

熟度の異なるカットフルーツにおける貯蔵中のエタノール、メタノールの生成

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Production of Ethanol and Methanol in Fresh-Cut Fruits at Different Maturity Stages during Storage

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Ethanol and methanol levels in fruits are fundamental to the production of fruit aroma. The levels of these chemicals in selected cut-fruits (bananas, pineapples, melons, tomatoes, kiwifruits, and strawberries) were measured immediately after cutting and 24 h after storage at 3 °C, at unripe, ripe, and overripe stages. Overripe fruits had higher levels of ethanol and higher ethyl acetate emission than those at the ripe stage, and all of them developed off-flavor, except kiwifruits. The ethanol content in one of the two cultivars of strawberry examined, increased dramatically 24 h after cutting, although it was at a good ripening stage. The methanol and ethanol contents in tomatoes increased sharply and gradually, respectively, after cutting. The strawberries and tomatoes had an unusual aroma 24 h after cutting. Higher alcohol dehydrogenase (ADH) was detected in bananas, pineapples, melons, and kiwifruits. These fruits were shown to have a quick increase in ethanol contents while ripening. The sudden increase in ethanol after cutting or aging of ‘Sagahonoka’ strawberry could not be explained by ADH activity. The gradual increase in ethanol content in tomatoes may be due to de novo induction of ADH. Tomatoes had very high levels of pectin methyl esterase and were shown to have a quicker increase in methanol levels after cutting. An ability of acetate ester production was detected in all fruits examined, except in kiwifruits. To conclude, the accumulation of ethanol and ethyl esters, especially ethyl acetate, seems to accelerate off-flavor in the overripe stage or during aging. An analysis of ethanol and methanol content of cut fruits over short time duration (2 h) was conducted at room temperature (23 °C) using bananas, tomatoes, and strawberries. The changes in alcohol levels were almost the same as those at 24 h after cutting (at 3 °C) in bananas. The two cultivars of strawberries used in this experiment showed equal increases in ethanol content at room temperature. This analysis also revealed that ethanol content in strawberries and tomatoes were at negligible levels immediately after cutting, then increased sharply in strawberries, while did so gradually in tomatoes.

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Key words : ethanol, methanol, fresh-cut fruit, esters, alcohol dehydrogenase

エタノール, メタノール, カットフルーツ, エステル, アルコールデヒドロゲナーゼ

Previous studies on the quality and safety of fresh-cut fruits have been mainly focused on their shelf-life for one to two weeks under refrigerated conditions and improved packaging to provide solutions for the industry requirements to supply

fresh-cut products regionally or nationally in many countries including USA¹⁾. However, in Japan, fresh-cut fruits are prepared at retail grocery stores and are ready for sale immediately after processing or at least until by the next day. Although some fresh

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cut producers have been attempting to distribute to a wider range, the marketable period of fresh-cut fruits is approximately one to three days. Another type of business is widely developing to serve fruits after fruit cutting at room temperature to answer the demand for party or catering services.

While research on fresh-cut banana has been conducted in Japan²⁾, the research has expanded to cut-persimmon^{3),4)}, and cut-citrus fruits for long term storage recently⁵⁾. Compared to cut vegetables, fresh-cut fruits normally have much higher water activity due to a much larger cut surface, and are more perishable and fragile. Therefore, the selection of fruits and their cultivar for fresh-cut processing must be done carefully^{1),6),7)}. A number of efforts have been made to improve the quality of the fresh-cut fruits and to extend the shelf-life, including prevention of softening, browning, off-flavor development, and reducing the growth of spoilage bacteria by means of physical and chemical treatments^{8)~15)}.

A number of studies have reported the changes of aromatic volatiles in fresh-cut fruits including melon^{16)~19)}, apple¹⁹⁾, pineapple²⁰⁾, mango^{21),22)} and watermelon²³⁾. Post-harvest formation of the endogenous alcohols, ethanol and methanol, in fruits are considered as fundamental compounds for aroma in fruits, yet the presence of an excess amount of ethanol may cause off-flavor development^{24),25)}.

The objective of this study was to analyze the concentrations of methanol and ethanol and release of their acetate esters from several fresh-cut fruits at different ripening stages immediately after cutting and after 24 h storage (at 3 °C) in connection with off-flavor development. Analysis of these alcohols immediately after cutting and up to 2 h at room temperature (at 23 °C) was also conducted for application in other types of businesses like cut-fruits decoration for catering or parties.

Materials and methods

1. Plant materials and treatments

The bananas and tomatoes used in this study were purchased from local wholesale markets under the guide of international color charts. Other fruits were purchased following by consumers favorite. The “ripe” fruits are chosen by consumers favorably and taste immediately. The “unripe” fruits are chosen by consumers who expect to eat them

several days after purchase. “Overripe” fruits are too soft and have off-flavor, and are typically avoided by consumers. Individual ripening changes of the fruits used in this experiment are as follows.

Bananas (*Musa paradisiaca* (Cavendish)) were from the Philippines and used at the following three stages under USDA color chart guide: yellow with green tips (unripe), yellow (ripe) and over ripe with brown flecks (overripe). Tomatoes (*Solanum lycopersicum* (Momotaro)) were from Hokkaido, and used at pink (unripe), light red (ripe) and red (overripe) as defined in the USDA color chart. The ripening stages of pineapples (*Ananas comosus* (Smooth Cayenne)) from the Philippines and melons (*Cucumis melo*, an orange flesh Cantaloupe) grown in Aomori, were determined by pressing and sniffing the butt of pineapples or blossom end of melons. When these parts are very hard and had no fruity smell, they are judged to be unripe. When these parts were slightly soft and had a characteristic good aroma, they were judged to be ripe. The overripe stage of both fruits was associated with a softer butt or blossom end, and we sensed some off-flavor development from the smell. Strawberries (*Fragaria ananassa* (Sagahonoka)) from Nara, and another berries strain (Sachinoka) from Nagasaki were purchased at the ripe stage, which showed the best quality at the time. Some of the berries were aged for 2 days at 3 °C. Strawberries have no ripening after harvest, so they were named aged fruits. The cultivar, (Sachinoka) have pretty hard flesh than did the other cultivar of berries (Sagahonoka). Kiwifruits (*Actinidia chinensis* (Golden Kiwifruit)) originated from Ehime, were purchased at the ripe stage, and some of them were ripened to an overripe stage which was indicated by fruits that were too soft for eating.

All the fruits except the strawberries were first washed with tap water and patted dry with paper towel. They were cut into round slices of 5–8 mm thickness along with the equatorial part, and the stem and calyx ends were discarded. Inedible peel, core, and seeds were also removed. Slices from pineapples and melons were further cut into quarters. At each ripening stage, the fruit slices from three fruits were individually packaged in three plastic containers with 2 holes (5 mm diameter) on the top, and then stored in a refrigerator (3 °C) for 24 h. The fruit slices were assessed for methanol and ethanol contents and their acetate

ester production immediately after cutting and 24 h after cold storage (3°C).

2. Determination of methanol and ethanol content

Methanol and ethanol concentrations in fruit tissues were determined using the method described by Bartolome and Hoff²⁶⁾ with slight modifications. Fruits pulp (1 g) was prepared quickly with a sharp knife and was dropped in a 17 ml-vial, which was precooled on ice with 2.5 ml of phosphoric acid (7%). The pulp in the vial was immediately crushed by a glass bar. After addition of 2.5 ml of cold potassium nitrite (5%), the vial was capped and shaken vigorously for 2 min on ice. The head space gas (1 ml) was taken by a glass syringe through a silicon cap and analyzed by gas chromatography (GC). Standard curves (ppm) of ethanol and methanol were developed by serial dilutions of the pure alcohols in water. Triplicate samples from three fruits (from different hand in the case of banana) were analyzed for alcohol contents and used in the other experiments. The timing of analysis was immediately after cutting and 24 h after cutting at each ripening stage. The data are shown as an average of three data sets with their standard deviation in the results.

The alcohol contents in these ripe fruits of bananas, tomatoes, and strawberries were also examined at short time intervals up to 2 h after cutting at room temperature (23°C) by the method above mentioned.

3. Analysis of ethyl and methyl acetate emission

The pulp slices (5 g) were put into a conical flask (150 ml), sealed and incubated at 23°C for 150 min. The head-space gas (1 ml) was taken through a silicon cap by a glass syringe, and the gas was injected into a GC for determination of ethyl acetate and methyl acetate. Concentrations of ethyl and methyl acetates were calculated by comparing the peak area to those of the pure standards.

4. Ester formation by addition of isobutyl alcohol

Determination of ester-forming ability was conducted by addition of external isobutyl alcohol to fruits slices. The pulp slices (5 g) in a conical flask (150 ml) were used in the same manner as mentioned in method 3. A small filter paper immersed in 10 μ l of isobutyl alcohol was put into the flask. After incubation for 150 min at 23°C, formation of isobutyl acetate was analyzed by GC.

5. Conditions of gas chromatography

A gas chromatograph equipped with a flame

ionization detector and a glass column (polyethylene glycol 9,000, 3 m \times 3 mm) was used for the alcohol analysis in fruit tissues and ester formation from the pulp of cut fruits. The column temperature was 23°C (room temperature) for alcohol analysis and 90°C for ester analysis. The flow rate of the nitrogen carrier gas and hydrogen gas were 20 ml min⁻¹ and 30 ml min⁻¹ respectively.

6. Extraction and assay of alcohol dehydrogenase (ADH) and pectin methyl esterase (PME)

Crude enzymes were prepared from one gram of fruit pulp by homogenization in a chilled mortar with pestle in 9 ml of a phosphate buffer (0.2 M, pH 7.7) containing an insoluble type of polyvinylpyrrolidone (PVP) (0.5 g), and centrifugation at 13,000 \times g for 20 min at 4°C. Since strong protease activity was expected in pineapple, melon, and kiwifruit, phenylmethylsulfonyl fluoride was added in the extraction solution (1 mM).

ADH activity was determined by monitoring the oxidation of NADH at 340 nm at 30°C using a spectrophotometer (BECKMAN DU 640). The reaction mixture was composed of 1.7 ml of MES buffer (0.1 M, pH 6.5), 0.1 ml of NADH (1.6 mM), 0.1 ml of acetaldehyde (80 mM), and 0.1 ml of crude enzyme in a total volume of 2 ml. The optical density of NADH was traced for 100 sec. The initial stable part of the reduction curve was chosen for calculations and translated to NADH extinction with a molecular extinction coefficient (6.22×10^3 M⁻¹ cm⁻¹) and indicated μ mol per gram fresh weight per min (gFW⁻¹ min⁻¹).

PME activity was measured by mixing 0.1 ml of crude enzyme and 0.9 ml of pectin solution (1%) in phosphate buffer (pH 7.7, 0.2 M) in a vial (17 ml) for 6 min at 30°C. Methanol formation during incubation was ceased by the addition of 2.5 ml phosphoric acid (7%), and methanol concentration was determined in the same way as for alcohol analysis by GC mentioned section 2. The activity was shown as μ g of methanol formation per gFW⁻¹ min⁻¹. A weak activity of PME was detected in strawberries. In that case, 0.1g of pulp was mixed as the enzyme to 0.9 ml pectin solution directly.

Results and Discussion

1. Methanol and ethanol content and their acetate ester production in fresh-cut fruits immediately after cutting and 24 h after cutting at three ripening stages

While a negligible amount of methanol was detected in the bananas and pineapples, ethanol concentration and ethyl acetate production at the overripe stage of the fruits were found to be about 4-5 times higher than at the ripe stage. No considerable difference either in ethanol levels or ethyl acetate production was observed between immediately after cutting (0 h) and 24 h after storage in both banana and pineapple at the ripe and overripe stages (Fig. 1, Fig. 2). Such a high emission of ethyl acetate at the overripe stage is considered to be the cause of off-flavor development in these fruits²⁷, even though ripe banana emits acetate esters of branched chain alcohols (isobutyl, isoamyl alcohols) and of butyl alcohol as the characteristic banana aroma²⁸, and ripe pineapple produces such as ethyl 3-(methylthio) propionate, ethyl butanoate, and ethyl (*E*)-3-hexenoate as the

volatile compounds of pineapple aroma²⁹. In general, the both fruits at the unripe stage have the problems of sour taste and weak aroma. Therefore, it is important to choose the proper maturity stage for use in fresh-cut fruit processing of these kinds of fruits.

In spite of some fluctuation in ethanol concentration in melon pulp, about 100 $\mu\text{g gFW}^{-1}$ of ethanol was detected at the ripe stage, and the ethanol content did not increase towards the overripe stage (Fig. 3). The methanol concentration in cut melon doubled 24 h after cutting over immediately after cutting (0 h). Ethyl acetate production increased two-fold at the overripe stage compared with the ripe stage of melon. Off-flavor development at the overripe stage seemed to be caused by high ethyl acetate. Although ethyl acetate itself is one of the characteristic aromas for melon, Beaulieu and Lancaster¹⁸ revealed that acetate esters cannot be correlated to the fruity aroma of melon. The melon used in this

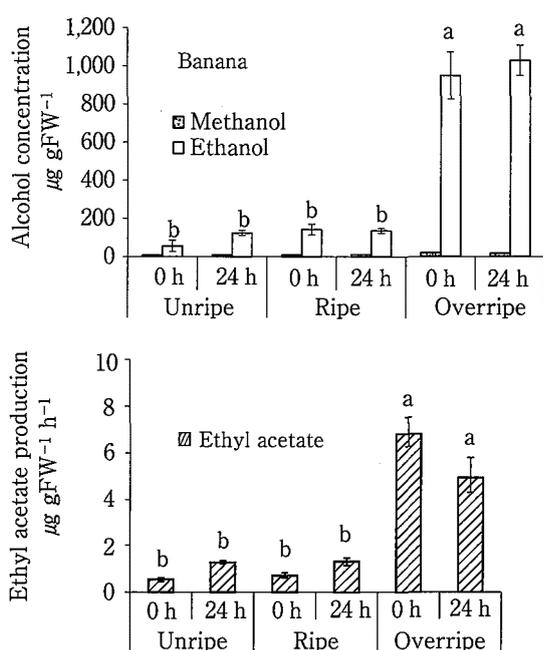


Fig. 1 Ethanol and methanol content (Above) and ethyl acetate production (Below) of banana pulp 24 h after cutting at different ripening stages

"0 h" means just after cutting. "24 h" means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation (n = 3). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey's range test after ANOVA test)

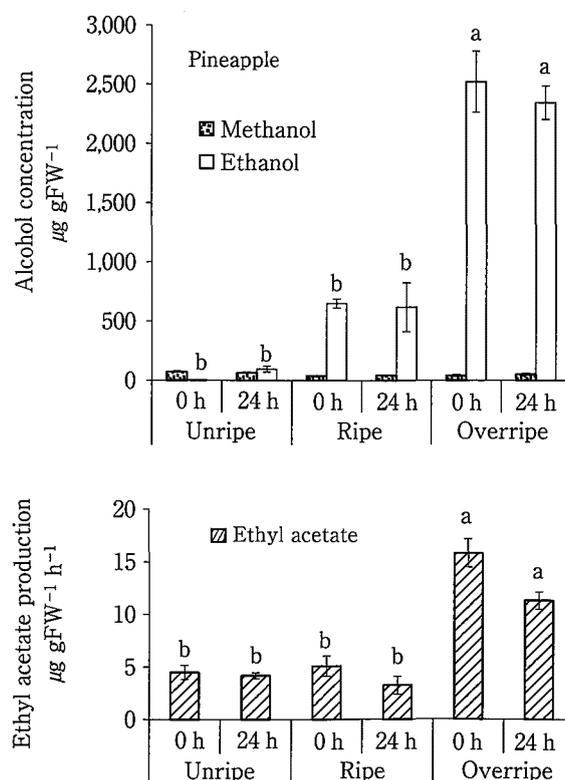


Fig. 2 Ethanol and methanol content (Above) and ethyl acetate production (Below) of pineapple pulp 24 h after cutting at different ripening stages

"0 h" means just after cutting. "24 h" means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation (n = 3). Different letters above the bar indicate significant difference at $p < 0.05$ (Tukey's range test after ANOVA test)

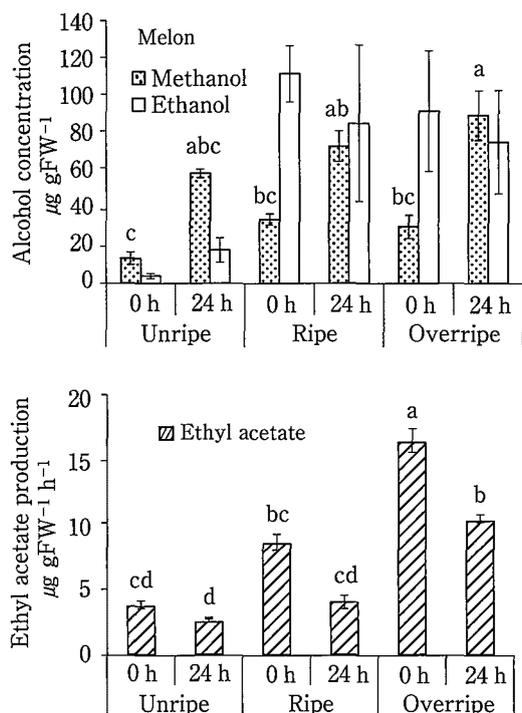


Fig. 3 Ethanol and methanol contents (Above) and ethyl acetate production (Below) of melon pulp 24 h after cutting at different ripening stages

“0 h” means just after cutting. “24 h” means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation ($n = 3$). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey’s range test after ANOVA test)

experiment has netted skin with reddish flesh (cantaloupe type). However, there are a lot of different varieties of melons and F1 hybrids. These fruits have wider climacteric ripening characteristics and have different ways of changes in hardness and aroma during ripening. We need more studies on aroma of different varieties after cutting.

Tomato is popularly used as cut slices in salads and considered as a vegetable instead of a fruit. Small levels of methanol were observed in tomato slices immediately after cutting (0 h), and the level increased 24 h after cutting (3 °C) at all ripening stages (Fig. 4). These changes became apparent in an overripe tomato. Regardless of the accumulation of high levels methanol in tomato after cutting (60 and 120 $\mu\text{g gFW}^{-1}$ in the ripe and overripe samples, respectively), off-flavor development in relation to methanol has not been reported. It may depend on the high threshold value of methanol compared with ethanol³⁰. Because of some accumulation of ethanol after cutting of tomato, freshness seems to be lost 24 h after cutting. Additionally, a high methanol

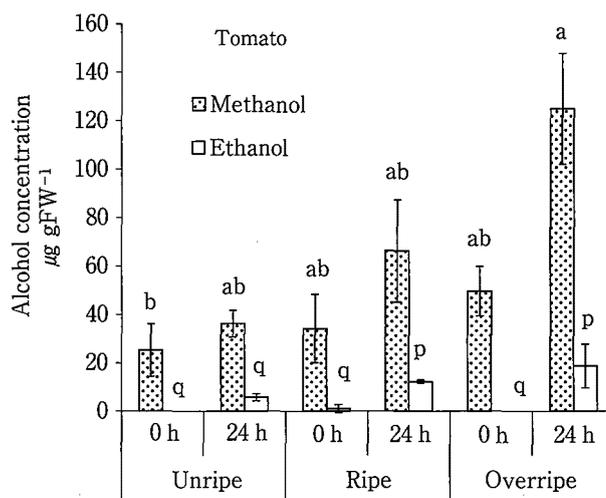


Fig. 4 Ethanol and methanol content of tomato pulp 24 h after cutting at different ripening stages

“0 h” means just after cutting. “24 h” means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation ($n = 3$). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey’s range test after ANOVA test)

content seems to mask the freshness of tomato slices after cutting. We need more studies at this point. Ethanol and acetaldehyde accumulation during long term storage of tomato were recognized as off-flavor forming compounds³¹. Ethyl acetate formation from tomato was not detected (data not shown).

Methanol and ethanol levels in kiwifruit were low compared with the other fruits used in this study. But these alcohol levels increased to a small extent at the overripe stages and further increased 24 h after cutting (Fig. 5). Fresh-cut kiwifruits may be stable in terms of holding their aroma, but softening may be the major concern with this cut fruits³². Ethyl acetate was not detected in kiwifruits (data not shown) during the experiment.

We used two types of strawberries in this study; one has softer flesh (Sagahonoka) than the other harder one (Sachinoka). The changes in ethanol content and ethyl acetate emission after cutting and ageing in these two strawberry types were quite different. Ethanol in softer flesh strawberry increased 24 h after cutting at the ripe stage, and also increased sharply at the aged stage and remained at a higher level 24 h after cutting (Fig. 6). Ethyl acetate emission from these softer flesh berries increased to some extent 24 h after cutting, but increased more sharply at the aged stage and continued to a higher level 24 h after cutting. These sharp changes make the softer berries to

have an unusual aroma similar to raspberries. In the harder type of strawberry (Sachinoka), changes in the alcohol contents and evolution of esters were similar to those in the softer type, but these

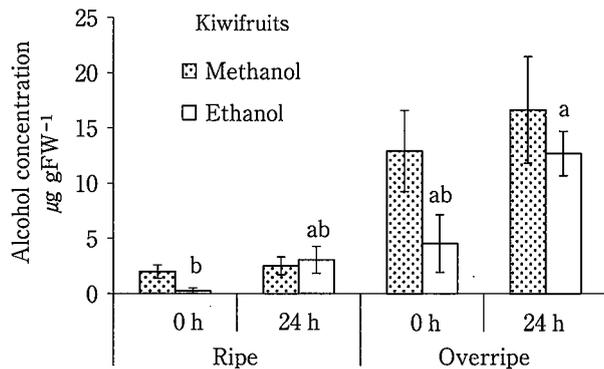


Fig. 5 Ethanol and methanol content of kiwifruits pulp 24 h after cutting at different ripening stages

“0 h” means just after cutting. “24 h” means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation (n = 3). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey’s range test after ANOVA test)

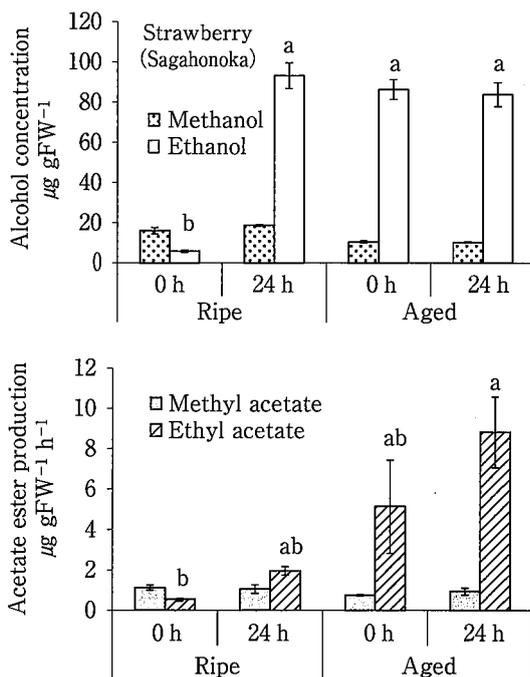


Fig. 6 Ethanol and methanol content (Above) and ethyl acetate and methyl acetate production (Below) of Strawberry (Sagahonoka) pulp 24 h after cutting at different ripening stages

“0 h” means just after cutting. “24 h” means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation (n = 3). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey’s range test after ANOVA test)

changes were very low when compared with the softer type (Fig. 7). Therefore, harder flesh type strawberries are better at retaining of fresh-cut aroma. Methanol and methyl acetate were detected to some extent, and remained low with aging and cutting in both berries (Fig. 6, Fig. 7). It is well known that, when strawberries are packed with a plastic film or subjected to high CO₂ treatment, such off-flavor (accumulation of ethanol and ethyl acetate) was developed^{33)~35)} because of fermentation condition. Among the varieties of strawberries, a difference has also been reported relating to their tolerance in high CO₂ conditions³⁵⁾.

2. Alcohol dehydrogenase (ADH), pectin methyl esterase (PME) and ester-forming activity after cutting

ADH and PME are important enzymes for ethanol and methanol formation, respectively. Higher ADH activity was detected in bananas, pineapples, melons, and kiwifruits at the ripe stage (Fig. 8). All of these fruits had high ethanol content at the ripe stage or when becoming overripe, except kiwifruits.

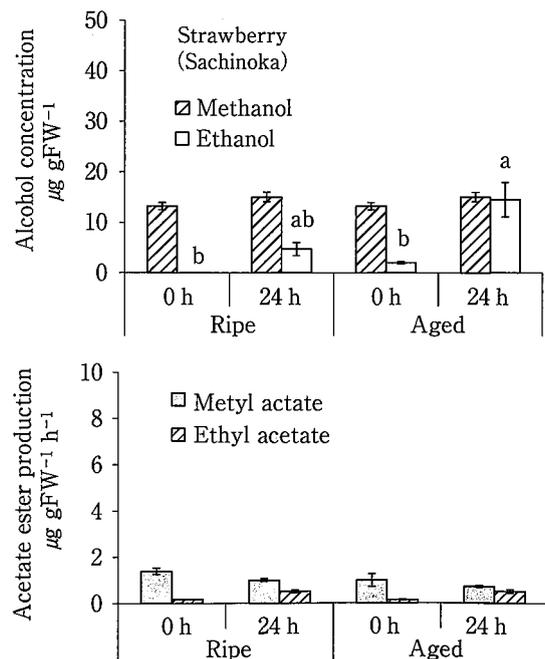


Fig. 7 Ethanol and methanol content (A) and, ethyl acetate and methyl acetate production (B) of Strawberry (Sachinoka) pulp 24 h after cutting at different ripening stages

“0 h” means just after cutting. “24 h” means 24 h after cutting (3 °C). Vertical lines on bar graphs show standard deviation (n = 3). Different letters above the bar indicate significant difference at $p < 0.05$ (Tukey’s range test after ANOVA test)

Kiwifruits had relatively high level of ADH activity at the ripe stage while the ethanol level in the pulp was quite low. An explanation for this discrepancy is very difficult right now. The ADH activity of tomatoes was relatively low at the ripe stage. The ethanol content of tomatoes was negligible just after cutting at any ripening stage and increased only after cutting (Fig. 4). ADH activities in the two cultivars of strawberries (Sagahonoka and Sachinoka) were almost the same at the ripe stage (Fig. 8), but ethanol contents showed much greater differences between them during aging or after cutting (Fig. 6 and Fig. 7). Additional research is needed to understand this further.

PME, which catalyzes methanol production from pectin, was detected about 10 times higher in tomato pulp than in the other fruits as shown in Fig. 9. The immediate increase of methanol in fresh-cut tomatoes could be attributable to this higher PME activity. A similar conclusion has been described previously where higher PME activity in tomato correlates with the methanol and also with the ethanol accumulation in tomato³⁶. The report states that off-flavor from tomato depends mainly on high levels of ethanol formed during long time storage after cutting.

Esterification of vaporized isobutyl alcohol on fruit slices is shown in Fig. 10. The esterification level must be reflective of the activity of alcohol

acyltransferase (AAT) and the background conditions of ester formation. The ester-forming ability of AAT was detected among fruits used in this study although only trace amount activity was detected in kiwifruit. The off-flavor at the overripe stages of bananas, pineapples, and melons were not only accompanied by high ethanol contents but also high ethyl acetate emission. This means that ester-forming ability seems to be one factor for off-flavor formation. We sometimes sensed an ester smell on kiwifruit at retail shops. According to a study³⁷, the kiwifruit is initiated ripening in two ways. One is initiated by ethylene and another is by rising temperature from chilled condition without any ethylene production. We are interested in which treatments make more ester production.

3. Alcohol content of fruits in a short time after cutting at room temperature

There are businesses to answer the demand for cut fruits decorations such as catering for a party or buffet style restaurant. So, the alcohol contents of some ripe fruits (banana, tomato and strawberry) were analyzed in a short time up to 2 h after cutting at room temperature (23 °C). The ethanol and methanol concentrations of bananas during the time course are shown in Fig. 11. A high concentration of ethanol was found in banana pulp at ripe stages and the ethanol level decreased slightly after cutting. The fruits had a good smell

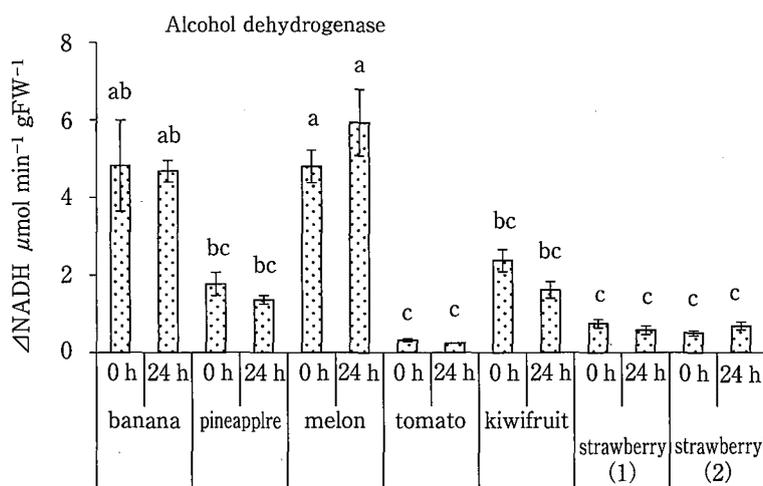


Fig. 8 Alcohol dehydrogenase (ADH) activities of cut fruits just after cutting (0 h) and 24 h after cutting at ripe stage

Strawberry (1) and strawberry (2) show Sagahonoka and Sachinoka respectively. Vertical lines on bar graphs show standard deviation ($n = 3$). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey's range test after ANOVA test)

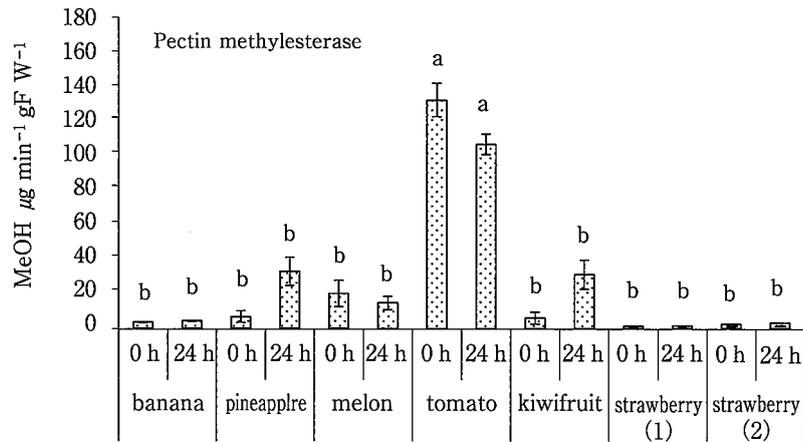


Fig. 9 Pectin methylesterase (PME) activities of cut fruits just after cutting (0 h) and 24 h after cutting at ripe stage

Strawberry (1) and strawberry (2) show Sagahonoka and Sachinoka respectively.

Vertical lines on bar graphs show standard deviation ($n = 3$). Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey's range test after ANOVA test)

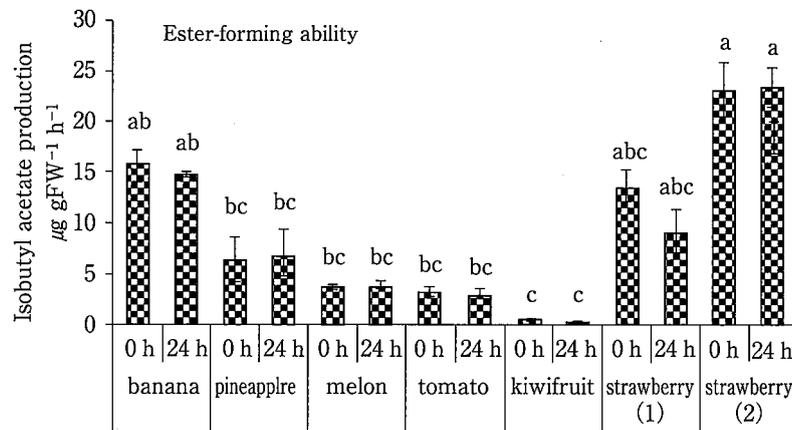


Fig. 10 Ester-forming activity of cut fruits just after cutting (0 h) and 24 h after cutting at ripe stage

Strawberry (1) and strawberry (2) show Sagahonoka and Sachinoka respectively. Vertical lines on bar graphs show standard deviation ($n = 3$).

Different letters above the bar indicate significant difference at $p < 0.05$. (Tukey's range test after ANOVA test).

after incubation for 2 h. No ethanol and a small amount of methanol were observed at 0 h of incubation of tomatoes (Fig. 11) with the quick analysis described at in materials and methods. Then, a sharp increase in methanol and a gradual increase of ethanol levels were detected during the 2 h incubation. In spite of a big change in the methanol concentration of tomatoes during a short time, significant off-flavor did not occur until 2 h incubation.

Negligible ethanol contents were detected at 0h incubation either in the softer (Sagahonoka) or the

harder (Sachinoka) strawberries with quick motion analysis of alcohols (Fig. 12). Then, the ethanol concentration increased sharply after cutting in both strawberries during incubation at room temperature ($23\text{ }^{\circ}\text{C}$). These berries showed off-flavor. This is different from the result at chilling condition ($3\text{ }^{\circ}\text{C}$) for 24 h, in which kept the good aroma of (Sachinoka). There are some reports published on the aroma of strawberry that describe the presence of certain amounts of ethanol and ethyl esters in the fresh berries. Although we understand that a wild strawberry (*Fragria nilgerrensis*) contain a high

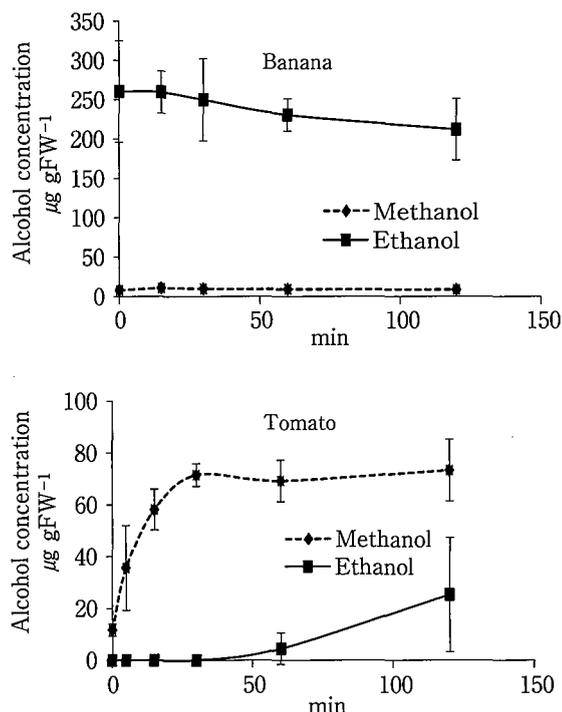


Fig. 11 Changes of ethanol and methanol content after cutting of banana (Above) and tomato (Below) during incubation (23°C)

Ripe stage of these fruits was used. Vertical lines on markers in graphs show standard deviation ($n=3$).

level of ethanol and ethyl esters³⁸⁾, some researchers may be hesitant to consider the sudden increase of ethanol after cutting of strawberry in the horticultural group of *Fragaria* × *ananassa* in connection with aroma analysis and may have overestimated the contents of ethanol and ethyl acetate in the strawberries.

We distinguished suitable fruits for fresh-cut application from unsuitable ones by measuring alcohols content after cutting. In general, fresh-cut fruits from the ripe stage, which is also the consumer's choice for buying, kept their aroma for at least 24 h (at 3°C) or 2 h (23°C). But some fruits (tomato and a cultivar of strawberry) showed an increase in ethanol, and aroma denaturing occurred at 24 h (at 3°C) or 2 h (23°C) after cutting. Further studies should be conducted for other fruits.

References

- 1) BEAULIEU, J. C. and GORNY, J. R.: Fresh-cut fruit, Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks. Edited by Gross, K. C., Wang Y. C. and Saltveit, M. (Agriculture Handbook

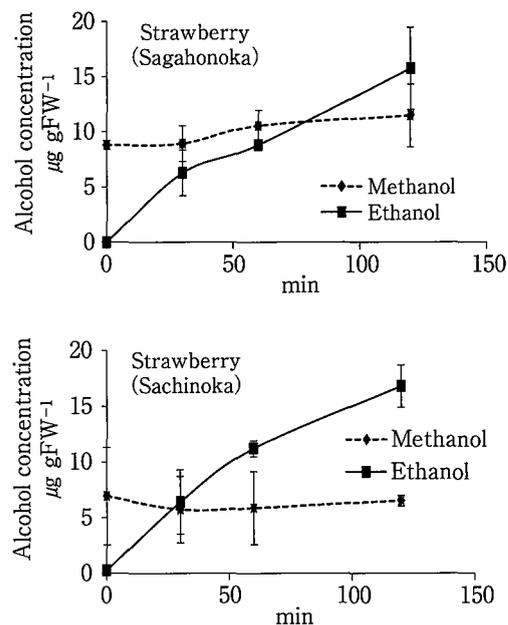


Fig. 12 Changes of ethanol and methanol content after cutting of strawberry (Above Sagahonoka and below Sachinoka) during incubation (23°C)

Ripe stage of these fruits was used. Vertical lines on markers in graphs show standard deviation ($n=3$).

- Number 66 Revised 2016 USDA), p.614 (2002)
- 2) ABE, K., TANASE, M. and CHACHIN, K.: Studies on physiological and chemical changes of fresh-cut bananas (part 1) Effect of cutting modes on the changes of physiological activity and deterioration in fresh-cut green tip bananas, *J. Japan. Soc. Hort. Sci.*, **67**, 123~129 (1998)
- 3) MURAKAMI, Y., OZAKI, Y. and IZUMI, H. : Microbiological quality and shelf life of enzyme-peeled fresh-cut persimmon slices stored in high CO₂ atmospheres, *HortScience*, **47**, 1758 ~ 1763 (2012)
- 4) ITAMURA, H., NAKAMOTO, T., HANAOKA, Y. and SUN, N. : Improving shelf life of cut persimmon fruit, *Acta Hortic.*, **833**, 295~298 (2009)
- 5) IZUMI, H., NAKATA, Y., INOUE, A. and OZAKI, Y.: Quality and shelf-life of enzymatically peeled and segmented citrus fruits in Japan, *Acta Hortic.*, **1141**, 245~250 (2016)
- 6) GORNY, J.R. CIFUENTES, R.A., HESS-PIERCE, B. and KADER, A. A.: Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime, *J. Food Sci.*, **65**, 541~544 (2000)
- 7) GORNY, J. R., HESS-PIERCE, B. and KADER A.A.:

- Quality changes in fresh-cut peach and nectarine slices as affected by cultivar, storage atmosphere and chemical treatments, *J. Food Sci.*, **64**, 429~432 (1999)
- 8) OMS-OLIU, G., ROJAS-GRAÜ, M. A., GONZÁLEZ, L. A., VARELA, P., SOLIVA-FORTUNY, R., HEMANDO, M. I. H., MUNUERA, I. P., FISZMAN, S. and MARTIN-BELLOSO, O.: Recent approaches using chemical treatments to preserve quality of fresh-cut fruits: A review. *Postharvest Biol. Technol.*, **57**, 139~148 (2010)
- 9) LUNA-GUZMÁN, I. and BARRETT D. M.: Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes, *Postharvest Biol. Technol.*, **19**, 61~72 (2000)
- 10) BAI, J-H., SAFTNER, R. A., WATADA, A. E. and LEE, Y. S.: Modified Atmosphere maintains quality of fresh-cut cantaloupe (*Cucumis melo* L.), *J. Food Sci.*, **66**, 1207~1211 (2001)
- 11) BAI, J-H., BALDWIN, E. A., FORTUNY, R. C. S., MATTHEIS, J. P., STANLEY, R., PERERA, C. and BRECHT, J. K.: Effect of pretreatment of intact 'Gala' Apple with ethanol vapor, heat, or 1-Methylcyclopropene on quality and shelf Life of fresh-cut slices, *J. Amer. Soc. Hort. Sci.*, **129**, 583~593 (2004)
- 12) ALVES, M. M., GONÇALVES, M. P. and ROCHA, C. M. R.: Effect of ferulic acid on the performance of soy protein isolate-based edible coatings applied to fresh-cut apples, *LWT - Food Sci. Technol.*, **80**, 409~415 (2017)
- 13) UKUKU, D. O., GEVEKE, D. J., CHAU, L. and NIEMIRA, B. A.: Microbial safety and overall quality of cantaloupe fresh-cut pieces prepared from whole fruit after wet steam treatment, *International J. Food Microbiol.*, **231**, 86~92 (2016)
- 14) BAI, J-H., PLOTTO, A., SPOTTS, R. and RATTANAPANONE, N.: Ethanol vapor and saprophytic yeast treatments reduce decay and maintain quality of intact and fresh-cut sweet cherries, *Postharvest Biol. Technol.*, **62**, 204~212 (2011)
- 15) MARTINDIÑON, M. E., MOREIRA, R. G., CASTELL-PREEZ, M. E., GOMES, C.: Development of a multilayered antimicrobial edible coating for shelf-life extension of fresh-cut cantaloupe (*Cucumis melo* L.) stored at 4°C, *LWT-Food Sci. Technol.*, **56**, 341~350 (2014)
- 16) LAMIKANRA, O. and RICARD, O. A.: Effect of storage on some volatile aroma compounds in fresh-cut cantaloupe melon, *J. Agric. Food Chem.*, **50**, 4043~4047 (2002)
- 17) AMARO, A. L., BEAULIEU, J. C., GRIMM, C. C., STEIN, R. E. and ALMEIDA, D. P. F.: Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons, *Food Chem.*, **130**, 49~57 (2012)
- 18) BEAULIEU, J. C. and LANCASTER, V. A.: Correlating volatile compounds, sensory attributes, and quality parameters in stored fresh-cut cantaloupe, *J. Agric. Food Chem.*, **55**, 9503~9513 (2007)
- 19) BEAULIEU, J. C.: Effect of cutting and storage on acetate and nonacetate esters in convenient, ready-to-eat fresh-cut melons and apples, *HortScience*, **41**, 65~73 (2006)
- 20) MONTERO-CALDERÓN, M., ROJAS-GRAÜ, M. A., AGUILÓ-AGUAYO, I., SOLIVA-FORTUNY, R. and MARTIN-BELLOSO, O.: Influence of modified atmosphere packaging on volatile compounds and physicochemical and antioxidant attributes of fresh-cut pineapple (*Ananas comosus*), *J. Agric. Food Chem.*, **58**, 5042~5049 (2010)
- 21) BEAULIEU, J. C. and LEA, J. M.: Volatile and quality changes in fresh-cut mangos prepared from firm-ripe and soft-ripe fruit, stored in clamshell containers and passive MAP, *Postharvest Biol. Technol.*, **30**, 15~28 (2003)
- 22) DEA, S., BRECHT, J. K., NUNES, M. C. N. and BALDWIN, E. A.: Occurrence of chilling injury in fresh-cut 'Kent' mangoes, *Postharvest Biol. Technol.*, **57**, 61~71 (2010)
- 23) SAFTNER, R., LUO, Y., MCEVOY, J., ABBOTT, J. A. and VINYARD, B.: Quality characteristics of fresh-cut watermelon slices from non-treated and 1-methylcyclopropene- and/or ethylene-treated whole fruit, *Postharvest Biol. Technol.*, **44**, 71~79 (2007)
- 24) PORAT, R., WEISS, B., COHEN, L., DAUS, A. and BITON, A.: Effects of polyethylene wax content and composition on taste, quality, and emission of off-flavor volatiles in 'Mor' mandarins, *Postharvest Biol. Technol.*, **38**, 262~268 (2005)
- 25) SOLIVA-FORTUNY, R. C., ALÒS-SAIZ, N., ESPACHS-BARROSO, A. and MARTIN-BELLOSO, O.: Influence of maturity at processing on quality attributes of fresh-cut conference pears, *J. Food Sci.* **69**, 290~294 (2004)
- 26) BARTHOLOME, L. G. and HOFF, J. E.: Gas chromatographic methods for the assay of pectin methylesterase, free methanol, and methoxy groups in plant tissues, *J. Agric. Food Chem.*, **20**,

- 262~266 (1972)
- 27) WENDAHOON, S. K., UEDA, Y., IMAHORI, Y. and ISHIMARU, M.: Effect of short-term anaerobic conditions on the production of volatiles, activity of alcohol acetyltransferase and other quality traits of ripened bananas, *J. Sci. Food and Agric.*, **86**, 1475~1480 (2006)
- 28) WYLLIE, S.G. and FELLMAN, J.K.: Formation of volatile branched chain ester in bananas (*Musa sapientum* L.). *J. Agric. Food Chem.*, **48**, 3493~3496 (2000)
- 29) WEL, C-B., LIU, S-H., LIU, Y-G., LV, L-L., YANG, W-X. and SUN, G-M.: Characteristic aroma compounds from different pineapple parts, *Molecules*, **16**, 5104~5112 (2011)
- 30) RUTH J. H.: Odor thresholds and Irritation levels of several chemical substances: A review. *Am. Ind. Hyg. Assoc. J.*, **47**, A142~A151 (1986)
- 31) MANEERAT, C. and HAYATA, Y.: Efficiency of TiO₂ photocatalytic reaction on delay of fruit ripening and removal of off-flavors from the fruit storage atmosphere, *ASABE* **49**, 833~837 (2006)
- 32) BEIRÃO-da-COSTA, S., STEINER, A., CORREIA, L., EMPIS, J. and MOLDÃO-MARTINS, M.: Effects of maturity stage and mild heat treatments on quality of minimally processed kiwifruit, *J. Food Eng.*, **76**, 616~625 (2006)
- 33) UEDA, Y. and BAI, J-H.: Effect of short term exposure of elevated CO₂ on flesh firmness and ester production of strawberry, *J. Japan Soc. Hort. Sci.*, **62**, 457~464 (1993)
- 34) KE, D., ZHOU, L. and KADER, A. A.: Mode of oxygen and carbon dioxide action on strawberry ester biosynthesis. *J. Amer. Soc. Hort. Sci.*, **119**, 971~975 (1994)
- 35) FERNÁNDEZ-TRUJILLO, J. P., NOCK, J. F., WATKINS, C. B.: Fermentative metabolism and organic acid concentrations in fruit of selected strawberry cultivars with different tolerances to carbon dioxide, *J. Amer. Soc. Hort. Sci.*, **124**, 696~701 (1999)
- 36) FRENKEL, C., PETERS J. S., TIEMAN, D. M., TIZNADO M. E. and HANDA, A. K.: Pectin methylesterase regulates methanol and ethanol accumulation in ripening tomato (*Lycopersicon esculentum*) fruit, *J. Biol. Chem.*, **273**, 4293~4295 (1998)
- 37) MWORIA, E. G., YOSHIKAWA, T., SALICON, N., ODA, C., ASICHHE, W. O., YOKOYANI, N., ABE, D., USHIJIMA, K., NAKANO, R. and KUBO, Y.: Low temperature-modulated fruits ripening is independent of

- ethylene in 'Sanuki Gold' kiwifruit, *J. Exp. Bot.*, **63**, 963~971 (2012).
- 38) UEDA, Y., ODA, Y., NOICHIDA, S. and DENG, H.: Difference of volatile aroma compound between wild and cultivated strawberries, *Appl. Biol. Sci.*, **3**, 67~73 (1997)

熟度の異なるカットフルーツにおける貯蔵中のエタノール、メタノールの生成

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果実中のアルコール含量を測ることはその果実の香気評価に重要である。果実(バナナ, パイナップル, メロン, トマト, キウイフルーツおよびイチゴ)の未熟果, 完熟果, 過熟果からカットフルーツを作り, カット直後および3℃, 24時間後のエタノールおよびメタノールの含量を測った。過熟のバナナおよびパイナップルから作ったカットフルーツは高いエタノール含量と酢酸エチルの生成も多く, オフフレーバーが発生していた。過熟のメロンからカットしたものも酢酸エチルの生成が多く, やはりオフフレーバーが発生した。一方トマトはカットすることにより3℃, 24時間後, 急激にメタノール含量が増え, エタノール含量もまたある程度増加し新鮮さが無くなった。イチゴは使用した栽培品種の内, 1品種は24時間後および老化後(3℃, 2日間)では高いエタノール含量を示し, 酢酸エチルの生成も多くオフフレーバーが感じられた。キウイフルーツは熟度やカットにかかわらずエタノール, メタノールが低含量でそのエステル生成もみられなかった。完熟果におけるアルコールデヒドロゲナーゼ活性を調べたところ, 高い活性を示す果実は, キウイフルーツを除き, アルコール含量も高かった。ペクチンメチルエステラーゼの活性はトマトが他に比べて非常に高く, トマトカット後のメタノール急増の原因と考えられる。エステルの生成能力はすべての果実で認められ(キウイフルーツは極低活性), 過熟果実のオフフレーバーを加速していると考えられる。

カット後すぐに供給され, 消費される業種形態もあるので, 室温でカット後, 2時間までのアルコール含量変

化を完熟のパナナ、トマト、イチゴで調べたところ、これらの果実は24時間冷蔵の結果と同じ傾向であった。完熟イチゴおよびトマトをカット後、直ちに測った場合、それぞれエタノールおよびメタノールは極少量検出されたのみであった。イチゴは両品種とも急激なエタノール

の増加が起こり、オフフレーバーが認められた。一方、トマトでは遅れてエタノールが増加し始めたが、2時間までは新鮮さが保たれていた。

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