % of the world market.

The crystallinity level of native starch granules ranges from about 15% for high-amylose starches to about 45–50 % for waxy starches. The granules have a hierarchical structure called growth rings with multiple concentric layers (Figure 2.1) with increasing diameter extend from the hilum (the centre of growth) towards the surface of granules that can be observed readily by light and electron microscopy (Copeland et al., 2009).

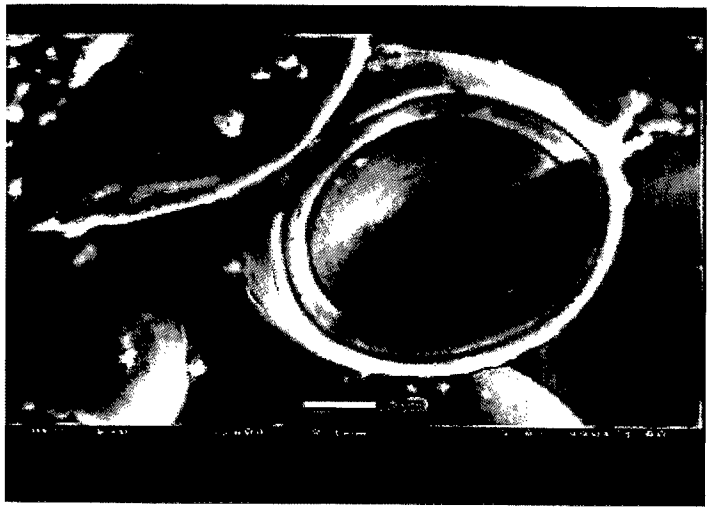

Figure 2.1: Growth ring as seen under ESEM (5 μm)

Source: Giannini

The concentric growth rings contained alternating crystalline and amorphous regions (Figure 2.2) of higher and lower density, respectively. The higher density regions have a lamellar structure of alternating crystalline and amorphous layers. (Donald et al., 2001; Donald, 2004; Yuryev et al., 2004; Copeland et al., 2009).

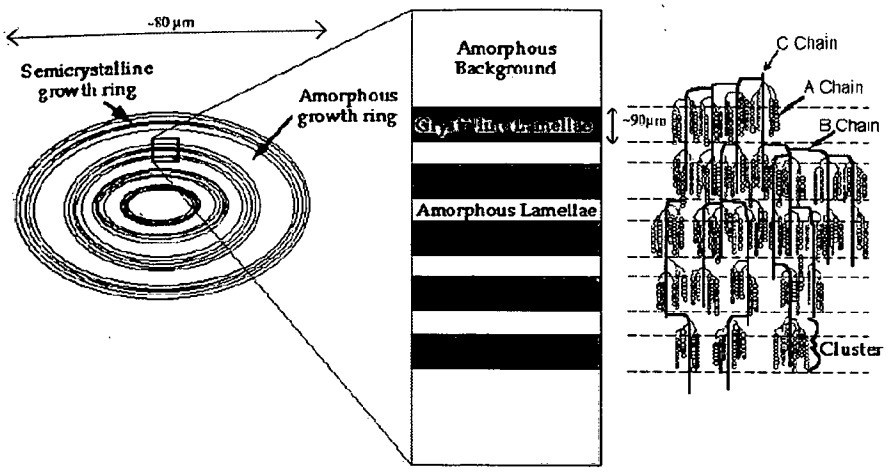

Figure 2.2: Inner structure of starch granule

Source: Giannini

2.1.3 Resistance Starch

According to Mustaza (2008), resistant starch is a complex carbohydrates which are resistant to be hydrolyze by acid and α - amylase and pullulanase eznymes. This was mainly due to the configuration of their osidic bonds. Most of the non-digestible oligosaccharides are hydrolyzed to small oligomers and monomers once they have reached the colon. The small oligomers and monomers are further metabolized by the anaerobic bacteria by fermentation process.

The functionality resistant starch that is, it has small particle size, white appearance and bland flavor. It also has low water holding capacity and desirable physicochemical properties such as swelling, viscosity increase and gel formation making it useful in a variety of foods. Resistant starch can be divided into four fractions which are R1, R2, R3 and R4 as in Table 2.2. As in banana, the available starch, resistant starch and retrograded resistant starch is 86.80 %, 44.01 % and 0.91 % respectively (Hernandez et al., 2008).

Table 2.2: Type of resistant starch

Types of RS	Description	Food sources	Resistance minimized by
RS1	Physically protected	Whole or partly milled grains and seeds, legumes	Milling, chewing
RS2	Ungelatinized resistant granules with type B crystalinity	Raw potato, green banana, legumes, high amylase corn	Food processing and cooking
RS3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, and food products with repeated moist heat treatment.	Processing condition
RS4	Chemically modified starches due to cross linking with chemical reagents	Foods in which modified starches have been used (breads, cakes)	Less susceptible to digestibility in vitro.

Source: Mustaza (2008)

2.2 BANANA STARCH

Starch is the principal component of green bananas, which undergoes important changes during ripening by reaction with amylase enzyme. The average starch content drops in the pre-climacteric from 80 % to less than 1 % at the end of the climacteric period due to starch breakdown. Meanwhile sugars, mainly sucrose will accumulate to more than 10 % of the fresh weight of the fruit.

Banana starch composition is differed in each stage of ripening process. The starch composition changes dramatically during ripening. The banana ripening was classified into eight stages according to the peel color (Von Loesecke., 1950; Zhang

et al., 2004). Table 2.3 summarize the proximate composition of the edible portion of banana at different stages as classified by the color of banana peel.

Table 2.3: Proximate compositions of the edible portion of banana at different stages as classified by the color of banana peel

Stage	Peel color	Starch (%)	Reducing sugar (%)	Sucrose (%)	Gelatinization temperature (°C)
1	Green	61.7	0.2	1.2	74–81
2	Green	58.6	1.3	6	75–80
3	Green/a trace of yellow	42.4	10.8	18.4	77–81
4	More green than yellow	39.8	11.5	21.4	75–78
5	More yellow than green	37.6	12.4	27.9	76–81
6	Yellow with a green tip	9.7	15	53.1	76–80
7	All yellow	6.3	31.2	51.9	76–83
8	Yellow/a few brown spots	3.3	33.8	52	79–83
9	Yellow/many brown spots	2.6	33.6	53.2	

Source: Zhang et al. (2005)

From Table 2.3, total soluble sugar content can reach from as low as 1.2 % until 53 % of the fruit fresh weight (about 80 % water content). This result indicates a high rate of conversion of starch to soluble sugar (Cordenunsi and Lajolo, 1995; Zhang et al., 2005). Amylases participate in starch hydrolysis, but they are probably not linked to sucrose synthesis. Starch–sucrose transformation during ripening of

bananas involves several enzymes and more than one pathway. In spite of the importance of this transformation in terms of fruit physiology, little is known about the mechanisms involved (Zhang et al., 2005)

Native raw banana starch is resistant to the attack of a-amylase and amyloglucosidae in which in vivo results showed that 75–84 % of the starch granules ingested reached the terminal ileum (Englyst and Cummings, 1986; Faisant, Buleon et al., 1995; P. Zhang et., al., 2005). However, the resistance of the native starch is largely can be overcome by cooking to gelatinize the starch.

2.2.1 Banana Starch Granule

In general, starch granules from various banana types, extinguish the same properties. All banana starch granules exhibits in irregular shape and appear microscopically as elongated ovals with ridges (Zhang and Whistler, 2002). They have major axes range from 6 to 80 mm, mostly between 20 and 60 mm. From Figure 2.1, eccentric birefringence or double refraction was observed under polarized light in starch granules of green Taiwan bananas.

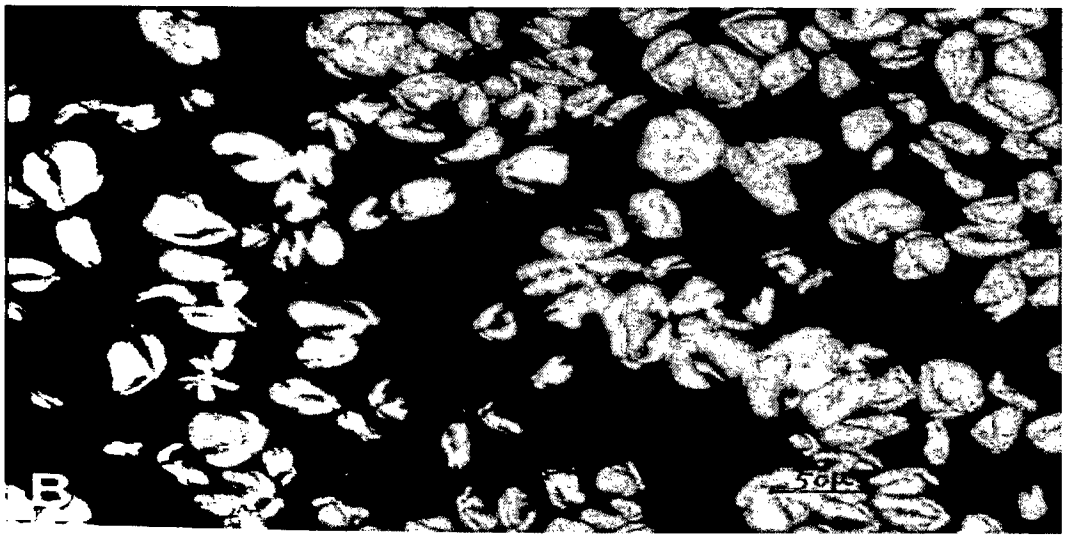

Figure 2.3: Starch granule under plane polarized light

Source: Zhang et al. (2005)

While in Cavendish type, based on report from Ling et al., (1982) and Zhang et al., (2005), the starch granule was highly irregular in shape and size. The same result obtained for Valery banana starch as scanning electron micrographs revealed irregularly shaped granules among the elongated and spheroid forms. The spheroid forms varied from 15 to 40 mm in diameter. Elongated granules were 7–25 mm in width and 20–50 mm in length.

2.2.2 Banana Starch Gelatinization

Starch, when heated in the presence of excess water, undergoes phase transition disorder known as gelatinization. The gelatinization process is differed over a temperature range characteristic of the starch source itself. In this phase, it begins to lose structures. This phase transition is related with diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of birefringence, loss of crystalline order, uptake of heat, uncoiling and dissociation of double helices in the crystalline regions and amylase leaching (Donovan, 1979; Evans and Haismann, 1982; Zhang et al., 2005). Gelatinization temperature is obtained by differential scanning calorimetry, DSC (Hernandez et al. 2008).

Based on report from Kayisu et al. (1981) and Zhang et al. (2005), the gelatinization temperature for banana starch was reported to be in range of (67-70) °C. Valery banana exhibits gelatinization temperature at 69.5 °C (Waliszewski et al., 2003). Meanwhile, the gelatinization temperature for Cavendish banana starch is in the range of (70.1–74.6) °C (Ling et al., 1982). For Macho and Criollo banana starches, gelatinization temperatures of 77 and 74 °C were reported, respectively (Bello-Perez, Agama-Acevedo et al., 2000). Table 2.4 shows pasting temperature of banana starch compared with other available starches (Zhang et al., 2005)

Table 2.4: Pasting temperature of different starch

Starch	Pasting temperature (°C)	Brabender viscosity units				
		Peak	95 °C	95 °C hold	50 °C	50 °C hold
Banana	67–70	~960	~860	~800	~1190	~1050
Potato	61	2500	850	340	600	630
Tapioca	59	1400	520	280	500	510
Waxy corn	69	1000	400	250	390	370
Corn	73	470	470	350	830	760
Wheat	77	65	60	60	300	270

Source: Zhang et al. (2005)

2.3 STARCH EXTRACTION AND HYDROLYSIS

2.3.1 Alkaline Extraction

Agama-Acevedo et al. (2000) stated that wet-milling process is suitable for banana starch isolation. The use of alkali can solubilize protein hence producing starch flour with low level of impurities. This was added by reports from Lii et al. (1982) and Zhang et al. (2004) that a starch isolation technique has been applied in green Taiwan dessert bananas. The method involves cutting the banana and macerating the pulp in a sodium hydroxide solution of concentration of 0.1 M or less. The slurry was then filtered through cheesecloth and bolting cloth and the starch obtained was washed with water and dried. These results are in approximate agreement with those of other workers analyzing different bananas. Ling et al. (1982) reported that the starch of green Cavendish bananas could be isolated easily by extraction of the pulp with 1 % sodium hydroxide solution.

According to Zhang et al. (2005), apparently, Chiang et al. (1987) was becoming the first team to produce banana starch on a pilot-plant scale. In their experiment, green Taiwan bananas (M. sapientum) were collected in between 112 to 116 days after petal fall. They weighed an average of 82 g per finger, had a pulp to peel ratio of 1.1, a pulp weight basis of 30% of the total solids, and a pulp starch content of 81 % (dry basis). Peeled bananas were sliced and milled with 0.5 M sodium hydroxide solution using a stone mill. The starch was washed on a shaker, collected by centrifugation, and then dried at 50 °C. The peel contained 5 % pectin and 21 % fiber. Immersion of fresh bananas in 0.5 M sodium hydroxide solution was deemed necessary for proper handling of the pulp and to obtain a 70% yield of starch with a purity of ca. 94 %.

A process to extract starch from green, un-gassed reject bananas was developed by Fichtali et al. (1999). Using disintegrated in a Fitzpatrick comminuting mill; the diced bananas were disintegrated in a 0.05 M solution of sodium hydroxide. The milled banana slurry was sequentially screened to pass 60, 80, and 200 mesh screens to remove peel fibers and pomace. After screening process, the crude starch was concentrated, counter-current washed with water, and then dewatered to a solids content of 55 %. Starch thus obtained had a brownish color (Zhang et al., 2005)

2.3.2 Non Alkaline Extraction

According to Zhang et al. (2005), Kayisu et al. (1981) have studied the extraction of starch from green bananas with water. Using a low speed, the sliced bananas were disintegrated in a Waring blender. The mixture was centrifuged, and the dark material on the top of the pellet was scraped off. Crude starch was washed with water. The starch-containing suspension was decanted and allowed to stand 15 min for the starch to settle. The purity of the thus-obtained starch was 99.5 %.

Zhang et al. (2005) also added that Whistler (1998) developed a cost-efficient process for producing starch from cull bananas. This process uses a minimum amount of processing chemicals, machinery, and processing time. Banana pulp was steeped in aqueous sodium bisulfite solution at pH of 3.5–5.5 for 2–8 h, more

preferably for about 4 h, at ambient to slightly elevated temperatures. During this steeping period, the endogenous banana enzymes, such as pectinase and polygalacturonase, disintegrate the cell walls, allowing starch granules to be released into the aqueous steeping solution. The mixture was passed through wire screens to remove cell walls and other non-starch pulp-mass material, and then centrifuged. Yields of dried starch (white in color) from green bananas

2.4 STARCH HYRDOLYSIS

2.4.1 Alpha Amylase Mechanism

Alpha-amylase is an enzyme which aids in the breakdown of starch to maltose Alpha-amylase hydrolyzes bonds between glucose repeats. The official name of Alpha-amylase is 1, 4-a-D-Glucan glucanohydrolase; EC 3.2.1.1. As in Figure 2.4, it can be observed of alpha-amylase started to hydrolyze the starch granules.

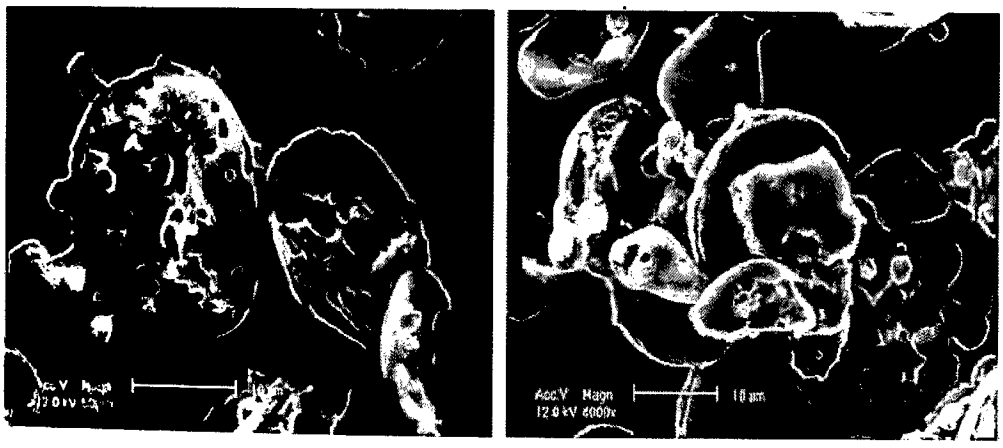

Figure 2.4: Partially degraded granules of waxy (left) and a high-amylose (right) wheat starch after 2 h with pancreatic alpha-amylase

Source: Blazek et al. (2009); Copeland et al. (2009)

Alpha-amylase is classified as family 13 of the glycosyl hydrolases and is present in archaea, bacteria, plants and animals. Alpha amylase is an essential enzyme in α-glucan metabolism, acting to catalyse the hydrolysis of α-1, 4-glucosidic bonds of glycogen, starch and related polysaccharides. Although all α-amylases possess the same catalytic function, they can vary with respect to sequence.

In general, they are composed of three domains: a TIM barrel containing the active site residues and chloride ion-binding site (domain A), a long loop region inserted between the third β strand and the α-helix of domain A that contains calcium-binding site(s) (domain B), and a C-terminal β-sheet domain that appears to show some variability in sequence and length between amylases (domain C). Amylases have at least one conserved calcium-binding site, as calcium is essential for the stability of the enzyme. The chloride-binding functions to activate the enzyme, which acts by a two-step mechanism involving a catalytic nucleophile base (usually an Asp) and a catalytic proton donor (usually a Glu) that are responsible for the formation of the β-linked glycosyl-enzyme intermediate.

2.4.2 Amyloglucosidase Enzyme Mechanism

Glucoamylase (amyloglucosidase, EC 3.2.1.3) are originally of fungal origin usually occurring in multiple forms. The enzyme is capable of converting starch completely to glucose. The enzymes of this group affect a hydrolysis of starch by a single chain mechanism in which glucose units are removed from the nonreducing end of a linear chain until complete hydrolysis of the chain has been affected. Amyloglucosidase was capable of hydrolyzing the α-D-(1→4), the α-D-(1→6) and the α-D-(1→3) glucosidic bonds of oligosaccharides