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CHANGE IN THE CHEMICAL COMPOSITIONS OF APPLE FRUIT

I. Ethylene Evolution in Apple Tissue and Seed at Various Stages of Maturity

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Introduction

We have been studying the relationship between harvest dates and suitability for storage of apple fruit grown in Hokkaido. Concerning this problem, NAKASHIMA and TAMURA of our laboratory previously conducted a study on the respiratory climacterics for the determination of the optimum harvesting time of 'McIntosh' apple fruit storage. They found that the respiratory climacteric minimum was a useful indicator for the determination of the time to harvest the fruit for storage (13, 14). The respiratory climacteric minimum is important for fruit storage and may also be extensively applied for the determination of fruit harvesting time and also may be applied to studies of physiological ripening of fruit.

It is known that ethylene evolution of the fruit starts immediately prior to the time of respiratory climacteric minimum (6, 7) or immediately after it (3) and that it plays an important role as a 'ripening promotor'. In recent years, it has been established that ethylene evolves immediately prior to the respiratory climacteric minimum.

The development of highly sensitive gas chromatographic technics has made it possible to determine the critically triggering concentration of ethylene for respiratory climacterics (7). In the case of the Anjou pear, WANG et al. found that the first small peak of ethylene concentration was related to the initiation of softening and the 2nd larger increase was associated with the respiratory climacterics (17, 18).

Although numerous papers have been published on the ethylene evolution and concentration of apple flesh slices (8, 9, 10, 12, 15, 16) and whole fruits (3, 6, 11, 17, 18), only a limited amount of papers are available concerning the core and seed.

The present report presents primary data as a preliminary step to clarify the relationship between ethylene evolution and the respiratory climacteric in several parts of a fruit

Materials and Methods

Plant Materials: Two varieties of apple fruits, 'McIntosh' and 'Jonathan' were used in the studies on the changes of ethylene evolution and internal ethylene concentration at various stages of maturity. The fruits were grown at the Yoichi Experiment Orchard of Hokkaido University, and were picked at 5 day intervals from September 12 to November 1 in 1972. At each harvest time, 40 fruits were picked from two trees. After picking, a minimum of 3 hours elapsed before measurement of ethylene evolution.

Tissue Slices: Tissue slices were prepared from samples of flesh and core in the vicinity of the fruit equator. Cylinders of 8 mm in diameter were cut from these tissues with a cork-borer, and were sliced at 1 mm in thickness with a microtome. The disk-shaped slices were rinsed in tap water for 5 minutes and excess water on the surface was removed by filter paper. Apple peel was pared at 10 mm in width, 1 mm in thickness.

Gas Sampling: Five grams of tissue slices were placed in a Warburg vessel, shaken and incubated at 120 r.p.m. at 25°C. Prior to ethylene determination, the vessels were flushed with ethylene free air after passage through KMnO_4 on silica gel (1), and the side arm was sealed with a serum cap. Gas samples obtained after 2 hours and a gastight syringe was inserted through the serum cap. The internal atmospheres was drawn from the core cavities of the fruits by means of a hypodermic syringe. The fruit was submerged in water immediately after harvest.

Ethylene Determination: Gas chromatography with a hydrogen flame ionization detector was used in this study (YANAGIMOTO Gas Chromatograph Model G 800-F). Ethylene was determined by the following procedure. After incubation, the gas in the vessel was transferred into a gastight syringe, and injected into the port of the gas chromatograph. The condition of detector was as follows: Analytical column, 0.3×75 cm stainless tube packed with activated aluminum (YANAGIMOTO MFG, 60~80 mesh); carrier gas, N_2 ; gas flow pressure, 4 kg/cm^2 and bath temperature, 80°C.

The gases, emerging from this chromatographic column, were characteristically fractionated into two separate bands on activated aluminum: the first was unknown; the second contained only ethylene.

Results and Discussion

The tissues, used in this experiment, were taken from various parts of an apple (Fig. 1).

Experiment I. The wounding effect on ethylene evolution was described in the first experiment. Cutting and wounding generally causes increased evolution of ethylene and other volatiles in fruit tissue. MCGLOSSON and PRATT (12) found that ethylene evolution in tissue slices was much more in volume than that of intact fruits. Although BURG and THIMANN (9) found an increase in the respiratory rate in tissue slices obtained from post-climacteric apples, the ethylene evolution of excised tissue was of the same extent as that in the intact fruit, and ethylene evolution in slices was appreciably lower. In the present experiments, the evolution of the ethylene was enhanced by increasing the area of cut the surface. As shown in Table 1, there was no significant difference in ethylene evolution between slices of 1 mm and 10 mm in thickness.

Fig. 2 shows the time course of typical ethylene evolutions in the tissue slices and peel sections. The gas evolved slightly describing a curve within 4 or 6 hours after cutting and the tendency was in accordance with the results of BURG and THIMANN (9).

Experiment II. Changes of ethylene evolution and internal concentration on 'McIntosh' and 'Jonathan' are shown in Fig. 3 and 4 respectively.

As shown in Fig. 3, because the values of ethylene level of 'McIntosh'

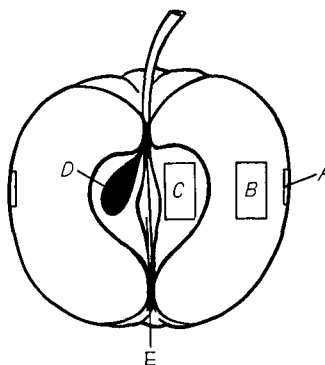


Fig. 1. A longitudinal section of an apple.

The following several parts were used in this experiments.

- A: peel B: flesh
- C: core D: seed
- E: core cavity

TABLE 1. Effect of cutting on ethylene evolution in Jonathan Apples at 25°C

Thickness (mm)*	0.5	1.0	5.0	10.0
Flesh (nl/g·hr)**	6.8	5.7	5.3	5.5
Core (nl/g·hr)**	30.3	21.1	17.7	18.6

* Cylinder of 8 mm in diameter and thickness are as in the table.

** Ethylene evolution is shown by nl per fresh weight gram at 25°C.

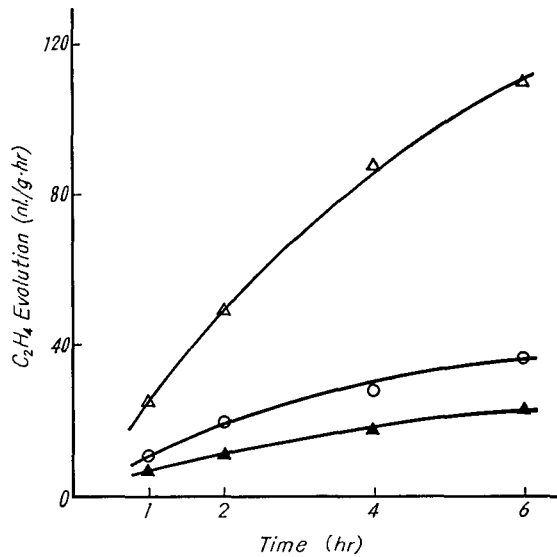


Fig. 2. Time course experiment of ethylene evolution in apple tissue, slices (8 mm in diameter and 1 mm in thickness) and peel sections (10 mm in width and 1 mm in thickness). (-○-; peel, -▲-; flesh, -△-; core, Samples were picked on October 22. Jonathan. at 25°C)

apple immediately prior and after the respiratory climacteric minimum were too small for determination using the scale of the graph, they were enlarged and presented in a small frame in the figure. Internal concentration of ethylene was maintained at an extremely low level until the onset of the respiratory climacteric rise. When the respiratory activity reached its climacteric minimum on September 17 (unpublished data), the ethylene level in the core cavity was approximately 1 ppm, at which concentration the respiration of these fruits was considered to be sufficient for stimulation. This seems to be in agreement with the results of BURG et al. (7) It is noted that various kinds of fruit show a respiratory rise under 1 ppm ethylene level.

Ethylene evolution in seeds and core slices were larger in amount than those of flesh slices. It seems possible that the ethylene in the core and seeds accumulates in the apple fruit and results in a climacteric rise in respiration.

The large increase in ethylene evolution in core slices commenced on October 7 and an extremely high concentration of ethylene was accumulated in the fruit on October 22 and subsequently the fruits dropped.

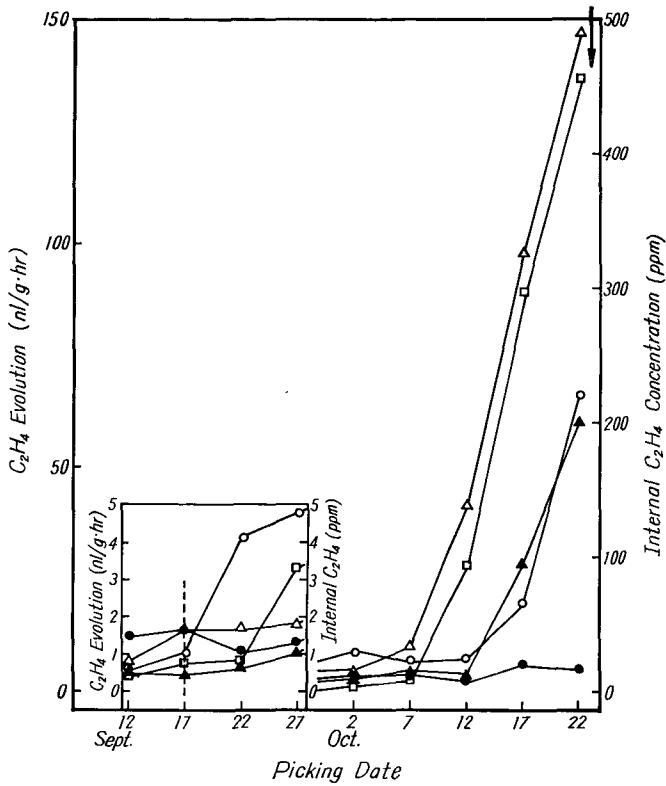


Fig. 3. Changes in ethylene evolution and internal concentration of apple fruit (McIntosh). Ethylene evolution nl per fresh weight gram at $25^{\circ}C$. Broken vertical line is the respiratory climacteric minimum. Arrow indicates fruit drop. ($-O-$; peel, $-▲-$; flesh, $-\triangle-$; core, $-●-$; seed, $-\square-$; internal concentration in core cavity)

According to BLANPIED and BARMORE et al. (2, 5) apple peel and pulp were considerably resistant to ethylene diffusion and extremely large amounts of the gas are accumulated in the fruit which results in fruit abscission and causes pre-harvest drop of fruit. It may be that the gas which induces the abscission might be mainly produced from the core in apple fruit.

As a result of our studies on 'Jonathan' (Fig. 4), ethylene concentration at the stage of the respiratory climacteric minimum was less than 1.3 ppm, and the gas evolution in the core sections and the seeds were larger in amount than those by the flesh. Due to the advance of the climacteric

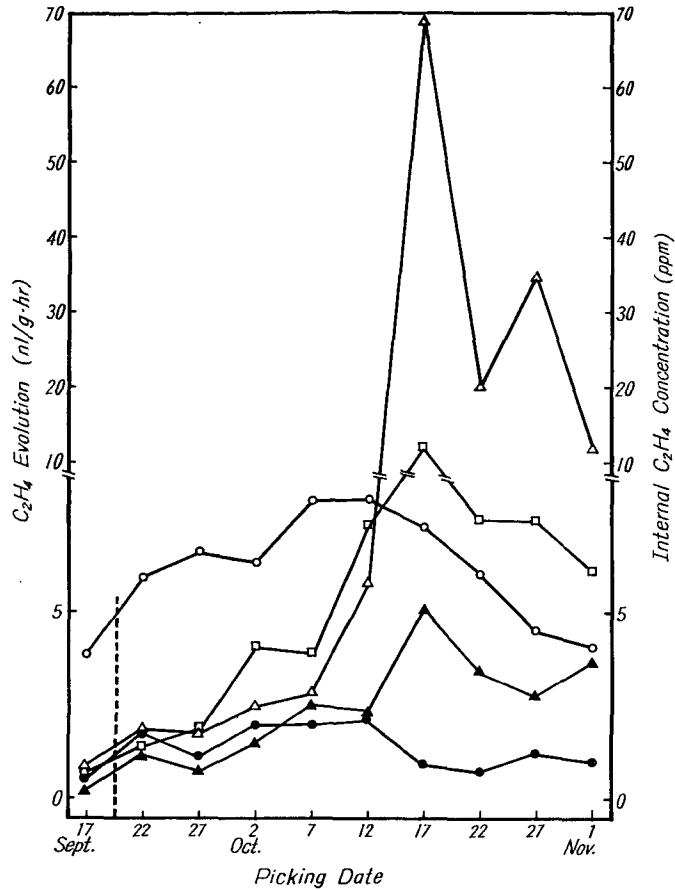


Fig. 4. Changes in ethylene evolution and internal concentrations of apple fruit (Jonathan).
See footnote in Fig. 3.

development, ethylene evolution in the core sections reached a peak at 69.01 $\text{nl/g}\cdot\text{hr}$ and at the same time the internal gas concentration was maximal on October 17 and thereafter decreased rapidly. The gas in the peel section increased steadily and reached its maximum on October 12 and thereafter decreased progressively.

At the stage of climacteric minimum, a large amount of ethylene was evolved from Jonathan apple peel section though only a small quantum of ethylene was evolved from the 'McIntosh' apple peel section, but it was not evident whether it was directly effective as the cause of the climacteric rise in respiration.

As for the internal ethylene concentration, while WANG et al. (17, 18) found 'the first small peak' using the method of BLANPIED (4) in the present work this could not be found.

Experiment III. Effect of inhibitor

As shown in Table 2, the ethylene evolution in the core section was greatly inhibited by 1/200 M malonate dissolved in 1/10 M potassium phosphate buffer solution (pH 5.0).

The method was as follows; five grams of the core section were soaked in the solution for five minutes and evacuated with an aspirator. After removing from the solution, excess liquid on the surface of the sections was removed with filter paper and were placed into WARBURG vessel. This was shaken and incubated in the same manner as in experiment I, II. The control was soaked in potassium phosphate buffer solution only.

As described above, malonate inhibited ethylene formation in the core sections. According to BURG et al. (7), ethylene formation in apple tissue was derived from acids of the TCA cycle. SHIMOKAWA et al. (15, 16) reported that pyruvate of the TCA cycle was decarboxylated to acetaldehyde

TABLE 2. Effect of malonate on ethylene formation in core section of Jonathan apple at 25°C

Concentration of malonate (Mol)	Ethylene formation (nl/g·hr)	Ratio of inhibition (%)
Control	23.3	0
10 ⁻³	16.4	29.6
5 × 10 ⁻³	3.8	83.2
10 ⁻²	1.3	94.6

Slices were treated with 1/10 M potassium phosphate buffer solution (pH 5.0) (control) or with the inhibitor dissolved in the same buffer. Each value in this table is the average of two or three experiments.

TABLE 3. Effects of aerobiosis and anaerobiosis of ethylene formation in core section of Jonathan apple at 25°C

Gas phase	Ethylene formation (nl/g·hr)	Ratio (%)
Air	23.3	100
Nitrogen	0	0
Oxygen	39.2	168.2

The ratio is calculated as 100 as the slice is treated with air. Each value in this table is the average of two or three experiments.

and converted to ethylene in apple tissue.

In anaerobic conditions, the ethylene formation of the core section was completely inhibited by pure N₂ gas. On the contrary, the ethylene formation was promoted by O₂ gas (Table 3).

The effects of inhibition by malonate and N₂ gas might suggest a possible correlation between the TCA cycle and ethylene formation by the core section.

On the other hand, LIBERMANN et al. (10), using apple flesh sections, investigated the mechanism of ethylene formation and reported that methionine was considered as a precursor of ethylene, and a copper enzyme system was related to ethylene evolution.

Concerning this problem, further studies are necessary.

Summary

The ethylene evolution by apple tissues sections and concentration in the core cavity in 'McIntosh' and 'Jonathan' apples were studied with highly sensitive gas chromatography.

At the respiratory climacteric minimum, the ethylene level in central cavity was approximately 1 ppm in the both varieties. The rate of ethylene evolution in the core section and seed was larger than that in the flesh section.

Accompanying the climacteric development, extremely large amounts of ethylene evolved in the core section, especially in 'McIntosh' it reached more than at 146.90 nℓ/g·hr and accumulated to 459.30 ppm in the core cavity and shortly after the fruit dropped.

Ethylene evolution in the core section was extremely inhibited by 1/200 M malonate and under anaerobic conditions (in pure N₂ gas) the ethylene evolution was terminated completely. The TCA cycle is possibly associated with the formation of ethylene.

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