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**PHD**

**A study of the effects of heating on banana and plantain fruit**

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**A STUDY OF THE EFFECTS OF HEATING ON  
BANANA AND PLANTAIN FRUIT**

Submitted by  
**Baoxiu Qi**  
for the degree of PhD.  
of the University of Bath  
**1997**

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## **Dedication**

**To my mother Zhou Zhifang, my family and late father, and  
especially to my dearest Dominic**

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## **ABBREVIATIONS**

AAS, atomic absorption spectrophotometry

AGA, anhydrous galacturonic acid

AIR, alcohol insoluble residue

CDTA, cyclohexandiamine tetra-acetic acid

CWM, cell wall material

D.e., degree of esterification

DMSO, dimethyl sulfoxide

FHIA, Fundacion Hondurena de Investigacion Agricola

GalA, (1→4) α-D-galacturonic acid

GDA, glutaraldehyde acid

GLC, gas liquid chromatography

GTC, guanidinium thiocyanate

HTC, hard-to-cook

IITA, International Institute for Tropical Agriculture

INIBAP, International Network for the Improvement of Banana and Plantain

KOH, potassium hydroxide

LM, light microscopy

NaCl, sodium chloride

Na<sub>2</sub>CO<sub>3</sub>, sodium carbonate

NRI, Natural Resources Institute

ODA, Overseas Development Agency

OsO<sub>4</sub>, osmium tetroxide

PAW, phenol:acetic acid:water

PGA, polygalacturonic acid

PIPES, 1,4 – piperazinebis (ethanesulphonic acid)

PM, plasmalemma

PME, pectin methylesterase

RG-I, rhamnosylgalacturonan I

SD, standard deviation

SEM, scanning electron microscopy

TEM, transmission electron microscopy

## SUMMARY

In order to establish the factors which are responsible for cooking-induced softening of banana and plantain fruits, experiments were carried out to detect the cell wall changes during cooking in terms of the cell wall composition, molecular weights of different cell wall components (pectin and hemicelluloses) and ultrastructure of the wall. Fractionation studies of CDTA- and  $\text{Na}_2\text{CO}_3$ -soluble pectic substances were also undertaken with raw and cooked materials from two Uganda cooking bananas Toro (soft) and Enkonera (hard). The effects of Soxhlet (hot) preparation of alcohol insoluble residues (AIR) on pectin solubilization and depolymerization were also investigated with comparison to the PAW (cold) preparation of AIR. In addition, the composition of starch and its composition, the content of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  were measured among different cultivars. Different treatments, with, for example,  $\text{CaCl}_2$ ,  $\text{NaCl}$  and EDTA present in the cooking solution, as well as different temperatures on softening rate of dessert banana and plantain pulp tissues were also studied.

In the raw pulp tissues, Enkonera (hard) cell walls were composed of much more galactose and slightly higher arabinose than Toro (soft), although there was no significant difference in overall galacturonic acid and cellulose contents. Enkonera also contained slightly higher water-, CDTA- and  $\text{Na}_2\text{CO}_3$ -soluble pectic substances, as well as GTC- and KOH-soluble hemicellulosic substances. The MWs of chelator-soluble pectins of plantain (hard) were much higher than that of dessert banana (soft). Cooking resulted in pectin solubilization and depolymerization in both Toro and Enkonera cooking bananas. However, the soft cultivar showed much more pronounced changes in terms of MW reduction of chelator-soluble pectin and increase in water-soluble pectin after cooking. Although  $\text{Na}_2\text{CO}_3$ -soluble pectins did not show a remarkable reduction in MWs, that from Enkonera CWM appeared to have a much higher proportion of the higher MW materials than that from Toro CWM both before and after heating. Cooking did not result in MW change in hemicellulosic substances. Anion exchange chromatography with phosphate buffer and buffer containing increasing concentrations of  $\text{NaCl}$  separated four components from the CDTA- and  $\text{Na}_2\text{CO}_3$ -soluble pectins, but the profiles were different between these two



fractions, as well as between different cultivars. Monomer analysis showed that of all the fractions studied galacturonic acid was the major component, and amounted to more than 50% of the total sugar content.

Hot alcohol refluxing to prepare the AIR caused substantial pectin solubilization and depolymerization from the raw Toro cooking banana pulp tissues. Therefore, a cold method for preparing the AIR, using phenol:acetic acid:water (2:1:1) was preferred and employed for all the cell wall studies.

SEM and TEM observations also revealed that middle lamella dissolution, intercellular space expansion and cell wall separation occurred at the onset of cooking in all four cultivars studied. Starch gelatinization and swelling were also observed during cooking. However, the soft varieties showed more advanced and extensive breakdown in both cell wall and starch than the soft ones.

Generally, hard cultivars contained more starch and amylose than the soft ones, and the starch content remained constant during cooking.  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  content were lower in the hard cultivar, and levels fell only slightly during cooking. Cooking in  $\text{CaCl}_2$  solution resulted in a much firmer texture and less water uptake in both dessert banana and plantain. Temperatures below  $70^\circ\text{C}$  only had little effect on loss of firmness in dessert banana. Therefore, starch,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents in the raw materials did not have a direct effect on firmness loss during cooking. However, cooking in  $\text{CaCl}_2$  solution and below  $70^\circ\text{C}$  had significant hardening effects on the pulp tissues.

**CHAPTER 1**  
**GENERAL INTRODUCTION**

## **1. GENERAL INTRODUCTION**

### **1.1 Botanical Background of Banana and Plantain**

Banana and plantain are monocotyledons and belong to the family of Musaceae, genus *Musa*. They are tree-like perennial herbs, two to nine metres tall, with an underground rhizome (or corm). A pseudostem is formed of the rolled leaf sheaths and a terminal crown of leaves. Some 7-9 months after planting a sucker (a shoot from the corm), an inflorescence is formed at the base of the pseudostem, and about 1 month later, this inflorescence emerges through the centre of the leaf crown after pushing up through the centre of the pseudostem. In edible cultivars, vegetative parthenocarpy results in the formation of fruits with edible pulp without pollination, and the fruits may be suitable for harvesting 90-150 days after emergence of the inflorescence (Stover and Simmonds, 1987; Marriott, 1980; Seymour, 1993).

### **1.2 Origins and Cultivars**

Most cultivated banana and plantain cultivars are triploid ( $2n=3x=33$ ). The genome of the cultivated types is derived from the diploid wild species *Musa acuminata* (AA genome,  $2n=22$ ) and *Musa balbisiana* (BB genome,  $2n=22$ ), which originated from Asia. The contribution of the two species are referred to as the A and B genomes, respectively. The development of edible bananas initially resulted from the human selection of diploid *M. acuminata* varieties that were parthenocarpic, i.e., they produced fruits without pollination. Diploid (AA) cultivars give rise through nuclear restitution during meiosis to triploid (AAA) cultivars. However, an important step in the evolution of the cultivated banana was the natural crossing that occurred between *M. balbisiana* and AA diploids to produce AB diploids, and AAB and ABB triploid

hybrids. Currently, the most important genome types are AAA, AAB and ABB (Stover and Simmonds, 1987).

It is not known how many different natural cultivars of *Musa* exist today. This is because methods are not yet available that will enable cultivars to be uniformly characterised and compared around the world to resolve synonymy. The most important cultivars vary in their genomic constitution: cultivars belonging to AAA group include the Cavendish dessert bananas, and East African highland bananas which are cooked. The former contribute the basis of international trade. Other types of bananas which are of importance, which are usually cooked prior to consumption, include plantains (AAB) and cooking bananas (ABB). Dessert bananas when green may be also cooked before use (Baldry and Dempster, 1976).

### **1.3 Distribution and Production**

The banana is a commercially important fruit crop in world trade. Bananas in a ripe stage are used as a dessert fruit, and plantains and cooking bananas are generally eaten either ripe or unripe after cooking. In parts of Africa and Southeast Asia, plantains and cooking bananas are the main staple food; daily consumption here may surpass 4 kg per head. The products derived from *Musa* are the “fig” which is dried slices of ripe bananas, powder which is ground from ripe bananas, chips and flour which are made from unripe fruits of bananas and plantains, flakes, juice, and puree (Marriott, 1980). Bananas and plantains contain mainly carbohydrates present in the form of starch in the green mature fruits. They are also fairly good sources of vitamins A, B<sub>1</sub>, B<sub>2</sub> and C and potassium.

Plantain (*Musa* spp., AAB group) and cooking banana (*Musa* spp., ABB group) are an important staple food and reported as being the fourth most important global food commodity after rice, wheat and milk in terms of the gross value of production (INIBAP Annual Report, 1992). They are major crops of the humid, lowland tropical zones of west and central Africa. These are grown in some 120 countries throughout the developing world. The crops develop as firm fruits when mature, with a nutrient composition comparable to potatoes, and are widely used as a starchy staple food after cooking (Marriott, 1980). The crop's status as a major source of sustenance does not come from the international trade in sweet dessert banana — which according to FAO (quoted by Wainwright, 1992) constituted only 10% of the total estimated world production in 1990 — but from the more than 60 million tonnes produced and consumed locally throughout the tropics (Table 1.1).

Most of the bananas and plantains grown for local consumption are cultivated by small farmers and the types of bananas and plantains differ from one region to another. In Uganda, bananas are the dominant staple for 50% of the population; the same is true for parts of Burundi, Kenya, Rwanda, Tanzania and Zaire. In all of these areas, a large part of the population depends on bananas for up to 80% of its carbohydrates (INIBAP Annual Report, 1993).

**Table 1.1** Production of bananas and plantains (major producing countries)

Total=64 673 400 tonnes

(a) By Region

REGION	% of Total
Latin America & the Caribbean	35.5
Asia/Pacific	29.0
East Africa	23.5
West & Central Africa	12.0

(b) By Type

TYPE	% of Total
Plantains AAB	23.0
Cavendish Bananas (Locally consumed)	19.5
AAA Highland Bananas	18.0
ABB & Other Cooking Bananas	16.5
Other Dessert Bananas	12.5
Cavendish Bananas (exported)	10.5

Source: INIBAP Annual Report (1993).

## 1.4 Biochemical and Nutritional Composition

### 1.4.1. Biochemical composition

The biochemical composition of the banana has been the subject of numerous studies concerned either with its utilisation either as a dessert or processed fruit or with the elucidation of the process of ripening. There are comprehensive reviews of the subject which provide much information (e.g. Marriott, 1980; Marriott and Lancaster, 1983; Stover and Simmonds, 1987).

The main carbohydrate of unripe banana and plantain pulp is starch, which forms 75-80% of the dry matter and 20 - 35% of the fresh weight. The amylose content of banana and plantain starches is in the range of 19.8 - 21.2%. Starch is converted

stoichiometrically converted to sugars during ripening with the formation of sucrose, glucose and fructose.

Unripe plantains contain 5.8% hemicellulose (as hexoses), and in addition 1% cellulose and 0.2% lignin. Whereas unripe bananas contain 8 - 10% hemicellulose. Pectin content ranges from 0.5-1% in bananas and plantains (all expressed as of fresh weight) (Stover and Simmonds, 1987; Marriott, 1980). Recently, Jarvis *et al* (1988) reported that bananas (monocots) had a wall pectin content comparable to dicots.

The main organic acids of banana and plantain pulp are malic, citric and oxalic. The titratable acidity of plantains is about twice that of bananas at an equivalent stage of ripening. Dessert bananas of the AAB group are also more acidic than Cavendish cultivars (AAA group) (Marriott, 1980).

About 1-1.2% of the fresh pulp is protein, which is a lower content than that of yam or sweet potato but higher than that of cassava. There are two predominate proteins in the pulp extracts with molecular weights 19,000 and 50,000 respectively (Askar, 1972, quoted by Stover and Simmonds, 1987). The amino acids composition of pulp tissue after hydrolysis has been examined for banana by Askar (1973, quoted by Stover and Simmonds, 1987) and plantain by Ketiku (1973). Banana proteins are rich in lysine and cystine but low in methionine. The most abundant free amino acids in a list of 17 were histidine, serine, valine, leucine and arginine with histidine comprising 31 per cent (Askar, 1973). The lipid content of bananas and plantains is very low, and the composition of banana and plantain lipid is similar (Stover and Simmons, 1987).

Phenolics, serotonin, dopamine and norepinephrine are present in a very high concentration in banana peel, and are also present in the pulp. Dopamine is the primary substrate in enzymatic browning (Griffiths, 1959). It is the concentration of dopamine rather than that of the polyphenoloxidases responsible for its conversion to pigment, which governs the rate of browning of bananas (Weaver and Charley, 1974).

#### ***1.4.2. Nutritional and therapeutic values***

Bananas have a special place in diets low in fats, cholesterol and salt. Sodium is present in trace amounts while the potassium level is 400mg/100g. Because of the low lipid and high energy value (Table 1.2) bananas are recommended for obese and geriatric patients (Gasster, 1963). Various preparations of dried unripe plantain were anti-ulcerogenic against aspirin-induced ulceration in rats and were effective as a prophylactic treatment and in healing ulcers (Best *et al.*, 1984).

**Table 1.2** Nutritional values of banana and plantain

Component (% fresh weight)	Ripe banana (AAA group)	Unripe plantain and cooking banana (AAB and ABB group)
Water	71-78	57-63
Carbohydrate	16-24	29-35
Fat	0.2	0.2-0.3
Crude protein	1.2	1.0-1.2
Fibre	3.0-4.0	6.0-7.0
Ash	0.8	0.8-1.0

**Source:** Marriott and Lancaster (1983).

Bananas and plantains are a good source of ascorbic acid (vitamin C). Exported bananas usually contain about 10 mg/100g, whereas plantain, cooking bananas, and also dessert banana of groups of AAB and ABB often contain up to 20 - 25mg/100g



fresh pulp. They are also an excellent dietary source of vitamin A (carotene) and Vitamin B (Marriott and Lancaster, 1983).

### **1.5. Strategies for Research on Bananas and Plantains**

Until the late 1970's, international research interest was limited to exported dessert bananas. Plantains and cooking bananas for local consumption received little attention. As a result cooking bananas and plantains are among the least studied of all the major food commodities, despite their importance as a staple and their vulnerability to a number of diseases and pests.

In recent years, the production of banana and plantain crops have been subjected to severe attack from "black Sigatoka" disease, which is a leaf spot disease caused by *Mycosphaerella fijiensis*. This disease results in a considerable reduction in photosynthetically effective foliage area and may reduce yields by 30-50% (Stover, 1983). To combat this destruction an active plant breeding and selection programme has been initiated at the International Institute for Tropical Agriculture (IITA) in Nigeria and the Fundación Hondureña de Investigación Agrícola (FHIA) in Honduras. The other disease is an infection to the soil called Panama disease which can be overcome only by growing banana varieties that are resistant to the infection. Therefore, these programmes have been primarily directed towards the development of new disease resistant hybrids.

It is known that consumers show distinct preferences for specific cultivars of cooking bananas and plantains for different purposes (Baldry and Dempster, 1976;

Wainwright, 1992). The International Network for the Improvement of Banana and Plantain (INIBAP) therefore recognised the need for postharvest appraisal as part of the overall breeding strategy for cooking bananas and plantains. Through funding from the Overseas Development Agency (ODA), INIBAP commissioned the Natural Resources Institute (NRI) to undertake a project to evaluate the post-harvest characteristics of some of the new disease resistant hybrids. In particular, it was considered important to determine the ripening, cooking and palatability characteristics of the new germplasm and to gauge its future potential for world-wide introduction. As a food which is consumed after cooking, it is essential to examine the differences in cooking behaviour and palatability between different types and to understand the basis for such differences in terms of starch characteristics, texture and nutritional value. Thus cultivars can be introduced that will be acceptable to the local consumers as well as possessing superior disease resistance characters.

#### **1.6. The Process of Cooking — Textural and Related Biochemical and Physical Changes in Fruits and Vegetables**

Cooking results in the softening of fruit and vegetable tissue. This change in texture and mechanical strength is (in most cases) the most important feature of a cooked product. The factors responsible for softening during cooking depend on several characteristics rather than one alone, and it may be regarded as the result of an interaction between several complex processes (Harada and Paulas, 1987). Softening in fruits and vegetables by heating involves the loss of turgor, a variety of chemical changes in the cell wall matrix polysaccharides (van Buren, 1979; van Buren and

Pitifer, 1992; Keijbets *et al.*, 1976; Fuchigami, 1987a, b), and the swelling and gelatinization of starch.

The main component of the cell wall which is affected by heating is the pectin, some of which acts as a cementing agent in the middle lamella region and can “glue” adjacent cells together (Fry, 1988). It is more easily brought into the cooking solution than other wall polymers, and is also chemically active. On the other hand, Warren and Woodman (1974) suggested that in potato the reduction in tissue strength which occurs on cooking is caused by an increase of the cell wall thickness due to water uptake by the cell wall polysaccharides, and this leads to a reduction in the viscosity of the cell wall matrix. In this way the stress required to separate cells has been reduced. Cellulose is not degraded by the cooking process.

Starch gelatinization has been frequently cited as another phenomenon that occurs on cooking of starchy tissues, such as potatoes. The loss of integrity of the starch grains, and the filling of the cell with amorphous starch gels is microscopically observable (Reeve, 1967; Reeve, 1977), and the swelling pressure could apply the force to separate the weakened middle lamella (Jarvis *et al.*, 1992). However, some researchers disagree with this hypothesis, and question the existence of the starch swelling pressure (Bartolome and Hoff, 1972; Bretzloff, 1970; Warren and Woodman, 1974).

In the majority of fruits and vegetables it is thought that the major causes of cooking-induced softening, and hence textural changes are modifications to the cell wall with starch swelling also playing a role in some tissues. Therefore, to understand what

factors may affect the susceptibility to cooking-induced softening it is necessary to have an understanding of plant cell wall structure, how it changes during cooking and other factors which may be involved.

## **1.7. Primary Cell Wall**

The cell walls of the edible portions of plants are predominantly primary cell walls. Edible fruit tissues are composed mainly of primary cell walls, and secondary cell walls are virtually absent from fruits (Nelmers and Preston, 1968).

### ***1.7.1. Primary cell wall composition***

The primary cell walls of both monocotyledon and dicotyledon are composed of four principal components: cellulose, hemicellulose, pectic polysaccharide (pectin) and glycoprotein. Table 1.3 shows the chemical composition of the cell walls isolated from suspension of cultured sycamore cells (a dicot) (Albersheim, 1976). However, the proportion and nature of the hemicellulose, pectic polysaccharide and glycoprotein contents of some monocots differ from the general composition of a dicot.

The pectic polysaccharides are made up of a group of polysaccharides rich in galacturonic acid, rhamnose, arabinose and galactose. They are some of the most complex polymers known, and are thought to perform many functions. The primary cell walls of dicots and some monocots contain roughly 35% of pectic polysaccharides (Table 1.3), which are characteristic of the middle lamella and primary wall. They are highly susceptible to degradation under relatively mild conditions.

**Table 1.3.** Chemical composition of the cell walls from isolated suspension-cultured sycamore cells.

Components	Weight (% composition)
TOTAL CARBOHYDRATE	80-90%
<i>Polysaccharides</i>	
Pectin	35%
Hemicellulose	25%
Cellulose	25%
Major sugars	
Glucose	27%
Arabinose	21%
Galactose	13%
Xylose	8%
Rhamnose	3%
Galacturonic acid	13%
<i>Minor sugars</i>	<5% in total
Apiose	
Fucose	
Mannose	
Aceric acid	
Glucuronic acid	
TOTAL PROTEIN	10%

**Source:** Albersheim (1976).

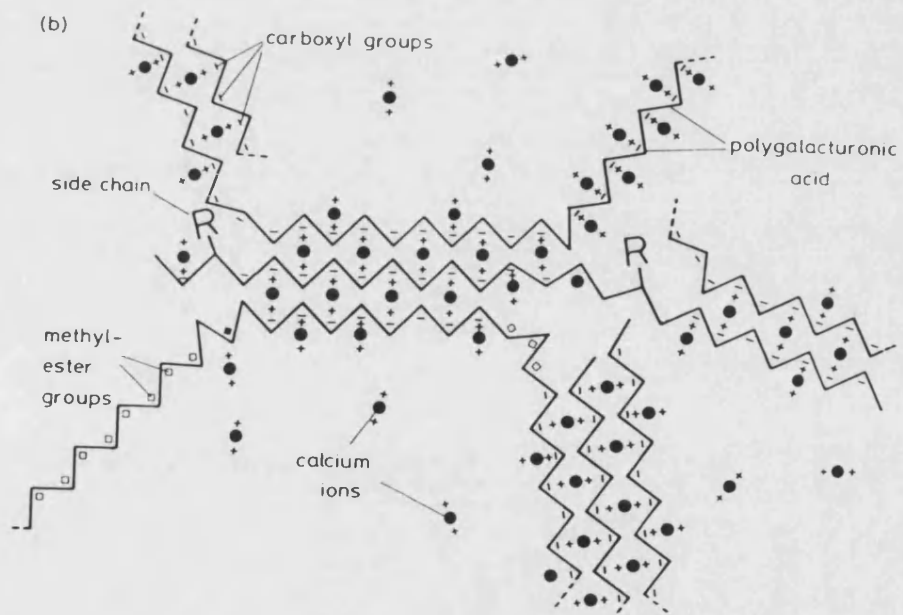
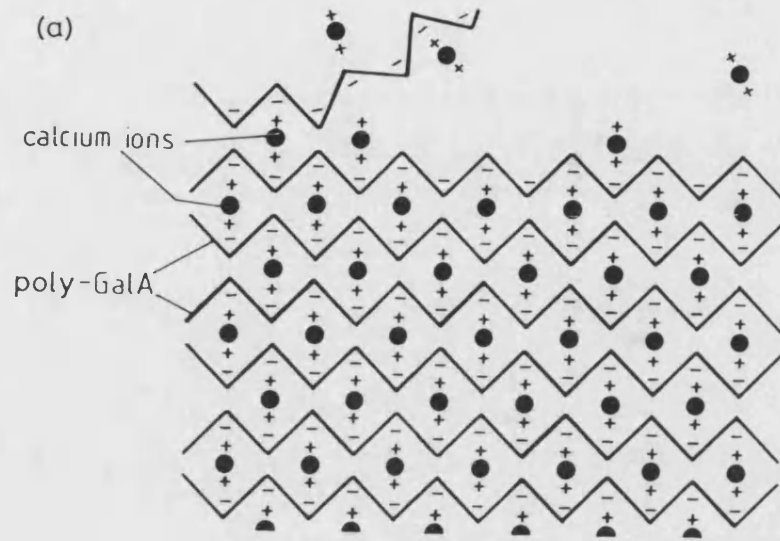
There are two fundamental constituents of pectins, polygalacturonic acid and rhamnosylgalacturonan I. Polygalacturonic acids (PGAs) or homogalacturonan, are helical homopolymers of (1→4) α-D-galacturonic acid (GalA), which may be partly methyl esterified. Rhamnosylgalacturonan I (RG-I), are contorted rod-like heteropolymers of repeating (1→2) α-L-rhamnosyl-(1→4) α-D-GalA disaccharides units (Lau *et al.*, 1985). An unusual minor component, RG-II, is also found in the primary walls of suspension-cultured dicot cells, which has a complex structure made up of galacturonic acid, rhamnose, arabinose and galactose in the ratio of 10:7:5:5,

together with a small amounts of some rare sugars (Brett and Waldron, 1990). Carpita and Gibeaut (1993) suggested that it is a signal molecule rather than a structural polymer.

The PGAs contain up to about 200 GalA units and are about 100 nm long, but the length of RG-I chains is unknown because there may be stretches of PGA on their ends (Lau *et al.*, 1985). The helical chains of PGAs can become cross-linked via Ca<sup>2+</sup> bridges to form 'junction zones', linking two anti-parallel chains (Figure 1.1) (Jarvis, 1984; Powell *et al.*, 1982; Morris *et al.*, 1982,).

Other pectin polysaccharides such as arabinans, galactans and highly branched arabinogalactans of various configurations and sizes are present as either independent molecules or as side chains attached to rhamnose of RG-I. In general, about half of the rhamnosyl units of RG have side chains, but this can vary with cell type and physical state (Carpita and Gibeaut, 1993; Brett and Waldron, 1990).

Using antibodies against specific polysaccharide epitopes viewed by immuno-electron microscopy, various wall polymers have been localised in the wall. For instance, RG-I has been localised to the outer surface of the primary cell wall plus the middle lamella (Moore *et al.*, 1986). In the carrot root apex cell walls, the de-esterified PGAs were found to be concentrated in the middle lamella, the outer surface of the wall facing in the intercellular spaces, and small layer at the interface between plasma membrane and the cell wall. In contrast, the methyl esterified PGAs were found to be distributed evenly throughout the wall (Knox *et al.*, 1990). (1→4)-β-D-Galactan were also recently immuno-localised in tomato fruit pericarp cell walls (Jones *et al.*, 1997). They are present in the primary cell walls but not in the middle lamella region.



**Figure 1.1.** Egg-box model of Morris *et al.* (1982) showing maximum cross-linking between unsubstituted polygalacturonic acid (a) and the factors that are likely to influence such cross-linking within the cell walls (b).

The precise functions of pectins are unclear, but it appears that one of their functions is to cement adjacent cells together, evidenced by the rapid release of single cells from plant tissues by pectinolytic enzymes and (in a few tissues) by chelating agents. Pectins are highly hydrophilic polysaccharides and when strongly hydrated they may loosen the wall (Rees and Wright, 1969), enabling the skeletal cellulose microfibrils to separate. Alternatively they can form cross-links via  $\text{Ca}^{2+}$  bridges and probably via covalent bonds (Fry, 1986; Jarvis, 1984; Morris *et al.*, 1982) and in this state they may serve the opposite function, resisting the expansion and separation of the wall.

Hemicelluloses, like pectins, are polysaccharides built up of a variety of different sugars. The principal hemicelluloses are xylans, xyloglucans and glucomannans. In contrast to pectins, the hemicelluloses vary greatly in different cell types and different species. In most cell types, one hemicellulose predominates, with others present in smaller amounts.

Xyloglucans (XGs) typically make up about 20% of the primary cell walls of dicots, and 1-5% of the primary walls of grasses. The XGs are linear chains of (1→4)- $\beta$ -D-glucan which is identical to cellulose, but unlike cellulose, there are number of xylosyl units added at regular sites to the O-6 position of the glucosyl units of the chain (McNeil *et al.*, 1984).

Xylans typically make up roughly 5% of the primary cell wall in dicots, 20% of the primary walls of grasses. They have a backbone of  $\beta(1\rightarrow4)$  linked xylosyl- residues, some of which carry side chains of single  $\alpha$ -L-arabinose and/or  $\alpha$ -D-glucuronic acid



attached to the 2- and/or 3-O position. Other hemicelluloses are found in much lower amounts (Bacic *et al.*, 1988; Darvill *et al.*, 1980).

Carpita and Gibeaut (1993) suggested that, in most flowering plant cell walls, XGs occupy two distinct regions *in muro*: one binds tightly to the exposed faces of glucan chains in the cellulose microfibrils, and a second spans the distance to the next microfibril or simply interlocks with other XGs (as a chain-like fence) to space and lock the microfibrils into places (Figure 1.3).

Cellulose is an unbranched polymer of D-glucan joined by  $\beta(1\rightarrow4)$  linkages. It usually comprises 20-30% of the dry weight of the primary cell wall. The degree of the polymerisation is usually at least 15,000. About 30-100 cellulose molecules are aligned side by side to form a microfibril. The microfibrillar phase is distinguishable from the matrix phase by its high degree of crystallinity in the wall. The latter phase is made up of pectic, hemicellulosic polysaccharides and glycoproteins. The microfibrils are also readily visible under the electron microscope. They are 5-15 nm wide and are spaced 20-40 nm apart (McCann *et al.*, 1990). The biological function of the cellulose is presumed to be skeletal, providing shape and strength to the cell wall.

### ***1.7.2. Cross-links between wall polymers***

The individual polymers of the primary cell wall (except cellulose) are intrinsically soluble. Once the polymers have been extracted from an intact cell wall, they tend to remain water-soluble (Selvendran *et al.*, 1985). However, the intact wall matrix is resistant to extraction in water. If water-soluble polymers are inextractable in water,

the only explanation is that the molecules are cross-linked *in situ* (Fry, 1986; Fry, 1988). The matrix of non-cellulosic polysaccharides and glycoproteins are linked together into a fabric of great strength, which is coupled with elasticity, plasticity and the ability to hold cellulose microfibrils together (and apart) as well as adhering to neighbouring walls. Cross-links between walls are responsible for cell adherence, and cell/cell bonding influences the texture of processed fruit and vegetables (van Buren, 1979).

These cross-links include non-covalent links such as hydrogen bonds, ionic bonds and  $\text{Ca}^{2+}$  bridges and covalent cross-links such as ester bonds, and all of these bonds play a role in building the wall. Figure 1.2 illustrates the possible cross-links (bonds) between primary wall polymers (Fry, 1986). Hydrogen bonds are believed to bind the hemicelluloses to the cellulose microfibrils of the wall (Northcote, 1972). Weaker H-bonds are also probably present between the pectic and hemicellulosic polysaccharides of the matrix.

$\text{Ca}^{2+}$  bridges are also a special case of ionic bonding;  $\text{Ca}^{2+}$ , being divalent, can bind ionically to two or more pectin molecules, which tends to hold them together. Thus strong cross-links can be formed between pectin molecules, probably in a well defined arrangement known as the 'egg-box' model (Figure 1.1; Morris *et al.*, 1982). These linkages only can occur between unesterified and unbranched polygalacturonic acid chains. However, just how many contiguous residues are needed to form stable junction zones and the extent to which several chains can stack to form the multiple 'egg-box' structures are not known both *in vivo* or *in vitro*. Jarvis (1984) suggested

that at low  $\text{Ca}^{2+}$  concentrations, two chains can form a stable junction with full binding strength at about 14 GalA units. With sufficient  $\text{Ca}^{2+}$  present, some interrupting esterified galacturonic acids can be tolerated in the stable junction zone. If  $\text{Ca}^{2+}$  is in excess, four chains or higher-order stacking of PGA chains is possible.

Good evidence for calcium binding has been obtained by treatment of walls with cold-cyclohexanediamine tetra-acetic acid (CDTA) at neutral pH, which can remove essentially all of the calcium and yield the pectin molecules without degradation (Jarvis, 1982). Conversely,  $\text{Ca}^{2+}$ -bridges can be broken by chelating agents.

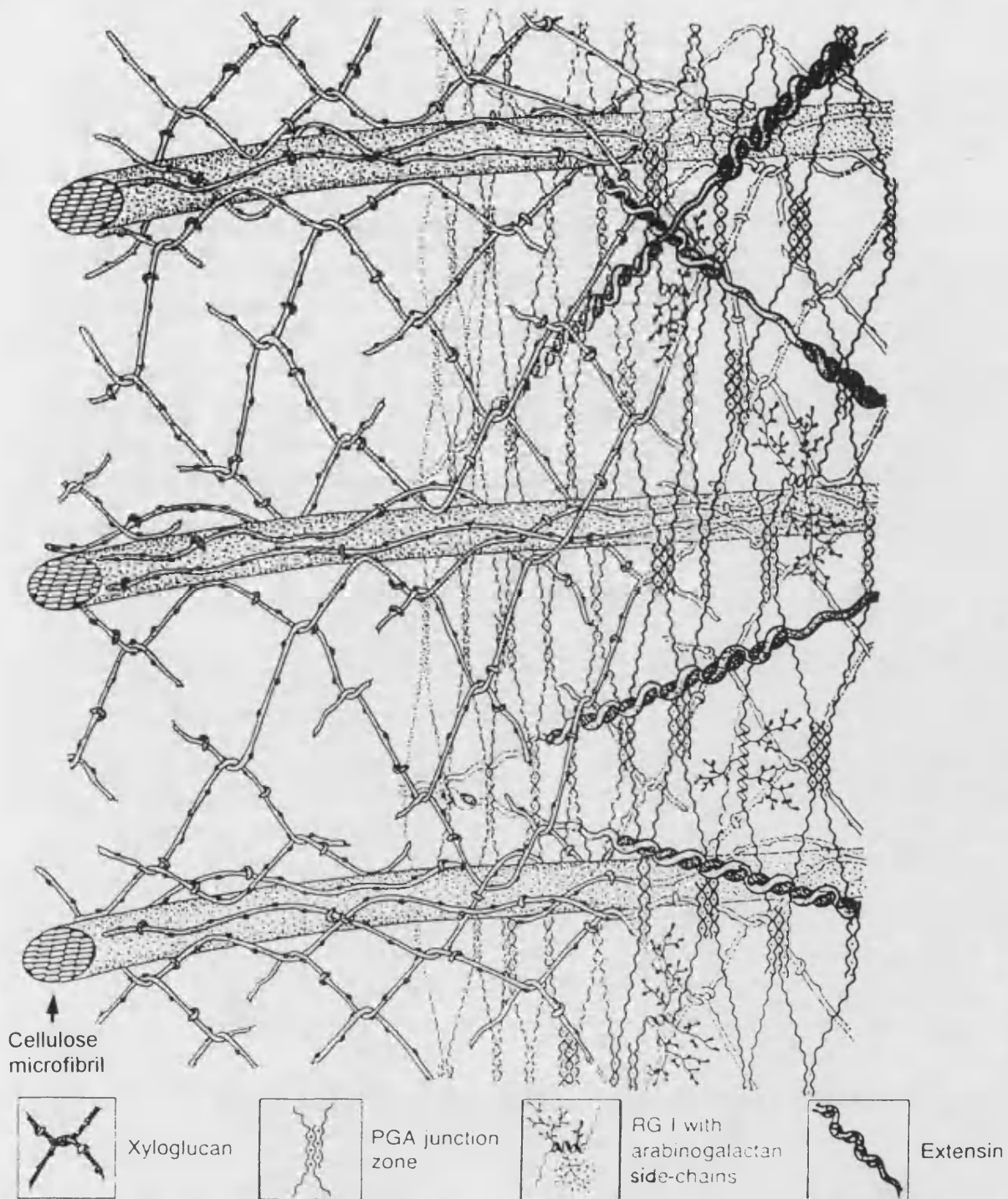
Pectins are synthesised in the Golgi apparatus in the fully esterified form which is soluble and thus readily transported. They are de-esterified as required at their destination in the wall. The unbranched and unesterified galacturonan blocks are bonded together via calcium bridges. The still esterified and/or partially esterified galacturonan blocks may be joined together by both  $\text{Ca}^{2+}$  junction zones and ester-acid bonds to form an intermediate gel (Gidley *et al.*, 1980).

### ***1.7.3. Wall structure***

The microscopic structure and molecular architecture of the cell wall has an important bearing on its function. After many polysaccharide and protein compositions had been characterised, hypotheses were proposed to explain how these different polymers were assembled to form an extensible wall.



A typical primary wall is about 0.1  $\mu\text{m}$  thick (Fry, 1988). It is a biphasic structure consisting of a relatively rigid skeleton of cellulose microfibrils and a gel-like structure composed of non-cellulosic polysaccharides and glycoproteins. The cellulose microfibrils establish the ultimate cell shape, whereas the dynamic interaction of the cellulose and non-cellulosic polysaccharides matrices dictate the rate of cell expansion and the extent that microfibrils are pulled apart. Early models of the cell wall were proposed by Cleland (1981) and Keegstra *et al* (1973). More recently, Carpita and Gibeaut (1993) have proposed a new cell wall model to account for the known structure and composition of the cell wall of most dicots and some monocot flowering plants (Figure 1.3). In this model, there are three structurally independent but interacting domains. The first one is the fundamental cellulose-xyloglucan framework, which is about 50% of the wall mass, and this is embedded in a second domain comprising a matrix of pectic polysaccharides (roughly 30% of the wall mass). The third independent domain consists of the structural protein extensin (Lamport, 1986). Xyloglucans, with (less often) other hemicellulosic polysaccharides, interlace and interlock the cellulosic framework. The pectins define the pore size of the wall possibly by their degree of esterification and branching which can govern the size and extent of the  $\text{Ca}^{2+}$ -PGA 'junction zones' (Figure 1.1). In the fully expanded cell wall extensin molecules, inserted radially, may interlock the separated microfibrils, which are generated after xyloglucans cleaved or dissociated by growth-relevant hydrolases. This process ceases further stretching of cell wall.



**Figure 1.3.** Model of the expanding primary cell wall of most flowering plants. Representation of a single stratum of the wall. Several strata such as this coalesce to form a wall. The cellulose microfibrils are interlaced with xyloglucan polymers, and this framework is embedded in a matrix of pectic polysaccharides, polygalacturonic acid and rhamnogalacturonan, the latter substituted with small polymeric side groups of arabinan, galactan, and arabinogalactan. Extensin molecules interlock the microfibrils into shape to prevent further stretching, and intramolecular covalent bonds among the extensin and other wall proteins signals the end of elongation (Ye and Varner, 1991).  
 Source: Carpita and Gibeaut (1993).

Most of the research on cell wall structure has been carried out on the primary walls of tissue other than fruit. However, it is generally thought that fruit cell walls have a three dimensional structure similar to that of other tissues (Knee and Bartley, 1981; Seymour *et al.*, 1993), though with a relatively high proportion of galacturonic acid, galactose and arabinose, suggesting that they are relatively rich in pectin. There is evidence that pectins from the middle lamella are less branched than those from the cell wall proper (Knee, 1978) and also in ripe tomato and apple, the pectic junction zones of their cell walls are single primary units because there is notably less calcium present (Jarvis, 1984).

### **1.8. Cell Wall Changes During Cooking**

Due to their domestic and economic importance the cooking behaviour of potatoes has been intensively studied for several decades by many workers (for example: Reeve, 1956, 1970, 1977; Keijbets and Pilnik, 1974; Loh *et al.*, 1982; Jarvis *et al.*, 1992). Other vegetables which have been studied, include Japanese radish root (Fuchigami, 1987a), snap beans (van Buren, 1983, 1984, 1986, van Buren and Pitifer, 1992; van Buren *et al.*, 1988), some legume seeds (Liu *et al.*, 1992; Bhatti, 1990). Virtually no information appears to be available for *Musa*. Since potatoes, bananas and plantains are cooked by similar methods (Marriot and Lancaster, 1983), and have a similarly starchy composition, the work with potatoes is likely to provide a most useful basis for comparison. However, it should be borne in mind that bananas and plantains are fruits whilst potatoes are derived from tubers (stem).

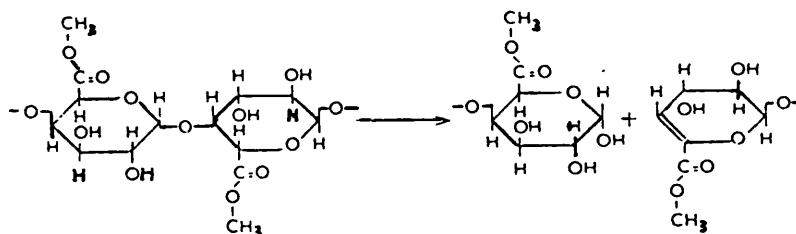
Texture (firmness, crispness, etc.) in fruits and vegetables is derived from the strength and thickness of the cell walls, the properties of the middle lamella and the content of the fibrous tissues (van Buren, 1979; Reeve, 1970). It is affected by such properties as the ease with which cells can be split open and the ease with which they can be separated from each other (Williams and Knee, 1980). These properties are determined predominantly by the cell wall and middle lamella respectively. Both cell separation and the cell wall softening contribute to diminishing the resistance of cooked tissue to cutting or biting, and their sum determine how soft the tissue will be.

Of the various components of the cell wall, pectic substances are particularly important in the adhesion which holds plant cells together. During the cooking of vegetables and fruits, pectin breaks down, leading to a release of pectic galacturonan from the cell walls, with accompanying cell separation and cell wall softening. The mechanism(s) of pectin breakdown are not fully understood.

Pectin depolymerisation resulting from heating under neutral and alkaline conditions is thought to take place by a  $\beta$ -elimination reaction with a transesterification via an E1cB mechanism (Pilnik and Voragen, 1970 and Keijbets and Pilnik, 1974; Figure 1.4). In this reaction the glycosidic bond between consecutive galacturonic acid molecules is split in conjunction with the formation of a double bond between the C<sub>5</sub> and C<sub>4</sub> of the newly formed non-reducing end group. The reaction requires that the carboxyl group of the residue undergoing  $\beta$ -elimination be esterified since this enhances the electron deficit in the C<sub>5</sub> position and hydroxyl ions speed the reaction by aiding the removal of H<sup>+</sup> from the C<sub>5</sub> position. Demethoxylated pectin substances



do not undergo the  $\beta$ -elimination and are relatively stable when heated under mild alkaline conditions, whereas  $\beta$ -elimination of methoxylated polygalacturonic acid is rapid even at room temperature in alkaline conditions. Heating speeds up the reaction so that even at pH 6.1 appreciable  $\beta$ -elimination takes place in 30 minutes at 100°C (Keijbets and Pilnik, 1974).



**Figure 1.4.** Splitting glycosidic bond by  $\beta$ -elimination (Pilnik and Voragen, 1970).

Hydrolysis of glycosidic bonds between neutral sugar residues occurs when pectic substances are heated under mild acid conditions. Splitting glycosidic bonds of pectic substances embedded in the wall matrix leads to increased solubility (van Buren, 1979).

The previous account described the  $\beta$ -elimination reaction *in vitro*. A similar reaction is thought to occur in the intact plant tissue, leading to softening of the tissue. The solubilization of the pectic materials during plant foodstuff cooking seems to involve two processes. The polymer molecules are first depolymerized by chain splitting via  $\beta$ -elimination. Once the chain length of the degraded parts is small enough and the

molecules are no longer bound in the framework of the cell wall gel by cations, pectic galacturonan is (partly) solubilized, resulting in the loss of texture (van Buren *et al*, 1988).

Sajjanantakul *et al* (1989) used isolated chelator-soluble pectin from carrot as a model component for an investigation of the *in vitro* heat degradation mechanism of pectin in an aqueous environment. The methyl ester content of the isolated pectin was modified from 0.45% to 96.69%. They confirmed that at pH 6.1 the pectin degraded primarily through  $\beta$ -eliminative cleavage of the polymer chain. The higher the ester content the greater the degradation. After having studied the cooking behaviour of Japanese radish root and 23 other kinds of vegetables including potatoes, Fuchigami (1987a, b) concluded that vegetables with higher methoxyl pectins soften more rapidly during cooking than those with lower methoxyl pectins, and concluded that this was because esterified pectins were more susceptible to the  $\beta$ -elimination depolymerization.

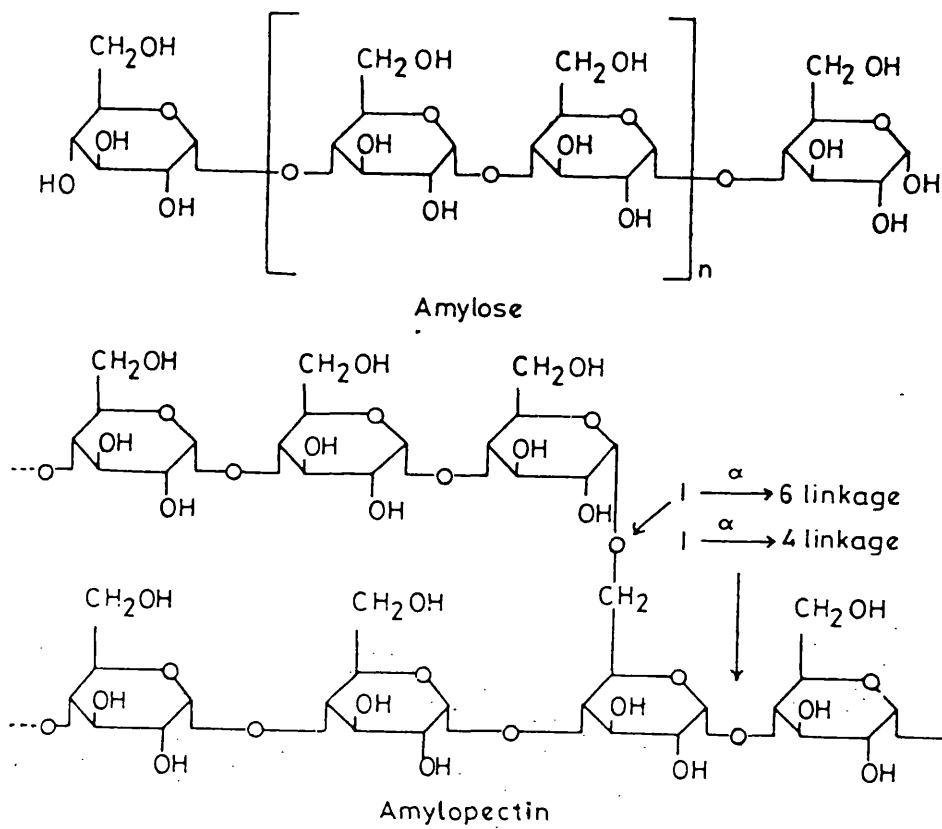
The finding that cold alkaline pectin de-esterification (1<sup>o</sup>C and pH12.5) before cooking can retard vegetable softening when cooked at neutral or slightly alkaline pH compared with control tissues (van Buren and Pitifer, 1992) is also consistent with the idea that  $\beta$ -elimination of methoxylated pectins is an important factor in thermal softening. The consensus view is that pectin makes a major contribution to heat-softening in snap beans, potatoes, apples and cauliflower.

## 1.9. Starch

### *1.9.1. Chemical composition and properties*

Starch consists largely of a mixture of two polysaccharides type, amylose and amylopectin, both of which are composed of D-glucose connected by  $\alpha$ -1,4 bonds. Amylose is essentially a linear polymer of (1 $\rightarrow$ 4)  $\alpha$ -linked D-gluco-pyranose residues with molecular weights ranging from 10-60 kD (Figure 1.5). In its native state amylose adopts a helical structure with 6 glucose residues per turn. It is in this form that amylose complexes with iodine to give a characteristic blue colour. Amylopectin consists of highly branched molecules, the branches occurring between C<sub>6</sub> of a glucose in the main chain ( $\alpha$ -1,4 glucan) and C<sub>1</sub> of the first glucose in the branch chain ( $\alpha$ -1,6 bonds) (Figure 1.5). The molecular weights of amylopectins vary from 10-500 million. Amylopectin reacts much less extensively with iodine, and exhibits a purple to red colour.

Starch is contained within discrete water insoluble granules, or grains in the native state. In storage organs, it accumulates in amyloplasts, and often one or more granules exists in one plastid-amyloplast. Most granules have an amylose core. Granules increase in size by adding concentric rings of material during growth and development. Within the layers, stacks of lamellae are formed comprising alternating crystalline (amylopectin) and amorphous (amylose) regions. The ratio of amylose and amylopectin is genetically controlled and varies between sources and with the maturity and development of the plant. The relative quantity of the two polymers is important in determining physical characteristics and the bioavailability of starches. Generally, amylopectin content in starch granules is about 75% and the rest being amylose



**Figure 1.5.** A schematic representation of the repeating units of starch molecules. Amylose and amylopectin are similar, except that amylopectin is much more branched (Phadnis and Jadhav, 1991).

One of the most important properties of starch is the formation of a viscous paste on cooking in water. Starch is insoluble in cold water but in the presence of heat ( $>50^{\circ}\text{C}$ ), the granules take up water, swelling to many times of their original volume, followed by rupture and starch solubilization. The critical temperature at which this occurs is known as the pasting or gelatinization temperature. During the gelatinization of a starch suspension the granules swell and impinge upon each other, causing the viscosity of the solution to increase. As swelling progresses, the inter-granule cohesive forces become weakened and the structure and viscosity of the paste are lost. The higher the amylose content, the stronger will be these cohesive forces, thus requiring higher temperature to complete the gelatinization (Banks *et al.*, 1970).

### ***1.9.2. Starch swelling during cooking of fruits and vegetables***

Cooking of potato tissue results in a series of structural changes which accompany the process of softening. Starch gelatinization *in situ* is observable by optical polarising microscopy during the early stage of cooking (Reeve, 1967; Shewfelt *et al.*, 1955) and it has been suggested that the pressure generated by starch swelling and gelatinization takes the place of turgor pressure when the integrity of the cell membrane is destroyed by heating. It has been suggested that tissue softening is a result of the pressure of the gelatinised starch tending to make the polyhedral shapes of the cells rounder, so that the cells tend to push apart or 'round-off' and hence separate (Reeve, 1970; 1977). However, Warren and Woodman (1974) suggested that the observed 'rounding-off' of the cells was the result, rather than the cause of cell separation. This hypothesis was supported by Bretzloff (1970) and Hoff (1972). Bretzloff (1970) observed that when an intact sample of potato tissue was examined during heating on a microscope stage,

the cells did not change their size although the individual cells in the cooking solution expanded and rounded up (Reeve, 1967). Hoff (1972) has also pointed out that “when a closed system consisting of water and suspended starch is heated to bring about starch gelatinization and then brought back to the original temperature, there is no observable increase in volume of the system”. This suggested that a substantial starch “swelling pressure” may not always develop when gelation of starch grains occurs in an intact tissue.

The reasons for these disagreements are perhaps due to the methods used for measuring cell size and starch swelling pressure in the cell tissue. Recently, Harada *et al* (1985a) measured the cell size changes of potato and other root vegetables before and after cooking and found that the cell size did increase due to heating. Jarvis *et al* (1992) used dialysis bags to heat the starch suspension inside at 100°C and proved that starch swelling pressure does exist, reaching around 100kPa. If a comparable starch swelling pressure may be generated within the cooked potato tissue, it would provide the force to separate the cells along the line of the weakened middle lamella between adjacent cells.

#### **1.10. Factors Influencing the Texture of Cooked Fruits and Vegetables**

The texture of fruits and vegetables is an important attribute of their quality. Interrelationships between tissue structure and composition provide a wide variety of textural qualities in processed fruits and vegetables (Reeve, 1970). The structural components of the tissue are altered by a number of factors, principally pH and the

amounts and type of salts present during the processing. The changes in structural components in turn leads to textural changes.

#### ***1.10.1. Pectin and its properties***

As discussed earlier, pectin is a very important cell wall component which determines the texture of a cooked plant foodstuff. Fuchigami (1987a, b) has studied the cooking behaviour of Japanese radish root and 23 other kinds of vegetables including potatoes. This worker concluded that vegetables with higher methoxyl pectins soften more rapidly during cooking than those with low methoxyl pectins, because esterified pectins are more susceptible to  $\beta$ -elimination depolymerization. Moreover, in some instances the increase in cell wall thickness caused by the hydration of pectins during cooking can be directly related to the degree of esterification of pectin (Warren *et al.*, 1975; Reeve, 1970). It has been suggested that methylation of cell wall pectin increases water uptake. This can reduce the cohesiveness of the wall matrix, soften the cell wall and decrease the intercellular adhesion (van Buren, 1979). This suggests that the presence of a high level of polyuronides (pectins) with high methoxylation in the cell wall material favour breakdown, and presumably *vice versa* (Warren and Woodman, 1974; Warren *et al.*, 1975; Sterling and Bettelheim, 1955).

A survey of several carrot genotypes by Greve *et al* (1994b) indicated that loss of texture in cooked carrots is accompanied by, and perhaps caused by, a substantial dissolution, depolymerization and destruction of cell wall pectins. Carrots with chelator-soluble pectins of relatively lower molecular weights tended to soften more extensively during heating. The viscosity of a pectin solution increases as the polymer

length increases. Also in the cell wall, increased pectin size might increase the possibility of an assortment of inter-polymer associations and, thus increase wall strength. This in turn could have an effect on tissue firmness. However, there have been no reports of varietal differences in overall pectin ester content. Therefore, pectin  $\beta$ -elimination cleavage may be only partially involved in carrot tissue softening during cooking.

It is possible to change the response of tissue to cooking by altering the pectin properties. For example in potatoes (Bartolome and Hoff, 1972; Hoff, 1972) and snap beans (van Buren and Pitifer, 1992) preheating (50-70<sup>0</sup>C) and cold alkaline treatments modify the cell wall pectin and reduce tissue breakdown. The firming by preheating at about 60<sup>0</sup>C is apparently due to the demethoxylation of pectin by pectin methylesterase (PME). This enzyme, present but rather inactive in most plant tissue, becomes active when the tissue is damaged by procedures such as heating to 50-80 <sup>0</sup>C, bruising or freezing. Bartolome and Hoff (1972) suggested that the cell membranes are made more permeable to cations during these treatments, thus allowing the salts to activate the cell enzyme. As a result of this an increase of demethylated, free carboxyl groups occur which increase the possibilities and the strength of Ca<sup>2+</sup> and Mg<sup>2+</sup>-induced binding between pectin polymers (Morris *et al.*, 1982; Jarvis, 1984). The decrease in the methyl ester content would also reduce the susceptibility to the degradation of pectin by  $\beta$ -elimination reaction during the subsequent cooking, therefore a firmer texture could be obtained.



Cold alkaline (NaOH, pH 12.5 at 1 °C) soaking of snap beans also results in pectin de-esterification by the same mechanisms, and hence the retention of a firmer texture after cooking. It has also been suggested that preheating-firming may be attributed to starch retrogradation and consequent reduction of “swelling pressure” (Reeve, 1972), though there is little evidence for this theory (e.g. Warren and Woodman, 1974).

### ***1.10.2. Starch and cellular structure***

Reeve (1967) reported that cellular structure and starch properties provide a distinction between varieties of potatoes and bear a strong relation to their culinary qualities. Potato varieties producing tubers with large cells, large starch granules, with higher content of starch and higher amylose composition are related to soft texture. These difference exist between different cultivars as well as within the different zones of one tuber.

High starch content may be associated with high “swelling pressure” due to starch gelatinization (Jarvis *et al.*, 1992), leading to mechanical separation of the rounded cells. So it has been suggested that the variation in starch “swelling pressure” in cultivars differing in starch content and composition, is the direct cause of their varying texture when cooked.

Conversely, Hoff (1972) suggested that high starch content would make less calcium available to the cell wall (Bretzloff, 1970) since a major portion of the total calcium of the potato tuber is present in the starch granules (Bartolome and Hoff, 1972). Therefore, the higher starch content is associated with soft texture because the starch

retains cell calcium, so that it cannot complex with the wall pectin, rather than because it is responsible for high 'swelling pressure'.

Starch may be important in other texture attributes. In both potatoes (Warren and Woodman, 1972, 1974) and cassava (Safo-Kantanka and Owusu-Nipah, 1992), the content and properties of starch are positively correlated with the quality known as "mealiness" when they are consumed after boiling. Mealiness is a textural character and refers to the feel in the mouth of the cooked tuber. It has been reported by many researchers that potato varieties which are mealy have relatively high starch content, high amylose content, and large starch granules (Gray, 1972; Warren and Woodman, 1974; Safo-Kantanka and Owusu-Nipah, 1992).

The cell size of the raw tubers is another important factor which is correlated with the texture of the cooked potatoes. Harada *et al* (1985b) pointed out that between the 21 potato cultivars examined, the smaller the cell size of the raw tissue, the firmer the tissue after cooking. This is because for the same relative volumetric expansion (Hoff, 1972), the stresses in the middle lamella of a 200  $\mu\text{m}$  diameter cell will be twice as large as the stresses developing in a 100  $\mu\text{m}$  diameter cell

### ***1.10.3. Inter-relationship of pectin and starch***

It seems unlikely that the thermal softening of potato and other starchy products such as cassava is due solely to the effects of heat on cell adhesion or due to starch swelling pressure. Reeve (1977) has suggested that the effects of heat on pectin and starch are both important in producing the structural changes that occur with cooking. Heating

causes cell wall adhesive components to break down, which leads to the cell adhesion becoming greatly diminished. Then, the 'swelling pressure' generated by the starch gelatinization within the cell content could apply the force to push the rounded cells apart along the weakened middle lamella (Jarvis *et al*, 1992). In this model, both the heat-lability of wall components and processes of starch swelling would interact to bring about these changes in tissue fracturability during cooking, and the differences in the balance between these components could be responsible for the difference in the effect of cooking on different tissues.

#### ***1.10.4. Cellular turgor***

Both cellular turgor and the integrity of cell walls are important components of the rigidity or firmness of plant materials (van Buren, 1979; Ilker and Szczesniak, 1990). On heating plant tissue above 60°C membrane integrity and consequently cell turgor is lost. The loss of turgor is particularly important in leafy vegetables due to heat-induced softening. Loh *et al* (1982) suggested that the loss of turgor pressure with heating appears less important in thick-walled parenchyma tissues usually derived from stems or roots where there is no replacement of turgor by starch gelatinization. However, Greve *et al* (1994a) suggested that the loss of turgor and membrane integrity could be significant in the early phase of firmness loss of carrot root disks, and showed that could be mimicked by plasmolyzing carrot tissues in mannitol solution. Therefore, the early rapid phase of cooking-induced firmness loss in carrots is a consequence of membrane disruption which eliminates the turgor component of texture.

#### ***1.10.5. Calcium and other salt treatments***

The addition of different salts to cooking water or their presence during a pre-cook treatment have the effects of enhancing (e.g., NaCl, EDTA, oxalate, etc.) or decreasing (e.g.,  $\text{Ca}^{2+}$ ) the rate of softening of plant tissues. It is generally accepted that such effects are due to changes in the degradation rate of the cell wall components (e.g., pectin) (Haydar *et al.*, 1980; Hughes *et al.*, 1975a; Keijbets *et al.*, 1976; van Buren, 1983, 1984, 1986; van Buren *et al.*, 1988). Although some workers (Reeve, 1970; Haydar *et al.*, 1980) have suggested that calcium simultaneously interacts both with cell wall pectin and gelled starch, the simplest explanation for the effect on cooking behaviour is the interaction of calcium with pectic substances of the cell wall and particularly the formation of calcium bridges (Morris *et al.*, 1982).

Displacement of calcium from pectin decreases its cementing efficiency and reduces the tissue firmness. Monovalent cations such as  $\text{Na}^+$  and  $\text{H}^+$  (low pH cooking), and also divalent cations such as  $\text{Mg}^{2+}$  which are capable of displacing calcium have shown to cause softening of cooked snap beans and other cooked vegetables including potatoes (van Buren, 1984). In addition, chelating agents such as EDTA and some organic acids, particularly citrate and phytate (which are probably released from the cell vacuole during cooking) can also cause softening due to their good calcium binding properties.

### **1.11. Goals and Objectives of this Project**

This project is concerned with the investigation of the biochemical and physical changes which are responsible for softening during cooking of bananas and plantains, and which may underlie the differences between cultivars in their response to cooking.

The approach is based on an understanding of the factors which influence the textural changes in other cooked plant materials. Thus a series of chemical measurements were undertaken before and after cooking, including cell wall and its components such as pectin and hemicelluloses both *in vitro* and *in vivo*, starch content and composition and calcium content in different genotypes. Microscopic observations with light, scanning and transmission electron microscopes were also made during cooking. By investigating the biochemical, cellular and cell wall structural changes during heating, it was hoped that certain pre-cooking characteristics could be identified which would provide predictors of the cooking properties of bananas and plantains, and provide specific breeding objectives for modification of the cooking quality of the fruit.

**CHAPTER 2**  
**GENERAL MATERIALS AND METHODS**

## **2. GENERAL MATERIALS AND METHODS**

### **2.1 Experimental Materials**

Green dessert banana (*Musa*, AAA group), plantain (*Musa*, AAB group) and Cocoye cooking banana (*Musa*, ABB group) were obtained from commercial sources. All were at their green mature stage, but no information was available on their history. They were stored at 14°C and used within 2-3 days. Different batches of dessert bananas had relatively little variations in terms of their origin, appearance, quality and maturity. However, the Big Ebanga plantains used for different salt solution treatments were slightly dehydrated (Figure 3.10 and 3.11).

Individual, undamaged fruits were selected. The middle region of each fruit was sliced into 10-mm thick sections and peeled. Six sections, at least, from 6 different fruits were used for each treatment.

### **2.2 Thermal Treatments**

The sections were immersed either in preheated distilled water or a salt solution in 250-ml beakers, using a ratio of about sample:water of 1:3 (by weight). The beakers were placed in a bath of boiling water and heated for various periods of time. At the end of each treatment the cooking solution was poured out and the sections were allowed to cool to room temperature. Compressive strength was then estimated, and the sections were then freeze dried for further chemical measurements.

### **2.3 Texture Measurements**

The compressive strength of the sections was measured by rupture force using a penetrometer fitted with a rounded 6 mm diameter probe. The probe was fitted to a

bench top pressure tester with a Salter 0-10 kg electronic force gauge. Two to three measurements were taken from each section. The value recorded for the rupture force was the average force for the probe to penetrate the pulp sections to depth of 5 mm.

#### **2.4 Alcohol Insoluble Residues (AIR) Preparation**

Known weights of either raw or cooked banana and plantain pulp sections were cut into small pieces and freeze dried to constant weight, then ground to fine powder and passed through a 250- $\mu\text{m}$  sieve. A sample (2 grams) of the powder was weighed into a cellulose extraction thimble and refluxed with 80% alcohol for 3 hours in a Soxhlet extractor. The resultant alcohol insoluble residues were dried in a warm oven (60 °C) to constant weight. The AIR were stored in a desiccator at -20 °C for subsequent determination of their chemical constituents.

#### **2.5 Scanning Electron Microscopy (SEM)**

The parenchyma tissue of raw and cooked dessert banana, plantain and cooking banana pulp were cut into approximately 3x5x5 mm pieces and frozen in liquid nitrogen before being fractured with razor blades according to Huang *et al* (1990). Fractured samples were then freeze-dried for 24 hours. The temperature of the condensing plate was maintained at -80 to -60 °C. The dried samples were mounted on aluminium dishes and sputter coated with gold for 3-5 minutes. The fractured surfaces of samples were examined with Joel T330 SEM and Joel 6310 microscopes operating at 10-15 kV.



## **2.6. Estimation of Uronic Acid**

The uronide content of the extraction was estimated colorimetrically by the m-phenylphenol method (Blumenkrantz and Asboe-Hansen, 1973) with neutral sugars correction (Kintner and van Buren, 1982). Galacturonic acid was used as a standard. A tube containing 0.4ml aliquot of the supernatant was cooled in an ice-water bath before 2.4 ml of chilled 0.0125M borax/conc.  $H_2SO_4$  was added. The contents were thoroughly vortex-mixed, then heated at  $100^{\circ}C$  for 5 minutes on a dri-heating block. The tube was then cooled in an ice-water bath. 40  $\mu$ l of 0.05% m-phenylphenol in 0.5% NaOH was added and mixed well. A blank was also prepared in the same manner except only 0.5% NaOH was added. The colour was allowed to develop for 20 minutes in the dark, then the reading was made at 520 nm using an UV visible spectrophotometer (see appendix 1).

## **CHAPTER 3**

### **TEXTURAL, CHEMICAL AND MICROSCOPIC CHANGES DURING *MUSA* COOKING: PRELIMINARY STUDIES**

### **3. TEXTURAL, CHEMICAL AND MICROSCOPIC CHANGES DURING *MUSA* COOKING: PRELIMINARY STUDIES**

#### **3.1. Introduction**

Although banana and plantain are very important staple food commodities which are consumed mainly in the cooked form, there is very little information available on their cooking behaviour. This chapter aims to examine the general pattern of effects of heating on different *Musa* genotypes. The effects of different temperature and different salt solutions during cooking on pulp texture changes were also investigated.

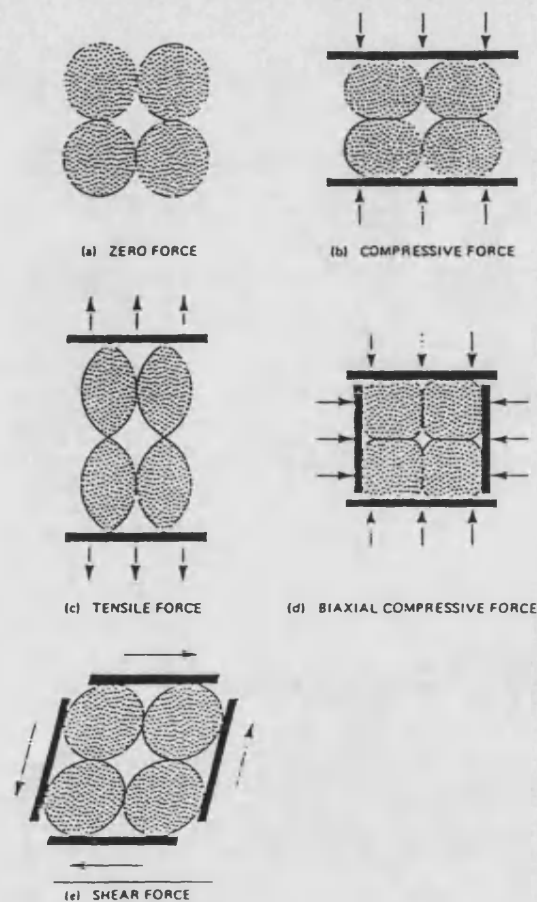
In order to examine the acceptability of seven new disease resistant tetraploid banana clones in Jamaica, Baldry and Dempster (1976) studied their cooking behaviours along with two well known Cavendish banana clones 'Lacatan' and 'Valery' as controls, using a taste panel of 12 people. They reported that among the clones tested the panellists greatly preferred the softer ones to the harder ones, and texture was considered to be more important than flavour. Similar work was also carried out on three plantains and two cooking bananas by Almazan (1990) to test if the palatability of cooking bananas and plantains is comparable. The main conclusion of these investigations is that texture is the most important attribute of the cooked product in determining a good cooking banana or plantain. A short cooking time to reach the adequate softness is an advantage where fuel is very scarce as is the case in many tropical countries. Nevertheless, the hardness of the uncooked fruit is obviously an advantage in the export trade, as it leads to less handling damage during transportation (Baldry and Dempster, 1976). However, no further studies, for example chemical and

microscopic observations of the effects of cooking on bananas and plantains, appear to have been conducted.

Texture is normally assessed subjectively by a taste panel, where preferences must depend on the interaction between a number of physical characteristics and consequently on a number of different chemical constituents (Linehan and Hughes, 1969a). Therefore, it is difficult to determine a causal relationship between texture and the level of any particular chemical constituent. To measure the texture objectively, a simple penetrometer or puncture tester (Sharma *et al.*, 1959; Linehan and Hughes, 1969b&c; Baldry and Dempster, 1976; Smith, 1989), and an instrument for measuring compressive strength (Sterling and Bettelheim, 1955; Hughes *et al.*, 1975a & b) are the two main types of instrument which have been used. It has been shown that both instruments determine the same physical characteristic of intercellular adhesion (Linehan and Hughes, 1969b & c).

Jarvis and Duncan (1992) and Freeman *et al* (1992) used a thin stainless steel wire to measure the softness of cooked potatoes to devise texture testing methods that could be used by Third World potato breeders and would require a minimum of complex apparatus. Harada *et al* (1985a & b), Harada and Paulus (1987) and McComber *et al* (1987) preferred a universal testing machine to measure the maximum shear force to characterise the effects of thermal treatment to the texture of potatoes, as well as other root vegetables.

Compressive force, shear force and tensile force all measure the external forces required to deform the plant tissue, i.e., tissue failure (Figure 3.1). Among them compression has been the preferred force mode for failure studies. Plant tissues appear to undergo at least three types of compressive failure: cell wall rupture, intercellular de-bonding and cell relaxation due to the migration of fluids out of the cells (Diehl *et al.*, 1979; Holt and Schoorl, 1982).



**Figure 3.1** Two-dimensional models of parenchyma tissue deformed under different external forces (McLaughlin, 1987)

The kinetics of thermal softening of fruits and vegetables, in particular potatoes, have been studied by many researchers, and the rate of softening of plant tissue during heating generally follows first-order kinetics (Loh and Breene, 1981; Harada *et al.*, 1985a & b; Huang and Bourne, 1983; Rao and Lund, 1986; Harada and Paulus, 1987). Loh and Breene (1981) and Harada *et al* (1985a) have clearly demonstrated a logarithmic relationship between objective textural parameters and heating time at a constant temperature. However, the measurements made to describe the textural behaviour of foodstuffs are highly subjective in nature, and the cooking kinetics have been found to be more complex than assumed so far (Huang and Bourne, 1983).

Having studied 11 selected fruits and vegetables, Loh and Breene (1981) found that, in general, the first-order kinetic model is adequate in describing their fracturability loss before reaching equilibrium texture, and is a better predictor for the textural changes on heating in tissues having thin cell walls (such as those derived from fruits) than in tissues having thick cell wall (such as those derived from stems and roots). This was confirmed later by Huang and Bourne (1983). It has been shown that, for a given species (potato), the first-order model can be applied regardless of sample size and morphological origin of the sample, heating temperature, variety, prior storage conditions, maturity and minor agronomic variations (Loh and Breene, 1981; Harada *et al.*, 1985b).

The aim of the experiments described in this chapter was to use a broad spectrum of *Musa* varieties to study their texture changes. These changes were measured by a penetrometer during heating. Various cytoplasmic components such as starch,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , as well as changes in the cell wall which may be responsible for differences in thermal softening were also assessed. Meanwhile, the effects on texture of different treatments, such as different salt solutions and different temperatures cooking were investigated.

## **3.2. Materials and Methods**

### ***3.2.1. Materials***

Dessert banana (*Musa*, AAA group), plantain (*Musa*, AAB group, possibly Big Ebanga, hereafter termed “Big Ebanga type”), horn plantain (*Musa*, AAB group) and Cocoye cooking banana (*Musa*, ABB group) were all obtained from commercial sources. Their history are unknown. Sample preparation for thermal treatments, textural measurements, SEM and alcohol insoluble residue (AIR) preparations were carried out as previously described (section 2.1-2.4).

### ***3.2.2. Calcium and magnesium measurement***

Calcium and magnesium in raw and cooked materials were determined by atomic absorption spectrophotometry (AAS).

### ***3.2.3. Different temperature and salt solution treatments***

Dessert banana pulp sections (or discs) were subjected to different temperature treatments using temperature controlled water baths. The temperatures were 60°C, 70°C, 80°C, 90°C and 100°C. The duration of cooking was 10 minutes for each temperature treatment. Dessert banana and plantain (Big Ebanga type) pulp sections were cooked in the same manner with 0.5% CaCl<sub>2</sub>, 0.5% NaCl and 0.5% EDTA at 100°C for 15 minutes.

### ***3.2.4. Starch determination***

The starch content of the AIR was determined using the ferricyanide colorimetric method (Rickard, 1992). The starch was acid-hydrolysed with 0.7 M hydrochloric

acid at 100°C for 2.5 hours and then neutralised with diluted NaOH solution. Solid materials were removed by filtering the hydrolysate through glass fibre filter paper. The starch in the original sample was determined as reducing sugar by boiling a 1 ml aliquot with 4 ml of potassium ferricyanide reagent for 15 minutes then cooled to room temperature. The reading was made at a wavelength of 380 nm using glucose as a standard (see appendix 2).

### ***3.2.5. Amylose and amylopectin determination***

Amylose was extracted from AIR and determined spectrophotometrically according to McCready *et al* (1950) and Jarvis and Walker (1993), respectively. 20 mg of AIR solid was weighed into a 15-ml test tube. To the residue, 1 ml of distilled water was added, the contents were cooled in an ice water bath, and 1.3 ml of 52% perchloric acid (cold) added while stirring. After stirring for about 5 minutes and occasionally thereafter for 25 minutes, keeping the mixture cold, 4 ml of water was added and the mixture centrifuged. The supernatant was poured into a 25-ml volumetric flask. 1.3 ml of perchloric acid reagent was added to the residue and allowed to hydrolyse as before for a further 30 minutes at 0 °C with occasional stirring. The contents of the tube were then washed into the same flask containing the first extract. The combined solution was diluted to 25 ml and filtered through GF-A glass fibre paper.

A 0.5 ml sample of the hydrolysate was pipetted into a 10-ml volumetric flask, and 5 ml of dH<sub>2</sub>O added and mixed well. 0.1 ml of iodine-potassium iodide reagent (0.2% I<sub>2</sub> in 2% KI) was added and, the whole diluted to 10 ml with constant stirring. After 15 minutes the absorbance was read at each wavelengths of 504, 548, 630, 700 and 800



nm, using an iodine blank containing the same amount of perchloric acid as that in the sample to be analysed.

Amylose (type III from potato, amylopectin free) and amylopectin (from potato) for use as standards were prepared in the same manner as the samples. Mixtures of amylose and amylopectin in ratios of 5:95, 10:90, 15:85, 20:80, 30:70, 40:60, 50:50, 60:40 and 70:30 totalling 10 µg/ml were used to determine the absorptivity of amylose and amylopectin at each wavelength (see appendix 3 for calculations).

### ***3.2.6. Estimation of total polyuronides (pectin) content of AIR***

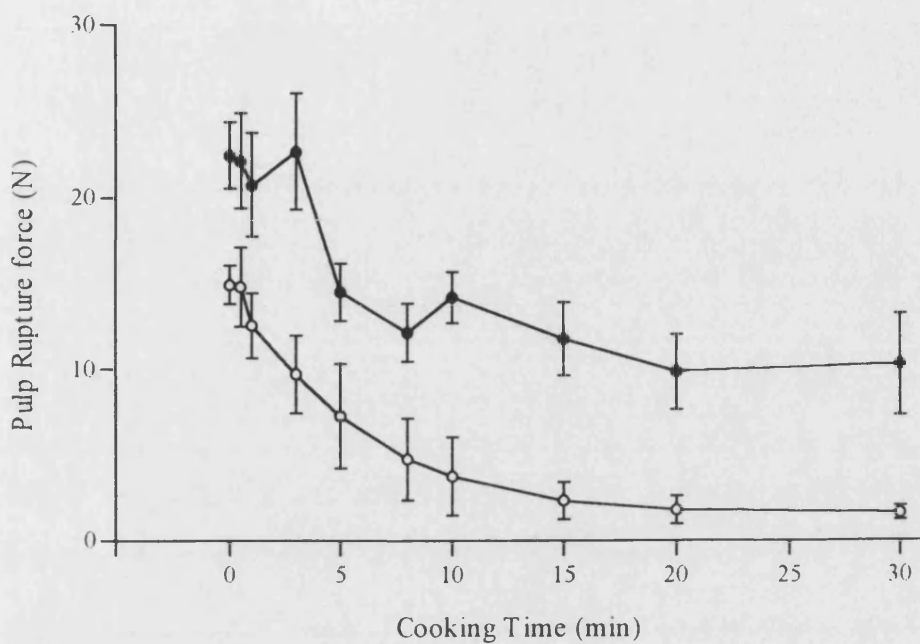
Total polyuronide content of AIR was estimated by stirring 40 mg of the AIR at 100°C with 1 M HCl acid for 2 hours, then neutralising with NaOH solution (Jarvis, 1982). An aliquot of the resulting solubilised material was then assayed for uronic acid content as described in section 2.6.

### 3.3. Results

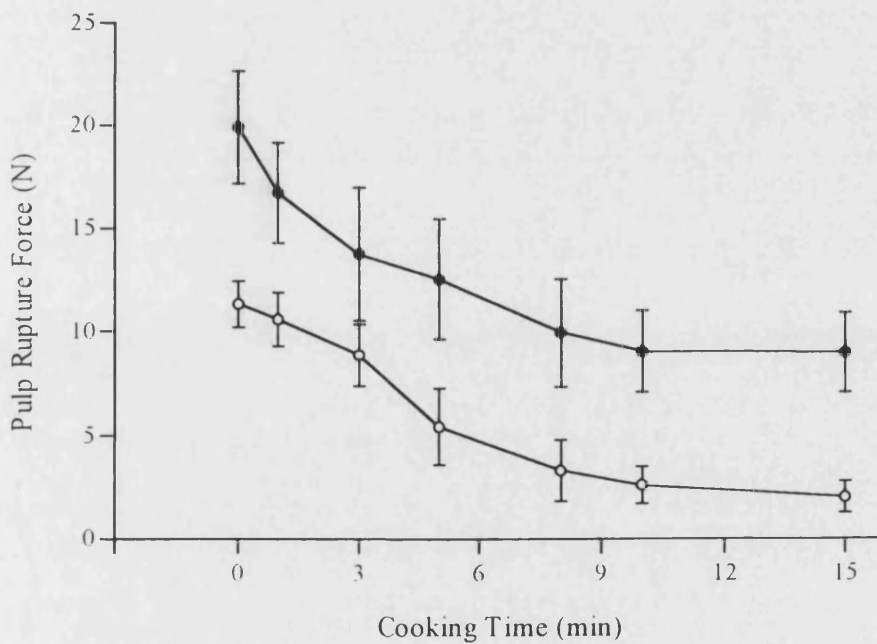
#### 3.3.1. Textural changes during cooking of *Musa*

The textural changes occurring during cooking (as measured by rupture force) of plantain (Big Ebanga type) and dessert banana, and also horn plantain and Cocoye cooking banana as pairs are shown in figure 3.2 and figure 3.3, respectively. The raw plantain pulp (Big Ebanga type) was much harder than raw dessert banana. The firmness of dessert banana pulp decreased sharply during the first 10 minutes of cooking, from 15N to 3.8N, i.e., 75% of the original firmness was lost after this period of time. Plantain showed a similar tendency, however firmness decreased from 22.5N to 14N, i.e., only 37% of the original firmness was lost after 10 minutes of cooking. Therefore, plantain softened at a slower rate than banana and the plantain pulp remained firmer throughout. Little change in firmness took place in either type of fruit after a further 10 min of cooking, i.e., after 20 min of cooking (figure 3.2).

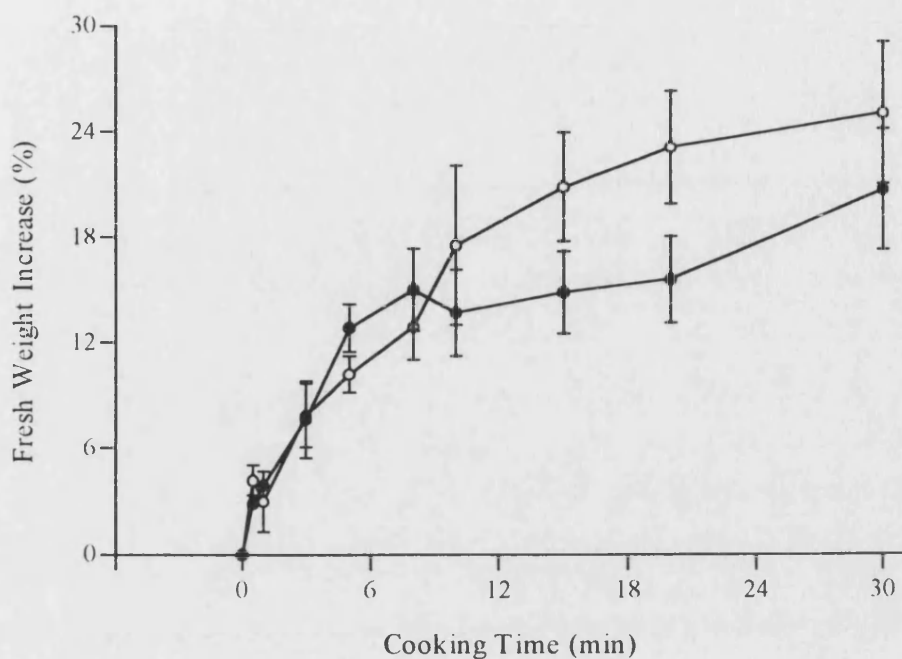
Figure 3.3 indicates that the raw Cocoye cooking banana was much softer than the raw horn plantain, and it softened much faster than horn plantain during cooking. After 10 minutes of cooking, 82% of the original firmness of Cocoye cooking banana was lost whilst only 55% of that of horn plantain was lost. These two figures show that there are significant difference between different *Musa* genotypes in their textural changes during cooking.



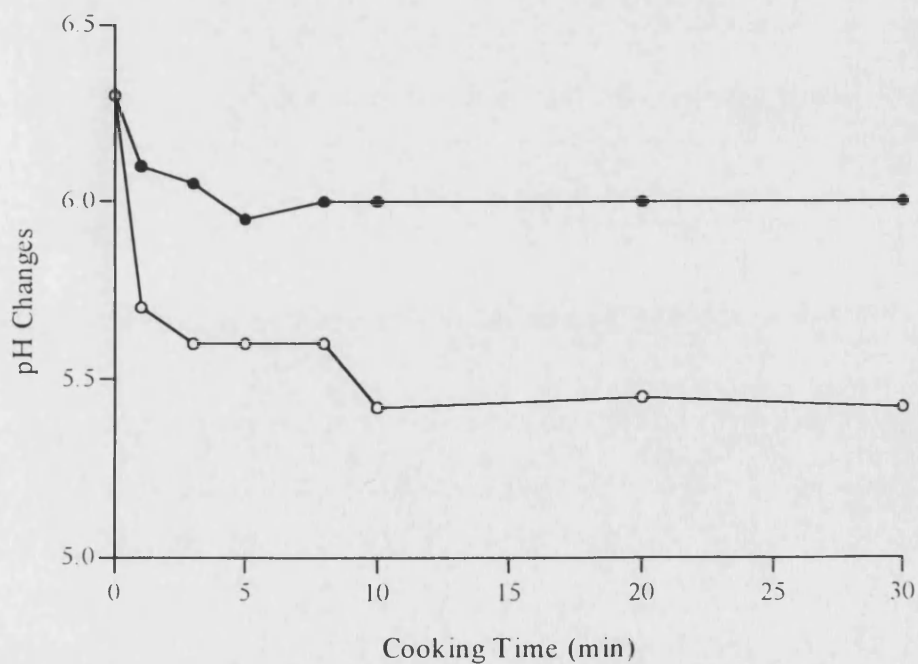
**Figure 3.2** Dessert banana and plantain (Big Ebanga type) pulp rupture force changes during cooking. Error bars show standard deviation on mean (mean±SD, n=12). N=Newton; -o - Dessert banana, -● - plantain (Big Ebanga type).



**Figure 3.3** Pulp rupture force changes during cooking of Cocoye cooking banana and horn plantain. Error bars show standard deviation on mean (mean±SD, n=20). -o -Cocoye cooking banana, -●- Horn plantain.



**Figure 3.4.** Dessert banana and plantain (Big Ebanga type) pulp water uptake during cooking. Error bars show standard deviation on mean (mean $\pm$ SD, n=6). -o- Dessert banana, -●-plantain (Big Ebanga type).



**Figure 3.5** pH value in the cooking solution changes during cooking. -o- Dessert banana, -●- plantain (Big Ebanga type).

### ***3.3.2. Changes of pulp water uptake and pH in the cooking solution during cooking***

Figure 3.4 indicates that dessert banana and plantain (Big Ebanga type) both took up water rapidly during cooking. In the first 3 minutes of cooking, they showed a similar water uptake rate with 7.9% and 7.6% increase of fresh weight, respectively. Between 5 and 8 minutes of cooking, plantain absorbed water more than banana, the former was 12% after 5 minutes and 15% after 8 minutes, but the latter 10.2% after 5 minutes and 12.9% after 8 minutes. Thereafter, dessert banana caught up and took up water much faster than plantain (Big Ebanga type) did. After 30 minutes of cooking, dessert banana pulp fresh weight had increased by 25% whilst plantain pulp fresh weight increased by 20.7%. Figure 3.5 shows the pH changes in the cooking solution of both dessert banana and plantain (Big Ebanga type). The values are seen to drop from 6.3 to 5.5 in banana, and from 6.3 to 6.0 in plantain after 30 minutes of cooking. pH drops quickly during the first 5 (plantain) or 10 (banana) minutes of cooking. Thereafter, no change occurred with either type of fruit.

### ***3.3.3. Changes of pectin content in the cooking solution and the remaining cooked materials during cooking***

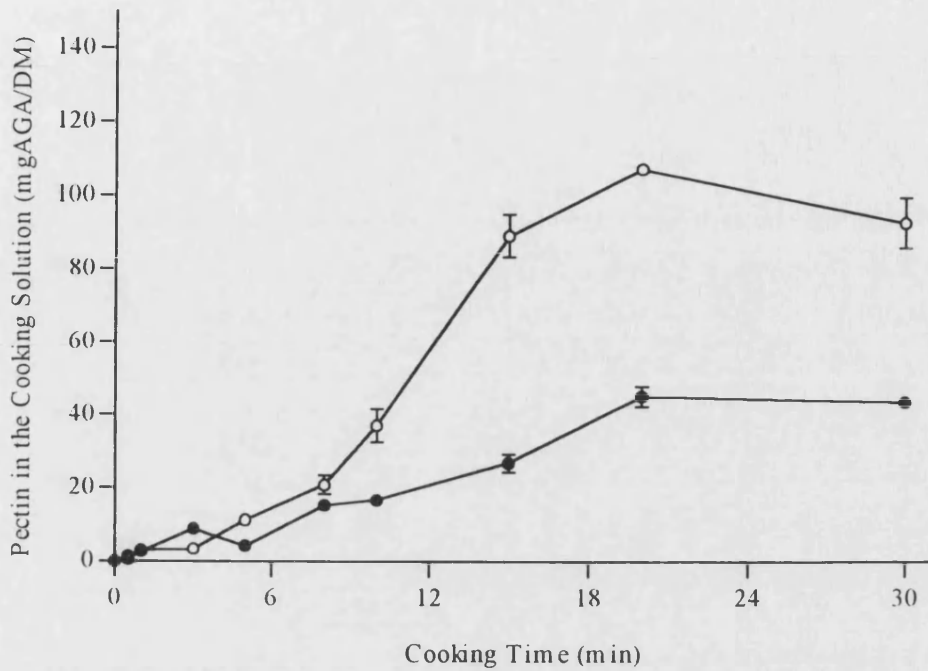
The amount of pectin (as measured by galacturonic acid) released into the cooking solution increased progressively throughout the first 20 minutes of cooking, then remained at the same level (Figure 3.6). In dessert banana, the rate of increase of the pectin in the cooking solution is larger between 8 and 20 min than that between 0 and 8 min of cooking. The rate of pectin release for plantain is however not so great between 8 to 20 minutes (Table 3.1). Dessert banana released approximately twice as

much pectin as plantain did into the cooking solution. After 20 minutes of cooking, the dessert banana had released 100mg anhydrous galacturonic acid per 100g dry matter into the cooking solution compared to only 45 mg from plantain.

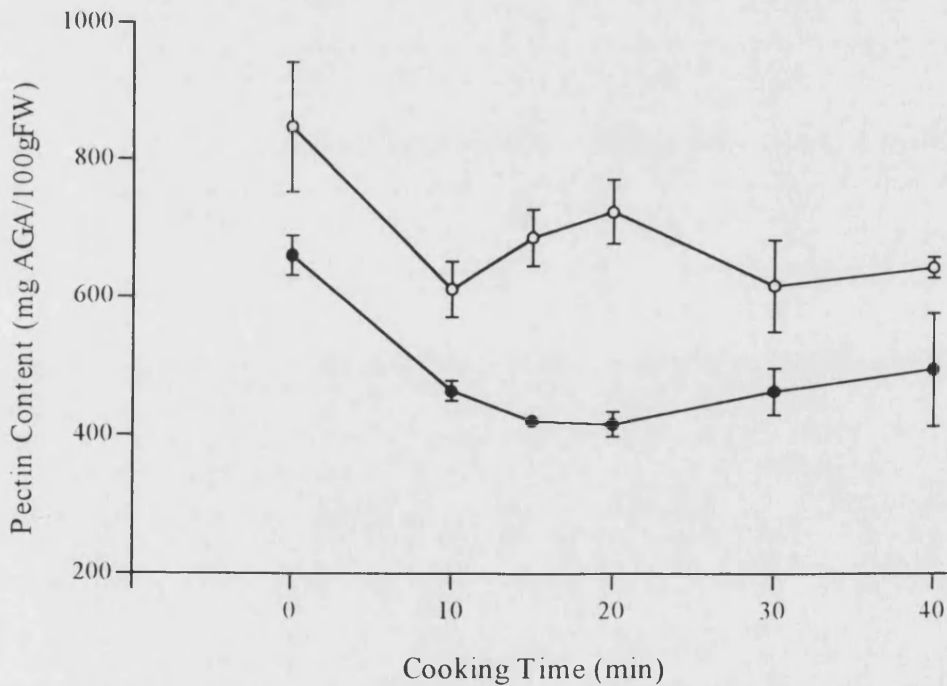
Cooking time (min)	Amount (mgAGA/100g DM)		Rate of release (mgAGA/min)	
	Dessert banana	Plantain	Dessert banana	Plantain
0	0	0		
8	20.96±2.54	15.25±0.64	2.62	1.91
20	107.37±0.39	45.03±2.78	5.37	2.25

**Table 3.1.** Rate of pectin release into the cooking liquor of dessert banana and plantain. AGA= anhydrous galacturonic acid; DM= dry matter.

Figure 3.7 show the changes in pectin of the pulp tissues of dessert banana and plantain (Big Ebanga type) during cooking. It appears that the total pectin content of raw banana pulp is much higher than that of plantain with 848 mg AGA/100g FW and 660 mg AGA/100g FW, respectively, and the pectin content remained higher in banana than plantain throughout. The pectin content decreased sharply in the first 10 minutes of cooking in both of banana (28%) and plantain (30%). Little change in pectin content was found during further cooking.



**Figure 3.6.** Changes pectin content in the cooking solution during cooking. Error bars show standard deviation on mean (mean $\pm$ SD, n=4). \*AGA=anhydrous galacturonic acid, DM=dry matter. -o- Dessert banana, -●-plantain (Big Ebanga type).



**Figure 3.7.** Effect of heating on total polyuronides content changes in the remaining cooked materials during cooking. Error bars show standard deviation on mean (mean $\pm$ SD, n=4). FW=fresh weight. -o- Dessert banana, -●-plantain (Big Ebanga type).

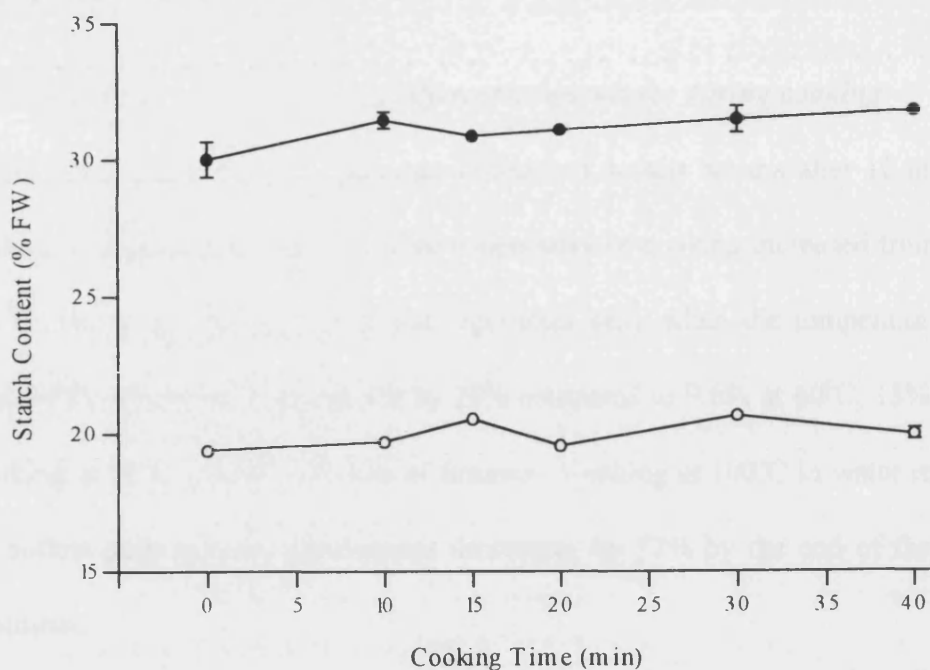
### 3.3.4. Starch, calcium and magnesium changes during cooking

Plantain (Big Ebanga type) pulp tissue contained much higher starch and dry matter than dessert banana. The dessert banana contained 19.4% starch and 26.3% dry matter, whilst plantain contained 30% starch and 37.6% dry matter (Table 3.2). Differences in composition were also found in Cocoye cooking banana and Horn plantain, i.e., horn plantain contained much higher starch and dry matter (32.2% and 37.6% respectively) than Cocoye cooking banana (22.2% and 28% respectively) (Table 3.2). Plantain (Big Ebanga type) contains higher amylose (33.3%) than dessert banana (27.4%). However, dessert banana contains more total polyuronides (HCl-soluble) than Big Ebanga plantain does. During cooking the starch content of both dessert banana and plantain (Big Ebanga type) pulp remained constant (Figure 3.8).

	Dessert banana	Plantain (Big Ebanga)	Cocoye cooking banana	Plantain (Horn)
Dry matter (% FW)	26.31±0.37	37.62±0.45	27.92±0.69	37.59±0.27
Starch (% FW)	19.39±0.69	30.02±0.63	22.24±0.15	32.15±0.02
Amylose (% total starch)	27.35±0.93	33.37±0.86	n.d. <sup>b</sup>	n.d. <sup>b</sup>
Polyuronide (AGA <sup>a</sup> % FW)	0.85±0.04	0.66±0.03	n.d. <sup>b</sup>	n.d. <sup>b</sup>

**Table 3.2.** Chemical composition of dessert banana, Big Ebanga plantain, Cocoye cooking banana and Horn plantain. Values are means±SD of four measurements (n=4). AGA<sup>a</sup>= anhydrous galacturonic acid; n.d.<sup>b</sup>= not determined





**Figure 3.8.** Changes of starch content in the remaining cooked materials during heating of dessert banana and plantain (Big Ebanga type). Error bars show standard deviation on mean (mean $\pm$ SD, n=4). -○-Dessert banana, -●- plantain (Big Ebanga type).

Table 3.3 shows that Cocoye cooking banana pulp tissue contains much higher levels of calcium and magnesium than horn plantain. After 15 minutes of cooking, magnesium decreased in both horn plantain and Cocoye cooking banana, and the latter showed a much greater decrease than the former. The calcium level remained the same during cooking in both fruits.

		Raw	1 min	3 min	5 min	8 min	10min	15min
Cocoye cooking banana	Ca (mg/100gDM)	24	25	25	25	27	26	23
	Mg (mg/100gDM)	116	120	119	115	103	101	94
Horn plantain	Ca (mg/100gDM)	6	7	7	6	7	7	7
	Mg (mg/100gDM)	60	65	65	62	60	60	58

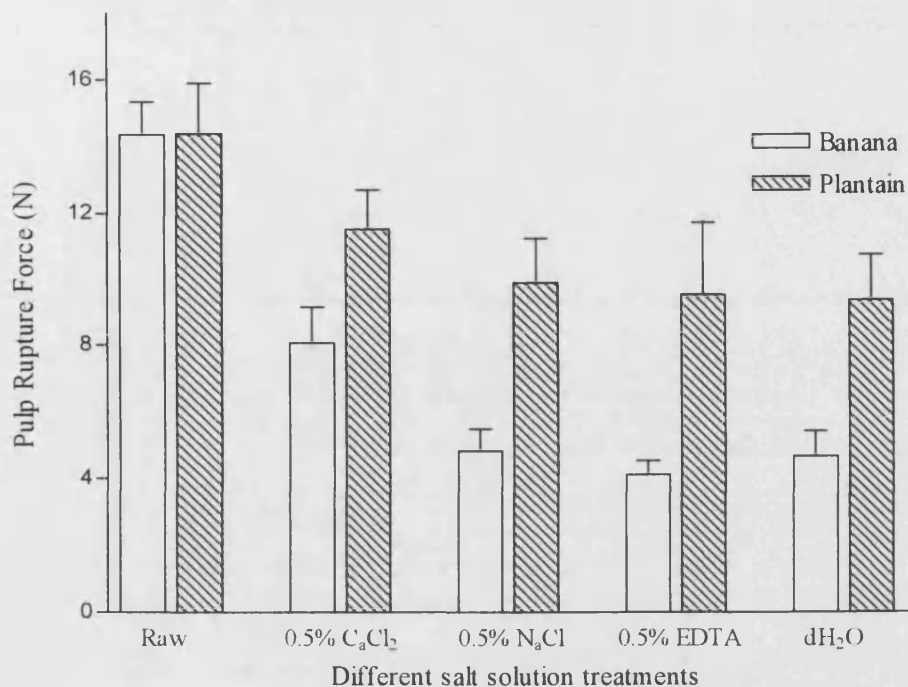
**Table 3.3.** Changes in calcium and magnesium during Cocoye cooking banana and Horn plantain cooking. Values are means of two measurements.

### ***3.3.6. The effect of different salt solutions on cooking of dessert banana and plantain (Big Ebanga type)***

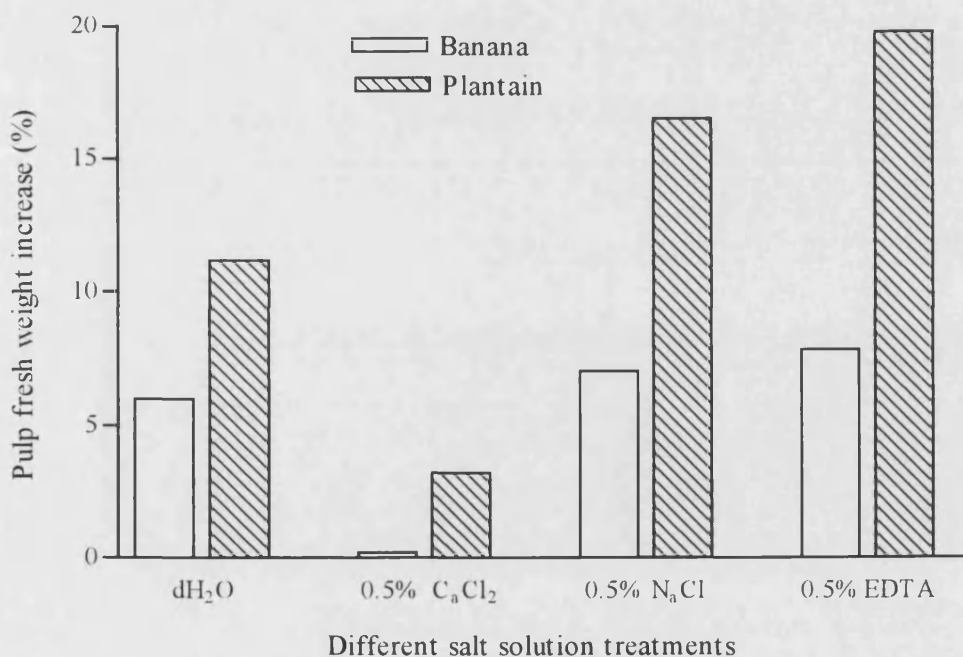
The use of different solutions for the cooking of dessert banana and plantain (Big Ebanga type) pulp showed that when 0.5% CaCl<sub>2</sub> was present in the cooking medium, the tissue retained significantly more firmness compared to cooking in distilled water, 0.5% NaCl or 0.5% EDTA solution (Figure 3.9). In dessert banana, cooking in CaCl<sub>2</sub> solution caused only 44% loss of the original firmness compared to 67.5% in distilled water, 66.4% in NaCl and 71.4% in EDTA solution. A similar tendency was also found in plantain where only 25.7% of firmness was lost after the pulp tissue was cooked in 0.5% CaCl<sub>2</sub> compared 35% in distilled water, 31.4% in NaCl and 34% in EDTA solution.

Cooking in 0.5% CaCl<sub>2</sub> solution also resulted in reduced water uptake in both banana and plantain (Figure 3.11). This was particularly apparent in banana in which the fresh weight of the pulp tissue increased by only 0.2% after 15 minutes of cooking in CaCl<sub>2</sub> solution compared 6% in water, 7% in NaCl and 7.8% in EDTA solutions, whilst in plantain the pulp fresh weight increased by 3.2%, 11%, 16.5% and 20% in CaCl<sub>2</sub>, dH<sub>2</sub>O, NaCl and EDTA, respectively. It seems that EDTA was the most effective agent in enhancing softening and increasing water uptake in both banana and plantain.

In each type of fruit, treatments that resulted in softening also resulted in most water uptake. However, although in each treatment plantain softened less than banana, the water uptake was greater. This is possibly due to the plantains being slightly dehydrated before cooking.



**Figure 3.10.** Effect of different salt solution on pulp rupture force changes during cooking of dessert banana and plantain (Big Ebanga type). The cooking time for all the treatments is 15 minutes. Error bars show standard deviation on mean (mean $\pm$ SD, n=15).

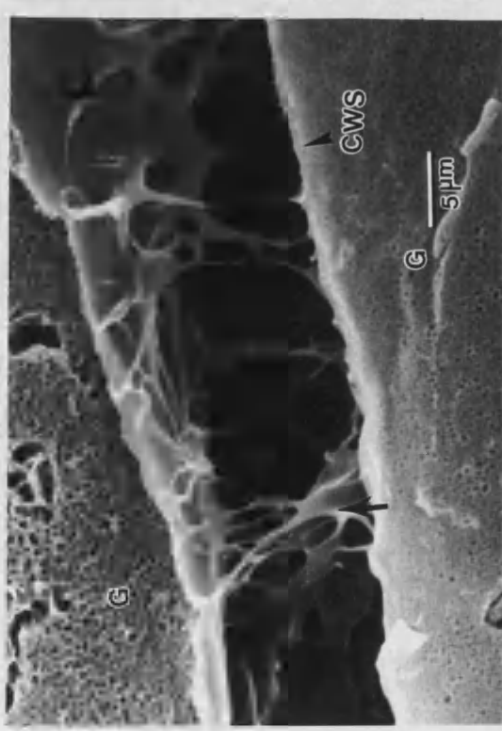
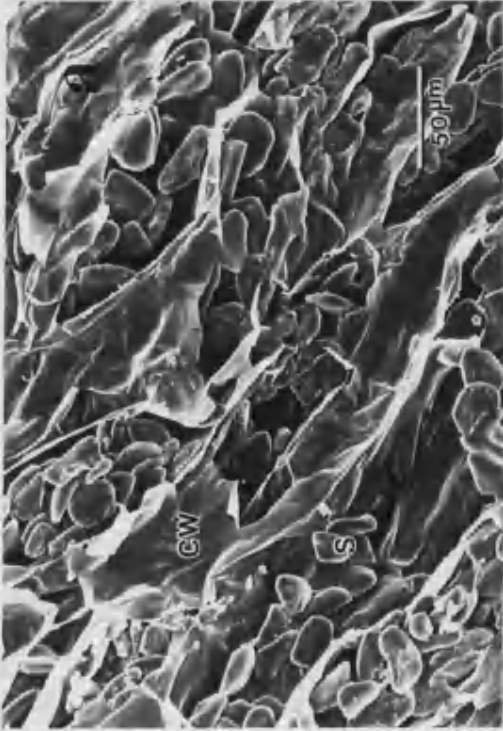
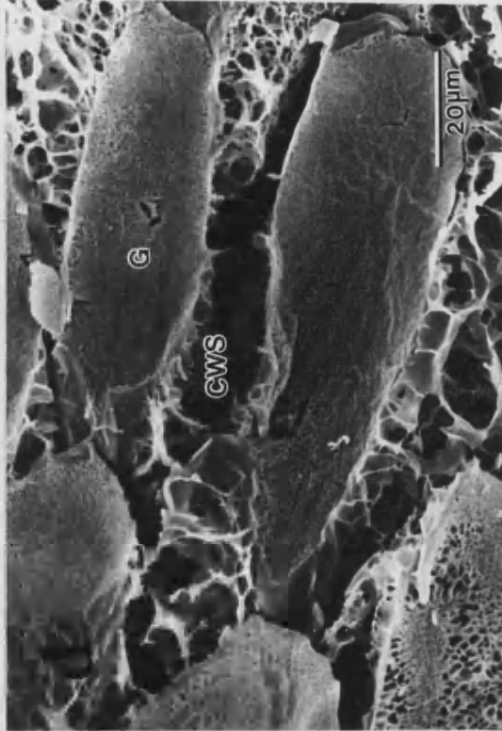


**Figure 3.11** Effect of different salt solution on water uptake by the pulp during cooking. The cooking time for all the treatments is 15 minutes. Each bar shows the mean of 5 measurements.

### ***3.3.7. SEM observations of dessert banana pulp tissue during cooking***

Figure 3.12 shows the gradual changes in dessert banana pulp parenchyma tissues of raw, 5, 10 and 20 minutes cooked materials. In the raw sample, the cell walls are smooth, and starch granules are also smooth and unfused (Figure 3.12a). As the tissue was subjected to heat for 5 minutes, cell wall separation became evident. The granular integrity of individual starch grains was replaced by large masses of gelatinized gels (Figure 3.12b). Prolonged cooking resulted in more cell wall separation, and meanwhile the presence of intercellular materials became apparent (arrows, Figure 3.12c & d). More detailed SEM studies were later carried out on both dessert banana and plantain (Big Ebanga type) using more intervals within the first 10 minutes of cooking. These ultrastructural studies are described in more detail in Chapter 4.

**Figure 3.12.** SEM micrographs of dessert banana pulp parenchyma tissue before and after cooking. Arrows in (c)&(d) indicate the presence of intercellular materials. (a), Raw; (b), 5 minutes cooked; (c), 10 minutes cooked; (d), 20 minutes cooked. CW=cell wall; S=starch; G=starch gel, CWS=cell wall separation.



### 3.4. Summary

There were two phases of firmness loss of the banana and plantain pulp tissues during cooking. The first phase, which was also the fastest, occurred within the first 10 minutes and more than half the original firmness was lost within this period of time. The second, much slower phase of firmness loss continued for a further 10-15 minutes. Plantains (Big Ebanga type and Horn type, *Musa* spp., AAB group) were found to be much firmer after the same periods of cooking than dessert banana (*Musa* spp., AAA group) and Cocoye cooking banana (*Musa* spp., ABB group).

Starch content was much higher in Big Ebanga plantain and horn plantain (30% and 32% respectively) than in dessert banana and Cocoye cooking banana (19% and 22% respectively). Dessert banana contained 30% more pectin than Big Ebanga plantain. Big Ebanga plantain which is higher in starch also contains higher amylose (33%) than dessert banana (27%). Higher Ca and Mg content is also found in Cocoye cooking banana than in horn plantain. During cooking, more pectin was released from dessert banana into the cooking liquor than from Big Ebanga plantain. A temperature below 70°C only had a little effect on thermal firmness loss in dessert banana. Cooking in 0.5% CaCl<sub>2</sub> solution resulted in a much firmer texture and less water uptake in both dessert banana and Big Ebanga plantain than by cooking in pure water, 0.5% EDTA and NaCl solutions. This was especially obvious with dessert banana compared to plantain.

## 3.5. Discussion

### 3.5.1. Firmness changes of *Musa* during cooking

#### 3.5.1.1 Cooking time

Dessert banana, plantains (both Big Ebanga type and Horn type) and Cocoye cooking banana softened with time during cooking as shown in Figures 3.2 and 3.3. The correlation coefficients ( $r$ ) between cooking time and pulp rupture force in the first 10 minutes of cooking are -0.98 ( $r^2=0.95$ ), -0.88 ( $r^2=0.78$ ), -0.98 ( $r^2=0.97$ ) and -0.96 ( $r^2=0.92$ ) for dessert banana, Big Ebanga plantain, Cocoye cooking banana and Horn plantain, respectively. There is a linear relationship between the force needed to penetrate (up to 5 mm) into the pulp tissues (firmness) of the cooked *Musa* and cooking time in the first 10 minutes of cooking. Less correlation was found after 10 minutes of cooking. This suggested that the cooking time has a big influence on the cooked *Musa* texture, however, the cooking kinetics were different before and after 10 minutes of cooking.

Harada *et al* (1985a) also reported a similar finding with cooked potatoes. They were able to use a first order equation to describe the change in shear force during short process times, but over prolonged process times, a second order equation provided a better fit to the data. Over prolonged cooking periods differences exist between the value of shear force calculated from the first order equation and real values, due to the fact that even overcooked samples still have measurable shear force. This is also supported by Huang and Bourne (1983) who postulated that there are two simultaneous first order kinetic mechanisms involved in vegetables softening during the early and prolonged cooking. Mechanism 1 is probably due to pectic changes in



the middle lamella layer, and responsible for approximately 95-97% of the firmness of the raw commodity, and the remaining firmness is contributed by mechanism 2 in which the biochemical nature is unknown.

#### 3.5.1.2. Cooking temperature

Heating dessert banana at 60-70°C for 10 minutes had very little effect on pulp firmness compared to heating at 80-100°C (Figure 3.9). This phenomenon was also observed by Loh and Breene (1981) with potatoes, and they suggested that this might be due to the retention of pectin methylesterase (PME) activity when the material was cooked below 70°C (Hoff, 1972). PME has the ability to de-esterify the methylated pectin into free carboxyl groups which are resistant to heating degradation. However, Sefa-Dedeh *et al* (1978) found that there was a linear decrease in firmness of cowpeas when cooked at different temperatures from 25-90°C although the SEM showed little microstructural change at the corresponding temperatures. The results obtained in this study indicates that temperature has a very important effect on the texture of the cooked banana pulp tissue. However, the mechanism leading to the tissue softening below 70 °C is different from that above 80 °C.

#### 3.5.1.3. Different salt solution treatments

Where an undesirable degree of softness results from heat processing, calcium salts are often added before cooking to improve texture (Hughes *et al*, 1975a; van Buren *et al*, 1988). The results of similar treatments showed that in cooking banana pulp tissue the presence of 0.5% CaCl<sub>2</sub> solution during cooking increased the tissue firmness after 15 minutes by approximately 25% compared to cooking in distilled water. It was

found that cooking in the presence of NaCl and EDTA solution had little effect on tissue firmness (Figure 3.10).

The dominant role of  $\text{Ca}^{2+}$  in promoting firmness can be attributed to the ability of  $\text{Ca}^{2+}$  to promote aggregation between pectin chains through formation of ionic  $\text{Ca}^{2+}$  bridges (Morris *et al*, 1982). This effect is enhanced when there are significant amounts of free carboxyl groups present. The new formed  $\text{Ca}^{2+}$ -pectic gels lead to the firming of tissue which may be via :

- 1) decreasing pectin solubilization and an improved cementing function of pectin (van Buren, 1986).
- 2) reduction of water uptake (Figure 3.11) which prevents the cell wall components from hydrating, thus increasing the cohesiveness of the cell wall matrix (Warren and Woodman, 1974). Both of these mechanisms could delay the cell wall softening and cell wall separation.

Sodium ions are considered to have a softening-inducing action by their displacement and competition with  $\text{Ca}^{2+}$  (van Buren, 1984; van Buren *et al*, 1988). EDTA, a well known  $\text{Ca}^{2+}$  chelator and pectin extractant, could remove  $\text{Ca}^{2+}$  from pectin effectively, resulting in pectin solubilization. Cooking in EDTA solution results in a very soft texture of snap beans (van Buren and Pitifer, 1992). However, the result of the present experiment, which showed that NaCl and EDTA solution had little effect on the texture of the cooked banana and plantain implies that the calcium-linked pectin may only represent a small proportion of the total, perhaps due to a shortage of calcium in

the pulp tissue (Table 3.3). Thus the natural level of calcium may contribute very little to the texture and this effect is easily overcome by heat during cooking. Therefore, mature fruits, which normally contain little calcium, would undergo a significant firming after calcium treatment (Jarvis, 1984), whereas Na<sup>+</sup> and EDTA only had little softening effects on the tissues.

### ***3.5.2. Chemical composition changes during heating***

#### ***3.5.2.1. Pectin solubilization***

Cooking in water released pectic substances into the cooking solution from both dessert banana and Big Ebanga plantain pulp disks (Figure 3.6). Figure 3.2 illustrates that the greatest loss of firmness occurred over the first 10 minutes; thereafter it only decreased slowly. However, the pectic substances continued to be released as the cooking progressed beyond 10 minutes, whereas tissue firmness decreased only slightly over this period. This suggests that the pectic substances released after this period of time play only a minor role in tissue softening. Similar results were also obtained with cooked potatoes (Hughes *et al.*, 1975b) where the authors stated that the pectic substances released into the solution indicate a change in solubility of the pectic substances, presumably through  $\beta$ -elimination.

The contents of HCl-soluble polyuronides ('total') decreased by approximately 28% and 30% in dessert banana and Big Ebanga plantain, respectively during the first 10 minutes of cooking (Figure 3.7). Less changes occurred after this time. Therefore, a major proportion of the 'total' pectin still remained in the cooked dessert banana and plantain pulp tissue disks. The HCl-soluble ('total') pectic substances may have

contained 'protopectin' which are not involved in the middle lamella structure (Hughes *et al.*, 1975b). Therefore, it seems that the initial solubilization of small amounts of pectic material which released into the cooking liquor may considerably weaken the intercellular cement together with a loss of cell adhesion and firmness. The pectic material released later during cooking may have been of different origin to that released earlier and may only have been partially concerned with cell wall strength as suggested by Hughes *et al* (1975c) from their studies with potatoes.

In a series of publications, Fuchigami *et al* have examined the relationship between cooking softening and pectic change with many kinds of vegetables, including East Indian lotus rhizomes (Fuchigami and Kishigami, 1991), Japanese radish roots (Fuchigami, 1987a) and 21 other materials including sweet potato and potato (Fuchigami, 1987a & b). It was concluded that the vegetables which soften more readily during heating have more highly methylated pectin which would be broken down by  $\beta$ -elimination in hot neutral solution and released into the cooking solution. Similar reasoning could be used to account for more pectic substances being released into the solution from dessert banana than from plantain (Figure 3.6) during cooking at near to neutral pH (Figure 3.5), From this it would be expected that dessert banana pulp would contain more methylated pectin than plantain. Further fractionation studies will enable this possibility to be examined further.

As the cooking progressed beyond 10 minutes, the tissue continued to soften although the total polyuronide content remained constant. This implies that the tissue softening is not necessarily or solely due to pectin solubilization and loss. Though those pectins

which were degraded and depolymerized, possibly by thermal  $\beta$ -elimination, were still trapped in the tissue (70% approximately, Figure 3.7) and had not diffused into the cooking medium, they appear to have very little effect on tissue firmness. Huang and Bourne (1983) suggested that 85-97% of the original tissue firmness loss during thermal softening of vegetables is probably due to pectic changes in the middle lamella layer, and its relative contribution to firmness decreases practically to zero during processing. Thereafter, an unidentified mechanism is responsible for the residual firmness of the vegetables after prolonged heating. Loh and Breene (1982) suggested that the neutral sugar component of wall polymers may play a structural role in maintaining cell wall integrity after galacturonan fractions have been broken down by heat. For a given vegetable species, the extractable neutral sugar content (i.e. those sugars which form hemicellulose and neutral side chains of pectin) content of the cell wall microstructure are the major determinant of the resistance of that plant tissue to fracturability loss during cooking. Thus the changes in total polyuronide content due to pectin solubilization during cooking cannot adequately explain the tissue textural response to heating (Warren and Woodman, 1974; Hughes *et al*, 1975b; Loh and Breene, 1982). Further studies on pectin fractionation and as well as pectin properties need to be done to characterise their contribution to the softening effect on *Musa* during cooking (see Chapter 5).

#### 3.5.2.2. Starch and other factors

Measurement of the starch content in Big Ebanga plantain, Horn plantain, dessert banana and Cocoye cooking banana showed some variation, values being 30%, 32%, 19.4% and 22%, respectively which were correlated with the dry matter content

(Table 3.2). Big Ebanga plantain which is higher in starch also contains higher amylose (33.4%) than dessert banana (27.4%) which is lower in starch. The higher starch content in plantain and horn plantain would be expected to produce a higher starch swelling pressure during heating (Jarvis *et al*, 1992) which in turn would lead to a softer texture. In practice it was found that Big Ebanga plantain and horn plantain pulp tissue remained much firmer than dessert banana and Cocoye cooking banana after cooking (Figure 3.2, 3.3). The relatively high calcium and magnesium contents of Cocoye cooking banana would be expected to decrease the swelling of gelled starch as found in potatoes (Reeve, 1967; Haydar *et al.*, 1980) (Table 3.3). The suggestion that high starch levels reduce intercellular adhesion by causing cell distension, is not supported by the available evidence for *Musa*, where the relationship between the starch content and firmness of the pulp tissues after cooking was found to be positive, rather than the predicted inverse relationship as reported in potatoes (Jarvis *et al.*, 1992). Nevertheless, the present result agrees with the findings of Sharma *et al* (1959), Linehan *et al* (1969a, b) and McComber *et al* (1994) who also found a positive relationship between the content of starch, or amylose and the firmness of the potatoes after cooking.

Linehan *et al* (1969c) found that when purified amylose and amylopectin solution were mixed with free potato tuber cells, amylose increased intercellular adhesion whilst amylopectin was almost without effect. Therefore, they suggested that amylose chains might act as a cement between potato tuber cells by formation of hydrogen bonds with polysaccharides of the cell walls. However, Keijbets *et al* (1976) found no other interactions between purified potato starch and pectic galacturonan other than

the transfer of  $\text{Ca}^{2+}$  ions to the galacturonan, by which they are much more strongly complexed resulting in a firmer texture. This might also occur in the intact cooked potato tuber.

During heating of the banana and plantain fruit, the starch content remained the same throughout (Figure 3.8). This implies that changes in starch content alone are not responsible for changes in the texture of the cooked banana and plantain pulp tissue although starch accounts for about 20% and 30% of the fresh weight (Table 3.2). This is similar to the result found with potatoes (Harada *et al*, 1985b; Harada and Paulas, 1987). Loh *et al* (1982) also found that the fracturability loss of potato and waterchestnut on heating did not have a direct relationship to starch content change.

### **3.5.3. SEM observations of dessert banana**

Thermal softening of banana pulp tissue increased with cooking time as shown in Figure 3.2. By the end of the heat treatment, 89% of the original firmness had been lost. These textural changes corresponded to the SEM data (Figure 3.12). In the early stages of cooking (before 10 minutes), cell wall separation and intercellular space expansion contributed to the 75% of the firmness loss. Further softening of the tissue during the later stages of cooking may be caused by starch "swelling pressure" along with thermal expansion of the cells, aiding further cell separation.

The observed intercellular strands in the early heating stages (Figure 3.12c) have not been cited in the literature before and may be a feature unique to banana/plantain. Such strands may build up to form the reticulated structure on the cell surfaces in the

later stages of cooking (Figure 3.12d). The strands may contribute to the stickiness or gumminess described by some workers as a component of the texture of cooked potatoes (e.g. Reeve, 1979). Further microscopic research, i.e., transmission electron microscopy might give more detailed information about the breakdown of the cell walls and middle lamella, and provide an explanation for thermal softening of *Musa* (see Chapter 4).

#### ***3.5.4. Dessert banana versus Big Ebanga plantain, Cocoye cooking banana versus Horn plantain***

Both banana and plantain are softened by heating, the latter softening at a lower rate (Figure 3.2 & 3.3). After the first 10 minutes of cooking, both dessert banana and Cocoye cooking banana lost 75% of their original firmness, whilst Big Ebanga plantain and Horn plantain lost only 37% and 55%, respectively. Such a remarkable difference in texture must be due to their very different chemical composition before cooking. Further experiments and analysis will be required to establish the reasons for such differences.

Table 3.2 and Figure 3.8 show that Big Ebanga plantain contained about 40% more starch than dessert banana and that in both types of fruit the starch content remained constant during cooking. It is unlikely that there is a direct relationship between firmness loss and starch content. A higher starch content would be expected to generate higher "swelling pressure" on heating, which in some tissues has been associated with softer tissue texture (Bettelheim and Sterling, 1955a; Sterling and Bettelheim, 1955; Jarvis *et al*, 1992). However, although plantain has a higher starch



as well as a higher amylose content than banana, it does not soften as fast during heating.

The data in Table 3.2 and Figure 3.7 show that dessert banana pulp tissue contained about 30% more polyuronide (pectin) than Big Ebanga plantain on a fresh weight basis (42% on a dry weight basis). Presumably higher polyuronide content leads to greater hydration of the cell wall matrix due to higher water uptake, and this results in cell wall softening and cell wall separation (Warren and Woodman, 1974). In fact, plantain pulp tissue took up water at a similar rate as banana before 10 minutes. With cooking subsequent to 10 minutes, banana increased water uptake and absorbed much more water than the plantain (Figure 3.4). This implies that the total polyuronide content is not adequate to explain the great differences observed in the textural response to heating of banana and plantain.

The remarkable pectin solubilization resulting from heating took place in both dessert banana and Big Ebanga plantain during the first 10 minutes (Figure 3.7). Plantain showed the more extensive solubilization. The amount of solubilized uronic acid after this period of time accounted for about 30% and 28% of total pectin in Big Ebanga plantain and dessert banana respectively. However, dessert banana released more pectin into the cooking solution than Big Ebanga plantain after 10 minutes of cooking (Figure 3.6). This suggests that a certain pectin fraction, although it is only a small proportion of the total, may be more important in determining the texture of a cooked *Musa* pulp than the total pectin.

## **CHAPTER 4**

### **EFFECT OF HEATING ON CHANGES OF CELL WALL ULTRASTRUCTURES AND CELLULAR STRUCTURES OF *MUSA***

## **4. EFFECT OF HEATING ON CHANGES OF CELL WALL ULTRASTRUCTURES AND CELLULAR STRUCTURES OF *MUSA***

### **4.1 Introduction**

The cell walls of edible fruit tissues are composed predominantly of primary cell wall, and secondary cell walls are virtually absent from fruits (Nelmes and Preston, 1968). The primary walls of adjacent cells are usually cemented together over part of their area. The 'glue' responsible for this, the middle lamella, may be visible as an electron dense layer under the transmission electron microscope (TEM). However, it is often difficult to define a precise boundary between the middle lamella and the rest of the cell wall.

Most of the research on cell wall structure has been carried out on the primary walls of tissues other than fruit. However, it is generally thought that fruit cell walls have a three-dimensional structure similar to that of other tissues (Knee and Bartley, 1981; Tucker, 1993). There is no information about banana pulp cell wall structure available at present.

Texture is a quality parameter of a cooked fruit or vegetable product. Although the physical parameters of texture are very complex, in its simplest description, texture can be said to vary from firm to soft. Scanning electron microscopy (SEM) has been applied to study potatoes and other plant edible portions during processing in order to understand why they soften when heated (Loh, *et al.*, 1982; Huang *et al.*, 1990; van Marle *et al.*, 1992., McComber *et al.*, 1994). Using cryo-SEM, van Marle *et al* (1992) revealed that cell wall and middle lamella breakdown did occur during steam-cooking of all the four cultivars of potatoes examined although the relative importance of these two factors, i.e., cell wall and middle lamella, varied between different cultivars. SEM studies on potato and waterchestnut (Loh *et al.*, 1982) also strongly suggested that factors responsible for cell adhesion and its heat resistance play a major role in

fracturability loss during cooking. Waterchestnut, which retained a firmer texture after cooking, showed a greater cell wall strength than potato cell wall (Loh *et al.*, 1982). This work was supported by McComber *et al* (1994) who concluded after SEM studies on four potato cultivars during steam-cooking that cultivars with a harder texture after cooking had a thickened cell wall-middle lamella complex after cooking which resisted deformation, although no cell wall separations were observed under SEM in their studies, neither in soft nor hard cultivars.

The hard-to-cook (HTC) phenomenon in which the seeds of some beans (Shomer *et al.*, 1990) and lentils (Bhatty, 1990) show poor softening in response to cooking is associated with a failure to undergo cell separation. In HTC cultivars the middle lamella remained clearly visible under TEM (Bhatty, 1990) whilst cultivars with good-cooking quality showed clear cell wall separation and middle lamella dissolution. This is another indication that cell wall and middle lamella are the important components that decide the degree of softness of a cooked plant product.

Additionally, investigations of tissue structure during cooking have shown that the gelatinization of starch can be clearly visualized under both light microscope and scanning electron microscope. Huang *et al* (1990) showed that heated potato cells became filled by gelatinized starch which had a reticulated structure. Reeve (1977) found that cell wall separation and cell wall softening along with starch gelatinization, contributed to the firmness loss of potatoes during heating.

In this study, SEM, LM and TEM were employed to study the effect of cooking (boiling) on the pulp tissues of dessert banana (soft) and plantain (hard), and steam-cooking on Uganda cooking bananas Toro (soft) and Enkonera (hard). The aim was to relate the timing of changes in the structure of the pulp tissue with the changes in tissue texture during heating.

## **4.2 Materials and Methods**

### ***4.2.1. Materials***

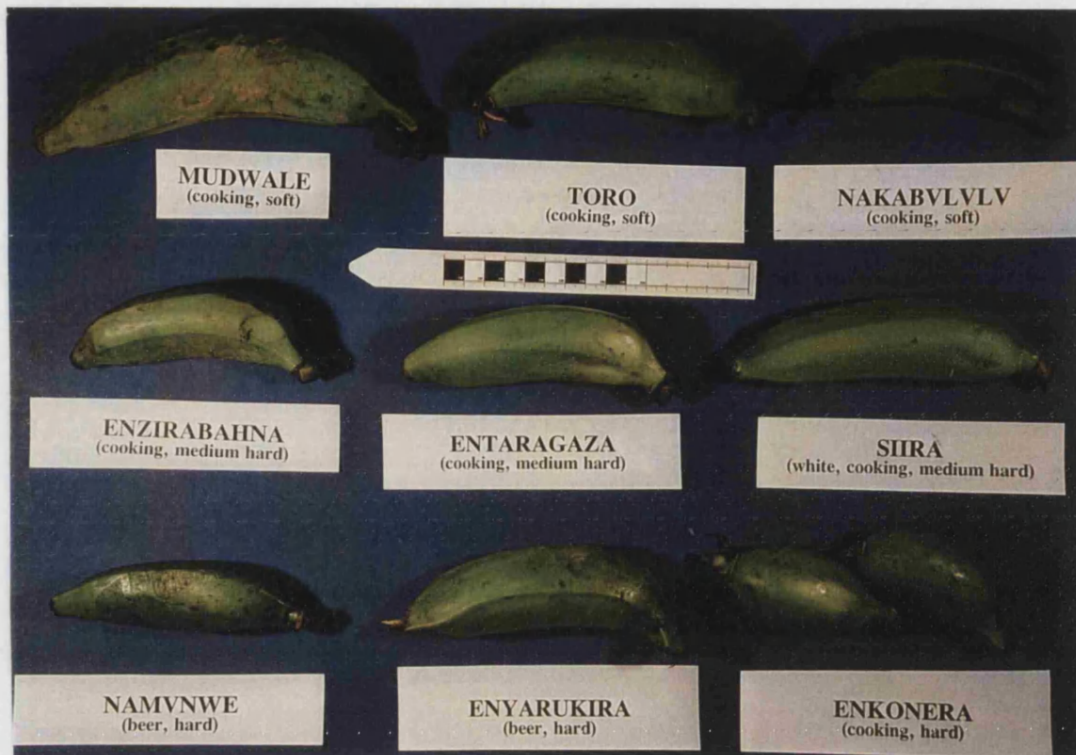
Nine known matooke cooking banana (*Musa*, AAA group) cultivars were obtained from Uganda (Figure 4.1). Details of their date of flowering and harvest dates are shown in table 4.1. They were harvested on January 18th, 1994, then transported back to UK by air on the same day. Experiments on these fruits were carried out on 19th and 20th. Of these nine cultivars, Toro as the softest and Enkonera as the hardest were selected for more detailed studies.

### ***4.2.2. Thermal treatment***

Sample preparation for thermal treatments was the same as previously described in section 2.2. Dessert banana and plantain (Big Ebanga type), which were obtained from commercial sources, were cooked in water in the same manner as previously described (section 2.2). In order to avoid the complications involved with a cooking solution which would contain solubilized materials and debris, the fruit discs from matooke cooking bananas were subjected to steam-cooking, rather than boiling, for 1, 3 and 10 minutes

### ***4.2.3 Optical and transmission electron microscopy***

The specimens were fixed with or without ruthenium red being present in the fixatives as Luft (1964) found that ruthenium red could stain pectin containing plant specimens strongly for TEM studies.



**Figure 4.1** Nine matooke cooking banana cultivars (*Musa*, AAA group) obtained from Uganda.

<i>Name</i>	<i>Date of flowering</i>	<i>Type</i>	<i>Hardness</i>
Mudude	11/10/93	Cooking	Soft
Nakabululu	25/10/93	''	''
<b>Toro*</b>	23/10/93	''	''
Enzirabahma	20/10/93	''	Med. Hard
Entaragaza	10/10/93	''	''
Siira	25/10/93	''	''
Enyarukira	21/10/93	Beer	Hard
Namunwe	20/10/93	''	''
<b>Enkonera*</b>	17/10/93	Cooking	''

**Table 4.1** Nine matooke cooking banana cultivars (*Musa*, AAA group) obtained from Uganda. Date of harvest was 18/01/94. \*Selected for further studies.

#### 4.2.3.1 With ruthenium red present

Small slices from the pulp parenchyma tissue of raw and cooked materials were prefixed in 1% glutaraldehyde acid (GDA) in 0.1 M sodium cacodylate buffer (pH 7.2-7.4), containing 500 ppm ruthenium red, for 1 hour at room temperature. The slices were then rinsed several times with the same buffer. Postfixing was conducted with 1% osmium tetroxide ( $\text{OsO}_4$ ) in 0.1 M sodium cacodylate buffer, containing 500 ppm ruthenium red, for 3 hours at room temperature followed by washing several times with the buffer, and finally distilled water. The fixed specimen were dehydrated gradually with a series of alcohol of 30%, 40%, 50%, 70%, 80%, 90%, 95%, and finally twice with absolute ethanol. Samples were then infiltrated with 1:3, 1:1, 3:1 of Taab Premix Epoxy Resin:ethanol, and finally pure resin. The specimens were embedded and polymerised in a 60°C oven for 24 hours in the same resin.

#### 4.2.3.2 Without ruthenium red present

The specimens taken from the raw and cooked materials were fixed in 3% GDA in 0.05M PIPES buffer (pH 7.0) for 5 hours at room temperature, then rinsed with the same buffer a few times followed with 0.1M PIPES containing 0.15M sucrose (pH 7.0). Postfixing was carried out with 1%  $\text{OsO}_4$  in the same buffer at room temperature for 1 hour. The subsequent procedures were the same as in section 4.2.3.1 except that acetone was used for the dehydration rather than ethanol.

For light microscopy, sections of about 0.5-1  $\mu\text{m}$  were sectioned with a Reichert ultra-microtome, mounted on a glass slide and stained with 10% toluidine blue in 1% borax. For transmission electron microscopy (TEM), ultrathin sections of 60-100 nm were cut with a glass knife and collected onto a formvar coated copper TEM grid (300 mesh). The sections were firstly stained with uranyl acetate in the dark for 10-15 minutes, rinsed with freshly boiled distilled water then stained with lead citrate for 4-5 minutes. When dried, the sections were examined with a Jeol 1200EX TEM operating at 80-100 kV.



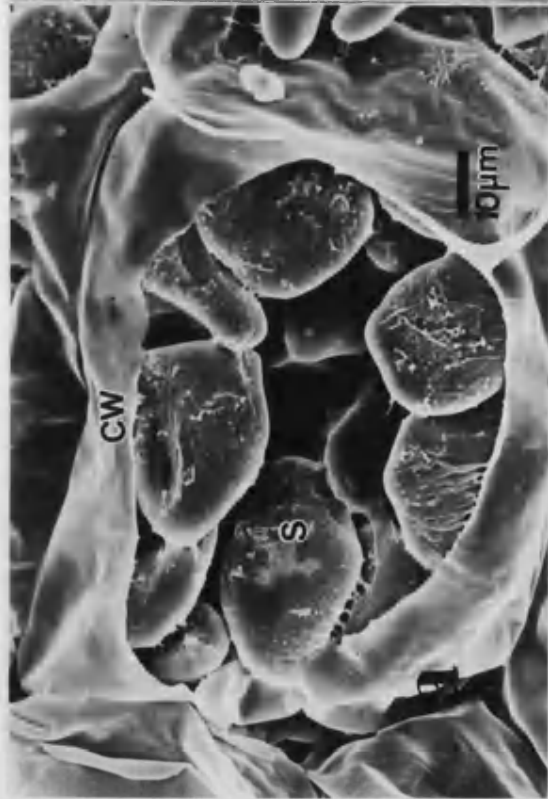
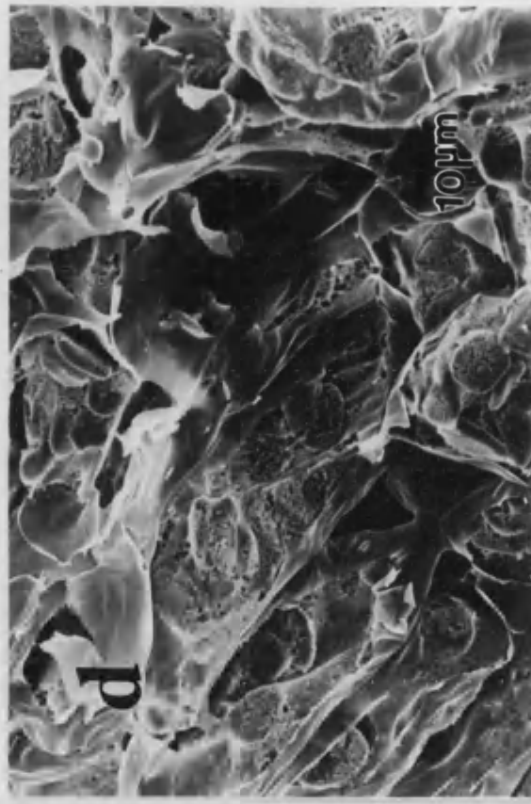
## 4.3 Results

### *4.3.1 Microstructural changes of dessert banana and plantain (Big Ebanga type) pulp tissue during boiling*

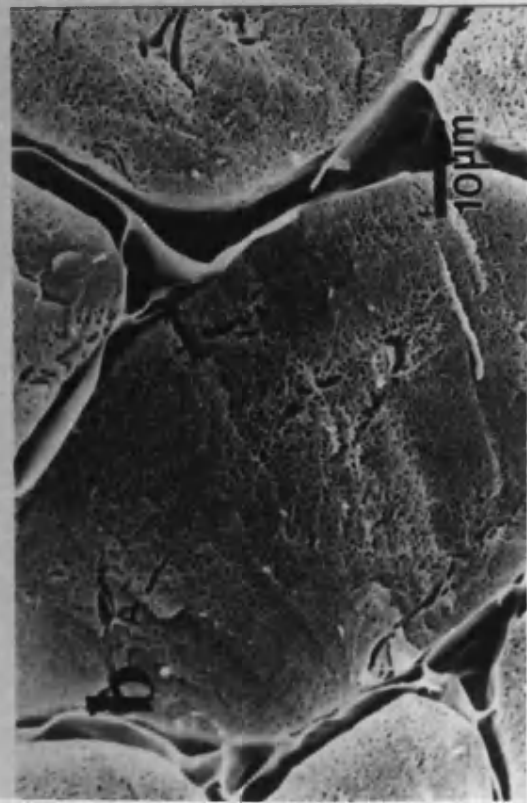
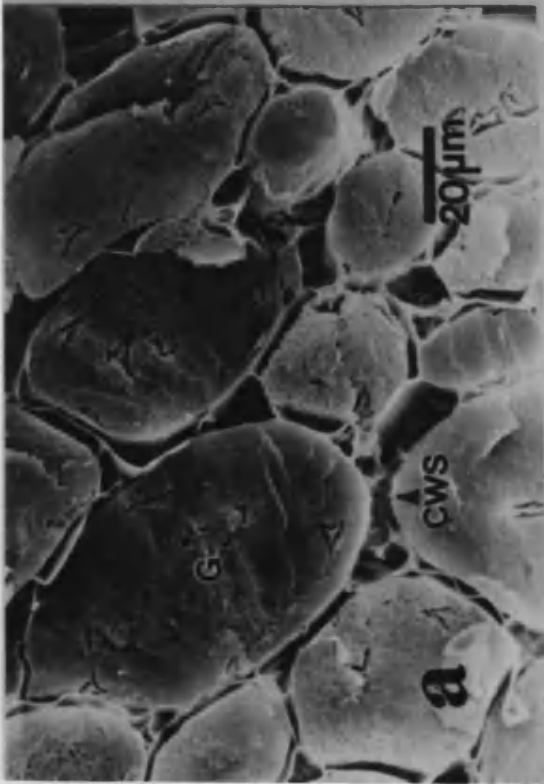
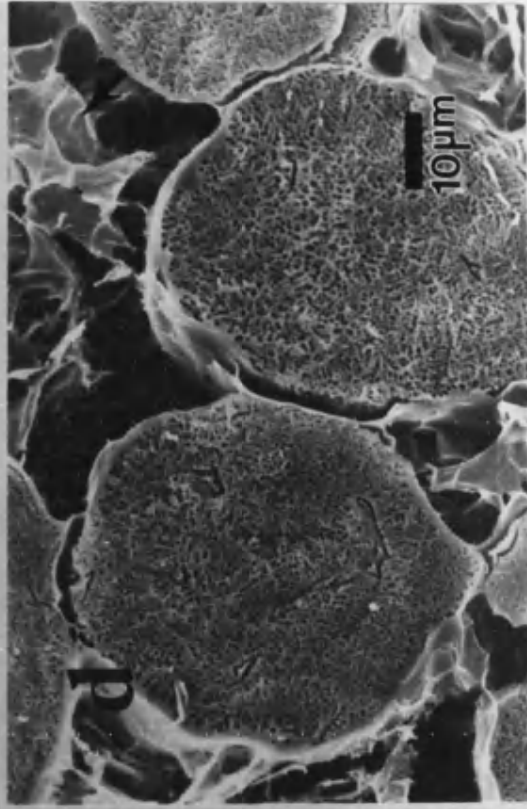
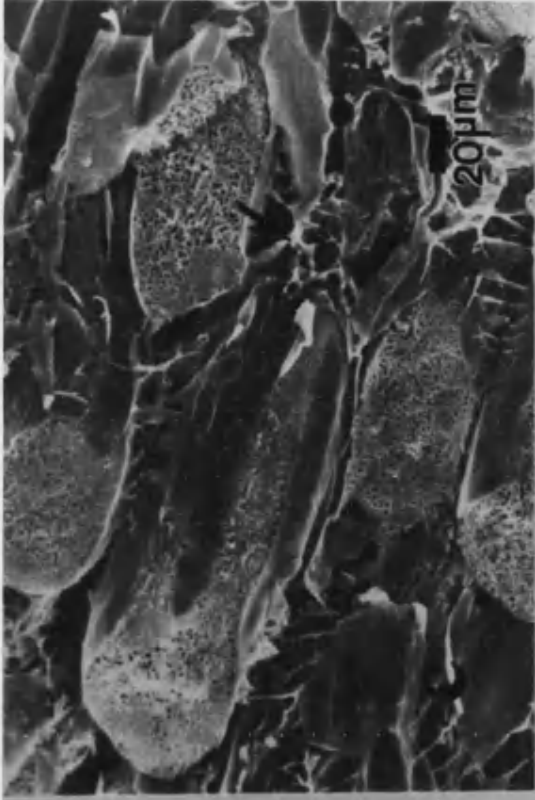
SEM observations of dessert banana and plantain (Big Ebanga type) pulp tissues before and after different periods of cooking by boiling in distilled water for 1 to 30 minutes are shown in figures 4.2-4.5 and 4.6-4.8, respectively. The raw pulp tissues of both banana and plantain were seen to have smooth cell walls, and the starch grains were distinct and well separated, showing a slightly angular morphology suggesting that the grains were hard (Figures 4.2a & b; Figure 4.6a). The shape of the plantain starch grain appeared to be more rounded and elongated than those of banana, which showed starch grains were more plate shaped. After 1 minute of cooking the starch grains of banana started to lose their distinct definition (Figure 4.2c & d), becoming impacted upon one another and distorted by mutual contact, and after two minutes of heating the starch grains became merged into a uniform reticulated structure (Figure 4.3 a& b). In plantain, very little loss of grain integrity was apparent after 1 minute of heating (Figure 6b). After 2 minutes the grains had started to gelatinize, apparently from the centre rather than the edge (Figure 4.6 c& d). After 3 minutes of cooking in both banana and plantain, the starch had developed a reticulated network appearance, which showed little difference in the different fruit types.

Accompanying these changes in the starch grains, there were changes both in cell wall and intercellular materials. In banana, the intercellular spaces appeared to expand and cell wall separation became evident in the 2 minute cooked samples (Figures 4.3 a& b). After 3 minutes of cooking reticulated material became obvious in the intercellular region (arrows, Figure 4.3c & d). Similar changes were found in plantain, but these became apparent only in 5 minute cooked materials (Figure 4.7 c). Further cooking of the two types of fruit resulted in more of these materials being formed, and after 30 minutes of cooking, it appeared that the masses of starch gel enclosed within the cell wall were now embedded within this reticulated material (figures 4.5 b and 4.8 d), and the distance between adjacent cells was increased. Thus cell wall separation became extensive after a longer cooking time. However, no cell wall rupture was found at any time throughout the cooking process.

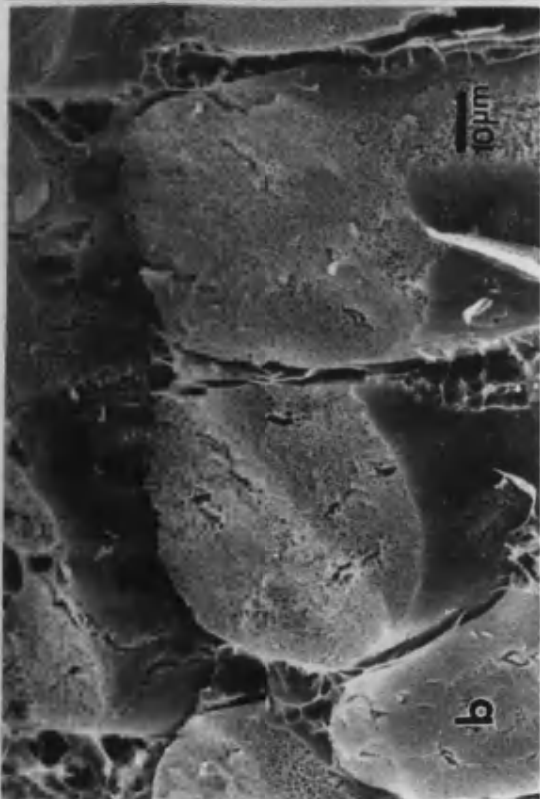
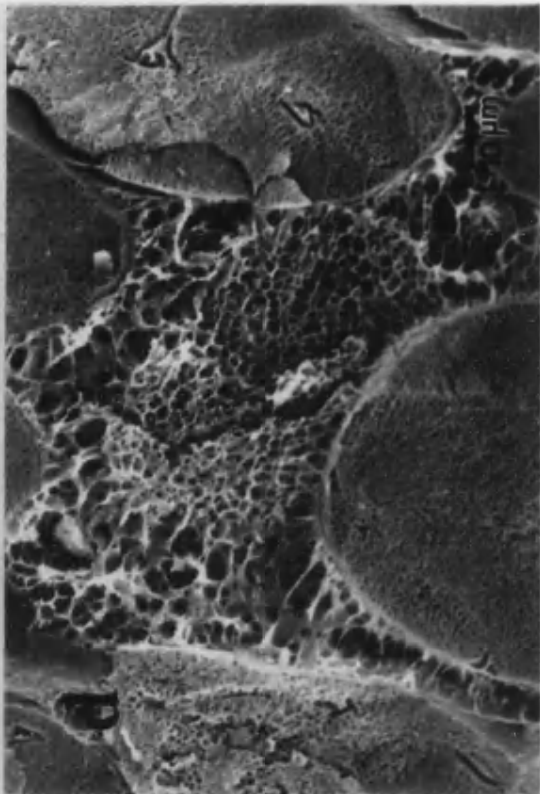
**Figure 4.2.** SEM mircographs of green mature dessert banana pulp parenchyma tissue before and after 1-minute cooking. (a) & (b) - raw; (c) & (d) - 1 minute cooked. S=Starch grains, CW=Cell Wall.



**Figure 4.3.** SEM micrographs of green mature dessert banana pulp tissue after 2 and 3 minutes of cooking. (a) & (b) - 2 minutes cooked; (c) & (d) - 3 minutes cooked. Arrow in (b) indicates the intercellular space expansion, and arrows in (c) & (d) show the intercellular reticulated structures. G=starch Gel; CWS=Cell Wall Separation.

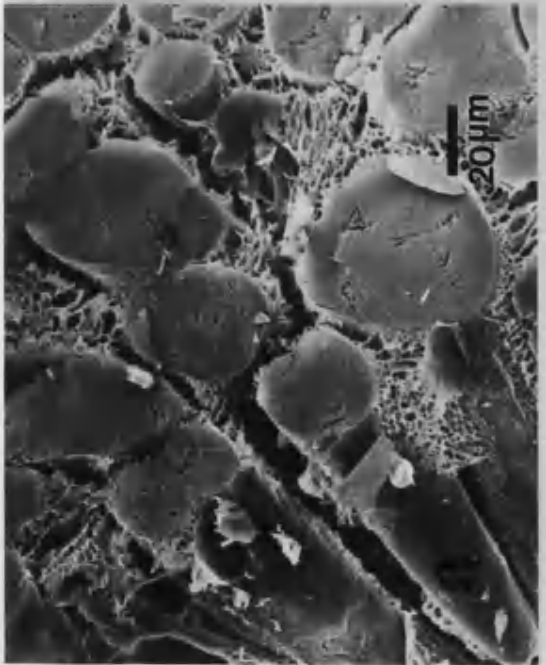
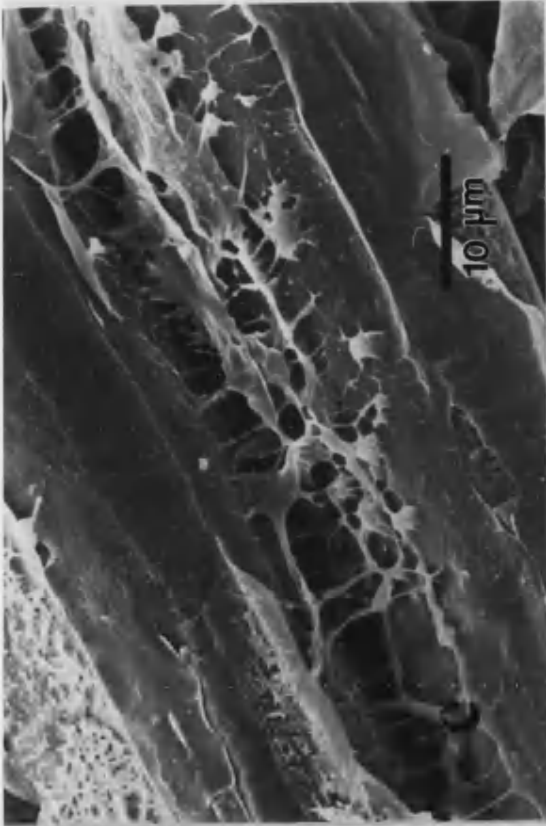


**Figure 4.4.** SEM micrographs of green mature dessert banana pulp tissue after 5-15 minutes of cooking. (a) - 5 minutes cooked; (b) - 8 minutes cooked; (c) - 10 minutes cooked; (d) - 15 minutes cooked. Arrows show the intercellular reticulated structures.

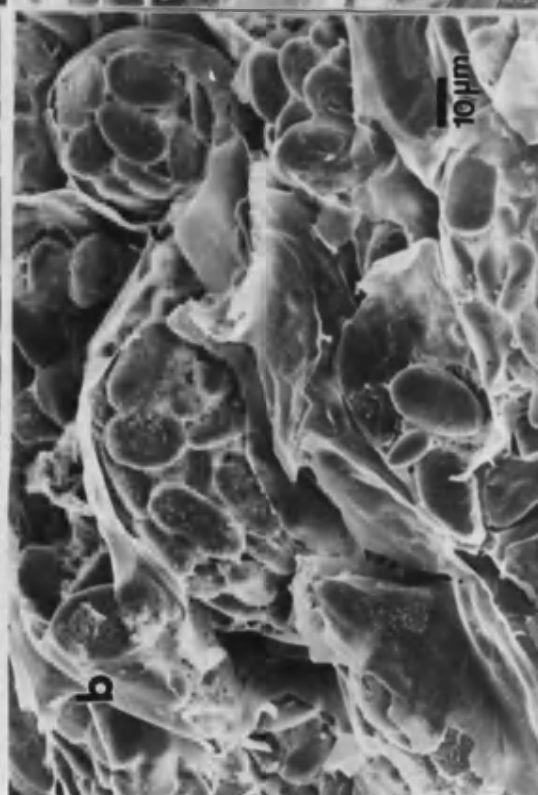
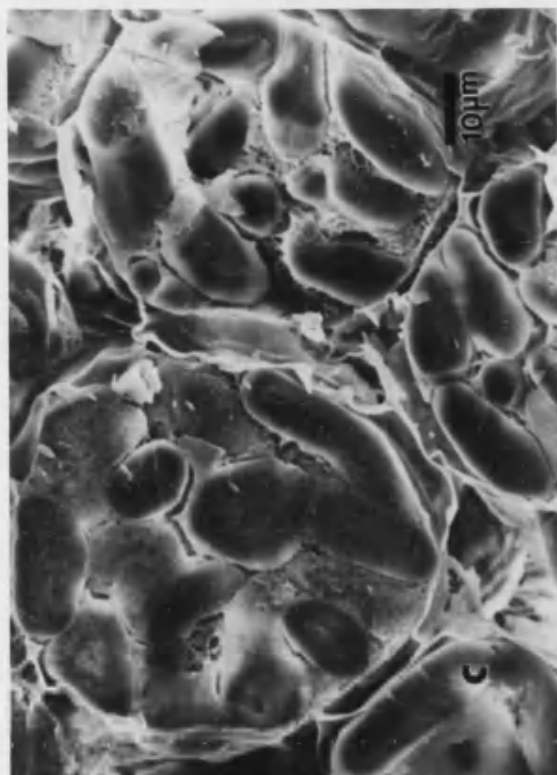




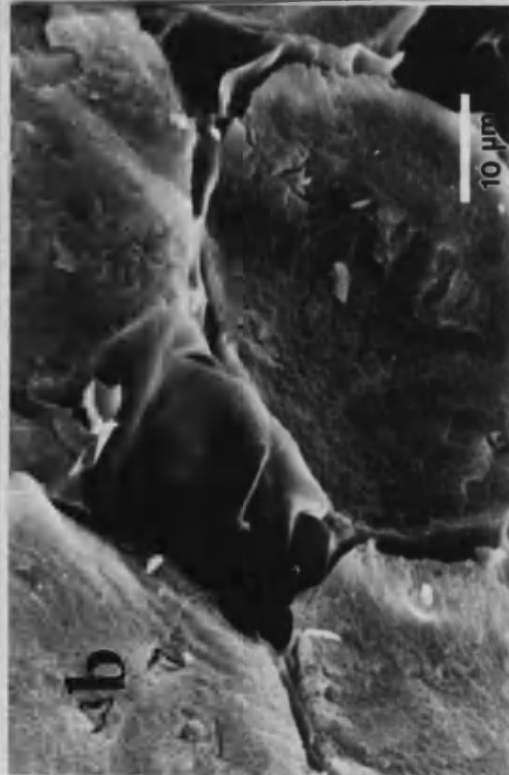
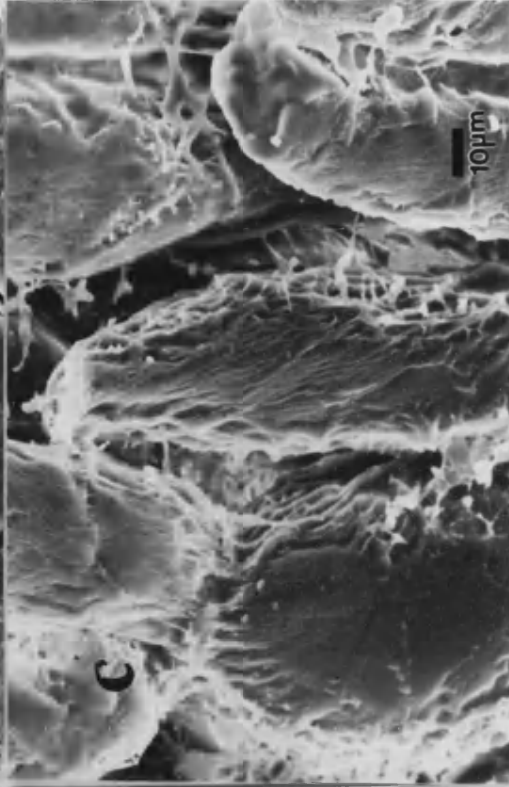
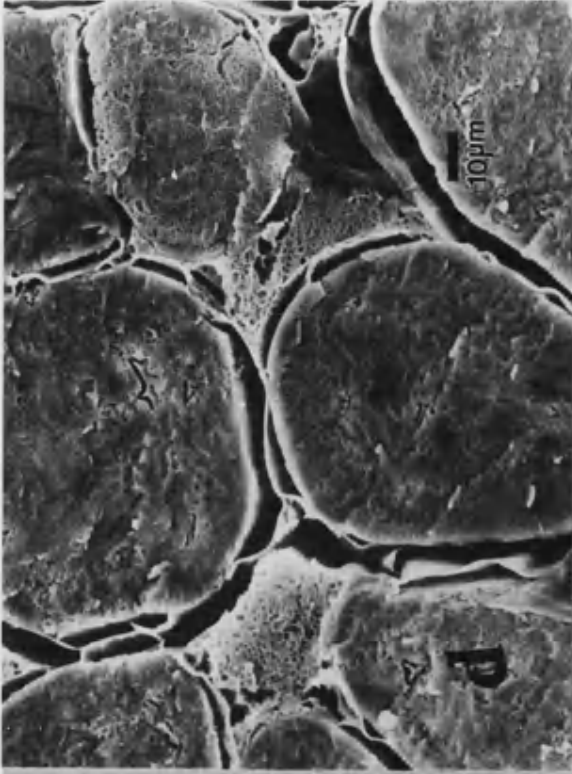
**Figure 4.5.** SEM micrographs of green mature dessert banana pulp tissue after 5-10, and 20-30 minutes of cooking. (a) - 20 minutes cooked; (b) - 30 minutes cooked; (c) - 5 minutes cooked; (d) - 10 minutes cooked. (c) & (d) indicate the progressive accumulation of intercellular materials and the separation of cell walls as cooking progressed.



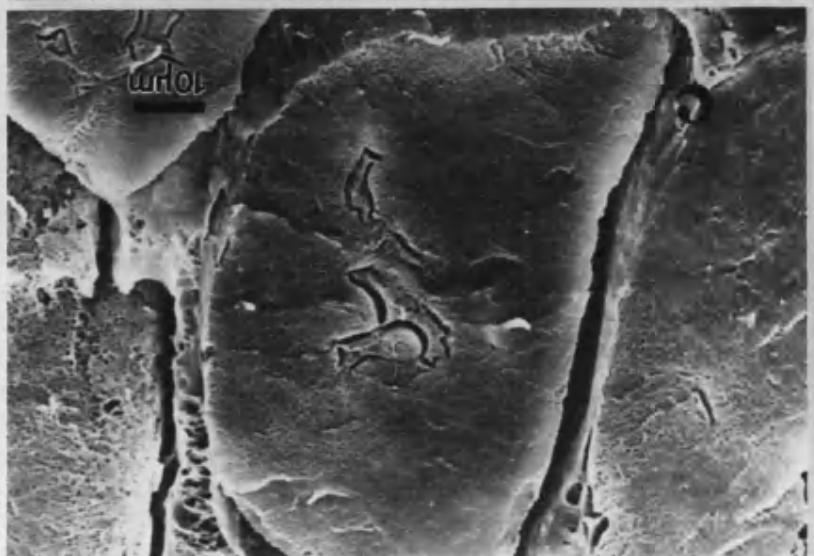
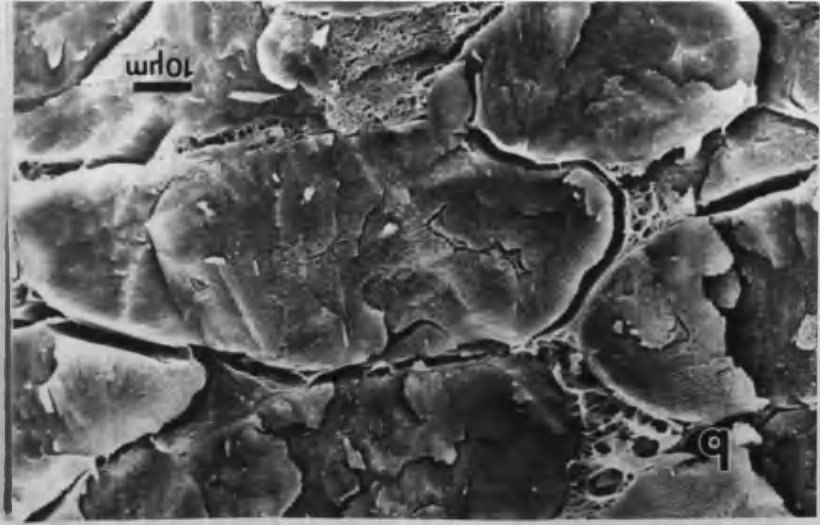
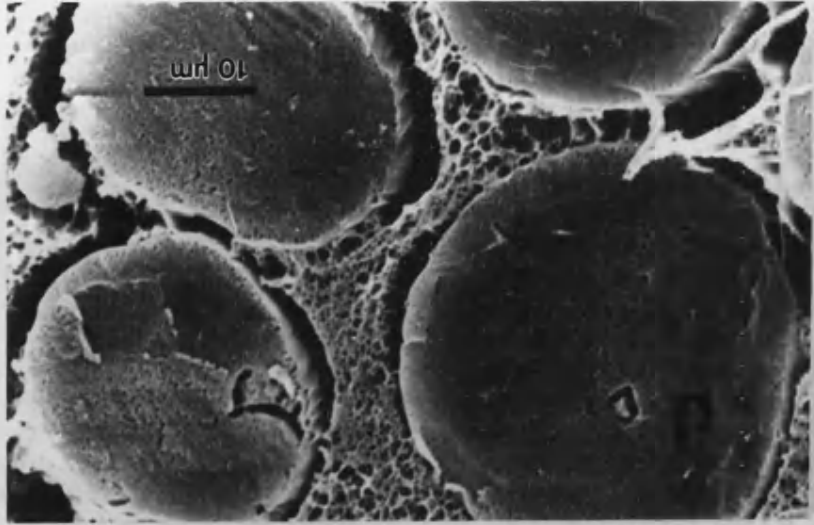
**Figure 4.6.** SEM micrographs of green mature plantain (Big Ebanga type) pulp parenchyma tissue before and after cooking. (a) - raw; (b) - 1 minute cooked; (c) & (d) - 2 minutes cooked. S=Starch grains, CW=Cell Wall.



**Figure 4.7.** SEM micrographs of green mature plantain (Big Ebanga type) pulp tissue after 3-8 minutes of cooking. (a) & (b) - 3 minutes cooked; (c) - 5 minutes cooked; (d) - 8 minutes cooked. Arrow in (b) indicates the expansion of intercellular space, and arrows in (d) show the formation of intercellular reticulated structures.



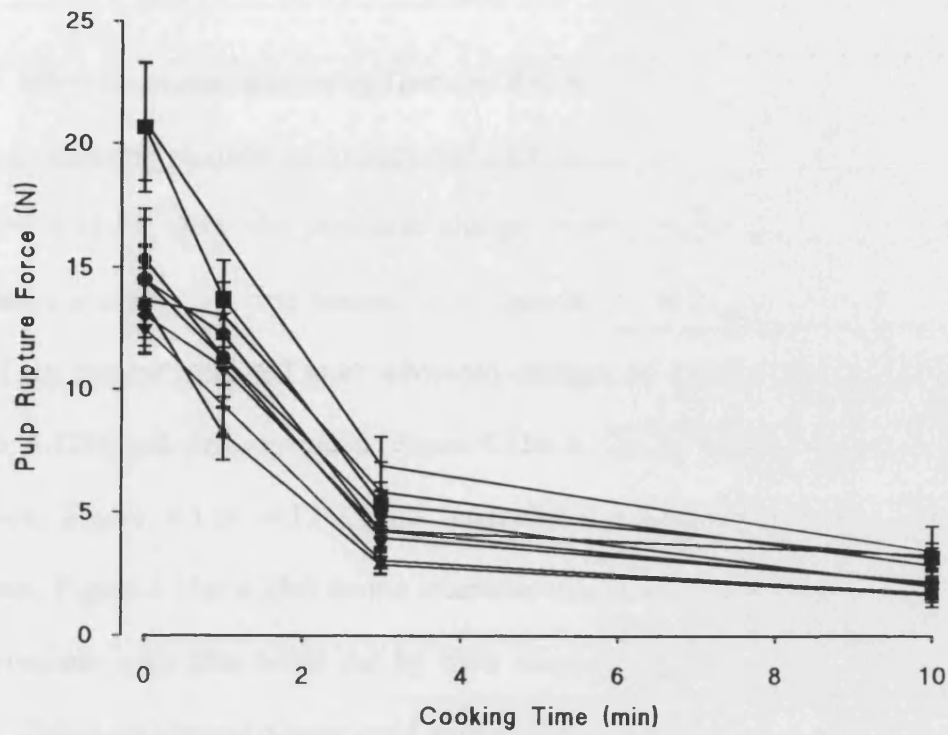
**Figure 4.8.** SEM micrographs of green mature plantain (Big Ebanga type) pulp tissue after 10-30 minutes of cooking. (a) - 10 minutes cooked; (b) - 15 minutes cooked; (c) - 20 minutes cooked; (d) - 30 minutes cooked. Arrows show the intercellular reticulated structures.



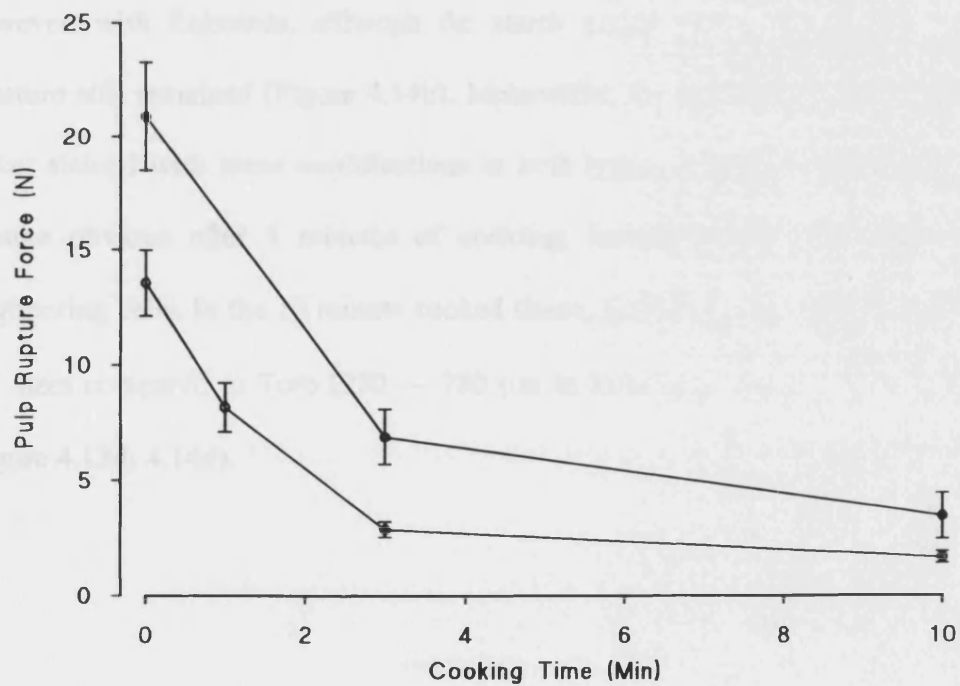


#### ***4.3.2. Textural changes during steam-cooking of matooke cooking banana from Uganda***

In order to avoid the possible complexity caused by the presence of cooking liquor due to boiling in water, the thermal treatments of the nine known matooke cooking banana cultivars were carried out by steam-cooking for 1, 3 and 10 minutes. Their pulp rupture force changes upon heating are shown in figure 4.9. The results indicate that a rapid decrease in pulp firmness occurred in all cultivars during the first 3 minutes of steaming. Of the 9 cultivars examined Toro was the softest and Enkonera the hardest (Figure 4.10). After 3 minutes of heating, the firmness of Toro decreased from 13.5N to 3N, e.g., 79% of the original firmness was lost after this period of time. However, in Enkonera, only 67% of the firmness was lost after same period of heating. So, Toro softened much faster than Enkonera. Due to their very different response upon cooking, a comparison of Toro and Enkonera was carried out by means of SEM and TEM observations and molecular studies of the cell wall components of these two cultivars was also undertaken with the aim of identifying the components which might be responsible for differences in softening during cooking.

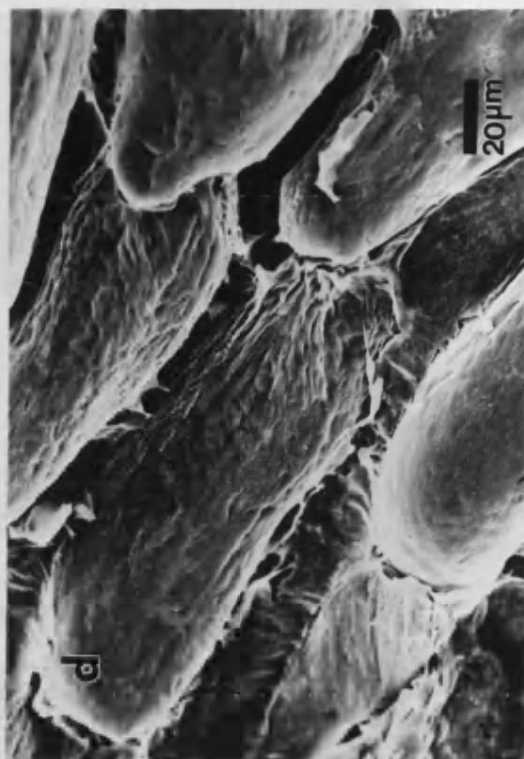
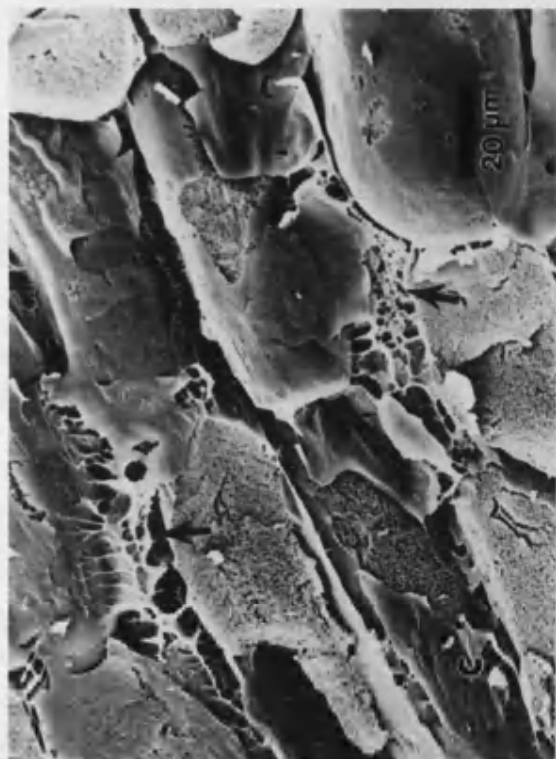


**Figure 4.9** Textural changes of matooke cooking bananas (*Musa*, AAA group) during steam-cooking. Error bars show standard deviation on mean (mean±SD, n=12).

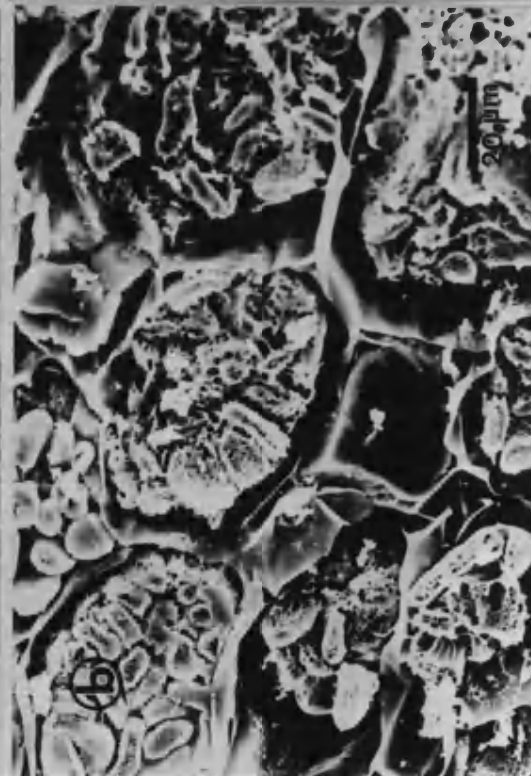
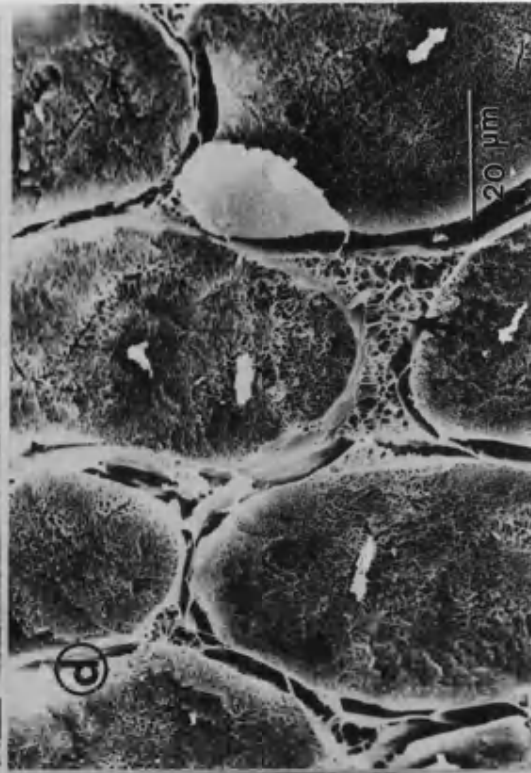


**Figure 4.10** Textural changes of Toro (softest) and Enkonera (hardest) during steam-cooking. Error bars show standard deviation on mean (mean±SD, n=12). -o- Toro (softest); -●- Enkonera (hardest).

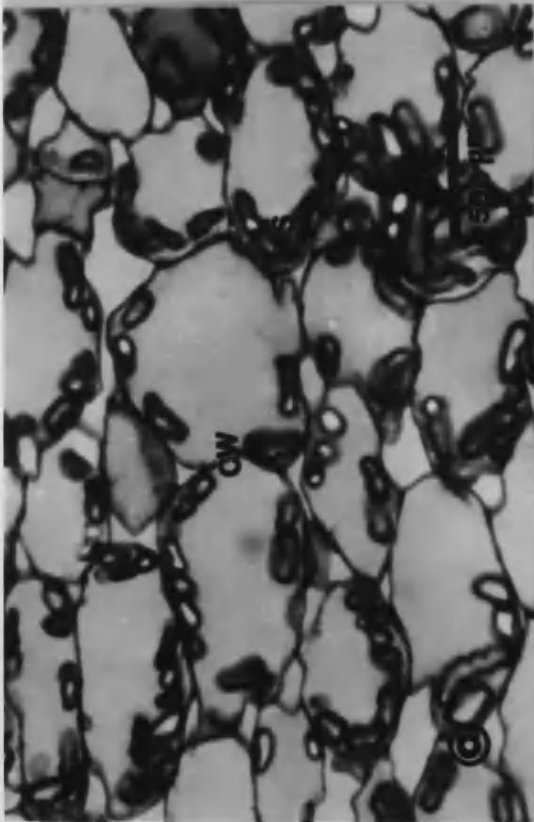
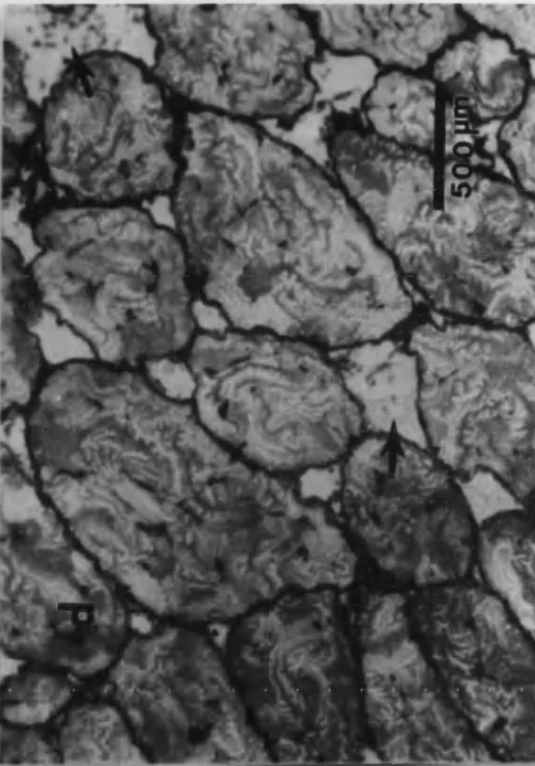
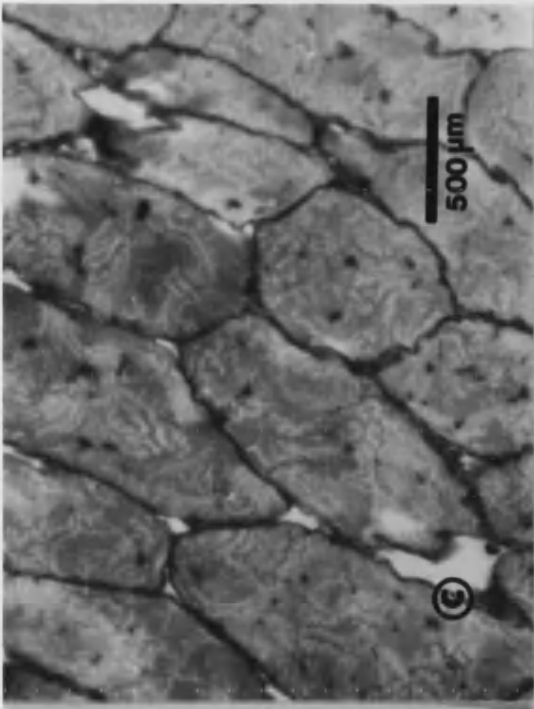
**Figure 4.11.** SEM micrographs of raw and steam-cooked pulp parenchyma tissue of the soft matooke cooking banana Toro. (a) - raw; (b) - 1 minute cooked; (c) - 3 minutes cooked; (d) - 10 minutes cooked. Arrows in b show the intercellular space expansion, and arrow in (c) shows the intercellular reticulated structures formation. S=Starch grains, CW=Cell Wall.



**Figure 4.12.** SEM micrographs of raw and steam cooked pulp parenchyma tissue of the hard Enkonera\_cooking banana. (a) - raw; (b) - 1 minute cooked; (c) - 3 minutes cooked; (d) - 10 minutes cooked. Arrow in (d) shows the intercellular reticulated structures. S=Starch grains, CW=Cell Wall, G=starch Gel.

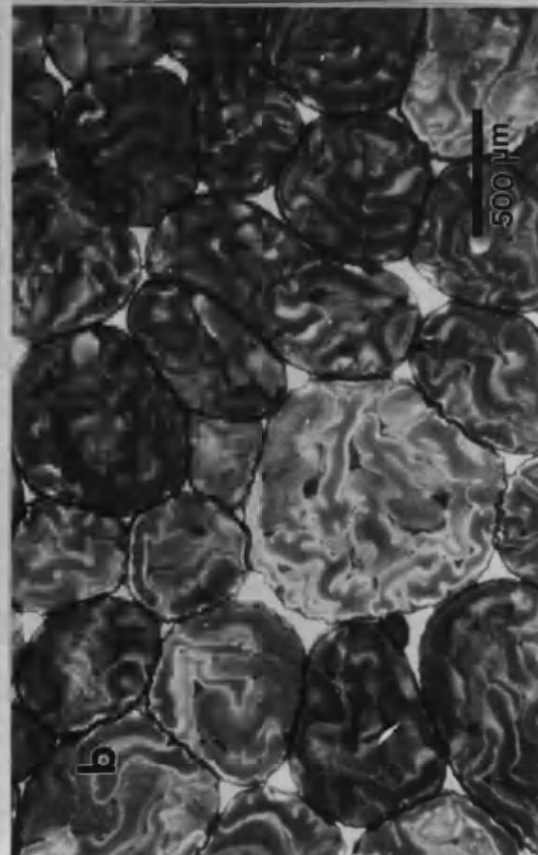
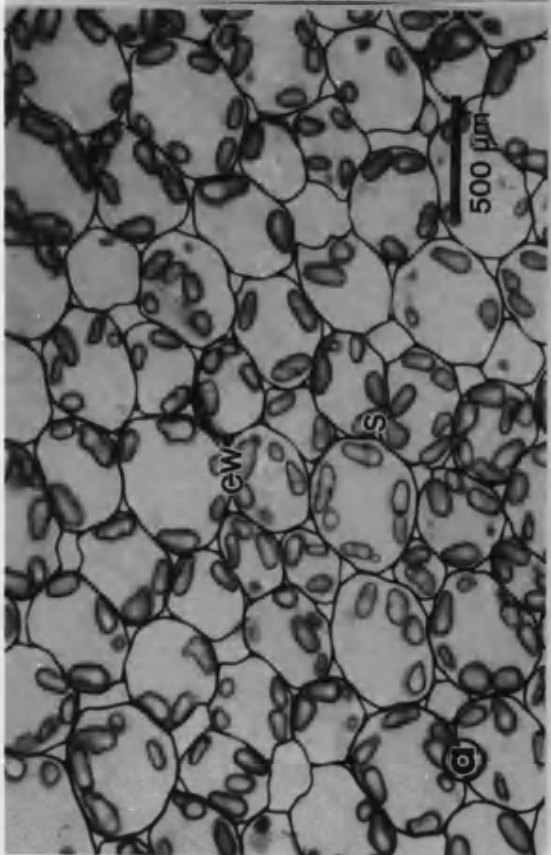
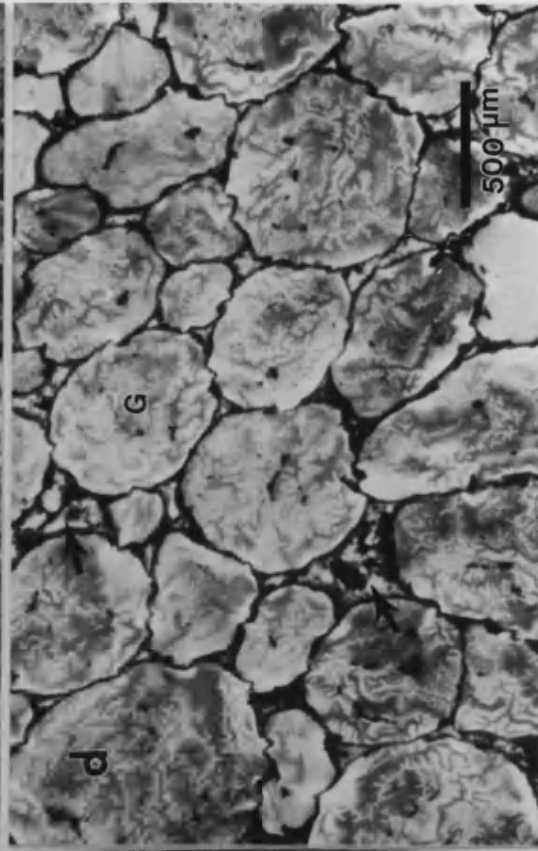
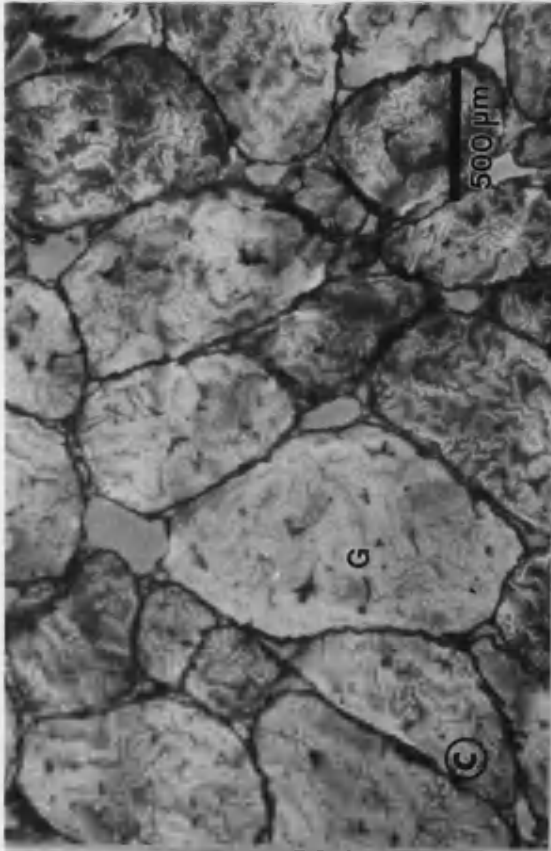


**Figure 4.13.** Light micrographs of raw and steam-cooked pulp parenchyma tissue of the soft Toro cooking banana. (a) - raw; (b) - 1 minute cooked; (c) - 3 minutes cooked; (d) - 10 minutes cooked. Arrows in (d) indicate the intercellular structures. S=Starch grains, CW=Cell Wall, G=starch Gel.





**Figure 4.14.** Light micrographs of raw and steam-cooked pulp parenchyma tissue of the hard Enkonera cooking banana. (a) - raw; (b) - 1 minute cooked; (c) - 3 minutes cooked; (d) - 10 minutes cooked. Arrows in (d) indicate the intercellular structures. S=Starch grains, CW=Cell Wall, G=starch Gel.

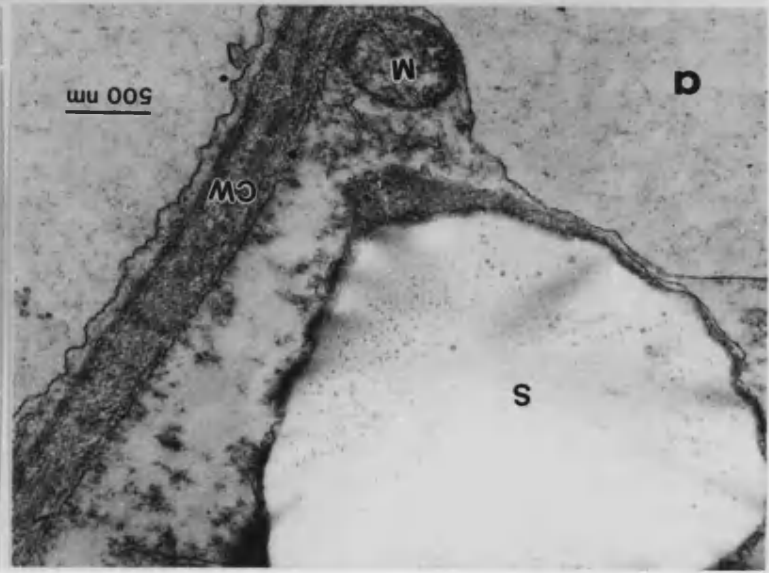
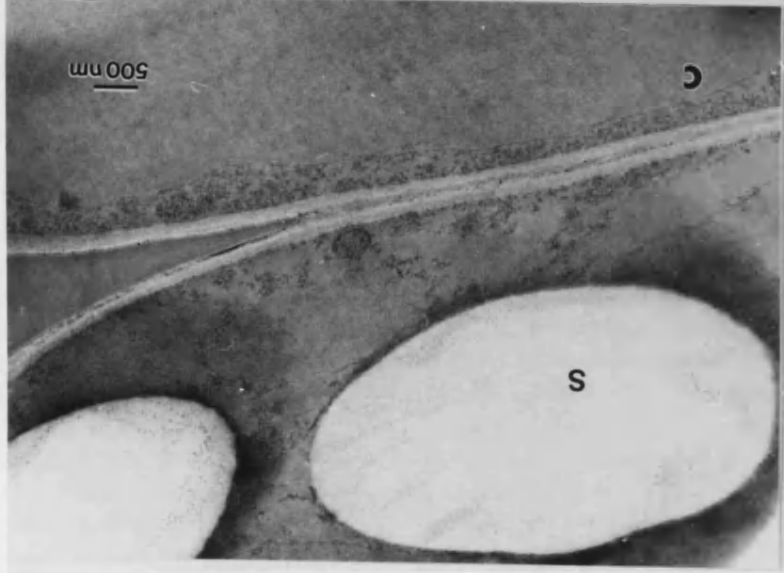
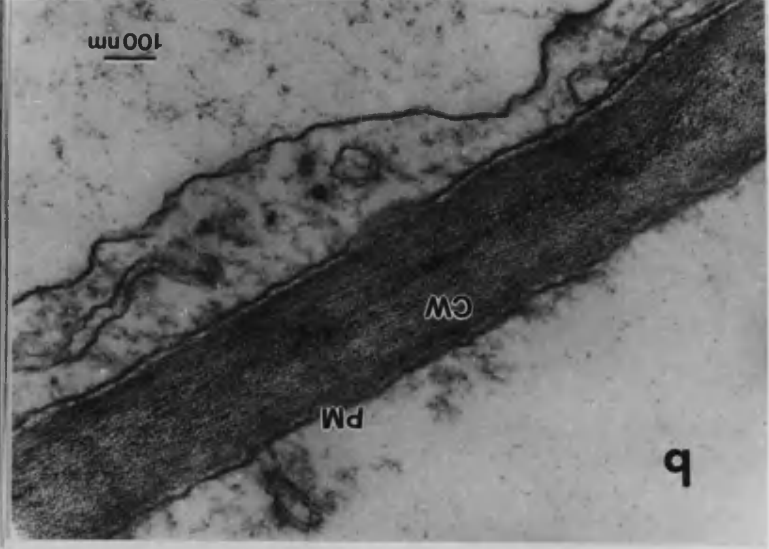


#### 4.3.3.2. TEM observations

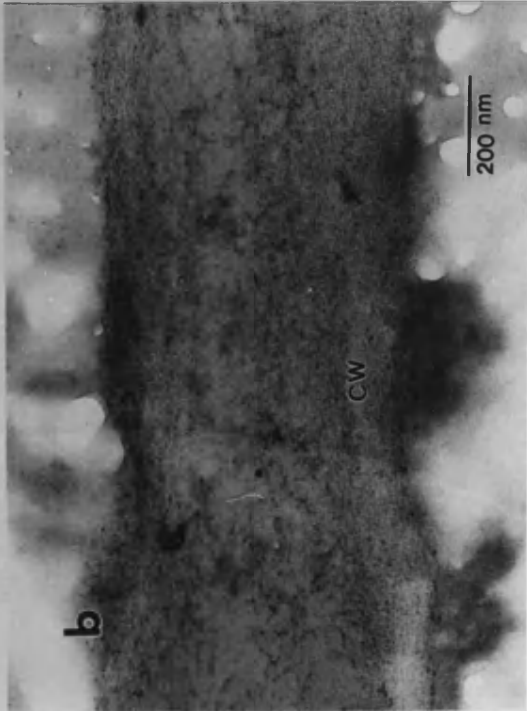
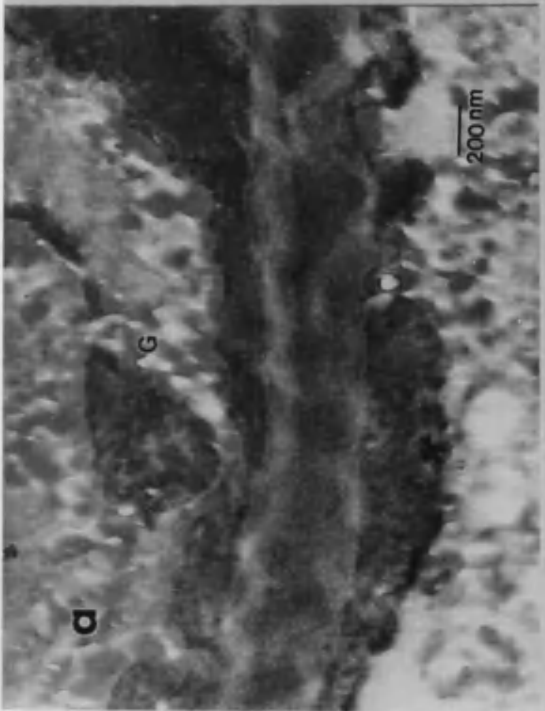
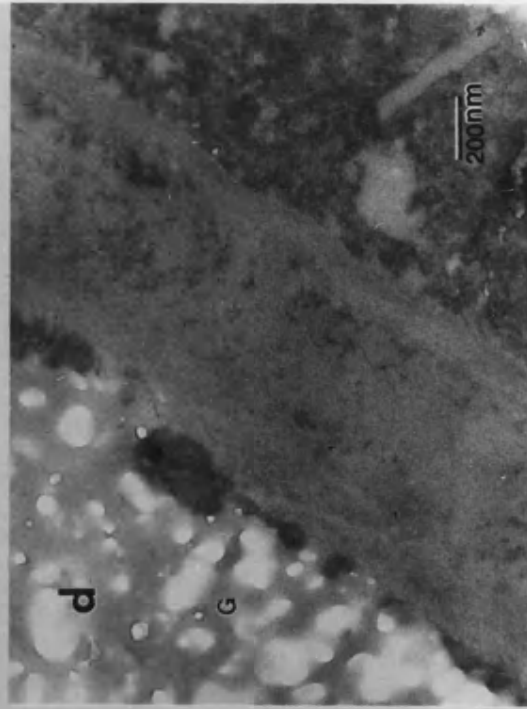
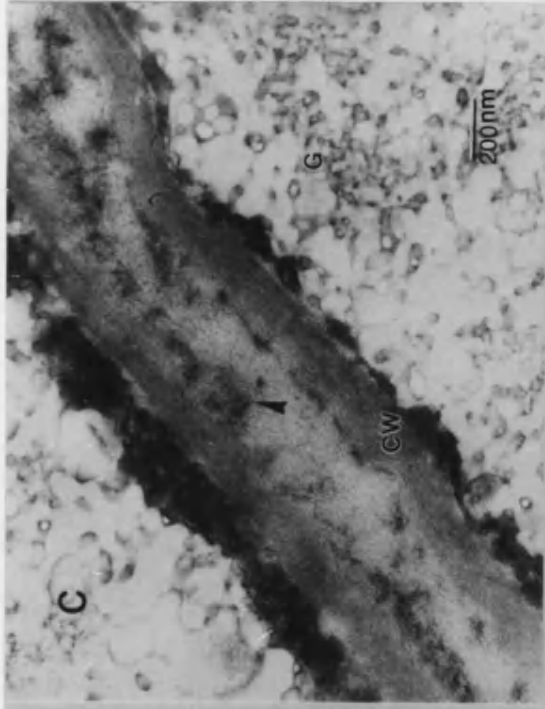
Transmission electron microscopy (TEM) was carried out on thin sections of both Toro and Enkonera cooking banana pulp tissues. The results are shown in figures 4.15-4.17 and figures 4.18-4.20, respectively.

The cell wall of Toro pulp parenchyma has the appearance of a typical primary wall with thickness of approximately 150 nm. The cellulosic microfibrils and the plasmalemma (PM) can be clearly observed. The cell wall appeared darker stained in sections fixed in the presence of ruthenium red (Figure 4.15a & b) compared to sections without it (c & d). After 1 minute of steaming, the plasmalemma membrane had disappeared. The cell wall was swollen in some areas (Figure 4.16a), and the middle lamella appeared loosened leaving an open network in the mid-zone between adjacent cells (Figure 4.16b). After 3 minutes cooking (Figure 4.16 c & d) the middle lamella appeared to have undergone more breakdown, and the cell wall had become less electron dense. Meanwhile, some dark stained particles appeared in the middle lamella region (Figure 4.16c). In the 10 minutes steamed pulp tissue (Figure 4.17), the middle lamella was almost totally dispersed with some dark stained particles present occasionally (arrows, Figure 4.17a). Adhesion between cells was limited to a few points of contact between the adjacent walls (Figure 4.17b). Meanwhile, some microfibrillar materials appeared to be separating away from the internal face of the cell wall (arrow, Figure 4.17b). In the intercellular spaces (Figure 4.17c), some microfibrillar (arrowheads) and gel-like materials (arrow) appeared.

**Figure 4.15.** TEM micrographs of raw matooke cooking banana Toro (soft) pulp tissue. (a) &(b) - with ruthenium red present in the fixatives. (c) & (d) - without ruthenium red present. S=starch, CW= cell wall, PM=plasmalemma, M=mitochondria.

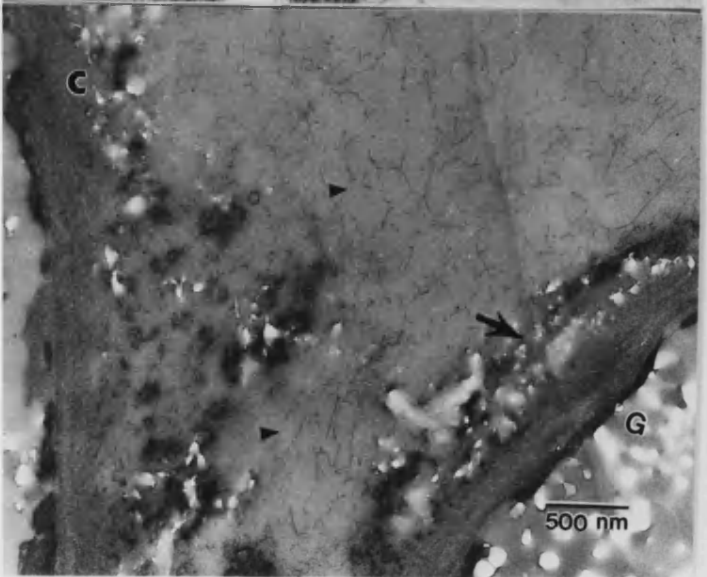
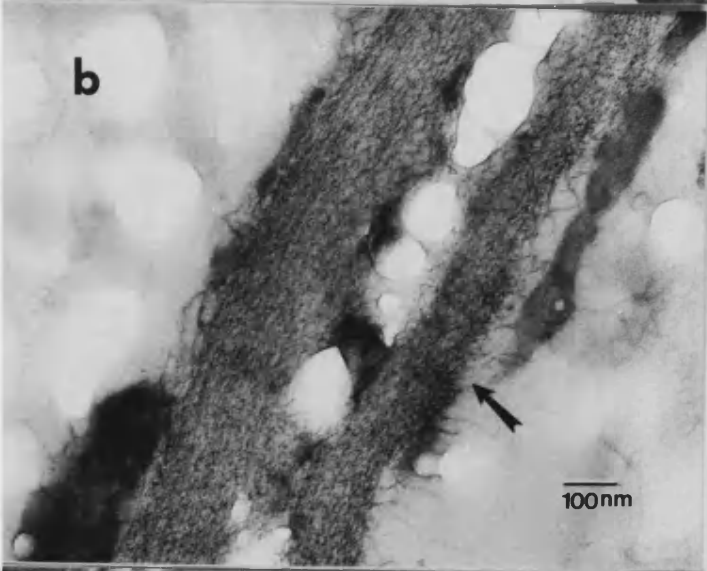
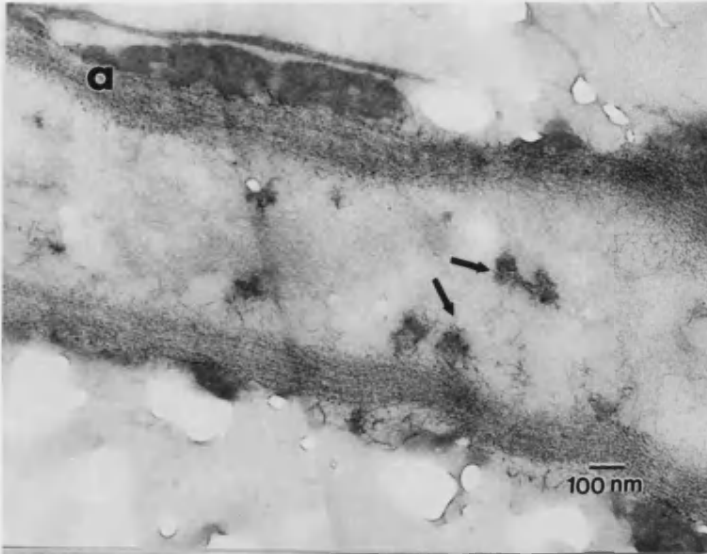


**Figure 4.16.** TEM micrographs of 1 and 3 minutes steam-cooked Toro cooking banana pulp tissue. All were with ruthenium present. (a) & (b) - 1 minute cooked, (c) & (d) - 3 minutes cooked. Arrowhead in (c) indicates the black particles present in the middle lamella region. G=starch gel, CW=cell wall.



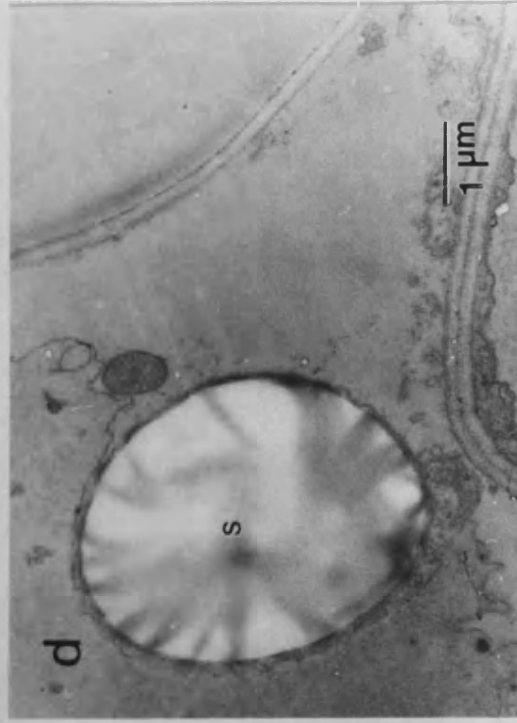
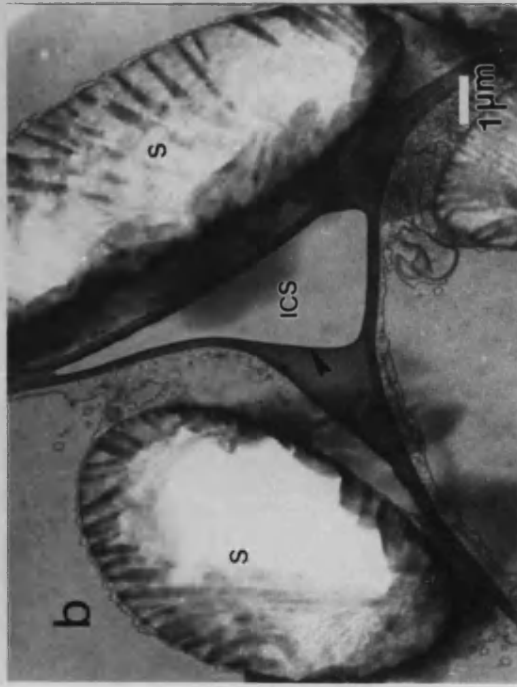
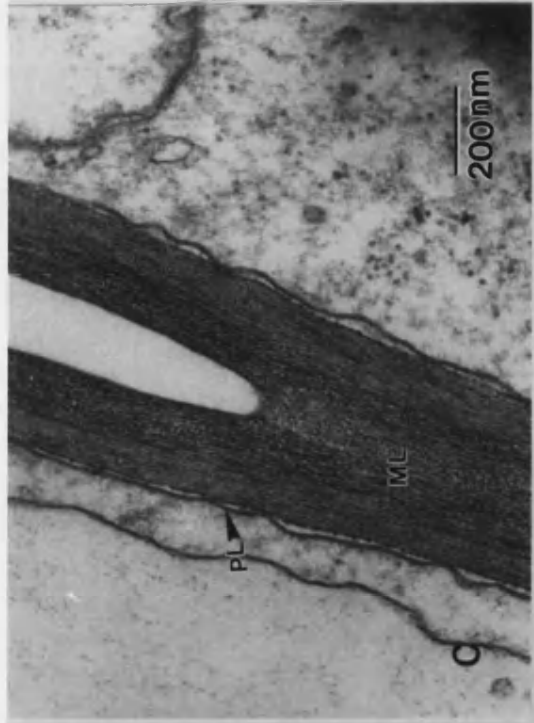
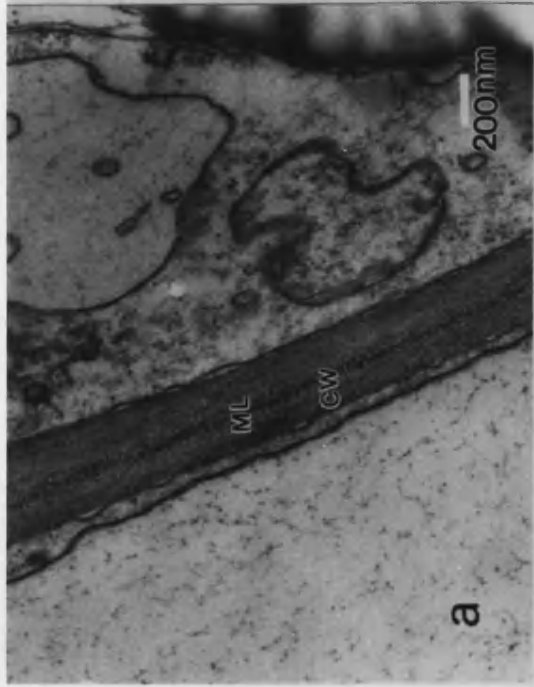
**Figure 4.17** TEM micrographs of 10 minutes cooked Toro cooking banana pulp tissue. Arrows in (a) show the black particles present; arrow in (c) indicates the microfibrils peeling away from the wall interface. Arrow in (c) shows the gel-like structure, and the small arrowheads show the microfibrils present in the intercellular space.



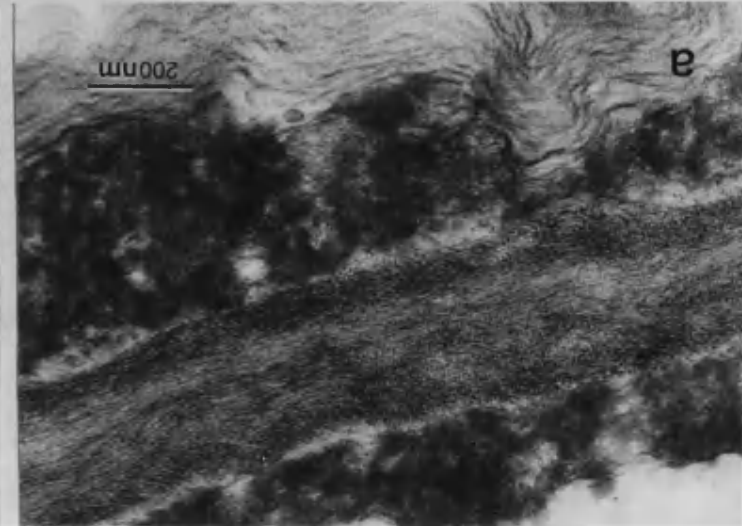
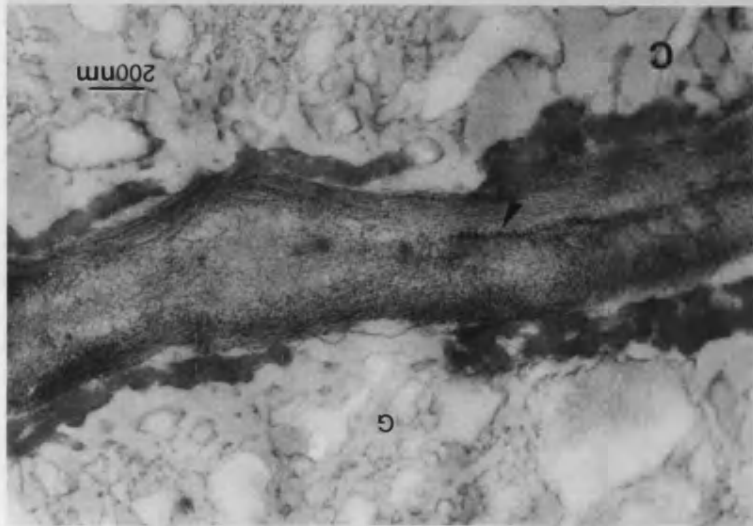
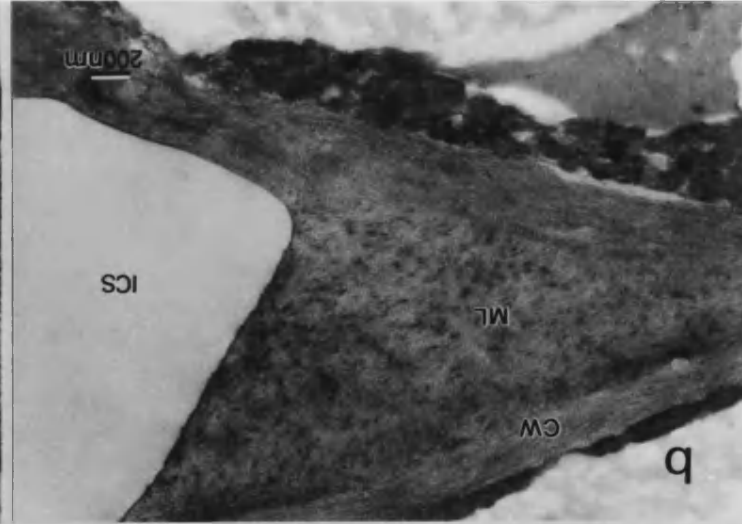
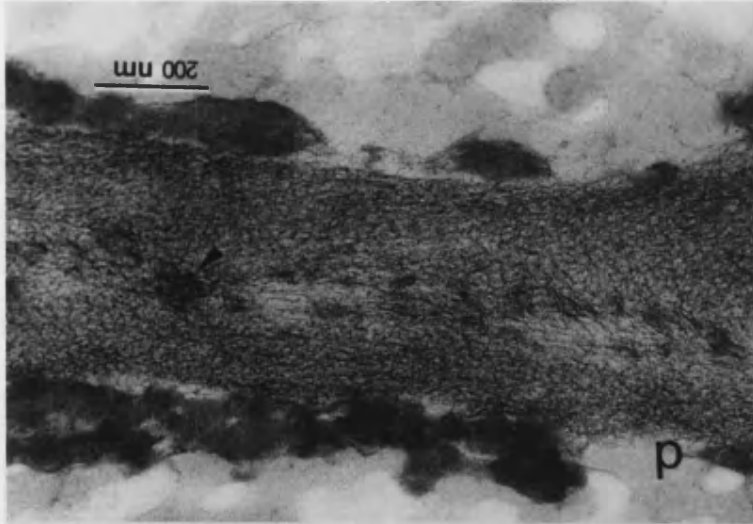


Figures 4.18-4.20 show the TEM micrographs of the pulp parenchyma tissues of Enkonera cooking banana before and after 1, 3 and 10 minutes of steam-cooking. The cell wall of the raw material shared most of the features of that from Toro (Figure 4.18). There was no difference in the cell wall thickness of the two cultivars. No structural materials were observed in the intercellular space (Figure 4.18b & c). In the 1 minute cooked pulp tissue (Figure 4.19a & b), the plasmalemma quickly vanished as in Toro, and the cell wall had swelled up. The middle lamella near the intercellular space (Figure 4.19b) appeared to have undergone some breakdown (compare with Figure 4.18c), but there were still no structures present in the intercellular space at this time. After 3 minutes of cooking (Figure 4.19c & d), the cell wall microfibrils appeared irregularly arranged, and some dark stained particles were also present in the middle lamella areas. However, the adjacent cell walls appeared to have more points of attachment than those of Toro. After 10 minutes of cooking (Figure 4.20), middle lamella dissolution and cell wall separation had occurred in a similar way as in Toro except there was less gel-like material present in the intercellular space (compare Figure 4.20d to Figure 4.17c).

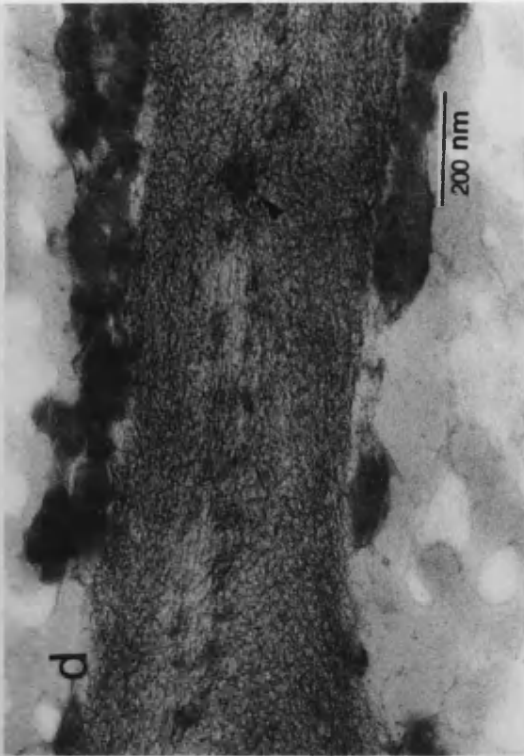
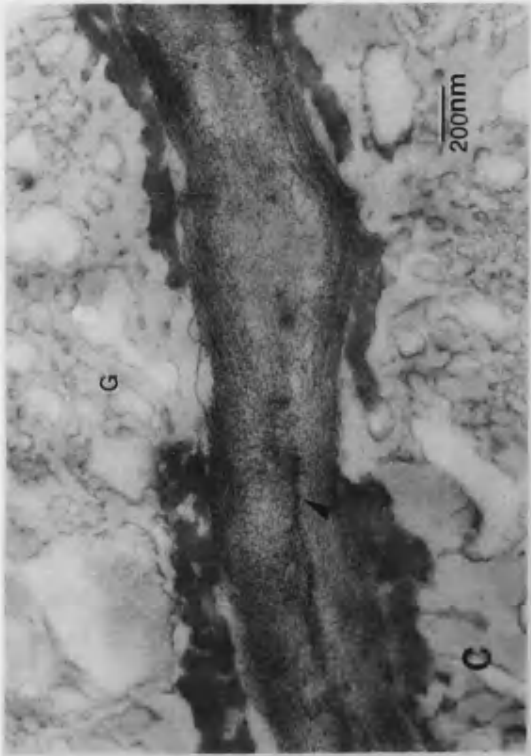
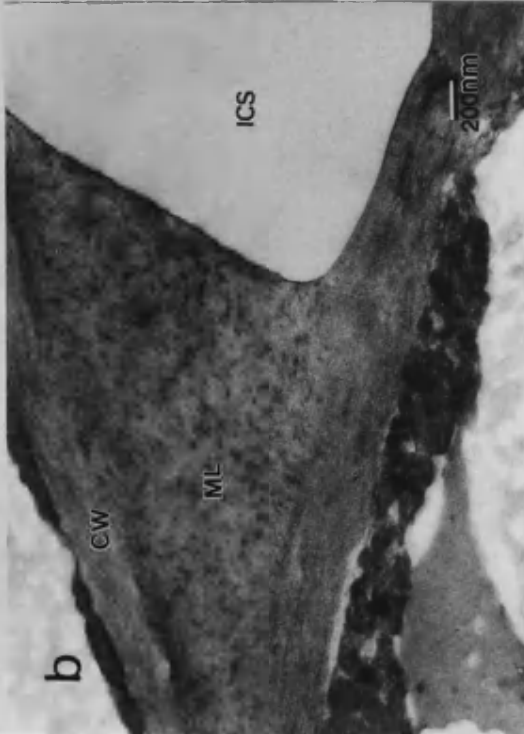
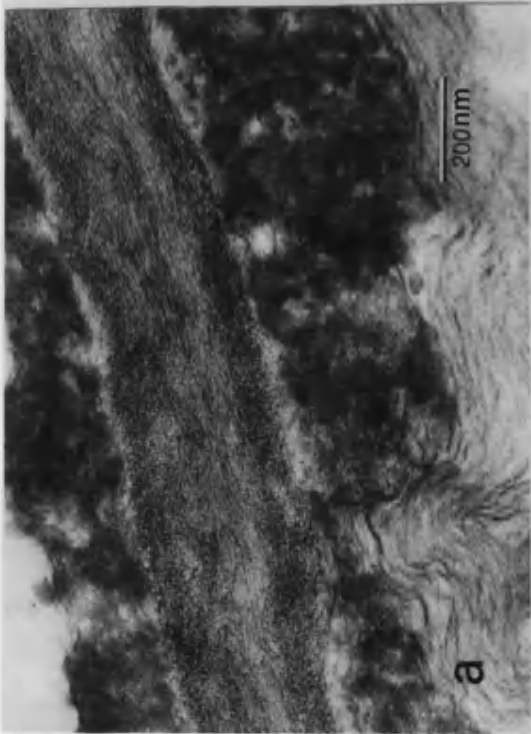
**Figure 4.18.** TEM micrographs of raw matooke cooking banana Enkonera (hard) pulp tissue. CW=Cell wall, ICS=intercellular space, ML=middle lamella, PM=plasmalemma, S=starch.



**Figure 4.19.** TEM micrographs of 1 and 3 minutes steam-cooked Enkonera pulp tissue. (a) & (b) - 1 minute cooked, (c) & (d) - 3 minutes cooked. Arrowheads in (c) & (d) indicate the black particles present at the middle lamella region. CW=cell wall, ICS=intercellular space, ML=middle lamella.

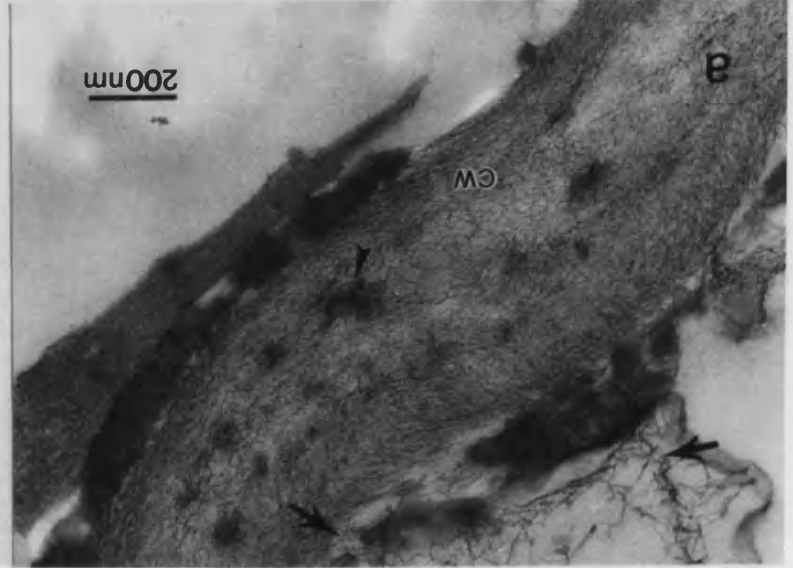
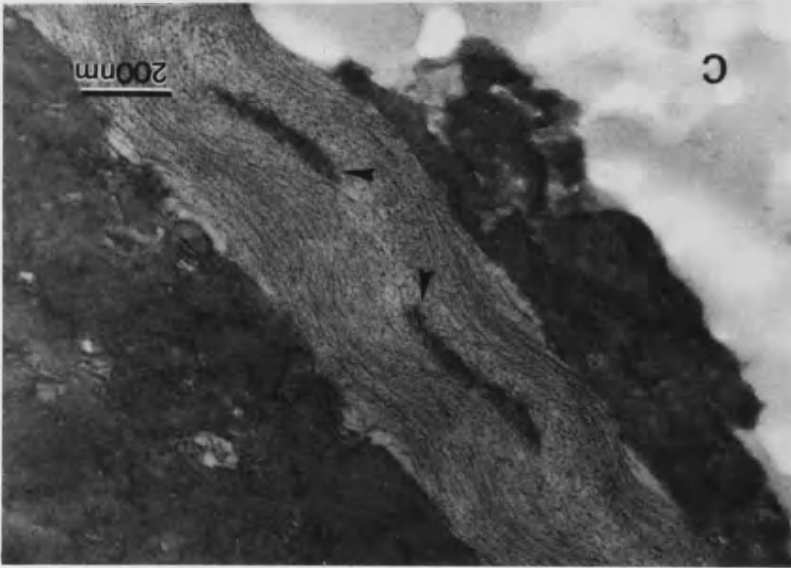
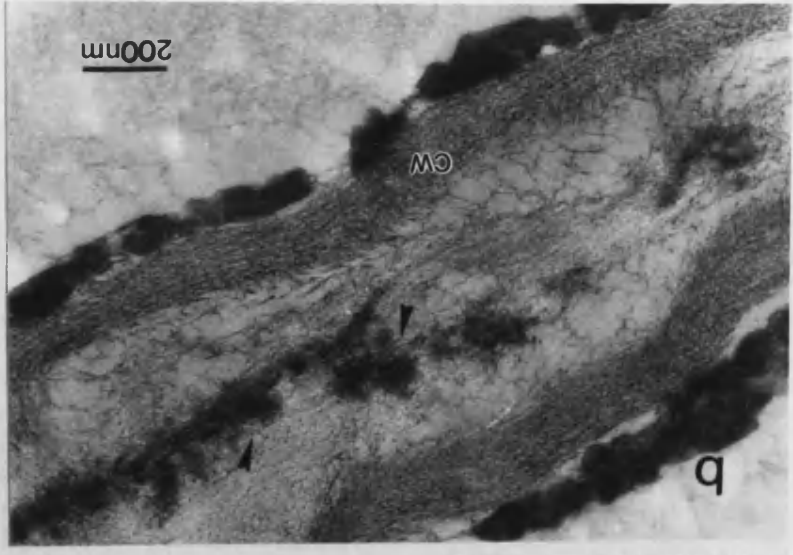
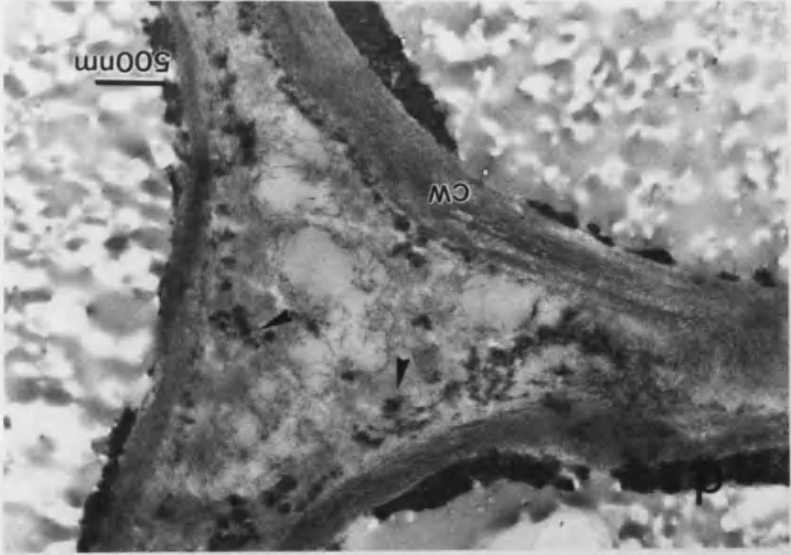


**Figure 4.19.** TEM micrographs of 1 and 3 minutes steam-cooked Enkonera pulp tissue. (a) & (b) - 1 minute cooked, (c) & (d) - 3 minutes cooked. Arrowheads in (c) & (d) indicate the black particles present at the middle lamella region. CW=cell wall, ICS=intercellular space, ML=middle lamella.





**Figure 4.20.** TEM micrographs of 10 minutes cooked Enkonera pulp tissue. Arrows in (a) shows the microfibrils peeling away from the wall interface; and arrowheads in (b), (c) and (d) indicate the black particles present in the middle lamella region and as well as the intercellular space. CW=cell wall.



#### **4.4. Summary**

SEM and TEM observations were made in pairs on dessert banana (soft)/plantain (hard) and Matooke cooking banana Toro (soft), Enkonera (hard) in order to study their different response to thermal softening. In general, starch gelatinization and cell wall separation were the two most noticeable changes during cooking in all the cultivars examined. However, dessert banana showed more advanced break-down than plantain. TEM studies of Toro and Enkonera cooking banana pulp tissues showed that the middle lamella dissolution in the intercellular space started as early as 1 minute into cooking. The dissolution then spread to the cell wall region, leaving a void space. Cell separation easily resulted from exertion of only a small force. The cell wall of the raw Enkonera cooking banana pulp tissue was more electron dense in the TEM than that of Toro.

## **4.5. Discussion**

Penetrometer measurements showed a decrease in firmness during heating of dessert banana, plantain and Toro and Enkonera cooking bananas using cooking by boiling or steaming. The rates of softening showed considerable variation between varieties. For example, dessert banana cooked in water for 10 minutes showed a 75% decrease in original firmness, whilst plantain only showed a 37% loss (Figure 3.2). Such a substantial difference in texture presumably results from large differences in the structural and chemical composition of the tissue before and after cooking. However, it is not a simple matter to determine which component(s) of the tissue are responsible for such textural differences.

### ***4.5.1 Starch changes during cooking***

Studies of raw dessert banana (soft) and plantain (hard) by SEM and Toro (soft) and Enkonera (hard) cooking bananas by both SEM and LM revealed that none of the cells from the four varieties is completely filled with starch (Figures 4.2 a & b, 4.6a, 4.11a, 4.12a, 4.13a, 4.14a). The starch grains varied in size, and the remaining cellular regions contained cell organelles and unstained spaces (Figures 4.15 & 4.18). Starch gelatinization occurred at a very early stage of cooking (2-3 minutes) in all varieties examined, and although there were slight differences in timing, the morphology of the gelatinised starch was similar in all the cultivars. Langton and Hermansson (1989) suggested that heat treatment of wheat starch dispersions gave rise to two stages of swelling and solubilization. Solubilization was observed in the centre of granules during the first stage of swelling. This also occurred with plantain (Figures 4.6 c& d) and Enkonera matooke cooking banana (Figures 4.12b and 4.14b) where the swelling started from the centre of each granule but did not result in the grains completely

coalescing in the first 1-2 minutes of cooking. This phenomenon was also observed with microwave cooked potato tissue (Huang *et al.*, 1990). However, these physical changes in starch appeared to occur at the onset of cooking with dessert banana and Toro cooking banana and were not seen in these experiments (Figures 4.2c & d, 4.11b and 4.13b). Further cooking caused the starch granules to become deformed and fused together to form a single starch gel network within each cell, separated from neighbouring cells by the heat modified cell wall and intercellular material.

In cooked material, the cells are supported by the masses of gelatinized starch, water and other cellular substances, which prevent the cooked cells from collapsing as the cell membrane is destroyed by heat in the very early stage of cooking (Greve *et al.*, 1994a). Therefore, one of the effects of the gelatinized starch is partially to maintain the integrity of the cooked tissue. However, no “rounding-off” of cells was observed in any of the four *Musa* varieties (dessert banana, plantain, Toro and Enkonera). This is in agreement with the findings made on potatoes by Hoff (1972) and McComber *et al.* (1994), although Reeve (1977) had observed the “rounding-off” phenomenon in cells which had sloughed off during cooking.

#### **4.5.2 Cell wall changes during cooking**

It is well known that middle lamella dissolution and cell wall separation during ripening can cause fruit softening and this has been studied extensively in many fruits including avocado (Platt-Aloa and Thompson, 1981), apple and pear (Ben-Arie *et al.*, 1979) and tomato (Crookes and Grierson, 1983). These changes can be observed by transmission electron microscopy (TEM). However, relatively few TEM studies have been conducted on cooked products including potato. TEM studies of hard-to-cook (HTC) legumes have been reported (Bhatty, 1990, Shomer *et al.*, 1990). However the

cells in these tissues are usually partially lignified, i.e., the walls are a mixture of primary and secondary cell walls. The banana cell wall is not closely comparable since banana pulp cells have typical primary cell walls (Figures 4.15 and 4.18). In this study detailed TEM observation has been undertaken on raw, 1, 3 and 10 minutes cooked Toro (soft) and Enkonera (hard) cooking banana pulp tissue (Figures 4.15-4.20). It was found that when ruthenium red was present in the fixatives during preparation, the cell walls were stained darker (Figure 4.15a & b), compared to when ruthenium red was absent (Figure 4.15 c& d). This suggested that ruthenium red preserves banana cell wall structure, maybe by complexing pectins.

Ruthenium red binds to ionizable carboxylic acid groups. Because of this it can be used in the fixation and visualisation of acidic mucopolysaccharides. It is often used to stain middle lamella and primary cell walls in light microscopy studies of pectin distributions within the cell wall (McComber *et al.*, 1994) although this stain is recognised to be non-specific for pectin (Albersheim *et al.*, 1960b) because it can react with certain lipids as well (Luft, 1964). However, Luft (1964) found that plant material specimens containing pectin stain strongly with ruthenium red when observed with transmission electron microscope. Therefore, the subsequent TEM observations were made using ruthenium red stained samples of Toro and Enkonera cooking bananas during steam-cooking.

After 1 minute of steam-cooking the cell walls of both Toro (Figure 4.16a & b) and Enkonera (Figure 4.19a & b) pulp became swollen and some middle lamella dissolution also appears to have taken place, especially in the area near the intercellular space (Figure 4.19b). The Toro cells showed more severe breakdown in both cell wall and the middle lamella than Enkonera. The plasmalemma disappeared

rapidly, and the various cellular organelles had become distorted and apparently pushed to the walls, presumably by the gelatinised starch, leaving an electron-dense layer next to the wall. Meanwhile, the intercellular space appears expanded in both Toro and Enkonera (Figures 4.11b, 4.13b, 4.14b). This is different from the behaviour of cooked potato tissues, where no intercellular space enlargement was observed by McComber *et al* (1994). The expansion of the intercellular space was also another indication of the middle lamella dissolution due to cooking.

In the 3-minutes cooked Toro pulp tissues, the middle lamella appeared to have broken down and the cell walls had separated from each other (Figure 4.19c & d). Enkonera cooking banana showed less extensive changes although some middle lamella dissolution had also taken place (Figure 4.19c & d). After 10 minutes of cooking the breakdown of the middle lamella and cell wall separation was observed in both Toro and Enkonera (Figures 4.17 & 4.20). This was taken as a clear indication that cooking had resulted in pectin solubilization as in potatoes (Hughes *et al.*, 1975a, b). The results agree with the studies on potatoes by van Marle *et al* (1992) and Loh *et al* (1982) who claimed that cell wall separation did take place during cooking when observed by SEM. However, based on SEM and LM studies, Huang *et al* (1990) and McComber *et al* (1994) stated that cell wall separation was not observable in cooked potatoes. The harder potato cultivar that resists deformation when mashed showed more thickened cell wall-middle lamella complexes instead of cell separation, in contrast to the soft cultivars (McComber *et al.*, 1994).

Some dark stained particles were visible in the middle lamella area (Figures 4.16-4.17, 4.19 - 4.20, arrowed). These particles may contain coagulations of the highly esterified pectin formed on cooking and cooling (Jarvis *et al*, 1984) or of protein

coagulated during cooking (Loh *et al* 1982). The cellulose microfibrils appeared to separate from the interior of the cell walls suggesting that the cross-links between them had become broken down. It seems that the layer of the 'melted' organelles may prevent the cellulose microfibrils from 'peeling' away from the walls as the 'peeling' can only be observed where there were no organelles present.

As the various cell membranes are destroyed by heat, the mobility of large molecules such as amylose and polyuronides would not be impaired and it seems likely that they could enter the intercellular space more easily. This effect may be responsible for the appearance in figure 4.17c and 4.20c, where the initially empty intercellular spaces (Figures 4.15c and 4.18b & c) have become filled with structures including microfibrils, presumably of cellulose (arrowhead), gel-like material (possibly amylose) (arrowed) and dark stained particles. All these materials may form the intercellular reticulated structure observed by SEM (Figures 4.11c & 4.12d, arrowed) and LM (Figures 4.13d & 4.14d, arrowed) of cooked Toro and Enkonera cooking banana tissues. Although it is difficult to assign their physical properties to the structures, it seems unlikely that the intercellular reticulated materials would resist deformation, since a large part of its volume is made up of spaces possibly containing soluble materials in the pre-fixation state of the tissue.

#### ***4.5.3. The relative importance of starch and cell wall in texture loss during cooking***

The cell wall first became apparently deformed when Toro and Enkonera cooking bananas were cooked in 100°C steam after as little as 1 minute (Figures 4.16a & b, 4.19a & b). This suggests that cell separation can take place before gelatinised starch



fills the entire cell space. Comparison of this finding with firmness loss, for instance in Toro cooking banana, indicates that as much as 40% of the original firmness had been lost before any starch “swelling pressure” effect would be likely to have become significant. Further softening of the tissue during the later stages of cooking may be caused by starch “swelling pressure” along with thermal expansion of the cells, aiding further separation. These results agree with the finding of Loh *et al* (1982) and Jarvis *et al* (1992) who stated that the starch “swelling pressure” could only separate cells when the middle lamella had become weakened by the pectin degradation, but not before that. However, no cell rupture was observed as claimed by Loh *et al* (1982) during potato cooking.

#### ***4.5.4 Comparison of cooking behaviour of dessert banana versus plantain and Toro versus Enkonera***

Dessert banana and Toro cooking banana showed a much faster rate of softening than plantain and Enkonera during cooking (Figure 3.2, Figure 4.10). It was observed that dessert banana cells appeared to separate from each other earlier and more fully during heating than was the case with plantain (Figures 4.2 - 4.18). Also more intercellular reticular materials accumulated during heating in banana than in plantain. Similar results were also found with Toro and Enkonera cooking bananas during cooking (Figures 4.11 & 4.12). This suggests that the cell walls of plantain and Enkonera cooking banana may be less rapidly affected than those of dessert banana and Toro cooking banana. This was proved by the TEM observations with Toro and Enkonera pulp tissue before and after cooking. Although there appears to be no difference in the wall thickness of the raw pulp tissue, the cell walls of the harder

Enkonera cooking banana pulp did appear more electron dense before cooking than those of the softer Toro (compare Figure 4.15 to Figure 4.18). Furthermore, the wall and middle lamella retained more integrity in Enkonera than in Toro after the same length of heating during the early stages of cooking (compare Figure 4.16 to Figure 4.19). The fact that the starch grains in the pulp tissues of plantain and Enkonera cooking banana were still recognisable in the onset of cooking when those of dessert banana and Toro cooking banana already became a gel suggests that there may be some linkage between the possession of a stronger cell wall and middle lamella and slower starch gelation in the intact cooked pulp. It seems unlikely that the physical properties of the wall would affect the rate of heat penetration into the cell, but the alternative conclusion, that cultivars with strong walls also have thermally stable starch is also difficult to explain.

However, it is possible that the textural changes in cooked starchy tissues may be affected by the total amount of starch which is present. If the swelled starch gel does not impart a strain on the cell wall by 'rounding off' the cells (e.g. because the cell wall-middle lamella complexes are strong enough to prevent this), then it is possible that the cells may actually be supported by the mass of gelatinised starch and may resist separation when shear is applied to them. Therefore, their pulp tissues would be hard when measured by the penetrometer. This was possibly why plantain and Enkonera cooking banana remained firmer after cooking than dessert banana and Toro cooking banana. The results support the suggestions of Reeve (1977) and McComber *et al* (1994) that textural qualities must be described by both the starch in the cells and the cell wall components.

## **CHAPTER 5**

### **CHANGES IN CELL WALL COMPONENTS DURING COOKING**

## 5. CHANGES IN CELL WALL COMPONENTS DURING COOKING

### 5.1 Introduction

A considerable amount of research has been directed toward understanding the tissue factors that contribute to the eating texture of fresh and cooked fruits and vegetables. The primary focus has been on changes in the plant cell wall as tissue firmness decreases during ripening, storage and processing.

The primary cell walls are composed of four types of structural macromolecule: the carbohydrates pectin, hemicellulose and cellulose and the protein extensin (see also Chapter 1). Chemical studies on the effect of processing on the mechanical properties of the cell wall have been concerned mainly with changes in pectin (a polyuronide), which is the most reactive component. It belongs to a class of compounds used by both plants and animals in cell adhesion (Ilker and Szczesniak, 1990). The principal chain of pectin consists mainly of polygalacturonic acid in which the carboxyl groups are methylated to various degrees. The middle lamella is believed to consist mainly of pectin, but to be intermeshed with other polymers in the primary wall (Albersheim *et al.*, 1960b). Cellulose gives rigidity and resistance to tearing, while pectic substances, extensin and hemicelluloses provide plasticity and elasticity and allow the cell wall to stretch.

Various pectic changes occur during heating resulting in the formation of soluble pectins and a decrease in firmness (Reeve, 1970; Fuchigami, 1987a,b). Van Buren and Pitifer (1992) suggested that pectin was the only heat labile middle lamella component making a major contribution to firmness in snap beans, carrots, potatoes,

apples and cauliflower.

It is generally thought that during heat-softening the pectic substances between as well as within the matrix of adjacent cell walls are depolymerized and degraded largely by thermal  $\beta$ -elimination. The pH condition found in most processed vegetables (5.0-6.5) would favour this reaction during cooking. Cell wall softening is due to the disintegration of the  $\text{Ca}^{2+}$ -pectic gel in the wall matrix by  $\beta$ -elimination (Jarvis, 1984). It allows teeth or a knife to slice through the cells, releasing their contents. Conversely, cell separation is due to the dissolution of the calcium-pectic gel in the middle lamella region where both  $\beta$ -elimination and chelation of  $\text{Ca}^{2+}$  ions by citrate released from within the cells by heating may be involved (Jarvis, 1984). Biting or cutting separates the cells without breaking them open.

The rate of  $\beta$ -elimination under neutral condition depends on the degree of pectin methylation (Sajjanantakul *et al.*, 1989). De-esterification of pectin using cold alkaline conditions (van Buren and Pitifer, 1992) or pre-heating at 50-70°C (Bartolome and Hoff, 1972) can reduce the susceptibility of pectin to breakdown, and retard tissue softening during the subsequent cooking. Conversely, higher pectin esterification may reduce adhesion of the wall matrix, and enhance cell wall softening and water uptake during cooking. This also leads to a soft texture in potatoes (Warren and Woodman, 1974).

However, there is evidence that some tissue softening may take place by mechanisms other than the  $\beta$ -elimination reaction. During the  $\beta$ -elimination reaction the cleavage

of the glycosidic bonds within the pectin macromolecules results in a rapid decrease in viscosity and release of breakdown products which can be measured by their absorption at 235 nm or 546 nm after reaction with thiobarbituronic acid (Albersheim *et al.*, 1960a). In studies of both potato and Chinese waterchestnut tissues, Loh and Breene (1982) reported that no detectable decrease in viscosity nor significant increase in reducing power were found when phosphate-soluble pectin (PSP) was subjected to brief heating treatments (up to 5 minutes) in deionized water, although fracturability was lost to an appreciable extent when raw tissues were subjected to these same heat treatments. Therefore the significance of the  $\beta$ -elimination mechanism of pectin breakdown to textural changes during brief heating was questioned.

Furthermore, although waterchestnut contained pectin with a slightly higher degree of esterification than potato pectin, and would be expected to show more breakdown by the transelimination reaction, gel filtration profiles of heat-treated pectin revealed that potato pectin showed a more rapid rate of depolymerization than waterchestnut pectin. These findings suggested that although the firmer structure of waterchestnut compared to potato after cooking could be related to the rate of depolymerization of pectin,  $\beta$ -elimination was unlikely to be the mechanism. This was also supported by studies made on carrots by Greve *et al* (1994b), in which the authors claimed that although the breakdown of pectins was more extensive in the soft type carrot than the firm one, there were no varietal differences in overall pectin ester content. Therefore, the significance of the more extensive pectin  $\beta$ -elimination from the soft type carrot is not clear.

Loh and Breene (1982) suggested that the thermal degradation of rhamnogalacturonan is unlikely to be directly involved in cell wall strength. They postulated that the unique composition, e.g., neutral sugars content, of the cell wall microstructure is the major determinant of the resistance to fracturability loss during cooking. In support of this is the fact that waterchestnut cell wall contained more neutral sugar than that of potato cell wall. However, Greve *et al* (1994b) did not find any difference in cell wall carbohydrate composition between firm and soft carrot genotypes. Their survey indicated that loss of texture in cooked carrots is accompanied by and perhaps caused by a substantial dissolution, depolymerization and destruction of cell wall pectins. Carrots with chelator-soluble pectins of relatively lower molecular weights tended to soften more extensively during heating. There are several reasons why polymer length might affect softening. The viscosity of a pectin solution increases as the polymer length increases. Also in the cell wall, increased pectin size might increase the possibility of an assortment of inter-polymer associations and, thus increase wall strength. This in turn could have an effect on tissue firmness.

Deducing the nature of physico-chemical changes involved in pectin solubilization requires characterisation of the *in situ* chemical changes in polyuronide structure that accompany cooking. Changes to individual polymer fractions isolated by chemical methods can be related to the *in situ* situation, providing precautions are taken to minimise inadvertent degradation of the cell wall during its preparation and fractionation. Cell walls are often prepared as alcohol or acetone insoluble residues (AIR) either by hot alcohol refluxing with a Soxhlet apparatus (Loh and Breeze, 1982), or by washing the insoluble pellet in acetone or alcohol (Fry, 1988). In both

methods the soluble sugar and other solubles are washed away. However, all insoluble polysaccharides and cytoplasmic proteins, including enzymes, are co-precipitated and recovered as part of the insoluble cell wall material (CWM). Chelator-soluble pectin is then extracted from the AIR by stirring in EDTA or CDTA. The content and properties of this fraction can then be studied and related to the texture changes of the cooked tissue upon cooking.

However, it is not certain that AIR preparations contain unmodified cell wall polysaccharides, nor that the polysaccharides subsequently solubilized from AIR by a water or CDTA extraction are representative of the polymers solubilized *in situ*. There is a possibility that the heat or enzyme action could already have had some effects on the properties of the polyuronide during the AIR preparation.

To avoid this problem, cold AIR preparations were obtained by phenol/ acetic acid/ water (PAW) extraction used in CWM preparation of the raw and cooked banana and plantain pulp tissues. PAW has the necessary property of differentially extracting soluble from insoluble cell wall polymers whilst inactivating endogenous wall enzymes (Selvendran *et al.*, 1985; Redgewell *et al.*, 1992).

The principal objectives of the work described in this chapter were firstly to identify the fraction(s) of the cell wall which were affected by the cooking process, secondly to determine the nature of the changes and thirdly to establish if the differences in susceptibility to softening between *Musa* genotypes could be related to differences in the amount, size or composition of particular wall fractions.



## **5.2. Materials and Methods**

### **5.2.1 'Cold' AIR preparation**

The freeze-dried powder was prepared as section 2.3. Two grams of each sample was dissolved into 20 ml of PAW (2:1:1, w/v/v, phenol:acetic acid:water) solution and stirred for 4 hours at 4°C. The suspension was then centrifuged and the residue washed with 80% alcohol 6 times or until it no longer gave a characteristic smell of phenol. The residue was freeze-dried and stored at -20°C until use. The AIR prepared in this way was referred to as 'cold' AIR, in contrast to the AIR prepared from Soxhlet refluxing (see section 2.4) which is referred to as 'hot' AIR hereafter.

### **5.2.2 Estimation of Water-Soluble, Chelator-Soluble and Sodium Carbonate-Soluble Polyuronides Content of AIR**

The water-soluble polyuronides were extracted by shaking 100 mg of the AIR with distilled water for 20 hours at 20°C on a orbital shaker. The residue from the water extraction was stirred with 50 mM CDTA (potassium salt, pH 6.5) for 20 hours at 20°C to solubilize the chelator-soluble polyuronides (Jarvis, 1982; Redgwell *et al.*, 1988). The residue was re-suspended in 50 mM Na<sub>2</sub>CO<sub>3</sub> containing 20 mM NaBH<sub>4</sub>, and stirred for 20 hours at 1-4°C, then 2 hours at room temperature. A few drops of toluene were added to all the solutions at the beginning of each extraction to inhibit micro-organisms growth. Each supernatant solution was obtained by centrifugation, then filtration through GF-A glass fibre filter paper. The Na<sub>2</sub>CO<sub>3</sub>-soluble fraction was neutralised with acetic acid under ice. Uronic acid in each fraction was estimated by the m-phenylphenol method (see section 2.6)

### **5.2.3 Cell Wall Purification**

Cell wall materials were purified from fresh or freeze-dried dessert banana, plantain

and cooking banana pulp tissue according to Selvendran *et al* (1985) with the modification of omitting Na deoxycholate in the initial preparation step (Redgwell *et al.*, 1992), since Na deoxycholate (SDC) is known to solubilize appreciable amounts of pectic materials from the cell wall.

Fresh sections of banana and plantain pulp were cut into small pieces and frozen in liquid nitrogen, then ground to a fine powder with an electric coffee grinder. 200g of the frozen powder was homogenised with 400 ml of PAW until it became a fine slurry, then stirred overnight at room temperature. For the Uganda matooke cooking banana Toro and Enkonera, the pulp tissue was freeze dried, ground and passed through a 250- $\mu$ m sieve. Ten grams of this powder was suspended in 100 ml of PAW solution and stirred for 4 h at 4<sup>o</sup>C to inactivate enzymes and to extract protein. The homogenate was centrifuged at 7,000g for 15 minutes and the residue washed with 80% alcohol in the same manner as for 'cold' AIR preparation (section 2.4.2). The residue was then extracted twice with 90% of dimethyl sulfoxide (DMSO) to solubilize the starch present at 4<sup>o</sup>C overnight. The suspension was centrifuged at 10,000g for 15 minutes, and the residue washed by centrifugation with 80% alcohol 6 times. The cell wall material (CWM) was recovered following dialysis and freeze drying, which yielded approximately 0.5 g dry weight for Toro and Enkonera matooke cooking banana (i.e. 5% yield/dry weight).

#### ***5.2.4. Solubilization of cell wall polymers from Toro and Enkonera CWM***

100 mg of CWM was extracted with 15 ml of each solvents of 50 mM CDTA, then 50 mM Na<sub>2</sub>CO<sub>3</sub> containing 20 mM NaBH<sub>4</sub> as described in section 5.2.2. The residue was then stirred for 18 h at room temperature in 6 M guanidinium thiocyanate (GTC) for 16 hours, and finally in 4 M KOH containing 20 mM NaBH<sub>4</sub> under nitrogen for 2

hours (Redgwell *et al.*, 1988). Uronic acid contents in pectic fractions were estimated by the m-phenylphnol method (Blumenkrantz and Asboe-Hansen, 1973), whilst the neutral sugar contents in hemicellulosic fractions were estimated by the phenol-H<sub>2</sub>SO<sub>4</sub> method (Dubois *et al.*, 1956) using galactose as a standard (see appendix 4). Each supernatant was then filtered (those containing alkali were neutralised), dialyzed and concentrated, than stored at -20°C for further heating experiments and gel filtration studies. The remaining materials were freeze-dried for sugar analysis by GLC.

### ***5.2.5 Measurement of the Degree of Pectin Esterification***

The degree of esterification of pectin was analysed by the enzymatic method of Klavons and Bennett (1986). To 5 ml of the sample solution, 2.5 ml of 1.5 N KOH solution was added, and incubated at room temperature for at least 30 minutes to hydrolyse the pectin. The mixture was then nearly neutralised with diluted H<sub>3</sub>PO<sub>4</sub> to pH 7.5 then 1 ml of this solution assayed for methanol content. 1 ml of this solution was pipetted into a test tube and mixed with 1 ml of alcohol oxidase solution (1 unit/1 ml), then incubated at 25°C for 15 minutes, and 2 ml of 0.02 M 2,4-pentanedione (freshly distilled) in 2.0 M ammonium acetate and 0.05 M acetic acid was added and mixed well. The tubes were placed in a dri-heating block at 58-60°C for 15 minutes and then allow to cool to room temperature. The absorbance was measured at 412 nm against a blank containing phosphate buffer at the appropriate concentration, pH 7.5, and dilute alcohol oxidase. HPLC grade methanol was used as a standard (see appendix 5). The percent esterification is expressed as the percent of the uronic acid present as methyl ester.

### 5.2.6 Gas-Liquid Chromatography (GLC) Measurement for Neutral Sugar

#### *Composition*

The neutral sugar composition of purified cell wall materials and other fractions was determined by GLC of their alditol acetates by the methods of Blakeney *et al.* (1983) and Harris *et al.* (1988), using myo-inositol as an internal standard. 5 mg of solids were suspended in 0.125 ml of 72% (w/w) H<sub>2</sub>SO<sub>4</sub> in Teflon-lined screw cap glass tube. The solid was dissolved with the aid of a vortex mixer and incubated at 30 °C for 90 minutes. The extract was diluted with distilled water (1.35 ml) so that the H<sub>2</sub>SO<sub>4</sub> concentration was molar, then heated under N<sub>2</sub> at 100°C for 3 hours. When cooled, the solution was made neutral and made molar with respect to ammonia by adding 15 M ammonia solution (0.32 ml). 50 µl of myo-inositol (20mg/ml) was added as an internal standard. 0.2 ml aliquots were reduced and acetylated according to the following procedure.

To 0.2 ml of the hydrolysate in M ammonia solution 1 ml of 2% sodium borohydride in dimethyl sulfoxide was added, and then reduced for 90 min at 40°C. 0.1 ml of 18 M acetic acid was added to decompose the excess of sodium borohydride. 0.2 ml of 1-methylimidazol and 2 ml acetic anhydride were then added to the reduced monosaccharides, mixed and left for 10 min at room temperature. 5 ml of distilled water was then added to decompose the excess of acetic anhydride. When cool, 1 ml of dichloromethane was added and agitated on a vortex mixer. The lower organic phase was removed with an acid-washed Pasteur pipette and stored in a 2-ml vial (Fisons) at -20°C. A mixture of sugars comprising rhamnose, arabinose, galactose, fucose, xylose, mannose and glucose (1 mg/ml) was also reduced and acetylated in the same way as the samples so that the response factor of each sugar could be determined (see appendix 6).

For the GLC separation of the alditol acetates, 1  $\mu$ l of sample was injected manually to a BPX 70 capillary column fitted to a Pye Unicam GCD chromatograph equipped with a flame-ionisation detector. The carrier gas was high purity helium, flow rate was approximately 1 ml/min (back pressure was 1.2 kg/cm<sup>2</sup>). The oven temperature was kept at 220°C. The injection port and detector were heated to 250°C. The peak areas were calculated by a TRIVector TR10 integrator.

#### ***5.2.7. Heating treatments of extracted cell wall polymers from Uganda cooking banana Toro and Enkonera***

Two ml portions of approximately 0.5% solutions of the CDTA, Na<sub>2</sub>CO<sub>3</sub>, GTC and KOH-soluble polymers in distilled water (pH 5.5) (see section 5.2.4) were distributed into 10 ml Teflon-lined screw cap test tubes for further heat treatments (Loh and Breene, 1982; Sajjanantakul *et al.*, 1989). No buffer was used to control the pH because most buffers used and described in the literature (including phosphate, acetate and tris buffers) are capable of enhancing degradation of potato pectin (Keijbets *et al.*, 1976).

Duplicate samples in tightly-capped tubes were heated in a heating block for 1, 3, 5 and 10 minutes, respectively. After heating the samples were immediately cooled in ice-water, then 2 ml of 50 mM acetate buffer containing 125 mM NaCl (pH 6.0) was added to each tube to aid in stabilizing the polymers. The samples were concentrated down to 2 ml, then kept at -20°C until tested for molecule weight studies by GPC.

#### ***5.2.8. Gel filtration chromatography***

Gel filtration chromatography was carried out using Sepharose CL-2B (fraction range 100,000 - 20,000,000 for linear dextrans) packed into a glass column (1.6x89 cm, bed

volume is 180 ml). The gel was equilibrated in 50 mM acetate buffer (pH 6.0) containing 125 mM NaCl and with 0.05% chlorobutanol as an anti-microbial agent.

The insoluble residue resulting from thawing was centrifuged down, and 0.5 ml of 0.2M imidazole-HCl (pH 7.0) for CDTA and Na<sub>2</sub>CO<sub>3</sub>-soluble pectic polysaccharides was added to solubilize the solid. For hemicellulosic polysaccharides from the GTC and KOH extraction, 0.5 ml of 0.2 M NaOH containing 20 mM NaBH<sub>4</sub> was added for the same purpose then neutralized with acetic acid. Each sample solution was loaded onto the column, eluted with 50 mM acetate buffer containing 125 mM NaCl and 0.05% chlorobutanol at 10 ml/h, and 2 ml fractions were collected by an automatic fraction collector (LKB), and monitored spectrophotometrically by the m-phenylphenol for galacturonic acid content (Blumenkrantz and Asboe-Hansen, 1973) and phenol-sulphuric acid method for total sugar content (Dubois *et al.*, 1956) at 520 and 485 nm, respectively.

#### **5.2.9. Ion-exchange chromatography**

CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions were isolated from 'cold' AIR of both Toro and Enkonera pulp tissue as previously described (section 5.2.1), and subjected to anion exchange chromatography on a glass column (2.6x20 cm) packed with DEAE Trisacryl M (Redgwell and Selvendran, 1986). The medium was equilibrated with phosphate buffer (pH 6.3). The CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions were concentrated over PEG 20,000 and dialyzed in distilled water for a few days at 4°C, and made to 0.05 M phosphate form with 0.2 M phosphate buffer, then the solution (about 100 ml) passed through the column at 40 mL/h. The fractions were eluted sequentially with 200 mL of 0.05 M phosphate buffer and 200 ml of 0.05 M buffer containing 0.125, 0.25, and 0.5 M NaCl. Fractions (8.0 ml) were collected and assayed for carbohydrate by the phenol-sulfuric acid method (Dubois *et al.*, 1956).

### 5.3 Results

#### 5.3.1. Fractionation CWM from raw fruit pulp

The CWMs of both Toro and Enkonera cooking bananas were sequentially extracted with CDTA, Na<sub>2</sub>CO<sub>3</sub>, GTC and KOH as described in sections 5.2.2 and 5.2.4. The relative amounts of polymers solubilised and their sugar compositions are given in Table 5.1 and 5.2.

	<i>Fractions (mg sugar/g CWM)</i>							
	<i>Water-sol</i>	<i>CDTA-sol</i>		<i>Na<sub>2</sub>CO<sub>3</sub>-sol</i>		<i>GTC-sol</i>	<i>KOH-sol</i>	<i>Residues</i>
		<sup>a</sup> <i>GA</i>	<sup>b</sup> <i>d.e.</i>	<sup>a</sup> <i>GA</i>	<sup>b</sup> <i>d.e.</i>			
Toro	4.43	56.65	47.95	155.23	75.58	120.93	96.2	566.56
Enkonera	5.21	66.84	65.68	160.36	68.61	124.46	100.9	542.23

**Table 5.1** Yields of fractions solubilized from CWMs isolated from the raw Toro and Enkonera pulp tissues. Values are means of two measurements. <sup>a</sup>Galacturonic acid, <sup>b</sup>Degree of esterification.

Table 5.1 shows that CDTA and Na<sub>2</sub>CO<sub>3</sub> together solubilised 21% and 23% of total pectins from Toro and Enkonera CWMs, respectively. Na<sub>2</sub>CO<sub>3</sub> solubilised substantial amounts of pectic polymers. Enkonera CWM is higher in both CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectic polymers and these fractions accounted for 66.8 and 160.4 mg galacturonic acid respectively compared to 56.7 and 155.2 mg GA/g CWM in Toro. Enkonera CWM also contains a little more of GTC- and KOH-soluble hemicellulosic polymers than Toro CWM. The CDTA-soluble pectin of Toro CWM is less esterified (48%) than that of Enkonera CWM (66%), whilst the Na<sub>2</sub>CO<sub>3</sub>-soluble pectin of the former is more highly esterified (76%) than that of the latter (69%).

Table 5.2 lists the sugar compositions of CWMs and two pectic fractions of Toro and Enkonera (see also appendix 7). The results indicated that Enkonera CWM is composed of a much higher proportion of galactose which accounted for 9.7 mol% compared to Toro CWM where the galactose content is 5.2 mol%. No difference in galacturonic acid content between the two was found owing to the big variation between replicates (see appendix 7). However, Enkonera had a lower ratio of galacturonic acid to galactose of 3.2:1 than Toro which had a ratio of 6.8:1. Enkonera CWM is also higher in arabinose and mannose. The sugar compositions of non-cellulosic fractions after 1M H<sub>2</sub>SO<sub>4</sub> hydrolysis generally followed the results of that of CWM, except the non-cellulosic fractions have much less cellulose (determined as glucose content) and galacturonic acid content is 50% of the total sugar, as expected.

The CDTA extraction solubilised a very viscous material having a higher ratio of galacturonic acid to galactose. The pectic fraction subsequently extracted with Na<sub>2</sub>CO<sub>3</sub> was less viscous. The CDTA- and Na<sub>2</sub>CO<sub>3</sub> soluble fractions of both Toro and Enkonera cooking banana CWMs were composed mainly of galacturonic acids (more than 81%). This confirms the results of Smith (1989).



<i>Sugars</i>	Fractions (mol%)							
	CWM <sup>a</sup>		Non-cellulosic <sup>b</sup>		CDTA-sol.		Na <sub>2</sub> CO <sub>3</sub> -sol.	
	<i>Toro</i>	<i>Enkonera</i>	<i>Toro</i>	<i>Enkonera</i>	<i>Toro</i>	<i>Enkonera</i>	<i>Toro</i>	<i>Enkonera</i>
<i>Rha</i>	t	0.6	0.8	t	3.3	2.9	t	t
<i>Fuc</i>	t	0.6	0.7	t	0.2	0.2	t	t
<i>Ara</i>	8	9.2	14.6	12.8	2.5	1.4	3.5	3.2
<i>Xyl</i>	8	7.8	15.4	12.7	2.5	1.5	0.7	0.7
<i>Man</i>	1.9	3.2	1.3	t	1.0	0.9	0.4	0.3
<i>Gal</i>	5.2	9.7	6.2	14.9	6.1	4.5	5.1	5.7
<i>Glc</i>	41.5	37.6	6.2	10.0	3.1	2.0	4.1	3.2
<i>GA</i>	35.4	31.3	54.7	49.7	81.4	86.6	86.2	87.0

**Table 5.2.** Sugar compositions of the purified cell wall materials (CWM), non-cellulosic cell wall materials and of fractions obtained by sequential extraction of CWM with CDTA and Na<sub>2</sub>CO<sub>3</sub>. <sup>a</sup>Anhydrous sugar values after Saeman hydrolysis. <sup>b</sup>Anhydrous sugar values after 1M H<sub>2</sub>SO<sub>4</sub> hydrolysis. t Trace.

### ***5.3.2 Effect of 'hot' and 'cold' methods of preparation of alcohol insoluble residue (AIR) on pectin composition and mol wt of Toro cooking banana pulp***

The AIR of Toro pulp tissue prepared from 'hot' alcohol refluxing (Section 2.4) and by the 'cold' method (Section 5.2.1) were extracted successively with water, CDTA and Na<sub>2</sub>CO<sub>3</sub> as described in Section 5.2.2.

The results in Table 5.3 indicated that far higher amounts of water-soluble pectin were extracted from AIR prepared from raw Toro pulp by the 'hot' alcohol method, than by the 'cold' method (490 and 90 mg/100 g AIR, respectively). During cooking, the amount of water soluble pectin (WSP) extracted from the 'cold' AIR increased remarkably after 10 minutes of cooking, but the WSP from the 'hot' AIR showed no obvious increase after the first 3 minutes of cooking, and only increased by 38% after 10 minutes. Thus the increase in pectin extracted from cooked material was much smaller when the AIR had been prepared by the 'hot' method than when it had been prepared by the 'cold' method, which gave a four fold increase in extraction as a result of cooking.

In the raw uncooked materials, both the contents of CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins were much lower in the 'hot' AIR than in the cold AIR. The content of CDTA-soluble pectin in the 'hot' AIR was 84% lower than in the 'cold' AIR, and that of Na<sub>2</sub>CO<sub>3</sub>-soluble pectin was 81% lower. After cooking, the Na<sub>2</sub>CO<sub>3</sub>-soluble pectin decreased in both 'hot' and 'cold' AIR, but the latter showed more significant change than the former. The CDTA-soluble pectin increased after 3 minutes, than decreased after 10 minutes of cooking in both 'hot' and 'cold' AIR, but changes were much

more severe in the 'hot' AIR than in the 'cold' AIR.

Gel filtration chromatography of the CDTA-soluble pectins isolated from the cold and hot AIR of the raw Toro pulp tissue were performed on a Sepharose CL-2B column. The results are shown in Figure 5.1. It appears that the CDTA-soluble pectin from the 'hot' AIR was of much lower mol wt (similar to that of blue dextran) than that from the cold AIR.

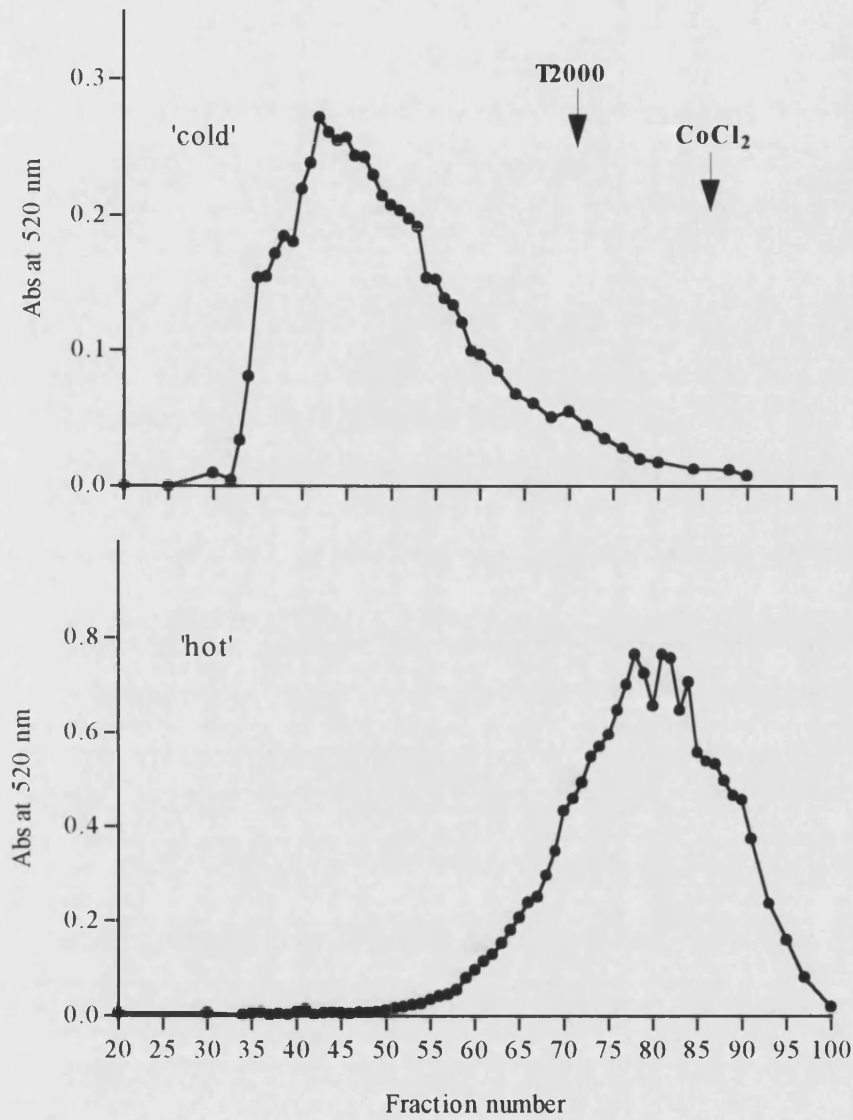
To summarise:

1. Using hot alcohol refluxing to prepare the alcohol insoluble residue (AIR) could cause an increase in the water-soluble pectin, and decrease in the CDTA- and  $\text{Na}_2\text{CO}_3$ -soluble pectin in the raw Toro pulp tissue;
2. Using the hot AIR for further fractionation studies, in order to determine the pectin composition changes during cooking, could lead to incorrect and wrong conclusions;
3. Hot AIR preparation could cause significant mol wt reduction of the CDTA-soluble pectin in the raw materials.

For this reason, the preparation of AIR by hot alcohol refluxing in a Soxhlet apparatus was not used in the studies of the *Musa* cell wall and cell wall polymers composition and mol wt distribution hereafter.

<i>Fractions (mg AGA<sup>a</sup>/100 g AIR<sup>b</sup>)</i>								
<i>Steaming time</i>	<i>Water-soluble</i>		<i>CDTA-soluble</i>		<i>Na<sub>2</sub>CO<sub>3</sub>-soluble</i>		<i>Total</i>	
	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
Raw	490.4± 10.2	90.9± 7.6	147.0± 3.4	269.7± 7.9	431.8± 2.8	784.0± 24.3	1069.2	1144.6
3 min	501.5± 19.3	193.9± 15.4	191.6± 12.1	276.5± 10.3	427.1± 11.5	560.7± 9.1	1121.2	1031.1
10 min	676.8± 59.9	435.6± 22.5	148.8± 12.6	315.6± 3.1	298.1± 12.3	354.9± 31.0	1123.7	1106.1

**Table 5.3** Effects of different AIR preparation on pectin composition of *Toro* cooking banana. <sup>a</sup>Anhydrous galacturonic acid. <sup>b</sup>Alcohol insoluble residue. Hot=hot alcohol refluxing prep. of AIR. Cold= cold alcohol prep. of AIR. Values are means±SD of three samples.



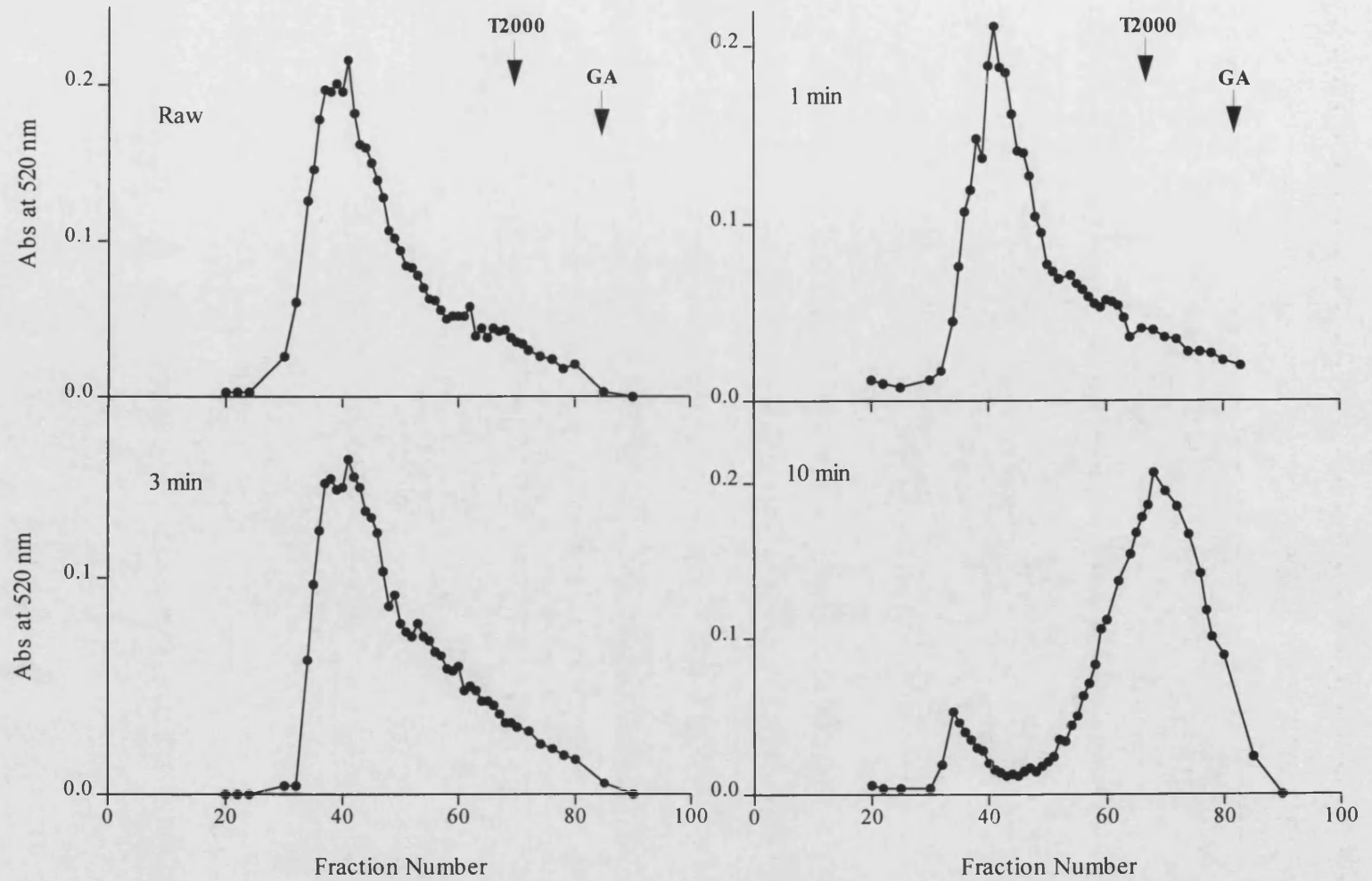
**Figure 5.1.** Gel filtration profiles of CDTA-soluble pectic polysaccharides isolated from 'cold' and 'hot' AIR on Sepharose CL-2B column. The galacturonic acid content in each fraction was monitored by the m-phenyl-phenol method. T2000=Blue dextran (Mw=2,000,000). CoCl<sub>2</sub>=cobalt chloride.

### ***5.3.3. Effect of heating on mol wt changes of the isolated pectic and hemicellulosic polymer fractions from purified cell wall materials***

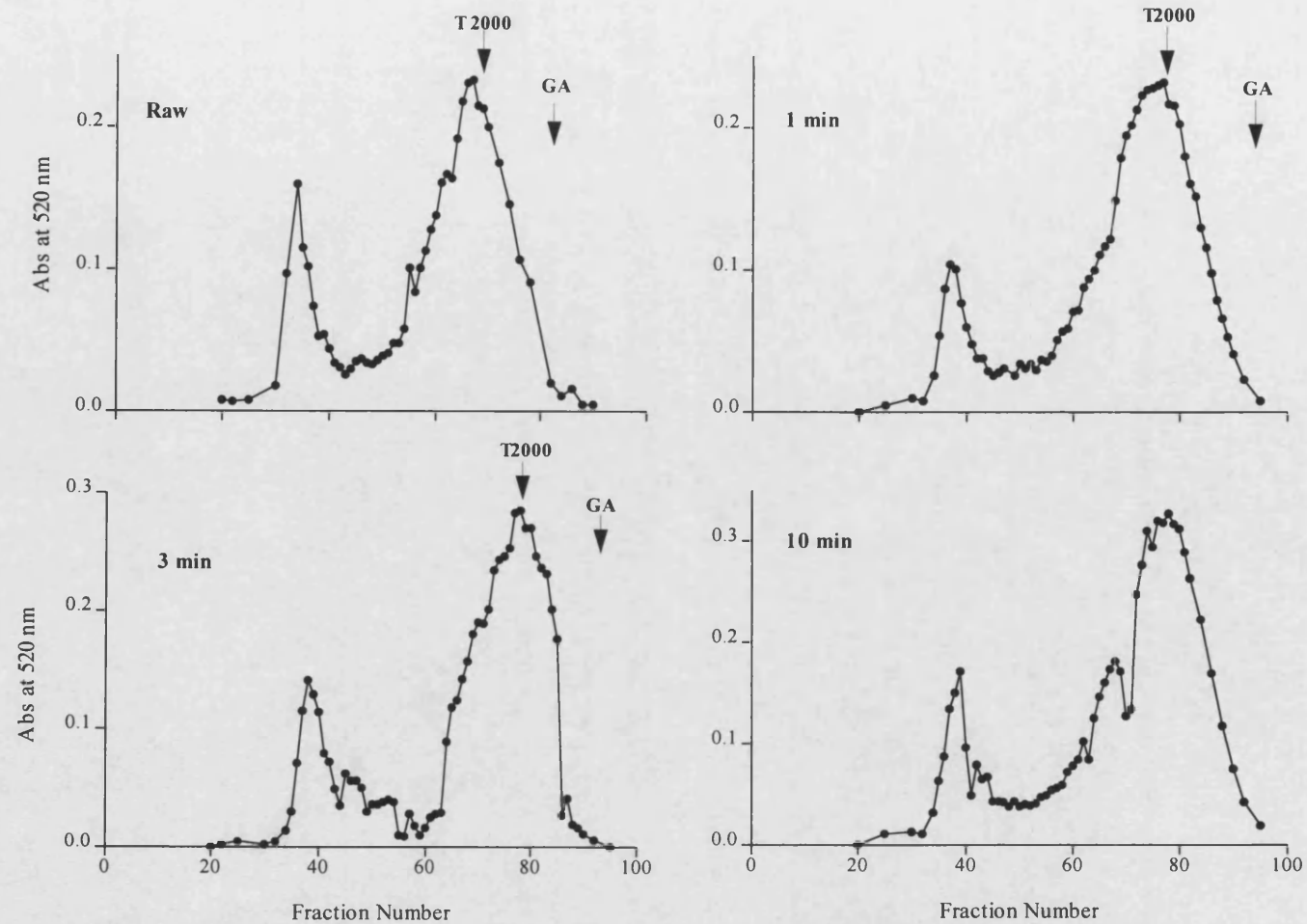
The isolated fractions resulting from sequential extraction of purified CWMs with CDTA, Na<sub>2</sub>CO<sub>3</sub>, GTC and KOH were dialysed, concentrated, then heated in test tubes for 1, 3, 5, 10 minutes. (Sections 5.2.4 & 5.2.7). Molecular wt distributions were determined by gel filtration chromatography performed on a Sepharose CL-2B column (fractionation range: 100,000-20,000,000).

Figures 5.2-5.5 are the gel filtration profiles of CWM polymer fractions from Toro cooking bananas. These indicated that the CDTA-soluble fraction was the only CWM pectic fraction to show a marked reduction in mol wt after 10 minutes heating. The unheated CDTA-soluble pectic polymers from the raw CWM were composed almost entirely of high mol wt material. After 1 minute heating there was little change in their mol wt. The peak broadened after 3 minutes of heating, indicating that more lower mol wt material had been formed. After 10 minutes of heating, almost the entire peak had shifted from the high mol wt side to the much lower mol wt side where the blue dextran is eluted (Mw=2,000,000), leaving a very small peak of higher mol wt materials behind (Figure 5.2).

Gel filtration profiles on Sepharose CL-2B of the Na<sub>2</sub>CO<sub>3</sub>-soluble fractions indicated that the mol wt profile of the unheated Na<sub>2</sub>CO<sub>3</sub>-soluble pectic fraction was different from that of CDTA-soluble pectic fraction. The former had more lower mol wt materials than the latter. There were no significant changes in the mol wt of the Na<sub>2</sub>CO<sub>3</sub>-soluble fraction during heating, except that an extra peak was developed on the higher mol wt side after 10 minutes of heating (Figure 5.3).

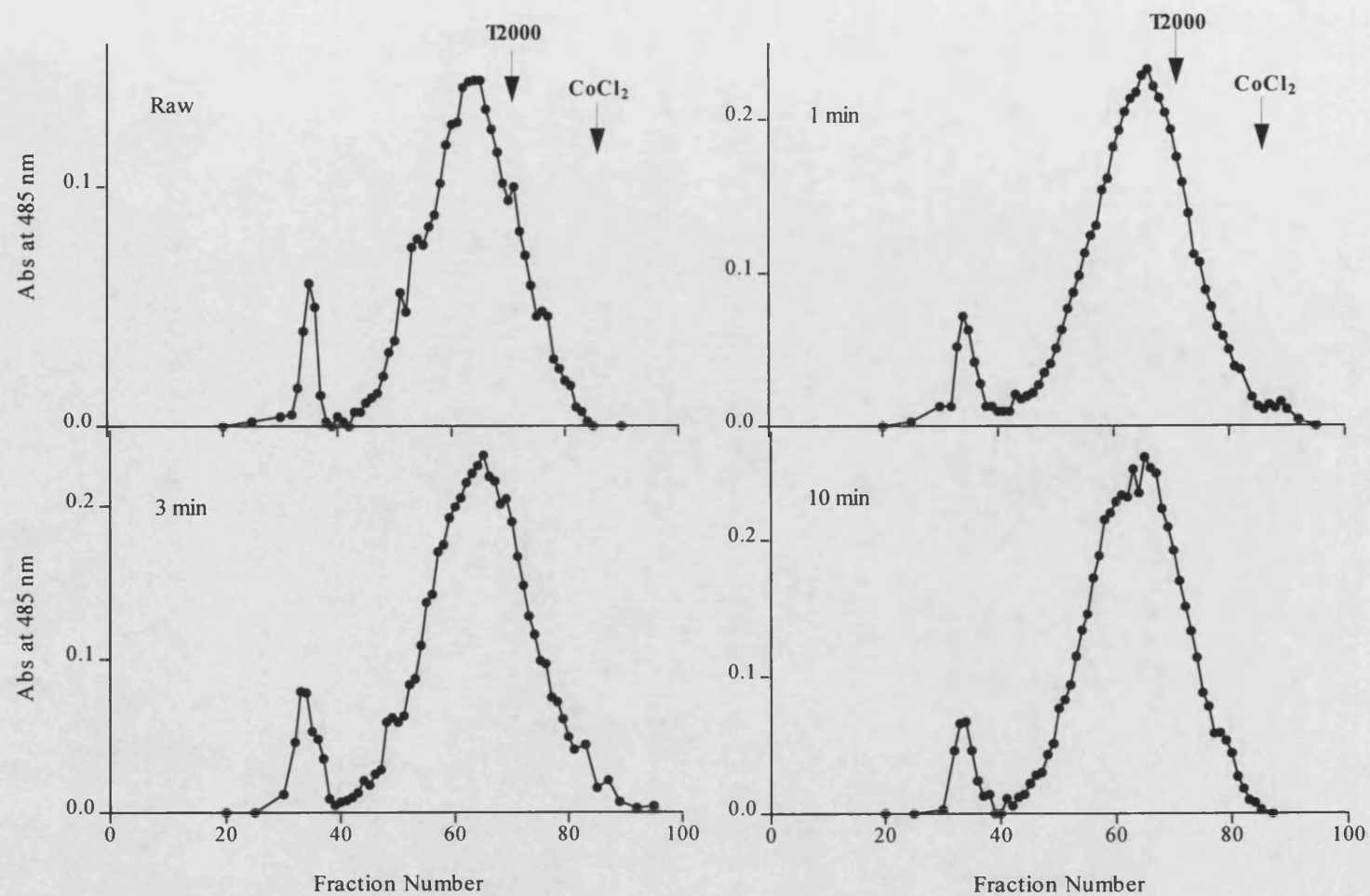


**Figure 5.2.** Gel filtration profiles of CDTA-soluble pectic polymers isolated from the CWMs of Toro cooking banana (soft) pulp tissue on Sepharose CL-2B column (1.6x90cm) before and after heating for 1, 3 and 10 minutes at 100 °C. The galacturonic acid in each collected fraction (2 mL) was monitored by the m-phenylphenol method (abs at 520 nm). T2000=Blue dextran (Mw=2,000,000); GA=galacturonic acid.

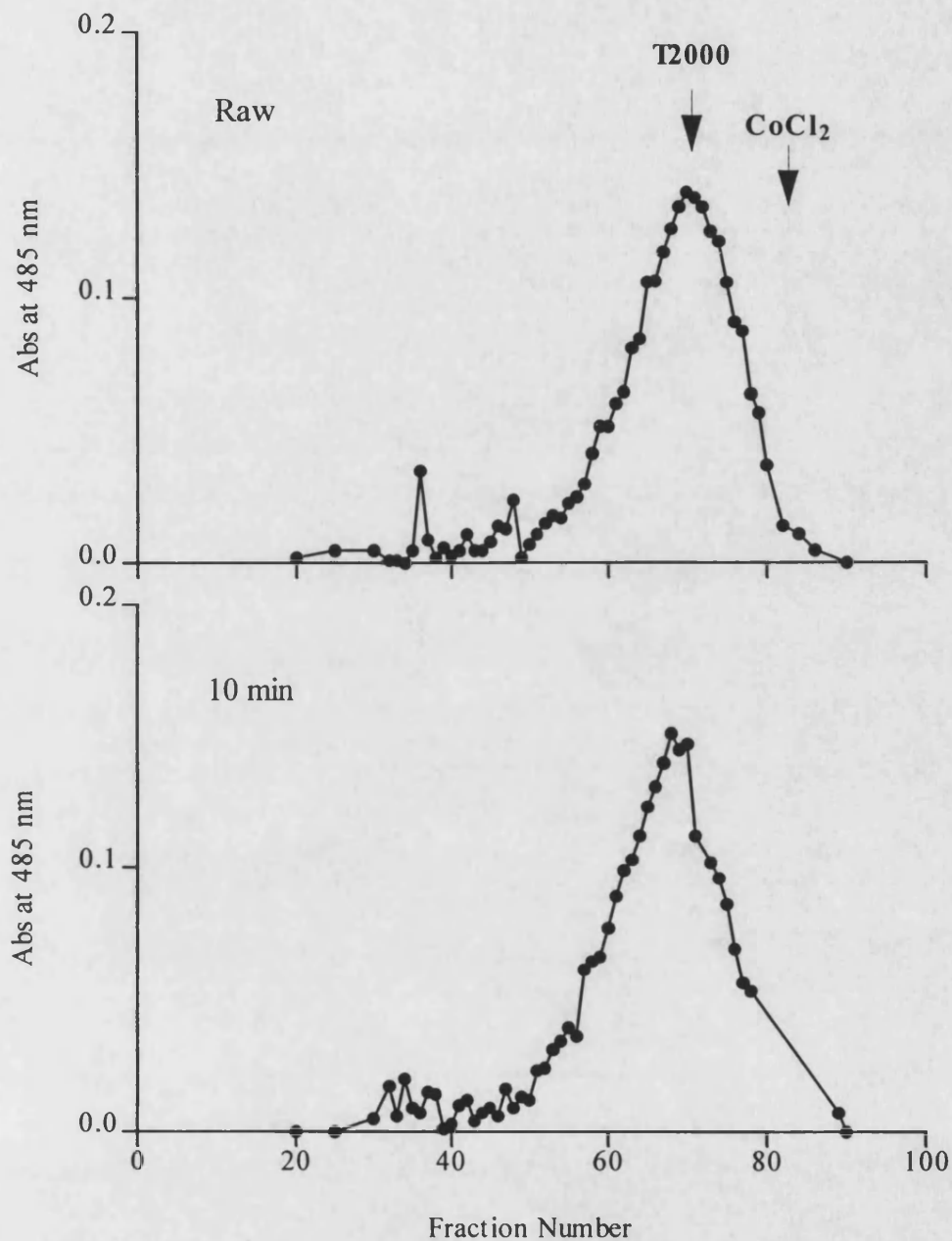


**Figure 5.3.** Sepharose CL-2B gel filtration profiles of  $\text{Na}_2\text{CO}_3$ -soluble pectic polymers isolated from the CWMs of Toro cooking banana (soft) pulp tissue before and after heating for 1, 3, 10 minutes at  $100^\circ\text{C}$ . The galacturonic acid in each collected fraction (2 mL) was monitored by the m-phenylphenol method (abs at 520 nm). T2000=Blue dextran ( $M_w=2,000,000$ ); GA=galacturonic acid.





**Figure 5.4.** Sepharose CL-2B gel filtration profiles of KOH-soluble hemicellulosic polymers isolated from the CWMs of Toro cooking banana (soft) pulp tissue before and after heating for 1, 3, and 10 minutes at 100 °C. The sugar content in each collected fraction (2 mL) was monitored by the phenol- $\text{H}_2\text{SO}_4$  method (abs at 485 nm). T2000=Blue dextran ( $M_w=2,000,000$ );  $\text{CoCl}_2$ =cobalt chloride (pink).

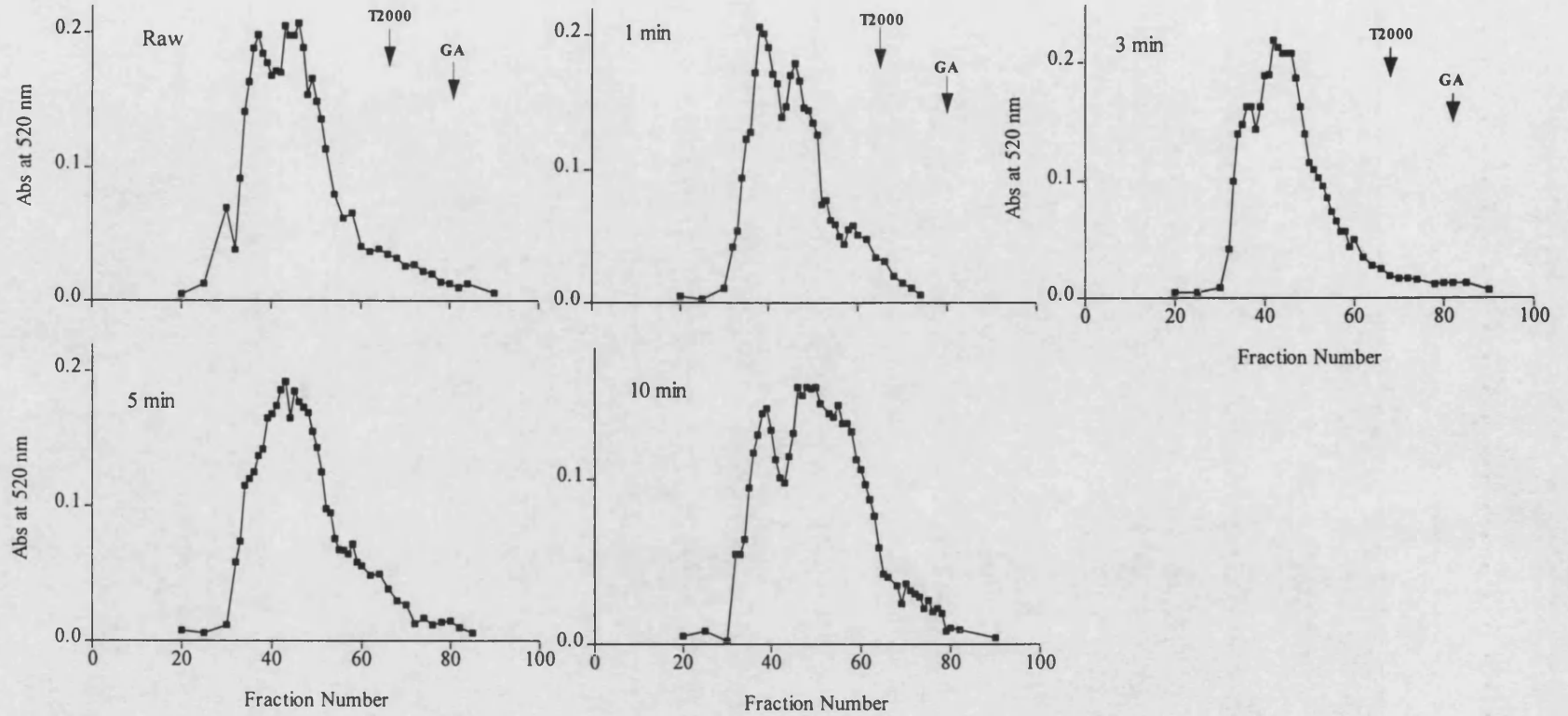


**Figure 5.5** Gel filtration profiles of GTC-soluble hemicellulosic polymers isolated from the CWMs of Toro cooking banana (soft) pulp tissue on Sepharose CL-2B column before and after 10 minutes of heating at 100 °C. The sugar content in each fraction was monitored by the phenol-H<sub>2</sub>SO<sub>4</sub> method. T2000=Blue dextran (Mw=2,000,000), CoCl<sub>2</sub>=cobalt chloride.

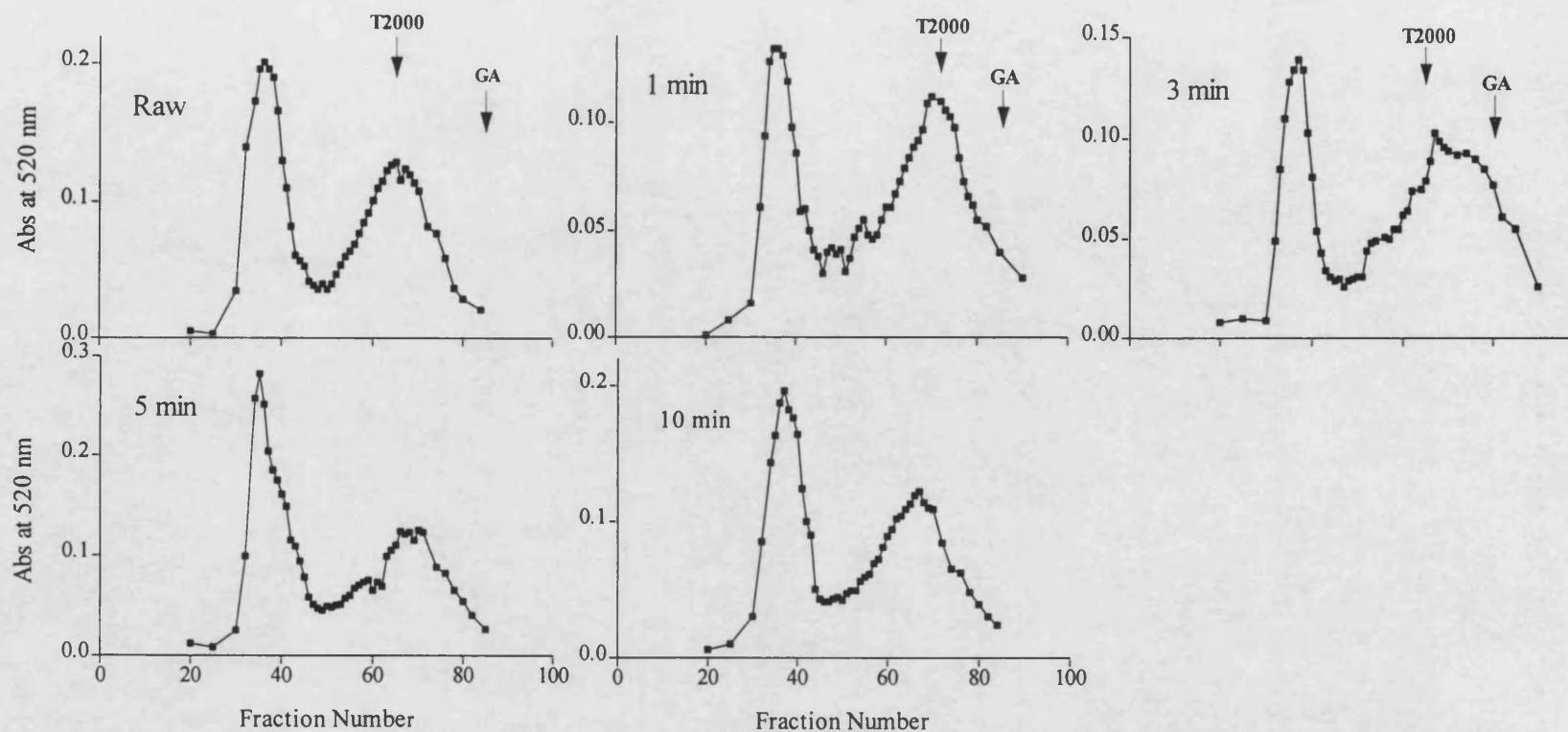
The mol wt of the GTC- and KOH-soluble hemicellulosic polymer fractions had no obvious changes upon heating (Figures 5.4 - 5.5). Both polymers had two distinct peaks with one on the high mol wt side and the other on the lower mol wt side which is similar to that of blue dextran ( $M_w=2,000,000$ ), although the KOH-soluble fractions had a slightly higher mol wt distribution compared to GTC-soluble fractions.

Figures 5.6 - 5.8 show the gel filtration profiles of Enkonera CWM polymer fractions on Sepharose CL-2B column before and after heating. Again, the CDTA-soluble pectic fraction was the only one showing a significant mol wt reduction upon heating (Figure 5.6). However, the profiles of CDTA- and  $\text{Na}_2\text{CO}_3$ -soluble pectic fractions were different from those from Toro CWM. The unheated CDTA-soluble pectic fraction isolated from Enkonera CWM had two peaks. Both are on the high mol wt side. After 1 minute of heating, the height of the second peak (lower mol wt) became lower, but after 3 minutes of heating, it was getting much higher than that of the first peak (higher mol wt). After 10 minutes of heating, the first peak was still much higher than that of Toro. Meanwhile, the second peak was getting much broader, but the mol wt was still much higher than that of blue dextran, i.e., higher than that of Toro (compare Figure 5.6 with Figure 5.2).

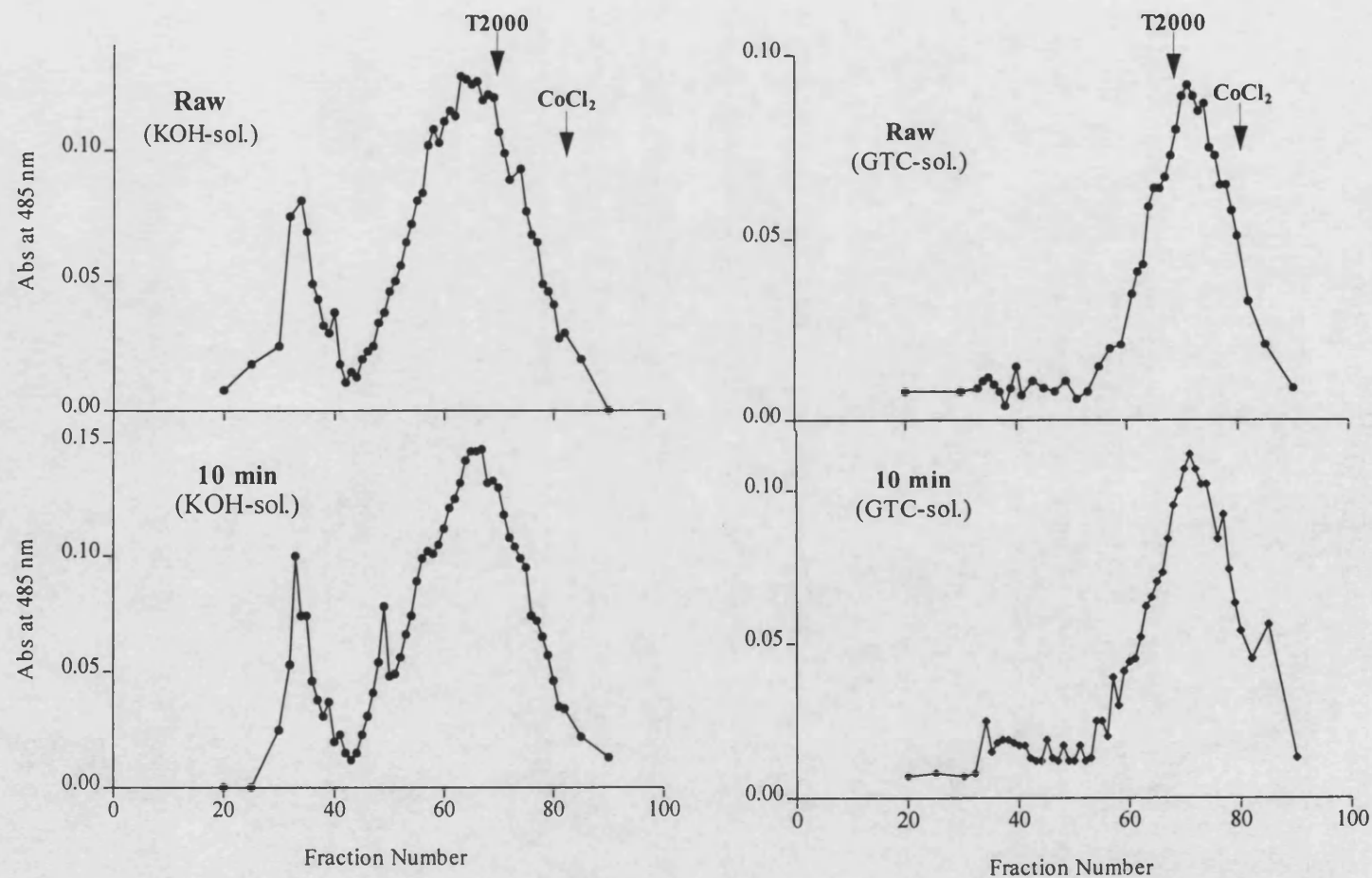
$\text{Na}_2\text{CO}_3$ -soluble pectic fraction from Enkonera CWM also shows no obvious mol wt change upon heating (Figure 5.7). However, the first peak (high mol wt) was much higher than the second one which was opposite to that of Toro (Figure 5.3), and it remained higher throughout the heating process.



**Figure 5.6** Sepharose CL-2B gel filtration profiles of CDTA-soluble pectic polymers isolated from the CWMs of Enkonera cooking banana (hard) pulp tissue before and after heating for 1, 3, 5 and 10 minutes at 100 °C. The galacturonic acid in each collected fraction (2 ml) was monitored by the m-phenylphenol method. T2000=Blue dextran (Mw=2,000,000); GA=galacturonic acid.



**Figure 5.7.** Sepharose CL-2B gel filtration profiles of  $\text{Na}_2\text{CO}_3$ -soluble pectic polymers isolated from the CWMs of Enkonera cooking banana (hard) pulp tissue before and after heating for 1, 3, 5 and 10 minutes at  $100^\circ\text{C}$ . The galacturonic acid in each collected fraction (2 mL) was monitored by the m-phenylphenol method (abs at 520 nm). T2000=Blue dextran ( $M_w=2,000,000$ ); GA=Galacturonic acid.



**Figure 5.8.** Sepharose CL-2B gel filtration profiles of KOH- and GTC-soluble hemicellulosic polymers isolated from the CWMs of Enkonera cooking banana (hard) pulp tissue before and after 10 minutes of heating at 100 °C. The sugar content in each collected fraction (2 mL) was monitored by the phenol-H<sub>2</sub>SO<sub>4</sub> method. T2000=Blue dextran (Mw=2,000,000); CoCl<sub>2</sub>=cobalt chloride (pink).

GTC- and KOH-soluble hemicellulosic fractions isolated from the Enkonera CWM show no changes in their mol wt upon heating which was similar to those from Toro CWM (Figure 5.8).

To summarise:

1. CDTA-soluble pectic polymers from both Toro and Enkonera CWMs are the only fractions which showed significant mol wt reduction due to heating. However, the former (soft type) showed much more severe change than the latter.
2. Although Na<sub>2</sub>CO<sub>3</sub>-soluble pectic polymers from both banana CWMs did not show mol wt changes during heating, that of Enkonera comprises a larger proportion of the higher mol wt components (bigger peak in the higher mol wt side) than that of Toro.
3. Both GTC- and KOH-soluble hemicellulosic polymers from Toro and Enkonera CWM showed no change due to heating although KOH-soluble fractions had a slightly higher mol wt distribution compared to GTC-soluble fractions.

#### ***5.3.4. Effect of cooking on pectin composition changes of AIR***

The yields of different pectin fractions from raw and steam cooked Toro and Enkonera cooking banana pulp tissues are listed in Table 5.4. This is based on the analysis of 'cold' AIR preparations. It revealed that in the raw pulp tissues, Enkonera AIR contained relatively higher amounts of water-, CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectic polysaccharides than Toro. After 3 minutes of cooking, the water-soluble pectin of Enkonera decreased, then increased after 10 minutes of cooking, whilst that of Toro increased 2 fold after 3 minutes, and 4 fold after 10 minutes of cooking. CDTA-soluble pectin of Enkonera increased by 60% after 3 minutes, then decreased by 30%

after 10 minutes of cooking although it was still higher than in the raw material. The CDTA-soluble pectin in Toro also increased during cooking, but in a much more modest manner. The Na<sub>2</sub>CO<sub>3</sub>-soluble pectin of both Toro and Enkonera decreased significantly after 10 minutes of cooking, and the former showed a slightly lower rate of decrease.

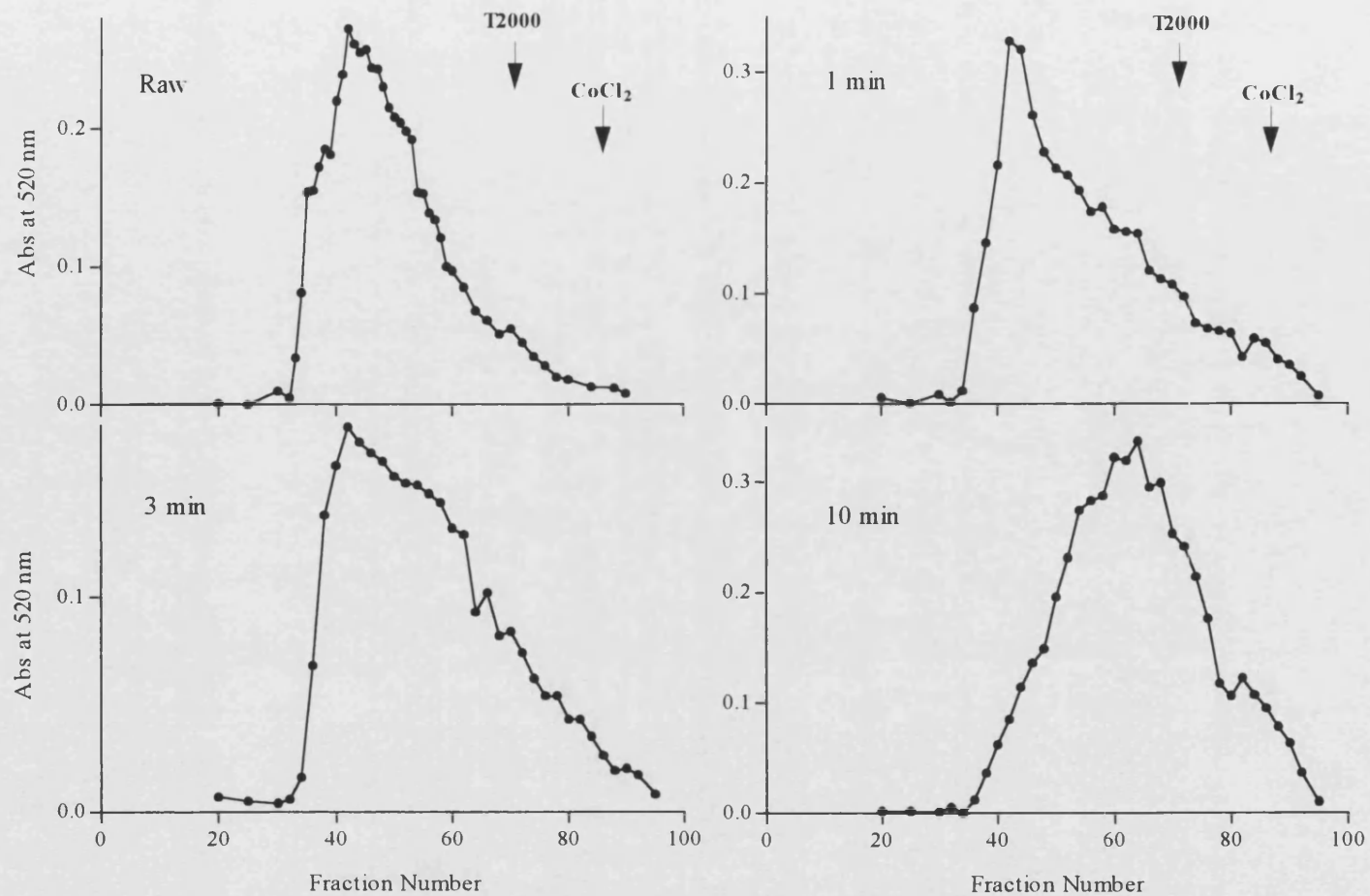
### ***5.3.5. Mol wt changes of the CDTA-soluble pectin of Toro pulp tissue during cooking***

The CDTA-soluble pectin isolated from the cold AIR of the raw and steamed Toro pulp tissues were subjected to gel filtration chromatography on Sepharose CL-2B column. The results are shown in Figure 5.9. This generally confirmed the studies of heating CDTA-soluble pectin which was isolated from the purified cell walls (Figure 5.2). However, the pectin isolated from the cooked pulp tissues showed more advanced mol wt reduction. After 3 minutes of cooking the high mol wt peak became much scattered to the lower mol wt side. By end of the cooking treatment (10 min), almost the entire peak had shifted to the lower mol wt side where the blue dextran was eluted, no trace of the high peak side was left as occurred in those heated isolated pectic polymers



<i>Fractions (mg AGA<sup>a</sup>/100 g AIR<sup>b</sup>)</i>								
<i>Steaming time</i>	<i>Water-soluble</i>		<i>CDTA-soluble</i>		<i>Na<sub>2</sub>CO<sub>3</sub>-soluble</i>		<i>Total</i>	
	Enkonera	Toro	Enkonera	Toro	Enkonera	Toro	Enkonera	Toro
Raw	164.4± 7.9	90.9± 7.6	295.2± 20.1	269.7± 7.9	838.7± 72.3	784.0± 24.3	1298.2	1144.6
3 min	144.5± 4.3	193.9± 15.4	482.8± 11.8	276.5± 10.3	601.7± 8.3	560.7± 9.1	1229.1	1031.1
10 min	223.9± 13.8	435.6± 22.5	371.7± 10.9	315.6± 3.1	273.0± 22.6	354.9± 31.0	868.6	1106.1

**Table 5.4** Effects of steam-cooking on pectin composition changes of Toro (soft) and Enkonera (hard) cooking banana pulp tissues. <sup>a</sup>AGA, anhydrous galacturonic acid; <sup>b</sup>AIR, alcohol insoluble residue; Values are means±SD of three samples.



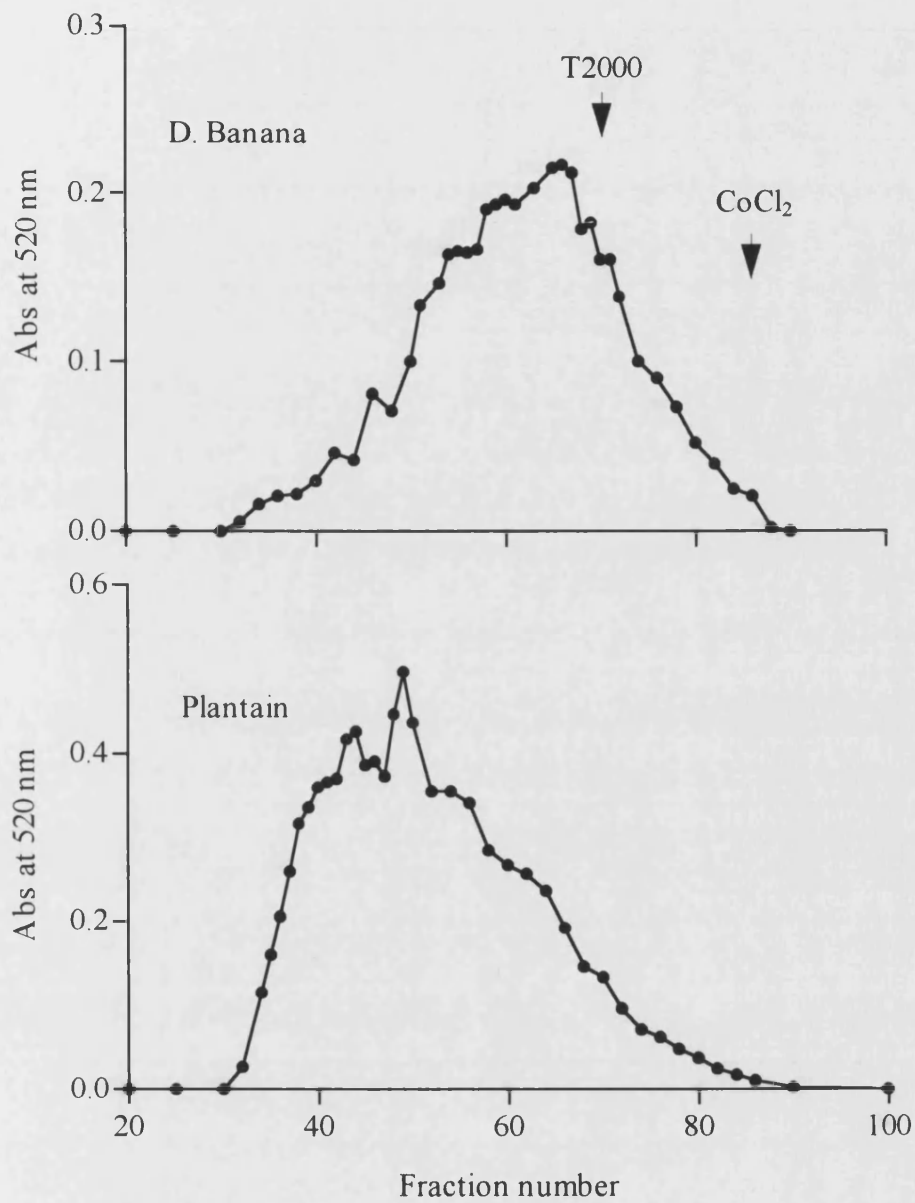
**Figure 5.9.** Gel filtration profiles of CDTA-soluble pectic polysaccharides isolated from the 'cold' AIR preparation of Toro cooking banana (soft) pulp tissue on Sepharose CL-2B column. The galacturonic acid in each fraction was monitored by the m-phenylphenol method. T2000=Blue dextran ( $M_w=2,000,000$ );  $CoCl_2$ =Cobalt chloride (pink).

### ***5.5.6 Mol wt of the CDTA-soluble pectins isolated from the raw dessert banana and plantain CWM***

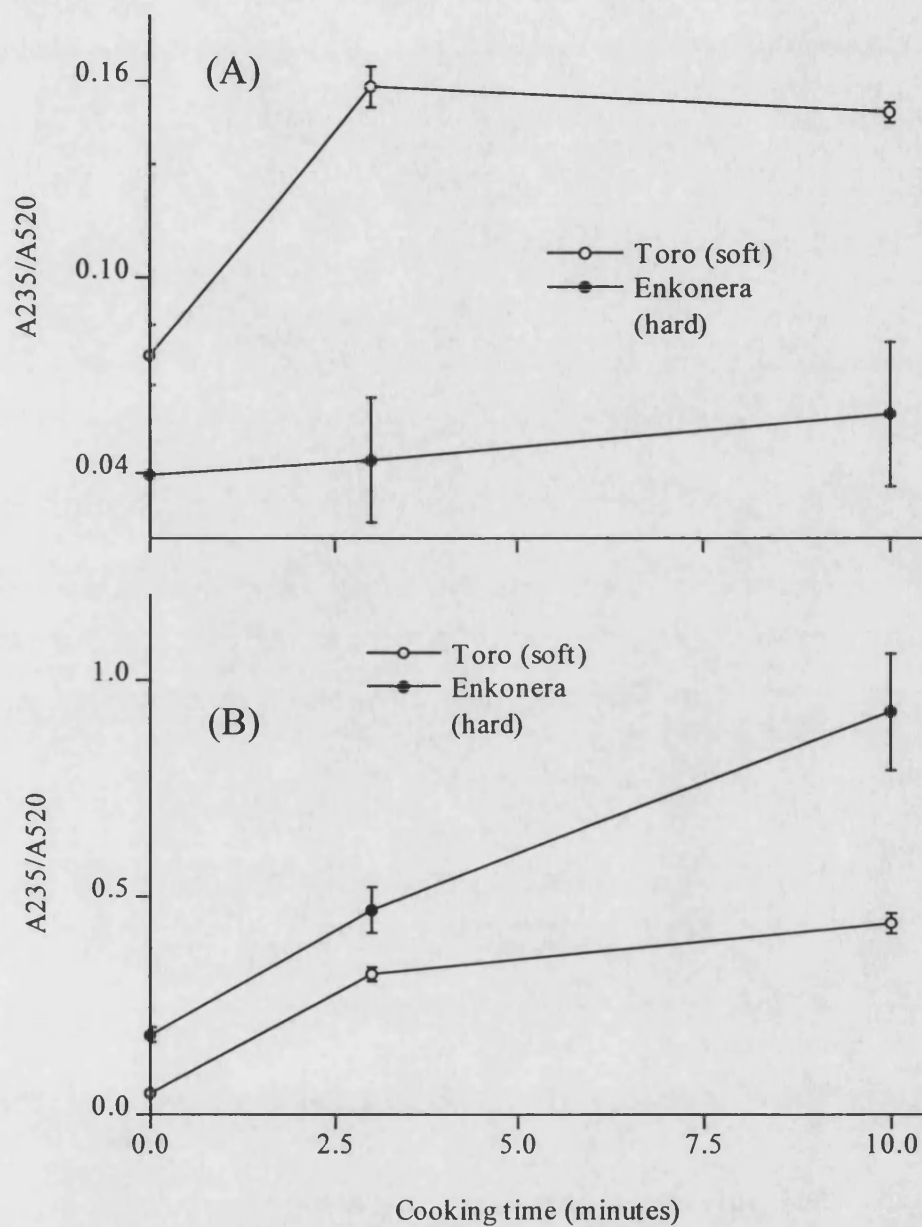
The gel filtration profiles of CDTA-pectic fractions isolated from the raw dessert banana (soft texture) and plantain (hard texture) CWM are shown in Figure 5.10. It indicated that the majority of molecules of the CDTA-soluble pectin were eluted between fraction number 50-75, just before the blue dextran. Thus, their mol wt was slightly higher than 2,000 KD. However, the CDTA-soluble pectin of the plantain CWM was eluted between the fraction 35-65, much earlier than blue dextran. Therefore, the mol wt of dessert banana chelator-soluble pectin was much lower than that of plantain.

### ***5.3.7 Effect of cooking on $\beta$ -elimination rate of CDTA-and $\text{Na}_2\text{CO}_3$ -soluble pectin***

The pectin  $\beta$ -elimination rate was measured by the ratio of the UV absorbance at 235 nm (as a measure of 4,5-unsaturated uronide) to the absorbance at 520 nm in the m-phenylphenol assay (as a measure of total uronic acids). The results are shown in Figure 5.11. It appeared that the CDTA-soluble pectin of Toro experienced much greater  $\beta$ -elimination compared to that of Enkonera during cooking (Figure 5.11A). However, the  $\text{Na}_2\text{CO}_3$ -soluble pectin of Enkonera showed a greater  $\beta$ -elimination rate during cooking than that of Toro (Figure 5.11B).



**Figure 5.10.** Gel filtration profiles of CDTA-soluble pectic polymers isolated from the CWM of raw dessert banana and plantain (big Ebanga type). The galacturonic acid in each fraction (2 mL) was monitored by the m-phenyphenol method. T2000=Blue dextran ( $M_w=2,000,000$ ),  $CoCl_2$ =cobalt chloride (pink).



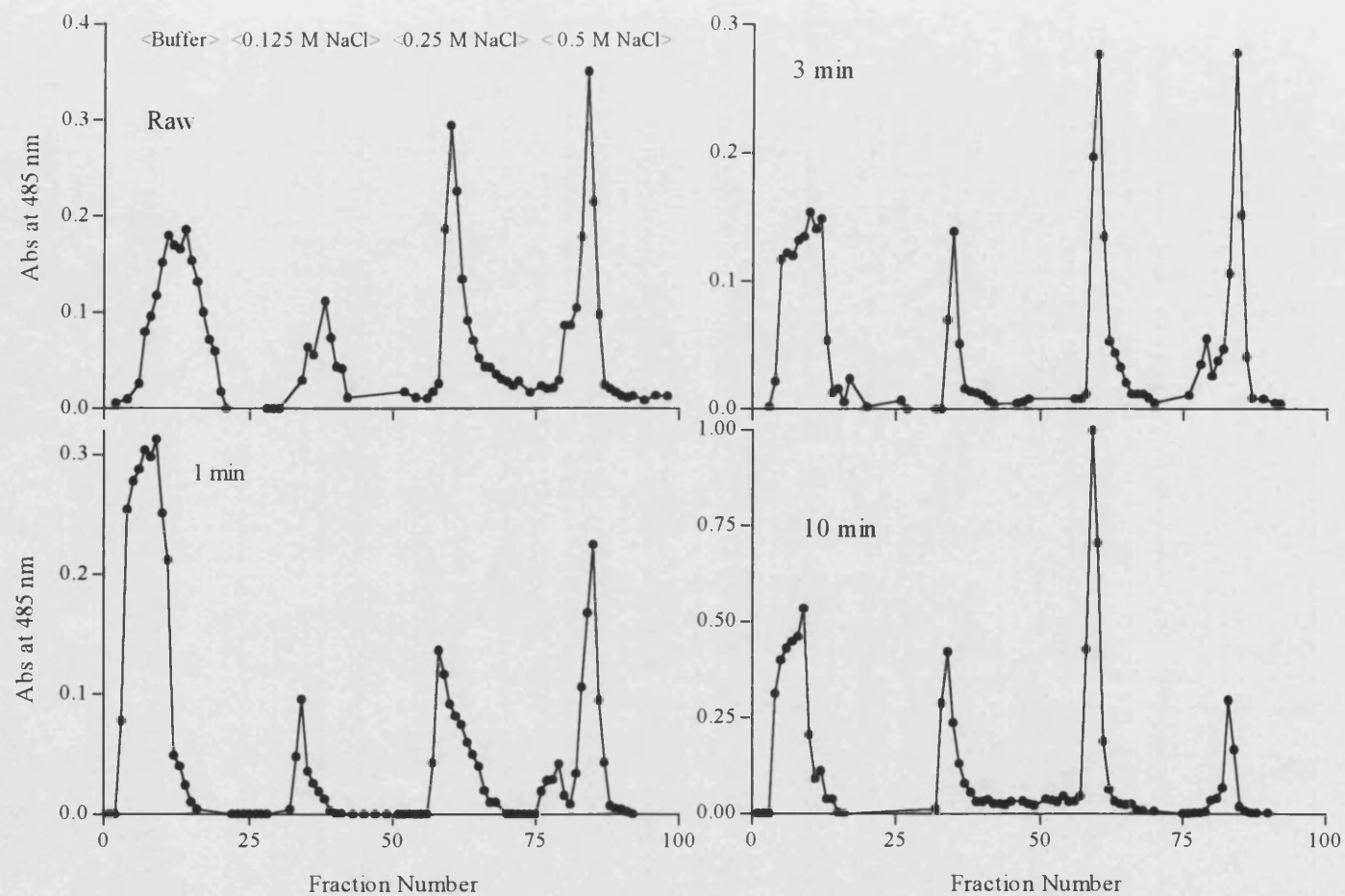
**Figure 5.11** Effect of heating on  $\beta$ -elimination rate (A235/A520) changes of CDTA- (A) and Na<sub>2</sub>CO<sub>3</sub>-soluble (B) pectic polysaccharides isolated from the cold AIR of Toro and Enkonera pulp tissues. Values are means  $\pm$  SD of three measurements.

### ***5.3.8 Anion exchange chromatography of the CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectic fractions isolated from Toro and Enkonera AIRs ('cold' preparations)***

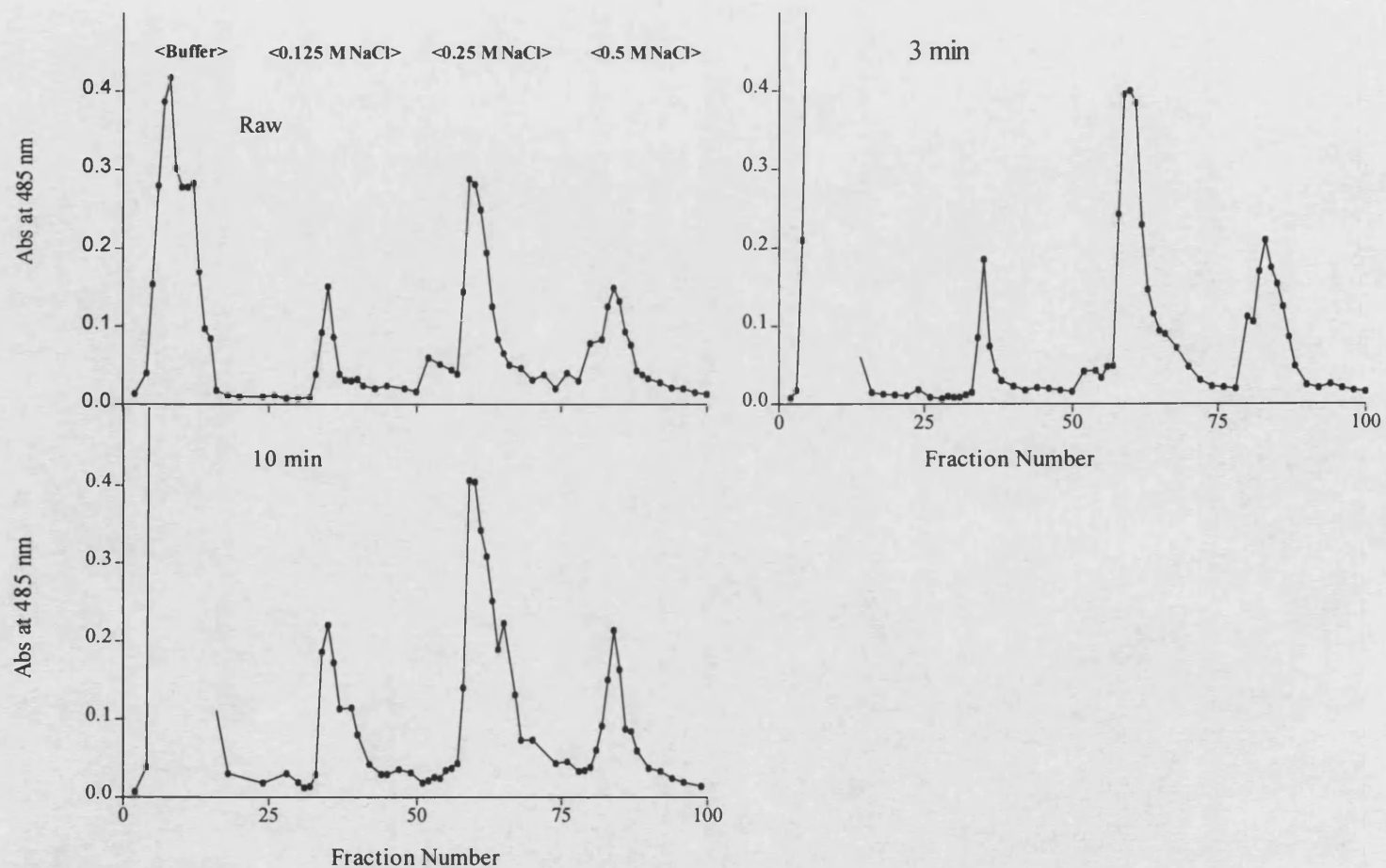
The sequential elution of the anion exchange column with phosphate buffer and buffer containing increasing concentrations of NaCl separated four components from the cell wall fractions. These components generally eluted as single peaks from the column. The size of the peaks varied with the cultivar from which the AIR had been prepared (Toro or Enkonera), the period of cooking (0, 1, 3 or 10 minutes) and the cell wall fractions used (CDTA and Na<sub>2</sub>CO<sub>3</sub>).

The ion exchange profiles of the CDTA fraction of the raw Toro banana (Figure 5.12) show that Toro has a major component which can be eluted with buffer alone, a much smaller peak eluted with buffer plus 0.125 M NaCl, and larger peaks eluted with buffer plus 0.25 M and 0.5 M NaCl respectively. With increasing periods of cooking, the relative contributions of the four peaks remained similar until 10 minutes, when the peak eluted by buffer plus 0.5 M NaCl became much smaller.

In the profiles of Enkonera CDTA-soluble fractions (Figure 5.13), the third peak was the major component (rather than the third and the fourth peaks as in Toro) and the first peak which is eluted with buffer alone was also large. It appears that there were no obvious changes in the proportions of the second to the third and the fourth peak upon cooking.



**Figure 5.12** Ion-exchange profiles of CDTA-soluble pectic polysaccharides isolated from the 'cold' AIR of raw and cooked Toro cooking banana (soft) pulp tissue. The anion exchanger is DEAE-Trisacryl M, and the sugar content of each collected fraction (8 mL) was monitored by the phenol-sulphuric acid method.



**Figure 5.13** Anion chromatography profiles of CDTA-soluble pectic polysaccharides isolated from 'cold' AIR preparation of Enkonera cooking banana ('hard') from Uganda. The medium is DEAE-Trisacryl M. 8 mL of each fraction was collected, and the sugar content was monitored by the phenol- $\text{H}_2\text{SO}_4$  method.

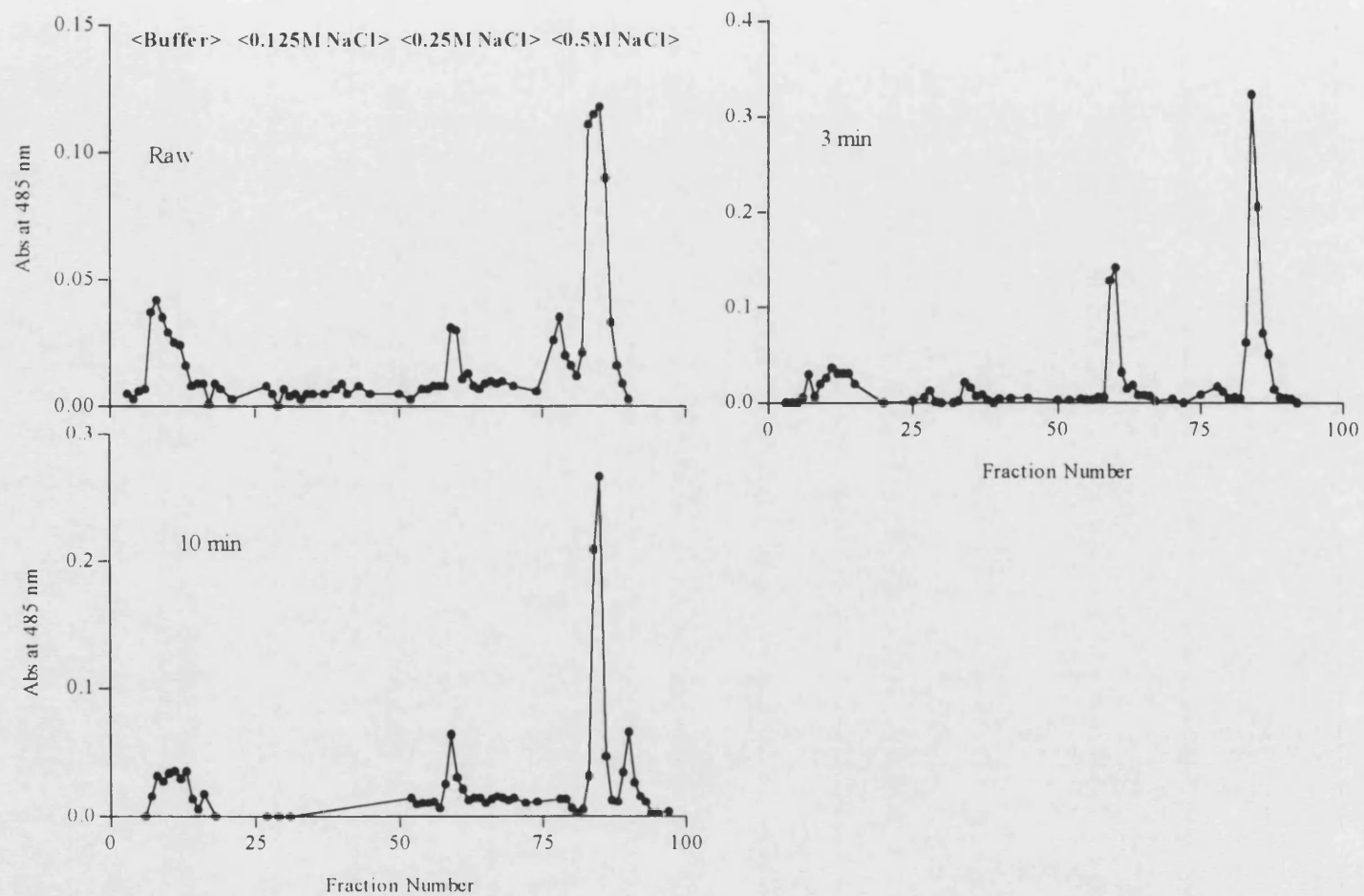


The anion exchange profiles of the Na<sub>2</sub>CO<sub>3</sub>-soluble fractions were generally different from those of the CDTA-soluble fractions. In these profiles, the fourth peak (eluted with buffer plus 0.5M NaCl) was the major peak. This was particularly obvious with the Na<sub>2</sub>CO<sub>3</sub>-soluble fractions isolated from the Toro AIR (Figure 5.14). The second peak (eluted with buffer plus 0.125M NaCl) was almost non-detectable, followed by a very small peak with buffer plus 0.25M NaCl. However in the profiles of the Na<sub>2</sub>CO<sub>3</sub>-soluble fractions isolated from the Enkonera AIRs, all the four peaks were detectable with the third and the fourth peaks prominent (Figure 5.15). Cooking had apparently little effect on the profiles, except a considerable increase in the buffer eluted fraction of Enkonera, which was not found in Toro.

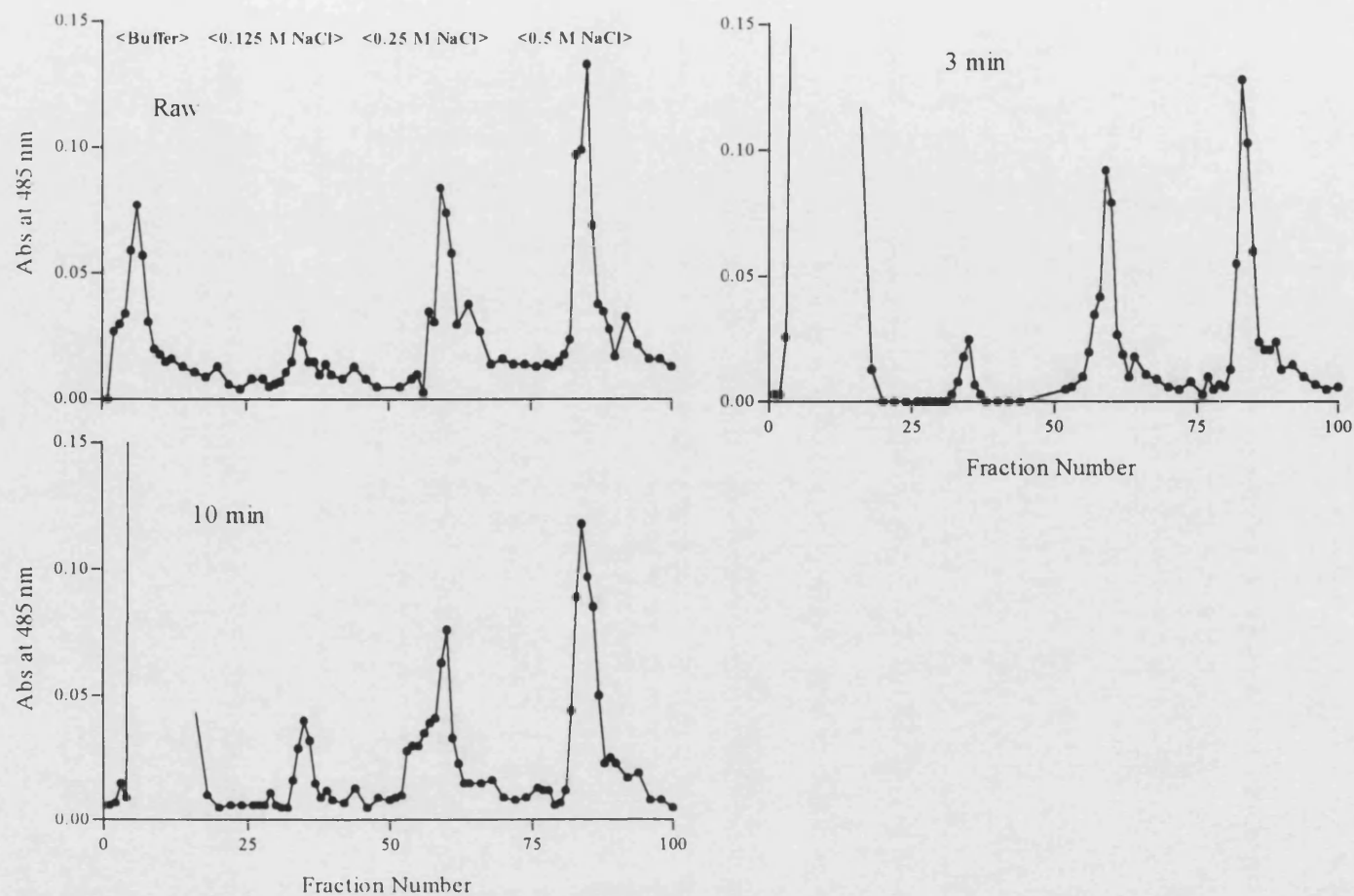
The sugar analysis of the component peaks is shown in Table 5.5 and 5.6. As the first peak, eluted with the buffer alone was within the void volume, having not been retained by the column, and did not contain any detectable galacturonic acid, these fractions were discarded. Generally, the uronic acid was the major component in both CDTA and Na<sub>2</sub>CO<sub>3</sub> fractions (>90% and >65%) in the raw material, and decreased to >82% and >54% after 10 minutes of cooking in both Toro and Enkonera AIRs (Tables 5.5 & 5.6). However, in most of the Na<sub>2</sub>CO<sub>3</sub>-soluble polymers, the contents of arabinose, xylose and galactose were much higher than in the CDTA-soluble polymers, leading to a reduced ratio of uronic acid to neutral sugars. This was particularly apparent in the Na<sub>2</sub>CO<sub>3</sub>-extracts from the Enkonera AIRs where the uronic acids only accounted for 65% in the fraction eluted with buffer plus 0.25 M NaCl, which reduced to 54% after 10 minutes of cooking, whereas, little difference was found between the fractions eluted with buffer plus 0.5 M NaCl before and after 10 minutes of cooking (Table 5.5).

The degrees of methyl esterification (d.e.) of the components of the CDTA-soluble polymers from Enkonera AIR are in the range 22-64% (The d.e. of that of Toro was not determined as the alcohol oxidase used in the assay was in-activated by the electricity failure in the department). Less esterified pectins were eluted with strong ionic strength buffer (high NaCl concentration). Meanwhile, cooking resulted in a decrease in the degree of methyl esterification of the pectins (Table 5.6). The Na<sub>2</sub>CO<sub>3</sub>-soluble fractions had low levels of methyl esterification. This may be partly due to de-esterification by the conditions of their extraction (Redgewell and Selvendran, 1986).

The above results clearly show the heterogeneity of the pectic polymers of Toro and Enkonera cooking bananas before and after cooking.



**Figure 5.14.** Ion exchange profiles of Na<sub>2</sub>CO<sub>3</sub>-soluble pectic polysaccharides isolated from the cold AIR preparation of Toro (soft) cooking banana pulp tissue. The anion exchanger used is DEAE-Trisacryl M, and the sugar content in each fraction (8 mL) was monitored by the phenol sulphuric acid method.



**Figure 5.15** Anion chromatography profiles of  $\text{Na}_2\text{CO}_3$ -soluble pectic polysaccharides isolated from cold AIR preparation of Enkonera cooking banana (hard). The exchanger is DEAE-Trisacryl M, and the sugar content in each collected fraction (8 mL) was monitored by the phenol-sulphuric acid method.

<i>Fraction</i>	<i>Sugar composition (% of total)</i>							
	<i>Rha</i>	<i>Fuc</i>	<i>Ara</i>	<i>Xyl</i>	<i>Man</i>	<i>Gal</i>	<i>Glc</i>	<i>GA<sup>a</sup></i>
<i>CDTA-sol</i>								
<b>Raw:</b>								
0.25M NaCl	0.46	0.48	0.62	<i>t</i>	0.67	1.65	1.80	94.34
0.5M NaCl	<i>t</i>	0.35	0.94	<i>t</i>	0.83	2.00	2.28	93.60
<b>10 min cooked:</b>								
0.25M NaCl	<i>t</i>	<i>t</i>	2.36	3.76	3.25	1.95	0.56	88.12
0.5M NaCl	<i>t</i>	1.87	1.13	3.00	5.24	4.13	1.36	83.28
<i>Na<sub>2</sub>CO<sub>3</sub>-sol.</i>								
<b>Raw:</b>								
0.5M NaCl	<i>t</i>	<i>t</i>	8.84	11.23	0.27	6.32	2.27	71.07
<b>10 min cooked:</b>								
0.5M NaCl	<i>t</i>	<i>t</i>	10.87	4.70	<i>t</i>	3.76	5.40	75.28

**Table 5.5** Sugar composition of components of CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions of AIR ('cold' preparation) isolated from Toro (soft) cooking banana pulp tissue separated on DEAE-Trisacryl M anion exchange column. <sup>a</sup>Galacturonic acid; <sup>t</sup>Trace.

<i>Fraction</i>	<i>D.e.</i> <sup>a</sup> (%)	<i>Sugar composition (% of total)</i>							
		<i>Rha</i>	<i>Fuc</i>	<i>Ara</i>	<i>Xyl</i>	<i>Man</i>	<i>Gal</i>	<i>Glc</i>	<i>GA</i> <sup>b</sup>
<b><i>CDTA-sol</i></b>									
<b>Raw:</b>									
0.25M NaCl	64.04	<i>t</i>	<i>t</i>	1.47	<i>t</i>	0.45	1.21	6.01	90.86
0.5M NaCl	28.32	0.52	<i>t</i>	1.43	0.52	0.74	1.21	3.01	93.10
<b>10 min cooked:</b>									
0.25M NaCl	44.47	0.17		0.64	2.82	0.29	1.04	9.64	85.41
0.5M NaCl	22.13	<i>t</i>	<i>t</i>	0.31	3.99	1.81	1.62	10.74	82.64
<b><i>Na<sub>2</sub>CO<sub>3</sub>-sol.</i></b>									
<b>Raw:</b>									
0.25M NaCl	<i>t</i>	<i>t</i>	1.84	1.96	15.77	5.23	1.96	7.52	65.72
0.5M NaCl	<i>t</i>	<i>t</i>	<i>t</i>	2.50	10.87	2.61	5.67	12.06	66.27
<b>10 min cooked:</b>									
0.25M NaCl	<i>t</i>	<i>t</i>	<i>t</i>	16.13	14.10	<i>t</i>	10.10	5.40	54.25
0.5M NaCl	<i>t</i>	<i>t</i>	2.20	9.47	3.64	2.54	4.70	11.89	65.55

**Table 5.6** Sugar composition of components of CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions of AIR ('cold' preparation) isolated from Enkonera (hard) cooking banana pulp tissue separated on DEAE-Trisacryl M anion exchange column. <sup>a</sup>Degree of esterification. <sup>b</sup>Galacturonic acid. <sup>c</sup>Trace.

#### 5.4. Summary

Enkonera cooking banana (hard) cell wall materials (CWM) is composed of much more galactose and slightly higher arabinose than Toro CWM. There is no significant difference in overall galacturonic acid and cellulose content. The former also contains slightly higher water, CDTA and Na<sub>2</sub>CO<sub>3</sub> soluble pectic polymers as well as GTC and KOH soluble hemicellulosic polymer compared to the latter.

The classic preparation of alcohol insoluble residues (AIR) which involves boiling the tissue in 80% alcohol or by refluxing with a Soxhlet apparatus was found to cause pectin solubilization and mol wt reductions in the raw Toro cooking banana pulp tissue. Therefore, a 'cold' method for preparing the AIR, using phenol:acetic acid:water (PAW, 2:1:1) which did not involve a heating step, was employed

Cooking caused pectin solubilization and depolymerization in both Toro and Enkonera cooking bananas and this was particularly significant in Toro compared to Enkonera. The water soluble pectin increased 4 fold in Toro and 2 fold in Enkonera after 3 minutes of steaming. Meanwhile, the Na<sub>2</sub>CO<sub>3</sub>-soluble pectin decreased at a similar rate in both fruits. CDTA-soluble pectin was the only pectin which experienced marked mol wt reduction during heating. Although Na<sub>2</sub>CO<sub>3</sub>-soluble pectin did not show a significant reduction in their MW, that from Enkonera CWM appeared to have much higher proportion of the higher MW materials than that from Toro CWM both before and after heating. The MW of plantain (Big Ebanga type) chelator-soluble pectin was much higher than that of dessert banana.

β-elimination occurred in both CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins in both fruits during steam-cooking. It appeared that the CDTA-soluble pectins of Toro experienced much greater β-elimination than that of Enkonera, whilst the Na<sub>2</sub>CO<sub>3</sub>-soluble pectins of Toro showed a lower β-elimination rate than that of Enkonera.

## 5.5. Discussion

### 5.5.1. Cell Wall Composition of Toro and Enkonera

The difference in overall cell wall carbohydrate composition in the fruit of different varieties of cooking bananas would seem to provide some explanation for differences in texture retention. Enkonera CWM contains slightly higher amounts of water, CDTA- and  $\text{Na}_2\text{CO}_3$ -soluble pectic polymers, as well as GTC- and KOH-soluble hemicellulosic polymers compared to Toro (Table 5.1). Individual sugar analysis by GLC also revealed that Enkonera CWM is composed of much more galactose and slightly higher arabinose and mannose than Toro although there is no difference in overall galacturonic acid and cellulose (measured as glucose) contents (Table 5.2). CDTA can solubilise rhamnogalacturonan from the middle lamella region (Jarvis *et al.*, 1981; Selvandran, 1985). These PGAs are supposed to be held in the middle lamella by  $\text{Ca}^{2+}$  linkages (Morris *et al.*, 1982; Jarvis, 1984). A less branched PGA chains as in Enkonera CDTA-soluble pectins with a ratio of Rha:GA 1:30 would form stronger  $\text{Ca}^{2+}$ -PGA 'egg-junction' zones than a more branched PGA chains found in the Toro CDTA-soluble pectins where the ratio of Rha:GA is 1:25. On the other hand,  $\text{Na}_2\text{CO}_3$ -soluble pectins contain more galactose and arabinose, which results in a lower ratio of Gal:GA. In Toro, it is 1:17, whilst in Enkonera, the ration is 1:15.  $\text{Na}_2\text{CO}_3$  is believed to contain primary wall pectins (Selvandran, 1985) where the pectic polymers are cross-linked to themselves as well as to other cell wall polymers, such as hemicellulosic polymers, via their neutral side-chains to maintain the great strength of the wall (Carpita and Gilbeaut, 1993; Fry, 1988). Therefore, more galactose in Enkonera  $\text{Na}_2\text{CO}_3$ -soluble pectins suggests more side-chains which result in stronger linkages between the primary wall polymers than in Toro. Therefore,



Enkonera possibly possess a stronger middle lamella as well as a stronger primary cell wall. This is possibly why Enkonera is firmer after cooking than Toro.

These results are in general agreement with the finding of Loh and Breene (1982) who claimed that vegetables, such as waterchestnut which remained firmer after cooking, were much higher in neutral sugar content than the softer species, such as potato. The authors suggested that this was due to the neutral sugars (i.e., galactose, rhamnose, arabinose and mannose) which can form as bridges between pectic polymers, maintaining some architecture when the susceptible galacturonic acids had already broken down.

#### ***5.5.2. Effect of different AIR preparation methods on the changes of different pectin fractions of Toro cooking bananas***

Pectin composition changes are often related to fruit or vegetable tissues softening during their ripening or cooking processes. The changes in different pectin fractions are used as a means of monitoring stages in plant foodstuff processing, and the amounts of these pectin fractions during different processing stages are often measured by using successive extraction with different solvents subject to their severity. The initial step involves acetone or alcohol in the preparation of insoluble residue (AIR). Generally, there are two different methods to prepare the AIR. One is the classic method, although it is still in use today, in which plant tissue is boiled in alcohol to inactivate the pectic enzymes, as well as to wash away the soluble components including any traces of galacturonic acid. This can be conducted by the use of a Soxhlet apparatus (For example, Bettelheim and Sterling, 1955b; Loh *et al.*,

1982). Another method was developed more recently by Redgwell *et al* (1992) in which phenol:acetic acid:water (PAW, 2:1:1) was used for the same purposes, as well as to avoid any possible pectin degradation caused by high temperature during preparation. However, it appears that no comparison has been done on how these two different preparation methods affect pectin composition.

In this study, Toro cooking banana pulp tissue was used to prepare 'hot' and 'cold' AIR before and after cooking as described in sections 2.4 and 5.2.1. The AIRs were then fractionated by sequential extraction with water, CDTA and Na<sub>2</sub>CO<sub>3</sub>, and the result is shown in Table 5.3. It appeared that hot alcohol preparation of AIR resulted in significantly high amount of water-soluble pectin in the raw materials, but the amounts of CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins were much lower compared to the 'cold' AIR. This suggests that hot alcohol solubilized significant amount of pectin from the raw pulp tissue. Changes in the yields of different pectin fractions due to cooking were also partially obscured by the use of the hot alcohol preparation of AIR. Furthermore, the mol wt of CDTA-soluble pectin was also significantly reduced by the hot alcohol boiling of the raw cooking banana tissue (Figure 5.1). This was possibly one of the reasons why other researchers (Loh and Breene, 1982; Fuchigami, 1987a; Sajjaanantakul *et al.*, 1989) did not always find a depolymerization of pectin with cooking, or pectin depolymerization became obvious only after a very long period of cooking (up to 240 minutes) because heating was employed to a lesser or greater extent during their AIR preparation or pectin extraction.

### ***5.5.3. Effect of cooking on the yields of different pectin fractions***

Successive extraction with water, CDTA and Na<sub>2</sub>CO<sub>3</sub> was carried out on 'cold' prepared AIR (see section 5.2.1). Table 5.4 showed that Enkonera which cooked hard contained higher water-soluble pectin in the raw materials than Toro (soft). This is in contrast with the finding of Greve *et al* (1994b) who claimed that carrot which cooked hard had lower water-soluble pectin in the raw tissue than the soft one. The amounts of CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins did not show clear differences between the two cultivars, relative to the big variations among the different measurements. However, in pulp tissue subjected to cooking, the changes in different pectic fractions differed dramatically in the two cultivars. The water-soluble pectin decreased only slightly in Enkonera, but increased 2 fold in Toro after 3 minutes of cooking, with further cooking the water soluble fraction continued to increase in both fruits with the increase in Toro being greatest (4 fold). Increase in the water-soluble pectin fraction during cooking or ripening of fruits and vegetables is often interpreted as an increase in the solubility of cell wall pectin, hence texture loss. Therefore, that is possibly why Toro had a softer texture after the same period of cooking than Enkonera.

Na<sub>2</sub>CO<sub>3</sub>-soluble pectins decreased in both fruits in a similar rate (28% decrease) after 3 minutes of cooking. After further cooking, there was a greater decline in Enkonera than in Toro. The probable explanation for the decrease in these wall pectins during cooking was either an increase of their solubility (i.e. conversion to soluble pectin fraction) or less extractibility due to an interaction between intracellular and/or cell wall proteins and the soluble anionic pectic polymers resulting in the formation of insoluble complexes during heating, or a combination of both processes.

The CDTA-soluble fraction, which is believed to contain the middle lamella pectin (Jarvis *et al.*, 1981; Jarvis, 1982; Redgewell and Selvendran, 1986), demonstrated a dramatic increase in its amounts during the first 3 minutes of cooking in Enkonera. Since both water soluble and Na<sub>2</sub>CO<sub>3</sub>-soluble fraction decreased markedly (by 12% and 28%, respectively) these fractions were the most likely sources of the extra polymers recovered in the CDTA-soluble fraction. Both water-soluble and Na<sub>2</sub>CO<sub>3</sub>-soluble pectin consist of higher methylated pectin (Table 5.1). A possible explanation for the rapid increase in CDTA-soluble fraction during the first 3 minutes of cooking is that this material had been increased by action of the enzyme pectin methylesterase (PME) on methylated pectin. The water- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins would provide ideal substrates for this enzyme which is active between 60-70°C (Bartolome and Hoff, 1972). This enzyme would cause the increase of the amount of free carboxyl groups in the cell wall pectin, which is the main characteristic of pectins extractable by CDTA.

The browning of the pulp tissue during the first minute of cooking suggests that some of the enzymes were not de-natured immediately. As the cooking lengthened, the temperature exceeded 70°C and the PME was destroyed (the pulp tissue was no longer browning after 3 minutes of cooking), thereafter exerting no effect on the cell wall material. Hence, the CDTA fraction decreased after continuously. However, the CDTA-soluble pectins in Toro only increased slightly throughout the cooking process. This could be due to the lower activities of PME in Toro bananas. These findings imply that the biochemical processes involved in cooking are complex, several chemical changes take place that may be interrelated. Therefore conclusions on the

softening of the cooked tissue based on a single aspect of chemical change cannot be drawn with any accuracy. It should also be pointed out that different extraction methods for pectic substances have been employed by different workers producing a variety of results. This makes comparison to this study difficult.

#### ***5.5.4. Effect of heating on changes of cell wall component molecular weight distribution***

Gel filtration profiles of different cell wall components isolated from purified CWM of Toro and Enkonera Ugandan cooking banana indicated that the CDTA-soluble pectic substances were the only polymers which showed a significant reduction in molecular weight after 10 minutes of heating (Figures 5.2-5.8). The CDTA-soluble pectic polymers are among the largest pectic molecules in both walls. It is suggested that CDTA can solubilize most of the pectic substances which are held in the walls by  $\text{Ca}^{2+}$  and localized in the middle lamella region (Jarvis *et al.*, 1981; Jarvis, 1982). The breakdown of this middle lamella pectin would cause the adhesion of adjacent cells to be greatly reduced, leading to greater cell separation, and hence the texture loss. This change is more extensive in Toro than in Enkonera. In another word, after the same period of cooking time, the soft genotype of cooking banana experienced more dissolution of CDTA-soluble pectin than the hard type. Greve *et al* (1994b) also found the same tendency with the soft and hard carrot genotypes. Later studies of the CDTA-soluble pectic fractions isolated from the raw and cooked Toro AIR also supported these studies although the reduction of the mol wt was more advanced (Figure 5.9). This may be because steaming could make the heat penetrate the pulp tissue much faster compared to heating test-tubes with cool solution of isolated pectic

polymers from the purified cell wall materials. Gel filtration profiles of the CDTA-soluble pectins (CSP) isolated from the CWM of raw dessert banana (soft) and plantain (hard) also showed that the CSP of plantain had much larger mol wt than that of dessert banana (Figure 5.10). These are also in agreement of Greve *et al* (1994b) who found that carrot which remained firmer after cooking contained much higher mol wt of CDTA-soluble pectic polymers than the soft varieties.

The bulk of the CDTA-insoluble pectic substances was solubilized by 0.05 M Na<sub>2</sub>CO<sub>3</sub> at 1<sup>o</sup>C and then at room temperature. These pectic substances were presumably held in the wall matrix by Ca<sup>2+</sup> and by ester linkages (Redgwell and Selvendran, 1986). Although the Na<sub>2</sub>CO<sub>3</sub>-soluble pectic polymers from both Toro and Enkonera cell walls did not show a large MW reduction even after 10 minutes of heating (Figures 5.3 & 5.7). However, the Na<sub>2</sub>CO<sub>3</sub>-soluble pectin from Enkonera had a much higher proportion of higher MW materials than those from Toro CWM, and they remained higher throughout the heating treatments. Presumably these higher MW materials could maintain some of the wall strength, hence the tissue firmness, as the CDTA-soluble middle lamella pectins progressively completely broke down. This may be one of the reasons why Enkonera remained harder even after a period of cooking which would have resulted in breakdown of middle lamella pectin.

The GTC- and KOH-soluble hemicellulosic polymers did not show any mol wt reduction during heating of both fruits. Therefore, hemicelluloses were not prone to depolymerization due to cooking of *Musa* fruits.

### ***5.5.5. Anion-exchange chromatography of the CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions***

As mentioned earlier, the first peak contained no galacturonic acid, therefore these fractions were discarded (Smith, 1989). Among the fractions studied, although uronic acid was the major sugar, the proportions of this sugar varied from 54% to 94% in different fractions from CDTA- and Na<sub>2</sub>CO<sub>3</sub>-extracts of both fruit tissues before and after cooking (Table 5.5 and 5.6). Generally, cooking resulted in a decrease of the uronic acid from all the fractions studied. The degrees of methyl esterification of the components of the CDTA-soluble polymers varied in the range 22-64% in Enkonera during cooking. The less methylated pectic polymers were eluted with buffer containing higher concentration of NaCl, suggesting that the less esterified pectic polymers combined more strongly with the column. Heating also could cause de-esterification of the pectic polymers. A similar finding of de-esterification by heating has also reported by Fuchigami (1987a) on the Japanese radish root during cooking.

The Na<sub>2</sub>CO<sub>3</sub>-soluble fractions were de-esterified by the conditions of their extraction (Redgwell and Selvendran, 1986; Redgwell *et al.*, 1988). However, based on the high methyl content of the CWM (Table 5.1), they were likely to be highly esterified. The above results clearly showed the heterogeneity of the pectic polymers of Toro and Enkonera cooking banana tissues. The heterogeneity of Kiwifruit cell wall polymers has been reported (Redgwell and Selvendran, 1986; Redgwell *et al.*, 1988).

The major components of the CDTA-soluble fractions were not retained on the column despite the high levels of galacturonic acid. This could be due to the

aggregation of the pectin molecules via inter-chain hydrogen bonding of the non-esterified carboxyl groups, as occurs in partially esterified pectins (Redgwell *et al.*, 1988). On the other hand, Fuchigami and Kishigami (1991) suggested this could be due to the high degree of esterification of the pectic components, preventing the polymers from combining with the anion exchange media. The  $\text{Na}_2\text{CO}_3$ -soluble fractions did not follow this pattern; each gave a small proportion of a neutral fraction, and the acidic components were retained on the column. There would be less tendency for aggregation in the  $\text{Na}_2\text{CO}_3$ -soluble acidic fractions because of the mutual repulsion of the more numerous carboxylate groups. Meanwhile the de-esterification by the extraction would also prevent the  $\text{Na}_2\text{CO}_3$ -soluble pectin from aggregation.

Due to only small amounts of each fraction recovered from the anion-exchange column, their neutral sugar composition showed in table 5.5 & 5.6 are only preliminary results. Further studies on a much larger scale need to be done to confirm the above results in the future.

The sequential elution of the anion exchange column with phosphate buffer and buffer containing increasing concentrations of NaCl separated four components from the CDTA and  $\text{Na}_2\text{CO}_3$  soluble pectins. Galacturonic acid was the predominant sugar in all the fractions determined.



**CHAPTER 6**  
**GENERAL DISCUSSION AND CONCLUSION**

## 6. GENERAL DISCUSSION AND CONCLUSION

Thermal softening of *Musa* had been studied in pairs (dessert banana vs. Big Ebanga plantain, Cocoye cooking banana vs. Horn plantain; and Matooke cooking banana Toro vs. Enkonera) by either boiling or steaming. Dessert banana, Cocoye cooking banana and Toro cooking banana lost their firmness at a much faster rate than Big Ebanga plantain, Horn plantain and Enkonera cooking banana, respectively.

### 6.1. Effect of cell wall components on texture

Cell wall sugar analysis indicated that the cell walls of Enkonera cooking banana, which cooked harder, contained more neutral sugars (galactose, arabinose and mannose) than that of Toro banana (Table 5.2). Rhamnose was only present in minute quantities in both fruits. Loh and Breene (1982) have suggested that neutral sugars could act as bridges between polymers in the wall, thus maintaining some of its integrity when most of the pectins have already broken down.

The molecular weight distributions of the CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins were very different with the firm and soft types of *Musa* fruits. In general, firm fruits had much higher mol wt pectins in the raw fruits than the soft types, and they also seemed to be less susceptible to heat-induced breakdown. A similar conclusion was also drawn with hard and soft carrot genotypes by Greve *et al* (1994b). Among the different polymers isolated from the raw purified cell walls, CDTA-soluble pectins were the only polymers which experienced a significant reduction in their mol wt after 10 minutes of heating (Figure 5.2 - 5.8). Later studies of these CDTA-soluble pectic polymers isolated from the raw and cooked pulp tissues also showed the similar

tendency of breakdown (Figure 5.9). As the CDTA-soluble pectins are localized in the middle lamella of the wall, which are less esterified and held by  $\text{Ca}^{2+}$  (Jarvis *et al*, 1981; Jarvis., 1982), the breakdown of these polymers would cause the adhesion of the adjacent cells to be greatly reduced, hence allowing separation from each other. However, TEM observations showed that the middle lamella modification and dissolution occurred as early as 1 minute into steam cooking of Toro and Enkonera cooking banana pulp tissues (Figure 4.16 & 4.19) whilst pulp firmness decreased by 40% in Toro (Figure 4.10). The MW of CDTA-soluble pectic polymers did not show a reduction in the same period of heating (Figure 5.2, 5.6 & 5.9). This suggests that the initial loss of firmness of the pulp tissue is not necessarily correlated to pectin depolymerization. It seems that the breakdown of cross links between the polymers may be a more important factor (Fry, 1988; McCann *et al*,1990). The results support the finding of Loh and Breene (1982) who claimed that loss of cell wall physical strength does not necessarily coincide with pectin depolymerization.

$\text{Na}_2\text{CO}_3$ -soluble pectic polymers did not show a large MW reduction even after 10 minutes of heating (Figures 5.3 & 5.7). As these polymers are much more esterified compared to CDTA-soluble pectic polymers (Table 5.1), it could be anticipated that the former would undergo a more extensive breakdown by  $\beta$ -elimination during heating (Sajjaanantakul *et al*, 1989). However, the TEM observations revealed that the breakdown of the cell walls started from the intercellular space and the middle lamella areas where the non-esterified pectins are localized (Knox *et al.*, 1990) rather than from the wall proper (Figures 4.16 & 4.19). The non-esterified pectic polymers were readily extracted by CDTA. Furthermore, although Enkonera contained higher

esterified CDTA-soluble pectins and lower esterified Na<sub>2</sub>CO<sub>3</sub>-soluble pectins than Toro, the β-elimination rate of the CDTA-soluble pectins of Enkonera was lower than that of Toro, whereas that of the Na<sub>2</sub>CO<sub>3</sub>-soluble pectins was higher in Enkonera than in Toro. These imply that β-elimination was not the predominant mechanism of the chemical change leading to softening as suggested by many others (Sajjanantakul *et al.*, 1989; Albersheim *et al.*, 1960a; Keijbets *et al.*, 1976). Similar findings were also made on other plant foodstuff (Greve *et al.*, 1994b; Loh *et al.*, 1982).

Both GTC and KOH-soluble hemicellulosic polymers showed no significant changes in their MW during heating. Therefore the peeling away of microfibrils (Figures 4.17&4.20) observed by TEM in the late stage of steaming may be another indication of the breakdown of cross-links between hemicellulosic polymers as well as between hemicellulose and cellulose.

The heterogeneity of the pectic polymers of both Toro and Enkonera pulp tissues was revealed by the preliminary fractionation studies of their CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins by anion exchange chromatography. Uronic acid was the major sugar in all fractions studied although its proportions were different with different fractions. The degrees of the pectin esterification were also different in different fractions, and they decrease after cooking.

## **6.2. Effect of starch on texture of *Musa***

Higher starch and amylose content found in the Big Ebanga plantain is positively correlated to the firmer texture after cooking rather than an inverse relationship as

reported in potatoes by Jarvis *et al* (1992). However, the result is in agreement with the findings made by Sharma *et al* (1959), Linehan and Hughes (1969a, b) and McComber *et al* (1994). Amylose might be able to cement the pulp cells by formation of hydrogen bonds with the cell wall polysaccharides (Linehan and Hughes, 1969c) which would increase the intercellular adhesion, hence the firmer texture.

The total starch content remained the same before and after cooking in both dessert banana and plantain (Figure 3.7) as found in potatoes (Harada *et al.*, 1985b; Harada and Paulus, 1987; Loh *et al.*, 1982). Therefore, the changes in total starch content alone are not responsible for the loss in the texture of the cooked *Musa* pulp tissues during heating. Mica and Brod (1985) reported that differences in behaviour of various starches (amylose and amylopectin) during heating may be more significant to the texture of cooked potatoes than differences in the amounts of starch among cultivars. Amylose has a greater tendency to retrograde following gelation on cooking, leading to increased firmness. Amylopectin is easier to gelatinize. Banks *et al* (1970) also claimed that higher amylose content resulted in higher inter-granule cohesive forces, therefore higher temperature is required to complete the gelatinization. In potatoes, hard strains have relatively higher amount of amylose, and 'waxy' strains contain high amounts of amylopectin. This has an impact on texture (Ilker and Szczesniak, 1990).

### **6.3. Cell wall and starch--their relative importance in texture loss during heating**

TEM observations made on Toro and Enkonera (Figures 4.15-20) showed that the cell wall started to dissolve as early as 1 minute into steam-cooking when some of the

starch integrity still remained (Figure 4.14b). After this period of cooking, as much as 40% of the original firmness of the Toro pulp tissue had been lost. This suggested that the initial cell wall separation is responsible for almost half of the texture loss before any starch swelling pressure has possibly become significant. Of course, starch swelling pressure together with thermal expansion of the cells in the later stages of cooking resulted in further cell wall separation, hence soft, desirable texture in both fruits.

#### **6.4. Effect of different treatments on texture of *Musa***

Heating dessert banana at 60-70°C for 10 minutes had very little effect on its pulp firmness loss compared to heating at 80-100°C (Figure 3.8). This is possibly due to the retention of pectinesterase activity which could result in an increase of the Ca<sup>2+</sup> binding non-esterified pectin when the material was cooked below 70°C (Hoff, 1972). Ca<sup>2+</sup> also had a big effect on the firmness retention of both dessert banana and Big Ebanga plantain (Figure 3.9) presumably due to the formation of Ca<sup>2+</sup>-pectic gels (Morris *et al.*, 1982), hence decreasing pectin solubilization and improving the cementing function of pectin (van Buren, 1986). Lower water uptake by cooking in CaCl<sub>2</sub> solution (Figure 3.10) also prevented the cell wall components (especially pectin) from hydrating, thus delayed the cell wall softening (Warren and Woodman, 1974).

#### **6.5. Conclusion**

Thermal softening of *Musa* pulp tissues appears to occur at very early stages of cooking. This is supported by the observations which show that cell wall separation

occurs at about the same time as thermal texture loss. Therefore, thermal degradation of intercellular and cell walls is believed to be a major cause of texture loss in *Musa* regardless of the cooking methods. As the texture loss becomes apparent before depolymerization of the CDTA-soluble pectin, the cross-links between these polymers seem more important in determining the texture loss in the early stages of cooking. *Musa* pectins might be depolymerized through a pathway or pathways other than  $\beta$ -elimination.

Enkonera (hard) contained more neutral sugars, as well as higher proportions of high mol wt pectins in its walls than Toro. The CDTA-soluble pectins from the former showed a less rapid rate of depolymerization than those from the latter. This agrees with the cell wall adhesion theory (Reeve, 1956; Loh and Breene, 1982) which holds that the physical strength of the tissue is determined by intercellular adhesive materials (e.g., pectin). The more unstable this material is to cooking, the greater will be the texture loss due to cooking.

Starch and its composition also play an important role in thermal softening on *Musa* pulp tissues. Higher starch content together with higher amylose proportion are positively associated with a firmer texture in plantain. Cooking time, temperature and medium also assert their influences on enhancing or decreasing the rate of tissue softening.

## **7. Future Work**

The future work should be concentrated on studies of the neutral sugar changes during cooking. In order to avoid the complication caused by high starch content in the pulp tissue, this should be done by heating the purified cell wall materials for various periods of time, then the sugar composition of the cell walls should be monitored. Possible cross-links in the cell walls which are responsible for the texture of different cultivars should also be determined. Immuno-labelling of esterified and non-esterified pectins by TEM studies in the wall before and after cooking should be employed to characterise the mechanism of pectin breakdown by  $\beta$ -elimination. Also, PME activities should be assessed to establish its contribution to the slow firmness loss in the early stages of cooking as well as the retention of firmness at lower temperature cooking (below 70°C). Finally, the proteins in the wall and their possible functions in cooking-induced softening should be studied.



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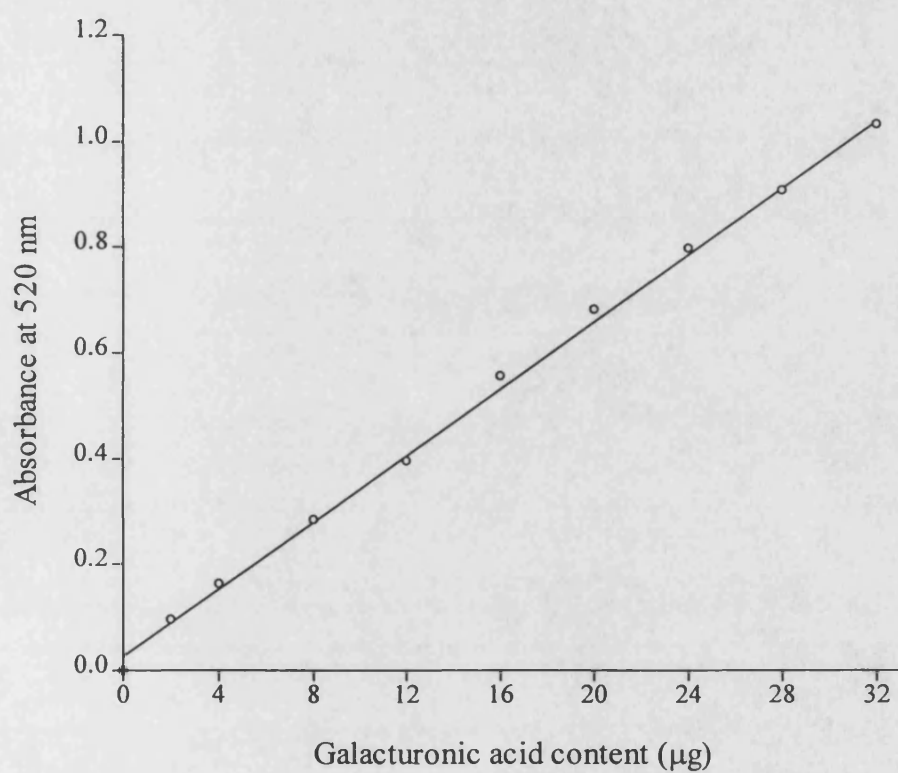
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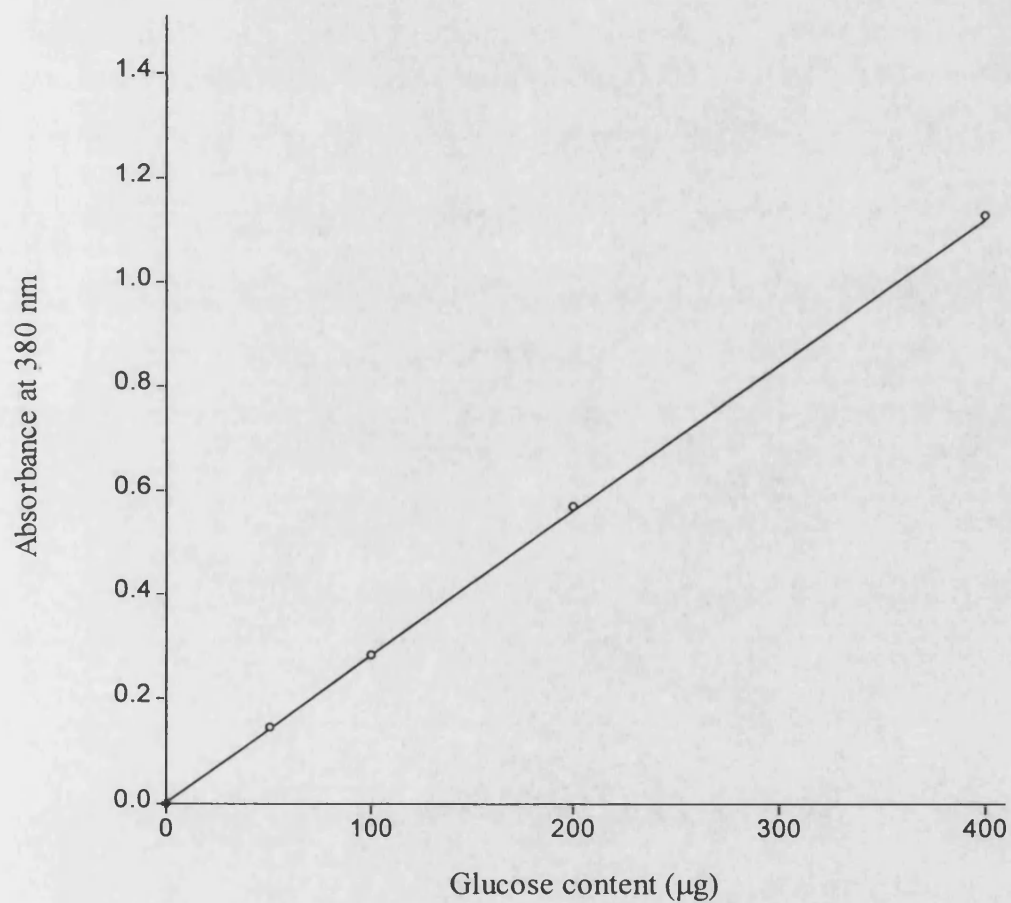
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**Appendix 1.** Galcturonic acid standard curve by the m-phenylphynol method (Blumenkrantz and Asboe-Hansen, 1973) for the measurement of pectin content.



$Y=31.3421X - 0.8156$  ( $r^2=0.9982$ ), where Y=galacturonic acid content (µg), X=absorbance at 520 nm.

**Appendix 2.** Glucose standard curve determined by ferrinide method (Richard, 1992) for the measurement of starch content.



$Y=354.9876 - 0.9507 (r^2=0.999976)$ , where  $Y$ =glucose content ( $\mu\text{g}$ ),  $X$ =absorbance at 380 nm.



**Appendix 3.** Simultaneous determination of amylose and amylopectin content of dessert banana and plantain (Jarvis and Walker, 1993)

$$A_1 = Eoam_1 \times Cam + Eoap_1 \times Cap \text{ (absorbance at 504nm)}$$

$$A_2 = Eoam_2 \times Cam + Eoap_2 \times Cap \text{ (absorbance at 548nm, amylopectin peak)}$$

$$A_3 = Eoam_3 \times Cam + Eoap_3 \times Cap \text{ (absorbance at 580nm)}$$

$$A_4 = Eoam_4 \times Cam + Eoap_4 \times Cap \text{ (absorbance at 630nm, amylose peak)}$$

$$A_5 = Eoam_5 \times Cam + Eoap_5 \times Cap \text{ (absorbance at 700nm)}$$

$$A_6 = Eoam_6 \times Cam + Eoap_6 \times Cap \text{ (absorbance at 800nm)}$$

$$Cam = \frac{A_2 \times (Eoap_1 / Eoap_2) - A_1}{(Eoap_1 / Eoap_2) \times Eoam_2 - Eoam_1}$$

$$Cap = \frac{A_2 - (Eoam_2 \times Cam)}{Eoap_2}$$

Where  $A_1$  = absorbance at first wavelength (504nm)

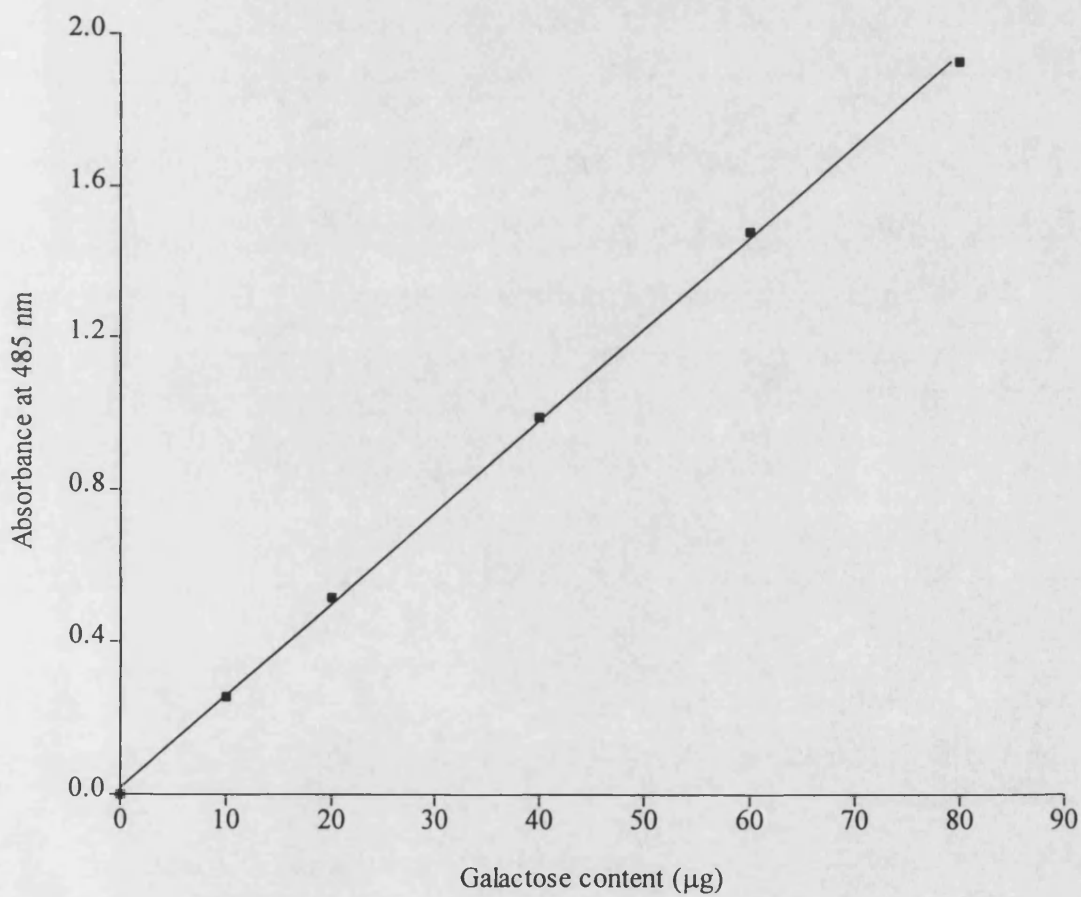
$A_2$  = absorbance at second wavelength (548nm)

$Cam$  = amylose content ( $\mu\text{g}$ )

$Cap$  = amylopectin content ( $\mu\text{g}$ )

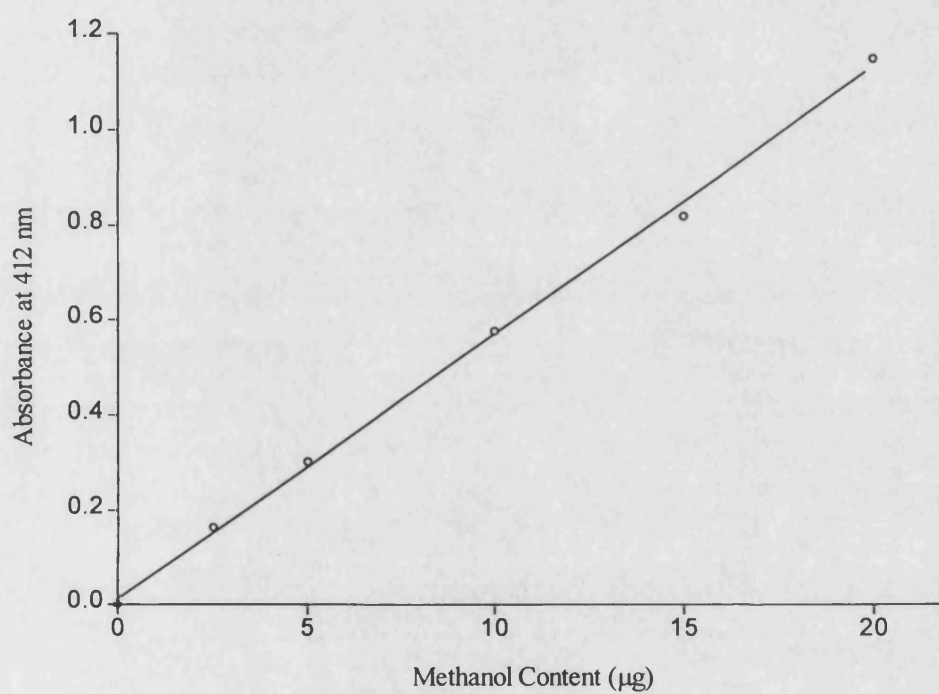
$Eoam_1$  and  $Eoap_1$  are the constants at the first wavelength of amylose and amylopectin, whereas  $Eoam_2$  and  $Eoap_2$  are the constants at the second wavelength of amylose and amylopectin, and so on.

**Appendix 4.** Galactose standard curve determined by the phenol-H<sub>2</sub>SO<sub>4</sub> method (Dubois *et al.*, 1956) for the measurement of neutral sugar content of cell wall.



$Y=41.6411X - 0.7281$  ( $r^2=0.9995$ ), where  $Y$ =galactose content ( $\mu\text{g}$ ),  $X$ =absorbance at 485 nm.

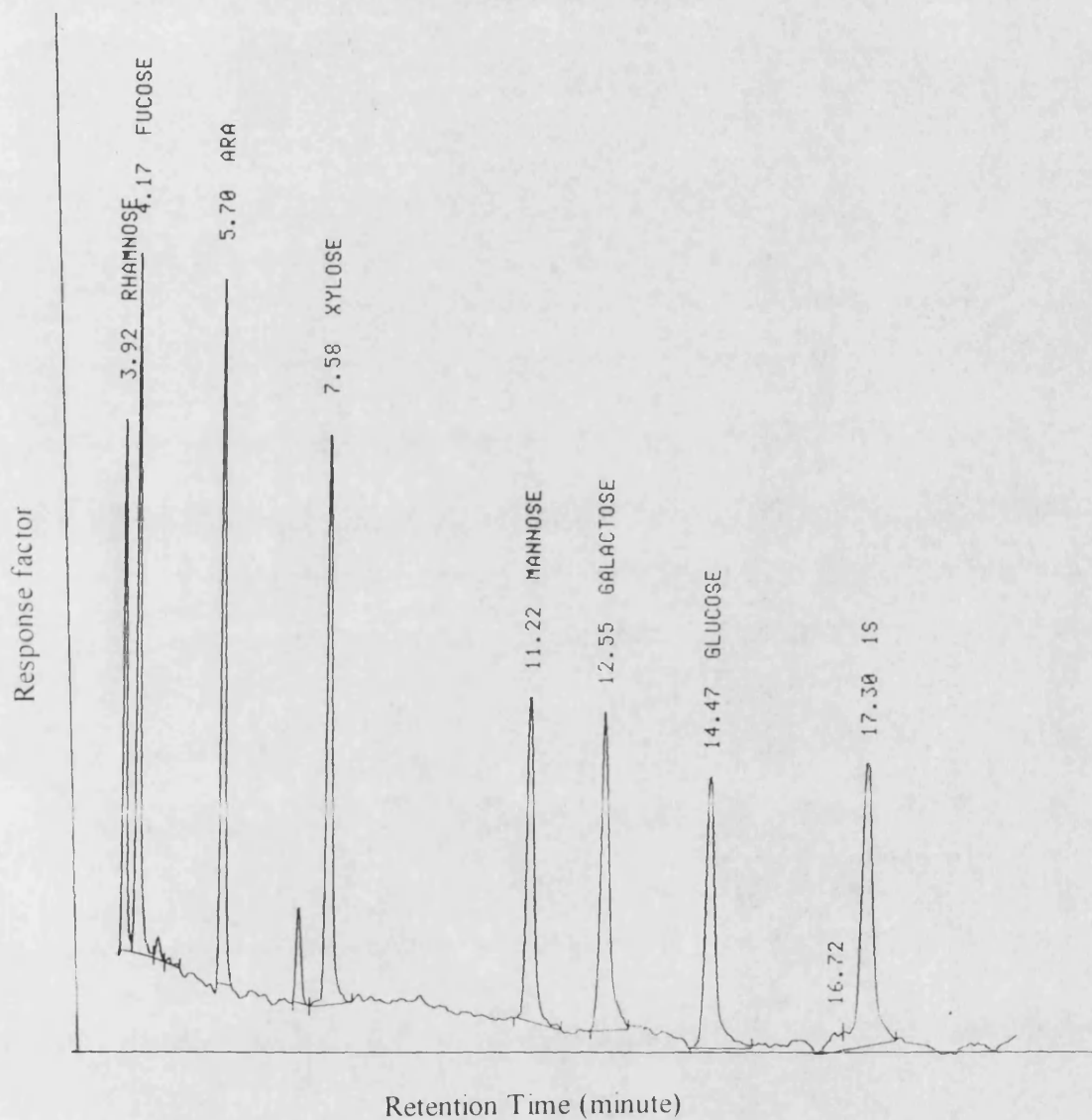
**Appendix 5.** Methanol standard curve determined by the enzymatic method of Klavons and Bennett (1986) for the measurement of pectin esterification.



$Y=17.9693X - 0.2376$  ( $r^2=0.9983$ ), where  $Y$ = methanol content ( $\mu\text{g}$ ),  
 $X$ =  
absorbance at 412 nm.

Degree of methylation (%) =  $100 \times (\mu\text{g of methanol} \div \mu\text{g of galacturonic acid}) \times 6.5$

**Appendix 6.** GLC separation of neutral sugars as their alditol acetates for the measurements of neutral sugar content in the cell wall and its components (Blakeney *et al.*, 1983; Harris *et al.*, 1988)



Response factor: rhamnose, 2.353; fucose, 2.402; arabinose, 1.977; xylose, 2.072; mannose, 2.921; galactose, 2.089; glucose, 3.03; IS as 1.00. IS: internal standard, myso-inisitol was used.

**Appendix 7** Sugar compositions of purified cell wall materials (CWM), non-cellulosic cell wall material and of fractions obtained by sequential extraction of CWM with CDTA and Na<sub>2</sub>CO<sub>3</sub>. Values are means±standard deviations of four measurements. <sup>a</sup>Anhydrous sugar values after Saeman hydrolysis. <sup>b</sup>Anhydrous sugar values after 1M H<sub>2</sub>SO<sub>4</sub> hydrolysis. <sup>c</sup>Degree of esterification. <sup>d</sup>Not determined; *t* Trace.

Sugars <sup>a</sup>	Fractions (µg mg solids)							
	TORO				ENKONERA			
	CWM	Non-Cellulosic <sup>b</sup>	CDTA-sol.	Na <sub>2</sub> CO <sub>3</sub> -sol.	CWM	Non-cellulosic <sup>b</sup>	CDTA-sol.	Na <sub>2</sub> CO <sub>3</sub> -sol.
<i>Rha</i>	<i>t</i>	4.1	9.0±0.34	<i>t</i>	5.0±0.4	<i>t</i>	12.5±1.4	<i>t</i>
<i>Fuc</i>	<i>t</i>	3.4	0.4	<i>t</i>	4.1±0.3	<i>t</i>	0.9	<i>t</i>
<i>Ara</i>	54.7±2.7	62.9±6.2	5.9±0.5	14.8±1.9	62.0±2.9	51.3±6.6	5.0±0.6	17.1±1.4
<i>Xyl</i>	54.4±6.7	65.7±7.4	5.8±1.7	2.8	52.1±2.6	50.5±6.7	5.9±0.5	3.5
<i>Man</i>	15.0±1.7	6.7	2.7±0.1	2.2	25.2±3.9	<i>t</i>	4.0±0.6	2.0
<i>Gal</i>	42.2±3.4	31.4±0.8	16.6±1.6	25.4±0.4	76.5±8.6	70.3±11.4	19.3±3.8	36.4±2.6
<i>Glc</i>	335.5±20.0	31.4±5.7	8.5±0.9	20.1	297.4±18.7	47.3±5.3	8.6±1.3	20.6
<i>GA</i>	314.4±18.5	304.7±16.0	244.7±10.9	471.7±31.8	271.9±26.5	258.6±5.8	410.7±41.5	613.5±28.7
<i>d.e. (%)</i> <sup>c</sup>	48.6	N.D. <sup>d</sup>	63.9	<i>t</i>	66.1	N.D. <sup>d</sup>	60.5	<i>T</i>
<i>Total</i>	815.2	509.7	293.5	536.8	783.9	430.7	466.9	693.1