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# Sugar Accumulation Types among Acid Citrus as might be Proposed by Seasonal Changes in Reducing and Non-reducing Sugar Ratios in the Juice

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In order to know if there is any differences in sugar accumulation pattern among acid citrus, the present experiment was preliminary carried out using Hanayu (*Citrus hanayu* Hort. ex Shirai, designated as H), Kabosu (*C. sphaerocarpa* Hort. ex Tanaka, designated as K), Sudachi (*C. sudachi* Hort. ex Shirai designated as S), 'Lisbon' lemon (C. *limon* Burm. f. Lisbon, designated as L), Yuzu (*C. junos* Sieb. ex Tanaka, designated as Y) and some of their F-l progenies. Seasonal changes in sugars and acids of these citrus were examined along with some physical fruit characteristics. If focussed to reducing sugar (RS)-to-non-reducing sugar (Non-RS) ratios in the juice, it seems there are 3 types among them, namely Non-RS Type I, Non-RS Type II and RS Type. K, S and H X Y 16 seemed to belong to Non-RS Type I, H and H X K 25 to Non-RS Type II, and L, Y, S X K 25 and L × Y 34 to RS Type. The ratios specifically assigned to each group seemed to be a good indicator of fruit, maturity for the acid citrus if only juice quality is taken into account.

## INTRODUCTION

To increase the flavor of cultivated tomato (*Lycopersicon esculentum*) which is a reducing sugar (RS) accumulating type (it accumulates predominantly glucose and fructose), the wild tomato species such as *L. chmielewskii* and *L. hirsutum* which are non-reducing sugar (Non-RS) accumulating types (they characteristically store sucrose), are used in tomato breeding programs as donor parents (Stommel and Haynes, 1993; Yelle *et al.*, 1991). Accordingly, the enzymes responsible for differentiating the RS and Non-RS accumulators have been intensively studied recently (Manning and Maw, 1975; Miron and Schaffer, 1991; Ohyama *et al.*, 1995; Stommel, 1992; Yelle *et al.*, 1988). Meanwhile, sugar accumulation was known to be a heritable factor in tomato (Stommel and Haynes, 1993; Yelle *et al.*, 1991) and in carrot (Freeman and Simon, 1983). Those indicate that knowing the sugar accumulating type is very important in breeding programs for an increased juice flavor quality.

RS and Non-RS accumulating types have been detected in tomatos (Davies, 1966; Manning and Maw, 1975; Miron and Schaffer, 1991; Ohyama *et al.*, 1995; Stommel, 1992; Yelle *et al.*, 1988), persimmon (*Diospyros kaki*) cultivars (Guo and Sugiura, 1990), and sugarcane (Hatch and Glasziou, 1963). In citrus, oranges (Harding *et al.*, 1940) and Satsuma mandarins (*Citrus unshiu* Marc.) (Daito and Sato, 1985) seem to be Non-RS accumulators as they accumulate sucrose during fruit maturation, whereas lemons (Sinclair, 1984) and grapefruit (Hilgeman and Smith, 1940; Sinclair, 1972) should be RS accumulators since the RS content is generally higher than the Non-RS one. No information on the sugar accumulation types of Japanese acid citrus, however, is available.

Of many Japanese acid citrus, Yuzu (*C. junos* Sieb. ex Tanaka), Kabosu (*C. sphaerocarpa* Hort. ex Tanaka), Sudachi (*C. sudachi* Hort. ex Shirai), and Hanayu (*C. hanayu* Hort. ex Shirai) are known for their high acidity and fragrance. From biochemical and enzymatic studies (Berhow et *al.*, 1994; Hashinaga and Hasegawa, 1989; Hirai et al., 1986; Ohta et *al., 1992;* Ozaki et *al.*, 1991; Rouseff and Nagy, 1982), they have been strongly suspected to be hybrids related to, at least, Ichang papeda (*C. ichangensis*) or Ichang lemon (*C. wilsonii*) as one of their suspected parents. Variabilities in sugar accumulation in their juice may then be expected. Stommel and Simon (1989) found variabilities in sugar accumulation among populations of carrot (*Daucus carota* L.) inbred lines.

The present study reports differences in sugar accumulation patterns among acid citrus, and seasonal changes of juice qualities and some physical fruit characteristics in order to see if there is any connection between sugar accumulation patterns and fruit maturation. Hilgeman and Smith (1940) proposed a somewhat low point of RS-to-Non-RS ratio as an index of maturity in grapefruit.

## MATERIALS AND METHODS

The parent acid citrus investigated were Hanayu (*Citrus hanayu)*, Kabosu (*C. sphaerocarpa*), Sudachi (*C. sudachi*), 'Lisbon' lemon (*C. limon*), and Yuzu (*C. junos*). The F-l progenies investigated were those of Hanayu X Kabosu, Hanayu × Yuzu, Sudachi X Kabosu and Lisbon X Yuzu; the first capital letters of parent names are, hereafter, used to designate the parent and progeny names. In all cases of the progeny names the female parent is mentioned first. The progenies were budded on Satsuma mandarin (*C. unshiu* Marc.). The progenies were chosen to represent the expected combinations of the sugar accumulation types. Sugar accumulation type was found to be a heritable character in tomato (Yelle *et al.*, 1991; Stommel and Haynes, 1993) and carrot (Freeman and Simon, 1983).

The fruits were tagged when they were about 1 cm in diameter. Ten fruits were monthly sampled from September 16, 1994 to December 16, 1994 from parent and F-l progeny trees grown in the Fruit Trees Experiment Station (FTES) of Kyushu University at Sasaguri, Fukuoka, Japan. The fruits of Sudachi were monthly sampled from the Fukuoka Agricultural Research Center (FARC) at Chikushino, Fukuoka, Japan. As a consequence of location effect that might bias the groupings, the fruits of Kabosu and Yuzu were sampled in both stations at the same sampling dates. It was conducted merely to show the location effect in connection with the RS and Non-RS groupings. The fruits were quickly carried to our laboratory, and were once kept refrigerated. Measurement of physical fruit characteristics and juice extraction were accomplished within l-3 days after sampling.

The diameter and height of the fruits were measured to calculate fruit shape index (diameter/height). The fruits were individually weighed and peeled. The juice of each fruit was extracted with hand-pressed juicer, and weighed. The combined juice of 10 fruits was'centrifuged at 2,500 rpm for 20 minutes. About 100 ml of the supernatant was quickly freezed for analyses.

Reducing sugar content was determined by a modified procedure of Bertrand’s method (1906) which is already used by Shiraishi (1993). Total sugar content was determined as follows: 4 ml of deionized water was added to 1 ml juice, and then 5 ml of 4% $H_2SO_4$ was added into it. The solution was boiled for 30 minutes, and was neutralized with 2 N NaOH. This solution was then treated the same as in the reducing sugar determination. The non-reducing sugar content was the difference between total and reducing sugar contents.

Free acid content (titratable acidity) was determined by a titration with 0.1 N NaOH and phenolphthalein as indicator. The titrated solution was then passed through a column (1.6 cm I. D. X 4 cm) of cation-exchange resin Amberlite IR-120 and its total acid content was determined the same as in the determination of free acid content.

## RESULTS AND DISCUSSION

As shown in Table 1, locations affected greatly sugar and acid contents of Kabosu and Yuzu. This effect was observed as a delay of phase change from RS to Non-RS accumulator in Kabosu, but it did not affect the groupings (Table 2). It seems that location affects the phase change followed by a delay of harvest time, but does not affect the groupings to which a given citrus species belongs.

Table 2 shows the reducing sugar (RS) and non-reducing sugar (Non-RS) ratios of the acid citrus examined and a possible grouping resulted from the ratios. The acid citrus examined might be grouped into three types: Non-RS Type I characterized by having a phase change from a RS accumulator to a Non-RS accumulator (Kabosu of the FTES and Sudachi around Nov./Dec., H X Y 16 around Oct./Nov.), Non-RS Type II characterized by relatively high portions of non-reducing sugar during maturation without a marked phase change (Hanayu and H X K 25) and RS Type (‘Lisbon’, Yuzu, S X K 25 and L X Y 34) characterized by relatively high portions of reducing sugar and having a minimum or maximum point in a certain moment during fruit maturation.

**Table 1.** Seasonal changes in acid and sugar contents (mg/ml) of Kabosu and Yuzu from different locations, 1994.

| | Samp. date | FARC[y] | | | | FTES[y] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | FA[z] | TA | RS | TS | FA | TA | RS | TS |
| Kabosu | Sep. 16 | 50.13±0.10 | 52.66±0.28 | 28.93±0.02 | 41.80t0.0’7 | 44.59±0.10 | 47.00±0.17 | 14.58±0.10 | 23.41kO.27 |
| | Oct. 17 | 53.91±0.25 | 56.76±0.28 | 26.18±0.34 | 39.39t0.32 | 52.62t0.09 | 55.40±0.08 | 20.49±0.03 | 35.69±0.67 |
| | Nov. 16 | 48.25±0.09 | 51.04±0.21 | 26.31kO.45 | 46.06±0.40 | 46.24kO.13 | 49.13±0.16 | 19.90k0.24 | 38.36±0.33 |
| | Dec. 16 | 47.51±0.07 | 50.18±0.05 | 24.49±0.33 | 48.49±0.39 | 46.18±0.04 | 49.OOkO.17 | 20.3%0.09 | 43.58±0.08 |
| Yuzu | Sep. 16 | 66.99±0.31 | 70.74±0.33 | 14.60±0.12 | 27.78±0.05 | 62.10±0.06 | 66.57±0.15 | 17.87±0.17 | 33.26±0.10 |
| | Oct. 17 | 59.97±0.24 | 62.79±0.25 | 12.75±0.12 | 23.01±0.32 | 56.54±0.18 | 59.74k0.25 | 11.40-t0.22 | 20.86±0.13 |
| | Nov. 16 | 64.94cO.15 | 67.7OkO.21 | 14.22±0.11 | 24.09±0.31 | 52.9OkO.28 | 56.27k0.29 | 16.57±0.10 | 27.78±0.23 |
| | Dec. 16 | 67.84t0.22 | 70.98±0.13 | 16.17±0.08 | 28.79±0.12 | 56.49±0.02 | 59.59±0.13 | 15.41kO.15 | 26.59±0.29 |

[y]FARC=Fukuoka Agricultural Research Center; FTES=Fruit Trees Experiment Station of Kyushu University.
[z]FA, TA, RS and TS are free acid, total acid, reducing sugar and total sugar, respectively; Mean±S.E.

Table 2. Reducing and non-reducing sugar ratios of sugar accumulation types among acid citrus.

| Citrus | Samp. date | RS/Non-RS | Group |
|---|---|---|---|
| Kabosu (K)" | 1994/09/16 | 1.65 (62.28 : 37.72)' | Non-RS Type I |
| | 1994/10/17 | 1.35 (57.41 : 42.59) | |
| | 1994/11/16 | 1.08 (51.89: 48.11) | |
| | 1994/12/16 | 0.88 (46.76 : 53.24) | |
| Kabosu (K)' | 1994/09/16 | 2.25 (69.21 : 30.79) | Non-RS Type I |
| | 1994/10/17 | 1.98 (66.47 : 33.53) | |
| | 1994/11/16 | 1.33 (57.11 : 42.89) | |
| | 1994/12/16 | 1.04 (51.06 : 48.94) | |
| Sudachi (S) | 1994/09/16 | 2.12 (67.99 : 32.01) | Non-RS Type I |
| | 1994/10/17 | 1.25 (55.52 : 44.48) | |
| | 1994/11/16 | 1.01 (50.23 : 49.77) | |
| | 1994/12/16 | 0.96 (48.92 : 51.08) | |
| H × Y 16 | 1994/09/16 | 1.49 (59.90 : 40.10) | Non-RS Type I |
| | 1994/10/17 | 1.29 (56.30 : 43.70) | |
| | 1994/11/16 | 0.91 (47.75 : 52.25) | |
| | 1994/12/16 | 0.64 (38.97 : 61.03) | |
| Hanayu (H) | 1994/09/16 | 0.38 (27.36 : 72.64) | Non-RS Type II |
| | 1994110117 | 0.48 (32.33 : 67.67) | |
| | 1994/11/16 | 0.37 (26.80 : 73.20) | |
| | 1994/12/16 | 0.70 (41.17 : 58.83) | |
| HXK25 | 1994/09/16 | 0.83 (45.40 : 54.60) | Non-RS Type II |
| | 1994/10/17 | 0.70 (41.23 : 58.77) | |
| | 1994111/16 | 0.53 (34.56 : 65.44) | |
| | 1994/12/16 | 0.64 (38.96 : 61.04) | |
| Yuzu (Y)" | 1994109116 | 1.16 (53.76 : 46.24)' | RS Type |
| | 1994/10/17 | 1.20 (54.65 : 45.35) | |
| | 1994/11/16 | 1.48 (59.65 : 40.35) | |
| | 1994/12/16 | 1.38 (57.96 : 42.04) | |
| Yuzu (Y)' | 1994/09/16 | 1.11 (52.57: 47.43) | RS Type |
| | 1994110117 | 1.24 (55.40 : 44.60) | |
| | 1994/11/16 | 1.44 (59.03 : 40.97) | |
| | 1994/12/16 | 1.28 (56.17 : 43.83) | |
| Lisbon (L) | 1994109116 | 1.60 (61.50 : 38.50) | RS Type |
| | 1994/10/17 | 1.61 (61.71 : 38.29) | |
| | 1994111116 | 1.40 (58.28 : 41.72) | |
| | 1994/12/16 | 1.09 (52.20 : 47.80) | |
| SXK25 | 1994/09/16 | 2.02 (66.92 : 33.08) | RS Type |
| | 1994/10/17 | 1.45 (59.12 : 40.88) | |
| | 1994/11/16 | 1.27 (55.99 : 44.01) | |
| | 1994112116 | 1.36 (57.57 : 42.43) | |
| LXY34 | 1994/09/16 | 2.22 (68.93 : 31.07) | RS Type |
| | 1994/10/17 | 2.46 (71.07 : 28.93) | |
| | 1994/11/16 | 3.23 (76.36 : 23.64) | |
| | 1994/12/16 | 2.61 (72.33 : 27.67) | |

“Sample from the Fruit Trees Experiment Station of Kyushu University.
‘Sample from the Fukuoka Agricultural Research Center.
‘Reducing sugar (RS): Non-reducing sugar (Non-RS) ratios as % of total.

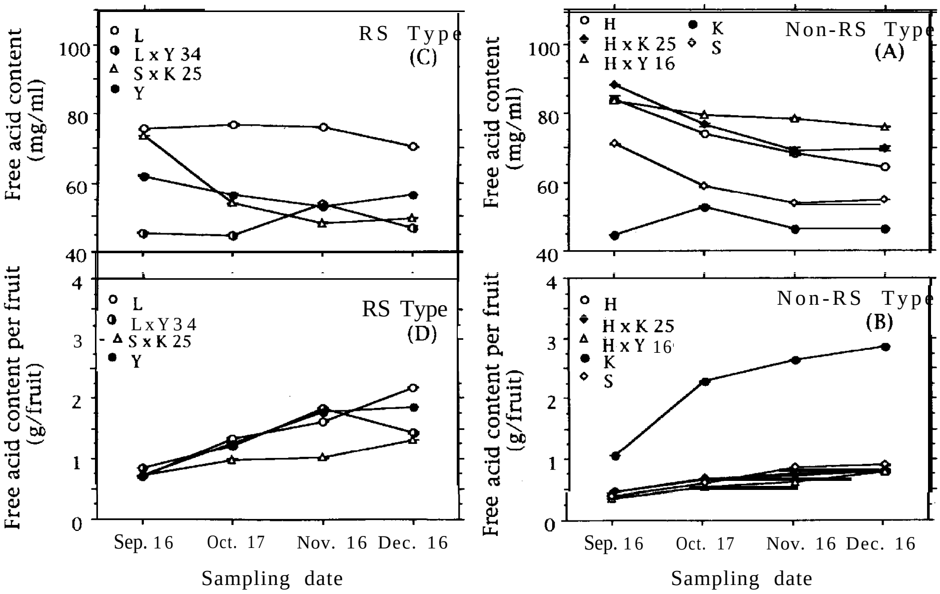


**Fig. 1.** Seasonal changes in absolute acidity and acidity per fruit of non-reducing (Non-RS) **and** reducing sugar (RS) typed acid citrus. H, K, Y, S and L are designated for Hanayu, Kabosu, Yuzu, Sudachi and 'Lisbon' lemon, respectively. Number after progeny names is the progeny's number. Bars represent standard errors.

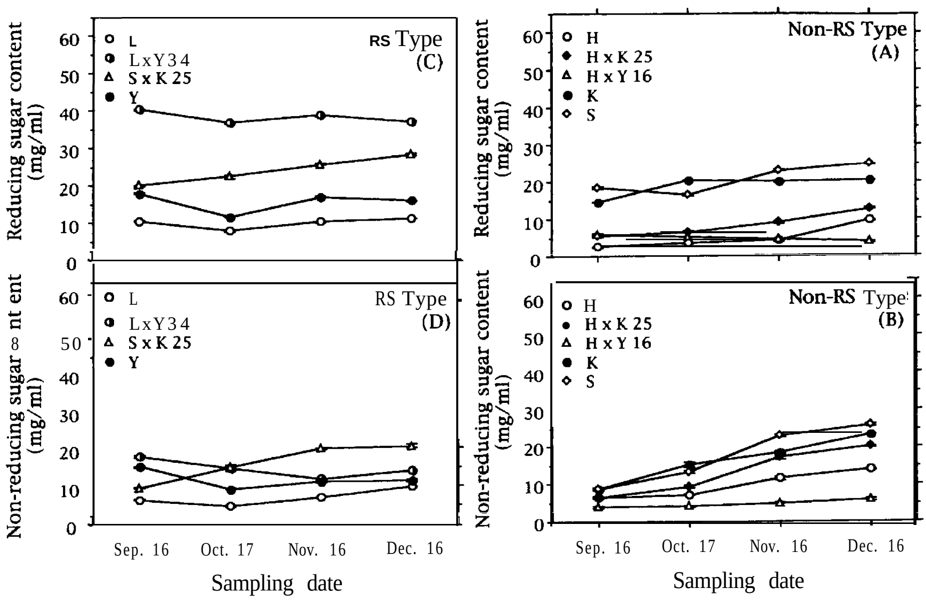


**Fig. 2.** Seasonal changes in sugar contents of non-reducing sugar (Non-RS) and reducing sugar (RS) typed acid citrus. See notes in Fig. 1.

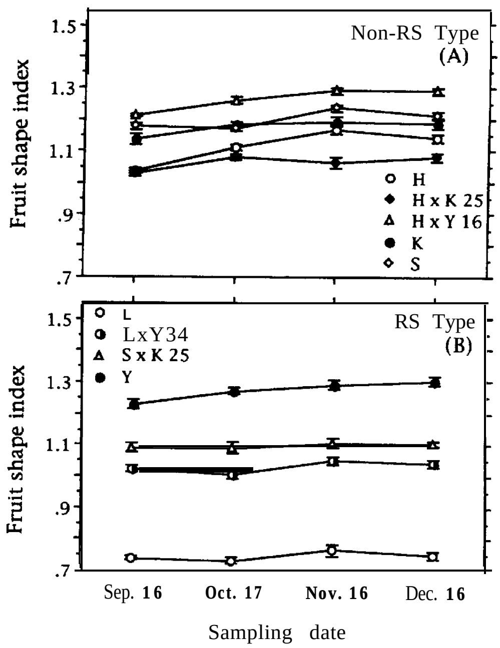


**Fig. 3.** Seasonal changes in fruit shape index of non-reducing (Non-RS) and reducing sugar (RS) typed acid citrus. See notes in Fig. 1.

The groupings were not clear by acidity (Fig. 1) and sugar components (Fig. 2). In general, acidity was decreasing while sugar content was increasing as the fruits increased in weight. A somewhat different pattern was observed through fruit shape index (Fig. 3). The RS Type tended to keep the index constant during fruit growth as was also observed in lemon (McDonald and Hillebrand, 1980), while Non-RS Type changed it larger until November 16. However, to correlate the groupings to this index seems too risky, because cultural practices and low night temperatures have been shown to produce more elongated fruits (Embleton et al., 1967; Wutscher, 1976).

Fig. 4 was drawn to show seasonal changes in relative sourness among the acid citrus investigated. In general, the ratio is decreasing during maturation. However, more attention should be paid to Hanayu and H × K 25, which belong to Non-RS Type II. The reason is that their ratios decreased sharply in comparison with slower rates of the Non-RS Type I and RS Type. Sugars, both kind and amount, are known to contribute to fruit and vegetable flavor (Freeman and Simon, 1983; Yelle et al., 1991; Stommel, 1992; Moriguchi et al., 1992). Therefore, the ratio in Fig. 4 will indirectly tell about the relative overall flavor. In other words, the overall relative flavor of the juice of Non-RS Type II will be greatly decreased during fruit maturation. These differences support separation within the Non-RS Type into Type I and Type II.

Here arises a question whether these groupings can be meaningful or not on a determination of fruit maturity. In this connection, some brief discussions will be tried as

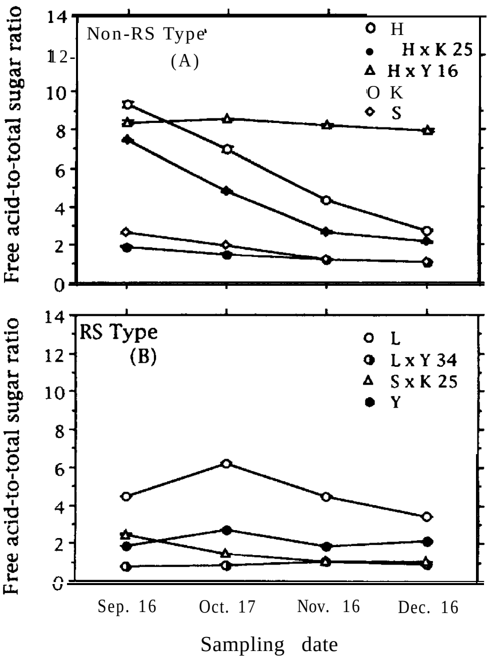


**Fig. 4.** Seasonal changes in free acid-to-total sugar ratios of non-reducing (Non-RS) and reducing sugar (RS) typed acid citrus. See notes in Fig. 1.

follows in accordance with the three types proposed.

*1. Non-RS Type I*

Kabosu, which is widely cultivated at Oita Prefecture, Japan, is the so-called green 'sour orange'. The fruit is preferably harvested in a green stage around September-October due to its quick natural degreening and loss of flavor (Murata, 1982; Yamauchi et c-cl., 1991). Our data show that in September both free acid content (Fig. 1, A) and juice weight (Fig. 5, A) of Kabosu were still low, and consequently free acid content per fruit was low (Fig. 1, B). A marked increase in free acid content in October (Fig. 1, A and B) along with a sharp increase in juice and fruit weights (Fig. 5, A and B) might be used to be an indicator of fruit maturity of Kabosu. Its juice weight (Fig. 5, A), however, was still increasing up to November and the decrease of its sourness was the slowest (Fig. 4, A). From these data, a high juice quality of Kabosu may still be expected up to November or even to December without worrying about the loss of its juice sourness or juice flavor. This is, interestingly, matched with its phase change around November/December (Table 2). The same is true for Sudachi and H X Y 16 (Table 2) which belong to this group. It should be mentioned, however, Kabosu and Sudachi are highly priced in Japan only when they are green, eventhough their juice quality may not be high at that time. For example, the harvesting time of Sudachi has long been decided to be in September because the quantity of its essential oil in the peel was found to be high in September (Waki and

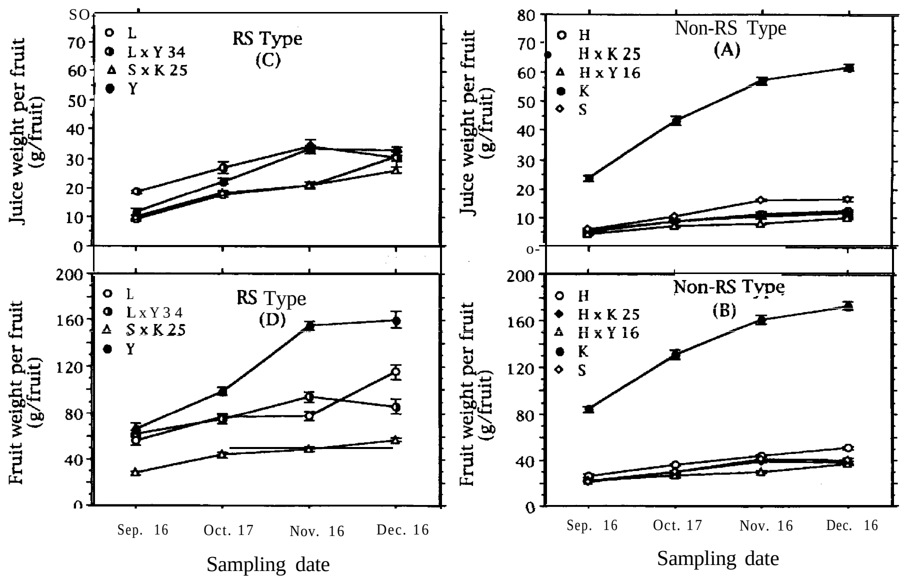


**Fig. 5.** Seasonal changes in juice and fruit weights of non-reducing (Non-RS) and reducing sugar (RS) typed acid citrus. See notes in Fig. 1.

Nakanishi, 1967), eventhough the acid content of its juice in September was less than half of that in November (Waki, 1959).

2. *Non-RS Type II*

This is the type to which more attention should be paid in determining maturity not only because there is no phase change detected as in the Non-RS Type I but also because its fruit juice is characterized by a sharp decrease in sourness or overall flavor (Fig. 4, A). Its juice flavor sharply decreased (Fig. 4, A) due to a sharp decrease in free acid content (Fig. 1, A) and a sharp increase in non-reducing sugar content (Fig. 2, B). Meanwhile, its juice and fruit weights increased slowly (Fig. 5, A and B). As there is no single indicator from the RS-to-Non-RS ratio, fruit color is the only indicator. The harvest season of Hanayu has been stated around December (Yamaki, 1988) when its fruit color has apparently reached a yellow stage. Our data suggest, however, that growers and consumers should be aware of decreased juice flavor during fruit maturation of Hanayu.

3. *RS Type*

RS Type is different from Non-RS Type II eventhough maintaining a high reducing sugar content during maturation. As to this group there is a high or low point on the RS/Non-RS ratio which differs depending on the species (Table 2). Similar to the Non-RS Type I, the point generally coincides with high free acid content per fruit (Fig. 1, D) due to high juice weight (Fig. 5, C) without worrying about loss of overall flavor (Fig. 4, B). The point, which in this study was generally around November, will be good enough to be an indicator of fruit maturity. The harvest season of Yuzu has been stated around

December (Yamaki, 1988). Hilgeman and Smith (1940) actually proposed a somewhat low point of RS/Non-RS ratio as an index of maturity.

The biochemical basis for differentiation in the three sugar accumulation types proposed is related to the mechanism of sugar accumulation. It is almost certain that an increase in the activities of sucrose-phosphate synthase (SPS) and/or sucrose synthase (SS) leads to sucrose accumulation (Cordenunsi and Lajolo, 1995; Giaquinta, 1979; Hubbard et al., 1989; Miron and Schaffer, 1991; Moriguchi et *al.*, 1992; Stommel, 1992). Whether a decline in the activity of acid invertase is obsolutely required for sucrose accumulation or not is still in argument (see Giaquinta, 1979; Hubbard *et al.,* 1989; Miron and Schaffer, 1991; Moriguchi et al., 1992; Stommel, 1992; Yelle *et al.,* 1991). High activity of acid invertase, however, has been shown to prevent sucrose accumulation (Ohyama *et al.,* 1995). Therefore, an increase in the activities of SPS, SS, and/or a decrease in the activity of acid invertase hypotetically characterize the Non-RS Types of the acid citrus investigated, and a high-level activity of acid invertase might be responsible for reducing sugar accumulation in the RS Type. Both alkaline and acid invertases are present in Satsuma mandarin (C. *unshiu* Marc.), and a decline in the activity of acid invertase has promoted its sucrose accumulation (Kato and Kubota, 1978).

As shown here, differences in sugar accumulation patterns are likely to exist even in acid citrus. By knowing which group they belong to, in addition to the other existing indicators such as total soluble solid-to-acid ratio, fruit maturity related to juice quality can be predicted objectively. More distinct characteristics will be expected a lot since research in flavor is developing by more sophisticated techniques and apparatus.

Our other data seem to indicate that there is an inheritance pattern of sugar accumulation. Sugar accumulation was known to be a heritable factor in tomato (Stommel and Heynes, 1993; Yelle *et al.,* 1991) and carrot (Freeman and Simon, 1983), controlled by a single gene dominant for a high percentage of RS. Furthermore, there have been some efforts to correlate sugar accumulation to cold hardiness (Parker, 1959 and 1962; Sakai, 1960 and 1966; Siminovitch et *al.,* 1953; Sieckmann and Boe, 1978; Li *et al., 1964)* along with its conflicting results (Fuchigami *et al.,* 1973; Lasheen and Chaplin, 1977).

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