Ethylene Formation during Ripening of Kiwifruit

Hiroshi HYODO, Saeko AIZAWA and Shinichi AO

*Department of Horticulture, Faculty of Agriculture,
Shizuoka University, Ohya, Shizuoka 422*

Summary

Kiwifruits (*Actinidia chinensis* Planch. cv. Hayward) began to produce ethylene at an increasing rate (exponentially) at 21°C after reaching the threshold level of $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$. The high rate of ethylene production is associated with fruit ripening. The time required for the fruits to reach the threshold level became much shorter and the wide range of variation in the time became less after fruits were stored for 4 months and longer at 2°C. Both 1-aminocyclopropane-1-carboxylic acid (ACC) and N-malonyl-ACC (MACC) levels increased in proportion to the rate of ethylene production in the range higher than $0.2 \text{ nmol g}^{-1}\text{h}^{-1}$, below which both ACC and MACC levels remained almost constant. Ethylene-forming enzyme (EFE) activity increased in parallel with the rate of ethylene production, which may indicate that EFE activity regulates ethylene production in the fruit tissue as well as the step of ACC formation.

Introduction

Kiwifruit is a climacteric fruit which shows a rise in ethylene production accompanied by an increase in respiration during ripening (3, 8, 9). The rise in ethylene production is followed by a decrease in flesh firmness. In a previous paper we reported that ethylene was produced at an increasing rate after exceeding a threshold level of $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$, that the increased rate of ethylene production was paralleled by the increased internal ethylene concentration in the fruit, and that ethylene biosynthesis in the kiwifruit may proceed along the methionine and 1-aminocyclopropane-1-carboxylic acid (ACC) pathway (3).

In the present study we investigated the changes in increasing patterns of ethylene production by individual kiwifruits after storage for various periods at a low temperature. We also studied the changes in ACC and N-malonyl-ACC (MACC) contents and the development of ethylene-forming enzyme (EFE) activity in relation to the increase in the rate of ethylene production.

Materials and Methods

Kiwifruits (*Actinidia chinensis* Planch. cv. Hayward) were harvested 7 November 1984 in Shizuoka at full maturity. They were stored at 2°C under high relative humidity until used. For determination of ethylene production by individual fruits, fruits were placed in 1.22-liter glass jars and sealed with plastic covers having one sampling port with a silicon rubber stopper. After 3 h, the head-space gas in the jars was sampled and assayed for ethylene as described elsewhere (3). The rate of ethylene production was measured every day at 21°C.

At various stages of ethylene production, the fruits were analyzed for ACC and MACC and assayed for EFE activity. Flesh tissue was homogenized with 10 times its volume (v/w) of 80% ethanol in a blender for 2 min. The homogenate was centrifuged at $8,000 \times g$ for 15 min at 2°C. The supernatant was concentrated to dryness in vacuo at 45°C in a rotary evaporator. The residue was taken up in 5 ml of water, and an aliquot of 0.5 ml was assayed for ACC by the method described by Lizada and Yang(7). MACC in the water extract was hydrolyzed in 2 N HCl at 100°C for 3 h. After hydrolysis the solu-

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tion was neutralized with 2N NaOH and centrifuged at $3,000 \times g$ for 10 min, and the supernatant was determined for total ACC content. The difference in the amount of ACC after and before acid hydrolysis was taken as that of MACC. ACC and MACC in the fruit extract were identified by paper chromatography.

EFE activity was assayed *in vivo* with flesh tissue in the presence of 1 mM ACC. Ten discs (9×2 mm) prepared from flesh tissue were placed in a 50-ml Erlenmeyer flask containing 6 ml of 10 mM phosphate buffer, pH 6.0, containing 1 mM ACC. The flasks were sealed with rubber serum caps and shaken at 120 strokes per min for 1 h at 24°C . The head-space gas in the flasks was sampled and assayed for ethylene by gas chromatography.

Results and Discussion

The rate of ethylene production was measured at 21°C for individual fruits immediately after harvest and during storage for 4, 6 and 9 months at 2°C . In every experiment each

fruit began to produce ethylene at an increasing rate after reaching the rate higher than $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$, which was considered to be the threshold level for kiwifruit(3). Fig. 1 shows the increasing patterns of ethylene production by single fruits which had been stored for 6 months at 2°C before they were measured at 21°C . Rates of ethylene production less than $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$ are not illustrated in the figure. Some fruits rapidly reached a level much higher than $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$ on transfer to the higher temperature at 21°C from the storage room at 2°C . The mean time and its standard deviation for exceeding the limit rate of $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$ were 2.1 ± 1.3 days ($n=19$). The values for fruits measured from 1 week after harvest in November were 15.9 ± 12.1 days ($n=19$) (3). Fig. 2 shows the changes in mean times and standard deviations for fruits to exceed the threshold level when measured after harvest and during storage for 4, 6 and 9 months at 2°C . After storage for 4 months and longer at 2°C , the time required to reach the increased rate became much shorter and the previous wide range of variation became less. It appears that exposure to a low temperature (2°C) or chilling stress may advance the onset of ethy-

Fig. 1. Time course of ethylene production by single fruits measured at 21°C after storage at 2°C for 6 months.

Fig. 2. The mean times and standard deviations for fruits to exceed the threshold level after harvest and storage at 2°C for various periods.

lene production at a greater rate in the fruit when transferred to a higher temperature (21°C). In cucumber fruit, chilling stress accelerates the induction of ACC synthase and the formation of ACC on transfer to a higher temperature, thus causing a high rate of ethylene production (10).

At various stages of ethylene production ACC and MACC contents were assayed in the flesh tissue (Fig. 3). Before the rate of ethylene production reached about $0.2 \text{ nmol g}^{-1} \text{ h}^{-1}$ ($4.8 \mu\text{kg g}^{-1} \text{ h}^{-1}$), the ACC level remained constant at 0.6 nmol g^{-1} . In the range higher than $0.2 \text{ nmol g}^{-1} \text{ h}^{-1}$ of the rate of ethylene production, ACC content rose progressively with the increasing rate ($\log Y = 0.084 + 0.514 \log X$, $r = 0.716$). The level of MACC was found to be much higher than that of ACC. The levels of MACC at the

Fig. 3. Relationship between rate of ethylene production by the fruits and ACC and MACC contents of the flesh tissue.

Fig. 4. Relationship between rate of ethylene production by the fruits and EFE activity in the flesh tissue.

different stages of ethylene production showed the same tendency as those of ACC, i.e. until the rate of ethylene production reached $0.2 \text{ nmol g}^{-1} \text{ h}^{-1}$, the level of MACC remained almost constant at 2 nmol g^{-1} and in the range exceeding $0.2 \text{ nmol g}^{-1} \text{ h}^{-1}$, MACC content increased in parallel with that of ACC ($\log Y = 0.816 + 0.486 \log X$, $r = 0.773$), being more than 5 times higher than that of ACC (Fig. 3). The fact that the ACC level increased in proportion to the rate of ethylene production at the higher rates may indicate that the rise in ACC is responsible for the increase in the rate of ethylene production. It is interesting that the level of MACC was much higher than that of ACC throughout the range of ethylene production, which suggests that ACC-malonyltransferase activity (5,6) is high enough in either unripe or ripe kiwifruit to convert ACC to the malonyl derivative (MACC).

EFE activity in the flesh tissue was assayed for the fruits producing ethylene at different rates. There was a close relationship (Fig. 4) between EFE activity and the rate of ethylene production by each fruit ($r = 0.927$), which may indicate that EFE activity is a limiting step regulating ethylene formation in the kiwifruit. It has been shown that EFE activity increases with the rise in ethylene production where ethylene is considered to be responsible for the induction and development of EFE (1, 2).

We reported previously that ethylene biosynthesis in the kiwifruit may proceed along the methionine and ACC pathway (3). The present data support the view that ACC may act as an intermediate in ethylene biosynthesis and EFE may play an important role in regulating ethylene biosynthesis in the kiwifruit. We also reported that in the wounded mesocarp tissue of winter squash fruit, ACC synthase and EFE were markedly induced in response to wounding and a large amount of ACC was formed in the wounded tissue as a result (4). The great difference between the amount of ACC in ripe kiwifruit and in the wounded mesocarp tissue of winter squash may be due to the much higher activ-

ity of ACC synthase found in the latter tissue.

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キウイフルーツ追熟中のエチレン生成の増大

兵藤 宏・相沢彩恵子・青 真一

静岡大学農学部 422 静岡市大谷

摘 要

キウイフルーツ (*Actinidia chinensis* Planch. cv. Hayward) のエチレン生成量は 21°C で $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$ の限界値を越えた後急激 (指数関数的) に増大した。顕著なエチレン生成は果実の追熟と密接な関連を有している。収穫直後の果実では $0.1 \mu\text{kg}^{-1}\text{h}^{-1}$ の限界値を越すのにかなりの時間を必要とし、また個体間の差異がかなりみられたが (15.9 ± 12.1 日)、果実を低温下 (2°C) で貯蔵することにより、常温 (21°C) に移した後のエチレン生成はかなり早まり、個体間のばらつきも小さくなった (6ヶ月貯蔵後, 2.1 ± 1.3 日)。キウイフルーツでエチレン生合成の中間体とみなされる 1-アミノシクロプロパン-1-カルボン酸 (ACC) および N-マロニル-ACC

(MACC) の果実中の含量は個体果実のエチレン生成量が $0.2 \text{ nmol}^{-1}\text{g}^{-1}\text{h}^{-1}$ ($4.8 \mu\text{kg}^{-1}\text{h}^{-1}$) まではほぼ一定値 (0.6 nmolg^{-1} と 2 nmolg^{-1}) であったが果実のエチレン生成量が上記の値を越すにつれ共に平行して増大した。この時 MACC の含量は ACC の含量より 5 倍以上高かった。エチレン生成酵素 (EFE) の活性は果実のエチレン生成量と密接な相関を有し、果実のエチレン生成量の増大と比例して著しく増加した。これらの結果は前報で述べたキウイフルーツにおけるエチレン生合成はメチオニン-ACC 経路を通るという結論を支持するものと思われる。