Changes in Abscisic Acid Content of Peel and Pulp of ‘Jonagold’ Apples during Pre- and Post-harvest Periods

Jamnong Uthaibutra and Hiroshi Gemma
Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba-shi, Ibaraki 305

Summary
The changes in the content of endogenous abscisic acid, membrane permeability and anthocyanin content of ‘Jonagold’ apples (Malus domestica Borkh.) were examined during the pre- and post-harvest periods. The endogenous ABA content of both peel and pulp did not change appreciably and remained less than 30 ng.g⁻¹ dry weight until 105 days after full bloom (DAFB). Thereafter, the ABA content tended to rise up through 160 DAFB parallel-ling the increase in anthocyanin content. After harvest, marked changes in fruit firmness, membrane permeability and ABA content were observed throughout the storage period. These results suggested that the presence of endogenous ABA is one of the factors which affect the maturation and senescence processes of the fruit.

Introduction
The changes in ethylene (C₂H₄) and carbon dioxide (CO₂) productions (17), fruit firmness (8, 17) and pigmentation (3) have been used as the markers to identify maturation stages of fruit, especially that of climacteric fruits. In climacteric fruits, including apple, the relationship between physiological and biochemical changes and fruit maturation as well as senescence has been examined.

It has been reported that the content of abscisic acid (ABA) increased markedly during the senescence of cut flowers (2, 4) and fruit maturation and senescence (7, 9, 13). The changes in the ABA content may be one of the factors affecting fruit maturation, ripening and senescence.

The present experiment was carried out to determine the endogenous ABA content in order to identify a possible effect of ABA on the physiological and biochemical changes of ‘Jonagold’ apples during the pre- and post-harvest periods. Additional data on fruit firmness, membrane permeability and anthocyanin content of peel were also obtained.

Materials and Methods
The study was conducted on ‘Jonagold’ apple fruit (Malus domestica Borkh.) collected during the pre- and post-harvest periods. The fruits were harvested at 10- and 15-day intervals between 25 June and 3 October 1989 from ‘Jonagold’/M.26 trees for the pre-harvest study. These trees were planted in the orchard of Agricultural and Forestry Research Center, University of Tsukuba, Ibaraki Prefecture in 1983~1984. For the post-harvest study, the fruits were harvested on 23 September (150 days after full bloom (DAFB)) and each batch of 15 fruits was placed in a corrugated cardboard carton, then kept at 20°C and 90~95% RH.

The relative humidity was controlled by a flow of humidified air over the fruits inside the cartons. Condensation of water under the high RH condition was prevented by placing 10 g of anhydrous copper sulfate bags into the cartons because the presence of water film on the fruit surface may lead to abnormal respiration. The storage conditions were monitored at 5-day intervals with a digital humidity meter (Model HN-K of Chino, Ltd.).

The following fruit characteristics were analyzed: 1. Fruit firmness: In 10 fruits for each sampling date the fruit firmness was measured with a Rheometer (Type NRM-2002 J, Fudokogyo Co., Ltd.) connected with a 3-mm dia. conical-tip plunger. Two readings were recorded per fruit at the opposite side of the equatorial diameter. 2. Membrane permeability: In this experiment,
membrane permeability was investigated for both peel and pulp tissues by measuring the electrolyte and K⁺ ion leakages from each portion. Peel and pulp disks which were removed and separated as described in the previous study (17), were weighed and rinsed with deionized-distilled water (DDW); free water was removed with a filter paper. The disks were incubated in a 100-ml beaker containing 50 ml DDW and shaken continuously at 20°C for 3 hr. Thereafter, the disks were collected and kept at −20°C for further analysis. Electrical conductivity of the incubated solution was measured to detect electrolyte leakage with a TOA Conductivity Meter-30 ET. The K⁺ ion leakage in the same solution was determined with a Hitachi 170-10 Atomic Absorption Spectrophotometer under the following conditions:

- **Lamp**: Hollow Cathode Lamp
- **Lamp current**: 15 mA
- **Wave length**: 766.5 nm
- **Gas for flaming**: Acetylene

The frozen disks were placed in DDW and boiled for 10 min in order to break down the membrane. Thereafter, the conductivity and K⁺ ion in the solution were also determined as above. The value was expressed as percentage of the total electrolyte leakage or K⁺ ion which leaked.

3. **Anthocyanin content**: Two grams of lyophilized peel was extracted with 20 ml of 2 M HCl and boiled for 40 min. The extract was filtered through an Advantec Toyo filter paper No. 2. The filtrate was fractionated two times with 20 ml each of ethyl acetate in order to remove flavones. The ethyl acetate fraction was discarded. The absorbance of the solution containing anthocyanin was determined at 530 nm with a spectrophotometer.

4. **Abscisic acid content**: Peel and pulp were separated and frozen in liquid nitrogen. The frozen samples were lyophilized and powdered and thereafter kept at −20°C. Initially, the moisture contents of the peel and pulp tissues were about 80 and 84%, respectively. The decrease in the moisture content of the peel was about 1% while that of the pulp was about 2% until the end of this investigation. Since there were some differences in moisture content of peel and pulp, the determination of ABA content in this study was based on dry weight.

Two grams of the lyophilized sample was extracted with 50 ml of 80% cold ethanol to which 0.5 g of polyvinylpyrrolidone (PVP) was added in order to remove the phenolic compounds and kept overnight at 4°C. The slurry was filtered through an Advantec Toyo filter paper No. 2. The filtrate was collected and stored at 4°C. The solid residue was re-extracted by the same procedure as above and the filtrates were combined and evaporated in vacuo to an aqueous phase. The aqueous phase was adjusted to pH 2.5 with 1 M HCl and extracted two times with 20 ml ethyl acetate. The organic fraction was collected and evaporated in vacuo until it was dry. Thereafter, the dried sample was dissolved in a few drops of methanol and 1 ml DDW. The dissolved sample was purified with a Sep-Pak C₁₈ Cartridge (Waters Associates, Milford, MA 01751) and eluted with 1 ml of 60% methanol. For the peel sample, the eluted sample was vigorously shaken after the addition of 0.03 g PVP in order to remove residual phenolic compounds, and then centrifuged at 3,000×g for 2 min. The supernatant was filtered through an Advantec Toyo type PTFF 0.5 μm membrane filter. Ten μl of the filtered sample was injected and the constituents separated by High Performance Liquid Chromatography (HPLC; Tosoh 8000 series) under the following conditions:

- **Column**: Zorbax ODS, 4.6 mm×25 cm
- **Column temperature**: 30°C
- **Mobile phase**: 40% methanol/60% DDW with 0.1% glacial acetic acid
- **Flow rate**: 1.0 ml/min
- **Detector**: UV (wave length at 254 nm)

In this experiment, we observed one peak which resembled ABA as it had the same retention time (22 min) as (+)-c,t-ABA (Sigma Co., Ltd.) which we used as standard. The fraction showing the peak in the HPLC-separation procedure was collected and identified by Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu GC-MS model QP 1000) after methyl esterification with diazomethane (10). The measuring conditions are shown in Fig. 4. The ABA conc. was calculated using a SIC integrator model 5000 E (System Instrument Co., Ltd.) as peak areas are proportional to the areas of standard ABA of known concentrations.

### Results

Fruit firmness decreased throughout the pre- and post-harvest periods (Fig. 1-A, B) whereas the electrolyte and K⁺ ion leakages increased during the
above periods (Fig. 2-A, B). Both the electrolyte and K⁺ ion leakages from the pulp tissues were higher than those from the peel tissues. During the storage of the fruits at 20°C, K⁺ ion leakage of the pulp tissues increased for the first 30 days and thereafter remained nearly constant. In case of the peel, the electrolyte leakage did not change appreciably whereas some K⁺ ions leaked during the post-harvest period (Fig. 2-B).

Anthocyanin content of the peel tissues, as determined by the absorbance at 530 nm, showed two peaks during the pre-harvest period (Fig. 3-A), the first at the young stage (60 DAFB) and the second at the mature stage of fruit (150~160 DAFB). Anthocyanin content in young fruits decreased between 60 to 105 DAFB, then peaked at the mature stage. When the fruits were stored at 20°C, the anthocyanin content gradually decreased until the end of the post-harvest period (Fig. 3-B).

The HPLC chromatograms of the peel and pulp tissues were compared with those of standard ABA under the same conditions and retention time (22 min). ABA in the sample collected from the HPLC-separation was identified based on the mass spectrogram and mass chromatogram (Fig. 4) obtained by GC-MS analysis.

ABA contents of both the peel and pulp tissues were less than 30 ng·g⁻¹ dry weight between 60 and 105 DAFB. After 105 DAFB, ABA contents increased during the pre-and post-harvest periods (Fig. 5-A, B). Those of the peel and pulp at 150 DAFB were about 2.5- and 3.0-folds, respectively, compared to levels at 60 DAFB. During the storage of the fruits at 20°C, the ABA content in the peel tissues increased and reached a peak 30 days after storage and remained constant thereafter up to 40 days; in the meantime ABA content of the pulp continued to increase until the end of the investigation period.

Discussion

A marked decrease in the fruit firmness was noted in 'Jonagold' apples during the pre- and post-harvest periods (Fig. 1-A, B). The changes in the fruit firmness are widely used as a marker of fruit stage (8, 17). We previously reported that fruit firmness decreased to a minimum value by the end of the storage period (17).

The membrane permeability was expressed as
electrolyte and $K^+$ ion leakages from the peel and pulp (Fig. 2-A, B). During fruit growth, the size of the cells increased while calcium content was low (data not shown), causing the cell wall to be weakened which resulted in the increase of electrolyte leakage (16). Electrolyte and $K^+$ ion leakages of the peel were not as high as those of the pulp. Differences in the tissue structure and composition such as the cuticle on peel (8, 16) may account for the differences in the changes in the membrane permeability. An increase in both electrolyte and $K^+$ ion leakages was also observed in the peel and pulp of fruits stored at 20°C (Fig. 2-B).

The anthocyanin content of the peel of ‘Jonagold’ apple showed two peaks during the pre-harvest period (Fig. 3-A). The cause of the appearance of the first peak at the young stage, which may be a specific characteristic of ‘Jonagold’ apple and may differ among cultivars, remains unknown. The second peak of anthocyanin content was observed at the mature stage. The increase in the anthocyanin content paralleled the increase in the ABA content (Fig. 3-A and 5-A). Adato et al. (1) reported that the ABA level in avocado flesh increased parallel to the rise in $C_2H_4$ production. Anthocyanin formation in grapevine fruit was increased by the application of ABA (11). Plich (13) suggested that ABA is one of the regulators in the last step of the $C_2H_4$ biosynthesis pathway and that $C_2H_4$ is involved in the anthocyanin formation (5, 8, 15). In our previous report (17), we also observed that an increase in $C_2H_4$ production paralleled the increase in red pigment of ‘Jonagold’ apple during the pre-harvest period.

Fig. 4. Mass spectrogram (left) and mass chromatogram monitoring $m/z$ 190 (right) of a standard of ABA (A, C) and apple peel extract (B, D) by GC-MS under the conditions of: Column; 2% OV-17 on Chromosorb W (80 – 100 mesh), 1 m x 2 mm internal dia. of glass column, Column temperature; 210°C. Carrier gas; Helium, 30 mL/min. Injection temperature; 270°C. Ion source temperature; 270°C. Ionized voltage; 70 eV. $M^+$ shows molecular ion of methyl esterified ABA.

$^x$ total ion intensity.

$^y$ monitoring $m/z$ 190.

Fig. 5. Changes in ABA content in peel (A) and pulp (B) of ‘Jonagold’ apples during the pre-harvest (A) and post-harvest (B) periods.
These evidences including the results of anthocyanin formation (Fig. 3-A) and ABA content (Fig. 5-A) as well as the previous data on C$_2$H$_4$ biosynthesis (17) suggest that C$_2$H$_4$ and ABA may be involved in the anthocyanin formation of 'Jonagold' apples during the pre-harvest period. On the contrary, we believe that anthocyanin formation at the young stage in this experiment is not associated with endogenous ABA.

ABA contents of the peel and pulp of the harvested fruits continued to increase throughout the post-harvest period (Fig. 5-B) which suggests that ABA may act as a promoter of the C$_2$H$_4$ biosynthesis (12, 13) which in turn stimulates fruit ripening and senescence. Ronen and Mayak (14) also reported that ABA stimulated the senescence processes of the carnation petal via C$_2$H$_4$. Alternatively, ABA may induce the sensitivity of the tissues to an advanced stage of senescence. Thereafter, the continued effect of C$_2$H$_4$ (2, 6) on fruit senescence is reflected by an increase in the membrane permeability, but this relationship may be coincidental.

In this study, we suggest that endogenous ABA may play a role in anthocyanin synthesis during the pre-harvest period and in the senescence process through the increase of the membrane permeability during the post-harvest period. The elucidation of the mode of action of ABA in the senescence process requires further studies.

**Literature Cited**


リンゴ‘ジョナゴールド’果実の発育および貯蔵期間における果皮と果肉の ABA 含量の変化

Jamnong Uthaibutra・弦間 洋
筑波大農林学系 305 つくば市天王台

摘 要
リンゴ‘ジョナゴールド’(Malus domestica Borkh.)果実の発育および貯蔵期間における、内生アプシジン酸(ABA)含量、膜透過性、およびアントシアニン含量の変化について調査した。収穫時に果実の果皮および果肉の ABA 含量は、収穫後 105 日までは少なかったが、(30 ng・g⁻¹ DW 以下)でほとんど変化しなかった。その後、収穫後 160 日まで内生 ABA 含量は僅かに増加し、果皮内アントシアニン含量も同様に増加した。
収穫後の果実においても、貯蔵中に果実硬さの低下、膜透過性の増大、および内生 ABA 含量の上昇が認められた。
以上の結果から、内生 ABA は果実の成熟と老化に関わる要因の一つであろうと考えられた。