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Ph. D. Thesis
**Relationship between physical parameters and
chemical composition of banana fruits during
ripening**

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Abbreviations

HB, hemicellulose B

NS, neutral sugar

PG, polygalacturonase

R, relaxation rate

T_0 , minimum stress-relaxation time

T_m , maximum stress-relaxation time

TS, total sugar

UA, uronic acid

Chapter I

General Introduction

(A) Physiology of fruit during ripening

(1) Introduction

The ripening process has been defined as changes that occur from the latter stage of growth and development through the early stage of senescence and resulting in characteristic aesthetic and/or food quality (Watada et al. 1984).

The first dominant theory of the mechanisms that regulate ripening appeared in the 1920s (Blackman and Parija 1928). This theory emphasized "organizational resistance" and suggested that the ripening events were the consequence of a breakdown in the resistances that kept cellular compartments contained. The mitochondrial fraction remains intact during ripening, suggesting that ripening is genetically controlled and not the result of the loss of cellular control (Haard and Hultin 1970). As evidence that protein and RNA synthesis played vital roles in the induction of ripening, a new modern theory of ripening as a process of tissue differentiation emerged (Brady 1987). Coombe (1976) treated briefly the ripening of fruit development and Sacher (1973) also reviewed it. Recently, reviews dealing generally with ripening or with the biochemistry and/or molecular biology of particular fruits have appeared (Marriott 1980, Brady 1987). The general changes associated with fruit ripening include softening, hydrolytic conversions of storage materials, and changes in the pigments and flavors in the fruit.

(2) Fruit softening

Softening is one of the most dramatic changes and an integral part of

the process of ripening of almost all fleshy fruits (Brady, 1987). It has immense commercial importance 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 wall degradation was suggested to be a major part of fruit softening (Bartley and Knee 1982, Huber 1983a). 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 evidence shows that softening of fruit is accompanied by an increase in the concentration of soluble pectic polysaccharides. However, considerably less is known regarding the role in fruit softening of cell wall polymers other than the polyuronides. Hemicelluloses are degraded during ripening in tomato (Huber 1983b), in strawberry (Huber 1984) and in muskmelon (McCollum et al. 1989). Cellulose metabolism has also been implicated as an important feature of fruit softening in peach (Hinton and Pressey 1974), avocado (Pesis et al. 1978) and apple (Abeles and Biles 1991).

(3) Banana study

Normal 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 (Charles and Tung 1973). Because banana is a more sensitive species, ripening is immediately induced, but the more immature the fruit, the higher the concentration of ethylene that is required (Brady et al. 1970b). In commercial practice, ethylene is used to "trigger" ripening while the rate of ripening is controlled largely by temperature regulation (Charles and Tung 1973). Ripening of green harvested banana in the commercial

practice is generally by exposing them to ca. 1000 ppm of ethylene with ripening schedules that enable ripening periods to be varied from 4 to 8 days at a temperature ranging from 15 to 21 °C at high humidity (Standard Fruit and Steamship Co. 1964, United Fruit Sales Corp 1964, Henze et al. 1983).

Biochemical and compositional changes associated with ripening have been reviewed in banana fruit (von Loesecke 1950, Palmer 1971, Marriott 1980, Stover and Simmonds 1987). Many investigators reported that starch content of pulp in banana fruit before ripening was higher than in other fruits, decreased dramatically during few days of ripening and then disappeared almost completely (Barnell 1943, Finney et al. 1967, Charles and Tung 1973, Kawabata and Sawayama 1974, Nakamura et al. 1979, Agravante et al. 1990, Nussinovitch et al. 1990). However, only a few have measured the degradation of cell wall components, such as pectin (Garces Medina 1968, Kawabata and Sawayama 1974, Marriott 1980), hemicellulose (Desai and Deshpande 1978, Barnell 1943) and cellulose (Desai and Deshpande 1978) during the ripening processes. Ripe banana pulp contained from 0.5 to 0.7% pectin (Garces Medina 1968). The water-soluble pectin fraction increased during ripening, while both the total and water-insoluble pectin decreased (Kawabata and Sawayama 1974). A very high correlation has been shown between alcohol-insoluble solid contents and taste panel texture scores in the ripening banana (Choo and Choon 1972). Although protein synthesis occurred during ripening of banana pulp, the RNA content and composition did not change (Brady et al. 1970). Six forms of banana pectinesterases could be detected by starch gel electrophoresis (Markovic et al. 1975). The activity of the pectinesterase of banana pulp remains constant during ripening (Brady 1976).

Ripeness and quality in banana are evaluated by subjective visual

examination based almost entirely upon the color of the peel (Finney et al. 1967). This single subjective criterion is assumed to indicate both flavor and texture, but texture is related to the mechanical sense of feel (Kramer et al. 1962). Texture may be related only casually to the sense of sight or the appearance of bananas (Finney et al. 1967).

The texture e.g. a softening of banana fruits must be attributed to the changes in physical or mechanical properties of the tissue, which are predominantly based on the changes in the chemical structure of starch grains (Finney et al. 1967) and/or cell walls of banana pulp. However, there are few direct measurements for assessing tissue texture changes in specific regions of the fruit. Hall (1987) pointed out that tissue softening of specific regions was a more precise means to assess structural changes than an analysis of intact fruit.

(B) Technique of measuring softness

(1) History of the technique of measuring softness

Softening has been usually measured by the force needed to press a plunger a given distance into the fruit. Firmness of cherries was assessed in terms of the deflection or deformation under a constant force (Parker et al. 1966). The smaller the deformation under the given load, the firmer the cherry. The Magness-Taylor (1925) pressure tester, which expresses firmness as the maximum force required to penetrate the fruit to a definite depth with a cylindrical metal rod, has been used to evaluate fruit firmness (Haller et al. 1941, Mohsenin et al. 1965). The mechanical thumb, which is similar to the Magness-Taylor pressure tester, could measure firmness without penetrating or cutting the fruit (Schomer et al. 1963). Ang et al. (1960) 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. Techniques have been reported for measuring firmness which is only a component of texture using a flat probe and a dissected tissue in apple (Bourne 1965), and in cherry fruit (Parker et al. 1966). Finney et al (1967) suggested the use of "Young's modulus of elasticity" as an objective definition of firmness. Texture was recognized as a major quality attribute in all fruits (Bourne 1967), but rheological properties of the fruit have been paid little attention.

(2) Technique of measuring softness in banana

In banana fruit, Charles and Tung (1973) have studied the maximum force at failure, the deformation under constant force and the linear limit of the force/deformation curve for pulps. Using a sonic technique, Finney et al. (1967) 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 reducing sugar and starch during ripening. Nissinovitch (1990) evaluated criteria suitable for following the progress of ripening banana and three criteria gave high correlation coefficients with parameters such as the Brix, starch content, age and color index.

One problem in measuring firmness of agricultural commodities is the lack of a suitable definition (Finney et al. 1967). Firmness, although important in marketing and in quality evaluation, has not been defined objectively, nor is a uniform technique accepted for its measurement.

(3) The physical technology of measuring softness (Stress-relaxation analysis)

Softening of fruit tissues must be attributed to the changes in physical or mechanical properties of the tissue, which are predominantly based on

the changes in chemical structure of the cell walls. Physical parameters thus obtained would predict not only the mechanical properties but would also allow the analysis of the expression of specific structural changes. The physical analysis must be based on a physical model. Several techniques for the physical measurement of tissue or cell wall have been developed (Taiz 1984). There is no agreed way to measure the elusive physiological cell wall parameters *in vitro*. Mechanical tests on isolated walls have been performed using either uniaxial or multiaxial stress. Uniaxial mechanical testing has been of three types: Instron analysis (constant strain rate), stress relaxation (constant strain), and creep (constant load). Of these, creep most closely resembles *in vivo* extension. 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 (Cleland and Haughton 1983). Stress-relaxation analysis developed by Yamamoto et al (1970), was based on the Maxwell viscoelastic model and included three parameters, T_0 , R and T_m . The T_0 is the time at which the stress starts with major decay, and corresponds to a Maxwell component with the lowest viscosity (Sakurai 1991). The R is the relaxation rate (%), and the reciprocal of R ($1/R$) corresponds to the number of relaxation components per unit volume. The T_m is the time at which the stress finishes with major decay, and corresponds to a Maxwell component with the highest viscosity. It has been applied to plant tissues to determine the mechanical properties of the stem cell walls during auxin-induced elongation. This analysis revealed an association between physical changes and the degradation of cell wall polysaccharides, such as xyloglucans in azuki bean epicotyls (Nishitani Masuda 1981), glucans in oat (Sakurai, Nevins and Masuda 1977, Sakurai, Nishitani and Masuda 1979), and barley coleoptiles (Sakurai and Kuraishi

1984, Sakurai and Masuda 1978a, Sakurai and Masuda 1978b). The analysis allows the prediction that the degradation of wall polysaccharides directly or indirectly leads to the decrease in wall viscosity, followed by cell extension. The original technique for stress-relaxation analysis was to extend plant specimens, and measure the relaxation of the initial applied stress. This technique, however, was applied to sliced tuber tissues of Jerusalem artichoke (Yamamoto et al. 1981). A tuber slice was subjected to compression by an Instron tensile tester. The parameters proved to be analogous to those obtained by the stretching method.

In order to examine the mechanism of softening with (1) a novel technique for measuring the softness based on a physical model and (2) simultaneous measurement of softness parameters and chemical components responsible for the softness, the following experiments were performed.

Firstly, a physically defined method (stress-relaxation analysis) was applied to analyze tissue softening in tomato fruit with a conical probe. The softness within a tomato fruit was measured with a newly controlled pressure device with a load cell.

Secondly, when the technique was applied to banana fruit, the plunging depth varied substantially between ripe and unripe fruits to attain the same initial stress. To get more precise data in stress-relaxation curves, an instrument which could control the plunging depth of the conical probe into the tissue was used. The optimum measuring conditions for the stress-relaxation analysis were determined, and the changes in the physical properties during softening of banana fruit pulp were analyzed.

Thirdly, for the index of ripening, the rate of ethylene production of fruit and color of peel were analyzed. The stress-relaxation parameters and the chemical components responsible for the firmness were determined to

investigate the chemical background of ripening of banana fruit. For the elucidation of the degradation of polysaccharides of pectin and HB fractions during ripening, the molecular weight distribution was analyzed by gel permeation chromatography

Finally, for technical discussion, the merit of this novel technique for measuring softness and the optimum conditions for measurement of softness of banana fruit pulp are discussed. As part of the physiological discussion, the physical properties of the banana pulp and their spatial distribution within the fruit, the relationship between softness and cell components and the mechanism of softening of the banana fruit are discussed.

Chapter II

Novel technique for measuring tissue firmness within tomato (*Lycopersicon esculentum* Mill.) fruit

Abstract

Developmental changes of tomato fruit tissues during maturation were analyzed by a physically defined method (stress-relaxation analysis). The tip of a conical probe connected to a load sensor was positioned on the cut surface of a sliced tomato fruit, and the decay of the imposed stress was monitored. Stress relaxation data thus obtained were used for the calculation of three stress-relaxation parameters. Different zones within tomato fruit harvested at six different ripening stages were analyzed. One of the stress-relaxation parameters, minimum stress-relaxation time (T_0), decreased as the fruits matured. The decrease in T_0 was first found in the core of the carpel junction within the endopericarp at the blossom end during the breaker stage. The decrease in T_0 progressed from the blossom end, through the equatorial region and finally throughout the shoulder, as the fruit matured. In mature green fruit, T_0 values within the placenta and the proximal carpel junction were lower than those by other parts of the fruit. In all measurements the maximum stress-relaxation time was not substantially changed during maturation nor were their changes observed in different regions of the fruit. The observed relaxation rate was therefore corrected with softening. The results indicate that fruit softening may be physically associated with the stress-relaxation parameter, T_0 , and the extent of softening is a function of position within the fruit. Decreases in T_0 value appear correlated with the reported regional variation in the appearance of polygalacturonase.

Introduction

Softening and ripening of fruits have been extensively studied with relation to hormonal control, pigment formation, metabolism of storage materials, and cell wall degrading enzymes. These biochemical and physiological events are of current interest because they are amenable to modification by molecular biological techniques. In tomato fruits, polygalacturonase (PG) is the best characterized enzyme associated with ripening events. Tucker and Grierson (1982) reported that PG activity is absent in green tomato fruits, but is expressed during ripening. PG mRNA increased by 2400-fold during ripening, indicating that PG is regulated by the PG mRNA levels (Bennet and DellaPenna 1987). Recently, using a technique of tissue blotting and immunocytolocalization of PG protein, Tieman and Handa (1989) demonstrated that PG appears first in the columella region followed by sequential appearance in the exopericarp and endopericarp, respectively. These results suggest a regional degradation of pectic substances in the fruits by PG. However, there are few direct measurements for assessing tissue texture changes in specific regions of the fruit. Hall (1987) pointed out that tissue softening of specific regions was a more precise means to assess structural changes than an analysis of intact fruit.

Softening of fruit tissues must be attributed to the changes in physical or mechanical properties of the tissue, which is predominantly based on the changes in chemical structure of the cell walls. Several techniques for the physical measurement of tissue or cell wall have been developed (Taiz 1984). Physical parameters thus obtained would predict not only the mechanical properties but would also allow the analysis of the expression of specific structural changes. The physical analysis must be based on a physical model. Stress-relaxation analysis developed by Yamamoto et al (1970), was based on the Maxwell viscoelastic model. It has been applied to plant tissues to determine the mechanical properties of the stem cell walls during auxin-induced elongation. This analysis revealed

an association between physical changes and the degradation of cell wall polysaccharides, such as xyloglucans in azuki bean epicotyls (Nishitani Masuda 1981) and glucans in oat, maize and barley coleoptiles (Sakurai and Kuraishi 1984, Sakurai and Masuda 1978a, Sakurai and Masuda 1978b, Sakurai, Nevins and Masuda 1977, Sakurai, Nishitani and Masuda 1979). The analysis allows the prediction that the degradation of wall polysaccharides directly or indirectly leads to the decrease in wall viscosity, followed by cell extension. The original technique for stress-relaxation analysis was to extend plant specimens, and measure the relaxation of the initial applied stress. This technique, however, was applied to sliced tuber tissues of Jerusalem artichoke (Yamamoto, Fujihara and Masuda 1981). A tuber slice was subjected to compression by an Instron tensile tester. The parameters proved to be analogous to those obtained by the stretching method.

If tomato softening is regulated by PG, the cell wall polysaccharides, especially pectin, must be degraded by PG, a process which should be detected by the stress-relaxation analysis. In the present study, we used the compression method with a conical probe to analyze the mechanical properties of the tomato tissues during the course of fruit softening.

Materials and Methods

Plant materials

Tomato (*Lycopersicon esculentum* Mill. cv. Zuishu) was grown in a green house of the Hiroshima Prefectural Agricultural Experimental Station (Hara, Higashi-Hiroshima, 739-01, Japan). Fruits were harvested at six selected developmental stages, as described by Ryall and Lipton (1972). The mature green stage was further divided into mature green stage 1 and 2. Full sized green fruit with a pinkish locule was designated as mature green stage 2. The other stages are breaker, turning, pink and red.

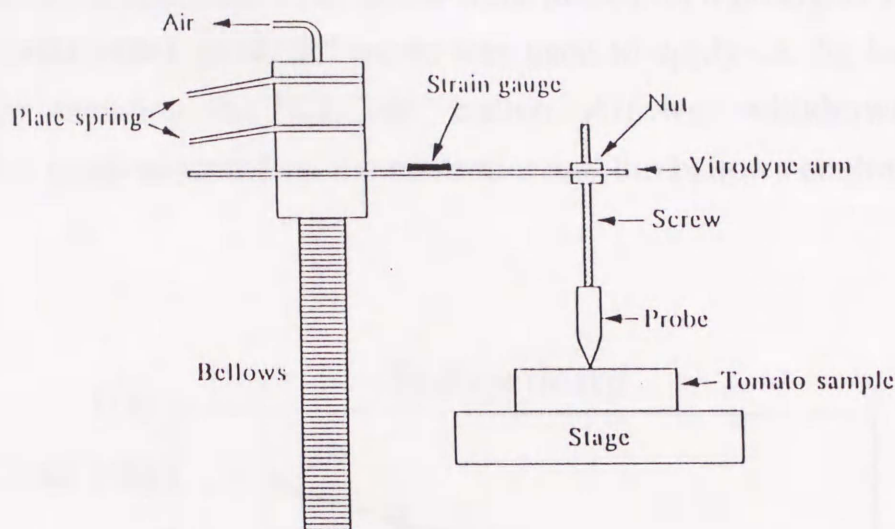


Figure 1. Schematic diagram of the probe adopted for the Vitrodyne instrument used for the measurement of tomato softening. The device was used in a vertical position. A tomato tissue slice (7–8 mm thickness) was placed on a Plexiglas stage. The probe (2.1 mm in diameter) cut to a 7-mm length was attached to the screw with adhesive. The probe cone was cut at an angle of 30°. The arm was lowered to the tissue surface by withdrawal of air through a tube connected to the bellows.

Measurements of fruit softening

Tomato fruit slices 7–8 mm thick taken along the equatorial or longitudinal plane were used. A diagram of the sensor component for the measurement of stress-relaxation of the tissues is shown in Figure 1. The forcing frame of Vitrodyne (Liveco, Inc., Burlington, VT 05401) was set in a vertical position, and one of two arms was used for the measurements. Calibration for the load was executed without tissue mounted on the arm in accordance with the instrument instructions.

A conical probe (2.1 mm in diameter) was cut 7 mm long. The point was polished by emery paper to an edge angle of 30°. The polished probe was secured by adhesive to a screw through the arm. The weight of the

mounted probe was 2.86g according to the controller's display. An additional weight of exactly 2.500g was applied to the top of the screw to ascertain the actual load. Fruit slices were placed on a plexiglas stage. The "HOLD PRESENT FORCE" mode was used to apply ca. 5g load to the tissue by pressing the "CLOSE" button. Air was withdrawn with a peristaltic pump mounted on the controller and the bellows contracted to

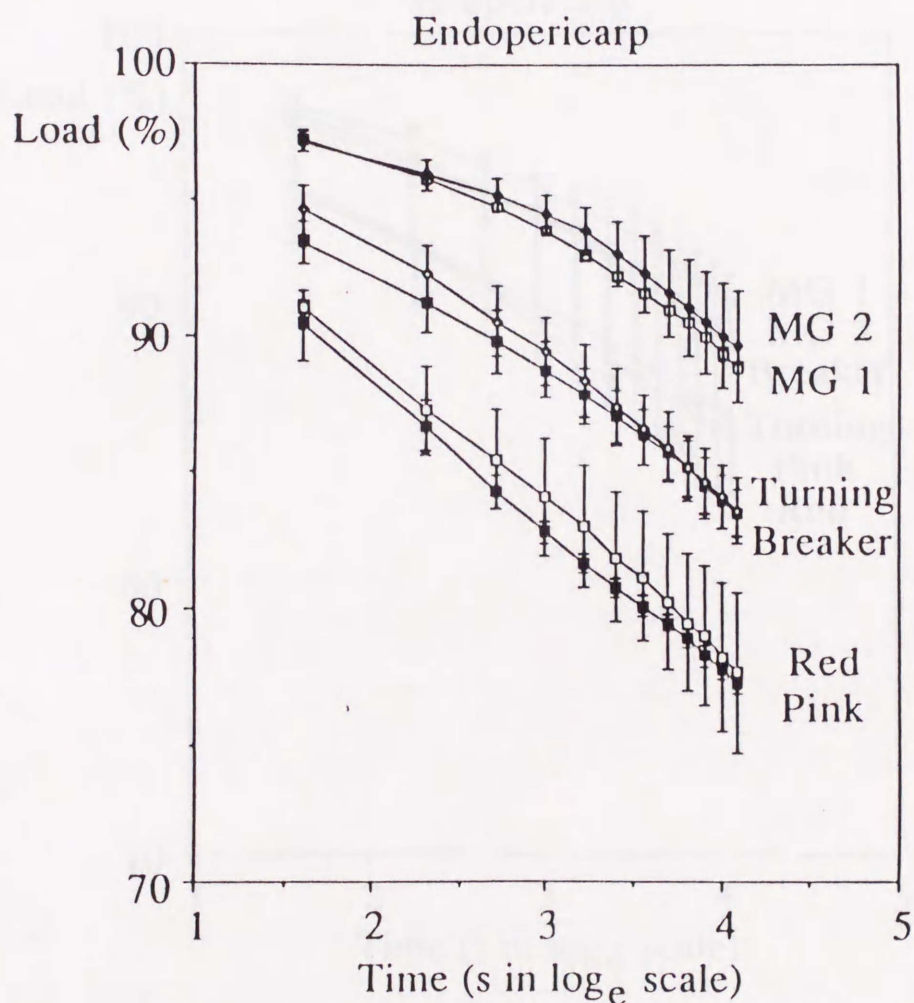


Figure 2. Stress-relaxation curve for endopericarp tissue of tomato fruits at different ripening stages. Tomato fruits harvested at six different ripening stages were cut at an equatorial plane. Endopericarp tissue was examined for stress-relaxation. Stress-relaxation data were recorded at 5-s intervals up to 60 s. Means and SE are shown ($n = 9$). MG 1, mature green stage 1; MG 2, mature green stage 2.

lower the probe. As the edge of probe was introduced into tissue surface, the arm was deflected. The extent of deflection was sensed by a strain gauge. Strain signals were converted to load values according to the calibration executed prior to the measurements. When the compressed load reached 5g, the arm position was held stationary and the decay of load

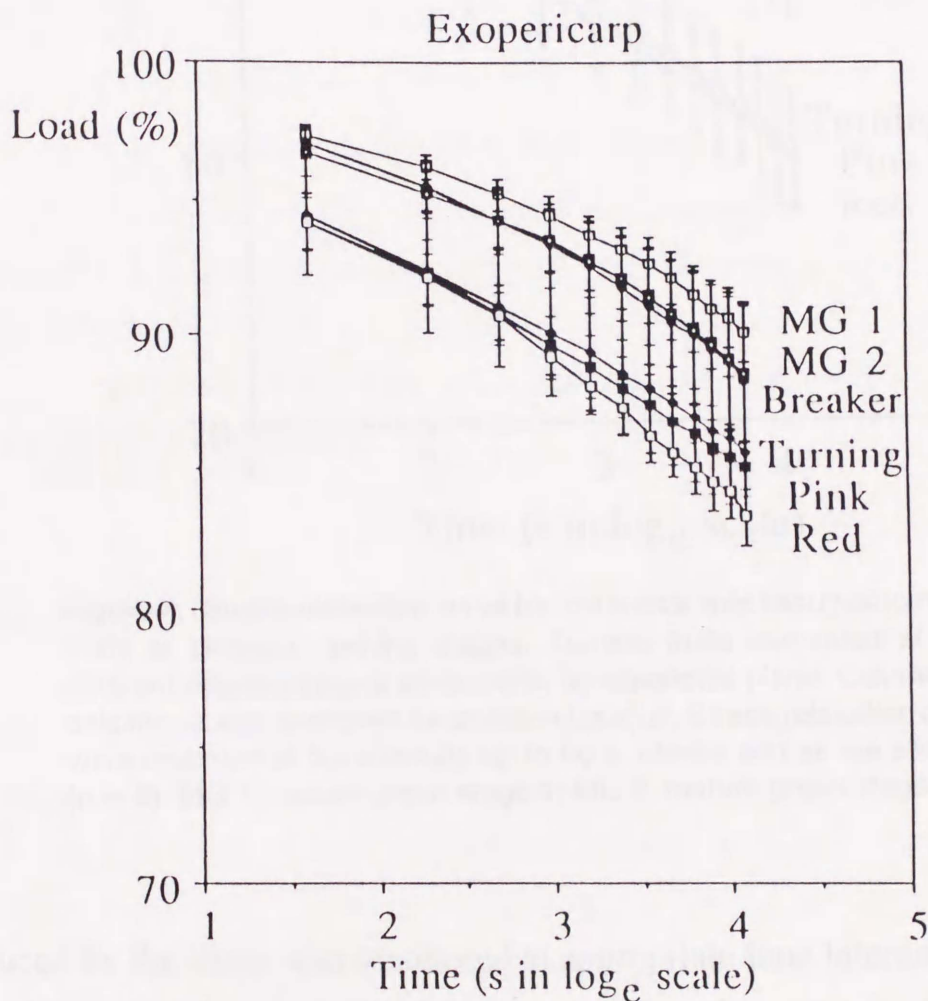


Figure 3. Stress-relaxation curve for exopericarp tissue of tomato fruits at different ripening stages. Tomato fruits harvested at six different ripening stages were cut at an equatorial plane. Exopericarp tissue was examined for stress-relaxation. Stress-relaxation data were recorded at 5-s intervals up to 60 s. Means and SE are shown ($n = 9$). MG 1, mature green stage 1; MG 2, mature green stage 2.

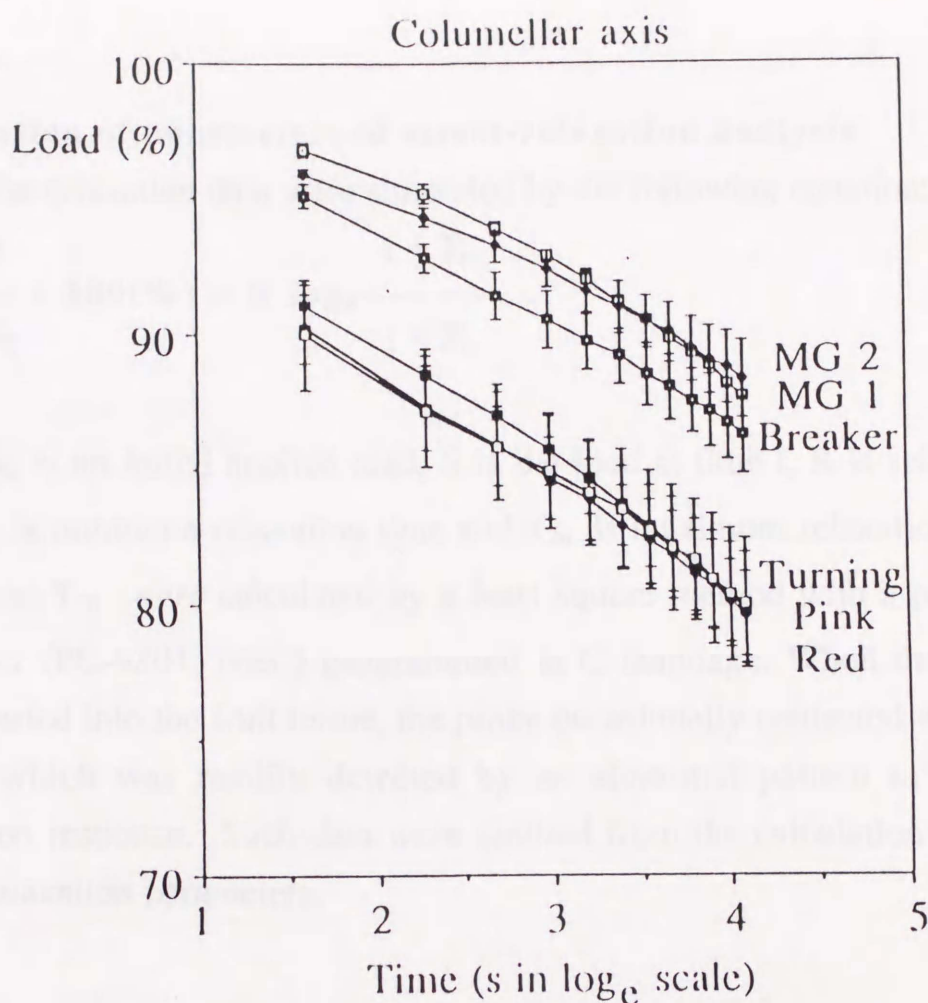


Figure 4. Stress-relaxation curve for columellar axis tissue of tomato fruits at different ripening stages. Tomato fruits harvested at six different ripening stages were cut at an equatorial plane. Columellar axis tissue was examined for stress-relaxation. Stress-relaxation data were recorded at 5-s intervals up to 60 s. Means and SE are shown ($n = 9$). MG 1, mature green stage 1; MG 2, mature green stage 2.

produced by the tissue was monitored at appropriate time intervals (usually 5 s). Normally an applied load 5 g was employed, except for the fruit at red stage. When a 5g load was applied to fruit at red stage, the probe sometimes penetrated excessively into the tissue slice. Friction between probe shaft and the tissue impeded smooth relaxation. Therefore, a lesser load was applied to ripe tissue to avoid penetration; i.e., only the tip of the probe was introduced into the tissues. The force applied to red fruit was

ca. 2 g.

Calculation of parameters of stress-relaxation analysis

The relaxation data were simulated by the following equation;

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

where S_0 is an initial applied load, S is the load at time t , R is relaxation rate, T_0 is minimum relaxation time and T_m is maximum relaxation time. R , T_0 and T_m were calculated by a least square method with a personal computer (PC-9801, NEC) programmed in C language. When the probe was inserted into the fruit tissue, the probe occasionally contacted vascular tissue, which was readily detected by an abnormal pattern in stress-relaxation response. Such data were omitted from the calculation for the stress-relaxation parameters.

Results

Tomato fruits harvested at different developmental stages were cut through the equatorial plane. Stress-relaxation was measured at 5 s intervals up to 60 s for endopericarp, exopericarp and the columellar axis. During longer measurements a small air leakage was observed ca. 5 to 10 min after stopping the peristaltic pump of the measuring apparatus. This leakage substantially disturbed a stress-relaxation curve. However the effects were negligible up to 60 s. The relaxation data were plotted as a function of time on natural logarithmic scale (Fig. 2-4). In all comparable tissues, relaxation was accelerated as the fruits matured. Table I summarizes three stress-relaxation parameters calculated by a least square method based on the equation in Materials and Methods. The tissue softening process was expressed most suitably by T_0 . T_m values were not

Table 1. Three Stress-Relaxation Parameters for Exopericarp, Endopericarp, and Columellar Axis Tissues of Tomato Fruits Harvested at Different Stages

Tomato fruits were harvested at six different maturation stages. The fruit was cut through the equatorial plane. The probe attached to a Vitrodyne arm was introduced into the sliced tissue (7–8 mm thickness). Stress-relaxation data were obtained at 5-s intervals up to 60 s. The data were used for the calculation of three stress-relaxation parameters, T_0 , R , and T_m . Correlation coefficients between measured values and calculated values based on the equation using obtained T_0 , R , and T_m (fitness of the equation) were >0.998 . Means and SE are shown ($n = 9$).

Tissue and Stage	T_0 s	R %	T_m $\times 10^6$ s
Exopericarp			
MG 1 ^a	31.6 ± 6.4	8.30 ± 0.13	4.6 ± 0.2
MG 2	35.7 ± 9.9	8.35 ± 0.16	4.7 ± 0.3
Breaker	31.8 ± 10.2	8.26 ± 0.15	4.6 ± 0.6
Turning	13.6 ± 6.1	7.24 ± 0.63	16.9 ± 11.7
Pink	10.3 ± 2.5 ^b	7.28 ± 0.27 ^b	8.0 ± 1.9
Red	7.9 ± 2.3 ^b	7.10 ± 0.42	11.0 ± 5.1
Endopericarp			
MG 1	22.5 ± 1.1	8.12 ± 0.02	4.6 ± 0.1
MG 2	35.1 ± 6.8	8.33 ± 0.11	4.9 ± 0.1
Breaker	7.9 ± 2.0 ^b	7.13 ± 0.34 ^b	9.3 ± 3.6
Turning	8.3 ± 2.2 ^b	7.39 ± 0.30	5.8 ± 1.8
Pink	1.6 ± 0.4 ^b	6.27 ± 0.42 ^b	29.9 ± 23.5
Red	3.3 ± 1.3 ^b	6.97 ± 0.23 ^b	6.7 ± 3.5
Columellar axis			
MG 1	17.7 ± 0.3	7.99 ± 0.05	4.4 ± 0.3
MG 2	20.5 ± 5.3	7.87 ± 0.11	5.2 ± 0.1 ^b
Breaker	14.8 ± 4.3	7.56 ± 0.19	6.1 ± 0.4 ^b
Turning	2.6 ± 1.1 ^b	6.02 ± 0.34 ^b	39.6 ± 14.7
Pink	4.0 ± 1.4 ^b	6.72 ± 0.33 ^b	11.6 ± 4.5
Red	3.4 ± 1.5 ^b	6.08 ± 0.42 ^b	45.7 ± 27.7

^aMG 1, mature green stage 1; MG 2, mature green stage 2.
^bSignificant difference from the data of MG 1 at 5% level.

substantially changed during maturation. Since we did not measure the relaxation data long enough to calculate the actual T_m value, the calculated T_m value may not directly show any characteristic of polymer changes in

Table II. T_0 Values of Different Regions of Tomato Fruits at Different Mature Stages

Tomato fruits were harvested at six different maturation stages. The fruit was cut in a vertical plane. Nine regions were examined for the stress-relaxation. Two regions were along the central axis (base and middle), three in endopericarp and exopericarp (blossom end, equatorial and shoulder), and one at the placenta. Mean and SE are shown ($n = 9$).

Tissue	Region	MG 1 ^a	MG 2	Breaker	Turning	Pink	Red
Axis	Base	10.3 ± 1.2	12.9 ± 1.7	12.8 ± 1.2	18.0 ± 2.3 ^b	12.1 ± 1.2	12.3 ± 1.1
	Middle	60.9 ± 6.2	66.8 ± 18.1	19.3 ± 5.6 ^b	3.4 ± 0.6 ^b	23.3 ± 4.1 ^b	5.9 ± 1.2 ^b
Endopericarp	Blossom end	52.3 ± 5.9	44.3 ± 5.41	11.9 ± 2.3 ^b	7.3 ± 2.2 ^b	3.2 ± 0.8 ^b	5.2 ± 1.1 ^b
	Equatorial	38.9 ± 6.1	61.2 ± 6.5 ^b	30.1 ± 8.4	6.4 ± 1.7 ^b	9.2 ± 2.2 ^b	4.8 ± 1.4 ^b
Exopericarp	Shoulder	45.0 ± 5.7	71.6 ± 10.0 ^b	37.0 ± 9.9	51.3 ± 8.6	10.2 ± 1.1 ^b	7.0 ± 2.5 ^b
	Blossom end	43.9 ± 4.7	44.8 ± 5.1	24.6 ± 4.8 ^b	16.4 ± 6.2 ^b	8.6 ± 2.6 ^b	7.0 ± 1.7 ^b
Placenta	Equatorial	37.4 ± 6.4	42.6 ± 7.3	25.8 ± 5.8	29.0 ± 5.9	7.3 ± 2.4 ^b	7.7 ± 1.9 ^b
	Shoulder	50.6 ± 4.9	74.3 ± 14.6	67.8 ± 8.8	29.1 ± 6.9 ^b	7.8 ± 1.7 ^b	6.7 ± 2.2 ^b
		17.4 ± 2.5	28.6 ± 12.8	8.5 ± 0.9 ^b	16.4 ± 3.3	6.4 ± 1.9 ^b	3.5 ± 0.6 ^b

^a MG 1, mature green stage 1; MG 2, mature green stage 2. ^b Significant difference from the data of MG 1 at 5% level.

Table III. R Values of Different Regions of Tomato Fruits at Different Mature Stages

Tissue	Region	MG 1 ^a	MG 2	Breaker	Turning	Pink	Red
Axis	Base	7.88 ± 0.08	7.75 ± 0.11	7.97 ± 0.06	8.06 ± 0.10	8.02 ± 0.05	7.99 ± 0.08
	Middle	8.63 ± 0.06	8.45 ± 0.23	7.94 ± 0.19 ^b	7.58 ± 0.28 ^b	7.84 ± 0.16 ^b	7.95 ± 0.60
Endopericarp	Blossom end	8.55 ± 0.07	8.44 ± 0.08	7.71 ± 0.15 ^b	7.12 ± 0.20 ^b	7.12 ± 0.28 ^b	7.20 ± 0.23 ^b
	Equatorial	8.34 ± 0.10	8.59 ± 0.10	8.10 ± 0.21	6.99 ± 0.24 ^b	7.49 ± 0.11 ^b	7.70 ± 0.36
Exopericarp	Shoulder	8.44 ± 0.07	8.70 ± 0.08	8.35 ± 0.11	8.53 ± 0.08	8.10 ± 0.24	7.48 ± 0.47
	Blossom end	8.43 ± 0.07	8.42 ± 0.07	8.13 ± 0.12 ^b	7.30 ± 0.33 ^b	7.24 ± 0.24 ^b	7.11 ± 0.33 ^b
Placenta	Equatorial	8.33 ± 0.10	8.32 ± 0.14	8.10 ± 0.14	8.18 ± 0.17	6.84 ± 0.23 ^b	7.34 ± 0.19 ^b
	Shoulder	8.51 ± 0.09	8.49 ± 0.26	8.70 ± 0.06	8.28 ± 0.08	7.80 ± 0.15 ^b	7.50 ± 0.26 ^b
		7.96 ± 0.07	8.04 ± 0.39	7.48 ± 0.08 ^b	7.90 ± 0.17	7.58 ± 0.19	7.71 ± 0.37

^a MG 1, mature green stage 1; MG 2, mature green stage 2. ^b Significant difference from the data of MG 1 at 5% level.

Table IV. T_m Values of Different Regions of Tomato Fruits at Different Mature Stages

Tissue	Region	MG 1 ^a	MG 2	Breaker	Turning	Pink	Red
Axis	Base	30.2 ± 1.9	44.8 ± 3.8 ^b	32.8 ± 2.4	39.2 ± 2.0 ^b	29.5 ± 2.2	31.9 ± 2.1
	Middle	57.8 ± 1.8	56.9 ± 4.6	32.4 ± 4.3 ^b	39.9 ± 16.2	59.2 ± 5.3	93.5 ± 46.7
Endopericarp	Blossom end	53.6 ± 2.2	50.8 ± 3.7	43.1 ± 4.7	64.2 ± 13.3	56.9 ± 20.5	75.4 ± 19.5
	Equatorial	51.2 ± 2.8	57.8 ± 1.9	47.7 ± 8.0	139.2 ± 62.5	45.5 ± 6.6	36.8 ± 10.8
	Shoulder	52.4 ± 1.5	57.8 ± 2.9	41.4 ± 5.6	52.1 ± 3.6	32.1 ± 8.1 ^b	79.9 ± 35.6
Exopericarp	Blossom end	52.6 ± 1.5	53.2 ± 1.3	41.8 ± 3.9 ^b	89.7 ± 19.9	58.9 ± 8.5	90.5 ± 33.3
	Equatorial	48.1 ± 2.3	54.8 ± 2.8	45.6 ± 3.5	42.7 ± 2.9	134.5 ± 29.9 ^b	64.6 ± 15.1
	Shoulder	54.5 ± 1.9	97.5 ± 37.7	57.6 ± 1.8	40.2 ± 3.9 ^b	28.4 ± 5.2 ^b	35.9 ± 8.5 ^b
Placenta	41.4 ± 2.9	44.0 ± 12.4	48.5 ± 4.6	41.7 ± 7.3	30.0 ± 5.1	58.7 ± 41.9	

^a MG 1, mature green stage 1; MG 2, mature green stage 2. ^b Significant difference from the data of MG 1 at 5% level.

the cell wall. The parameter R was an intermediate index. Endopericarp showed a significant decrease in the T_0 value after the breaker stage, while the exopericarp and columellar axis was delayed until after the turning stage.

Next, the fruits at different stages were cut in a vertical plane. Nine regions were examined for the stress-relaxation. Two regions were along the central axis, three in endopericarp and exopericarp, and one at the placenta. The three stress-relaxation parameters for each zone were summarized in Table II - IV. Most of the T_0 values of immature or mature green tomato were significantly higher than those of tomato at other mature stages. Even in the same immature green stage, the basal axis (near the calyx) and placenta showed lower T_0 values than other regions. T_0 of the basal axis was stable throughout maturation. The placenta showed lower T_0 values after pink stage. T_0 for the middle axis region decreased after the breaker stage. T_0 for the equatorial and shoulder regions of endopericarp decreased after turning and after the pink stage, respectively. In exopericarp tissues, the pattern of decrease in T_0 during maturation was similar to that in the endopericarp. Therefore, the decrease in T_0 was first found in middle axis and blossom end, then followed by equatorial and shoulder regions. This changing pattern was similar to the changes in red color within the fruit. Changes in R value were similar to those in T_0 , although the decrease in R was not always found in pink and red fruits. T_m values did not substantially differ as a function of stage or region.

Discussion

Tieman and Handa (1989) clearly showed that increases in PG levels in different sections of tomato fruit are not uniform during tomato fruit ripening using tissue blotting and immunocytolocalization. They found that PG protein appeared first in the columella and by the turning stage of

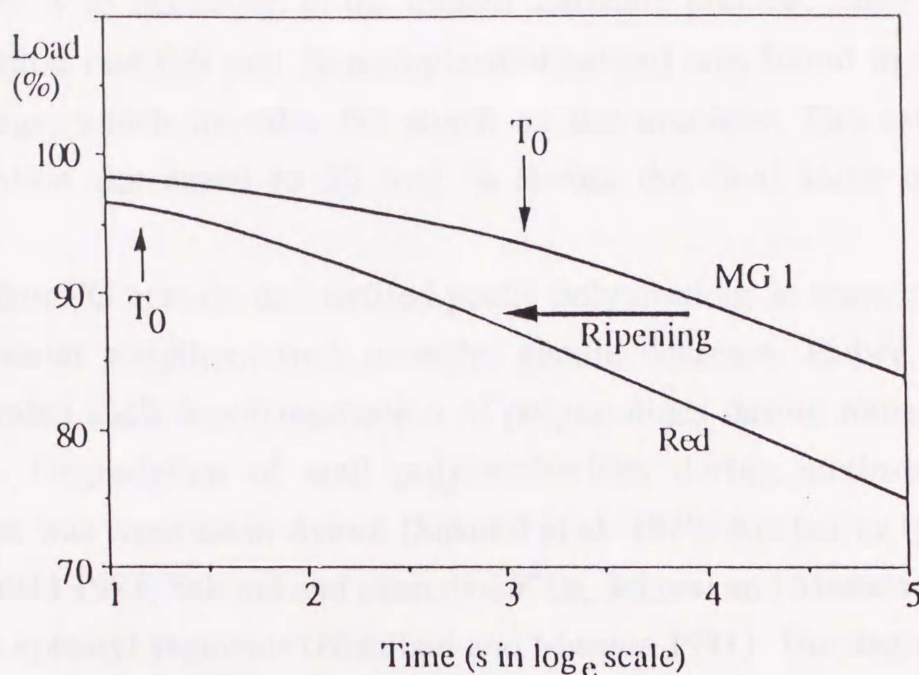


Figure 5. Schematic representation of changes in stress-relaxation curve during tomato fruit ripening. The stress-relaxation curves were drawn by the computation with three parameters calculated using the data from "MG 1" and "Red" in Figure 2. The endopericarp of mature green stage 1 (MG 1) showed greater T_0 than that of red stage fruit (Red). During ripening, the relaxation curve shifts from right to left.

ripening PG appeared in exocarp at the blossom end of tomato fruit. The present results revealed that the decrease in T_0 appeared first in middle columella and blossom end of pericarp tissues at breaker stage, then in the equatorial and shoulder regions of pericarp. PG protein levels did not appear in any region of the fruit at the breaker stage (Tieman and Handa 1989), while we note that the decrease in T_0 was already significant at the breaker stage. The discrepancy may be due to different cultivars. Alternatively we suggest that actual initiation of tissue softening might not be induced by the action of PG. Pectin degradation is dependent on several enzymes (Baldwin and Pressey 1989). Tomato fruit contains PG and pectinmethylesterase (Koch and Nevins 1989). Koch and Nevins (1990)

have claimed that pectinmethylesterase is a prerequisite to enhanced susceptibility to PG action in the tomato softening process, since a high esterification rate (90 mol % methylesterification) was found in mature green stage, which impedes PG attack on the uronides. The extent of esterification decreased to 30 mol % during the final stage of fruit ripening.

When PG acts on deesterified pectic polyuronides in tomato fruits, the molecular weight of such uronides should decrease. Huber (1983) demonstrated such depolymerization of polyuronides during tomato fruit ripening. Degradation of wall polysaccharides during auxin-induced elongation was reported in *Avena* (Sakurai et al. 1979) and barley (Sakurai and Kuraishi 1984, Sakurai and Masuda 1978a, Sakurai and Masuda 1978b) and bean epicotyl segments (Nishitani and Masuda 1981). The degradation was involved in wall-loosening, leading to cell extension growth and the wall loosening process was physically monitored by a stress-relaxation technique (Yamamoto et al. 1970, Yamamoto et al. 1981).

Essential changes in stress-relaxation curve during tomato fruit ripening is shown in Figure 5 using three parameters calculated from the data in Figure 2. Both in equatorial and vertical planes, T_0 was the most indicative parameter for fruit softening, followed by R. In studies of stem tissues a decrease in T_0 was involved in decrease in viscosity resulting from the depolymerization of wall polysaccharides (Sakurai et al. 1979). Therefore the decrease in T_0 found in specific regions of tomato fruits suggests a zonal pattern in the degradation of the cell wall polysaccharides, which probably results from the action of wall degrading enzymes including PG and PE or others as that have not yet been disclosed.

Conventional techniques for the measurement of tissue softening fail to resolve changes in softening within a specific tissue or organ. Ahrens and Huber (1990) improved the technique using excised tissue upon which a known weight was applied. Although the data revealed tissue softening in tomato fruit more effectively than those obtained by a conventional

techniques, the data does not predict physical changes in cell wall architecture. The Vitrodyne system with a flat probe may also reveal such values, but our preliminary attempts with such probe failed to establish a basis for determining stress-relaxation parameters. The cause may be related to the geometry and variations in the dimension of tissue segment taken from the fruits. Very small variation in dimensions of dissected tissues affected the calculated parameters. Therefore a probe was devised with known dimensions. The technique described allows measurements of the stress-relaxation at any specific region within the fruit.

Introduction

Softening of the fruit is an integral part of the process of ripening in almost all fleshy fruits (Grady 1957). It has immense commercial importance because the marketing life of the fruit is to a large extent limited by increasing softness. The chemical changes involved in softening and the enzymes constituting it have been studied (Haber 1963). In banana fruit, biochemical and compositional changes associated with ripening have been reviewed (Marriott 1962; Hoover and Simmons

Chapter III

Physical measurement of firmness of banana fruit pulp : Determination of optimum conditions for measurement.

Abstract

Stress-relaxation curves were obtained by plunging a conical probe into the pulp of green and yellow banana fruits [*Musa* (AAA group, Cavendish subgroup) 'Giant Cavendish']. Three stress-relaxation parameters, minimum stress-relaxation time (T_0), relaxation rate (R), and maximum stress-relaxation time (T_m), were calculated from the stress-relaxation curve. Plunging depth and plunging speed varied the parameters. When parameters were fixed, with a plunging speed of 0.5 mm/sec and the plunging depth of 0.6 mm, the yellow bananas showed significantly lower T_0 and T_m than green bananas. The lower T_0 and T_m can predict the degradation of polymers responsible for the pulp texture. Measurements of stress-relaxation parameters in different parts of banana pulp revealed that the physical properties were not uniform within the same fruit.

Introduction

Softening of the fruit is an integral part of the process of ripening of almost all fleshy fruits (Brady 1987). It has immense commercial importance because the postharvest life of the fruit is to a large extent limited by increasing softness. The chemical changes involved in softening and the enzymes contributing to the changes have been studied (Huber 1983). In banana fruit, biochemical and compositional changes associated with ripening have been reviewed (Marriott 1980, Stover and Simmonds

1987). The softening of banana fruits must be attributed to the changes in physical or mechanical properties of the tissue, which are predominantly based on the changes in the chemical structure of starch grains (Finney et al. 1967) and/or cell walls of banana pulp. However, there have been few direct physical analysis of the banana fruit (Finney et al. 1967, Charles and Tung 1973, Nussinovitch et al. 1990). If the physical analysis is based on a rheological model, parameters obtained afford not only the indices of tissue softening but also indices of the chemical and physical changes underlying the softening process. The stress-relaxation analysis developed by Yamamoto et al. (1981) to examine the mechanical properties of plant cell walls includes three parameters, T_0 , R and T_m . T_0 , the time at which the stress begins to decay, has been extensively studied in terms of the index of cell wall loosening (Masuda et al. 1974, Sakurai et al. 1977, Sakurai and Masuda 1977, 1978a, 1978b).

Kojima et al. (1991) applied the technique to analyze tissue softening in tomato fruit with a conical probe. They measured the softness of a small region within a tomato fruit to assess the structural changes in a specific region. An analysis of tissue slices using a conical probe provides more precise data than using a dissected tissue and a flat probe. Furthermore, a small variation in dimensions of excised tissues affected the calculation of the physical parameters. The pattern of spreading of the softening region appeared to be correlated with the presence of polygalacturonase localized by Tieman and Handa (1989). However, an instrument was not available to control the depth of plunge of the conical probe into the tissue. Since plunging depth varies substantially between ripe and unripe fruits, to get more precise data in stress-relaxation curves, it is necessary to have constant conditions for the probe.

The purpose of this study was to determine the optimum measuring conditions for stress-relaxation analysis and to investigate the distribution

of the physical properties of banana fruit pulp.

Materials and Methods

Plant materials

Bananas [*Musa* (AAA group, Cavendish subgroup) 'Giant Cavendish'] (Chiquita brand) of special grade from the Philippines were obtained from a local wholesaler (Nishiyama Seika Inc., Hiroshima) in Japan. The fruits at the green unripe stage were brought to the laboratory. Hands were selected for uniformity of size and freedom from blemishes. "Green bananas" (color index 2) were used immediately after arrival or stored in polyethylene bags (0.06 mm in thickness) at 15 °C until used (a maximum of 3 days). Peel color was evaluated by comparison with a color plate on a banana ripening chart (United Fruit Sales Corp 1964). Ripening was carried out in a biotron at 25 °C with a relative humidity of 90% during 14 days to obtain "Yellow bananas" (color index 7) (Inaba et al. 1984).

Sample preparation

Positions of five slices sampled along the long axis of a banana were designated from stem end to distal end as follows: stem end (SE), stem (S), middle (M), distal (D), distal end (DE). Two measuring points in a cross section were the central point of a loculus (Center) and near the placenta (Inner).

A banana pulp sample from five parts of a fruit was sliced in a radial direction with a sharp knife. The thickness was 7 mm. Sliced pulps were analyzed immediately after excision. During measurement, the samples were wrapped with a wet filter paper to prevent desiccation.

Stress-relaxation analysis

The test of stress-relaxation analysis was carried out using the Creep Meter (RE-33005, Yamaden Inc., Tokyo, Japan) equipped with a sample-height counter (HC-3305, Yamaden Inc., Tokyo, Japan). Stress was measured by a 200 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 was adjusted to contact the probe at the measuring point. The stage was moved upward to plunge the probe into the sample. Plunging speeds were 0.05, 0.1, 0.5 and 1.0 mm/sec, and plunging depths were 0.2, 0.4 and 0.6 mm. The stress decay after stopping movement of the stage was recorded from 0 to 180 sec by a microcomputer (PC-9801, NEC). Time schedule for the data acquisition had thirty sampling times with 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.

Calculation of parameters of stress-relaxation analysis

The relaxation data were simulated by the following equation (Yamamoto et al. 1970);

$$S_t / S_0 \times 100 (\%) = R \log_e \{ (t + T_m) / (t + T_0) \} \text{ -----(1)}$$

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

Sum of deviation of calculated stress data from the measured ones was calculated by the following equation;

$$\text{Sum of deviation} = 100 \times \sum (|S_t - \text{Scal}_t| / S_0) \text{ -----(2)}$$

where S_t and Scal_t are the measured and calculated stress at time t ,

respectively.

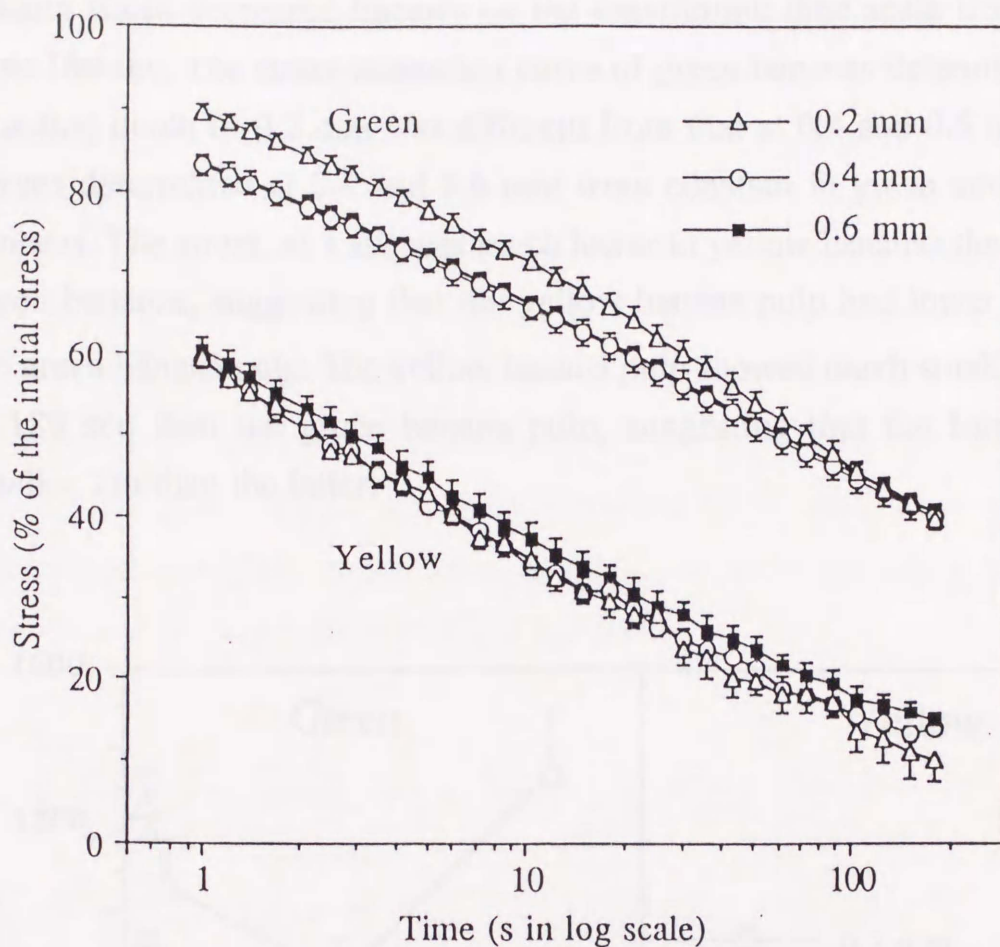


Fig. 1. Stress-relaxation curves for the center region of M slices from green and yellow bananas, plunging speed 0.5 mm/sec with different plunging depths. The initial stress is expressed as 100 % on the ordinate ($n=12$).

Results

Pulps of green and yellow bananas were cut into slices in the middle region. The stress-relaxation curves of the slices were measured at "Center" region for 180 sec at a plunging speed of 0.5 mm/sec for three plunging depths. The stress-relaxation curves were plotted against as a

function of time on a common logarithmic scale (Fig. 1). The initial stress was expressed as 100 % on the ordinate. The stress of green and yellow banana slices decreased linearly on the logarithmic time scale from 1 sec until 180 sec. The stress-relaxation curve of green bananas determined at a plunging depth of 0.2 mm was different from that at 0.4 and 0.6 mm. The curves determined at 0.4 and 0.6 mm were constant in green and yellow bananas. The stress at 1 sec was much lower in yellow bananas than that in green bananas, suggesting that the yellow banana pulp had lower T_0 than the green banana pulp. The yellow banana pulp showed much smaller stress at 180 sec than the green banana pulp, suggesting that the former had smaller T_m than the latter.

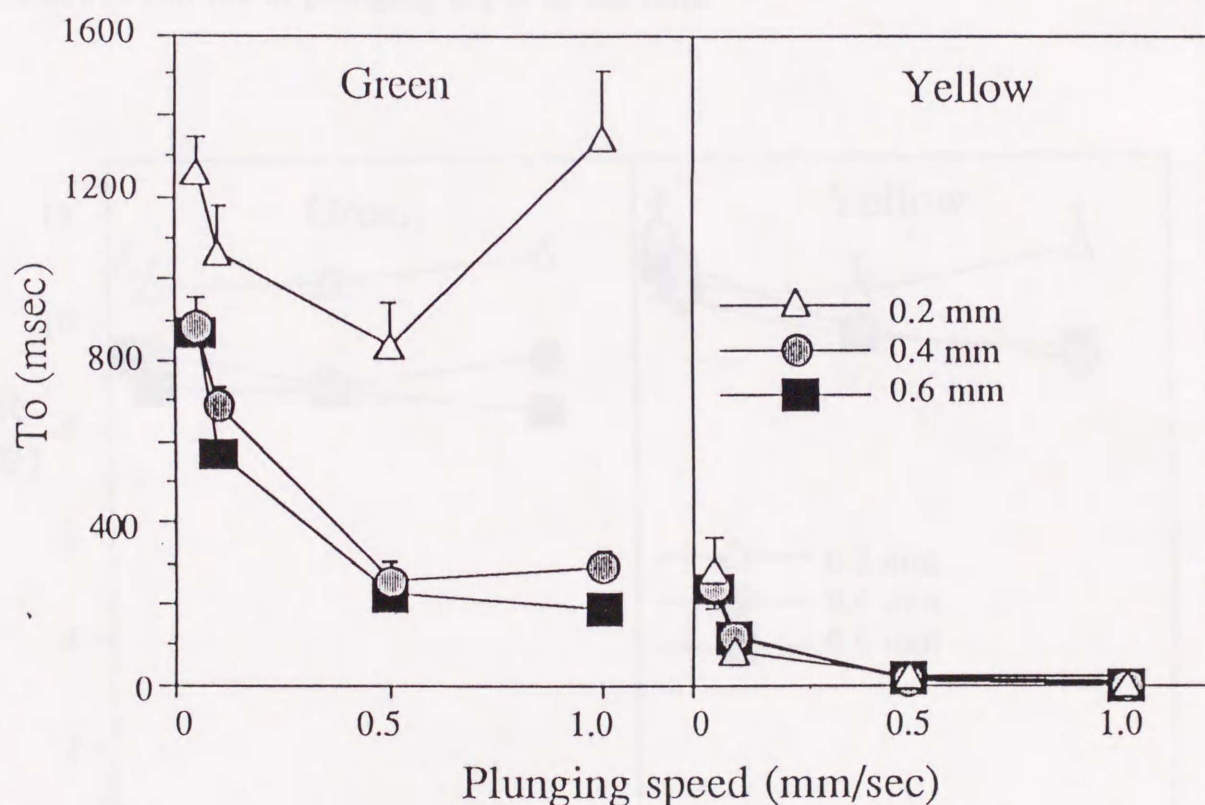


Fig. 2. Effects of plunging speed and depth on T_0 values for the center region of M slices from green and yellow bananas ($n=12$).

Three stress-relaxation parameters were calculated from the stress-relaxation curves under various plunging speeds and depths by a least square method based on the equation in "Materials and Methods". Figure 2 shows the T_0 values of green and yellow bananas. The T_0 values of green and yellow bananas tended to decrease as plunging speed increased up to 0.5 mm/sec, and were almost constant beyond 0.5 mm/sec. When the plunging speed was 1 mm/sec, a significant overshooting of stress was detected and it affected the calculation of stress-relaxation parameters. The T_0 values of green bananas decreased as plunging depth increased, while plunging depths did not significantly change T_0 values of the yellow bananas. The T_0 value of the green bananas (223 msec) was ten times higher than that of the yellow bananas (19 msec) at the plunging speed 0.5 mm/sec and the at plunging depth of 0.6 mm.

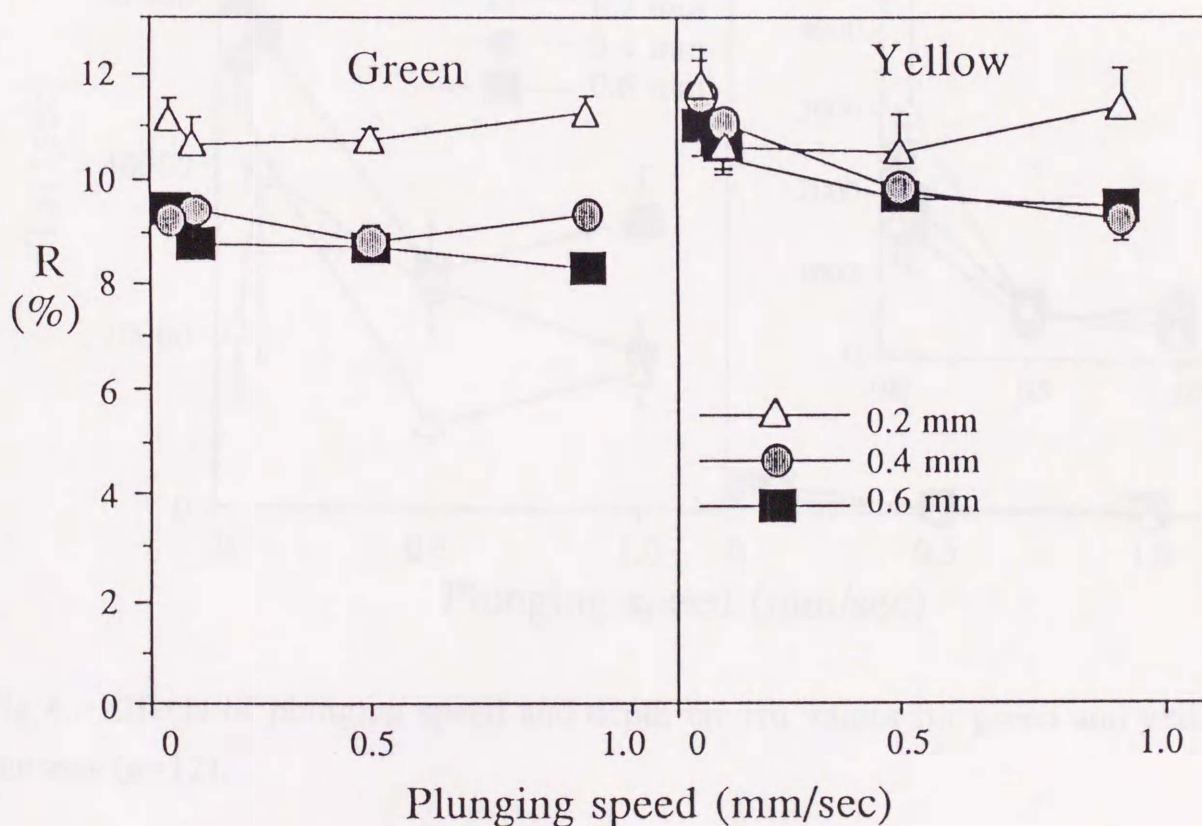


Fig. 3. Effects of plunging speed and depth on R values for green and yellow bananas (n=12).

The plunging speed did not remarkably change R values (Fig. 3). The R values of green and yellow bananas decreased as plunging depth increased up to 0.4 mm, and remained more or less constant beyond 0.4 mm. The R values of green and yellow bananas showed little difference at plunging speeds of 0.5 and 1.0 mm/sec with plunging depths of 0.4 and 0.6 mm.

The T_m values of green bananas were nearly one hundred times higher than those of yellow bananas (Fig. 4). The T_m values of yellow bananas at plunging speeds of 0.5 and 1.0 mm/sec were nearly constant in spite of changes in plunging depths (see insert in Fig. 4).

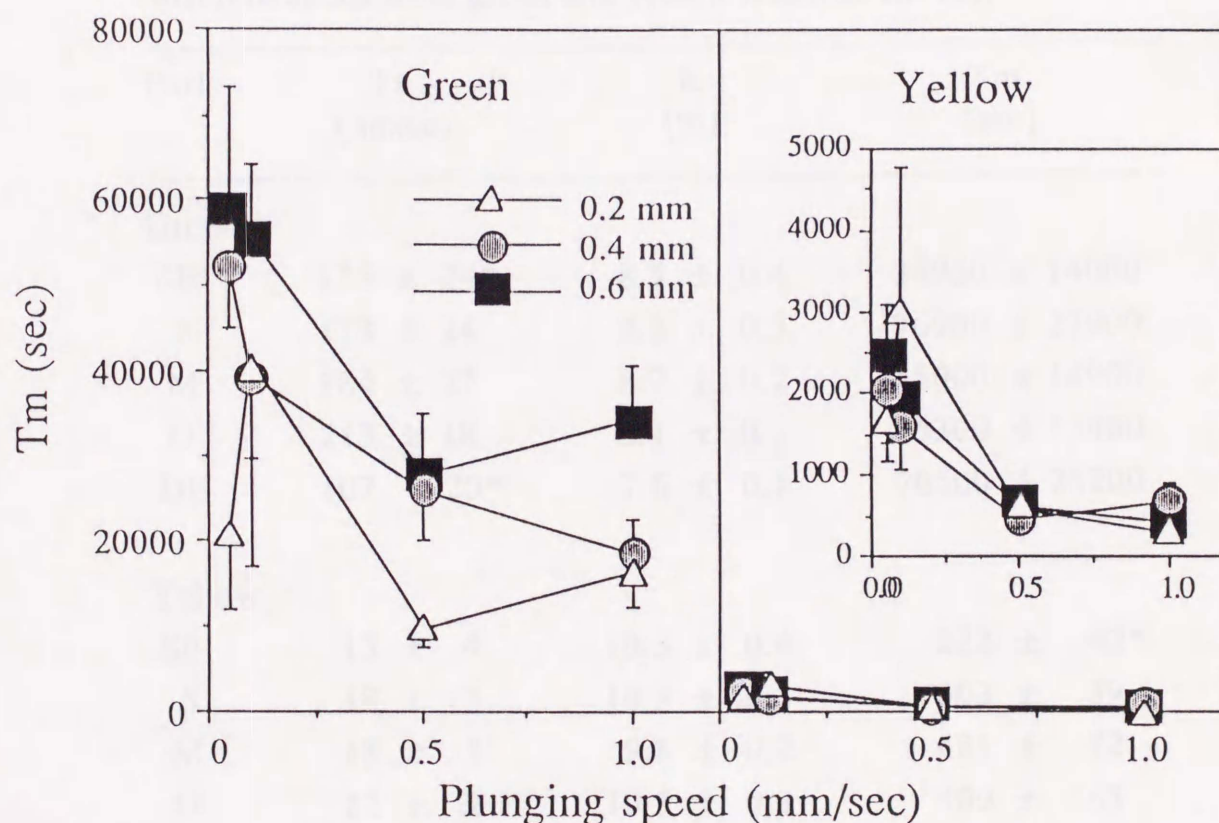


Fig 4. Effects of plunging speed and depth on T_m values for green and yellow bananas ($n=12$).

The sums of deviation under different measuring conditions were calculated (data not shown). The sum of deviation is the magnitude of difference between measured and calculated stress values. Lower sum of deviation is desirable. The sums of deviation of yellow bananas were always higher than those of green bananas, and they were independent of plunging speed but considerably decreased as plunging depth increased.

TABLE 1

Three stress-relaxation parameters for the center region of different slices from green and yellow Bananas (n=12).

Part	To (msec)	R (%)	Tm (sec)
Green			
SE	125 ± 24*	8.5 ± 0.4	38900 ± 14000
S	173 ± 24	8.2 ± 0.3	76900 ± 27900
M	184 ± 27	8.2 ± 0.2	48000 ± 14900
D	213 ± 18	8.1 ± 0.2	65300 ± 15400
DE	107 + 20*	7.6 ± 0.1	70500 ± 25200
Yellow			
SE	13 ± 4	10.3 ± 0.4	222 ± 42*
S	18 ± 3	10.3 ± 0.3	303 ± 39
M	18 ± 3	9.8 ± 0.2	481 ± 72
D	22 ± 2	10.3 ± 0.3	409 ± 65
DE	16 + 3	10.0 ± 0.2	369 ± 54

*Significant difference from the highest value in each banana at $P < 0.05$.

The distribution of the three stress-relaxation parameters along the long axis in green and yellow bananas was examined (Table 1). Plunging speed of 0.5 mm/sec and plunging depth of 0.6 mm were used for measuring the distribution of these parameters in banana pulp. The T_0 values for SE and DE slices in green bananas were significantly lower (at 5% level) than those for D slices. T_m values for SE slices in yellow bananas were significantly lower than those for M slices. Table 2 shows the data for the three stress-relaxation parameters in cross sections from M slices in green and yellow bananas. The T_0 and R values for the "Inner" region of green banana slices were lower than those for the "Center" region. The R value for the "Inner" region of yellow banana slices was lower, but the T_0 value was higher than that for the "Center" region.

TABLE 2

Three stress-relaxation parameters for the center and inner region of M slices from green and yellow bananas (n=12).

Part	T_0 (msec)	R (%)	T_m (sec)
Green			
Center	188 ± 19	8.1 ± 0.2	49000 ± 8300
Inner	115 ± 18*	7.3 ± 0.2*	192000 ± 87800
Yellow			
Center	14 ± 2	9.9 ± 0.3	437 ± 130
Inner	30 ± 7*	8.6 ± 0.4*	5960 ± 3080

*Significant difference from Center part in each banana at $P < 0.05$.

Discussion

The physical property of yellow bananas was substantially different from that of green bananas. The T_m values for green bananas were about one hundred times higher than those for yellow bananas. The T_0 values for green bananas were about ten times higher than those for yellow bananas. The T_m value is the most indicative parameter to determine softening of banana fruits, followed by T_0 . The R value did not substantially differ between the green and the yellow bananas. A considerable decrease in T_m was found in banana pulp associated with ripening. The decrease in T_m values might be the result of the degradation of high molecular weight polysaccharides in fruit pulp, but the physiological aspects of this observed decrease in T_m have not been thoroughly investigated. On the other hand, T_0 has been extensively studied in terms of the index of cell wall loosening (Masuda et al. 1974, Sakurai et al. 1977, Sakurai and Masuda 1977, 1978a, 1978b). Masuda et al. (1974) reported that the T_0 values were smaller in the cell wall of segments which were capable of undergoing greater extension than in those where it was more. In studies of stem tissues, a decrease in T_0 was associated with a decrease in viscosity resulting from the depolymerization of wall polysaccharides (Sakurai et al. 1979). Therefore, the decrease in T_0 of yellow banana suggests a degradation in the chemical structure of starch grains and/or cell walls. The lower T_0 value expresses softer texture of the pulp.

Lower T_0 values of the parts SE and DE in a green banana imply that softening of pulp commences from both ends, but a critical analysis of banana pulp with varying maturity is necessary to draw a final conclusion. Lower T_0 value for the "Inner" region in a green banana suggests that the

physical properties of the pulp are not uniform in a cross section.

Conventional techniques for the measurement of tissue softening failed to resolve changes in the softening of a small region, because the dissected tissue was measured with a flat probe (Charles and Tung 1973, Nussinovitch et al. 1990). Kojima et al. (1991) used a conical probe and measured stress-relaxation curves for small regions of tomato fruits. Charles and Tung (1973) have studied the maximum force at failure, the deformation under constant force and the linear limit of the force/deformation curve for banana pulps. Since a dissected tissue was measured with a flat probe, parameters obtained might be influenced by the dimension of the dissected tissue. Nussinovitch et al. (1990) evaluated criteria suitable for following the progress of ripening banana. Although three criteria gave high correlation coefficients with parameters such as Brix, starch content, age and color index, they gave no information about physical changes in tissue architecture.

During long-term measurements, increases in stress were observed at approximately 10 to 15 min. This may have been due to changes at the surface of banana slice (e.g. drying, oxidation, activation of enzymes). However, the effects were negligible up to 180 sec. The calculation of the stress-relaxation parameters are required till 50% of the stress decay. At the plunging speed of 0.5 mm/sec with a plunging depth of 0.6 mm, a sampling time of 180 sec is sufficient to calculate the stress-relaxation analysis in banana pulp. A higher plunging speed is desirable, because a longer plunging time increases T_0 values (Fujihara et al. 1978). However, when plunging speed is higher, an overshooting phenomenon occurs with a sharp, marked increase, especially at plunging speed of 1.0 mm/sec. Therefore, the plunging speed of 0.5 mm is practical. When different plunging depths were used for both green and yellow bananas, the plunging depth of 0.6 mm showed the lowest sum of deviation compared to that of

0.2 and 0.4 mm. Thus the plunging depth of 0.6 mm was most suitable for measuring. The conditions described above allow measurements of stress-relaxation at any ripening stage and at any region of the banana.

Chapter IV

Fruit Softening of Banana

Correlation among stress-relaxation parameters, cell wall components and starch during ripening

Abstract

Bananas [*Musa* (AAA group, Cavendish subgroup) "Giant Cavendish"] were used for the study of physical and chemical process of fruit softening. Changes in mechanical properties of the pulp were detected by a stress-relaxation technique, suggesting that the decrease in viscosity and elasticity of pulp is a crucial physical event of the pulp softening. Amount of starch and pectic and hemicellulose polysaccharides of the cell walls decreased and amount of soluble sugars increased, while cellulose content unchanged. Sugar composition of pectins and hemicelluloses more clearly revealed that the partial degradation of hemicelluloses preceded the breakdown of starch. The results suggest that the coordinated degradation process of pectic and hemicellulosic polysaccharides and starch is a main cause for the pulp softening process.

Introduction

In fruit softening, the chemical changes involved and the enzymes contributing to the changes during ripening have been extensively investigated (Bartley and Knee 1982, Huber 1983a). There is evidence that softening of fruit is accompanied by an increase in the concentration of soluble pectic polysaccharides. However, considerably less is known regarding the role in fruit softening of cell wall polymers other than the polyuronides. Hemicelluloses are degraded during ripening in tomato (Huber 1983b), in strawberry (Huber 1984) and in muskmelon (McCollum et al. 1989). Cellulose metabolism has also been implicated as an important feature of fruit softening in apple (Abeles

and Biles 1991), peach (Hinton and Pressey 1974) and avocado (Pesis et al. 1978).

In banana fruit, many investigators reported that starch content of pulp before ripening was higher than in other fruit, decreased dramatically during few days of ripening and disappeared almost completely (Agravante et al. 1990, Charles and Tung 1973, Finney et al. 1967, Nussinovitch et al. 1990, Barnell 1943, Nakamura et al. 1979, Kawabata and Sawayama 1974). However, only a few measured the degradation of cell wall components, such as pectin (Marriott 1980, Kawabata and Sawayama 1974), hemicellulose (Desai and Deshpande 1978, Barnell 1943) and cellulose (Desai and Deshpande 1978) on the ripening processes. Since pectic and hemicellulosic polysaccharides are composed of complex polymers, precise determinations of cell wall components are necessary for elucidating the biochemical events of ripening of banana fruit. Furthermore, to clear the causal relationship among chemical changes in fruits and firmness, it is necessary to measure softness with a reliable physical measurement.

There have been few direct physical analysis of softening of the banana fruit (Charles and Tung 1973, Finney 1967, Nussinovitch 1990). If the physical analysis is based on a rheological model, parameters obtained afford not only the indices of tissue softening but also indices of the chemical and physical changes lying under the softening process. The stress-relaxation analysis was developed by Yamamoto et al. (1981) to examine the mechanical properties of plant cell walls and reviewed (Sakurai 1991). Kojima et al. (1991) applied this technique to analyze tissue softening in tomato fruit with a conical probe. They measured the softness within a tomato fruit with a newly controlled pressure device with a load cell and demonstrated that softening progressed from the inner columella region to the shoulder of fruit via distal end. An analysis of tissue slices using a conical probe provides more precise data than using a dissected tissue and a flat probe, because a small variation

in dimensions of excised tissues affected the calculation of the physical parameters. When the technique was applied to banana fruit, plunging depth varied substantially between ripe and unripe fruits to attain the same initial stress. To get more precise data in stress-relaxation curves, it was necessary to have optimum conditions for the measurement. Therefore, an instrument capable of controlling the plunging depth of the conical probe into the tissue was used (Kojima et al. 1992). They determined the optimum measuring conditions for the stress-relaxation analysis, and showed the changes in the physical properties during softening of banana fruit pulp.

The purpose of this study was to determine the stress-relaxation parameters and the chemical components responsible for the firmness, and to investigate the chemical background of ripening of banana fruit.

MATERIALS AND METHODS

Plant Materials

Bananas [*Musa* (AAA group, Cavendish subgroup) 'Giant Cavendish'] (Chiquita brand) of special grade from the Philippines were obtained from a local wholesaler (Nishiyama Seika Inc., Hiroshima) in Japan. The fruit at green unripe stage were brought to the laboratory. Five hands were selected for uniformity of size and maturity, and freedom from blemishes. Fingers of bananas were separated from the hands and end fingers (larger fruit) were discarded. Ethylene treatment (1000 ppm) was carried out in sealed plastic box (50 L) for 24 h at 25 °C. Ripening was carried out in a biotron at 25 °C with a relative humidity of 90% during 3 days (Inaba et al. 1984). Ripening stage was expressed by day from exposing ethylene.

Measurement of Color Index and Moisture Content

Peel color was evaluated by comparison with a color plate on a banana ripening chart (United Fruit Sales Corp 1964). Moisture content

was determined by drying 5 g of banana pulp in an oven at 70 °C for 1 d and then by keeping roughly dried pulp in a vacuum desiccator over P₂O₅ for 3 d.

Measurements of Fruit Softening

A banana pulp sample from middle portion along the long axis of a fruit was sliced in a radial direction with a thickness of 7 mm. The measuring point in a cross section was at the center of a locule. Sliced pulps were immediately analyzed by the stress-relaxation technique. During measurement, samples were surrounded with wet filter papers to prevent desiccation.

The stress-relaxation analysis was carried out using a Creep Meter (RE-33005, Yamaden Inc., Tokyo, Japan) equipped with a sample-height counter (HC-3305, Yamaden Inc., Tokyo, Japan). Stress was measured by 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 impose the probe into the sample. Plunging speed was 0.5 mm/s and plunging depth was 0.6 mm. The stress decay after stopping a 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 thirty 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.

Calculation of Parameters of Stress-Relaxation

The relaxation data were simulated by the following equation (Yamamoto et al. 1970);

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

where S_0 is an initial applied load, S_t is a load at time t , R is a

relaxation rate, T_0 is a minimum stress-relaxation time and T_m is a maximum stress-relaxation time. The R , T_0 , and T_m were calculated by a least square method with a personal computer (PC-9801, NEC) programmed in C language.

Cell Wall Fractionation

Tissue of banana fruit was homogenized for 5 min in 80% ethanol with a blender (Universal homogenizer, Nihon Seiki Seisakusyo Co., Tokyo) at the maximum speed. The homogenate was centrifuged for 10 min at 1000 g. The supernatant was designated as EtOH fraction. In preliminary experiment, as the washed residue was treated with 10 mL of 20 units/mL porcine pancreatic α -amylase (type I-A, Sigma, St. Louis) for 2 h at 37 °C, starch was not digested apparently. Therefore, the precipitate in deionized water was treated in boiling water bath for 10 min 3 times. The solution was centrifuged for 10 min at 1000 g. The supernatant was collected and designated as hot water soluble (HWS) fraction. The residue was treated in α -amylase in 100 mM sodium-acetate buffer (pH 7.0) as in the preliminary experiment. The solution was centrifuged for 10 min at 1000 g. The residue was treated with 10 mL of 0.6 units/mL β -amylase (type I-B Sigma, St. Louis) and iso-amylase (Nacalai tesque Inc., Kyoto, Japan) in 100 mM sodium-acetate buffer (pH 4.5) for 2 h at 37 °C. The supernatant solution after α -amylase and β - and iso-amylase treatment, and water washings were combined and designated as Starch fraction.

Residual pectic substances were extracted from the cell walls by three treatments with 50 mM EDTA in 50 mM sodium-phosphate buffer (pH 6.8) at 95 °C and designated as EDTA soluble pectin (ESP). Next, hemicellulose was extracted for 18 h with 17.5 % NaOH containing 0.02 % NaBH₄. The hemicellulosic fraction was neutralized with glacial acetic acid in an ice-cold water bath. The pectic and neutralized hemicellulosic fractions were dialyzed in a Visking cellulose tubing

(18/32) against deionized water for 18 h at $22\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. The dialyzed hemicellulosic fraction was centrifuged for 20 min at 10,000 g. The supernatant were designated as HB fraction, and the alkali-insoluble fraction after extraction of hemicellulose designated as cellulose fraction.

The cellulose fraction was washed three times with 0.03 N acetic acid, 4 times with 10 mL of 80% aqueous 1,4-dioxane with continuous stirring at $22\text{ }^{\circ}\text{C}$ for 10 d, and then three times with ethanol:ether (1:1, v/v). The washed residue was dried at $40\text{ }^{\circ}\text{C}$ for 3 d and kept at room temperature in a vacuum desiccator on P_2O_5 for 3 d. The completely dried material was hydrolyzed with 15 N H_2SO_4 for 1 h at room temperature and 2 N H_2SO_4 for 5 h at $100\text{ }^{\circ}\text{C}$.

Measurement of Sugar Contents and Compositions

Total sugar content of each fraction was determined by the phenol-sulfuric method (Dubois et al. 1956) and UA contents by the carbazole method (Galambos 1967). NS contents were calculated as follows:

$$\text{NS } (\mu\text{g/ml}) = 91[A_{490} - (148/239)A_{525}]$$

where A_{490} is the absorbance at 490 nm in the phenol-sulfuric method, A_{525} is the absorbance at 525 nm in the carbazole method (Sakurai et al 1987 1).

The neutral sugar compositions of pectin and HB fraction were determined by the method of Albersheim et al. (1967).

RESULTS

Peel Color and Moisture Analysis

For an index of ripening, the color of peel were evaluated (Fig. 1A). Index of peel color increased gradually during ripening and reached 6 at day 4.

Moisture content of pulp was nearly constant level from day 0 to

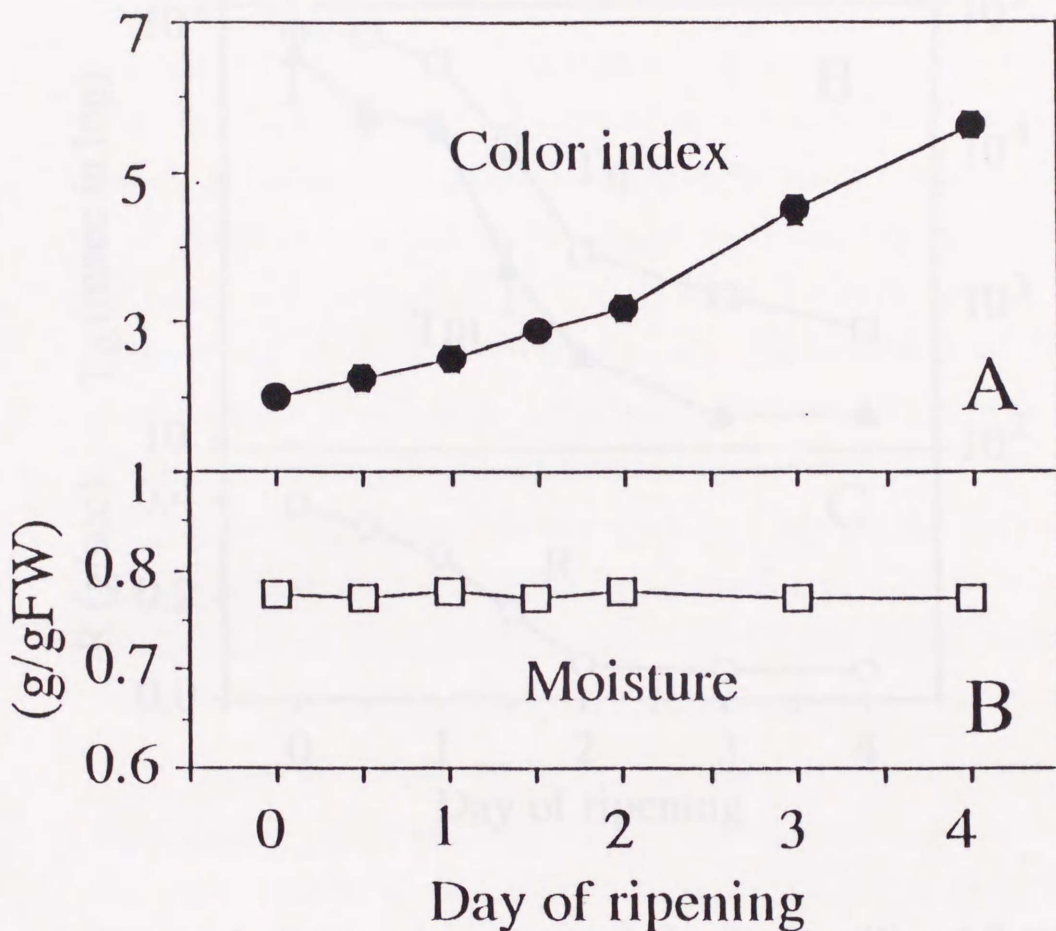


Figure 1. Color index of peel (A) and moisture content of pulp (B) during ripening of banana at 25 °C. Peel color was evaluated by comparison with a color plate on a banana ripening chart. Moisture content was determined by drying 5 g of banana pulp in an oven at 70 °C for 24 h and by keeping roughly dried pulp in a vacuum desiccator with P₂O₅ for 3 days.

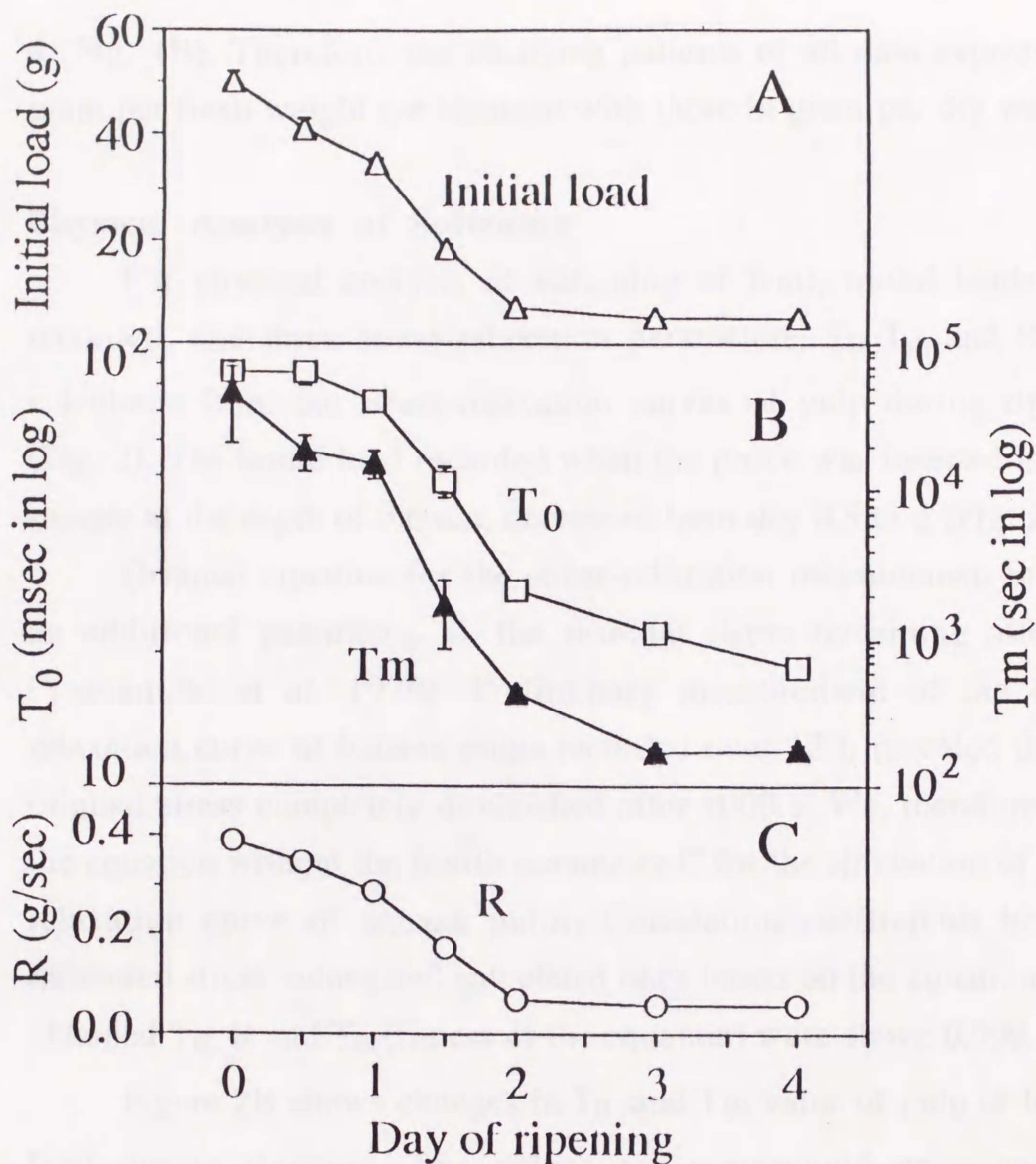


Figure 2. Changes in initial load (A), T_0 , T_m (B) and R value (C) of banana pulp during ripening at 25 °C. The pulp was sliced in a radial direction (7 mm thickness). The probe attached to a load cell was introduced into the sliced tissue. The initial load was recorded when the probe was inserted into the pulp at the depth of 0.6 mm. Plunging speed was 0.5 mm/s. Stress-relaxation data were obtained from 0 to 100 s. The data was used for the calculation of three stress-relaxation parameters, minimum stress-relaxation time (T_0), relaxation rate (R) and maximum stress-relaxation time (T_m). Correlation coefficients between measured stress values and calculated ones based on the equation using obtained T_0 , R and T_m (fitness of the equation) were above 0.999. Means and SE are shown ($n=50$).

4 (Fig. 1B). Therefore, the changing patterns of all data expressed in gram per fresh weight are identical with those in gram per dry weight.

Physical Analysis of Softening

For physical analysis of softening of fruit, initial loads were recorded, and three stress-relaxation parameters, T_0 , T_m and R were calculated from the stress-relaxation curves of pulp during ripening (Fig. 2). The initial load recorded when the probe was inserted into the sample at the depth of 0.6mm, decreased from day 0.5 to 2 (Fig. 2A).

Original equation for the stress-relaxation measurement includes an additional parameter, C , the residual stress remaining after T_m (Yamamoto et al. 1970). Preliminary measurement of the stress-relaxation curve of banana pulps recorded over 12 h revealed that the original stress completely diminished after 1000 s. We, therefore, used the equation without the fourth parameter C for the simulation of stress-relaxation curve of banana pulps. Correlation coefficients between measured stress values and calculated ones based on the equation using obtained T_0 , R and T_m (fitness of the equation) were above 0.999.

Figure 2B shows changes in T_0 and T_m value of pulp of banana fruit during ripening. The values are represented on a common logarithmic scale. T_0 is the time at which the stress starts with major decay, and corresponds to a Maxwell component with the lowest viscosity (Sakurai 1991). T_0 value did not decrease significantly till day 1 and decreased dramatically till day 2. T_m is the time at which the stress finishes with major decay, and corresponds to a Maxwell component with the highest viscosity (Sakurai 1991). T_m value also did not decrease significantly till day 1 and decreased dramatically till day 2. R is relaxation rate, and predicts the number of component of visco-elastic properties (Sakurai 1991). The R value decreased from day 0.5 to 2 (Fig. 2C).

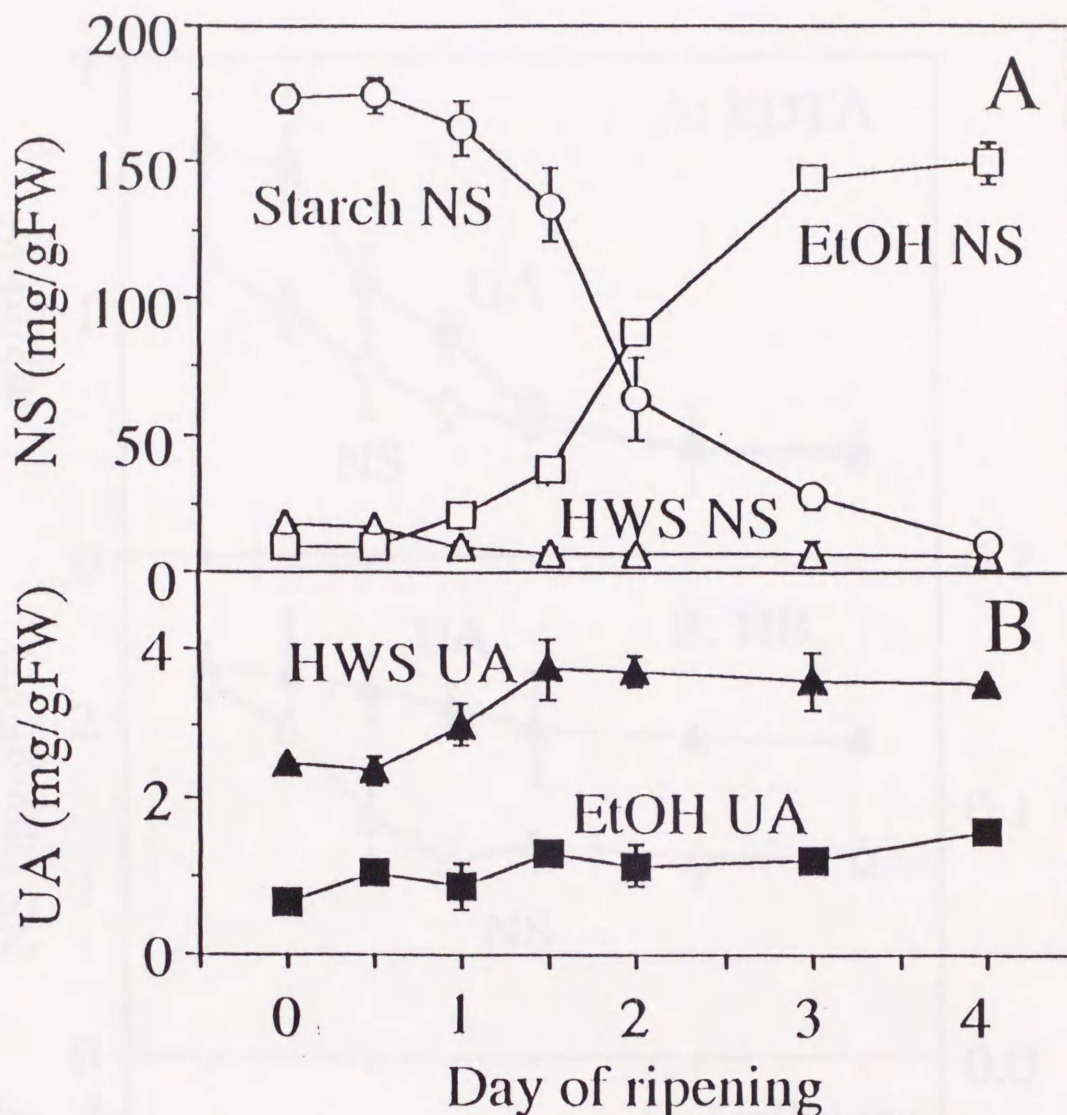


Figure 3. Changes in sugar content of EtOH soluble, hot water soluble (HWS) and Starch fractions from banana pulp during ripening. EtOH soluble fraction was obtained after the homogenization of banana pulp with 80% EtOH. HWS was obtained after 3 times boiling bath for 10 min. Rehydrated residue was treated with α -, β - and iso-amylase. NS in the digest supernatant was designated as Starch fraction. UA was determined by a carbazole method. NS was determined by phenol-sulfuric acid method. Means and SE are shown (n=5).

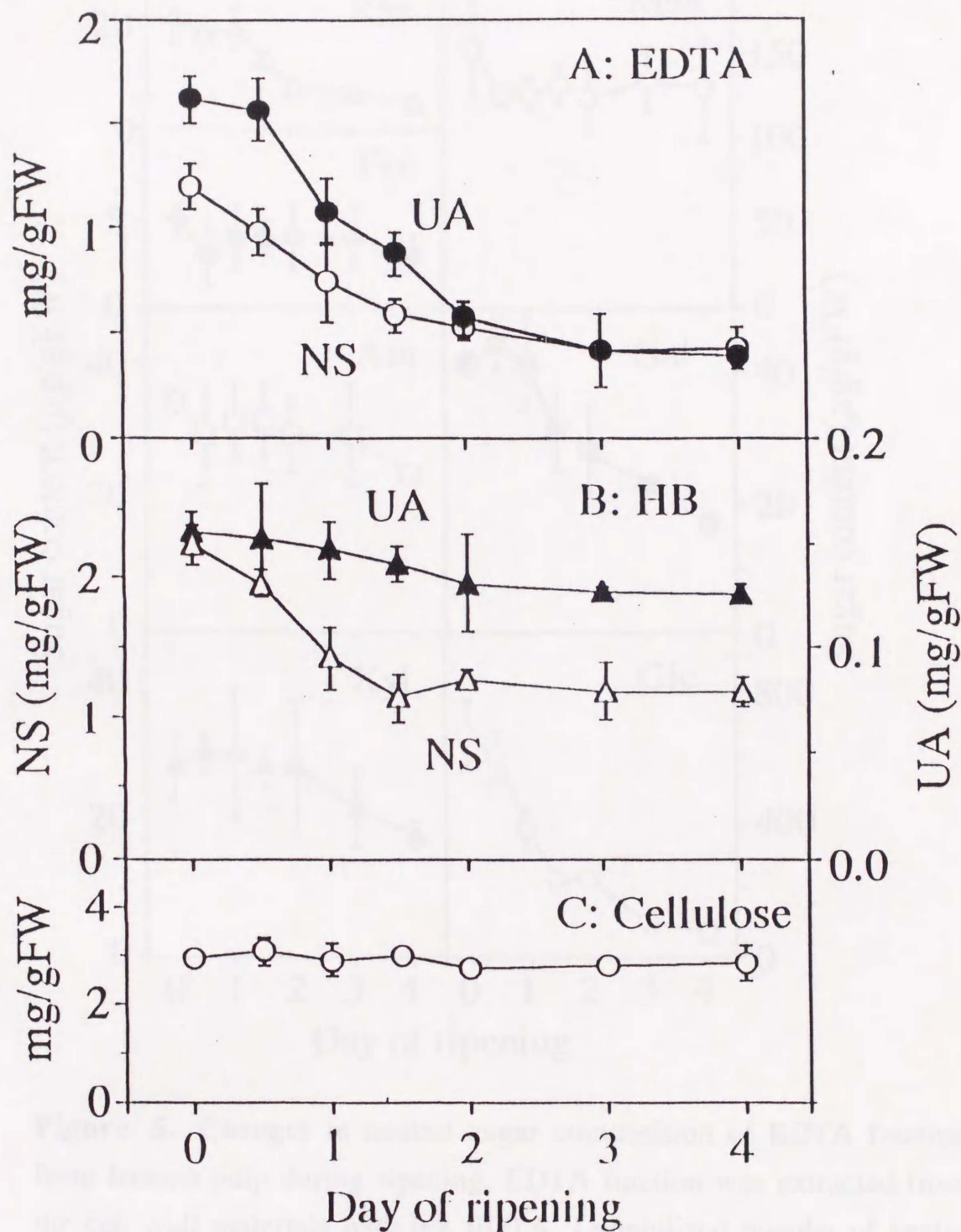


Figure 4. Changes in NS and UA of EDTA (A), HB (B) and cellulose (C) fraction of banana pulp during ripening. EDTA fraction was extracted with hot EDTA (pH 6.8) and hemicellulose with 17.5% NaOH. Residue was washed with 80% aqueous 1,4-dioxane and designated as cellulose fraction. Means and SE are shown (n=5).

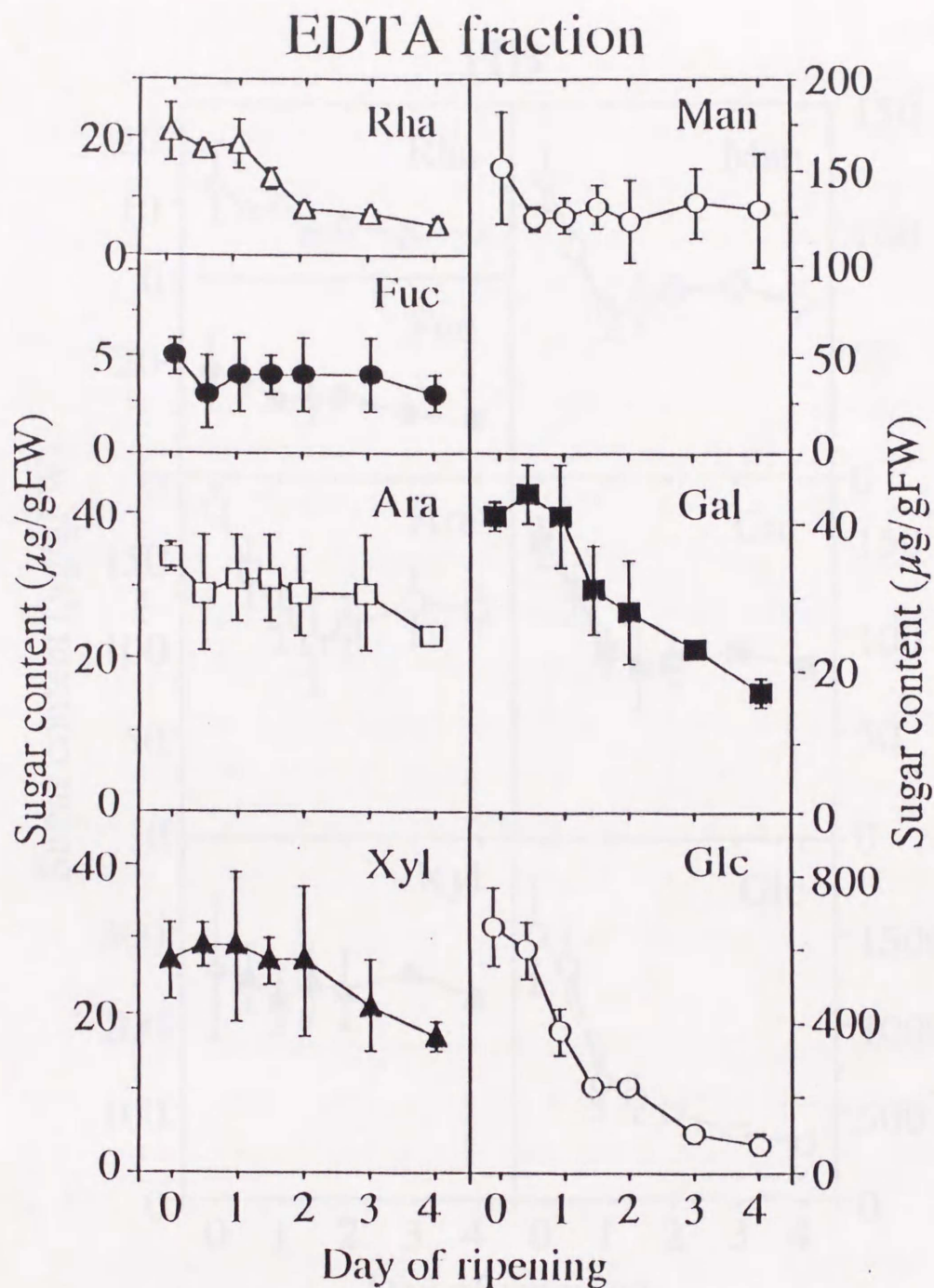


Figure 5. Changes in neutral sugar composition of EDTA fraction from banana pulp during ripening. EDTA fraction was extracted from the cell wall materials with hot EDTA. Lyophilized powder of pectic substances was hydrolyzed for 1.5 h with 2 M trifluoroacetic acid. Neutral sugar compositions of hydrolysate were determined by GLC. Means and SE are shown (n=5).

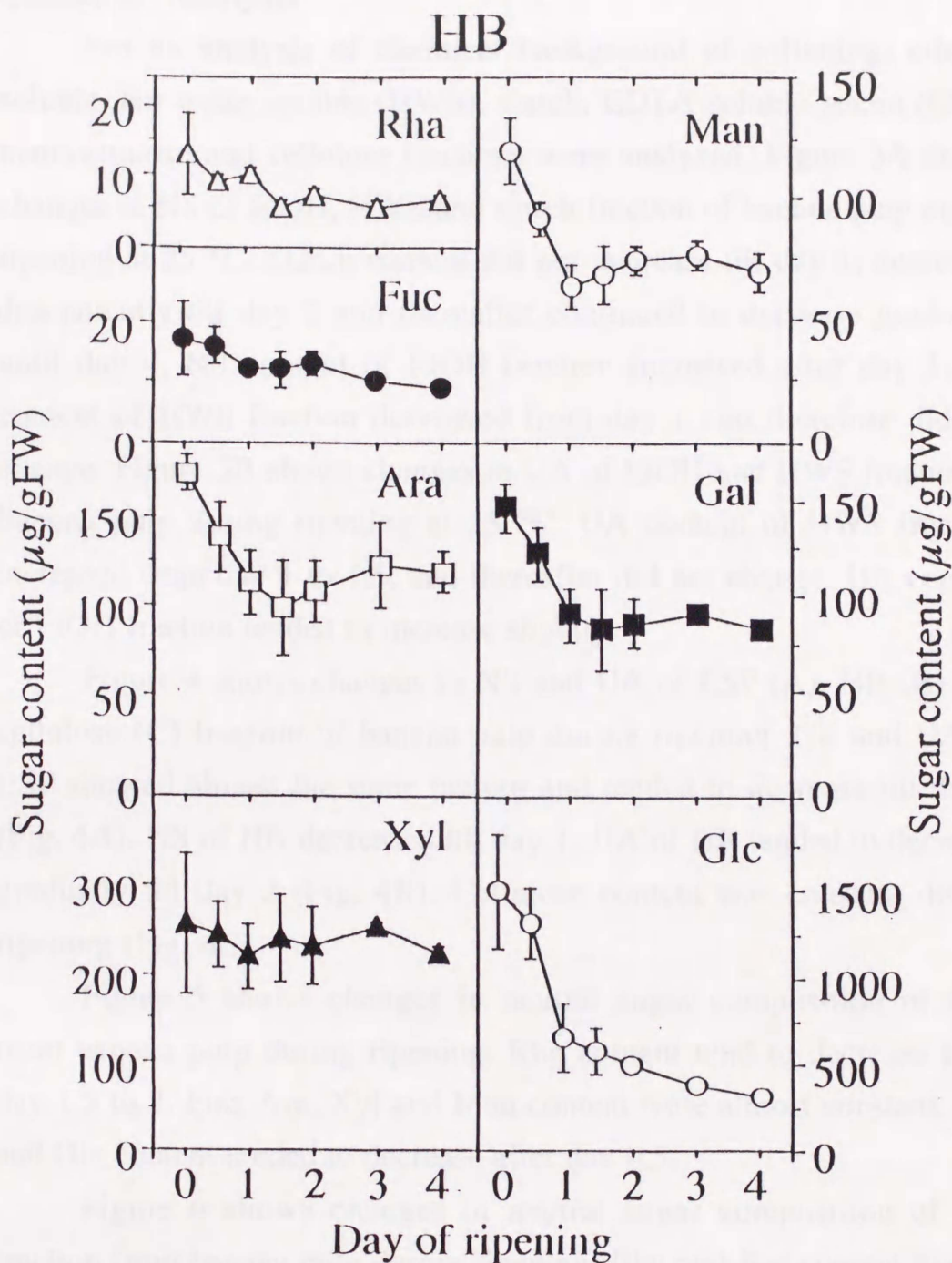


Figure 6. Changes in neutral sugar composition of HB fraction from banana pulp during ripening. Hemicellulose was extracted with 17.5% NaOH from the cell wall materials which had been treated with hot EDTA. Hemicellulose fraction was neutralized with glacial acetic acid. The neutralized solution was centrifuged for 20 min at 10,000g. Supernatant (HB) was subjected to GLC analysis to determine the sugar composition. Means and SE are shown (n=5).

Chemical Analysis

For an analysis of chemical background of softening, ethanol soluble, hot water soluble (HWS), starch, EDTA soluble pectin (ESP), hemicellulose and cellulose fractions were analyzed. Figure 3A shows changes in NS of EtOH, HWS and starch fraction of banana pulp during ripening at 25 °C. Starch content did not decrease till day 1, decreased dramatically till day 2 and thereafter continued to decrease gradually until day 4. NS content of EtOH fraction increased after day 1. NS content of HWS fraction decreased from day 1 and therefore did not change. Figure 3B shows changes in UA of EtOH and HWS fraction of banana pulp during ripening at 25 °C. UA content of HWS fraction increased from day 1 to 1.5, and thereafter did not change. UA content of EtOH fraction tended to increase slightly.

Figure 4 shows changes in NS and UA of ESP (A), HB (B) and cellulose (C) fraction of banana pulp during ripening. NS and UA of ESP showed almost the same pattern and tended to decrease till day 3 (Fig. 4A). NS of HB decreased till day 1, UA of HB tended to decrease gradually till day 2 (Fig. 4B). Cellulose content was constant during ripening (Fig. 4C).

Figure 5 shows changes in neutral sugar composition of ESP from banana pulp during ripening. Rha content tend to decrease from day 1.5 to 2. Fuc, Ara, Xyl and Man content were almost constant. Gal and Glc content tended to decrease after day 0.5.

Figure 6 shows changes in neutral sugar composition of HB fraction from banana pulp during ripening. Rha and Fuc content tended to decrease slightly. Ara, Man, Gal and Glc content tended to decrease till day 1, and thereafter to be almost same level. Xyl content was almost constant.

DISCUSSION

In the previous study (Kojima et al. 1992), the optimum measuring condition for the stress-relaxation analysis and the

distribution of the physical properties of banana fruit pulp were determined. In this study, the location of physical and chemical analysis was focused on the middle part along the long axis of a banana.

The changes in mechanical properties of banana pulps were detected by the stress-relaxation technique. Decrease in initial load during ripening suggests that the elastic component of the pulp also changed. The decreases in T_0 and T_m suggested that the viscosities of the mechanical components with lowest and highest viscosity decrease (Sakurai 1991). Moreover, the decreases in T_0 and T_m suggest that highest and lowest mol wt of polysaccharides which contribute to the mechanical hardness in tissues might decrease. Decreasing pattern in elasticity of banana pulp is different from that in viscosity. Thus stress-relaxation method may provide more useful information than only measurement of initial load. The decrease in viscosity and elasticity detected by the stress-relaxation method results from the breakdown of biopolymers responsible for the firmness of pulps. Viscosity decrease in the cell wall detected by the stress-relaxation method has been reported in auxin-induced elongation, where the degradation of β -1,3:1,4-glucan of hemicelluloses is responsible for the decrease in viscosity of cell walls (Sakurai 1991). Viscosity results from the friction and entanglement of polymers. Since there was no prominent change in moisture content and fresh weight during banana ripening, the decrease in viscosity predicted by the decrease in T_0 and T_m should attribute to the breakdown of high mol wt of polymers, such as cell wall polysaccharides and starch.

Cellulose metabolism has been implicated as an important feature of fruit softening in apple (Abeles and Biles 1991), in peach (Hinton and Pressey 1974) and in avocado (Pesis et al. 1978). In Dwarf Cavendish banana, Desai and Deshpande (1978) also reported that the cellulose content continued to decrease during ripening. However, our results show that cellulose content is constant during ripening. Although the

discrepancy may be due to difference of cultivar used, at least in Giant Cavendish banana the cellulose content appears not to be involved in softening of the pulp during ripening.

Many investigators reported that starch of banana pulp before ripening was higher than other fruit, decreased dramatically during few days of ripening and disappears (Agravante et al. 1990, Charles and Tung 1973, Nussinovitch et al. 1990). It was suggested that softening of banana fruit was related to starch content (Finney et al. 1967). However, in our results, the decrease in pectin and HB content seems to precede the decrease in starch content. The results suggest that softening of banana fruit is more likely initiated by the changes in pectin and hemicellulose structure rather than by those in starch.

The significant changes in initial load was detected after day 0.5 and stress-relaxation parameters were almost constant till day 1, while degradation of the HB polysaccharides was detected after day 1. It suggests that the initial breakdown of HB polysaccharides does not contribute to viscosity but to elasticity. The decreases in the bulk amount of the polysaccharides and starch correlate more with the changes in mechanical properties of the fruit.

Present results clearly separated the sequential events during banana pulp ripening; first, the partial degradation of polysaccharides in hemicelluloses, then that in residual pectin (ESP) and starch breakdown. The measurement of mechanical properties of the pulp suggests that the loss of firmness is more likely due to the latter event. Causal relationship between the ethylene production and polysaccharide degradation is open to the further investigation.

Chapter V

General Discussion

A physically defined method (stress-relaxation analysis) was applied to analyze tissue softening in tomato fruit with a conical probe. The softness within a tomato fruit was measured using a newly controlled pressure device with a load cell. The obtained results showed that softening progressed from the inner columella region to the shoulder of the fruit via the distal end. An analysis of tissue slices using a conical probe provided more precise physical data than using a dissected tissue and a flat probe (Chapter II).

When the technique was applied to banana fruit, plunging depth varied substantially between ripe and unripe fruits to attain the same initial stress. To get more precise data in stress-relaxation curves, it was necessary to have optimum conditions specific for the banana fruits. Therefore, an instrument which could control the plunging depth of the conical probe into the tissue was newly devised. Using the instrument, the optimum measuring conditions for the stress-relaxation analysis were determined, and the changes in the physical properties during softening of banana fruit pulp were measured (Chapter III).

Both the stress-relaxation parameters and the chemical components responsible for the firmness were determined to investigate the chemical background of ripening of banana fruit. Changes in mechanical properties of the pulp were detected by the stress-relaxation technique, suggesting that the decrease in viscosity of the pulp is a crucial physical event of pulp softening. The amount of starch and pectic and hemicellulose polysaccharides of the cell walls decreased and the amount of soluble sugars increased, while cellulose content was unchanged. Gas chromatography of pectins and hemicelluloses more

clearly revealed that the degradation of some species of these polysaccharides preceded the prominent decrease in their amounts (Chapter IV).

(A) Technical discussion

(1) Merit of novel technique for measuring softness

1. Prediction of structural change

Charles and Tung (1973) studied the maximum force at failure, the deformation under constant force and the linear limit of the force/deformation curve for banana pulp. Nissinovitch (1990) evaluated criteria suitable for following the progress of ripening banana, giving high correlation coefficients with parameters such as Brix, starch content, age and color index. Ahrens and Huber (1990) improved the technique using excised tissue upon which a known weight was applied, revealing that tissue softening in tomato fruit is more effectively measured than those obtained by conventional techniques. However, these data do not predict physical changes in cell wall architecture. If the physical analysis is based on a physical model, physical parameters thus obtained would predict not only the mechanical properties but would also allow the analysis of the expression of specific structural changes. In the present study, stress-relaxation analysis developed by Yamamoto et al. (1970), based on the Maxwell viscoelastic model was used. Its parameters can predict a mechanical component with the lowest viscosity and the highest viscosity, and the number of relaxation components per unit volume (Sakurai 1991).

2. Measuring softness in a small region

Conventional techniques for the measurement of tissue softening failed to resolve changes in the softening of a small region, because the dissected tissue had been measured with a flat probe (Charles and Tung

1973, Nissinovitch et al. 1990). On the other hand, in the present study a conical probe was used to measure stress-relaxation curves for small regions of fruits (Chapter II, Fig. 1). This technique allowed measurements of stress-relaxation at any small region within the fruit.

3. Non-error in preparing a sample

Conventional techniques for a measurement used a flat probe and dissected tissue. Because a tissue is fragile especially in the more ripe fruit, dissected tissue has an error in shape and/or dimension. In our preliminary attempts, the Vitrodyne system with a flat probe failed to establish a basis for determining stress-relaxation parameters. The cause may be related to the geometry and variations in the dimension of tissue segment taken from the fruits. Very small variations in the dimensions of the dissected tissues affected the calculated parameters. Therefore, a conical probe was devised with known dimensions and the sliced tissue was used in this study.

(2) Optimum conditions for measurement of softness of banana fruit pulp

A higher plunging speed is desirable, because a longer plunging time increases T_0 values (Fujihara et al. 1978). However, when plunging speed was higher, an overshooting phenomenon occurred with a sharp, marked increase, especially at plunging speed of 1.0 mm/sec (Chapter III, Fig. 2). Therefore, the plunging speed of 0.5 mm was practical. When different plunging depths were used for both green and yellow bananas, a plunging depth of 0.6 mm showed the lowest sum of deviation compared to those of 0.2 and 0.4 mm. Thus the plunging depth of 0.6 mm was most suitable for the measure event.

During long-term measurements, increases in stress were observed at approximately 10 to 15 min, which may have been due to

changes at the surface of banana slice (e.g. drying, oxidation, activation of enzymes). However, the effects were negligible up to 180 sec. The calculation of the stress-relaxation parameters was required till 50% of stress decay. In conclusion, at the plunging speed of 0.5 mm/sec with a plunging depth of 0.6 mm, a sampling time of 180 sec is sufficient to calculate the stress-relaxation analysis in banana pulp.

(B) Physiological discussion

(1) Physical property and its distribution within banana pulp tissue

In preliminary experiments, we measured stress-relaxation of pulp slices of unripe and ripe banana enclosed in polyethylene bag to prevent drying during 12 h. Stress of the slice of ripe banana decreased to zero within 1000 sec. However, stress of the slice of unripe banana decreased constantly to about 1000 sec, thereafter tended to increase a little. This small increase was due to changes of the surface of banana slice (e.g. activation of enzymes, oxidation and/or drying). As this small increase in surface change was removed, the modified stress-relaxation curve of unripe banana indicated that stress was decreasing to zero. These results suggest that banana pulp may be almost completely viscous in structure rather than being elastic.

In long axis, lower T_0 values of the parts SE and DE in a green banana imply that softening of pulp commences from both ends, but a critical analysis of banana pulp with varying maturity is necessary to draw a final conclusion (Chapter III, Table 1). In cross section, Garcia and Lajolo (1988) reported that starch degradation started at the central core of the fruit advancing toward the outer borders as ripening proceeded. In my study, lower T_0 value for the "Inner" region in a green banana confirms that starch degradation started at the central core

of the fruit. (Chapter III, Table 2).

(2) Relationship between softness and cell components

Cellulose metabolism has been implicated as an important feature of fruit softening in peach (Hinton and Pressey 1974), in avocado (Pesis et al. 1978) and in apple (Abeles and Biles 1991). In dwarf Cavendish banana, Desai and Deshpande (1978) also reported that the cellulose content continued to decrease during ripening. However, our results showed that cellulose content was constant during ripening (Chapter IV, Fig. 5). Although the discrepancy may be due to the different cultivar used, at least in Giant Cavendish banana the cellulose content may not be involved in softening of the pulp during ripening.

The changes in mechanical properties of banana pulp were detected by the stress-relaxation technique (Chapter IV, Fig. 2). The decreases in T_0 and T_m suggested that the viscosities of the mechanical components with lowest and highest viscosity decreased (Sakurai 1991). This viscosity decrease detected by the stress-relaxation method results from the breakdown of biopolymers responsible for the firmness of the pulp. Viscosity decrease in the cell wall detected by the stress-relaxation method has been reported in auxin-induced elongation, where the degradation of β -1,3:1,4-glucan of hemicelluloses is responsible for the decrease in viscosity of cell walls (Sakurai 1991). Viscosity results from the friction and entanglement of polymers. Since there was no prominent change in moisture content and fresh weight during banana ripening, the decrease in viscosity predicted by the decrease in T_0 and T_m should be attributed to the breakdown of high mol wt polymers, such as cell wall polysaccharides and starch.

The significant changes in initial load was detected after day 0.5 and stress-relaxation parameters were almost constant till day 1, while degradation of the HB polysaccharides was detected after day 1 (Chapter

IV, Fig. 2 and 6). It suggests that the initial breakdown of HB polysaccharides does not contribute to viscosity but to elasticity.

What contribute to the changes in mechanical properties of the fruit? Many investigators reported that the starch of banana pulp before ripening was higher than the other fruits, decreased dramatically during few days of ripening and then disappeared almost completely (Agravante et al. 1990, Charles and Tung 1973, Nussinovitch et al. 1990). It was suggested that softening of banana fruit was related to starch content (Finney et al. 1967, Leopold 1964). Figure 1 shows diagram of the sequential events during banana ripening in my study. The initiation of breakdown of pectin and hemicellulose B is earlier than that of mechanical properties of the fruit, while the initiation of breakdown of starch correlates well with that of mechanical properties of the fruit. Thus the changes in mechanical properties of the fruit may be mainly contributed by starch rather than pectin or hemicellulose B, although the end of breakdown of starch does not correlate with that of mechanical properties of the fruit.

(3) Mechanism of softening of banana fruit

In my study, the decrease in pectin and HB content seems to precede the decrease in starch content (Chapter IV, Fig. 3 and 4). The results suggest that softening of banana fruit is more likely initiated by the changes in pectin and hemicellulose structure rather than by those in starch.

Then which is the key function for the induction of fruit softening, ethylene evolution or degradation of polysaccharides? Ahrens and Huber (1990) proposed the possibility that the release of polyuronide fragments due to the degradation of pectic polysaccharides may cause ethylene evolution, since pectin-fragment-mediated increases

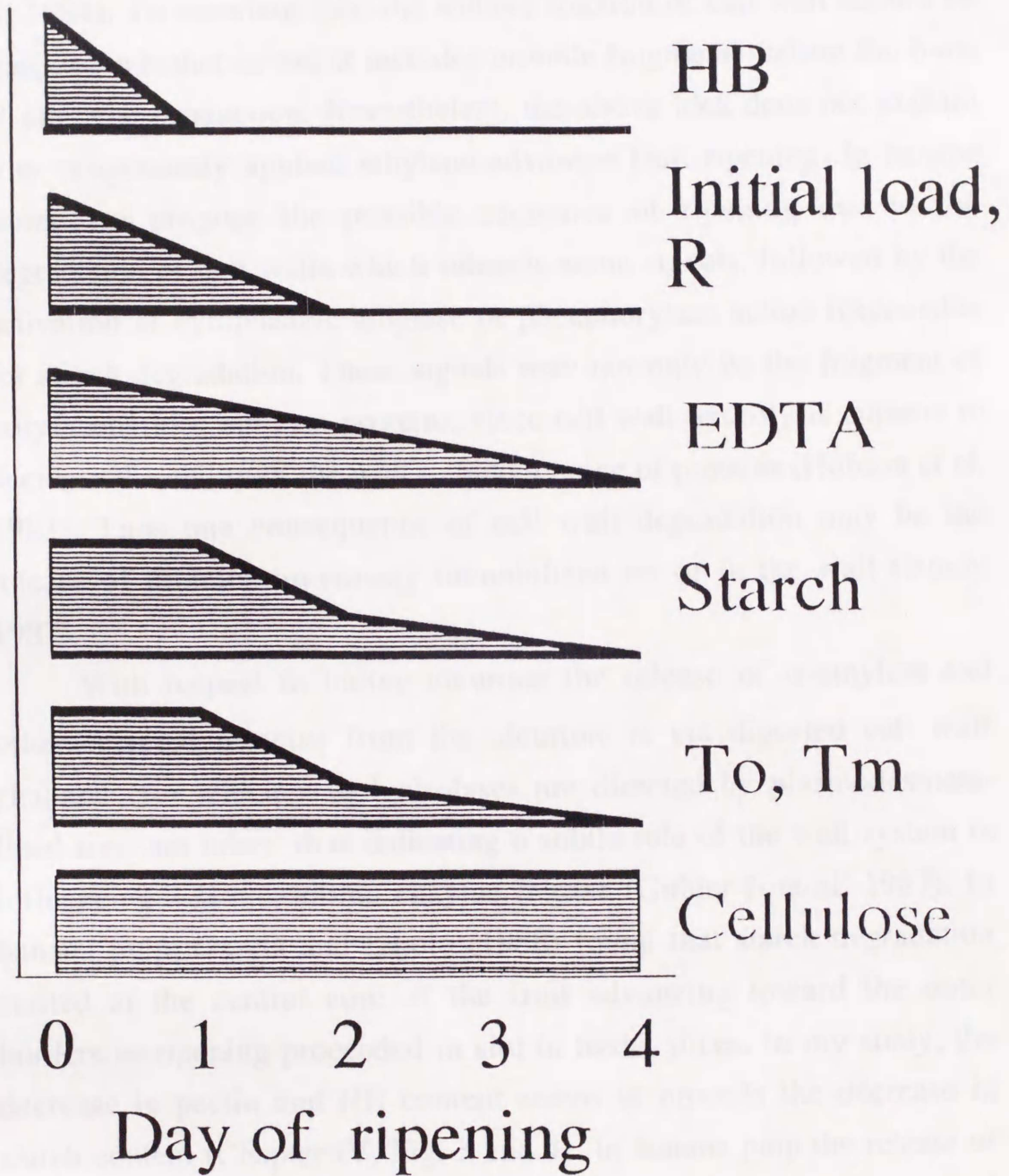


Figure 1. Diagram of the sequential events during banana pulp ripening

in ethylene production were reported in host-pathogen interactions (Roby et al. 1985a, Roby et al. 1985b, VanderMolen et al. 1983, West et al. 1984). To ascertain this, the soluble fraction of cell wall should be analyzed whether or not it includes uronide fragments before the burst of ethylene production. Nevertheless, the above idea does not explain why exogenously applied ethylene advances fruit ripening. In banana fruits, we propose the possible sequence of ripening due to the degradation of cell walls which releases some signals, followed by the activation of cytoplasmic amylase or phosphorylase action responsible for starch degradation. These signals may not only be the fragment of polysaccharides but also proteins, since cell wall hydrolysis appears to decrease the ability of the wall to bind a range of proteins (Hobson et al. 1983). Thus one consequence of cell wall degradation may be the release of proteins previously immobilized on or in the wall (Brady 1987).

With respect to barley aleurone the release of α -amylase and other secreted proteins from the aleurone is via digested cell wall channels, for which wall hydrolases are directed by plasmodesmata-lined resistant tubes, thus indicating a subtle role of the wall system in influencing and controlling enzyme release (Gubler F et al. 1987). In banana pulp, Garcia and Lajolo (1988) found that starch degradation started at the central core of the fruit advancing toward the outer borders as ripening proceeded in situ in tissue slices. In my study, the decrease in pectin and HB content seems to precede the decrease in starch content (Chapter IV, Fig. 3 and 4). In banana pulp the release of α -amylase and other proteins, which might be secreted from the central core of the fruit, may be also via digested cell wall channels.

(4) Conclusion

Present results clearly separated the sequential events during

banana pulp ripening; first, the partial degradation of polysaccharides in hemicelluloses, then that in residual pectin (EDTA fraction) and starch breakdown. The measurement of mechanical properties of the pulp suggests that the loss of firmness is more likely due to the latter event. The measurement of mechanical properties of the pulp suggests that the loss of firmness is more likely due to the breakdown of starch. The causal relationship between ethylene production and polysaccharide degradation is open to further investigation.

Chapter VI

Acknowledgements

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Chapter VII

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