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Variations of Acidity in the Peel of Acid Citrus. II. Organic Acids Detected in the Flavedo and Albedo of Acid Citrus by HPLC

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To elucidate organic acid(s) responsible for differences in acid accumulation in the flavedo of Kabosu (*C. sphaerocarpa* Hort. ex Tanaka), Yuzu (*Citrus junos* Sieb. ex Tanaka), 'Lisbon' lemon (*C. limon* Burm. f. Lisbon), Hanayu (*C. hanayu* Hort. ex Shirai), and Sudachi (*C. sudachi* Hort. ex Shirai), seasonal changes in organic acid composition of their flavedo and albedo were investigated by a high performance liquid chromatography (HPLC). While malate was a predominating acid only in the first sampling date (Sep. 16) in the flavedo and albedo of Japanese acid citrus and the albedo of Lisbon as it dropped quickly, malonate increased tremendously in the later sampling dates. Oxalate was present in the flavedo and albedo of all citrus investigated, and mostly decreased slightly. In the flavedo of Lisbon, malate and malonate were relatively stable, citrate was barely detected. Citrate increased and was found to be a major acid only in the flavedo of Kabosu and Yuzu.

INTRODUCTION

Differences in acid accumulations in the flavedo of Kabosu (*C. sphaerocarpa*), Yuzu (*Citrus junos*), 'Lisbon' lemon (*C. Limon* Burm.), Hanayu (*C. hanayu*), and Sudachi (*C. sudachi*) have been detected previously in our study, further reconfirmed our gas chromatographic determinations (Widodo *et al.*, 1995). While the free acid (FA) contents in the flavedo of Kabosu and Yuzu were continually increasing and the combined acid (CA) contents were decreasing, resulting in high FA contents in later sampling dates, the CA contents in the flavedo of Hanayu, 'Lisbon' lemon and Sudachi was kept higher than the FA contents.

In citrus peel extracts, malate, malonate, oxalate, and citrate are frequently reported to be present in detectable amounts (Clements, 1964a and 1964b; Monselise and Galily, 1979; Sasson and Monselise, 1977; Sinclair and Eny, 1947). Beard *et al.* (1972) found that the presence of citric acid reduced the rate of oxygen absorption by orange oil. Organic acid composition in peel extract is not only chemically and nutritionally informative, but such information is also technically needed as acidity is often expressed as the quantity of one acid assumed to be the only acid present per unit of extract volume or per unit fresh weight of fruit portion.

To further elucidate organic acid(s) responsible for the differences in acid accumulation in the flavedo of Kabosu, Yuzu, 'Lisbon' lemon, Hanayu, and Sudachi, seasonal changes in organic acid composition of their flavedo and albedo were investigated by a high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Ten fruits of Hanayu (*Citrus hanayu*), Kabosu (*C. sphaerocarpa*), 'Lisbon' lemon (*C. limon*), and Yuzu (*C. junos*) were monthly sampled beginning from September 16, 1994 to December 16, 1994 from trees grown in the Fruit Trees Experiment Station (FTES) of Kyushu University at Sasaguri, Fukuoka, Japan. They were tagged when they were about 1 cm in diameter. At the same sampling dates, 10 fruits of Sudachi (*C. sudachi*), Kabosu and Yuzu were also monthly sampled from the Fukuoka Agricultural Research Center (FARC) at Chikushino, Fukuoka, Japan. The fruits were quickly brought to our laboratory and were once kept refrigerated.

Flavedo and albedo of the fruits were separately peeled. Into as many as 50 g of each fractions, about 150 ml water was added. After homogenizing, they were centrifuged at 2,500 rpm for 20 minutes. The supernatant was decanted into a 200 ml flask and mixed up with water. Extractions of flavedo and albedo were accomplished in 1-3 days after sampling. The water used was distilled-deionized one.

Five ml of each flavedo and albedo extracts were separately passed through a column (1.6 cm I. D. X 4 cm) of cation-exchange resin Amberlite IR-120. The first 5 ml of water was slowly drained and discarded to minimize dilution on the collected solution. Samples for HPLC determinations were obtained from the first 20 ml of solution passed through the resin at 2 ml/min. Washing with distilled-deionized water was conducted little by little to get most of the extracts passed down the resin. The collected solutions for HPLC samples were passed through a 0.45 μ m filter prior to injection.

Organic acid composition was detected with an HPLC of Shimadzu LC-6A Liquid Chromatograph equipped with a Shimadzu SPD-GA UV Spectrophotometric Detector. The column (4.6 mm I.D. X 15 cm L, Tosoh TSK-Gel ODS-80 Tm) was operated at 40°C. The mobile phase was 5 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 2.4), and flowed at 0.4 ml/min. Eluting compounds were detected at 210 nm. The retention times of the compounds were compared with those of standard solution composed of oxalate, malate, malonate, and citrate. The injection volume was 10 μ l. Injection was run in triplicate. The results were presented as % of total detected organic acids.

RESULTS AND DISCUSSION

One peak significantly differentiating the flavedo of acid citrus investigated was detected to be citrate (Fig. 1). Its percentage was generally increasing and it became a major organic acid in the flavedo of Kabosu and Yuzu in December 16 (Fig. 2 and 3). Eventhough citrate was also increasing in the flavedo of Hanayu and Sudachi (Fig. 4 and 5), its percentage was always lower than malate. In the flavedo of Lisbon, citrate was barely detected even in the last sampling date (Fig. 4). Citrate was also barely detected in the albedo of all acid citrus investigated. Its presence and fluctuation in the albedo of Kabosu and Yuzu (Fig. 2 and 3) might be due to contamination from flavedo during peeling, or translocation from flavedo. Translocation of labelled photosynthates into albedo from highly active flavedo tissues has been noted by Bean and Todd (1960).

In the first sampling date, malate was the predominating acid found in the flavedo and

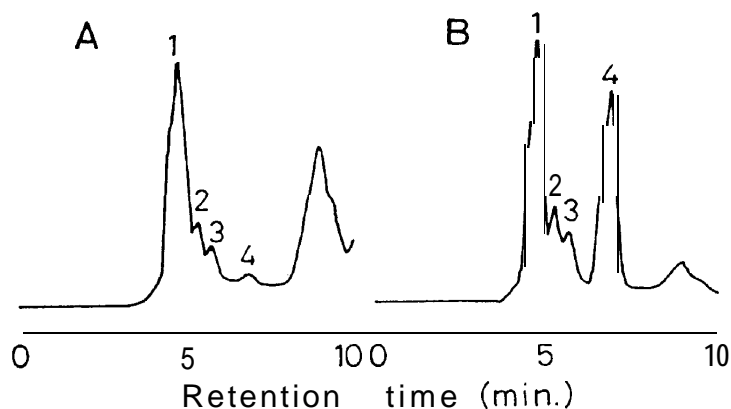


Fig. 1. Typical profiles of organic acid composition in the flavedo of Hanayu (A) and Yuzu (B). Samples were taken in December 16, 1994. Peak numbers on the chromatograms : 1 Oxalic acid, 2 Malic acid, 3 Malonic acid, 4 Citric acid.

albedo of all acid citrus investigated (Fig. 2 to 5). Except in the flavedo of Lisbon, its percentage decreased tremendously and was undetected in the flavedo of Kabosu and Yuzu of FARC and in the albedo of all citrus investigated, especially in the later sampling dates. In the flavedo and albedo of Valencia orange, malate has been noted to exhibit a general decrease during sampling period (Clements, 1964b).

A tremendous increase of malonate seems to be a general phenomena found in the flavedo and albedo of Japanese acid citrus investigated (Fig. 2 to 5). It is the only organic acid that exceeded citrate in the flavedo of Yuzu in the later sampling dates (Fig. 3). Malonic acid, as a competitive inhibitor of acid respiration, has been noted to be an indicator of fruit tissue senescence because its accumulation in the peel always begins at fruit maturity (Sasson and Monselise, 1977). Moreover, it is always present in a measurable amount in various citrus fruits (Clements, 1964a and 1964b; Monselise and Galily, 1979; Sasson and Monselise, 1977).

Oxalate was present in the flavedo and albedo of all citrus investigated, and mostly decreased slightly (Fig. 2 to 5). Like malate and malonate, it is one of the major acids detected in the peel of various citrus fruits (Clements, 1964a and 1964b; Monselise and Galily, 1979; Sasson and Monselise, 1977). Clements (1964b) also observed that oxalate decreased in the flavedo and albedo of Navel orange at maturity.

As shown here that a significantly different pattern of organic acid changes in the flavedo of acid citrus investigated was observed on citrate. Citrate occupied a high portion in the flavedo extract of Kabosu and Yuzu in the later sampling dates. The unusual citrate accumulation in the flavedo of Kabosu and Yuzu is both physiologically interesting and technically meaningful. A significant, increase of citric acid can be an advantage because

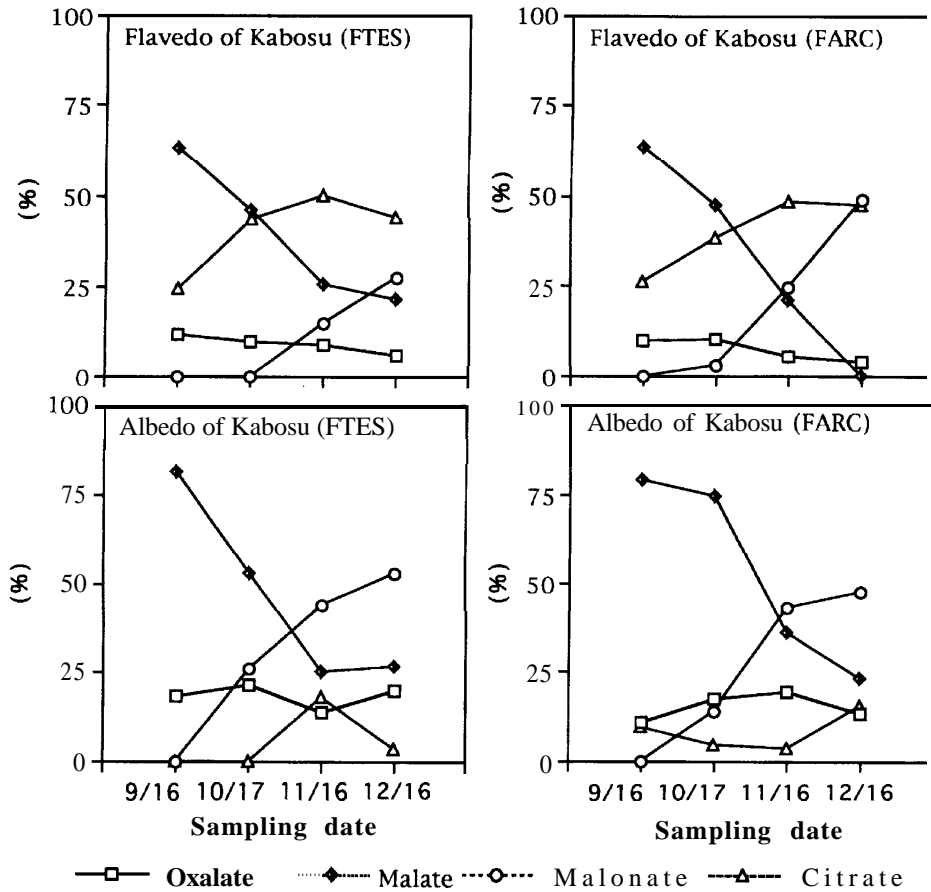


Fig. 2. Seasonal changes in organic acid composition in the flavedo and albedo of Kabosu. FTES = Fruit Trees Experiment Station of Kyusyu University, FARC = Fukuoka Agricultural Research Center.

of its synergistic effect with α -tocopherol as an antioxidant (Sinclair, 1984). Beard et al. (1972) reported that the presence of citric acid reduced the rate of oxygen absorption by orange oil. Meanwhile, acidity is often expressed as the quantity of one acid assumed to be the only acid present per unit of extract volume or per unit fresh weight of fruit portion. Our data presented here show that expressing acidity in the flavedo of Kabosu and Yuzu as citrate is undoubtedly technically valid.

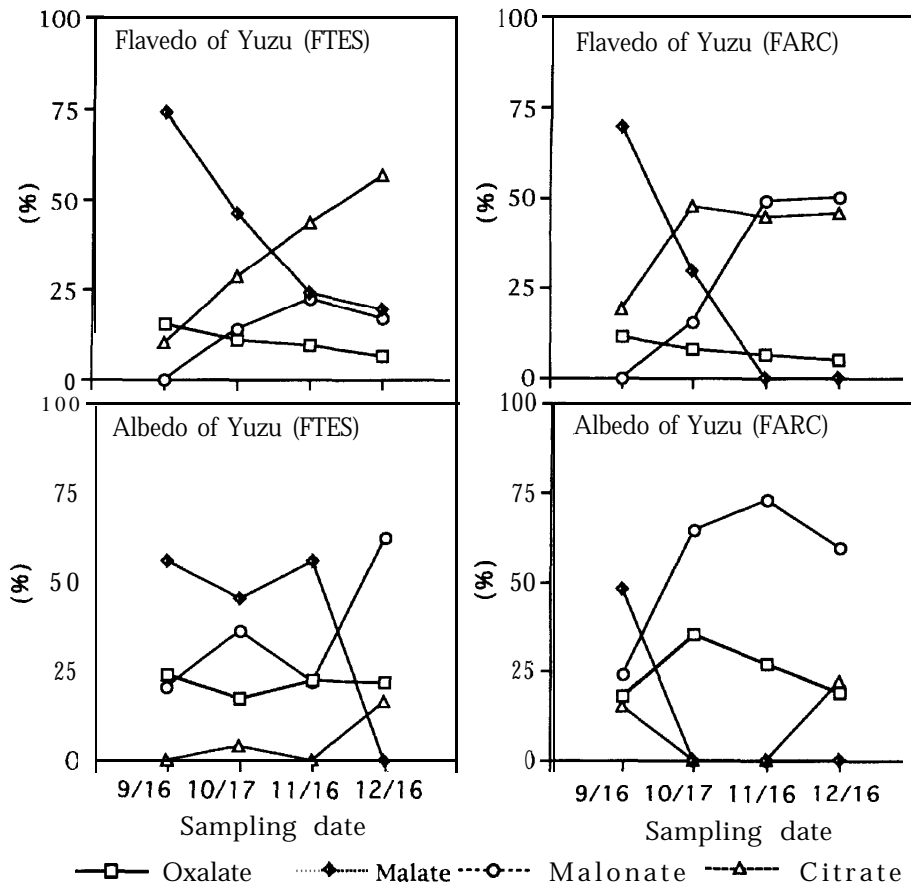


Fig. 3. Seasonal changes in organic acid composition in the flavedo and albedo of Yuzu. See note in Fig. 2.

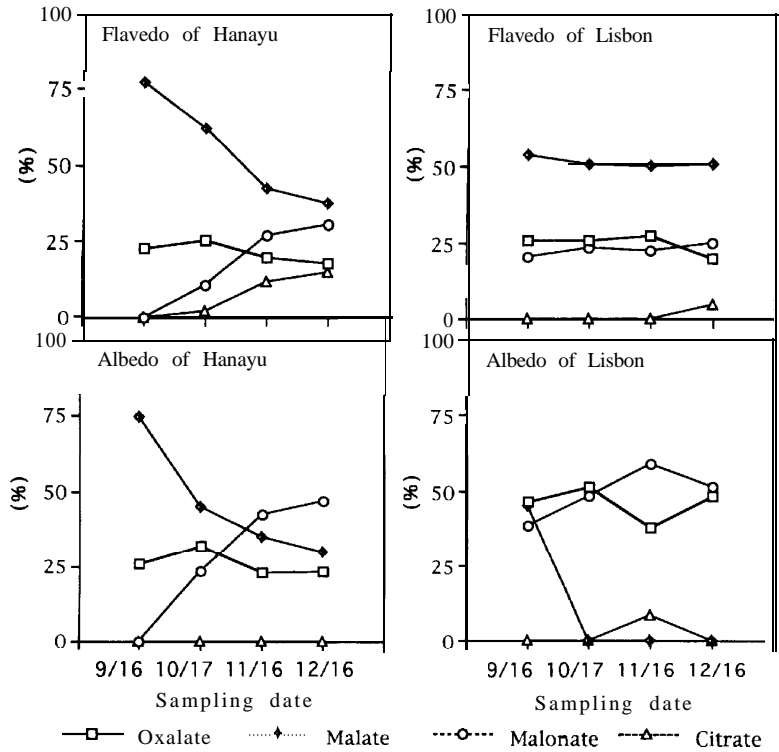


Fig. 4. Seasonal changes in organic acid composition in the flavedo and albedo of Hanayu and 'Lisbon' lemon.

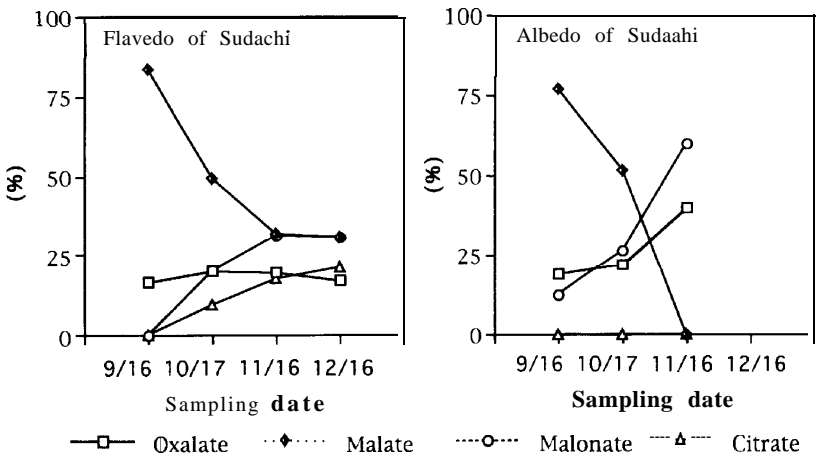


Fig. 5. Seasonal changes in organic acid composition in the flavedo and albedo of Sudaahi. The albedo in Dec. 16 was too thin to be separated from the flavedo, so it was combined to the flavedo.

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