

## Softening of Banana Fruit: Relationship between Firmness and Chemical Composition

Kiyohide KOJIMA

Faculty of Agriculture, Kyoto Prefectural University (Shimogamo, Sakyo-ku, Kyoto, 606 Japan)

### Abstract

Stress-relaxation analysis was applied to examine tissue softening in tomato fruit using a conical probe. Next, the technique was applied to banana using an instrument capable of controlling the plunging depth. When bananas were ripened in a biotron at 25°C, index of peel color increased after the burst of ethylene production, while moisture and cellulose contents did not change during ripening. Next, bananas were ripened with ethylene during 4 days. Changes in the mechanical properties of pulp were detected by a stress-relaxation method, suggesting that the decreases in elasticity and viscosity of the pulp are major physical components of pulp softening. Results of chemical analysis clearly revealed that the partial breakdown characterized by a decrease in arabinose, mannose and galactose contents in the hemicelluloses of the cell wall preceded the breakdown of starch. These observations suggested that the associated process whereby the contents of pectic and hemicellulosic polysaccharides and starch decreased is the main cause for the pulp softening process. In plantains (cooking banana) the physical and chemical properties were analyzed during fruit softening. The results obtained suggest that cell wall polysaccharides, pectin and hemicelluloses are the chemical components responsible for the changes in firmness rather than starch in plantain pulp.

**Discipline:** Experimental apparatus and methods/Postharvest technology

**Additional key words:** cell wall, plantain, postharvest, ripening, stress-relaxation

### Introduction

Softening is one of the major changes and an integral part of the ripening process in almost all fleshy fruits<sup>7)</sup>. It is important in terms of commercial value because the postharvest life of the fruit is to a large extent limited by increasing softness. The chemical changes involved in fruit softening and the enzymes contributing to these changes during ripening have been extensively investigated, and it was suggested that cell wall degradation was a major component of fruit softening<sup>5,14)</sup>. In some fruits, considerable contributions to softening occur through the hydrolysis of cell contents, e.g. the hydrolysis of starches in squash and fats in avocado. Accumulated evidences showed that softening of fruit was accompanied by an increase in the concentration of soluble pectic polysaccharides. However, information about the role in fruit softening of cell wall polymers other than the polyuronides is limited. Hemicelluloses were degraded during ripening in strawberry<sup>15)</sup> and in muskmelon<sup>22)</sup>. Cellulose

metabolism has also been considered to play an important role in fruit softening in peach<sup>13)</sup>, avocado<sup>25)</sup> and apple<sup>1)</sup>.

To clarify the causal relationship between softening and the chemical constituents in fruits, it is necessary to measure the softness with a reliable physical method based on rheology. However, the development of a rheological method for fruit softening has not been emphasized, although texture was recognized as a major quality attribute in all fruits<sup>6)</sup>. Firmness has been usually measured by the force required to press a plunger at a given distance into the fruit. Ang et al.<sup>3)</sup> compressed onion bulbs between flat plates, measured the force applied to the bulbs as a function of its compression, and took the ratio of the applied force to the amount of compression of the bulb as a measure of firmness. Recently, stress-relaxation analysis was developed by Yamamoto et al.<sup>31)</sup> to examine the mechanical properties of plant cell walls.

Because banana is cultivated on a large scale and distributed in a cold line, fruits with uniform size and properties before the beginning of ripening can

be obtained. Thus in order to examine the mechanism of fruit softening, using the banana fruit as a material, a series of experiments were conducted with (1) a novel technique for measuring the firmness based on a physical model and (2) simultaneous measurement of firmness parameters and chemical components responsible for the softening.

### Studies on banana fruit

#### 1) *Physiology of banana fruit softening*

Ripening of banana can be controlled within limits by temperature regulation, ethylene application and modification of oxygen and carbon dioxide concentrations in the storage environment<sup>8)</sup>. In the commercial practice, ripening of green harvested bananas occurs generally by exposing them to about 1,000 ppm of ethylene with ripening schedules that enable ripening periods to be varied from 4 to 8 days at temperatures ranging from 15 to 21°C at a high humidity<sup>12)</sup>.

Softening of banana fruit is associated with changes in the mechanical properties of the tissues, which are predominantly based on the changes in the chemical structure of starch grains<sup>11)</sup> and/or cell walls of banana pulp. Biochemical and compositional changes associated with ripening have been reviewed in banana fruit<sup>21,24,28)</sup>. Many investigators reported that the starch content of the pulp in banana fruit before ripening which was higher than in other fruits, decreased drastically during the short period of ripening and then starch was no longer detected<sup>2,4,8,11,23)</sup>. However, only a few researchers have analyzed the degradation of cell wall components, such as pectin<sup>21)</sup>, hemicelluloses<sup>4,10)</sup> and cellulose<sup>10)</sup> during the ripening process.

#### 2) *Methods of measuring firmness of banana fruit*

Charles & Tung<sup>8)</sup> have studied the maximum force at failure, the deformation under constant force and the linear limit of the force/deformation curve for pulps in banana fruit. Using a sonic technique, Finney et al.<sup>11)</sup> measured the resonant frequencies of cylindrical specimens of the pulp and calculated Young's modulus of elasticity, which is defined as the ratio of stress to strain. The modulus of elasticity was closely related to the amount of reducing sugars and starch during ripening. Nussinovitch<sup>23)</sup> evaluated the criteria suitable for following the progression of ripening in banana and 3 criteria gave high correlation coefficients with parameters such as the Brix value, starch content, age and color index.

### Development of a method of measuring firmness

#### 1) *Physical methods of measuring firmness*

Several techniques for the physical measurement of tissues or cell walls have been developed<sup>29)</sup>. There is no agreed way to measure the elusive physiological cell wall parameters *in vitro*. Mechanical tests applied on isolated walls have been performed using either uniaxial or multiaxial stress. Uniaxial mechanical testing has consisted of 3 types: instron analysis (constant strain rate), stress relaxation (constant strain), and creep (constant load). Usually, analyses of higher plant walls have been based on the Instron or stress relaxation methods because of the ease and rapidity of the measurements<sup>9)</sup>. Stress-relaxation analysis developed by Yamamoto et al.<sup>31)</sup> was based on the Maxwell viscoelastic model and included 3 parameters,  $T_0$ ,  $R$  and  $T_m$ . The  $T_0$  parameter is the time at which the stress starts with major decay, and corresponds to a Maxwell component with the lowest viscosity<sup>26)</sup>. The  $R$  parameter is the relaxation rate (%), and the reciprocal of  $R$  ( $1/R$ ) corresponds to the number of relaxation components per unit volume. The  $T_m$  parameter is the time at which the stress ends with major decay, and corresponds to a Maxwell component with the highest viscosity. The analysis enables to predict that the degradation of wall polysaccharides directly or indirectly leads to the decrease in wall viscosity. The original technique for stress-relaxation analysis aimed at extending plant specimens, and measuring the relaxation of the initial stress applied. This technique, however, was applied to sliced tuber tissues of Jerusalem artichoke<sup>32)</sup>. A tuber slice was subjected to compression by an Instron tensile tester. The parameters were found to be analogous to those obtained by the stretching method.

#### 2) *Application of stress-relaxation analysis to fruit firmness*<sup>16,17)</sup>

Stress-relaxation analysis was applied to the analysis of tissue firmness in tomato fruit using a conical probe<sup>16)</sup>. The softness within the tomato pulp was measured using a controlled pressure device with a load cell and softening progression from the inner columellar region to the shoulder of the fruit via the distal end was demonstrated. An analysis of tissue slices using a conical probe provided more precise data than the use of dissected tissues and a flat probe, because small variations in the dimensions of the excised tissues may affect the calculation of

the physical parameters. When the technique was applied to banana, plunging depths varied substantially between ripe and unripe fruits to obtain the same initial stress. To obtain more precise data in stress-relaxation curves, it was necessary to set up optimum conditions for the measurement. Therefore, an instrument capable of controlling the plunging depth of the conical probe into the tissues was used<sup>17)</sup>. Optimal conditions for measuring stress-relaxation were determined and the changes in the physical properties during softening of banana fruit pulp were described.

### 3) Method of analysis of stress-relaxation<sup>17-20)</sup>

A banana pulp sample from the middle portion of the long axis of a fruit was sliced in a radial direction at a thickness of 7 mm. The measuring point in a cross section was the center of a locule. Sliced pulps were immediately analyzed by the stress-relaxation technique.

The stress-relaxation analysis was carried out using a Creep Meter (RE-33005, Yamaden Inc., Tokyo, Japan) (Fig. 1) equipped with a sample-height counter (HC-3305, Yamaden Inc., Tokyo, Japan). Stress was measured using a 200 gf (gram force) load cell. The conical probe with an edge angle of 60° was attached to the load cell. A slice of banana pulp was placed on a vertical-moving stage, which adjusted the probe at the measuring point. The stage was moved upward to introduce the probe into the sample. Plunging speed was 0.5 mm/s and plunging depth was 0.6 mm<sup>17)</sup>. The stress decay after the

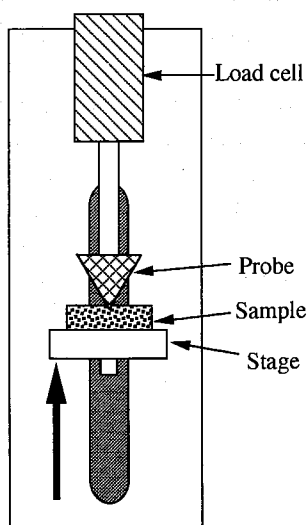


Fig. 1. Schematic diagram of the Creep Meter used for the measurement of banana softening

end of the movement of the stage was recorded from 0 to 100 s by a microcomputer (PC-9801, NEC). The time schedule for the data acquisition accommodated 30 sampling points at equal intervals on a logarithmic scale. The stress and time data were stored in the microcomputer and used later for the calculation of the stress-relaxation parameters.

### 4) Calculation of stress-relaxation parameters

The relaxation data were simulated by the following equation<sup>31)</sup>;

$$St/S_0 \times 100(\%) = R \log_e \{ (t + T_m) / (t + T_0) \}$$

where  $S_0$  is the initial applied stress,  $S_t$  is the stress at time  $t$ ,  $R$  is the relaxation rate,  $T_0$  is the minimum stress-relaxation time and  $T_m$  is the maximum stress-relaxation time. The  $R$ ,  $T_0$ , and  $T_m$  parameters were calculated by the least square method with a personal computer (PC-9801, NEC) programmed in C language.

### Changes of banana fruit during natural ripening<sup>18)</sup>

When bananas were ripened in a biotron at 25°C, index of peel color increased after the burst of ethylene production (Fig. 2A). Moisture content of the

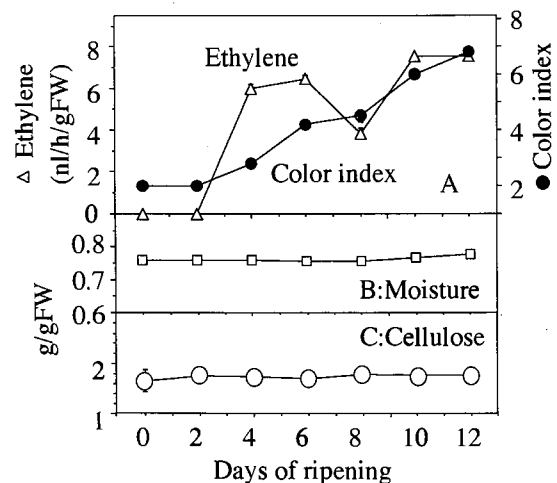


Fig. 2. Color index of peel and ethylene production (A), moisture (B) and cellulose contents (C) of banana pulp during ripening of banana at 25°C

Peel color was evaluated by comparison with a color plate on a banana ripening chart. Moisture content was determined by drying 5 g of banana pulp. Residue after extraction of pectin and hemicellulose was washed with 80% aqueous 1, 4-dioxane and designated as cellulose.

pulp remained nearly constant (Fig. 2B). Thus, the changing patterns of all the data expressed in gram per fresh weight were identical with those in gram per dry weight.

Cellulose metabolism has been considered to play an important role in fruit softening in apple<sup>11</sup>, peach<sup>13</sup> and avocado<sup>25</sup>. In dwarf Cavendish

banana, Desai and Deshpande<sup>10</sup> also reported that the cellulose content continued to decrease during ripening. However, the results clearly show that the cellulose content is absolutely constant during ripening (Fig. 2C). The cellulose content does not appear to be involved in the softening of the pulp at least in Giant Cavendish banana, although the discrepancy may be due to differences in the cultivars used.

### Correlation between softness and constituents in dessert banana<sup>19</sup>

Bananas were ripened in a biotron at 25°C with ethylene treatment during 4 days. The changes in the mechanical properties of the banana pulp during ripening were clearly detected by the stress-relaxation technique (Fig. 3A, B). Decrease in initial stress indicates that the elastic component of the pulp changed during ripening. The decreases in the  $T_0$  and  $T_m$  values suggested that the viscosity of the mechanical components with lowest and highest viscosity decreased<sup>26</sup>. Timing of decrease in elasticity of banana pulp represented by the initial stress was different from that in the viscosity revealed by  $T_0$  or  $T_m$ . Thus the stress-relaxation method may provide more useful information than a simple measurement of initial stress.

Viscosity results from the friction and entanglement of polymers. The decrease in viscosity which was predicted from the decrease in the  $T_0$  and  $T_m$  values should be attributed to the breakdown of polymers, such as cell wall polysaccharides and starch, but not to the increase in the water content, because there was no significant change in the moisture content and fresh weight during banana ripening.

The initial changes in viscosity (Fig. 3B) and starch content (Fig. 3C) occurred between day 0.5 and 1, and decreasing patterns of these changes were associated. On the other hand, elasticity started to decrease immediately after ethylene treatment (Fig. 3A). Of the neutral sugar components of hemicellulose B, arabinose, mannose, and galactose contents started to decrease immediately after ethylene treatment (Fig. 4). Arabinose and galactose may consist of arabinogalactan polymers that either are attached or not attached to acidic polysaccharides such as rhamnogalacturonans. Mannose is probably a component of galactomannan. The decrease in the mannose content during the ripening process of banana suggests the breakdown of mannose-containing polymers such as galactomannan. Such a breakdown of galactomannan was reported in

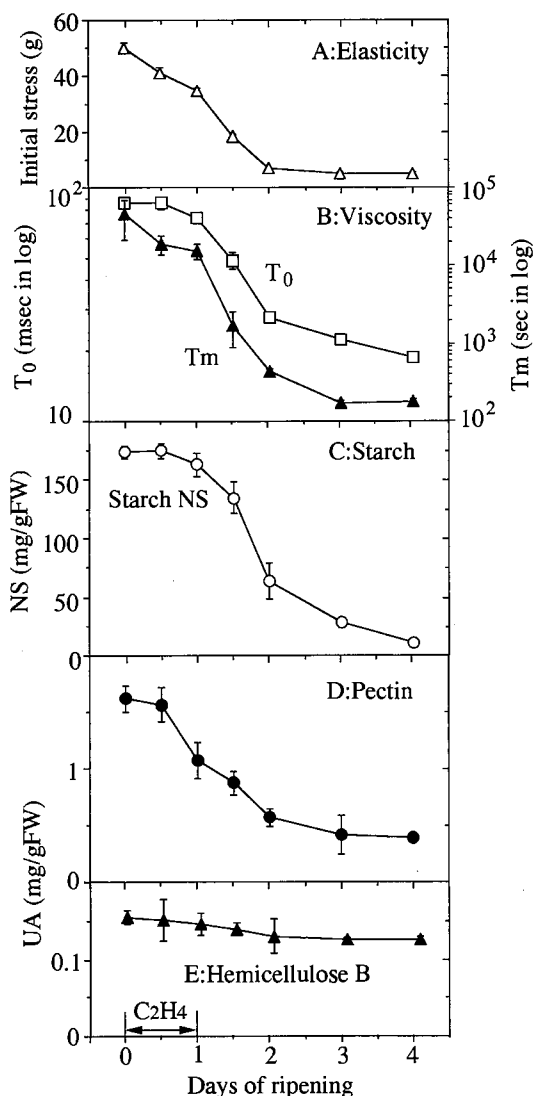


Fig. 3. Changes in elasticity (A), viscosity (B), starch content (C), uronic acid content in pectin (D), uronic acid content in hemicellulose B (E) of banana pulp during ripening at 25°C

Ethylene treatment was applied between day 0 and 1. The pulp was sliced in a radial direction (7 mm thickness). Initial stress was recorded when the probe was inserted into the pulp. Neutral sugar in the supernatant treated with  $\alpha$ -,  $\beta$ - and iso-amylase was designated as starch. Pectin was extracted with hot EDTA (pH 6.8) and hemicellulose B with 17.5% NaOH.

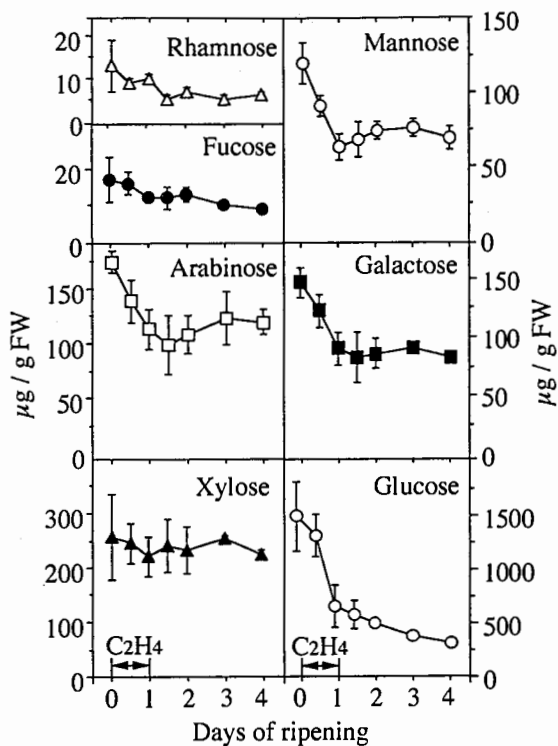


Fig. 4. Changes in neutral sugar composition of hemicellulose B from banana pulp during ripening. Neutral sugar composition of hydrolysate was determined by gas-chromatography. Means and SE are shown (n = 5).

endosperm cell walls during germination of *Datura* seeds<sup>27</sup>). These results indicate that the decrease in the amount of pectin and hemicellulose constituents may precede the decrease in the starch content and they further imply that softening is more likely to be initiated by changes in the pectin and hemicellulose structure in the cell walls than by those in starch.

**Correlation between softness and constituents in cooking banana<sup>20</sup>**

Which constituents contribute to the firmness of the banana fruit? It was suggested that the firmness of banana was related to the presence of starch<sup>11</sup>) and polyuronides<sup>30</sup>). Based on the results of analysis of dessert bananas, the chemical components responsible for the firmness had not yet been identified because there was no appreciable difference in the decreasing pattern among firmness parameters, cell wall constituents and starch<sup>19</sup>). As a related variety whose starch content does not decrease sufficiently could be used, it may become possible to identify the constituents responsible for the firmness.

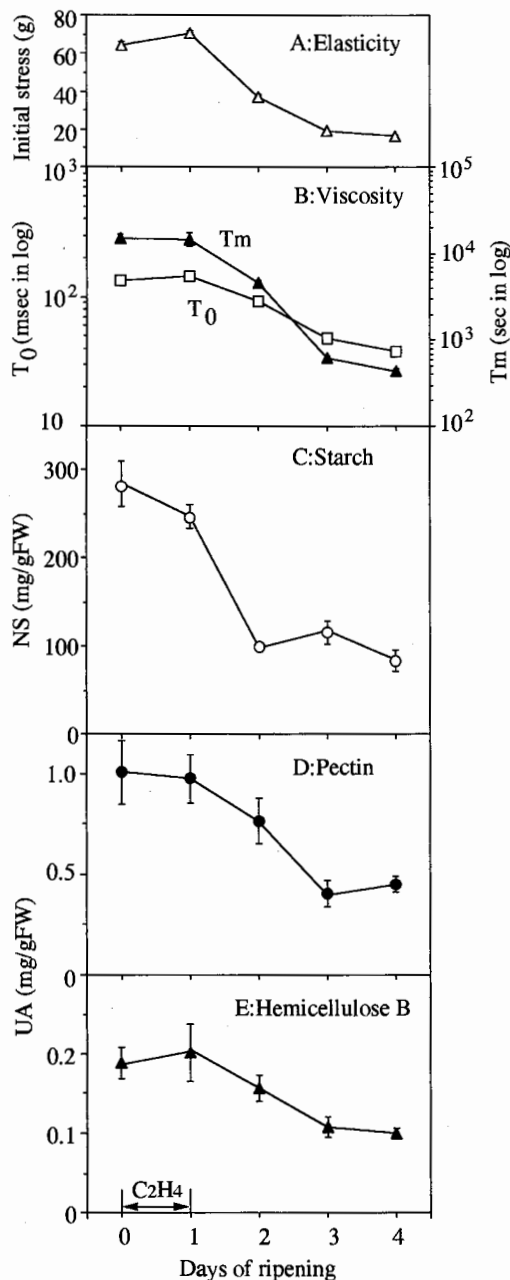


Fig. 5. Changes in elasticity (A), viscosity (B), starch content (C), uronic acid content in pectin (D), uronic acid content in hemicellulose B (E) of plantain pulp during ripening at 25°C

Plantains (cooking banana), which are relatives of dessert bananas, contain a larger amount of starch than dessert bananas when ripe, and are assumed to display a slower rate of starch hydrolysis<sup>28</sup>). Plantains were ripened in a biotron at 25°C with ethylene treatment during 4 days<sup>20</sup>). The elasticity and viscosity of the plantain pulp started to decrease after day 2 (Fig. 5A, B). The decreasing pattern

of the pectin and hemicellulose B contents (Fig. 5D, E) was comparable to that of the elasticity and viscosity, but the starch content decreased rapidly on day 2 (Fig. 5C). Thus, in the plantain pulp, it was suggested that the cell wall polysaccharides, pectin and hemicelluloses rather than starch are the chemical components responsible for the firmness, because the decreasing pattern of the firmness parameters was similar to that of the pectin and hemicellulose B contents rather than to that of the starch content.

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