Variations in the soluble sugar and organic acid contents in radish (Raphanus sativus L.) cultivars

Running title

Sugars and organic acids in the radish

Title

Variations in the soluble sugar and organic acid contents in radish (*Raphanus sativus* L.) cultivars

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Summary

The radish is a root vegetable of the Brassicaceae family that is mainly consumed in Far Eastern Asian countries. Because chemical indexes indicating the quality of a radish have not been established, we investigated the variations in the contents of the soluble sugars and organic acids that influence taste among 7 radish cultivars. In terms of the total soluble sugar content, which is a strong index of sweetness, Koshin, Kouto, and Shogoin were the 3 highest among the 7 cultivars. Sobutori, which is the most common radish cultivar in Japan, was the lowest. The total organic acid contents varied among the 7 cultivars, although they were 5-13 times lower than the total soluble sugar contents. These results indicate that there are variations in the soluble sugar and organic acid contents among radish cultivars; therefore, these contents may be used as chemical indexes for the quality of radishes.

Keywords Ascorbate, citrate, fructose, glucose, malate, organic acids, radish, *Raphanus sativus*, soluble sugars, taproot.

Introduction

The radish (*Raphanus sativus* L.) is a Brassicaceae tuber vegetable produced throughout the world. The major consumption area includes the Far Eastern Asian countries, such as Japan, Korea, and China. Recently, researchers have focused on radishes because radishes have been found to have health benefits. Radishes contain fair amounts of soluble dietary fibres (Khanum et al., 2000) and phenolic acids (Mattila and Hellström 2007). The radish produces glucoraphasatin as a major radish glucosinolate (Lim et al., 2009; Montaut et al., 2010). The glucoraphasatin and the corresponding isothiocyanate 4-methylthio-3-butenyl isothiocyanate showed antioxidative and anticarcinogenic activities in several studies (Barillari et al., 2008; Papi et al., 2008; Hanlon et al., 2007; Beevi et al., 2010). These data demonstrate that the 4-methylthio-3-butenyl isothiocyanate which is derived from the glucoraphasatin is an important bioactive compound in radishes. On the other hand, researchers have not thoroughly investigated the contents of soluble sugars and organic acids in radishes. In fruits, the contents of soluble sugars and organic acids have been well investigated, because they influence the taste (Malundo et al., 1995; Harker et al., 2002; Versari et al., 2002; Kafkas et al., 2007). If the contents of soluble sugars and organic acids in radishes can be determined, they will provide information regarding the taste of radishes.

In Japan, 1.6 million tons of radishes were harvested in 2008 (Preliminary Statistical Report on Agriculture, Forestry and Fisheries of Japan, 2009). From the 1970s to today, the Sobutori (also called the Taibyo-Sobutori) cultivar has made up a large fraction of the radish production in Japan. Thus, we first analysed the soluble sugars and organic acids in Sobutori. We also analysed the soluble sugars and organic acids in 6 cultivars including 2 European radishes (French Breakfast and Kuromaru), 2 Chinese radishes (Kouto and Koshin), and 2 Japanese radishes (Shinhasshu and Shogoin), whereas these cultivars have not been produced in large numbers in Japan. In conclusion, we found variations in the soluble sugar and organic acid contents in radish cultivars.

Materials and Methods

Plant materials

The seeds of 7 radish cultivars were obtained from seed companies as follows: French Breakfast, Sobutori, and Shinhasshu were obtained from Takii (Kyoto, Japan), Kouto and Koshin were obtained from Sakata (Yokohama, Japan), Kuromaru was obtained from Fujita (Osaka, Japan), and Shogoin was obtained from Takayama (Kyoto, Japan). French Breakfast radishes were grown in a plastic planter containing Peatban (Sakata Seed, Yokohama, Japan) at a greenhouse at Shizuoka University, Japan, in 2010. The plants were watered every week with Hyponex solution (500 times dilution; Hyponex, Tokyo, Japan) and were harvested on the 20th day (young stage) or the 30th day (mature stage) after sowing. The other 6 cultivars were cultivated in a field at Fujieda, Shizuoka, Japan, from September 2010 to November or December 2010. A fertilizer was applied to the soil before planting.

Radishes were harvested at 2 different stages, i.e. the young and mature stages. In this study, the young stage was the growing stage in which the peel (consisting of the epidermis and cortex) of the young hypocotyl was peeling off. The mature stage was the period when the taproot was fully expanded. The cultivation periods of the 6 cultivars were as follows. Sobutori radishes were harvested on the 20th day (young stage) or the 70th day (mature stage) after sowing; Kouto, Koshin, and Kuromaru radishes were harvested on the 21st day (young stage) or the 83rd day (mature stage) after sowing; and Shinhasshu and Shogoin radishes were harvested on the 23rd day (young stage) or the 73rd day (mature stage) after sowing. After harvest, the taproot was weighed and immediately analysed for sugars and organic acids as described below.

Sample preparation for assays

A whole hypocotyl of the young radish was used to test metabolites. To analyse metabolites in the mature radish, a plate sliced off from the taproot was prepared as described below. Preliminary experiments showed that, when Sobutori and Koshin were tested, the contents of soluble sugars (glucose, fructose, and sucrose) and organic acids (malate, citrate, ascorbate, lactate, and pyruvate) were not significantly different between the use of plates and the use of whole taproots. By this method, a large number of mature taproots (around 1 kg per taproot) could be handled in a short time.

A fresh taproot was vertically sliced through the centre of the taproot to form a thin plate (approximately 2 mm in thickness) with a cooking knife. The thin plate, which was almost symmetrical, was further cut at the central vertical line of the plate to obtain 2 tissue portions whose shapes were similar. We used the 2 tissue portions to analyse soluble sugars and organic acids, respectively.

Assays of metabolites

Soluble sugars such as glucose, fructose, and sucrose were analysed by high-performance liquid chromatography (HPLC) as described previously (Noichinda et al., 2007) with modifications. The procedure, described below, was applied to 1 g samples of the tissue portions. The tissue portion was cut into small pieces (approximately 1 cm x 1 cm plates) and then extracted twice with boiling ethanol (20 ml), each time for 15 min. After the extracts were combined, the ethanol was evaporated. Residues were taken up in deionized water (1 ml) and then centrifuged at 10,000 x *g* for 5 min at room temperature. The supernatant was directly passed through a Sep-Pak Cl8 cartridge (Waters, MA, USA), which was activated with 10 ml of methanol and then equilibrated with water. This process was done to remove pigments and phenolics. The sample was subjected to the isocratic HPLC system (LC-2000, Jasco, Tokyo, Japan) with an Asahipak NH_2P-50 4E column (250 mmx4.6 mm id; Showa Denko, Kawasaki, Japan) and an RI-2031 RI detector (Jasco). The solvent system was acetonitnile/water (75:25, v/v). Lactose was used as an internal standard.

The organic acid contents were determined by enzymatic reaction methods. The procedure for 1 g of the tissue portion is described below. The tissue portion was ground to powder in liquid nitrogen. The sample was transferred to 3% metaphosphoric acid (5 ml) to extract the organic acids. After being incubated on ice for 30 min, the sample was centrifuged at 10,000 x *g* for 10 min at 4 °C. The supernatant was used for the following tests. Malate, citrate, lactate, and pyruvate were quantified by enzymatic reaction methods using an F-kit (Roche Diagnostics, Tokyo, Japan). Ascorbate was determined by an enzymatic method (Foyer et al. 1983) with slight modifications. A reaction mixture (total volume; 1 ml) containing 100 mM sodium phosphate pH 5.6 (975 µl), sample solution (pH was adjusted to 5.6 with NaOH, 20 µl), and 5 U ascorbate oxidase (5 µl) was incubated at 25°C. Absorption at 265 nm was monitored.

Statistical analysis

To determine any significant differences between Sobutori and the other 6 cultivars, Student's t test was performed. A P value < 0.05 was considered significant.

Results

First, we grew 7 kinds of radish cultivars, i.e., Sobutori, French Breakfast, Kuromaru, Shinhasshu, Shogoin, Kouto, and Koshin. The young and mature radishes were harvested to determine their soluble sugar and organic acid contents. Photographs of the 7 cultivars used in this study are shown in Fig. 1. The characteristics of the 7 cultivars at the mature stage are noted below. Sobutori is a white radish and is the most commonly produced radish in Japan. This cultivar is used as a standard in the present report. French Breakfast is a European small radish that is two-tone in colour (red and white). Kuromaru is a black radish that originated from Europe. Shinhasshu and Shogoin are Japanese radishes. Shinhasshu is a white radish produced for pickled vegetables. Shogoin is a local cultivar which is mainly cultivated in the Kansai area of Japan. Kouto and Koshin are Chinese radishes which have been recently introduced in Japan. Kouto is a two-tone (green and white) cultivar. Koshin is a white radish whose inside is red.

The fresh weights, soluble sugar contents, and organic acid contents of the taproots of the 7 cultivars are shown in Tables 1 and 2. We investigated not only mature radishes but also young radishes. In Japan, young radishes called "Oronuki-daikon" are also sold in the local market, although their production is low. The data of the young and mature taproots are given in Tables 1 and 2, respectively. Although the fresh weights of the young taproots of all the cultivars were less than 3 g/plant (Table 1), the fresh weights of the mature taproots reached 0.8 to 1.7 kg/plant except in the French Breakfast radish (15 g/plant) (Table 2).

Regarding soluble sugars, glucose, fructose, and sucrose were analysed because only these 3 sugars were detected using the present HPLC system. The glucose, fructose, and sucrose contents of young Sobutori were 6.9, 3.1, and 5.0 μmol/g fresh weight, respectively (Table 1). The glucose, fructose, and sucrose contents of other cultivars at the young stage were not significantly different from the corresponding contents of young Sobutori except in the case of the glucose and fructose contents of French Breakfast (28 and 13 μmol/g fresh weight, respectively) and the sucrose content of Kouto (1.0 μmol/g fresh weight). Mature radishes showed remarkably higher glucose and fructose contents than young radishes. The highest glucose and fructose contents were recorded in mature Kouto (63 μmol/g fresh weight) and mature Shogoin (55 μmol/g fresh weight), respectively (Table 2). On the other hand, mature Sobutori contained glucose and fructose at 33 and 39 μmol/g fresh weight, respectively.

Tables 1 and 2 show the contents of 5 organic acids, including malate, citrate, ascorbate, lactate, and pyruvate, in the young and mature radish taproots of the 7 cultivars. The dominant organic acids in the radishes were malate, citrate, and ascorbate, as in other vegetables (Belitz et al., 2009). Among the 7 cultivars at the mature stage, Sobutori showed the lowest, second-lowest, and third-lowest ascorbate, malate, and citrate contents, respectively (Table 2). The highest malate, citrate, and ascorbate contents were recorded in mature French Breakfast (13 μmol/g fresh weight), mature Koshin (1.8 μmol/g fresh weight), and mature Shogoin radishes (2.2 μmol/g fresh weight), respectively. In some metabolites, the SD values in Tables 1 and 2 were large. Preliminary experiments indicated that these great differences were not due to the sampling method described above, but rather to the large variations in the contents of the corresponding metabolites between individual plants.

In order to comprehensively grasp the sugar and acid contents of the radishes, we produced graphs showing “total soluble sugars” and “total organic acids” (Fig. 2) in the various cultivars based on the data in Tables 1 and 2. The total soluble sugar contents are the sums of the glucose, fructose, and sucrose contents for each cultivar. The total organic acid contents are the sums of the malate, citrate, and ascorbate contents for each cultivar. The total soluble sugar content of young Sobutori was not significantly different from the total soluble sugar contents of the other 6 cultivars. However, mature Sobutori contained the lowest total soluble sugar content among the 7 cultivars. The total soluble sugar contents of mature Shogoin, Kouto, and Koshin were significantly higher than that of mature Sobutori. The proportions of the total soluble sugars made up of fructose tended to be higher in the mature radishes than in the young radishes. The total organic acid contents of young Kouto and young Koshin were significantly higher than the total organic acid content of young Sobutori. In the mature radishes, Sobutori had a lower organic acid content than French Breakfast, Kuromaru, Shogoin, Kouto, and Koshin. Mature radishes tended to have lower total acid contents than young radishes except in the case of French Breakfast.

Discussion

In the practical market, size and fresh appearance are the primary selection standards for good-quality radishes. However, no chemical index regarding the quality of radishes has been established. Here we report the variations in the contents of soluble sugars and organic acids that are related to taste among radish cultivars. Seven radish cultivars including Sobutori, which has an overwhelming share of the radish market in Japan, were tested for their contents of soluble sugars and organic acids. The contents in Sobutori were then compared with those in other 6 cultivars.

At the mature stage, the total soluble sugar contents of Koshin, Kouto, and Shogoin were significantly higher than that of Sobutori (Fig. 2). Moreover, mature Koshin, Kouto, and Shogoin contained significantly more fructose, which is believed to be sweeter than glucose and sucrose, than mature Sobutori (Table 2). These results indicate that Koshin, Kouto, and Shogoin should be considered potentially sweeter cultivars than Sobutori.

Our previous study showed that there is variation in the starch content among radish cultivars (Hara et al., 2009a). Koshin is a starch-rich cultivar (18 mg/g fresh weight), but Sobutori is a starch-poor cultivar (1.4 mg/g fresh weight). Intriguingly, cultivars with higher starch content tended to show higher amylase activity (Hara et al., 2009a). It was reported that most amylase activity in the radish taproots was due to the thermostable β-amylase RsBAMY, whose amylolytic activity was not reduced even at 70°C (Hara et al., 2009b). These results suggest that, when cooked, Koshin may generate more soluble sugars by degrading more starch with higher amylase activities than Sobutori.

We noticed that cultivars with high total soluble sugar contents at the mature stage such as Kouto and Koshin also exhibited high total organic acid contents at the young stage (Fig. 2). Thus, we plotted the total soluble sugar contents of the mature radishes against the total organic acid contents of the young radishes in Fig. 3; the former values appeared to be positively correlated with the latter values ($R^2 = 0.71$). This indicates that cultivars which contain more organic acids at the young stage accumulate more soluble sugars when they mature.

In the case of fruits, it has been documented that the sour taste that is contributed by organic acids is attenuated when the total soluble sugar contents overwhelm the total organic acid contents (Malundo et al., 1995; Harker et al., 2002). Mature radishes possess higher total soluble sugar contents than total organic acid contents (Fig. 2). The total soluble sugar contents were more than 10 times greater than the total organic acid contents in Shinhasshu (12.8 times), Shogoin (10.1 times), Kouto (11.9 times), and Koshin (11.0 times), showing that these cultivars may exhibit little sourness.

In conclusion, we found that there are intraspecies variations in the soluble sugar and organic acid contents between radishes. Our results suggest that Shogoin, Kouto and Koshin are sweet cultivars. The contents of soluble sugars and organic acids may be chemical indexes for the taste of radishes, and such indexes will also provide useful information for the production, use, and breeding of high-quality radishes.

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Table 1 Fresh weight, soluble sugar contents, and organic acid contents in the young taproots of 7 radish cultivars

Cultivar	Fresh weight (g/plant)	Soluble sugars (μmol/g fresh weight)			Organic acids (μmol/g fresh weight)				
		Glucose	Fructose	Sucrose	Malate	Citrate	Ascorbate	Lactate	Pyruvate
Sobutori	0.92±0.08 a	6.85± 5.66 a	3.08±2.97 a	5.00±3.22 a	8.38±0.42 a	1.85±0.42 a	2.89±0.58 a	0.15±0.07 a	0.16±0.01 a
French Breakfast	2.85±1.17 b	27.85± 18.25 b	13.35±9.21 b	3.50±3.47 a	14.09±0.28 b	0.86±0.28 b	2.26±0.56 a	0.16±0.07 a	0.11±0.01 b
Kuromaru	0.30±0.07 b	4.24± 1.69 a	0.96±0.32 a	3.24±2.09 a	10.02±1.17 a	3.31±1.17 b	3.32±0.65 a	0.07±0.04 a	0.12±0.04 a
Shinhasshu	1.06±0.10 a	7.28± 4.34 a	1.69±0.82 a	4.69±3.18 a	8.82±0.82 a	3.71±0.82 b	3.08±0.83 a	0.13±0.10 a	0.11±0.02 b
Shogoin	0.85±0.20 a	5.66± 2.51 a	1.93±0.80 a	9.15±5.01 a	6.11±0.61 a	6.46±0.61 b	4.33±0.41 b	0.12±0.06 a	0.12±0.06 a
Kouto	0.56±0.20 b	3.50± 2.62 a	1.06±0.77 a	0.98±0.67 b	15.81±1.52 b	3.36±1.52 a	3.74±0.45 b	0.16±0.06 a	0.10±0.03 b
Koshin	0.56±0.08 b	9.34± 5.30 a	2.01±1.19 a	5.56±3.46 a	19.97±0.72 b	3.81±0.72 b	3.84±0.52 b	0.03±0.08 b	0.20±0.06 a

Values are expressed as means ± SD of 5 plants. Roman letters "a" and "b" represent values that are not significantly different and are significantly different from the values of Sobutori (Student's t test, $P < 0.05$) within each column, respectively.

Table 2 Fresh weight, soluble sugar contents, and organic acid contents in the mature taproots of 7 radish cultivars

Cultivar	Fresh weight (kg/plant)	Soluble sugars (μmol/g fresh weight)			Organic acids (μmol/g fresh weight)				
		Glucose	Fructose	Sucrose	Malate	Citrate	Ascorbate	Lactate	Pyruvate
Sobutori	1.386±0.095 a	32.71± 2.33 a	38.99±2.40 a	5.08±4.41 a	5.86±0.57 a	0.79±0.57 a	1.19±0.16 a	0.07±0.04 a	0.13±0.03 a
French Breakfast	0.015±0.003 b	42.84± 7.07 b	37.11±4.58 a	2.66±1.22 a	12.87±0.19 b	0.43±0.19 a	1.89±0.93 a	0.17±0.12 a	0.16±0.02 a
Kuromaru	0.812±0.070 b	51.90± 15.16 b	25.93±9.01 b	5.97±1.37 a	9.12±0.31 b	1.31±0.31 a	1.45±0.19 b	0.08±0.04 a	0.09±0.02 b
Shinhasshu	1.700±0.276 b	48.27± 7.77 b	38.66±8.77 a	3.93±1.13 a	5.18±0.24 a	0.42±0.24 a	1.53±0.12 b	0.07±0.03 a	0.11±0.01 a
Shogoin	1.578±0.365 a	55.22± 5.37 b	54.57±3.77 b	5.57±1.96 a	8.44±0.16 b	0.94±0.16 a	2.16±0.32 b	0.10±0.07 a	0.13±0.03 a
Kouto	0.870±0.173 b	63.28± 18.51 b	47.70±7.76 b	11.95±2.10 b	7.45±0.22 b	0.89±0.22 a	1.87±0.39 b	0.07±0.03 a	0.11±0.04 a
Koshin	1.023±0.201 b	53.45± 10.28 b	53.46±7.46 b	21.38±6.00 b	7.76±0.45 b	1.80±0.45 b	1.58±0.12 b	0.22±0.11 b	0.11±0.00 a

Values are expressed as means ± SD of 5 plants. Roman letters "a" and "b" represent values that are not significantly different and are significantly different from the values of Sobutori (Student's t test, $P < 0.05$) within each column, respectively.

Figure legends

Figure 1 Photographs of the 7 radish cultivars used in this study at the young (upper line) and mature (lower line) stages. White bars represent 1 cm (young radishes) and 5 cm (mature radishes), respectively.

Figure 2 Total soluble sugar contents and total organic acid contents of the 7 radish cultivars at the young and mature stages. Values and error bars show the means ± SD of 5 plants. Roman letters “a” and “b” represent values that are not significantly different and are significantly different from the values of Sobutori (Student's t test, $P < 0.05$) within each graph, respectively.

Figure 3 Relationship between the total soluble sugar contents in the mature radishes and the total organic acid contents in the young radishes in 7 radish cultivars. Values and bars represent the means ± SE of 5 plants. The dotted line is the regression line ($R^2 = 0.71$).

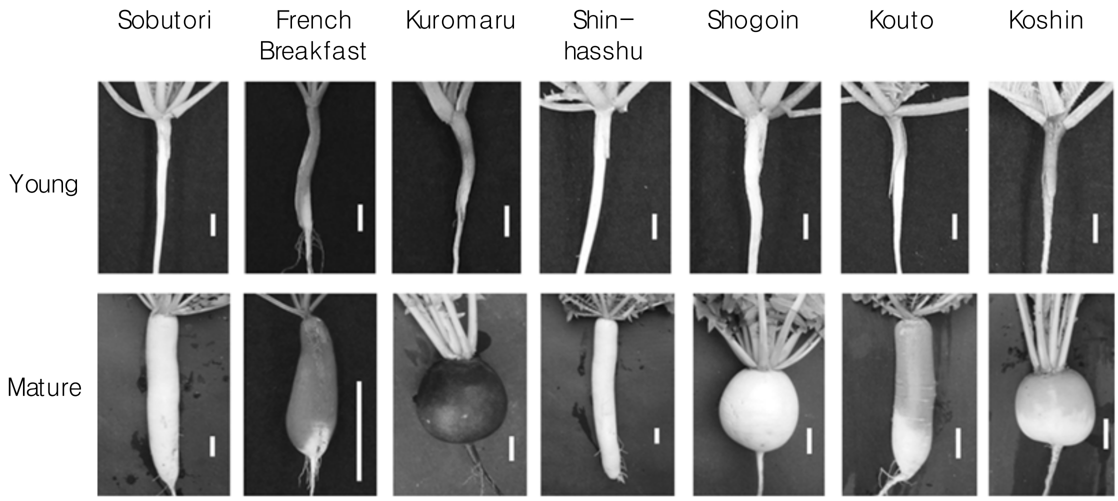

Fig. 1 Hara et al.

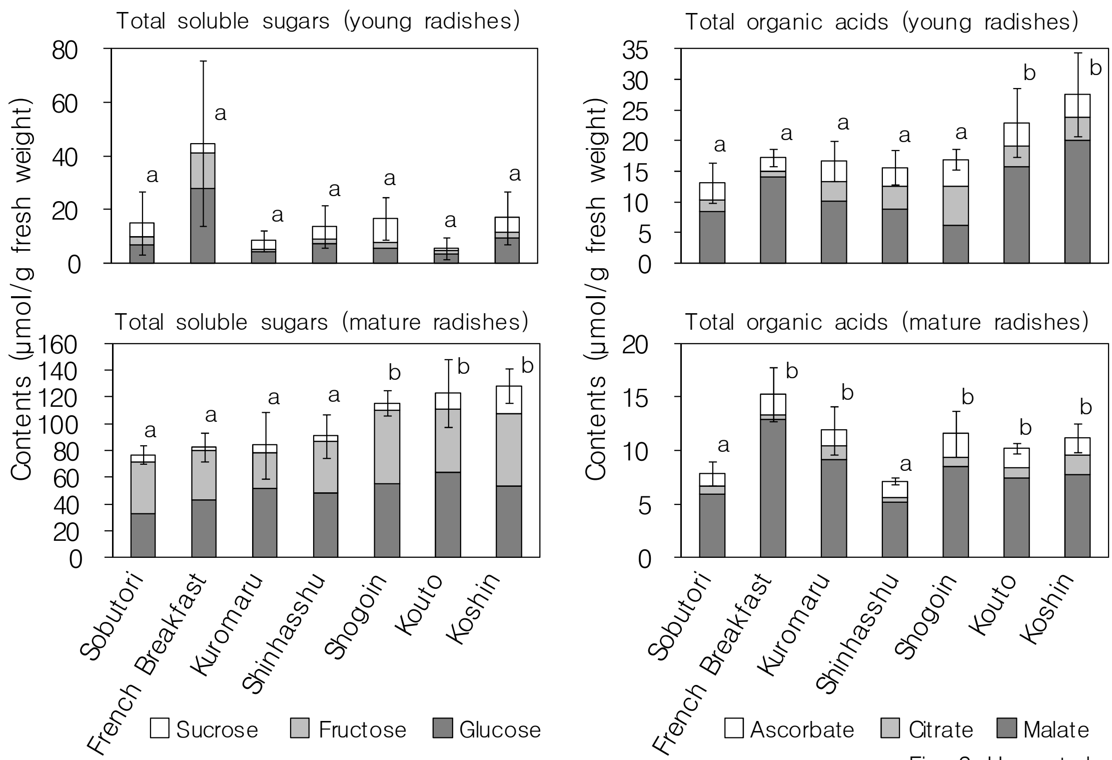

Fig. 2 Hara et al.

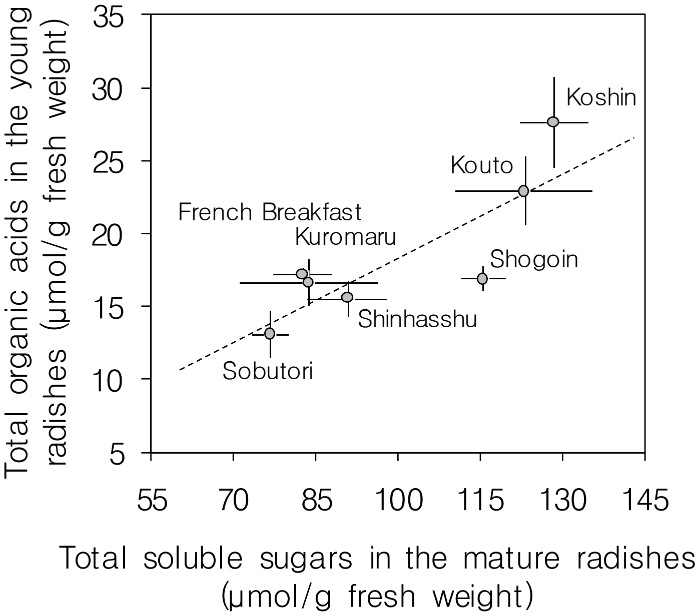

Fig. 3 Hara et al.