# Effect of Heating on the Cell Wall Components of Matooke Cooking Bananas (*Musa* spp., AAA group)

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Abstract

Changes of cell wall carbohydrate composition of cooking banana (*Musa* spp., AAA group) were examined before and after cooking in order to investigate the factors responsible for their textural properties. Cell wall materials (CWM) and alcohol insoluble residues (AIR) were isolated from two Matooke cooking banana varieties, Toro (soft) and Enkonera (firm). Two pectic and two hemicellulosic fractions were subsequently extracted and their sugar composition and relative molecular weight distributions were determined. It was found that the fresh pulp AIR of cv. Enkonera contained higher water-, cyclohexane-trans-1,2-diaminetetraacetate- (CDTA) and $Na_2CO_3$-soluble pectic polymers than that of Toro. Enkonera CWM also contained much more galactose and slightly higher arabinose than cv. Toro although there was no significant difference in overall uronides and cellulosic glucose content. The majority of the pectins in the fresh pulp tissues of both cultivars were $Na_2CO_3$-soluble (~ 70%). This implies that a high proportion of the pectins was most likely covalently linked to other cell wall polymers. The major changes during steam cooking were concerned with the pectic polysaccharides where both pectin depolymerization

and solubilization from the middle lamella and cell wall in the fruit of both types had occurred. This was particularly significant in the softer variety Toro as compared to Enkonera. Hemicellulosic polymers were, however, not affected by heating in terms of their molecular weights.

Keywords: Banana, *Musa*, cooking, texture, cell wall components, pectin.

## Introduction

A significant proportion of the population of Uganda, Burundi, Kenya, Rwanda and Tanzania depends on bananas, particularly cooking varieties, for up to 80% of their carbohydrates (1). Importantly, cooking bananas are less susceptible to a serious leaf disease of banana, black Sigatoka, which can reduce productive potential by 30-50% (2). Producers and consumers preferences for specific cultivars of cooking bananas are based on a number of intrinsic factors and desired end-use (3). Texture is viewed as one of the most important attributes of the cooked product in determining a good cooking banana (4).

A considerable amount of research has been undertaken on plant cell walls and their components in relation to tissue firmness. Pectic substances have been the main subject of research aimed at understanding the tissue factors that contribute to the texture of fresh and cooked fruits and vegetables as tissue firmness decreases during ripening, storage and processing. This is due to the role of pectins in determining the mechanical strength of the primary cell wall and adhesion between cells (5, 6). Studies have indicated that during heat-induced softening the pectic substances between, as well as within, the matrix of adjacent cell walls are depolymerized and degraded, largely by thermal β-elimination (7,8). The rate of β-elimination depends on the degree of pectin methylesterification (9,10).

Other cell wall components, such as xyloglucan hemicellulosic polymers (11,12) and phenolic esters (13,14) may also be affected by the processing treatments. There have been studies on the chemical characterisation of alcohol insoluble solids extracted from banana cell walls (15), but little, if any, information exists on compositional changes in the cell walls during cooking. The purpose of the study reported here is to determine changes in component(s) of the cell walls of cooking banana during cooking in order to establish if differences in susceptibility to softening between genotypes could be related to differences in the amount, size or composition of these particular wall fractions.

## Materials And Methods

*Plant Material*

Toro and Enkonera cooking bananas (*Musa* spp., AAA group) were harvested at their green mature stage in Uganda and transported back to the UK by air within the same day of harvesting. Transverse, 10-mm thick sections were taken from the mid-regions of six fruits and peeled before they were steamed for 1, 3 and 10 minutes. All the samples were then frozen in liquid nitrogen and freeze-dried to constant weight. They were ground to a fine powder to pass through a 250-μm sieve.

*Firmness Measurement*

The compressive strength of pulp sections was measured by rupture force using a penetrometer fitted with a rounded 6 mm diameter probe (16). 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.

77

78 *Alcohol Insoluble Solids (AIS) Preparation*

79 Two grams of each freeze-dried sample was dissolved into 20 mL of phenol:acetic acid:water
80 (PAW, 2:1:1, w/v/v) solution and stirred for 4 hours at 4 °C. The suspension was then centrifuged
81 at 7,000g for 15 minutes and the residue washed with 80% ethanol six times to remove phenol.
82 The alcohol insoluble solids (AIS) were then freeze-dried and stored at -20°C until use.

83

84 *Estimation of Water-Soluble, Chelator-Soluble and Sodium Carbonate-Soluble Polyuronides*
85 *Content of AIS*

86

87 Water-soluble polyuronides were extracted by shaking 100 mg of the AIS sample with 10 mL
88 distilled water at 20°C for 20 hours on an orbital shaker. After shaking, the aqueous extract was
89 recovered by centrifugation at 4000 rpm for 30 minutes. The supernatant, containing the water-
90 soluble polyuronides, was then filtered through GF-A glass fibre filter paper.

91

92 The residue from the water extraction was stirred with 50 mM carboxyethylene-diamine-tetraacetic
93 acid (CDTA) (potassium salt, pH 6.5) at 20°C for 20 hours to extract the chelator-soluble
94 polyuronides (17,18). The extract was centrifuged (using the same conditions as above) to recover
95 the CDTA-soluble polyuronides in the supernatant which was then filtered through GF-A glass
96 fibre filter paper.

97

98 The residue remaining after the above step was re-suspended in 50 mM $Na_2CO_3$ containing 20 mM
99 $NaBH_4$, and stirred for 20 hours at 1-4 °C, then for two hours at room temperature. The $Na_2CO_3$-
100 soluble polyuronide solution was obtained by centrifugation, then filtration through GF-A glass

101 fibre filter paper. The $Na_2CO_3$-soluble fraction was neutralised by the drop wise addition of glacial
102 acetic acid on ice.
103
104 A few drops of toluene were added to all the solutions at the beginning of each extraction to inhibit
105 microbial growth.
106
107 Uronic acid in each fraction was estimated colorimetrically by the m-phenylphenol method (19),
108 with a correction for neutral sugars (20). Galacturonic acid was used as a standard. The selection
109 of galacturonic acid as the standard is justified based on sugar analysis reported here, which found
110 that galacturonic acid was the major component of CDTA- and $Na_2CO_3$-soluble fractions isolated
111 from cooking banana (90% and 76%, respectively).
112
113 *Cell Wall Purification*
114 Cell wall materials were purified from freeze-dried pulp tissues according to the method used by
115 Selvendran (21) with the modification of omitting sodium deoxycholate (SDC) in the initial
116 preparation step (22), since SDC is known to solubilize appreciable amounts of pectic materials
117 from the cell wall. Ten grams of the freeze-dried powder were treated with 100 mL of PAW
118 solution in the same manner as for AIR preparation. The residue was then extracted twice with
119 100mL of 90% of dimethyl sulfoxide (DMSO) at 4°C overnight to solubilize the starch present.
120 The suspension was centrifuged at 10,000g for 15 minutes, and the residue washed by
121 centrifugation with 80% alcohol 6 times. The cell wall material (CWM) was recovered following
122 dialysis and freeze drying, which yielded approximately 0.5 g dry weight CWM.
123

*Solubilization of Cell Wall Polymers From Toro and Enkonera CWM*

CWM (100 mg) was extracted with 15 mL of 50 mM CDTA, then 50 mM $Na_2CO_3$ containing 20 mM $NaBH_4$, as described above for AIR. The residue from the $Na_2CO_3$ extraction was then stirred for 18 hours at room temperature in 6 M guanidinium thiocyanate (GTC), and finally in 4 M KOH containing 20 mM $NaBH_4$ under nitrogen for 2 hours (18). Uronic acid contents in each fraction were estimated by the m-phenylphenol method (19) using galacturonic acid as a standard, whilst the neutral sugar contents in hemicellulosic fractions were estimated by the phenol-$H_2SO_4$ method (23) using galactose as a standard.

Each supernatant was then filtered (those containing alkali were neutralised), dialyzed and concentrated, then stored at –20 °C for further heating experiments and gel filtration studies. The remaining materials were freeze-dried for sugar analysis by gas liquid chromatography (GLC).

*Measurement of the Degree of Pectin Esterification*

The degree of esterification of pectin was analysed by the enzymatic method of Klavons and Bennett (24). HPLC grade methanol was used as a standard. The percent esterification is expressed as the percent of the uronic acid present as methyl ester.

*Heating Treatments of Extracted Cell Wall Polymers*

Two-mL portions of approximately 0.5% solutions of the CDTA, $Na_2CO_3$, GTC and KOH-soluble polymers in distilled water (pH 5.5) were distributed into 10-mL Teflon-lined screw cap test tubes. Duplicate samples were heated in a heating block for 1, 3 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

148 samples were concentrated down to 2 ml, then kept at -20°C until tested for molecular weight by
149 gel filtration chromatography.
150
151 *Gel Permeation Chromatography (GPC)*
152 Gel filtration chromatography was carried out using Sepharose CL-2B (fraction range 100,000 -
153 20,000,000 for linear dextrans) packed into a glass column (1.6 x 89 cm, bed volume of 180 mL).
154 The gel was equilibrated in 50 mM acetate buffer (pH 6.0) containing 125 mM NaCl and 0.05%
155 chlorobutanol as an anti-microbial agent. Each sample solution was loaded onto the column, eluted
156 with the same buffer at 10 mL/hour. Two-mL fractions were collected by an automatic fraction
157 collector (LKB), and monitored spectrophotometrically by the m-phenylphenol for uronic acid
158 content (19).
159
160 *Gas-Liquid Chromatography (GLC) Measurement for Neutral Sugar Composition*
161 The neutral sugar composition of purified cell wall materials and other fractions was determined by
162 GLC of their alditol acetates by the methods of Blakeney *et al.* (25) and Harris *et al* (26), using
163 myo-inositol as an internal standard.
164
165 **Results and Discussion**
166 Measurement of tissue firmness revealed that uncooked Ekonera pulp tissue was firmer than Toro
167 and that this difference in firmness was maintained during streaming (Table 1). To evaluate the
168 changes of cell wall composition in relation to cooking banana texture after cooking, cell walls and
169 their components of fresh and steam cooked bananas of these two cultivars were compared.
170
171 The main types of polysaccharides of the cell wall material (CWM) isolated from the pulp tissues
172 of both cooking banana cultivars were pectic polysaccharides and cellulose (Table 2). The

presence of pectic polysaccharides can be deduced from the relatively large amounts of uronic acids, arabinose, galactose, and to a minor extent rhammose. The occurrence of cellulose is inferred from the fact that the bulk of glucose could be released only after Saeman hydrolysis. In addition, the presence of xylose and non-cellulosic glucose suggested the possible presence of xylans and xyloglucans, hemicellulosic polysaccharides. Although there were no significant difference in wall pectin and cellulosic glucose content between the two cultivars, the overall pectin compositions were different where the amounts of neutral sugars (rhammose, arabinose and galactose) appeared higher in cv. Enkonera (GA/Ara+Gal = 1.7) whilst the amount of uronic acids appeared relatively higher in cv. Toro (GA/Ara+Gal = 2.7). This suggested that pectin from pulp tissues of cv. Enkonera contained more or longer neutral side chains than pectin from cv. Toro which result in stronger linkages between the primary cell wall polymers. These results are in agreement with the finding of Loh and Breene (27) that vegetables, such as waterchestnut, which remained firmer after cooking, were much higher in neutral sugar content than softer species, such as potato.

In fresh banana pulp of cv. Enkonera there seemed to be relatively more water-, CDTA- and $Na_2CO_3$-extractable pectic materials than in that of cv. Toro (Table 3). Water extracts free and high methoxyl pectin whilst CDTA extracts $Ca^{2+}$ bound pectins of which the majority originated from the middle lamella region (17, 21, 28). The $Na_2CO_3$-soluble fractions contain pectic substances, which are probably linked to other pectins, hemicelluloses and cellulose by weak ester bondings (5,6). Because these fractions represented the major cell wall pectins of both fresh banana cultivars, these bananas appear to contain mainly these insoluble, covalently bound pectic substances. Similar results were reported with green beans (29) although in carrots, CDTA-soluble pectins were the main constituents of wall pectins (13, 14).

198 The CDTA extracts isolated from cv. Enkonera consisted of fewer neutral pectic sugars and more
199 galacturonic acids whilst the $Na_2CO_3$ extracts from both cultivars were comparable. Since overall
200 wall pectin of cv. Enkonera contained much more neutral pectic sugars than cv. Toro (Table 2),
201 therefore, the extra neutral sugars in cv. Enkonera cell wall may be bound to the hemicellulosic
202 polysaccharides in a more branched pectin form. Although there were a number of studies that
203 showed a good correlation between the amount of neutral sugar side chains of pectin to cell-cell
204 attachment (30), the functions of such neutral sugars to rheological properties has not been studied
205 in depth (29).
206
207 During cooking pectins were depolymerised and solubilised (Table 3). Analysis of the different
208 pectic fractions revealed that substantial more pectin became water soluble after cooking, and the
209 mol wt of the chelator soluble pectins were greatly reduced. Pectins were shifted from the $Na_2CO_3$
210 fractions to the water- and CDTA-fractions. However, in pulp tissue subjected to cooking, the
211 changes in different pectic fractions differed dramatically in the two cultivars. The water-soluble
212 pectin increased in both fruits with the increase in Toro being greatest (5 fold). Increase in the
213 water-soluble pectin fraction during cooking or ripening of fruits and vegetables is often interpreted
214 as an increase in the solubility of cell wall pectin, hence texture loss. $Na_2CO_3$-soluble pectins
215 decreased in both fruits in a similar rate (28% decrease) after 3 minutes of cooking. After further
216 cooking, there was a greater decline in Enkonera than in Toro. The probable explanation for the
217 decrease in these wall pectins during cooking was either an increase of their solubility (i.e.
218 conversion to soluble pectin fraction) or less extractability due to an interaction between
219 intracellular and/or cell wall proteins and the soluble anionic pectic polymers resulting in the
220 formation of insoluble complexes during heating, or a combination of both processes. The fact that
221 more electron dense materials have been observed in cv. Enkonera heated cell walls than in Toro

might be interpreted as more aggregations between pectins and wall proteins being formed which results in less extractability of the pectins (16).

The CDTA-soluble fraction demonstrated a dramatic increase in its amounts during the first three minutes of cooking in cv. Enkonera. Since both water- and $Na_2CO_3$-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, and this was possibly due to the action of the enzyme pectin methylesterase (PME) on these highly methylated pectins. This enzyme is activated between 60-70 °C (31) and denatured after 90 °C. During the time before the entire pulp disk reaches 90 °C the enzyme would have demethylated some of the pectins resulting in an increase of free carboxyl groups in the cell wall pectin, which is the main characteristic of pectins extractable by CDTA. This hypothesis is supported by the browning of the pulp tissue during the first minute of cooking that suggests that some of the enzymes were not denatured immediately. As the cooking lengthened, the temperature exceeded 70°C and the PME was deactivated (the pulp tissue was no longer browning after 3 minutes of cooking) exerting no effect on the cell wall material. Hence, the CDTA fraction decreased thereafter continuously. A similar result was reported for the blanched beans (29). 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.

GPC profiles of the two pectic fractions were also very different between the two cultivars. The CDTA-soluble pectic substances were the only polymers that showed a significant reduction in molecular weight after 10 minutes of heating (Figures 1-2). The CDTA-soluble pectic polymers are among the largest pectic molecules in both walls. The breakdown of this middle lamella pectin would cause the adhesion of adjacent cells to be greatly reduced, leading to greater cell separation, and texture loss. This change is more extensive in cv. Toro than in cv. Enkonera. In other words,

247 after the same period of cooking, the soft genotype of cooking banana experienced more
248 dissolution of CDTA-soluble pectin than the firm type. Greve *et al.* (32) also found the same
249 tendency with soft and hard carrot genotypes. These pectic substances were presumably held in the
250 wall matrix by $Ca^{2+}$ and ester linkages (21). Although the $Na_2CO_3$-soluble pectic polymers from
251 both Toro and Enkonera cell walls did not show a large MW reduction even after 10 minutes of
252 heating (Figure 2), the $Na_2CO_3$ fraction from cv. Enkonera had a much higher proportion of higher
253 MW materials than those from cv. Toro CWM, and they remained higher throughout the heating
254 treatments. Presumably these higher MW materials could maintain some of the wall strength, hence
255 the tissue firmness, as the CDTA-soluble middle lamella pectins progressively completely broke
256 down. This may be one of the reasons why cv. Enkonera remained harder even after a period of
257 cooking which would have resulted in breakdown of middle lamella pectin.
258 It is suggested that heat-induced degradation of pectic polysaccharides may be caused by a ß-
259 elimination process, which breaks the methylated polygalacturonic acids into smaller fragments.
260 However, this would not account for the dramatic heat-induced molecular weight reduction of the
261 CDTA-soluble pectic polymers from cv. Toro CWM which have lower levels of methylation than
262 cv. Enkonera (Table 4). Therefore, it seems that there may be more than one mechanisms involved
263 in pectin breakdown during the heating process of cooking bananas.
264
265 The GTC- and KOH-soluble hemicellulosic polymers did not show any molecular weight reduction
266 during heating of both fruits (16). Therefore, hemicelluloses of *Musa* fruits were not prone to
267 depolymerization due to cooking.
268
269 In conclusion, thermal softening of cooking banana pulp tissues were accompanied by both
270 depolymerization of the linear polygalacturonic acid chains, and solubilization of highly
271 methylated pectic substances. The former is localised in the middle lamella and responsible for cell

to cell adhesion, and the latter in primary cell walls. Therefore, both cell wall separation and softening have occurred during the cooking process. There were some differences in cell wall carbohydrate composition and their response to cooking between the soft and firm fruits of the two cultivars. Cultivar Enkonera fruit, which remained much firmer after cooking, appeared to have (1) more branched wall pectins, (2) less branched middle lamella pectins, (3) high proportion of large sized cell wall pectins, and (4) less severe depolymerisation of middle lamella pectins upon cooking.

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382 FIGURE LEGENDS

383 Figure 1. GPC profiles of CDTA-soluble fractions from Matooke cooking banana cvs Toro (left)
384 and Enkonera (right) before and after steam cooking. A and C, fresh; B and D, 10 minutes steam
385 cooked. T2000, blue dextran (Mw = 2,000,000). GA, galacturonic acid (Mw = 194). Column
386 fractions were monitored for UA.

387

388 Figure 2. GPC profiles of carbonate-soluble fractions from Matooke cooking banana cvs. Toro
389 (left) and Enkonera (right) before and after steam cooking. A and C, fresh, B and D, 10 minutes
390 steam cooked. . T2000, blue dextran (Mw = 2,000,000). GA, galacturonic acid (Mw = 194).
391 Column fractions were monitored for UA.

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**Table 1. Firmness Changes (in Newtons) During Steam Cooking Of Cultivars Toro and Enkonera**

| | fresh | 3 min | 10 min |
|---|---|---|---|
| Toro | 13.5[a] ± 1.4 | 2.9 ± 0.3 | 1.7 ± 0.3 |
| Enkonera | 20.9 ± 2.4 | 6.9 ± 1.2 | 3.4 ± 1.0 |

[a] $n = 12$, ± SD.

**Table 2. Sugar Composition[a] of the Purified CWM, and of Fractions Obtained by Sequential Extraction of CWM with CDTA and $Na_2CO_3$.**

| | fractions (mol % of total cell wall sugars) | | | | | |
|---|---|---|---|---|---|---|
| | CWM[b] | | CDTA[c] | | $Na_2CO_3$[c] | |
| Sugars | Toro | Enkonera | Toro | Enkonera | Toro | Enkonera |
| Rha[d] | *tr*[e] | 0.6 | 3.3 ± 0.1 | 2.9 ± 0.3 | *tr* | *tr* |
| Fructose | *tr* | 0.6 | 0.2 | 0.2 | *tr* | *tr* |
| Ara | 8 ± 0.4 | 9.2 ± 0.4 | 2.5 ± 0.2 | 1.4 ± 0.2 | 3.5 ± 0.4 | 3.2 ± 0.1 |
| Xylose | 8 ± 1.0 | 7.8 ± 0.4 | 2.5 ± 0.7 | 1.5 ± 0.1 | 0.7 | 0.7 |
| Man | 1.9 ± 0.2 | 3.2 ± 0.5 | 1.0 | 0.9 ± 0.1 | 0.4 | 0.3 |
| Gal | 5.2 ± 0.4 | 9.7 ± 1.1 | 6.1 ± 0.6 | 4.5 ± 0.9 | 5.1 ± 0.1 | 5.7 ± 0.4 |
| Glucose | 41.5 ± 2.5 | 37.6 ± 2.4 | 3.1 ± 0.3 | 2.0 ± 0.3 | 4.1 | 3.2 |
| GA | 35.4 ± 2.1 | 31.3 ± 3.1 | 81.4 ± 3.6 | 86.6 ± 8.8 | 86.2 ± 5.8 | 87.0 ± 4.1 |
| GA/ Ara+Gal | 2.7 | 1.7 | 9.5 | 14.7 | 10.0 | 9.8 |

[a] $n$= 4, ± SD. [b] Anhydrous sugar values after Saeman hydrolysis. [c] Anhydrous sugar values after 1M $H_2SO_4$ hydrolysis.
[d] Rha - rhamose; Ara - arabinose; Man - mannose; Gal - galactose; GA - galacturonic acid. [e] Trace.

**Table 3. Pectin Composition[a] of AIR from Cultivars Toro and Enkonera Cooking Banana Pulp Tissues during Steam Cooking**

| fractions (mg AUA/100 g AIR) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| steaming time | water | | CDTA | | $Na_2CO_3$ | | Total | |
| | ***Toro*** | ***Enkonera.*** | ***Toro*** | ***Enkonera.*** | ***Toro*** | ***Enkonera.*** | T[b] | E[c] |
| fresh | 90.9 ± 7 | 164.4 ± 8 | 269.7 ± 8 | 295.2 ± 20 | 784.0 ± 24 | 838.7 ± 72 | 1145 | 1298 |
| 3 min | 193.9 ± 15 | 144.5 ± 4 | 276.5 ± 10 | 482.8 ± 12 | 560.7 ± 9 | 601.7 ± 8 | 1031 | 1229 |
| 10 min | 435.6 ± 22 | 223.9 ± 14 | 315.6 ± 3 | 371.7 ± 11 | 354.9 ± 31 | 273.0 ± 27 | 1106 | 869 |

[a] $n$=3, ± SD. [b] Cv. Toro. [c] Cv. Enkonera.

**Table 4. Degree of esterification of pectins from CDTA- and $Na_2CO_3$-fractions isolated from fresh Toro and Enkonera pulp tissues**

| | CDTA | $Na_2CO_3$ |
|---|---|---|
| | Degree of esterification | Degree of esterification |
| Toro | 48.0 | 75.6 |
| Ekonera | 65.7 | 68.6 |

Values are means of two measurements.