# EFFECTS OF PRE-TREATMENTS ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF *Musa acuminata* x *balbisiana* cv. Awak FLOUR

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by

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## LIST OF SYMBOLS / ABBREVIATION

| Symbol / Abbreviation | Caption |
|---|---|
| a* | redness |
| $a_W$ | water activity |
| AA | ascorbic acid |
| AAS | Atomic Absorption Spectroscopy |
| ANN | annealing |
| ANOVA | one-way analysis of variance |
| b* | blueness |
| BD | breakdown |
| $BF_3H_2O$ | N-halosuccinimide |
| BFA | flour prepared from *Awak* slices pre-treated by ascorbic acid |
| BFA0.5 | flour prepared from *Awak* slices pre-treated by 0.5% ascorbic acid solution |
| BFA1 | flour prepared from *Awak* slices pre-treated by 1 % ascorbic acid solution |
| BFA2 | flour prepared from *Awak* slices pre-treated by 2 % ascorbic acid solution |
| BFB | flour prepared from *Awak* slices pre-treated by blanching |
| BFB60 | flour prepared from *Awak* slices pre-treated by blanching at $60^oC$ |
| BFB6010 | flour prepared from *Awak* slices pre-treated by blanching at $60^oC$ for 10 min |

| | |
|---|---|
| BFB6020 | flour prepared from *Awak* slices pre-treated by blanching at 60$^{o}$C for 20 min |
| BFB65 | flour prepared from *Awak* slices pre-treated by blanching at 65$^{o}$C |
| BFB6510 | flour prepared from *Awak* slices pre-treated by blanching at 65$^{o}$C for 10 min |
| BFB6520 | flour prepared from *Awak* slices pre-treated by blanching at 65$^{o}$C for 20 min |
| BFB70 | flour prepared from *Awak* slices pre-treated by blanching at 70$^{o}$C |
| BFB7010 | flour prepared from *Awak* slices pre-treated by blanching at 70$^{o}$C for 10 min |
| BFB7020 | flour prepared from *Awak* slices pre-treated by blanching at 70$^{o}$C for 20 min |
| BFB75 | flour prepared from *Awak* slices pre-treated by blanching at 75$^{o}$C |
| BFB7510 | flour prepared from *Awak* slices pre-treated by blanching at 75$^{o}$C for 10 min |
| BFB7520 | flour prepared from *Awak* slices pre-treated by blanching at 75$^{o}$C for 20 min |
| BFL | flour prepared from *Awak* slices pre-treated by L-cysteine |
| BFL0.5 | flour prepared from *Awak* slices pre-treated by 0.5% L-cysteine solution |

| | |
|---|---|
| BFL1 | flour prepared from *Awak* slices pre-treated by 1 % L-cysteine solution |
| BFL2 | flour prepared from *Awak* slices pre-treated by 2 % L-cysteine solution |
| BFN | native *Awak* flour |
| BI | browning index |
| C | Chroma value |
| Ca | calcium |
| Cu | cuprum |
| $Cu^{2+}$ | ion cuprum |
| DDT | dough development time |
| DF | dietary fibre |
| DHAA | dehydroascorbic acid |
| DMSO | dimethyl sulfoxide |
| DP | degree of polymerization |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| DSC | Differential Scanning Calorimetry |
| Ea | activation energy |
| EB | enzymatic browning |
| EDTA | Ethylenediaminetetraacetic acid |
| FAO | Food and Agriculture Organization |
| FDA | U.S. Food and drug Administration |
| Fe | ferum |
| $Fe^{3+}$ | ion ferum |
| $[Fe(CN)6]^{3-}$ | ferricyanide |

| | |
|---|---|
| $[Fe(CN)6]^{4-}$ | ferrocyanide |
| $FeSO_4.7H_2O$ | ferrous sulphate |
| FRAP | Ferric reducing antioxidant power |
| FTIR | Fourier transform infrared spectroscopy |
| FV | Final viscosity |
| GI | Glycemic index |
| GRAS | Generally recognized as safe |
| $\Delta H$ | Gelatinized enthalpy |
| $H_2O_2$ | hydrogen peroxide |
| $H_2SO_4$ | sulfuric acid |
| HCl | Hydrochloric acid |
| HI | Hydrolysis index |
| HMF | Hydroxymethylfurfural |
| HMT | Heat moisture treatment |
| $HNO_3$ | nitric acid |
| HPLC | High Performance Liquid Chromatography |
| Hsp | heat –shock protein |
| $I_2$ | iodine |
| IDF | Insoluble Dietary Fibre |
| IVSD | In-vitro starch digestibility |
| K | kalium / potassium |
| KBr | potassium bromide |
| KI | potassium iodine |
| KOH | potassium hydroxide |
| L | Brightness |

| | |
|---|---|
| LCMS | Liquid Chromatography Mass Spectrometry |
| L-Cys | L-Cysteine |
| MFM | Malayan Flour Mill |
| Mg | magnesium |
| MTI | tolerance index |
| Na | natrium / sodium |
| $Na_2CO_3$ | sodium carbonate |
| NaCl | sodium chloride |
| NaOH | sodium hydroxide |
| NEB | Non enzymatic browning |
| ORD | oxidative reduction depolymerization |
| PME | pectin methylesterase |
| $POCl_3$ | phosphoryl chloride |
| POD | polyphenol peroxidise |
| PPO | polyphenol oxidase |
| pPPO | plantain crude polyphenol oxidase |
| PV | Peak viscosity |
| RH | Relative humidity |
| RS | Resistant starch |
| RVA | Rapid Visco Analyzer |
| SB | setback |
| SCFA | short chain fatty acid |
| SDF | soluble dietary fibre |
| SEM | scanning electron microscope |
| SH – SS | sulfhydryl – disulfides bonding |

| | |
|---|---|
| SMI | small medium industry |
| SN2 | bimolecular nucleophilic substitution |
| SP | Swelling power |
| STMP | sodium tripolyphospahate |
| TDF | Total Dietary Fibre |
| $T_o$ | gelatinization temperature |
| $T_p$ | peak temperature |
| TPC | total phenolic compound |
| TPTZ | 2,4,6-tris(1-pyridyl)-5-triazine |
| TS | total starch |
| WF_30BFA | noodle incorporated with 30 % BFA1 |
| WF_30BFB | noodle incorporated with 30 % BFB6510 |
| WF_30BFL | noodle incorporated with 30 % BFL1 |
| WF_30BFN | noodle incorporated with 30 % BFN |
| WHC | water holding capacity |
| YAN | yellow alkaline noodle |
| Zn | Zinc |

**KESAN PRA-PENGOLAHAN TERHADAP SIFAT-SIFAT FIZIKO-KIMIA DAN FUNGSIONAL TEPUNG PISANG *Musa acuminata x balbisiana* cv. Awak**

## ABSTRAK

Penyelidikan ini dijalankan untuk menghalang tindak balas pemerangan dengan kaedah pra-pengolahan kepingan pisang *Awak* dengan cara konvensional: asid askorbik (AA) (0.5 %, 1 %, 2 %), L - sisteina (L-cys) (0.5 %, 1 %, 2 %) dan penceluran (60 $^{o}$C / 10 min, 60 $^{o}$C / 20 min, 70 $^{o}$C / 10 min, 70 $^{o}$C / 20 min, 75 $^{o}$C / 10 min dan 75 $^{o}$C / 20 min), selaras dengan objektif untuk menjelaskan kesan cara-cara pra-pengolahan yang berlainan ke atas sifat-sifat fiziko-kimia, dan fungsianol tepung *Awak* yang dihasilkan. Pra-pengolahan secara kimia (dengan mengguna AA dan L-cys), terutamanya L-cys (1 %) mengekalkan kompaun fenolik dan sifat antioksidan tepung. Suhu penceluran yang tinggi (70 – 75 $^{o}$C) memusnahkan sebahagian kompaun fenolik dan merendahkan potensi antioksidan. Pra-pengolahan secara kimia (AA dan L-cys, terutamanya 2 % larutan) didapati mendorong depolimerisasi pada cabangan rantai amilopektin dan meningkatkan daya ikatan air kanji pisang. Fenomena ini telah terbukti dengan aktiviti air, kebolehan pembengkakan, sifat pengaliran dan kelikatan pes yang tinggi dalam tepung *Awak*. Pengamatan yang sama ditemui dalam tepung *Awak* yang diperolehi daripada pra-pengolahan secara penceluran suhu rendah (60 $^{o}$C and 65 $^{o}$C), yang menyebabkan ikatan kanji dan polisakarida dilemahkan dan menjadi lebih fleksibel. Struktur granul kanji pisang yang diekstrak daripada kepingan-kepingan *Awak* dipra-olah secara kimia didapati masih sempurna walaupun rekahan didapati wujud dalam kajian SEM. Perubahan struktur ini dapat dicerminkan dari segi kebolehcernaan kanji in-vitro (IVSD) yang lebih tinggi dan penurunan sifat termal tepung *Awak*. Dalam ujian 'Fourier transform infrared (FTIR) spectroscopy', spektrum IR untuk tepung Awak

yang dipre-olah tidak berubah dengan ketara. Keputusan ini membuktikan depolimerisasi cabangan amilopektin daripada kemusnahan seluruh granul kanji. Kelakuan aliran semua sampel juga menunjukkan kelakuan pseudoplastik yang mana sesuai dengan Herschey-Bulkley model. Dalam fasa aplikasi kajian, 30 % tepung pisang yang dihasil daripada kepingan-kepingan pisang yang pra-olah dengan kaedah berbeza (BFA1, BFL1 dan BFB6510) dan BFN (kawalan) digabungkan ke dalam formulasi mi. Dengan menggabungkan BFA1, BFL1 dan BFB6510, doh komposit (WF_30BFA, WF_30BFL dan WF_30BFB) yang dihasilkan adalah kurang fleksibel yang mana ditunjukkan oleh nilai indeks toleransi (MTI) yang lebih tinggi dalam ujian reologi. Nilai 'pasting viscosity' (PV) dan 'breakdown' (BD) yang lebih tinggi diperhatikan dalam pes WF_30BFA dan WF_30BFL juga mengambarkan rintangan yang lebih rendah terhadap daya ricih yang disebab oleh struktur pes yang lemah. Penggabungan BFA1 dan BFL1 menggalakkan penyerapan air dalam tepung komposit yang mengakibatkan mi dengan tekstur lebih lemah dihasilkan. Tepung-tepung ini memperbaiki ciri indeks glisemik (GI) mi dengan merendahkan kebolehcernaan mi yang terhasil. Secara kesimpulan, pra-pengolahan kepingan-kepingan *Awak* secara kimia dengan mengguna AA dan L-cys (walaupun pada tahap rendah) dan penceluran menunjuk perubahan signifikan ke atas ciri-ciri tepung yang dihasilkan. Tahap pra-pengolahan harus dikawal untuk mengelakkan kemerosotan pada sifat – sifat fungsional dan nilai nutrisi tepung yang dihasilkan.

**EFFECTS OF PRE-TREATMENTS ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF *Musa acuminata x balbisiana* cv. Awak FLOUR**

## ABSTRACT

This research was carried out to pre-treated the plantain *Awak* slices with conventional methods: ascorbic acid (AA) (0.5 %, 1 %, 2 %), L-cysteine (L-cys) (0.5 %, 1 %, 2 %) and blanching (60 $^{o}$C / 10 min, 60 $^{o}$C / 20 min, 70 $^{o}$C / 10 min, 70 $^{o}$C / 20 min, 75 $^{o}$C / 10 min dan 75 $^{o}$C / 20 min), in line with the objective of elucidating the effect of pre-treatments on the physico-chemical and functional properties of *Awak* flour produced. Chemical pre-treatments (AA and L-cys), especially L-cys (1 %) retained the highest phenolic compounds and antioxidant properties in the flour. High temperature blanching (70 $^{o}$C – 75 $^{o}$C) destroyed certain amount of phenolic compounds and reduced the antioxidant power. AA and L-cys pre-treatments, especially at 2 % solution were found to induce depolymerization of amylopectin chains and facilitated the water binding ability of plantain starches. This phenomenon was indicated by higher water activity, swelling power, flow behaviour index and higher paste viscosity of *Awak* flour. Similar observation was found in *Awak* flour obtained from low blanching temperature pre-treatment (60 $^{o}$C and 65 $^{o}$C), which resulted in weakening of starch flexibility and polysaccharide bonding. Granular structure of starches extracted from pre-treated *Awak* slices were shown to remain intact but cracks were observed in the SEM study. These structural changes reflected higher in-vitro starch digestibility (IVSD) and lower thermal behaviour of *Awak* flour. In Fourier transform infrared (FTIR) spectroscopy test, IR spectra of pre-treated *Awak* flour did not change significantly. This observation indicated that depolymerisation occurred at amylopectin branches instead of destroying the whole

starch granule. Flow behaviour of all samples also showed pseudoplastic behaviour which fitted the Herschey-Bulkley model. In the application phase of the study, 30 % of *Awak* flour produced from different pre-treated *Awak* slices (BFA1, BFL1 and BFB6510) and BFN (control) were incorporated into the noodle formula. By incorporating BFA1, BFL1 and BFB6510, the composite dough (WF_30BFA, WF_30BFL and WF_30BFB) produced were found to be less stiff as indicated by higher tolerance index (MTI) values obtained in the rheological test. Higher pasting viscosity (PV) and breakdown (BD) observed in WF_30BFA and WF_30BFL composite flour paste also reflected lower resistant to shear force caused by weakening of paste structure. Incorporation of BFA1 and BFL1 encouraged water absorption in composite flour which resulted in noodles with weaker texture. These flours also improved the Glycemic index (GI) properties of noodle by lowering digestibility of the resultant noodle. In conclusion, *Awak* slices those were chemically pretreated with AA, L-cys (even at low level) and blanched showed significant changes in the flour properties. The level of chemical used in pre-treatment must be controlled to avoid reduction in functional and nutritional properties of the flour produced.

# CHAPTER 1
# INTRODUCTION

## 1.1 Background and Rationale

Browning is the phenomenon occurring during food processing and storage, especially in fruits, vegetable products and in minimally processed (Bolin *et al*., 1989), dehydrated and frozen foods (Sapers, 1993). The reaction resulted from both enzymatic and non-enzymatic oxidation of phenolic compounds. It is generally difficult to ascertain whether the mechanism is enzymatic or non-enzymatic (Wedzicha, 1984). Yet, both enzymatic browning (EB) and non-enzymatic browning (NEB) are mostly undesirable due to their deleterious effects in term of appearance, sensory, nutritional profiles and shelf life (Martinez & Whitaker, 1995; Lyengar & McEvily, 1992). These cause drawbacks to the consumers resulted also in economic losses.

EB is the main browning mechanism which involves polyphenol oxidase (PPO) oxidative, exacerbated by tissue damage during fruit processing (Lyengar & McEvily, 1992) while NEB occurs without involvement of enzymes. Consequently, chemical or physical pre-treatments are widely applied and studied in an attempt to preserve the quality of fresh and processed fruits. In term of chemical pretreatment, application of sulfating agents is the most widespread inhibiting agent. However, this had caused contradiction due to adverse health effects (Martinez-Castellanos *et al*., 2009; Lyengar & McEvily, 1992). Therefore, other non-sulfite anti-browning agents including reducing agent (ascorbic acid, L-cysteine and glutathione), chelating agents (EDTA, organic acids, phosphares) and acidulants (citric acid) compounds have been widely used in food industry (Ozoglu & Bayindirh, 2002; Walker, 1995). Currently,

pre-treatment researches are more focusing on evaluating enzymes such as catechol transferase, proteases, ring-cleaving oxygenases (Arnold *et al*., 1992; Lyengar, 1992) and natural products, such as dog rose and pomegranate extracts (Zocca *et al*., 2011), honey and propolis (de la Rosa *et al*., 2011), and chitosan (Martinez-Castellanos *et al*., 2009) as alternative and novel browning inhibitors. On the other hand, inhibition of fruit browning can be also achieved effectively by physical pre-treatment involving thermal treatment (blanching, steaming), refrigeration (freeze drying, blast freezing), and exclusion of oxygen (nitrogen and vacuum atmosphere). However, most of these latest and novel techniques used may not be practical in food industry due to their high production cost and inconveniences. Therefore, conventional methods such as addition of reducing agent and heat treatment by blanching are still the most widespread, and affordable methodologies applied, especially in the local or small and medium industries.

Most studies have been primarily focused on the effectiveness of browning inhibitor and treatments used and also their mechanisms on the raw fruits or products. There are numerous studies being reported on the effect of adding reducing agents to different extracted starch or flour and their reaction with components in food systems, such as starch granules (Valler-Pamise *et al*., 1997; Paterson *et al*, 1994, MatHashim *et al*., 1992), polysaccharide (Majzoobi *et al*., 2011; Sriburi *et al*., 1999) and protein matrix (Angioloni & Rosa, 2007; Elkhalifa & El-Tinay, 2002). These small interactions will then affect the rheological, functional properties and digestibility of starch or flour produced and as well as the end product quality. However, not much study have been carried out and reported on the effects of the browning inhibition pretreatment on flour properties produced from the pre-treated fruit slices.

Plantain flour is gaining more interest among researchers and studies have been carried out by incorporating plantain flour into food product due to its high nutritional values and health benefit. Plantain flour produced from unripe plantain pulp provide a good source of dietary fibre (Juarez-Gatcia *et al*., 2006, da Mota *et al*., 2000), resistant starch (Zhang *et al*., 2005), essential minerals such as potassium and magnesium (Chong, 2007; Kreb, 2002) and it is notably rich in phenolic compounds with great antioxidant properties (Someya *et al*., 2002; Kanazawa, 2000).

Therefore, attempts has to be taken to control the destructive effects caused by food browning on the significant health benefits found in plantain flour. Nevertheless, the effects and suitability of pre-treatment used must be studied and understood to prevent or improve other physico-chemical and functional properties of the flour produced.

## 1.2 Objectives

The main objective of this research was to study the effect of conventional browning inhibition pre-treatments on the functional properties and nutritional qualities of plantain *Awak* flour. The specific objectives were:

1. To determine the feasibility of selected pre-treatments to prevent browning of process and to preserve the antioxidant properties of *Awak* flour.
2. To study the effect of selected pre-treatments on functional and digestibility properties of starches from *Awak* flour.
3. To determine the suitability of conventional browning inhibition pre-treatment in *Awak* flour production.
4. To assess the physico-chemical and nutritional qualities of different anti-browning pre-treated *Awak* flour in noodle.

# CHAPTER 2

# LITERATURE REVIEWS

## 2.1 Plantain

Plantain is a giant perennial plant, belongs to the genus *Musa* of the family *Musaceae*. Plantains are regarded as the triploid banana, originating from hybridization of two wild diploid species, *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Langhe *et al*., 2005; Stover & Simmonds, 1987; Robinson, 1996). The genome groups are mostly AAB, ABB or BBB.

Plantain is a member of banana family and regarded as cooking bananas since their outward appearance is very similar to unripe dessert banana. However, in term of morphological characteristics, plantains are larger and longer than bananas. They also have thicker skins with natural brown spot and rough areas on them (Figure 2.1a,b). Green plantains are firm, contain more starch and bland in flavor. On top of that, plantains tend to be cooked as a type of vegetable or otherwise processed before being eaten.

(a) (b)

Figure 2.1: (a) Mature green plantain (*Musa acuminate x balbisiana cv. Awak*) on the tree. (b) Cross section of green plantain with starchy pulp (Robinson, 1996).

### 2.1.1 Plantain plantation

Plantains originated in Southeast Asia and are grown in 52 countries amounting to 12.8 million acres (FAO, 2007) which production of banana in Malaysia is 283,220 MT in 2010 (Nationmaster, 2010). According to FAO's statistics database, world production of banana and plantain in 2007 was 100 million MT and among them 33 million MT was plantains. This form about one-third of the total banana production (FAO, 2007). Plantains are among the world's leading fruit crops and the four leading plantain producing countries are Uganda, Ghana, Nigeria and Colombia. Banana remains the second most important fruit crop in Malaysia and produced about 283,220 metric tonnes (MT) in 2010 (NationMaster, 2010). Almost ninety percent of plantain production is consumed domestically and is the staple food for over millions people in these developing countries (Arias *et al*., 2003). Plantains also provide food security and important income for local small-scale farmers.

### 2.1.2 Plantain flour and starch production

Almost ninety percent of plantain production is consumed in primitive forms such as boiled, deep-fried, roasted or porridge. Due to the perishable nature of the fruit which subjected to fast deterioration, the rate of plantain post-harvest losses is high (Demirel, 2003). The post-harvest losses of plantain are heavy due to poor handling, transport conditions and inadequate market access routes. Consequently, latest research and development programmes for the post-harvest of plantain had been focused on the improvement of conservation and transformation techniques of the products and reduction of post-harvest losses. Therefore, the production and export of plantain flour is gradually gaining market. According to Adeniji and

Empere (2001) and Ogazi (1998), processing of fresh unripe plantain into flour has a number of advantages, which included:

i. Extending shelf life
ii. Price stability
iii. Wider availability
iv. Stimulation of agricultural production through market expansion, and
v. Facilitating transportation

Traditional milling methods (Figure 2.2) have been used since ancient times in the processing of ripe or unripe local cultivar plantain into stable flour in Nigeria and in other West and Central African countries (Ukhum & Ukpebor, 1991). The flour obtained is then mixed with boiling water to prepare an elastic pastry (as *amala* in Nigeria, and *foufou* or *fufu* in Cameroon) which is eaten with various sauces. Despite there are some major constraints in the traditional plantain processing. These included high labor input, unhygienic practices, uneconomical operations and lack of quality assurance. Furthermore, the lack of automatic peeling devices also limits the development of plantain flour for SMEs. To remedy this situation, agricultural technologies, material, and knowledge should be provided to smallholder producers or rural population to improve the indigenous processing technologies and to establish the good grade flour.

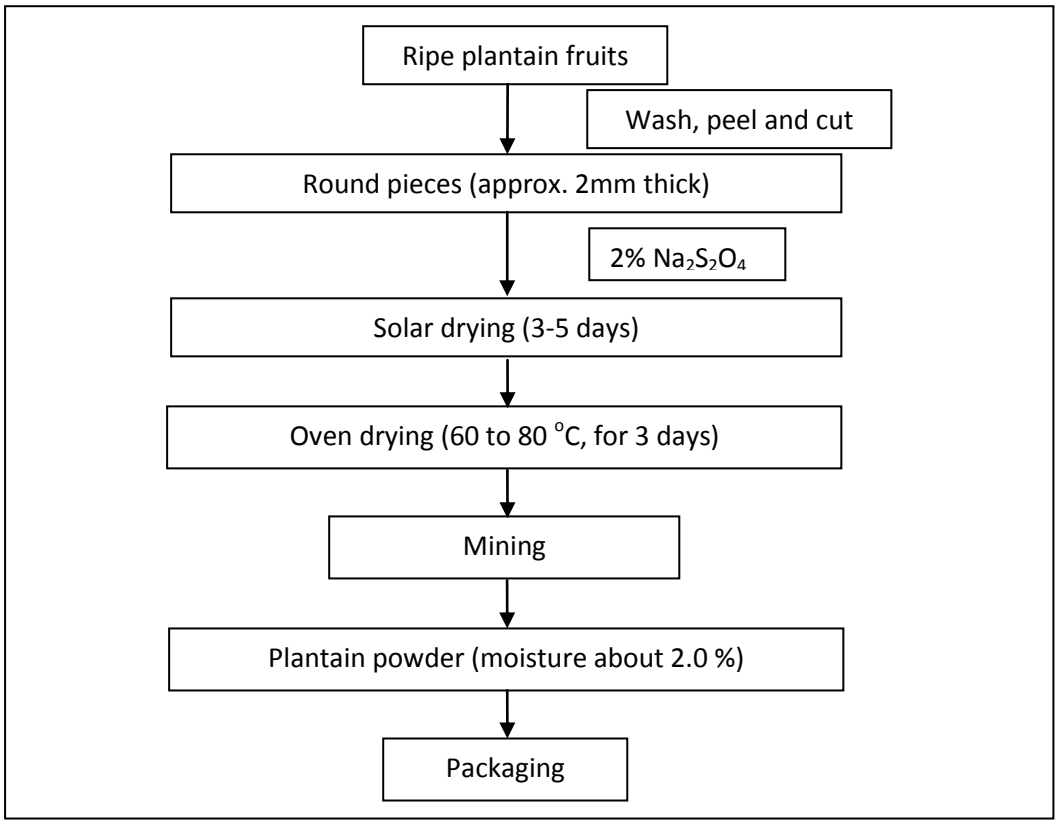



Figure 2.2: Flow chart of plantain flour preparation in Ghana (adapted from Zakpaa *et al*., 2010).

The understandings of fundamental scientific and technological principles are needed to upgrade the indigenous plantain flour processing techniques. This would be useful in developing the downstream sector by contributing the emergence and growth of small and medium scale agro enterprises in banana and plantain production countries (Table 2.1). When plantain is dried and ground, it gives an equivalent of wheat flour. This flour can help food manufacturers in providing healthier and more wholesome products. Plantain flour is also being exploited in baking and complementary weaning food in Nigeria (Adeniji & Empere, 2001; Baiyeri, 2004). Therefore, industrialization of plantain had helped in tampering the important of wheat and other related farinaceous products which is costly in third world countries.

Table 2.1: The percentage of banana produced which is converted into processed products for resale varies with the type of cultivars. (adapted from IPGRI, 2006)

| | Type of banana | Total area | Volume produced (MT) | % sold fresh nationally | % sold processed nationally | % exported fresh | % exported processed |
|---|---|---|---|---|---|---|---|
| **Nigeria** | Dessert | 68000 | 701000 | 98.5 | 1.5 | - | - |
| | Cooking | 34000 | 362000 | 35 | 65 | - | - |
| | Plantain | 161000 | 1224000 | 85 | 13 | 1.5 | 0.5 |
| **Cameroon** | Dessert | 65000 | 630000 | 55 | 5 | 38 | 2 |
| | Cooking | - | 150000 | - | - | - | - |
| | Plantain | 200000 | 1200000 | 75 | 15 | 10 | 1 |
| **Malawi** | Dessert | 180000 | 900000 | 90 | 5 | 5 | - |
| | Cooking | 40000 | 120000 | 25 | 75 | - | - |
| | Plantain | 15000 | 30000 | 80 | 20 | - | - |
| **India** | Dessert | 421400 | 14952000 | 96 | 0.1 | 0.1 | 0.8 |
| | Cooking | 343300 | 1008000 | 60 | 40 | - | - |
| | Plantain | 34300 | 840000 | 45 | 53 | 1.2 | 0.8 |
| **Malaysia** | Dessert | 27000 | 150000 | 69 | 1 | 30 | - |
| | Cooking | - | 210000 | - | - | - | - |
| | Plantain | 4000 | 20000 | 50 | 50 | - | - |
| **Costa Rica** | Dessert | 45000 | 2035000 | 10 | 1 | 75 | 15 |
| | Cooking | 300 | 650 | 100 | - | - | - |
| | Plantain | 11000 | 75000 | 35 | 10 | 43 | 12 |

### 2.1.3 Nutritional values of plantain and plantain flour

#### 2.1.3.1 Nutritional values

Plantains are claimed as a multipurpose crop, as the fruit can be used at all stage of ripeness. Plantains also provide a good source of nutrient for human consumption. The green plantains are mainly composed of 61 – 63 % moisture (Zakpaa *et al*., 2010) and are low in calorie, protein (3.2 %) and fat (0.5%, Zakpaa *et al*., 2010; 1.3 %, Suntharalingam & Ravindran, 1993). They are noted as a good source of vitamins A, B6, C and also rich in iron, potassium, magnesium and phosphorus. The chemical composition of plantain varies with the variety, maturity,

degree of ripeness and the soil type (Giami & Alu, 1994; Lee, 2007; Zakpaa *et al.*, 2010). The proximate values per 100 grams of edible plantain at different stage of ripeness are indicated in Table 2.2.

Table 2.2: The proxiamte values of plantain per 100 g of edible portion.

| Components | Stage of ripeness | | |
|---|---|---|---|
| | Unripe | Fully ripe | Overripe |
| Moisture | 60.0 ±1.10 | 62.9 ±1.80 | 68.2 ±2.10 |
| Crude Fibre | 3.50 ±0.12 | 2.01 ±0.04 | 1.15 ±0.06 |
| Total ash | 1.65 ±0.03 | 2.65 ±0.05 | 3.20 ±0.13 |
| Crude protein (Nx6.25) | 1.89 ±0.06 | 2.50 ±0.02 | 2.84 ±0.04 |
| Carbohydrate (by difference) | 32.6 ±1.20 | 29.6 ±1.00 | 24.5 ±1.00 |

(Adapted from: Giami & Alu, 1994)

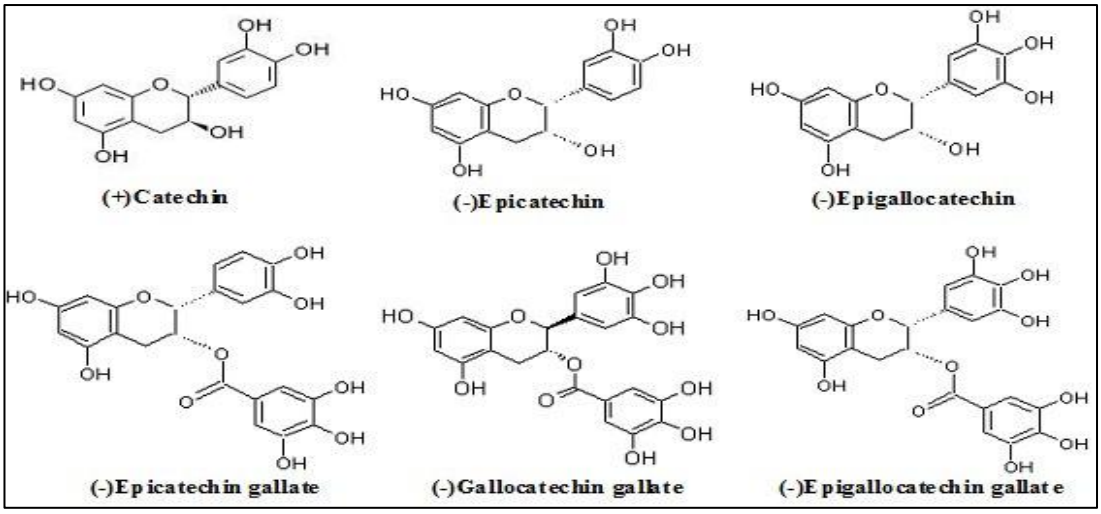
### 2.1.3.2 Major chemical components

#### a. Polyphenolic compounds

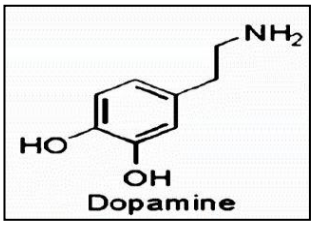
Research has proven that banana and plantain possess anti-oxidant, anti-inflammation, hypolipidemic, and hypoglycemic (Vijayakumar *et al*., 2008) protective effects against diseases such as cancer and cardiovascular diseases (Kawasaki *et al*., 2008; Wright *et al*., 2008; Boey & Liu, 2004). These health beneficial effects are attributed to the high content of phytochemical, and among them, phenolic compounds and flavonoid deserve special mention due to their cytotoctoxicity and antioxidant abilities. Lewis *et al*. (1999) and Beil *et al*. (1995) have demonstrated flavonoids extracted from unripe plantain can reduce acid secretion from gastric parietal cells and protects the gastric mucosa from aspirin-induces erosions. The biological role of flavonoids is attributed to the aromatic -OH

groups and the availability of the phenolic hydrogens as hydrogen-donating radical scavengers (Heijnen *et al.* (2001).

The antioxidant activity in banana and plantain is notably rich in phenolic compounds and flavonoid, of which catechin, epicatechin and gallocatechin (Figure 2.3a) and dopamine (Figure 2.3b) are the greatest proportion (Someya *et al*., 2002). Kanazawa and Sakakibara (2000) claimed that dopamine and vitamin C contents in bananas contributed to a high antioxidant capacity. According to Du *et al*., (2009), the protections mechanisms of antioxidants involve in inhibiting the formation of free radical species, act as breakers of radical-chain reaction, and help in repairing oxidative damage.




(a)




(b)

Figure 2.3: (a) Chemical structures of the naturally occurring catechins groups found in banana and plantain. (adapted from Nagarajan *et al*., 2008). (b) Chemical structure of dopamine.

**b. Dietary fibre**

Dietary fibre (DF) is defined as edible parts of plants that resistant to human gastrointestinal enzymes digestion and absorption in human small intestine and with partial or complete fermentation in large intestine. Unripe plantain pulp constitutes a good source of dietary fibre, ranging from 6.28 – 15.54 % (Juarez-Garcia *et al*., 2006; Da Mota *et al*., 2000). These non starch polysaccharides included crude fibre, cellulose, hemicelluloses, pectin substances, lignin, β-glucan and gums (Figuerola *et al*., 2005; Gallaher & Schneeman, 2001) which are collectively known as fibre. Dietary fibre from plantain is classified to soluble and insoluble form. Consumption of dietary fibre has been proven related to many positive physiological and metabolic effects on health. The role of dietary fibre in lowering cholesterol (Jenkins *et al*.,1998), coronary heart disease (Wolk *et al*., 1999) and other metabolic diseases, such as diabetes, treatment of obesity and colorectal cancer (Terry, 2001; Peter, 2003) have been proven and reported.

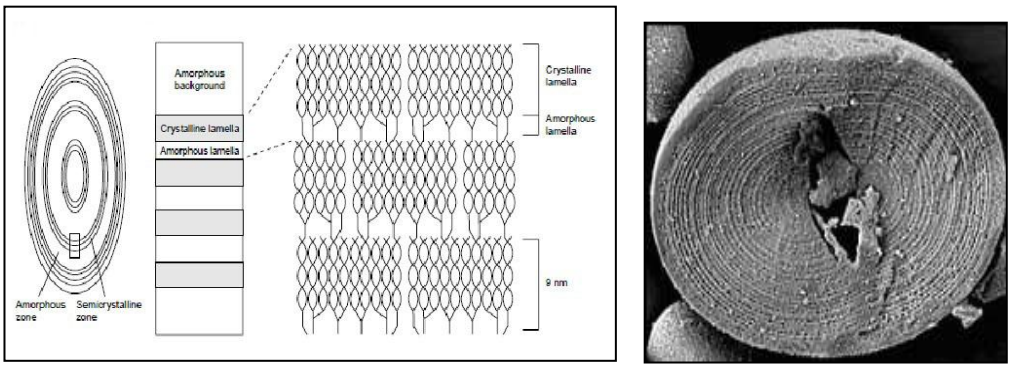
Soluble fibre refers to fibre which dissolves in water to become a gelatinous viscous substance and fermented by anaerobic bacteria in digestive tract (Anderson *et al*., 2009, Nancy *et al*., 2001). The fermentable soluble fibre to short chain fatty acids (SCFA) has a prebiotic effect, stimulating the production of beneficial bacterial in colon. In small intestine, soluble fibre binds to bile acids, decreases the production of cholesterol from liver and thus, lowering blood cholesterol levels (Cornfine *et al*., 2010). Studies published by Skrbic and Cvejanov (2011) and Figuerola *et al*. (2005) found that soluble fibre may reduce inflammation associated with obesity diseases and help in strengthen human immune system. The sponge like form of soluble fibre slow down the rate of digestion and help to control blood sugar levels (Brownlee, 2011).

Insoluble fibre does not dissolve in water but shows passive hydrophilic properties throughout the digestive system, helping in increasing bulk, soften stool and shorten transit time along intestinal tract. Therefore, the insoluble fiber is then related to the intestinal regulation, and alleviates constipation. Several epidemiological studies have established that consumption of insoluble fibre will reduce risk of large bowel cancer, and colonic diverticulosis (Kim, 2000; Davidson & McDonald, 1998).

**c. Starch**

**i. Starch granule structure**

Starch is the major storage polysaccharide found in plants and it is also the principal component of green bananas and plantain (61 – 76 %, Tribess *et al*., 2009; 70 – 80 %, Zhang *et al*., 2005). Native green plantain starches are oval to ellipsoidal in shape, 20 – 50 μm in sizes and in the form of partially crystalline granules with ordered radial arrangement (Figure 2.4). However, this large amount of starches is rapidly degrading during ripening and converted into soluble sugars.

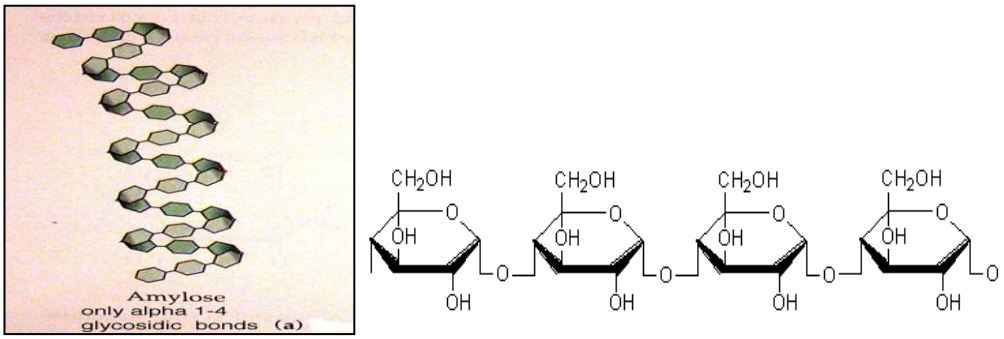



(a) (b)

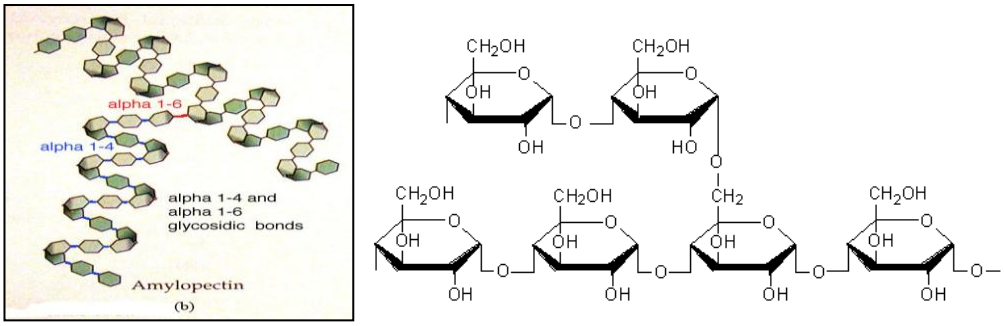
Figure 2.4: (a) Schematic view of the structure of a starch granule, with alternating amorphous and semi-crystalline zones. (adapted from Jenkins & Donald, 1995). (b) TEM image of starch granule showing the alternating crystalline and amorphous lamellae (adapted from Lopez & Gilbert., 2007).

**ii. Starch composition: amylose and amylopectin**

Plantain starches consist of two types of molecules: amylose (linear α-D-glucose) and amylopectin (branched α-D-glucose) (Figure 2.5), with the latter is the major component and comprises about 70 – 80 %. However, their compositions vary and are depending on the botanic origin (Kurakeke *et al*., 2009; Garcia-Alonso *et al*., 1999). Amylose content in plantain is reported to be 10 – 11% (Eggleston *et al*., 1992) and this range is much lower than cereal starches which typically consist of 20 – 25 % (Zhang *et al*., 2005). Due to its amylose content, characteristics of banana or plantain are very different from those of other normal starch such as corn although plantain starch is placed within group of 'normal' starches.




(a)




(b)

Figure 2.5: Two type of polysaccharide components in starch. (a) amylose (b) amylopectin (adapted from http://alevelnotes.com/content_images/i16_amylose.jpg, 2011)

Both amylose and amylopectin characterize and determine the physicochemical properties of starch, such as pasting, gelatinization and retrogradation (Kurakake, *et al*., 2009; Thitipraphunkul, 2003; Mua & Jackson, 1997). Amylose consists of long linear chains, are linked with (1→4)-α-D-glucosidic bonds, with a low level of α-1,6 branches (Hizukuri *et al*., 1981). Amylose chains appear in helical shape (Figure 2.5a) with hydrogen atoms are positioned in the interior of coils, while the exterior of coil reveals with some hydroxyl groups (Lee, 2003).

Amylopecyin , the major polysaccharide present in starch is a highly branched molecule with molecular mass in the range of $10^7 – 10^8$ g/mol (Murphy *et al*., 2008). Similar to amylose, amylopectin also contain chains sequences linked with (1→4)-α-D-glucosidic bonds, however, it has extensive branching through α-1,6 linkages (Juna *et al*., 2011; Lee, 2003).

**iii. Debranching of amylopectin**

Indeed, amylopectin - debranching activities might occur in the process of raw material manufacturing or during the process of starch production. During debranching of amylopectin, intact branch residue such as 1→6 linked branches of (1→4), (1→6)-α-D-glucan might occur. The rate or degree of debranching are greatly depending on the starch properties and the reaction conditions (Jayakody & Hoover, 2002; Singh & Ali, 2000) or the present of debranching enzyme (pullulanase, galactosidase, isoamylase) (Miao *et al*., 2009; Repellin *et al*., 2008; Thurn & Burchard, 1985; McCleary *et al*., 1981). Guilbot and Mercier (1985) also reported that the length of branching and the specificity of enzymes are greatly contributing to the rate of hydrolysis or liberating linear chains.

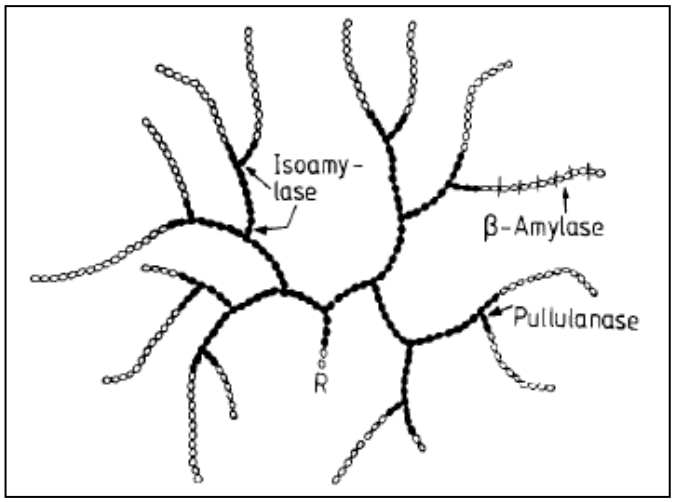



Figure 2.6: Schematic structure of amylopectin and action of the enzymes β-amylaseisoamylase and pullulanase. (adapted from Thurn & Burchard, 1985)

The debranching or depolymerisation of amylopectin by amylolytic enzymes acts in the manner depicted in Figure 2.6. The action of enzymes toward the amylopectin are specific which will act on the α-(1→6)-D-glycosidic interchain linkages but not on the major α-(1→4)-D-glycosidic bonds (Ong *et al*., 1994; Bello-Perez *et al*., 1996).

However, debranching of amylopectin by other chemically and physically treatment methods might not be specific reaction and normally encounter with functional groups. This selective degradation of polysaccharide has been commonly limited to structural modification applications through some mechanisms, such as oxidation, oxidative reduction depolymerization (ORD) and reductive aminations (Yalpani, 1985).

The basic principal of this amylopectin debranching reaction involves the attack of hydroxonium ions towards oxygen atom at the glycosidic bonds in amorphous sites and this resulting free hydroxyl function on the newly exposed

terminal residues (Hoover, 2000). Tsao *et al*. (2011) proposed that depolymerization mechanism after protonation of glycosidic linkages, hydrolysis take places as these protonated glycosodic linkages react with water molecules (Figure 2.7). Walker and Whelan (1960) also showed that hydrolysis of amylopectin would produce maltose, maltotriose, and a series of branched oligosaccharides, which is equivalent to the release of maltose and maltotriose during hydrolysis of amylose.

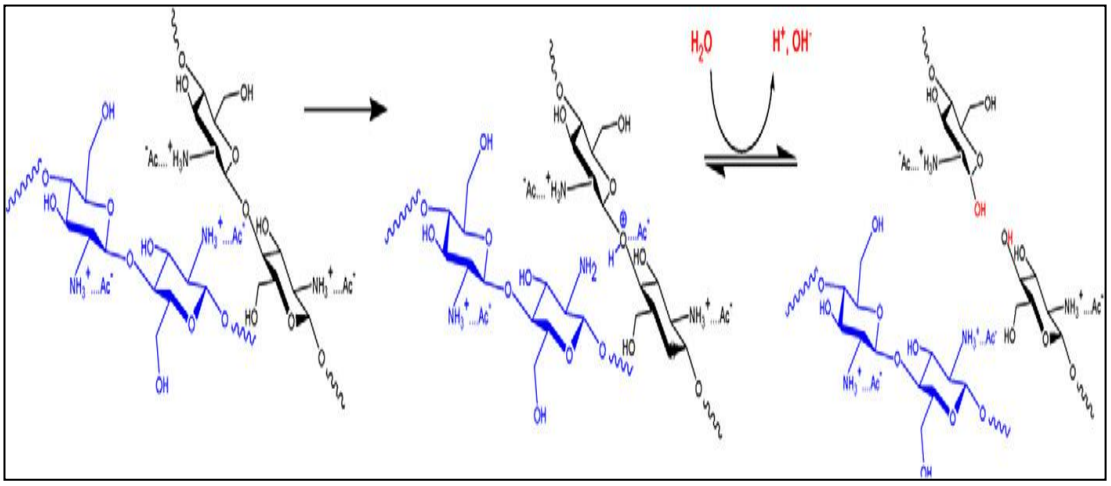



Figure 2.7: Mechanisms for acid depolymerization of protonation chitosan (adapted from Tsao *et al*., 2011)

**d. Resistant starch**

Green banana pulp contains about 70 – 80 % of starch on a dry weigh basis and the great part of the starch is the resistant starch (RS). Englyst *et al*. (1996) defined RS as 'the sum of starch and starch degradation products that, on average, reach the human large intestine'. It can be classified as RS1, RS2, RS3 and RS4, in which RS1 is physically inaccessible starch found in partly milled grains due to structural rigidity; RS2 refers to native starch granules with B-type crystals found in raw foods that resistant to digestion; RS3 is retrograded starch which formed during cooling of

gelatized starch and is found as major type of RS in processed food products (Englyst *et al*., 1996). Seib and Woo (1999) distinguished chemically modified starches as RS4 and these cross-linked starches are produced from wheat, maize, potato, tapioca, mung bean etc treated with chemicals such as sodium tripolyphospahate (STMP) and phosphoryl chloride ($POCl_3$). Many reports revealed that phosphorylation, acid hydrolysis using diluted HCl, $H_2SO_4$ and acetic acid (Ahmed & Auras, 2011; Ozturk *et al*., 2011; Zieba *et al*., 2011) managed to produce RS4.

Resistant starch (RS) from banana or plantain is classified as RS2 which is referred as native raw ungelatinised starch. Native RS2 is resistant to hydrolysis of α – amylase and glucoamylase in human small intestine (Zhang *et al*., 2005; Faisant *et al*., 1995, Eggleston *et al*., 1992, Englyst & Cummings 1986). The smooth and dense surface of the starch (Figure 2.8) account for their resistance to enzymes hydrolysis and the thick external layer of starch also impede the action of enzyme which reduce the rate of hydrolysis (Gonz âlez-Soto *et al*., 2007; Zhang *et al*., 2005). Besides, the low digestibility of starch is also due to the high amylopectin contents that contribute to the high degree and uniqueness in type of crystallinity present in plantain starch.

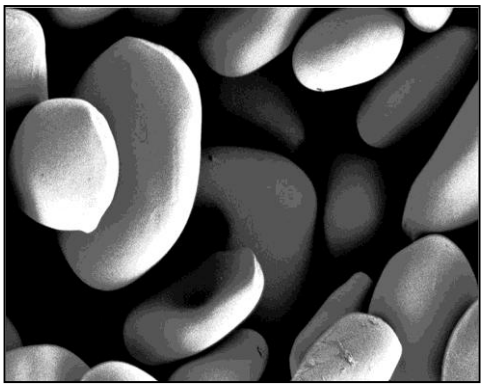


Figure 2.8: Scanning electron micrographs of native plantain starches. Magnification 2000x (adapted from Lawal *et al*., 2008)

The interest of RS in food industry and nutritionist is increasing because of the health benefits in protecting colonic disease (Topping & Clifton, 2001; Haralampu 2000; Hylla *et al*., 1998). The RS is fermented by the intestinal microflora to short chain fatty acids (SCFA), primarily acetate, propionate, and butyrate which provide an importance substrate for colonocytes (Sharp & Macfarlane, 2000). The presence of SCFA helps in inhibiting the growth of pathogenic bacteria in the large intestine by lowering intestinal pH. They also help to increase the absorptive of minerals, inhibit the absorption of toxic compounds and stimulate colonic blood flow (Murphy *et al*., 2008; Brown, 2004; Bird *et al*., 2000).

**i. Formation of resistant starch (RS)**

As mentioned in previous section, RS3 and RS4 are respectively referring to retrograded starch and chemically modified starch which formed during food processing. Formation of RS is of great interest in processed food industry due to its impact on human health. Moreover, the presence of RS improved the functional properties of food products such as emulsion properties (Koksel *et al*., 2008), swelling, solubility, and water binding capacity (Koksel *et al*., 2008; Mohan *et al*., 2005). Processing techniques and storage conditions are the two main factors influencing both gelatinization and retrogradation processes which subsequently affected the formation of RS formation (Garcia-Alonso *et al*., 1999).

Researchers had proven that heat-moisture treatment (HMT) managed to enhance RS3 content in different starches (Chung *et al*., 2009; Luo *et al*., 2003; Sievert & Pomeranz, 1989). For example, RS in corn, lentil, and pea starches respectively increased from 5, 9 and 10 % to 12 %, 15 % and 15 % after undergoing heat treatment at 120 $^{o}$C (Chung *et al*., 2009). Thermal processing enhances

formation of RS by enhanced leaching of starch fragments (amylose and amylopectin) from swollen granules, hydrogen bonds are then formed between aligned molecular chains and these strong associations formed tight structure within starch granules. Therefore, Berry (1986) showed that de-branched starch which contributes to high RS3 content due to the increased of interaction between starch components.

RS4 is usually formed by partial enzyme or acid hydrolysis (Ozturk *et al*., 2011) and chemical modification (Charalampopoulos *et al*., 2002). Generally, chemical modifications are achieved by etherification, esterification, cross-linking and grafting of starch by introducing functional groups of the starch molecule (Singh *et al*., 2007). Such modifications have changed the physico-chemical properties of native starches including gelatinization, retrogradation and pasting properties of the starches produced (Tharanathan, 2005; Hung & Morita, 2005; Choi & Kerr, 2003; Perera *et al*., 1997). Among the modifications, cross-linking is one of the most common methods used. According to Chung *et al*. (2009) and Wurzburg (1986), besides provide stability to acidic conditions, cross-linking also provides heat and mechanical shear resistances to starches. Therefore, RS4 produced are more rigid and shows low swelling property which might not be suitable to be used as thickeners in food system (Chung *et al*., 2009).

Most of the studies on RS formation were done on various pure starches, including wheat, corn, potato and other cereal grains (Faraj *et al*., 2004; Shin *et al*., 2002, Adamu, 2001). However, RS3 formation during flour production is also important and might be given more attention. Kim and colleagues (2000) had shown that extrusion, a thermal processing also increased RS content in wheat flour while Tribess and colleagues (2009) had reported higher RS content is obtained in green banana flour at different drying condition.

### 2.1.4 Physico-chemical and functional properties of plantain flour and starch

#### 2.1.4.1 Water holding capacity, swelling power and solubility

At lower temperature (40 $^{o}$C to 60 $^{o}$C), banana and plantain flour both show low water-holding capacity (WHC). Zakpaa *et al*. (2010) had shown that unripe plantain flour contains higher –OH groups to form hydrogen bonding which indicating higher ability of water binding. From their studies, with this mentioned property, unripe plantain flour might not suitable to be used in infant foods products since bulky paste might forma and hard to be digested. Moreover, it was also suggested that plantain flour should be stored in moisture–proof places due to its ability to absorb moisture upon storage and to extend the storage life.

Generally, the swelling power of solubility of native green plantain starch is lower as compared to potato, tapioca, mung bean and is closely to the behavior of sorghum starch (Zhang *et al*., 2005). In term of solubility, banana starches show solubility range from 0.14 – 21.7 % within the temperature of 65 – 95 $^{o}$C. The swelling pattern of plantain flour or starch is affected by condition of different processing. Pacheco-Delahaye *et al*. (2008) showed that drum-drying flour exhibits higher swelling pattern which caused by higher degree of starches disruption while tray dryer and freeze-drier plantain flours showed lower values swelling and solubility properties. Moreover, substitution certain levels of plantain flour in formulations of various bakery products had also produced dough with less extensibility and extension, lower resistance to mechanical work and higher dough deformation (Mepba *et al*., 2007).

### 2.1.4.2 Pasting properties and retrogradation

Pasting properties have been identified as an important index in determining the cooking and baking qualities of flours (PBIP, 1995). According to Ratnayake *et al*., (2002), pasting properties of flour were greatly depending on the ratio of amylose with amylopectin, degree of branching, length of branches and conformation of starch granule in flour. At low concentration, noticeable resistance of mechanical fragmentation is shown by banana or plantain paste, however, a pronounced peak viscosity, breakdown and set back are observed for viscosity profiles at higher paste concentration (7 – 8 %) (Zhang *et al*., 2005). Kayisu *et al*. (1981) also reported that banana starch paste showed highest viscoammylograph viscosities among other common food starches (potato, tapioca, waxy corn, corn and wheat) when heating above 95$^{o}$C. Literature also showed that swollen plantain granules were resistant to breakdown even after prolonged cooking (Zhang *et al*., 2005). These cited studies have indicated that property of native plantain starch is behaving fairly like a lightly cross-linked starch.

### 2.1.4.3 Thermal behavior

It is important to understand that thermal characterization of green banana flour prior to its application in food products (Tribess *et al*., 2009). According to Mangala *et al*. (1999) and Garcia-Alonso *et al*., (1999), process condition and RS content has been shown to respectively affect the thermal behavior of rice and cereal flour by influencing the gelatinization and retrogradation of starches in these flours. However, in the study of Torre-Gutierrez *et al*. (2008), native banana starch exhibits high gelatinization temperature ($T_o$), peak temperature ($T_p$) and also gelatinization

enthalpy (ΔH) as compared to mango, corn and potato starches. These authors also suggested that banana flour or starch has great potential to be used in products desired to delay pasting such as in retorted canned food.

### 2.1.5 Potential and important uses of plantain flour in future

Starches have gained interest in modern food industry and the annual worldwide starch production is 66.5 million tons (FAOSTAT, 2005). Plantain and banana starch has the potential to be one of the non-conventional starches in food and polymer science applications as unripe plantain or banana pulp has high starch concentration (70 – 80 % on dry weight basis, Zhang *et al*., 2005; 81 %, Chaing *et al*., 1987). The technologies of plantain starch production included alkaline extraction (Bello-Perez *et al*., 2000; Fichtali *et al*., 1999) and non-alkaline extraction (Waliszewski *et al*., 2003; Bello-Perez *et al*., 1999; Whistler, 1998).

However, using native starches in industrial applications has caused some drawbacks. According to Lawal *et al* (2008) and Sánchez-Rivera *et al*. (2005), extreme process conditions (e.g. temperature, pH and pressure used) during extraction steps has produced native starches with low shear stress resistance, low decomposition, high retrogradation, and syneresis. To overcome these shortcomings, recent studies tendency are towards looking for alternative or improved methods for obtaining banana and plantain starches with better physiochemical and functional properties. Native plantain starches have been modified chemically, physically or by enzymatic methods (Sánchez-Rivera *et al*., 2005).

In order to meet the requirement of specific industrial processes, the evolution of the starch sector starch modification is generally achieved by chemical derivatisation such as esterification, cross-linking, grafting, decomposition, acid hydrolysis and degradation (Hung & Morita, 2005; Tharanathan, 2005; Choi & Kerr, 2003).

## 2.2 Effects of processing on flour quality

Banana or plantain flour is easily susceptible to flavor deterioration and undergo chemical and nutritional changes during the processing or cooking (Di Scala & Crapiste, 2008). According to Tortoe *et al*. (2009), discolouration of the flour has caused a major drawback for commercial availability of plantain flour. The major processing problems for plantain flour processing are the browning resulting not only from enzymatic browning but also non-enzymatic browning. This phenomenon has been reported to occur during the processing of sweet potato and yam (Krishnan *et al*., 2010). Generally, browning may take place when endogenous ascorbic acids (AA) in fruits or vegetables is oxidised to dehydroascorbic acid (DHAA), which then undergo Maillard reaction by reacting with free amino acids (or protein) to yield deep brown colours (Capuano *et al*., 2009).

### 2.2.1. Browning reactions

#### 2.2.1.1 Enzymatic browning (EB)

Enzymatic browning is one of the most destructive reaction and widespread colour reaction occurring in fruits and vegetables, when cell integrity is affected during processing or storage. It involved the reaction of oxygen, phenolic compounds

and enzymes that appeared in fruits and vegetables. Phenolic compounds are widely distributed in plant kingdom, such as green leafy vegetables; starchy staples included potato, yam, apples, bananas and many other tropical and subtropical fruits. Phenolic compounds are considered as secondary metabolites which contain an aromatic ring with one or more substitution groups such as hydroxyl, methyl and glycosyl groups (Marshall *et al*., 2000). Phenolic compounds in food materials are mostly appear in flavonoid type. According to Yoruk and Marshall (2003), the affinities of enzymes for their phenolic substrates are determined by its active site and the stereochemistry of the substrate. Thus, enzymes are very specific in terms of their substrates. 3, 4-dihydroxyphenylethylamine (dopamine) has been identified as the main substrate for banana PPO (Palmer, 1963; Yang *et al*., 2000; Danyen *et al*., 2009).

**a. Mechanisms and factors of enzymatic browning (EB)**

With the presence of oxygen, oxidation of phenolic compounds to quinones is catalysed by three major enzymes, laccase (EC 1.10.3.2), catechol oxidase (EC 1.10.3.1) and peroxidase (EC 1.11.1.7) (Pourcel *et al*., 2006; Mayer & Staples, 2002). Laccase and catechol oxidase belong to the PPO category. This copper (Cu) prosthetic group of polyphenol oxidases must be present for the enzymatic browning to happen (Figure 2.9). PPOs catalyses two basic reactions of melanin synthesis: 1) the hydroxylation of monophenol to *ortho*-diphenols (*o*-diphenol) and 2) oxidation of *o*-diphenols to *o*-quinones which then rapidly polymerize to produce high-molecular-weight compounds or dark pigment melanin as illustrated in Figure 2.10. These melanins are then undergoing further non-enzymatic reaction (autoxidation and chemical oxidation) with amino acids and protein, yielding dark brown pigment (He & Luo, 2007; Wuyts *et al*., 2006; Marshall *et al*., 2000).