Biochemical Changes of Cell Wall Elements with Fruit Development and Ripening, and the Harvesting Period in Japanese Pear Fruit (*Pyrus serotina* Rehder var. *culta* Rehder)

By SHOHEI YAMAKI

Fruit Breeding Division, Fruit Tree Research Station
(Yatabe, Ibaraki, 305 Japan)

The quality of fruit is mainly influenced by flesh texture, sweetness, acidity, flavor and color. The time when the above factors in fruit become most favorable for table use or processing is termed a good harvesting period for fruit. Therefore, it is of prime importance to make clear biochemical or physiological elements of these factors for setting exactly good harvesting periods. Until now, much were reported about post-harvest physiology or biochemistry of fruit,[4] especially about mechanism of climacteric rise[1] and alteration of cell wall constituents related to fruit softening.[3] However, since the ripening process of fruit has been already programmed in a fruit life composed of setting, development, maturation and senescence, we will be unable to understand the essential mechanism of ripening by investigating fruit only after harvest. Nevertheless, less information is available on pre-harvest physiology or biochemistry of fruit, which seems to be closely related to physiological characters of harvested fruit. Often delay or stimulation of ripening, or appearance of physiological disorders at ripening, is closely related to physiological characters during early development. Therefore, more information is necessary on biochemical or physiological characters during fruit development and ripening.

The flesh texture is the most important element among the above factors in the quality of Japanese pear fruit, since it exerts a great effect upon storage and shelf life after harvesting. The flesh texture is known to be much depending upon chemical or physical properties of cell wall, especially quantity and quality of cell wall polysaccharides, and strength of cell-cell adhesion. In general, cell wall polysaccharide is composed of pectin, hemicellulose and cellulose. The pectic substance is mainly localized on the outer surface of cell wall and takes an important part for the adhesion between cells, while the hemicellulose substances and cellulose fiber are mainly located in the inner layer of cell wall as characterized by prominent deposition of cellulose in the secondary wall, and take a part for integrity and firmness of cell wall. Albersheim proposed an attractive model of cell wall structure based on molecular compositions and their steric construction of each cell wall constituent in the primary wall of *sycamore* cell, and showed detail properties of each cell wall constituent.[2] This model also seems to be essential for the alteration of cell wall in the fruit softening. Thus, we tried to determine biochemically a good harvesting period based on the character of flesh texture of Japanese pear fruit, by investigating the alterations of cell wall polysaccharides and cell wall-degrading enzyme activities throughout fruit development and ripening periods.

Distinction of fruit development and ripening periods[6]

It is of prime importance to accurately determine, using biochemical methods, the periods of cell division, cell enlargement, maturation and senescence in relation to the following subjects: 1, treatment with growth regulators; 2, administration of fertilizers; 3, protection from physiological disorders; and 4, harvest of ripened fruit.

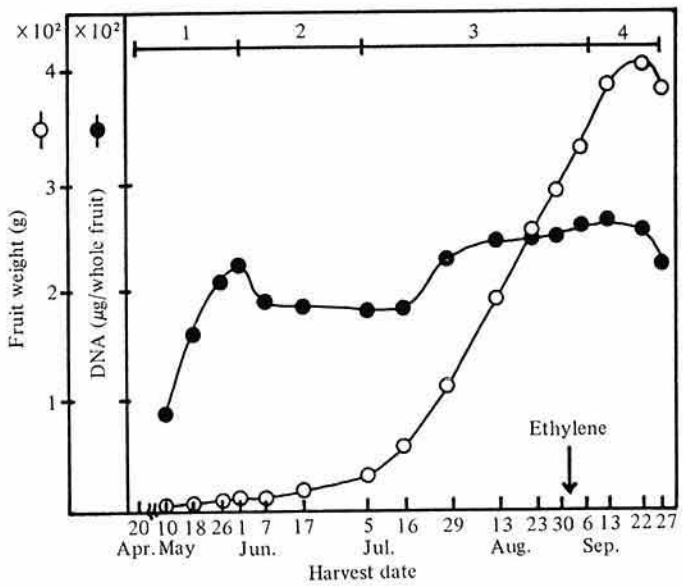

Fig. 1. Changes in DNA content and fruit weight during development and ripening of Japanese pear (var. Hosui)
The abscissa shows the dates of fruit picking after blossoming around Apr. 20, 1975. 1, 2, 3 and 4 at the top of the Figure indicate cell division (stage 1), pre-enlargement (stage 2), enlargement (stage 3) and ripening stages (stage 4), respectively. -●-: DNA content per whole fruit (μg DNA/whole fruit), -○-; Fresh weight of whole fruit (g). An arrow indicates start of ethylene evolution.

So that, we divided the growth periods into 4 stages, using DNA contents, potency of callus formation in flesh, prominent fruit enlargement, abrupt evolution of ethylene and the increase of RQ value. As shown in Fig. 1, after blossoming at about Apr. 20, DNA content per fruit increased sharply until about May 25. This indicates that active cell division occurred. This period was termed the cell division period (or stage 1). Then, up to about July 10, fruit weight did not increase and the cells did not enlarge significantly. However, in this stage, the density of fruit was the largest among all stages and the fruit flesh maintained the potency of callus formation under natural condition. This period corresponded to the pre-enlargement stage (stage 2). A remarkable increase in fruit weight began from about July 10 and continued to Aug. 25 (enlargement stage; stage 3). The fruit flesh in this stage maintained no more potency of callus formation. Around Aug. 30, the rapid enlargement of fruit ceased, evolution of ethylene started and the value of RQ raised to more than 1. After these changes, fruits ripened, overripened and fell from the tree (ripening and senescent stage, stage 4).

Alteration of cell wall polysaccharides during 4 stages of fruit development[8)]

Changes in quantity and quality of cell walls during cell division, elongation and softening in fruit flesh were investigated by analyzing the polysaccharide and monosaccharide components. Wall polysaccharide in ripening stage was separated into 6 fractions: water-soluble carbohydrate (F1, 10% of total polysaccharide contents*), $NaClO_2$-soluble polysaccharide (F2, few %), EDTA-soluble polysaccharide (F3, few %), acid-soluble hemicellulose (F4, 35%), alkali-soluble hemicellulose (F5, 15%) and cellulose (F6, 35%). Cell wall monosaccharides (calculated as the sum of monosaccharides in the above 6 fractions) consisted of glucose (40%), xylose (10%), arabinose (10%), galactose (5%), uronic acid (30%), mannose (few %), rhamnose (few %)

* Percentage of each fraction is that at the stage 4.

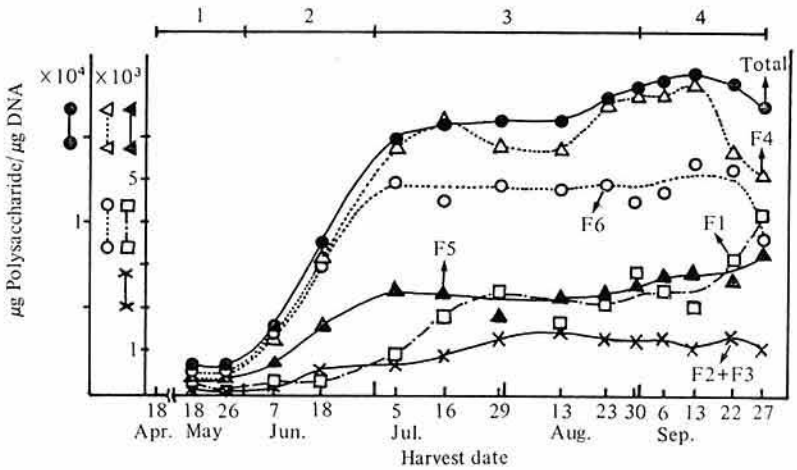

Fig. 2. Changes in amount of each cell wall polysaccharide expressed by μg/μg of DNA in fruit flesh tissue during development and ripening of Japanese pear (var. Hosui)
□ : F1 (water-soluble polysaccharide), × : F_2 ($NaClO_2$-soluble polysaccharide) +F3 (EDTA-soluble polysaccharide), △ : F4 (acid-soluble hemicellulose), ▲ : F5 (alkali-soluble hemicellulose), ○ : F6 (cellulose), ● : Total polysaccharide (the sum of 6 fractions). The amount of each fraction was calculated in terms of monosaccharides determined by GLC analysis after acid hydrolysis of each fraction and successive alditol acetylation of the hydrolyzates.[8]

and fucose (few %). These compositions of polysaccharide and monosaccharide changed drastically during fruit development and ripening.

Japanese pear fruit enlarges up to about 80 fold (from 5 to 400 g) during 3 months after completion of its cell division. Active degradation and regeneration of cell wall are expected to be related to cell enlargement. So that, the amount of cell wall polysaccharides and monosaccharides was calculated on the cell number basis (DNA content basis) to get further information about the cell wall changes per cell during the cell division, cell enlargement and ripening periods. As shown in Fig. 2, total polysaccharide content per cell (based on DNA) remained constant during the stage 1. As soon as the stage 1 was finished, the total polysaccharide content started to increase greatly during the stage 2. After about 30 days, the total polysaccharides content per cell increased 10 fold compared with a fruit weight increase of only 3 fold. It suggests that the most cell wall constituents required for cell enlargement in the stage 3 have already been prepared quantitatively in the stage 2. Thereafter, the total polysaccharide content almost stopped its increase when extensive enlargement started (stage 3), and remained constant although it showed a little rise in the pre-climacteric period and a little reduction in the maturation or over-ripening period (stage 4).

The seasonal change in cellulose content (F6) was very similar to that of the total polysaccharide except for the decrease at over-ripening (Fig. 2). Acid and alkali-soluble hemicelluloses, also, had already been prepared in the cell wall before fruit enlargement. Then, the latter remained roughly constant and the former increased in the late stage 3 and decreased in the stage 4 due to changes in arabinan and pectin (Fig. 2). F4 included arabinose, galactose, xylose and uronic acid as major monosaccharides, and glucose, fucose and rhamnose as minor ones and seemed to be composed of pectin, arabinan galactan etc. (Fig. 3). Arabinose and uronic acid showed the transient increase in the late stage 3 and then decreased in the stage 4 with ripening. Galactose decreased markedly in the stage 4 with ripening. F5 contained xylose and glucose as major monosaccharides, and uronic acid, galactose, rhamnose and fucose as minor ones (Fig. 4). Xylose content per DNA increased greatly in the stage 2 and decreased till it remained constant in the late stage 3 and stage 4. Contrariwise, non-cellulosic glucan content continued to increase with fruit enlargement after the stage 2 and became constant in the late stage 3 and stage 4. This prominent increase with enlarge-

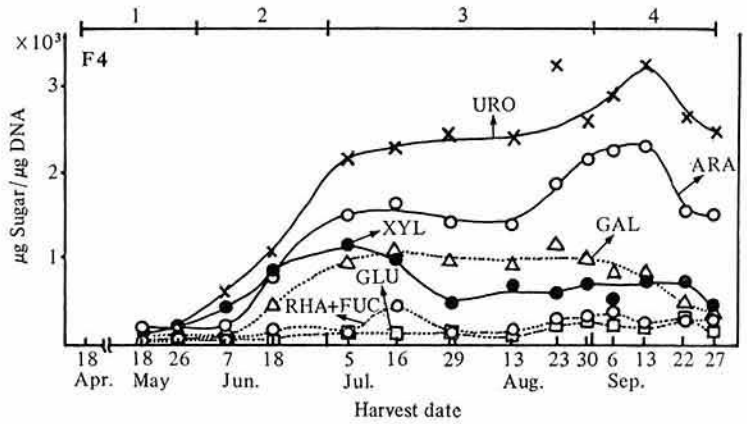

Fig. 3. Changes in amounts of monosaccharide of acid-soluble hemicellulose (F4) expressed by μg/μg DNA in fruit flesh tissue during development and ripening of Japanese pear (var. Hosui)
● : Xylose, × : Uronic acid, ○ : Arabinose, △ : Galactose, □ : Glucose, ○ : Rhamnose + fucose.

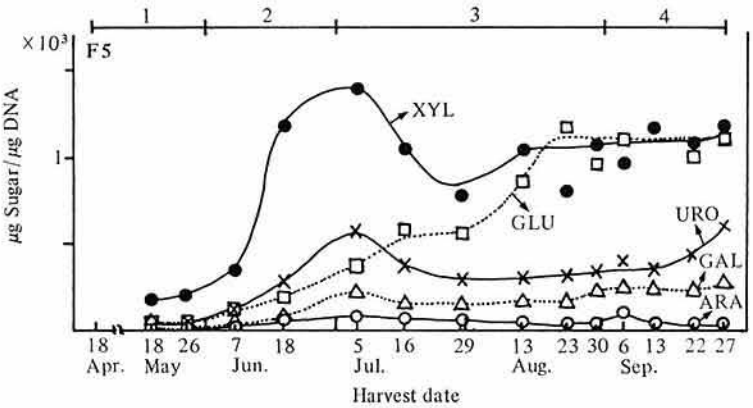

Fig. 4. Changes in amount of monosaccharide of alkali-soluble hemicellulose (F5) expressed by μg/μg of DNA in fruit flesh tissue during development and ripening of Japanese pear (var. Hosui)

ment may imply the rearrangement of cell wall polysaccharides. From all results described above, it can be thought that the breakdown or solubilization of arabinan, galactan or pectin, contained in acid-soluble hemicellulose, plays important roles in fruit softening, the increase in non-cellulosic glucan (β-1,3-glucan, xyloglucan) contained in alkali-soluble hemicellulose in fruit enlargement and the rapid degradation of cellulose in fruit breakdown with over-ripening.

Alteration of some cell wall-degrading enzyme activities during 4 stages of fruit development[7]

Seasonal changes in the activities of polygalacturonase, cellulase and some hemicellulose-degrading enzymes in Japanese pear fruit were studied in connection with development, softening and over-ripening. These enzyme activities per fruit flesh weight were very high during the cell division and pre-enlargement stages, and greatly decreased in the enlargement stage. Thereafter, they again exhibited clear increase with ripening. High enzyme activities during the cell division and pre-enlargement stages may reflect the dynamic turnover of cell wall. The great decrease of enzyme activities in the enlargement period seemed to indicate that the dramatic turnover of cell wall was not required for the enlargement because the polysaccharide of cell wall material had mostly been provided during the pre-enlargement stage.

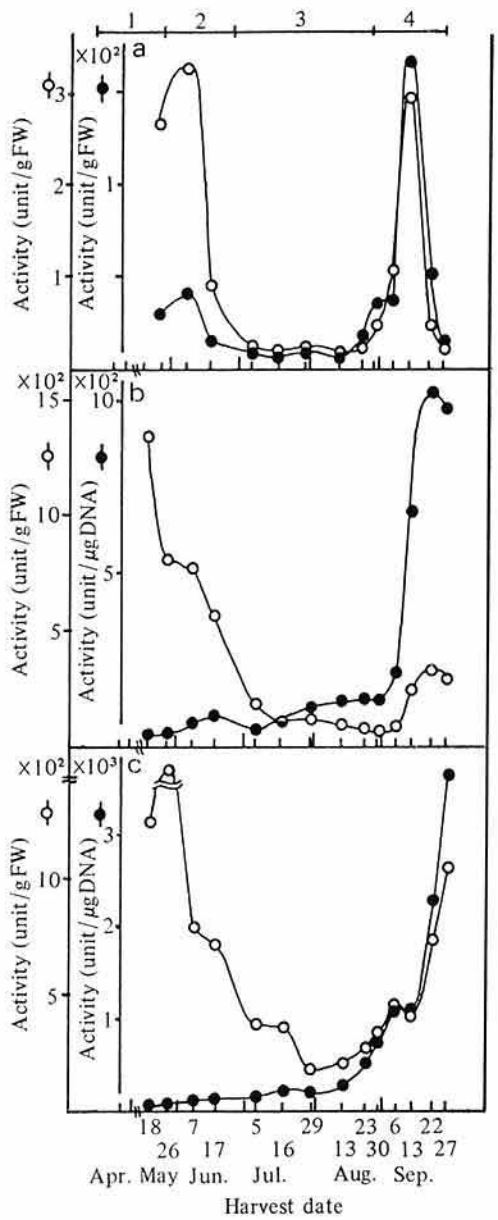

Fig. 5. Changes in cell wall degrading enzyme activities during fruit flesh development and ripening of Japanese pear (var. Hosui)
a: Polygalacturonase (-○-), exo-cellulase (-●-), b: Arabanase, c: β-galactosidase. In b and c,-○-and-●-indicate the activity per fresh weight (units/g fresh weight) and the activity per DNA content (units/μg DNA), respectively. Enzyme preparation and assay are described in a previous paper.[7]

By their alteration pattern with ripening, these cell wall-degrading enzymes were divided into the following three groups. The first group consisted of polygalacturonase, exo-cellulase and mannanase which showed maximum activities on Sep. 13 after beginning to rise on Sep. 5, as shown typically in polygalacturonase and exo-cellulase activities (Fig. 5a). The second one consisted of arabanase, xylanase, β-glucosidase and endo-cellulase which showed peaks of their activities on Sep. 22 after beginning to rise on Sep. 13, as typically shown in arabanase activity (Fig. 5b). The third one consisted of β-xylosidase and β-galactosidase which continued to increase with over-ripening after beginning to rise on Sep. 13, as typically shown in β-galactosidase activity (Fig. 5c). The activity increase in the first enzyme group seems to be parallel with fruit softening succeeding the climacteric rise. This parallelism was also found in the increase of water soluble pectin. So that, it is possible that the increase in these enzyme activities plays an important role in fruit softening. On the other hand, the hemicellulase and cellulase activities belonging to groups 2 and 3 were enhanced with over-ripening, which degrades the cross-linkage of neutral sugar linked to poly-galacturonic acid or cellulose. With the occurrence of cellular breakdown such as pithiness,[5] enhancement of these enzyme activities was observed. The high correlation between cellular breakdown and these enzymes may further be confirmed by the fact that arabinose and galactose were released from cell wall material with ripening. These hemicellulases and endo-cellulase are, hence, considered to play an important role in cellular breakdown with over-ripening.

Physiological characters of developing fruit and harvesting period

We divided the fruit life of Japanese pear fruit into 4 stages: the cell division, pre-enlargement, enlargement, and ripening stages. Each stage is accompanied with some peculiar biochemical changes and takes an essential part for obtaining desirable fruits. In particular, the role of the pre-

enlargement and ripening stages should be noticed.

In the pre-enlargement period, the fruit accumulated most of cell wall polysaccharides necessary for cell enlargement, held the high activities of cell wall-degrading enzymes, kept the highest density, and retained the potency of cell division of flesh tissue. Therefore, if the accumulation of polysaccharides in this stage was insufficient, the fruit cells seem to be unable to bear with the rapid enlargement of themselves without their breakdown. The cracking occurring in fruit flesh may be caused by this reason. So that, in case of insufficient accumulation of polysaccharides, the rate of enlargement of fruit has to be slowed down by some growth retardants to reduce the fruit damage, whereas the stimulation of enlargement rate by application of etherel should be avoided. Furthermore, as cells of the flesh in this stage can divide themselves secondarily, the fruit will recover to the level of ordinary cell numbers by exogenous hormonal treatments, for example gibberellic acid, at this stage, even if the cell division proceeded insufficiently in the cell division period. Thus, the determination of polysaccharide accumulation and cell numbers, and exogenous hormonal treatments based on its results may make it possible to protect fruit flesh from some physiological disorders which appear at the enlargement or ripening period.

When the ripening period starts, the fruit flesh is softened by a rapid enhancement of polygalacturonase and exo-cellulase activities, and then is broken down by a gradual rising of hemicellulases and endo-cellulase activities with over-ripening. Thus, the most desirable harvesting period of Hosui lasts for about 10 days from the start of an extensive increase in activity of enzyme group 1 to the beginning of clear increase in activity of enzyme group 3 (Fig. 6). On the other hand, soluble sugars of fruit on the tree were kept to increase gradually until over-ripening. Especially, accumulation of sucrose with ripening was notable.[9] Whereas, in the detached fruit, further sugar increase never occurred because of less accumulation of starch in ripening period. Therefore, if we want to obtain the most sweet fruit, fruit should be kept on trees as long as possible. In the case of

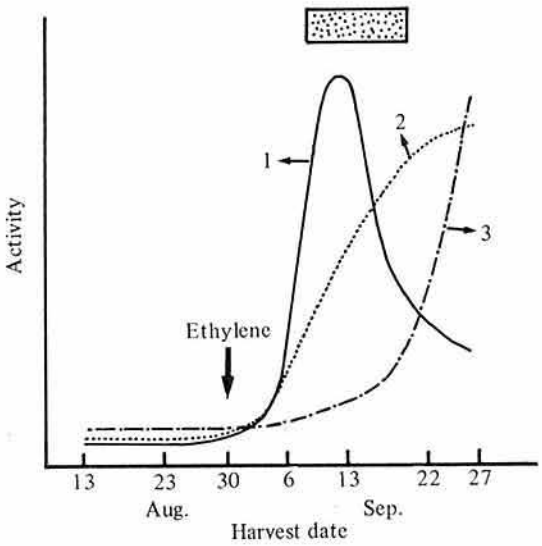

Fig. 6. Typical alteration of activity of enzyme group 1, 2 or 3 during fruit ripening of Japanese pear (var. Hosui)
Group 1 contains polygalacturonase, exo-cellulase and mannanase, group 2 arabanase, xylanase and β-glucosidase, and group 3 β-galactosidase and β-xylosidase. ▒ and ⬇ show the harvesting period for good texture of flesh and the beginning of ethylene evolution, respectively.

organic acids, their content is inclined to decrease gradually with stimulation of decarboxylation by a rapid rise of malic enzyme activity after climacteric rise. So that, acidity of fruit lowered gradually with the ripening. As described above, the best periods for harvesting fruit of good texture, good sweetness and good acidity, respectively, never coincided with each other. Thus, we must select the best harvesting period for each purpose such as long storage, short storage, or other utilizations.

With fruit ripening, various abnormalities are well known to appear in flesh, such as mealy breakdown, water core, pithiness or Ishinashi (like early symptom of black end of pear) fruit. These symptoms are accompanied by other degradation process of cell wall polysaccharides or/and other activation process of cell wall-degrading enzymes than those in the ordinary softening of fruit. There also existed great differences of flesh texture among fruit varieties, which were suggested to prominently correlate with the differences among polygalacturonase,

cellulase and arabanase activities. That is, the various textures of fruit seem to be formed by the genetically controlled various induction processes of some cell wall-degrading enzyme activities with ripening. In future, we can exclude some troubles based on the fruit texture, if the induction of these enzyme activities can be controlled artificially such as by treatments of some plant growth substances.

References

1) Hobson, G. E.: What factors are involved in the onset of ripening in climacteric fruit ? *Cur. Adv. Plant Sci.*, **11** (37), 1–11 (1979).
2) Keegstra, K., Talmadge, K. W., Bauer, W. D. & Albersheim, P.: The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Physiol.*, **51**, 188–196 (1973).
3) Labavitch, J. M.: Cell wall turnover in plant development. *Ann. Rev. Plant Physiol.*, **32**, 385–406 (1981).
4) Sacher, J. A.: Senescence and post harvest physiology. *Ann. Rev. Plant Physiol.*, **24**, 197–224 (1973).
5) Yamaki, S. et al.: Watercore in Japanese pear. III. Changes in the activities of some enzymes relating to the degradation of cell walls and the accumulation of sugar. *Scientia Hort.*, **6**, 45–53 (1977).
6) Yamaki, S. & Matsuda, K.: Changes in the activities of some cell wall-degrading enzymes during development and ripening of Japanese pear fruit (*Pyrus serotina* Rehder *var.* culta Rehder). *Plant & Cell Physiol.*, **18**, 81–93 (1977).
7) Yamaki, S. & Kakiuchi, N.: Changes in hemicellulose-degrading enzymes during development and ripening of Japanese pear fruit. *Plant & Cell Physiol.*, **20**, 301–309 (1979).
8) Yamaki, S., Machida, Y. & Kakiuchi, N.: Changes in cell wall polysaccharides and monosaccharides during development and ripening of Japanese pear fruit. *Plant & Cell Physiol.*, **20**, 311–321 (1979).
9) Yamaki, S., Kajiura, I. & Kakiuchi, N.: Changes in sugars and their related enzymes during development and ripening of Japanese pear fruit (*Pyrus serotina* Rehder *var.* culta Rehder). *Bull. Fruit Tree Res. Sta.*, **A6**, 15–26 (1979).

(Received for publication, July 31, 1982)