Contribution of Katsura-uri (Japan’s Heirloom Pickling Melon, Cucumis melo var. conomon) at the Completely Ripe Stage to Diabetes Control

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Summary  The fruit of Katsura-uri (Japan’s heirloom pickling melon, Cucumis melo var. conomon) possesses a fruity aroma and moderate sweetness. The fruit juice has potential to minimize human postprandial blood glucose levels. This study provides information regarding the health benefits of Katsura-uri and its utility in treating diabetes. The study methodology involved measuring the color and firmness of Katsura-uri fruit at five ripening stages, and quantitation of the aroma substances, proximate composition, and sugars. Significant changes were detected in the color, firmness, and level of aroma substances with ripening of Katsura-uri fruit, albeit with no major changes in proximate composition, with the exception of dietary fiber, and sugars. To determine the effects of Katsura-uri juice, the blood glucose levels of ten diabetic volunteers aged 46–75 y were monitored after its consumption, and compared with after consumption of muskmelon juice equivalent to the total weight of Katsura-uri juice. The blood glucose area under the curve level was significantly lower after consumption of Katsura-uri juice (16±5 h · mg/dL) than after consumption of muskmelon juice (55±6 h · mg/dL; p<0.05). The level of the glucose spike was also significantly lower after consumption of Katsura-uri juice (22±5 mg/dL) than after consumption of muskmelon juice (64±6 mg/dL; p<0.05). The completely ripe Katsura-uri fruit provides the best results for diabetic subjects, which is the first case of fruits sweetened with the addition of zero-calorie sweeteners.

Key Words  blood glucose, sugar, ripening stage, diabetic, heirloom vegetable, aroma

The Kyoto Prefecture has an extensive collection of various heirloom vegetables that have been termed “Kyo-yasai.” The 37 varieties of heirloom vegetables in Kyoto are referred to as “Kyo-no-dento-yasai” (traditional vegetables in Kyoto). These probably originated prior to 1868 (end of the Edo period), and were described by the Kyoto Prefecture in 1988. Katsura-uri (Japan’s heirloom pickling melon, Cucumis melo var. conomon) is one of the Kyo-no-dento-yasai and has been cultivated in a limited area, namely the Katsura-quarter in Kyoto. Unfortunately, the cultivation area has been decreasing since its peak in 1942, and in 2011, a single septuagenarian farmer was the sole cultivator of Katsura-uri (1). Traditionally, immature and semi-ripened Katsura-uri fruits are used for preparing pickles, “kasu-zuke,” by soaking the fruit for several months in the lees of Japanese liquor and Japanese sweet seasoning liquor. The immature and semi-ripened fruits are suitable for pickling, because the flesh is firm and the shape is retained during the pickling process. In contrast, the completely ripe fruit is unsuitable for pickling because of its softer flesh. However, the fruit has stronger fruity aroma than immature and semi-ripened Katsura-uri fruits.

Certain compounds responsible for the aroma of the completely ripe Katsura-uri are also involved in other

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physiological functions (1–4). Six aroma substances (methylthioacetic acid ethyl ester: MTAE; acetic acid 2-methylthio ethyl ester: AMTE; 3-methylthiopropionic acid ethyl ester: MTPE; acetic acid 3-methylthio propyl ester: AMTP; benzyl acetate: BA; eugenol) were identified in completely ripe Katsura-uri fruits, and five of them (except BA) possessed at least one or more anticarcinogenic effects as determined by differentiation-inducing, antimutagenic, and antioxidative activities (2). MTAE-derived methylthioacetic acid, which MTAE is acid-hydrolyzed to in stomach, improves glucose aerobic metabolism in skeletal muscle during exercise (3). We previously identified Katsura-uri fruit as a low-carbohydrate-content food material which was unique in the character of fruits kingdom, and had therefore potential benefit for obese people and diabetic subjects. The juice of the completely ripe Katsura-uri fruits possesses low-carbohydrate properties (small amounts of fructose, glucose, and sucrose) and a fruity aroma (1). In the questionnaire-based sensory evaluation of Katsura-uri juice with zero-calorie sweeteners, the assessment values for taste and aroma were high, which meant the juice met the demands of human taste in satisfaction, and in human trials, the Katsura-uri juice minimized postprandial blood glucose levels in healthy humans. Then, we proposed that the Katsura-uri juice was an acceptable and novel functional drink with potential to minimize human postprandial blood glucose levels (4).

Currently, diabetes is serious health issues because the population of diabetic subjects is reported to reach 425 million in 2017 (http://diabetesatlas.org/IDF_Diabetes_Atlas_8e_interactive_EN/). Basic treatment for diabetes includes controlling the postprandial blood glucose levels by diet, therapeutic agents, and exercise. For management of postprandial blood glucose levels, International Diabetes Federation (Brussels, Belgium) recommends the target level for postprandial blood glucose measured 1–2 h after a meal is 160 mg/dL as long as hypoglycemia is avoided (https://www.idf.org/our-activities/advocacy-awareness/resources-and-tools/82:management-of-postmeal-glucose.html). To control blood glucose levels, certain effective strategies can be implemented: strategy 1, reducing carbohydrate intake; strategy 2, delaying carbohydrate absorption from the gastrointestinal tract; strategy 3, stimulating insulin secretion; strategy 4, suppressing glucagon secretion; strategy 5, improving insulin sensitivity; strategy 6, preventing renal glucose reabsorption; strategy 7, inducing insulin-independent glucose uptake in skeletal muscle. Several therapeutic agents are currently available to preferentially lower postprandial blood glucose. Alpha-glucosidase inhibitors execute strategy 2, whereas sulfonylureas, glinides, dipeptidyl peptidase-4 (DPP-4) inhibitors, and glucagon-like peptide-1 GLP-1 derivatives execute strategy 3; DPP-4 inhibitors and GLP-1 derivatives also execute strategy 4 (5, 6). Thiazolidine derivatives execute strategy 5, and sodium glucose cotransporter 2 inhibitors execute strategy 6 (6). Exercising can address strategy 7 (7). Although therapeutic agents and exercise form the basis of strategies 2 to 7, strategy 1 (reduction in carbohydrate intake) can be implemented only by consuming low-carbohydrate-content food and reducing food intake. However, sufficient varieties of low-carbohydrate-content food products are not available and reduction in food intake may be stressful for diabetic subjects. Thus, increasing the variety of low-carbohydrate-content food products will be helpful for controlling diabetes. Furthermore, preparation of food items with low carbohydrate content can be beneficial for diabetic subjects.

To utilize the Katsuni-uri fruit for diabetes control, information regarding its ripening process and optimally ripe stage are required prior to promoting it as a low-calorie food ingredient, such as sweet fruits substitute for diabetic subjects. Additionally, changes in blood glucose level post-consumption of the juice have to be confirmed, and to the best of our knowledge, this is the first study to verify this observation. This will generate a wide variety of food materials for daily consumption by diabetic subjects, which will potentially improve their quality of life. In this study, we classified Katsura-uri fruit ripening into five stages and attributed physical properties (fruit color and firmness) and compositional characteristics (aroma substances, proximate composition, and sugars) to each stage of the fruit. Based on this classification, we determined that fruit at ripening stage 5 (the completely ripe stage), was most suitable and effective for diabetes control.

MATERIALS AND METHODS

Chemicals. Fructose (99% pure grade), glucose (98% pure grade), sucrose (99% pure grade), benzyl acetate, and eugenol were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). MTPE and MTAE were purchased from Avocado Research Chemicals Ltd. (Lancashire, England). AMTE and AMTP were prepared according to a previously reported method (2). Pal sweet diet® (Ajinomoto Co. Inc., Tokyo, Japan) was purchased at supermarket.

Plant samples. Katsura-uri plants were harvested in July and August 2011, in an open field culture system at the Kyoto Prefectural Agricultural Research Institute, Kameoka, Kyoto, Japan. The variability between the plants harvested in different years was judged to be small because of the preliminary results that the variability of carbohydrate amount in the plants harvested in 2010 and 2011 was small (2.5%). Muskmelon (Cucumis melo var. reticulatus; cultivar name Raiden red) was supplied by a wholesale market in Kyoto, Japan. Katsura-uri fruits were classified into five stages of ripening based on length, fruit color, and aroma. The classification was as follows: fruit at the stage 1 was less than 30 cm in length and yellow-green in color; fruit at the stage 2 was 30–40 cm long and light green in color; fruit at the stage 3 was more than 40 cm in length, and did not grow further (30±1 d after fruit-bearing); fruit at the stage 4 had light yellow color skin and weak fruity aroma (35±1 d after fruit-bearing); fruit at the stage 5 emitted intense fruity aroma (40±1 d after fruit-bearing) (Fig. 1). Prior to recording fruit color and firmness, the
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Katsura-uri fruits were thoroughly washed with water. After the measurements, the fruits were longitudinally cut into four equal pieces, each of which was peeled and the seeds were removed. One piece was used for the quantification of aroma substances, and another piece was freeze-dried and stored at −25°C for analysis of proximate composition and sugar content. The remaining pieces of ripening stage 5 were vacuum-packed in plastic bags and stored at −25°C for estimation of blood glucose levels. The muskmelon was subjected to a similar procedure for analysis of proximate composition and estimation of blood glucose levels.

Measurement of fruit color and firmness. Fruit color was measured on the top, middle, and bottom sides of a Katsura-uri fruit using a colorimeter (Minolta, Osaka, Japan), and was expressed using a CIELAB color system (L*, a*, b*). Fruit firmness was measured using a texture analyzer (Rheotech, Tokyo, Japan). Each fruit was compressed on the top, middle, and bottom portion at the rate of 6 cm/min, and the maximum force developed during the tests was recorded.

Quantification of aroma substances. Aroma substances were analyzed using gas chromatography-electron ionization-mass spectrometry (GC-EI-MS; GCMS-QP2010 Ultra, coupled to a GC-2010 Plus, Shimadzu, Kyoto, Japan). Twenty grams of fresh Katsura-uri fruit was homogenized and extracted four times with 4 mL n-hexane. The n-hexane extracts were volume-filled up to 20 mL and subjected to chromatographic analysis. Two microliters sample was injected into the GC-EI-MS equipped with a Rtx-5MS capillary column (0.25 mm ID×30 m, 0.25 μm film thickness; Restek, Pennsylvania, USA). The column oven temperature was held at 60°C for 5 min and then increased to 100°C at a rate of 5°C/min. The GC-EI-MS in the selected ion monitoring (SIM) mode was used to quantify each aroma substance. The EI-MS m/z (relative intensity) of MTAE, AMTE, MTPE, AMTP, BA, and eugenol identified in the Katsura-uri fruit are as follows: MTAE 134 [M]+ (68, confirmatory ion: CI), 88 (56, CI), 70 (10), 61 (100), AMTE 134 [M]+ (2, CI), 75 (15), 74 (100, QI), 61 (50), 47 (12, CI); MTPE 148 [M]+ (90, CI), 103 (34, CI), 75 (54), 74 (100, QI), 61 (86), 47 (18); AMTP 148 [M]+ (50, CI), 105 (8), 88 (100, QI), 73 (100, CI), 61 (68), 47 (15); BA 150 [M]+ (70, CI), 108 (100, QI), 107 (34), 91 (90), 90 (70), 89 (28), 79 (50, CI), 65 (24), 51 (14); and eugenol 164 [M]+ (100, QI), 149 (50, CI), 137 (26), 131 (42), 121 (24), 103 (34, CI), 91 (26), 77 (30), 55 (25).

Analysis of proximate composition. Proximate composition was analyzed following the methods described in the Standard Tables of Food Composition in Japan in 2015, Seventh edition (8). Moisture content was determined gravimetrically using a dry lyophilized powder. Protein content was estimated by multiplying the total nitrogen concentration determined using the Kjeldahl method and a nitrogen conversion factor of 6.25 (9). Fat content was determined using Soxhlet extraction method and diethyl ether (9). Ash content was determined gravimetrically after incineration of the lyophilized powder at 550°C. Dietary fiber content was determined using the modified Henneberg-Stohmann method (10). Carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash from 100 g. Energy content was determined as the sum of calculated values of protein and carbohydrate (4 kcal/g), and fat (9 kcal/g).

Quantification of sugars. The sugar content was determined using HPLC according to a previously reported method (1). Sugars (fructose, glucose, sucrose) in Katsura-uri fruit had retention times of 9.0, 11.2, and 15.4 min for fructose, glucose, and sucrose, respectively.

Preparation of fruit juice. Katsura-uri juice was prepared as follows: 100 g of frozen fruit (stored at −25°C) was partially thawed, chopped, and mixed in an electric automatic mixer (Tescosm, Tokyo, Japan), with 2.25 g of zero-calorie sweeteners (ingredients: erythritol, aspartame, acesulfame-K; product name: Pal sweet diet®, equivalent to the sweet taste of muskmelon juice. Muskmeon juice was prepared in a similar manner, except that zero-calorie sweeteners were not added.

Measurement of blood glucose levels. Ten diabetic volunteers (6 men and 4 women, aged 47–75 y) who had been hospitalized for diabetes at the University Hospital in Kyoto Prefectural University of Medicine since 1–34 y before were recruited as volunteer outpatients. They usually controlled blood glucose levels by diet and/or oral drug. Their recruitment was approved by the Ethics
Committee of Kyoto Prefectural University (113), and it was in compliance with the declaration of Helsinki. Informed consent was obtained from all volunteers. They were advised to fast, but were allowed to consume water from 22:00 h on the day prior to the trial until the end of the trial. They were advised not to take medication for lowering postprandial blood glucose levels in the morning of the trial day. At 10:00 h on the day of the trial, blood samples were drawn using a finger-stick sampler, and the peripheral blood glucose level was measured using an automated portable glucose meter (Sanwa Kagaku Kenkyusho, Nagoya, Japan). After sampling, the volunteers were provided a cup of Katsura-uri juice (240 g), and their blood glucose levels were measured. The results were considered significantly different at p<0.01. Student’s t-test was performed to assess the differences in the blood glucose levels. The results were considered significantly different at p<0.05.

RESULTS

Fruit color and firmness of Katsura-uri

The color of Katsura-uri fruit at different ripening stages was expressed as L*, a*, b* values as shown in Fig. 2A. The value “L*” refers to lightness, ranging from 0 (dark color) to 100 (white color). The values of “a*” and “b*” indicate chromaticity, with a* ranging from −60 (green color) to 60 (red color), and b* ranging from −60 (blue color) to 60 (yellow color). During ripening stages 1 to 3, the value of L* increased from 70.4 to 82.9. During ripening stages 1 to 4, the loss of green and yellow colors was evident from the increase in a* values from −16.8 to −3.1 and the decrease in b* values from 34.8 to 15.9. Statistical analysis of these results revealed significant effects among stages 1, 2, and 3 (p<0.01). As a result, the appearance of fruit surface color changed from light-green to light-yellow during ripening (Fig. 1).

Katsura-uri fruit gradually softened during ripening (Fig. 2B). A significant decrease in firmness was observed between stage 3 (1.33 kg) and stage 4 (0.50 kg)

Table 1. Concentration of aroma substances in Katsura-uri fruit analyzed at five ripening stages.

| Substance | Stage 1     | Stage 2     | Stage 3     | Stage 4     | Stage 5     |
|-----------|-------------|-------------|-------------|-------------|-------------|
| MTAE      | ldlb        | ldlb        | ldlb        | 0.19b       | 1.05b       |
| (μg/100 g)| (0.05–0.28) | (0.02–0.14) | (0.05–0.28) |
| AMTE      | ldlb        | ldlb        | ldlb        | 0.29b       | 1.87b       |
| (μg/100 g)| (0.16–0.41) | (0.14–0.28) | (0.16–0.41) |             |
| MTPE      | ldlb        | lqla        | ldlb        | 0.45b       | 1.51c       |
| (μg/100 g)| (0.17–0.70) | (0.14–0.28) | (0.17–0.70) |             |
| AMTP      | ldlb        | lqla        | ldlb        | 4.52b       | 6.58b       |
| (μg/100 g)| (2.81–6.92) | (2.51–5.68) | (2.81–6.92) |             |
| BA        | ldlb        | ldlb        | lqlb        | 9.71b       | 25.56b      |
| (μg/100 g)| (9.12–10.43)| (8.51–11.72)| (9.12–10.43)|             |
| Eugenol   | ldlb        | ldlb        | ldlb        | 0.16b       | 0.33b       |
| (μg/100 g)| (0.09–0.36) | (0.07–0.21) | (0.09–0.36) |             |

Each value represents mean of five individual Katsura-uri fruits sampled at the same ripening stage, and their range is shown in parentheses. Different letters in the same row indicate significant differences (Tukey-Kramer test, p<0.01). ldl: lesser than the detection limit (<0.01 μg/100 g); lql: lesser than the quantification limit (<0.05 μg/100 g).
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1.87, 1.51, 6.58, 25.56, and 0.33 (0.01). In fruits that were at stage 5, which is the tasting, significantly increased between stages 4 and 5.

AMTE, MTPE, AMTP, BA, and eugenol were 1.05, (0.01). At stage 5, the firmness was 0.27 kg (the least and therefore the softest among the five stages), and the values remained in a narrow range, indicating similarity in firmness. Katsura-uri fruit at stage 5 was the softest in the fruits during stages, which could be the juice having smoother texture. As the skin color of the fruit changed during stages 1 to 3, the Katsura-uri fruit started softening at the advent of stage 4.

Concentration of aroma substances in Katsura-uri

The aroma substances of Katsura-uri fruit at stage 5 were detected by GC-El-MS, resulting in six peaks of standards. Table 1 shows the quantity of the aroma substances detected in Katsura-uri fruit at each ripening stage. The amounts of all aroma substances during stages 1 to 3 of fruit ripening were lower than the detection limit (0.01 μg per 100 g fresh weight) or the quantification limit (0.05 μg per 100 g fresh weight). During stages 3 to 5 of fruit ripening, the amount of aroma substances gradually increased, as the amounts of MTAE, AMTE, MTPE, AMTP, BA, and eugenol were 1.05, 1.87, 1.51, 6.58, 25.56, and 0.33 μg per 100 g fresh weight, respectively. Each of the six aroma substances had unique aromas as determined by the human sense of smell: MTAE, apricot-like odor; AMTE, flowery odor; MTPE, melon-like odor; AMTP, fruity odor; BA, jasmine-like odor; eugenol, clove-like odor. The combination of these ingredients generates a fruity aroma that is a characteristic of Katsura-uri fruit at the stage 5 of ripening.

Concentration of proximate composition in Katsura-uri

Table 2. Concentration of proximate composition in Katsura-uri fruits at five ripening stages and muskmelon fruits.

| Katsura-uri | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
|-------------|---------|---------|---------|---------|---------|
| Energy (kcal/100 g) | 17 (15–19) | 17 (15–18) | 19 (17–20) | 18 (16–21) | 18 (12–20) |
| Water (g/100 g) | 95.9 (95.3–96.3) | 95.9 (95.7–96.2) | 95.4 (95.1–95.7) | 95.5 (94.9–96.1) | 95.6 (95.0–97.0) |
| Protein (g/100 g) | 0.2 (0.2–0.3) | 0.2 (0.2–0.3) | 0.3 (0.2–0.3) | 0.2 (0.1–0.3) | 0.3 (0.2–0.5) |
| Fat (g/100 g) | 0.05 (0.04–0.05) | 0.05 (0.03–0.06) | 0.03 (0.02–0.04) | 0.02 (0.02–0.04) | 0.04 (0.02–0.05) |
| Carbohydrate (g/100 g) | 3.8 (3.4–4.3) | 3.9 (3.5–4.0) | 4.3 (4.1–4.6) | 4.2 (3.6–4.8) | 4.0 (2.6–4.7) |
| Mineral (g/100 g) | 0.37 (0.34–0.41) | 0.39 (0.37–0.44) | 0.45 (0.38–0.52) | 0.43 (0.39–0.52) | 0.41 (0.35–0.49) |
| Dietary fiber (g/100 g) | 1.2 a (1.0–1.4) | 1.0 b (0.9–1.0) | 0.8 b (0.6–1.1) | 0.7 b (0.6–0.9) | 0.8 b (0.7–0.9) |
| Total dietary fiber (g/100 g) | 0.2 (0.2–0.3) | 0.3 (0.2–0.5) | 0.3 (0.2–0.3) | 0.3 (0.2–0.3) | 0.3 (0.2–0.3) |
| Soluble dietary fiber (g/100 g) | 0.9 a (0.7–1.2) | 0.7 ab (0.6–0.8) | 0.5 (0.3–0.8) | 0.5 (0.4–0.6) | 0.5 (0.4–0.6) |
| Insoluble dietary fiber (g/100 g) | 0.3 (0.02–0.04) | 0.4 (0.38–0.52) | 0.4 (0.39–0.52) | 0.4 (0.35–0.49) | 0.4 (0.35–0.49) |

Each value represents mean of five individual Katsura-uri fruits sampled at the same ripening stage and two individual muskmelon fruits, and their range is indicated in parentheses. Different letters in the same row in Katsura-uri indicate significant differences (Tukey-Kramer test, p<0.01).

Concentration of sugars in Katsura-uri

The sugar fractions of Katsura-uri fruit were assessed using HPLC with refractive index resulting in three peaks: standard fructose, glucose, and sucrose. No significant differences were observed between the ripening stages of Katsura-uri fruit (Table 3). Sucrose was not detected in the immature and semi-ripened fruits (from the stage 1 to 3), whereas the amounts of sucrose in 100 g of Katsura-uri fruit at the stage 5, which is suitable for producing the fruity aroma and preparation of juice, were 18 kcal, 95.6 g, 0.3 g, 0.04 g, 4.0 g, 0.41 g, and 0.8 g, respectively.

Concentration of the blood glucose levels

Blood glucose levels of ten diabetic volunteers were measured after consuming either 240 g of muskmelon
Table 3. Concentration of sugars in Katsura-uri fruits at five ripening stages and muskmelon fruits.

|                | Stage 1 (g/100 g) | Stage 2 (g/100 g) | Stage 3 (g/100 g) | Stage 4 (g/100 g) | Stage 5 (g/100 g) | Muskmelon (g/100 g) |
|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------------------|
| Fructose       | 1.3 (1.1–1.5)     | 1.2 (1.0–1.3)     | 1.3 (1.2–1.6)     | 1.2 (1.0–1.6)     | 1.2 (0.9–1.3)     | 2.7 (2.5–3.0)       |
| Glucose        | 1.3 (1.1–1.3)     | 1.3 (1.2–1.4)     | 1.4 (1.3–1.8)     | 1.2 (1.0–1.4)     | 1.1 (0.8–1.7)     | 1.8 (1.7–2.0)       |
| Sucrose        | LDL               | LDL               | LDL               | 0.4 (0.1–0.8)     | 0.5 (0.2–0.8)     | 4.4 (3.9–4.9)       |
| Total          | 2.7 (2.4–3.1)     | 2.5 (2.3–2.7)     | 2.8 (2.4–3.4)     | 2.8 (2.2–3.7)     | 2.8 (1.8–3.3)     | 8.9 (8.8–9.1)       |

Each value represents mean of five individual Katsura-uri fruits sampled at the same ripening stage, and their range are indicated in parentheses. The values of muskmelon fruits are reported previously (1). No significant difference was observed between ripening stages in Katsura-uri fruits (p<0.01). LDL: lesser than the detection limit (<0.015 g/100 g).

Table 4. Profile of human volunteers.

| Volunteer | Gender | Age | Height (cm) | Weight (kg) | BMI (kg/m²) | HbA1c (%) |
|-----------|--------|-----|-------------|-------------|-------------|-----------|
| A         | Male   | 75  | 166         | 55          | 20.0        | 6.5       |
| B         | Female | 63  | 153         | 41          | 17.5        | 6.6       |
| C         | Female | 57  | 163         | 66          | 24.8        | 7.2       |
| D         | Male   | 75  | 167         | 62          | 22.2        | 6.0       |
| E         | Female | 60  | 163         | 45          | 16.9        | 6.6       |
| F         | Male   | 46  | 165         | 72          | 26.4        | 6.4       |
| G         | Female | 64  | 152         | 57          | 24.7        | 7.1       |
| H         | Male   | 47  | 183         | 86          | 25.7        | 7.1       |
| I         | Male   | 54  | 175         | 84          | 27.4        | 6.8       |
| J         | Male   | 64  | 172         | 70          | 23.7        | 6.7       |
| Average±SE|        | 61±3| 166±3       | 64±5        | 22.9±1.2    | 6.7±0.1   |

HbA1c values were measured using the standard certified by the Japan Diabetes Society (JDS), and converted to National Glycohemoglobin Standardization Program (NGSP) values using the formula provided by the JDS: HbA1c (NGSP)=HbA1c (JDS)+0.4 (12).

Fig. 3. Profiles of blood glucose response curve in ten diabetic volunteers (A-J) after oral administration of two drinks. (○) 240 g of Katsura-uri juice, (●) 240 g of muskmelon juice.
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The levels of glucose, Katsura-uri juice than after consumption of muskmelon juice. The levels of the six aroma substances in Katsura-uri juice were significantly different after oral administration of two drinks in ten diabetic volunteers. The AUC range of the ten volunteers who consumed 240 g of Katsura-uri juice was 16 ± 5 mg/dL in the muskmelon trial; however, the level was 22 ± 5 mg/dL in the Katsura-uri trial. The level was significantly lower after consumption of Katsura-uri juice than after consumption of muskmelon juice (p < 0.05).

**DISCUSSION**

Generally, the amount of aroma substances and sugars increase simultaneously during fruit ripening. Interestingly, the amount of aroma substances showed a marked increase in the Katsura-uri fruit, whereas the sugar content remained constant during the ripening process (Tables 1 and 3). We showed the benefits of Katsura-uri fruit juice, prepared from the fruit having strong fruity aroma and low-sugar content at ripening stage 5 and sweetened with the addition of zero-calorie sweeteners, as a low-calorie beverage for diabetic subjects. This recipe can be used without any concerns regarding increase in postprandial blood glucose levels, and can potentially improve the quality of life of diabetic subjects.

Currently, aroma and sweetness are important criterions for evaluating quality of fruit. Aroma substances in fruits generally increase during fruit ripening (13–15). The levels of the six aroma substances in Katsura-uri fruit dramatically increased at ripening stage 4, and peaked at stage 5 (Table 1). The results of aroma in Katsura-uri are similar to those observed in muskmelon. Only ethanol is detected in immature muskmelon fruits, although other aroma volatiles (e.g., ethylacetate, ethylvpropionate, 2-methylbutylacetate) are produced during the final growth stage (13). In sugar, the content in general fruits also increase during fruit ripening (13, 14, 16). However, change of sugars in Katsura-uri was different from the general change. Additionally, the chemical analyses in this study show that Katsura-uri is a unique fruit as only a few components vary during ripening, unlike other ordinary fruits that show large variations in aroma substances, proximate composition, and sugars during ripening (13–19). In terms of aroma substances and sugar content, Katsura-uri fruits at ripening stage 5 had the strongest fruity aroma and low sugar content similar to that seen at stage 1, which is the best stage for consuming the fruit for controlling diabetes. This is because zero-calorie sweeteners improved the palatability of Katsura-uri at stage 5 without altering its low-sugar properties and fruity aroma (4). The low-sugar characteristic having fruity aroma of Katsura-uri fruit is invaluable from a health perspective as it can be a good source of low-calorie and low-sugar food for obese people and diabetic subjects.

A human trial was conducted to assess blood glucose levels after diabetic volunteers consumed Katsura-uri juice prepared from the fruit at ripening stage 5 supplemented with zero-calorie sweeteners. Recently, glucose spike as well as AUC level is used as an important indicator for assessment of postprandial hyperglycemia. The AUC levels and glucose spike were significantly
lower after consumption of Katsura-uri juice than after consumption of muskmelon juice (Fig. 4). The AUC levels and glucose spike after consumption of Katsura-uri juice were both improved. Previously, we performed similar preliminary trials to measure blood glucose levels in healthy volunteers after consistent consumption of Katsura-uri juice (4). In the present study with diabetic volunteers, a trend similar to the previous trial was observed for the blood glucose levels. For treatment of diabetes, fasting and postprandial blood glucose levels and HbA1c are monitored for glycemic control. Controls for fasting and postprandial hyperglycemia are necessary for achieving the recommended HbA1c level (20). Postprandial hyperglycemia is a strong risk factor for diabetic microangiopathy and cardiovascular disease in type-2 diabetes (21, 22). The blood glucose levels after consumption of Katsura-uri juice were less than the target level for management of postprandial blood glucose levels (160 mg/dL, recommended value by IDF). In contrast, the level of 60 min after consumption of muskmelon juice (167 mg/dL) did not decrease below the target level (Fig. 4A). Volunteers with diabetes could control postprandial blood glucose levels after consumption of Katsura-uri juice. Previously, sensory evaluation of Katsura-uri juice by 531 panelists showed a high satisfaction level in taste and aroma (4). In this study, thus, diabetic volunteers consumed the same amounts of the juices (240 g), which could give the same level of satisfaction in human trial. The results showed that Katsura-uri juice did not raise blood glucose levels in diabetic subjects with keeping high satisfaction muskmelon juice gave to them. Katsura-uri fruit could be a unique fruit utilized positively as the materials in the juice which can be consumed without any concerns regarding increase in postprandial blood glucose levels in diabetic subjects. We consider that Katsura-uri juice has potential to replace other sweet drinks in the daily consumption of diabetic patients and improve their quality of life.

In conclusion, significant changes in physical parameters and concentrations of aroma substances were observed during the ripening process of Katsura-uri fruit, with no change in the levels of proximate composition, except for that of dietary fibers, and sugars. Certain aspects, such as the strong fruity aroma and low-sugar content of stage 5 Katsura-uri fruit, are favorable for preparing beverages for diabetic subjects, as the sweetness can be enhanced by adding zero-calorie sweeteners. These observations may contribute to public health, especially diabetes management. In addition, the current aim of fruits selective breeding toward more sweetness has been successful in meeting the demands of human taste (but probably resulted in a lower benefit to human health). If we could find mildly sweet or non-sweet fruits with keeping the individual aroma, the fruits can be good candidate to be the available low-calorie fruit by adding sweet taste with zero-calorie sweeteners artificially. We might start to consider the direction of selective breeding in the taste of fruits that should go toward mildly sweet for better health promotion.

Authorship
Research conception and design: YN, YK, WA and TN; experiments: AS, YN, KS, NS and MF; interpretation of the data: AS, YN, TM, SO, YT, EYP and KS; writing paper: AS and YN.

Disclosure of state of COI
No conflicts of interest to be declared.

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