Original paper

Daily Radical Scavenging and Singlet Oxygen Quenching Capacity Intake from Fruits and Vegetables in Japan

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Radical scavenging and singlet oxygen quenching activities of 32 kinds of fruits and vegetables typically consumed in Japan were evaluated using both the oxygen radical absorbance capacity (ORAC) and singlet oxygen absorption capacity (SOAC) methods. Total-ORAC and SOAC values correlated with total polyphenol ($r = 0.92$) and carotenoid ($r = 0.85$) contents. From the data of daily fruit and vegetable intake (110.0 and 286.4 g, respectively) in the National Health and Nutrition Survey in Japan, daily ORAC and SOAC intakes were estimated to be 3177.7 μmol trolox equivalents/day and 1610.3 μmol α -tocopherol equivalents/day, respectively. The major contributors to ORAC intake were apples, Satsuma mandarins, edible burdock, and onions, accounting for 44.3 % of the total; whereas Japanese squash, carrots, tomatoes, and spinach accounted for 73.2 % of total SOAC intake. Total antioxidant capacity assessment of foods may facilitate the identification of relationships between antioxidant intake and disease risk reduction.

Keywords: ORAC, SOAC, polyphenol, carotenoid, fruit, vegetable, daily antioxidant capacity intake

Introduction

Epidemiological studies have suggested that the consumption of fruits and vegetables rich in antioxidants is associated with a reduced risk of chronic diseases (Hu *et al.*, 2014; Wang *et al.*, 2014). This is thought to be due in large part to the elimination of reactive oxygen species (ROS) by these foods and their components, as excessive ROS generation in human tissues causes oxidative stress, which in turn may represent one of the causes of chronic diseases such as cancer (Valko *et al.*, 2006), cardiovascular disease (Madamanchi *et al.*, 2005), metabolic disease (Hotamisligil, 2006), and neurodegenerative disease (Jenner, 2003). However, the optimal levels of antioxidant consumption required to maintain good health and/or prevent chronic disease have not yet been established. Accordingly, comprehensive understanding of the total antioxidant capacities of foods is essential to reveal the relationships between antioxidant intake and risk reduction for various diseases.

Peroxyl radicals and singlet oxygen constitute two well-

known representative ROS generated in biological systems. As an evaluation tool, the oxygen radical absorbance capacity (ORAC) assay method (Huang *et al.*, 2002b) can measure not only hydrophilic (H-ORAC) but also lipophilic (L-ORAC) antioxidants (Huang *et al.*, 2002a), and is therefore widely used to evaluate the radical scavenging activity of many kinds of foods (Wu *et al.*, 2004). However, a convenient assay method to evaluate the singlet oxygen quenching activity of antioxidants had not been established. Therefore, we developed a singlet oxygen absorption capacity (SOAC) assay method in previous studies (Aizawa *et al.*, 2011; Mukai *et al.*, 2012; Ouchi *et al.*, 2010). In this method, disappearance of the absorption of 2,5-diphenyl-3,4-benzofuran (DPBF), a UV-vis absorption probe, at 413 nm by singlet oxygen generated by the thermal decomposition of endoperoxide (EP) is measured, and the SOAC value is assessed from the difference in half-life of DPBF decay in the presence and absence of antioxidants. The SOAC assay method can evaluate food samples such as fruits and vegetables (Iwasaki *et al.*, 2015). Furthermore, a modified

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method using a microplate reader has been established, enabling SOAC to be applied as a high throughput assay (Takahashi *et al.*, 2016). This modified SOAC method has been validated in an inter-laboratory test (Wakagi *et al.*, 2017).

Previous studies have reported ORAC values of various foods as their antioxidant capacity; however, the values provide only the radical scavenging activity within the total antioxidant activity. In order to understand the comprehensive antioxidant capacity of foods, it is necessary to measure not only the radical scavenging, but also the singlet oxygen quenching activity. Therefore, measurement using both ORAC and SOAC methods is necessary. Although the activity obtained from *in vitro* assays may not be simply extrapolated to *in vivo* activity, knowledge of the appropriate antioxidant capacity of foods would likely contribute to clarifying the relationship between antioxidant intake and risk reduction for various diseases. For example, several epidemiological studies have shown a significant association between ORAC intake and health (Holtan *et al.*, 2012; Kobayashi *et al.*, 2012; Kobayashi *et al.*, 2014; Kobayashi *et al.*, 2017; Rautiainen *et al.*, 2012; Rautiainen *et al.*, 2013; Rautiainen *et al.*, 2014); whereas other studies showed no significant association (Mekary *et al.*, 2011; Vece *et al.*, 2015). This discrepancy may be a consequence of only the association with radical scavenging activity having been investigated. Therefore, in the present study, we evaluated not only H-ORAC and L-ORAC values, but also SOAC values of fruits and vegetables typically consumed in Japan. In addition, we estimated the daily ORAC and SOAC intakes of Japanese individuals from fruits and vegetables. Furthermore, we measured total polyphenol and carotenoid contents to evaluate the correlation with the ORAC and SOAC value, respectively. To our knowledge, this is the first study that simultaneously measured both radical scavenging and singlet oxygen quenching activities of many kinds of fruits and vegetables, and estimated SOAC intakes from foods.

Materials and Methods

Chemicals and apparatus 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein (sodium salt) (FL), gallic acid, and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), sodium carbonate, 4-methyl-1,4-etheno-2,3-benzodioxin-1(4H)-propanoic acid (endoperoxide, EP), dimethyl sulfoxide (DMSO), α -tocopherol (α -Toc), pyrogallol, triethylamine (TEA), and butylhydroxytoluene (BHT) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methyl- β -cyclodextrin (MCD) was obtained from Junsei Chemical (Tokyo, Japan). 2,5-Diphenyl-3,4-benzofuran (DPBF) was obtained from Tokyo Chemical Industry (Tokyo, Japan).

Extractions of samples were performed on an ASE-200 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA).

ORAC, SOAC, and total polyphenol analyses were carried out using an SH-9000Lab microplate reader (Corona Electric, Ibaraki, Japan). Carotenoid content was analyzed with a Shimadzu Prominence high performance liquid chromatography (HPLC) system consisting of a CBM-20A system controller, an SPD-M20A photodiode array detector, an SIL-20ACHT auto sampler, a CTO-20AC column oven, LC-20AC pumps, and a DGU-20A5 degasser (Shimadzu, Kyoto, Japan). An eVol-XR digital analytical syringe (SGE Analytical Science, Melbourne, Australia) was used for sample addition to the well.

Sampling and sample preparation All fruit and vegetable samples of three or more different crops were purchased from retail stores in Nasushiobara city, Japan in 2015–2018 (Table 1). For each sample, the edible portion was freeze-dried and stored at -30°C . Moisture content was calculated from fresh weight and dry weight, and all values were converted and expressed on a fresh weight basis.

The procedures of sample preparation for ORAC analysis were based on a previous study (Watanabe *et al.*, 2014) with some modifications. A freeze-dried sample (0.3 to 2.0 g) was mixed with approximately 5 g of sea sand (Wako). The mixture was transferred to an 11-mL extraction cell and initially extracted with hexane/dichloromethane (1:1, v/v; Hex/Dc) at 70°C , followed by methanol/water/acetic acid (90:9.5:0.5, v/v/v; MWA) at 80°C using an ASE-200 accelerated solvent extractor. Hex/Dc extracts were dried under nitrogen gas flow in a 30°C water bath, and the residue was reconstituted in 5 mL DMSO and diluted 10-fold with dilution buffer (7 % MCD (w/v) in a 50 % acetone-water mixture (v/v)). This solution was used to measure the L-ORAC value following dilution as necessary. MWA extracts were transferred to a 25-mL brown volumetric flask and diluted with MWA to a total volume of 25 mL. This solution was used to measure the H-ORAC value after appropriate dilution.

The procedures of sample preparation for the SOAC analysis were performed according to previous studies (Aizawa *et al.*, 2011; Iwasaki *et al.*, 2015). A freeze-dried sample (0.3 to 2.0 g) was mixed with approximately 5 g of sea sand. The mixture was transferred to an 11-mL extraction cell, and extracted with ethanol/chloroform/ D_2O (50:50:1, v/v/v; ECD) at 70°C using an ASE-200 accelerated solvent extractor. ECD extracts were adjusted to 25 mL with ECD in a 25-mL brown volumetric flask. This solution was used to measure the SOAC value after dilution as necessary.

H-ORAC and L-ORAC assay H-ORAC and L-ORAC values were obtained following 2-step measurement according to previous studies (Watanabe *et al.*, 2012; Watanabe *et al.*, 2016). The first-step measurement was performed to calibrate approximate H-ORAC and L-ORAC values for each test sample, and the second-step measurement was performed to measure the net area under the curve (AUC) at two optimal dilution ratios settled by the value in the first-step

Table 1. The list of 32 fruits and vegetables samples, survey item number, item number, data of purchase, and place of production.

Category Sample name	Survey item No. ^a	Item No. ^b	Data of purchase					Place of production				
			N = 1	N = 2	N = 3	N = 4	N = 5	N = 1	N = 2	N = 3	N = 4	N = 5
Fruits												
Apples	300	07148	2015/4/14	2016/11/9	2017/7/12	-	-	Aomori	Yamagata	Aomori	-	-
Bananas	312	07107	2016/8/19	2017/7/7	2017/7/7	-	-	Philippines	Philippines	Ecuador	-	-
Grapefruit, pink	314	07164	2016/8/19	2017/7/7	2017/9/13	-	-	South Africa	South Africa	South Africa	-	-
Grapes	306	07116	2015/4/16	2015/8/7	2017/7/7	-	-	Chile	Yamanashi	Tochigi	-	-
Japanese persimmons	307	07049	2016/8/19	2016/11/9	2017/9/8	-	-	Nara	Wakayama	Nara	-	-
Kiwifruit, green	316	07054	2015/8/3	2016/11/9	2017/7/7	-	-	New zealand	New zealand	New zealand	-	-
Muskmelon, orange	310	07174	2015/8/7	2017/7/7	2017/7/12	-	-	Yamagata	Ibaraki	Chiba	-	-
Oranges, Valencia	315	07041	2015/4/14	2017/7/7	2017/7/12	-	-	USA	USA	Australia	-	-
Peaches, white	308	07136	2015/8/7	2017/7/7	2017/9/8	-	-	Nagano	Yamanashi	Yamagata	-	-
Pears, sand pears	305	07088	2016/8/19	2017/9/8	2017/10/6	-	-	Tochigi	Ishikawa	Tochigi	-	-
Satsuma mandarins	301	07027	2015/4/14	2016/11/9	2017/9/8	-	-	Kagawa	Kumamoto	Aichi	-	-
Strawberries	311	07012	2015/4/14	2018/1/12	2018/1/12	-	-	Tochigi	Tochigi	Fukuoka	-	-
Watermelon, red	309	07077	2015/8/7	2017/7/7	2017/7/11	-	-	Tochigi	Niigata	Chiba	-	-
Vegetables												
Bamboo shoots ^d	25X	06149	2016/8/19	2017/7/7	2017/7/7	-	-	China	Kyusyu	Yamagata	-	-
Bean sprouts, mung bean sprouts	245	06291	2016/8/19	2017/7/11	2017/9/8	-	-	Tochigi	Tochigi	Tochigi	-	-
Broccoli	247	06263	2015/4/14	2015/7/2	2015/7/2	2016/11/9	-	Aichi	Iwate	Tochigi	Tochigi	-
Cabbage	240	06061	2016/4/1	2016/11/9	2017/7/11	-	-	Kanagawa	Tochigi	Aomori	-	-
Carrot, orange	254	06212	2015/4/14	2017/7/7	2017/7/11	-	-	Tokushima	Chiba	Hokkaido	-	-
Chinese cabbage	242	06233	2015/4/14	2017/7/7	2017/7/12	-	-	Ibaraki	Nagano	Ibaraki	-	-
Cucumber	262	06065	2015/8/3	2015/8/3	2017/7/11	-	-	Tochigi	Iwate	Tochigi	-	-
East Indian lotus root	258	06317	2016/8/19	2017/7/7	2017/7/11	-	-	Ibaraki	Ibaraki	Tokushima	-	-
Edible burdock	255	06084	2015/8/3	2016/11/9	2017/7/11	-	-	Tochigi	Tochigi	Aomori	-	-
Eggplant, Japanese type	263	06191	2015/4/14	2017/7/7	2017/7/12	-	-	Kumamoto	Tochigi	Gunma	-	-
Japanese radishes, Daikon	253	06134	2016/4/1	2016/11/9	2017/7/12	-	-	Kanagawa	Tochigi	Aomori	-	-
Japanese squash	261	06046	2015/4/14	2015/7/2	2015/7/2	2015/7/31	2016/11/9	New zealand	Kanagawa	Kagoshima	Tochigi	Hokkaido
Kidney beans, "Sayaingen"	260	06010	2015/7/31	2015/8/3	2015/8/3	-	-	Tochigi	Iwate	Wakayama	-	-
Lettuce, head lettuce, crisp type	244	06312	2016/4/1	2016/11/9	2017/7/13	-	-	Ibaraki	Tochigi	Nagano	-	-
Onions	256	06153	2015/4/14	2017/7/11	2017/7/11	-	-	Hokkaido	Tochigi	Kumamoto	-	-
Spinach	241	06267	2015/4/14	2015/7/2	2017/7/11	-	-	Gunma	Tochigi	Tochigi	-	-
Sweet peppers, green	265	06245	2015/4/14	2015/7/31	2017/7/12	-	-	Miyazaki	Tochigi	Tochigi	-	-
Tomatoes	264	06182	2015/11/9	2015/11/9	2015/11/9	2016/8/19	-	Tochigi	Hokkaido	Tochigi	Hokkaido	-
Welsh onions, "Nebuka-negi"	243	06226	2016/8/19	2016/11/9	2017/7/12	-	-	Ibaraki	Tochigi	Tochigi	-	-

^a Survey item numbers in the annual report on the Family Income and Expenditure Survey.^b Item numbers in the Standard tables of Food Composition in Japan 2015, Seventh Revised Edition.

measurement. For the H-ORAC assay, trolox calibration solutions (6.1, 12.1, 24.2, and 48.4 μM), FL solution (110.7 nM), and AAPH solution (31.7 mM) were made with 10 % (v/v) MWA in the assay buffer (75 mM phosphate buffer, pH 7.4). For the L-ORAC assay, trolox calibration solutions (19.4, 38.8, 77.5, and 155.0 μM), FL solution (77.5 nM), and AAPH solution (82.4 mM) were prepared with 10 % (v/v) DMSO in the dilution buffer. Extracted samples for H-ORAC and L-ORAC assays were diluted as necessary with 10 % (v/v) MWA in the assay buffer and 10 % (v/v) DMSO in the dilution buffer, respectively. Trolox calibration solution or two concentration samples (35 μL) were added to the wells of the 96-well microplate in duplicate using a digital analytical syringe. Then, FL solution (115 μL) and AAPH solution (50 μL) were added to all wells, and a sealing film for real-time polymerase chain reaction amplification was placed on the plate. The fluorescence intensity (excitation at 485 nm and emission at 528 nm) was monitored every 2 min for 120 min at 37 °C with a microplate reader. The net AUC was calculated by subtracting the AUC for the blank from that for the sample or standard. The H-ORAC and L-ORAC values for each sample were calculated on the basis of the standard curve for trolox. These values are expressed as micromoles of trolox equivalents (TE) per gram fresh weight of the edible portion ($\mu\text{mol TE/g FW}$).

SOAC assay The SOAC assay was carried out using the method of our previous study (Takahashi *et al.*, 2016). α -Toc calibration solution (1.50 mM), DPBF solution (0.19 mM), and EP solution (1.2 mM) were prepared with ECD. ECD-extracted samples were measured for absorbance at 413 nm using a UV-1800 UV-Vis spectrophotometer (Shimadzu) and diluted with ECD as necessary; these were used as stock solutions. Stock solutions were further diluted to 2/10, 3/10, and 6/10 with ECD. α -Toc calibration solution or four concentration samples (300 μL) were transferred to the wells of a 24-well glass microplate FU-24 (NGS Precision, Tokyo, Japan) or a 24-well microplate made of polytetrafluoroethylene (PTFE; iTEC SCIENCE, Ibaraki, Japan) using a digital analytical syringe. Then, DPBF solution and EP solution (300 μL each) were added to all wells except for the baseline wells, which were used to correct the absorbance. The final volume of each well was adjusted to 900 μL with ECD. Immediately after completion of these additions, the 24-well microplate was tightly sealed with a quartz glass cover and transferred to the microplate reader, which had been pre-incubated to 35 °C. Absorbance at 413 nm was measured every 30 s for 120 min at 35 °C. According to previous reports (Takahashi *et al.*, 2016), the absorbance in the time range $40 < t < 70$ min was used for analysis. The SOAC value was calculated from each half-life ($t_{1/2}$) of four concentration samples, and these values were averaged. If the difference between the $t_{1/2}$ values of the sample and the blank was smaller than 5 min, this value was excluded from the average SOAC value. When all four concentration

samples did not satisfy this condition, the SOAC value was defined as not detected (nd). The SOAC value is expressed as micromoles of α -Toc equivalents per gram fresh weight of the edible portion ($\mu\text{mol } \alpha\text{-TE/g FW}$).

Carotenoid quantification The carotenoid analysis was performed by reverse phase HPLC according to a previously reported method (Aizawa and Inakuma, 2007) with some modifications. Freeze-dried sample (0.1 to 0.3 g) and pyrogallol (1.0 g) were added to a 50-mL brown volumetric flask. After the addition of 3 mL distilled water, the mixture was mixed well. Hexane/ethanol/acetone/toluene (10:7:6:7, v/v/v/v; HEAT, 20 mL) was added to a volumetric flask and then sonicated for 10 min in a bath sonicator. The extract was adjusted to 50 mL with ethanol. The extract (10 mL) was mixed with HEAT (20 mL), pyrogallol (0.2 g), 40 % (w/v) methanolic KOH (2 mL), and saponified for 20 min at 56 °C. After the addition of 3 % (w/v) NaCl solution (10 mL), the pigment was extracted with HEAT several times and evaporated to dryness. Subsequently, the residue was dissolved in 10 mL HEAT/ethanol (40:60, v/v) and filtered through a 0.45- μm PTFE filter. An aliquot of 5 to 10 μL was injected into the HPLC. A YMC Carotenoid C30 column (S-5 μm , 250 $\times$ 2.0 mm I.D.; YMC, Kyoto, Japan) with a C30 cartridge pre-column (S-5 μm , 10 $\times$ 2.1 mm I.D.; YMC, Kyoto, Japan) was used, and the column temperature was set at 30 °C. The composition of solvents was as follows: A = methanol/methyl *tert*-butyl ether/water (75:5:20) with 0.5 % TEA and 0.01 % BHT; B = methanol/methyl *tert*-butyl ether/water (8:90:2) with 0.5 % TEA and 0.01 % BHT. The gradient procedure at a flow rate of 0.2 mL/min was as follows: a linear gradient from 0 % to 100 % B for 25 min, a hold at 100 % B for 3 min, a linear gradient back to 0 % B for 2 min, and a hold at 0 % B for 10 min. UV/Vis spectra were recorded in the range from 250 to 550 nm and a chromatogram was acquired at 460 nm. Using this method, (all-*E*)-capsanthin, (all-*E*)-lutein, (all-*E*)-zeaxanthin, (all-*E*)- β -cryptoxanthin, (all-*E*)- α -carotene, (all-*E*)- β -carotene, (all-*E*)-lycopene, (5-*Z*)-lycopene, (9-*Z*)-lycopene, and (13-*Z*)-lycopene were adequately separated. These carotenoids were quantified at 460 nm using a calibration curve for each carotenoid except for (Z)-lycopene isomers, which were quantified using the calibration curve of (all-*E*)-lycopene. Each carotenoid concentration was expressed as milligrams per 100 gram fresh weight of the edible portion (mg/100 g FW).

Total polyphenol analysis The total polyphenol analysis was based on the Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). For total polyphenol analysis, a freeze-dried sample (0.25 g) and 80 % methanol (8 mL) were added to a glass test tube, and the mixture was incubated in a water bath at 80 °C for 10 min. After the sample was cooled to room temperature, the sample was centrifuged at 1000 $\times g$ for 20 min. The supernatant was transferred to a 10-mL brown volumetric flask and adjusted to 10 mL with 80 % methanol. The extract was diluted 5-fold with 80 % methanol. Diluted

extract or gallic acid calibration solutions (100 µL) were transferred to separate plastic tubes, and 1/10 diluted Folin-Ciocalteu reagent (200 µL) was added to each tube. After 5-min incubation at room temperature, 7 % sodium carbonate solution (800 µL) was added and incubated in a water bath at 25 °C for 2 h. Then, 200 µL of supernatant after centrifugation at 1000 × g for 10 min was added to the 96-well microplate and the absorbance at 750 nm was measured at 25 °C using a microplate reader. The total polyphenol concentration was calculated from the standard curve for gallic acid ranging from 12.5 to 400 µM. Total polyphenol content was expressed as milligrams of gallic acid equivalents (GAE) per gram fresh weight of the edible portion (mg GAE/g FW).

Estimation of daily ORAC and SOAC intake from fruits and vegetables The 13 fruits and 19 vegetables surveyed in the annual report on the Family Income and Expenditure Survey in Japan were used for the daily ORAC and SOAC intake estimation. According to the method of Takebayashi *et al.* (2013), the percentage of intake for each fruit and vegetable was calculated as follows:

$$\text{Percentage of intake (\%)} = \frac{W \times \frac{(100-R)}{100}}{\sum [W \times \frac{(100-R)}{100}]} \times 100 \dots \text{Eq. 1}$$

where W (g) is the 5-year average of the annual purchase weights per household of each fruit and vegetable from the annual report on the Family Income and Expenditure Survey 2012–2016 (Statistics Bureau, Ministry of Internal Affairs and Communications, Japan), R (%) is the discard (or inedible) ratio described in the Standard Tables of Food Composition in Japan 2015, Seventh Revised Edition (Council for Science and Technology, Ministry of Education, Culture, Sports, Science and Technology, Japan), and $\sum \{W \times (100 - R) / 100\}$ (g) is the sum of the estimated annual intake per household of the 13 fruits and 19 vegetables. The estimated daily intake of each fruit and vegetable was calculated as follows:

$$\text{Estimated daily intake (g/day)} = \frac{P}{\sum P} \times D \dots \text{Eq. 2}$$

where P (%) is the percentage of intake for corresponding fruits and vegetables; $\sum P$ is the sum of the percentage of intake of fruits or vegetables (in case of fruits, $\sum P$ is the sum of fruits; in case of vegetables, $\sum P$ is the sum of vegetables), and D (g) is the 5-year average of daily intake of fruits or vegetables for adults (≥ 20 years old) reported in the National Health and Nutrition Survey in Japan 2012–2016. Thereafter, daily ORAC and SOAC intakes from fruits and vegetables were estimated from Total-ORAC values and SOAC values, and the above daily intake of each fruit and vegetable.

Statistics All analyses were evaluated in three or more independent samples. H-ORAC, L-ORAC, SOAC, and total polyphenol analyses were performed in triplicate and average values were adopted. The data were expressed as mean $\pm$ standard deviation of independent samples. Correlations were tested by Pearson's product-moment correlation analysis using

EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

Results

H-ORAC, L-ORAC, and Total-ORAC values and total polyphenol contents Table 2 shows H-ORAC, L-ORAC, Total-ORAC values, and total polyphenol contents of 32 typically consumed fruits and vegetables in Japan. H-ORAC and L-ORAC values were obtained in all samples. The H-ORAC values of each sample broadly ranged from 1.34 (cucumber) to 83.77 (edible burdock) µmol TE/g FW. In comparison, L-ORAC values were consistently lower, and ranged from 0.02 (grapes) to 3.76 (spinach) µmol TE/g FW. The total-ORAC value, which is the sum of H-ORAC and L-ORAC values, paralleled H-ORAC values because the contribution of L-ORAC values was quite low. Among 32 fruits and vegetables, the highest Total-ORAC values were observed for edible burdock (84.56 µmol TE/g FW), followed by East Indian lotus root (24.83 µmol TE/g FW), strawberries (20.23 µmol TE/g FW), and spinach (20.15 µmol TE/g FW). Total polyphenol contents ranged from 0.09 (carrot and lettuce) to 2.40 (edible burdock) mg GAE/g FW. The relationships between H-ORAC, L-ORAC, or Total-ORAC value and total polyphenol content are presented in Fig. 1A–C. Total-ORAC or H-ORAC value significantly and strongly correlated with total polyphenol content ($r = 0.92$ and 0.922 , respectively); however, a significant correlation between L-ORAC value and total polyphenol content was not observed ($r = 0.114$).

SOAC values and carotenoid contents SOAC values and carotenoid contents of 32 fruits and vegetables are presented in Table 3. SOAC values were obtained in 25 of the 32 samples. The range of SOAC values was from 0.18 (bamboo shoots) to 47.43 (Japanese squash) µmol α -TE/g FW among the detectable 25 samples. The SOAC value of Japanese squash was more than double that of carrot (19.50 µmol α -TE/g FW), which was the second highest. The highest total carotenoid levels were found in spinach (9.51 mg/100 g FW), carrot (9.42 mg/100 g FW), and Japanese squash (8.90 mg/100 g FW). In regards to individual carotenoids, capsanthin was not detected among these samples. Lycopene was detected in only 4 samples: pink grapefruit (2.35 mg/100 g FW), Japanese persimmons (0.07 mg/100 g FW), watermelon (3.49 mg/100 g FW), and tomatoes (2.75 mg/100 g FW). β -Cryptoxanthin was detected in 11 samples, with the highest level detected in Satsuma mandarins (1.28 mg/100 g FW). Lutein, zeaxanthin, α -carotene, and β -carotene were detected in 26, 16, 11, and 27 samples, respectively. No carotenoids were detected in 4 samples (East Indian lotus root, Japanese radish, onions, and pears), and SOAC values were also not obtained from these. The relationship between SOAC value and carotenoid content was evaluated in the 25 samples from which both SOAC values and carotenoids were detected (Fig. 1D). SOAC values were

Table 2. H-ORAC values, L-ORAC values, total ORAC values, total ORAC values, and polyphenol contents of fruits and vegetables^a

Category Sample name	N	ORAC value (μmol TE/g FW) ^b			Polyphenol content (mg GAE/g FW) ^d
		H-ORAC	L-ORAC	Total ORAC ^c	
Fruits					
Apples	3	16.71 ± 2.76	0.46 ± 0.28	17.17 ± 2.90	0.57 ± 0.16
Bananas	3	4.64 ± 0.94	0.87 ± 0.24	5.52 ± 0.94	0.89 ± 0.68
Grapefruit, pink	3	14.28 ± 2.26	0.39 ± 0.04	14.67 ± 2.26	0.47 ± 0.31
Grapes	3	5.44 ± 0.21	0.02 ± 0.04	5.46 ± 0.25	0.28 ± 0.07
Japanese persimmons	3	2.62 ± 1.88	0.05 ± 0.09	2.67 ± 1.91	0.45 ± 0.26
Kiwifruit, green	3	5.91 ± 1.31	0.34 ± 0.09	6.25 ± 1.26	0.54 ± 0.30
Muskmelon, orange	3	2.24 ± 0.59	0.24 ± 0.15	2.48 ± 0.67	0.21 ± 0.10
Oranges, Valencia	3	18.92 ± 11.28	0.41 ± 0.05	19.32 ± 11.30	0.88 ± 0.27
Peaches, white	3	13.99 ± 4.41	0.33 ± 0.14	14.32 ± 4.27	0.52 ± 0.07
Pears, sand pears	3	1.63 ± 0.58	0.13 ± 0.02	1.76 ± 0.59	0.10 ± 0.04
Satsuma mandarins	3	17.24 ± 4.36	0.30 ± 0.15	17.54 ± 4.43	0.49 ± 0.18
Strawberries	3	19.65 ± 3.17	0.58 ± 0.24	20.23 ± 2.94	1.16 ± 0.09
Watermelon, red	3	1.39 ± 0.26	0.22 ± 0.07	1.62 ± 0.21	0.11 ± 0.02
Vegetables					
Bamboo shoots ^e	3	9.67 ± 1.87	1.80 ± 0.09	11.46 ± 1.80	0.42 ± 0.10
Beans prouts, mung bean sprouts	3	6.07 ± 3.76	0.42 ± 0.08	6.49 ± 3.84	0.19 ± 0.14
Broccoli	4	12.09 ± 3.92	2.06 ± 0.41	14.15 ± 4.27	0.62 ± 0.26
Cabbage	3	4.29 ± 1.08	0.69 ± 0.30	4.98 ± 0.89	0.27 ± 0.11
Carrot, orange	3	1.65 ± 0.53	0.49 ± 0.02	2.14 ± 0.55	0.09 ± 0.02
Chinese cabbage	3	2.53 ± 0.84	0.31 ± 0.06	2.84 ± 0.90	0.13 ± 0.02
Cucumber	3	1.34 ± 0.10	0.50 ± 0.04	1.84 ± 0.09	0.12 ± 0.01
East Indian lotus root	3	24.18 ± 7.06	0.65 ± 0.28	24.83 ± 7.22	1.06 ± 0.51
Edible burdock	3	83.77 ± 53.15	0.79 ± 0.34	84.56 ± 53.41	2.40 ± 1.46
Eggplant, Japanese type	3	19.32 ± 7.66	0.44 ± 0.06	19.76 ± 7.69	0.58 ± 0.19
Japanese radishes ,Daikon	3	2.38 ± 1.75	0.27 ± 0.11	2.65 ± 1.65	0.10 ± 0.03
Japanese squash	5	4.12 ± 1.14	1.57 ± 0.67	5.70 ± 1.08	0.30 ± 0.07
Kidney beans, "Sayaingen"	3	4.81 ± 2.27	1.36 ± 0.63	6.17 ± 1.69	0.25 ± 0.01
Lettuce, head lettuce, crisptype	3	2.45 ± 0.85	0.62 ± 0.19	3.08 ± 1.04	0.09 ± 0.02
Onions	3	6.96 ± 2.21	0.33 ± 0.10	7.29 ± 2.23	0.36 ± 0.19
Spinach	3	16.39 ± 4.79	3.76 ± 0.54	20.15 ± 4.84	0.49 ± 0.17
Sweet peppers, green	3	7.93 ± 5.85	0.75 ± 0.47	8.68 ± 6.31	0.50 ± 0.24
Tomatoes	4	3.19 ± 0.58	0.38 ± 0.08	3.57 ± 0.58	0.26 ± 0.06
Welsh onions, "Nebuka-negi"	3	2.71 ± 0.78	0.56 ± 0.23	3.26 ± 0.72	0.13 ± 0.06

^a Data are presented as mean ± standard deviation of three or more independent samples.^b ORAC values are expressed as micromoles of Trolox equivalents (TE) per gram fresh weight of edible portion (μmol TE/g FW).^c Total ORAC = H-ORAC + L-ORAC.^d Polyphenol content are expressed as milligrams of gallic acid equivalents (GAE) per gram fresh weight of edible portion (mg GAE/g FW).^e Bamboo shoots, canned in water, were used for the measurement.

significantly and strongly correlated with carotenoid contents ($r = 0.85$).

Daily ORAC and SOAC intakes of Japanese individuals from fruits and vegetables Table 4 presents an estimation of daily ORAC and SOAC intakes from fruits and vegetables in Japan. Daily ORAC and SOAC intakes were estimated using the average daily fruit and vegetable intakes for adults (≥ 20

years old) in the National Health and Nutrition Survey in Japan 2012–2016, which were 110.0 and 286.4 g, respectively. For fruits, the estimated ORAC intake was 1214.9 μmol TE/day, and the SOAC intake was 228.8 μmol α-TE/day. The estimated ORAC and SOAC intakes from vegetables were 1962.8 μmol TE/day and 1381.5 μmol α-TE/day, respectively. Total-ORAC and SOAC intakes from fruits and vegetables were estimated

Fig. 1. Relationship between H-ORAC (A), L-ORAC (B) and total-ORAC values (C), and total polyphenol content, and between SOAC value and carotenoid content (D). The correlation was assessed by using Pearson's product-moment correlation coefficient (r).

to be 3177.7 μmol TE/day and 1610.3 μmol α-TE/day, respectively.

Discussion

A large number of studies have indicated that the consumption of whole fruits and vegetables, rather than of certain individual components contained therein, is more effective to maintain overall health and prevent chronic diseases. Fruits and vegetables contain various antioxidants that have different effects on various ROS; therefore, evaluation of the scavenging activity of foods against several types of ROS is thought to be important. Peroxyl radicals and singlet oxygen are considered as representative ROS among various kinds of ROS generated in biological systems. To evaluate the radical scavenging activity of foods, H-ORAC and L-ORAC methods were developed (Huang *et al.*, 2002a; Huang *et al.*, 2002b), and modified H-ORAC and L-ORAC methods have been validated (Watanabe *et al.*, 2012; Watanabe *et al.*, 2016). Recently, we developed a SOAC method to evaluate the singlet oxygen quenching activity of antioxidants and foods (Aizawa *et al.*, 2011; Iwasaki *et al.*, 2015; Mukai *et al.*, 2012; Ouchi *et al.*, 2010; Takahashi *et al.*, 2016). Furthermore, Wakagi *et al.* (2016) reported the validation of a SOAC method using a microplate reader. In the present study, we therefore measured not only H-ORAC and L-ORAC values, but also SOAC values of 32 kinds of fruits and vegetables commonly

consumed in Japan using these validated methods.

The H-ORAC, L-ORAC values, and total polyphenol content for many kinds of foods have been determined in previous studies (Mikami-Konishide *et al.*, 2013; Takebayashi *et al.*, 2013; Wu *et al.*, 2004). In the current study, we found that edible burdock, East Indian lotus root, strawberries, and spinach showed the highest Total-ORAC value as calculated from individual H-ORAC and L-ORAC measurements, which was consistent with previous findings (Takebayashi *et al.*, 2013). In particular, it has been reported that edible burdock has a relatively high ORAC value among vegetables (Mikami-Konishide *et al.*, 2013) and contained caffeoylquinic acid derivatives such as chlorogenic acid and dicaffeoylquinic acid, which have antioxidant activity (Maruta *et al.*, 1995). However, the H-ORAC and L-ORAC values obtained in this study are generally slightly lower than the values reported previously (Mikami-Konishide *et al.*, 2013; Takebayashi *et al.*, 2013; Wu *et al.*, 2004), which may be attributable to the specific cultivar, climate, and growing conditions (Mikami-Konishide *et al.*, 2013).

Polyphenols are known as the main contributors to the radical scavenging effect; therefore, we examined the correlations between H-ORAC, L-ORAC, or Total-ORAC values with total polyphenol contents. We found that the H-ORAC value had the highest correlation with total polyphenol content, followed by Total-ORAC and L-ORAC

Table 3. SOAC values and carotenoid contents of fruits and vegetables^a

Category	Sample name	N	SOAC value ^b ($\mu\text{mol } \alpha\text{-TE/g FW}$)	Carotenoid content (mg/100g FW) ^c						
				Capsanthin	Lutein	Zeaxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	Lycopene
Fruits										
	Apples	3	0.48 $\pm$ 0.12	nd ^e	0.04	nd	nd	0.03 $\pm$ 0.00	nd	0.05 $\pm$ 0.03
	Bananas	3	0.48 $\pm$ 0.03	nd	0.15 $\pm$ 0.02	nd	nd	0.05 $\pm$ 0.03	0.05 $\pm$ 0.02	0.25 $\pm$ 0.03
	Grapefruit, pink	3	5.94 $\pm$ 2.37	nd	nd	0.04	0.07	0.70 $\pm$ 0.27	2.35 $\pm$ 0.23	3.09 $\pm$ 0.44
	Grapes	3	nd	nd	0.07 $\pm$ 0.04	0.00	nd	0.03 $\pm$ 0.01	nd	0.10 $\pm$ 0.04
	Japanese persimmons	3	2.93 $\pm$ 0.82	nd	0.06 $\pm$ 0.05	0.13 $\pm$ 0.00	0.26 $\pm$ 0.08	0.06 $\pm$ 0.00	0.07 $\pm$ 0.03	0.52 $\pm$ 0.08
	Kiwifruit, green	3	0.80 $\pm$ 0.37	nd	0.14 $\pm$ 0.03	0.02	nd	0.04 $\pm$ 0.01	nd	0.19 $\pm$ 0.02
	Muskmelon, orange	3	5.99 $\pm$ 1.69	nd	0.06 $\pm$ 0.03	nd	0.03 $\pm$ 0.01	2.87 $\pm$ 1.03	nd	2.98 $\pm$ 1.06
	Oranges, Valencia	3	2.52 $\pm$ 0.83	nd	0.10 $\pm$ 0.02	0.08 $\pm$ 0.05	0.21 $\pm$ 0.06	0.02 $\pm$ 0.01	nd	0.41 $\pm$ 0.09
	Peaches, white	3	0.32	nd	nd	0.02 $\pm$ 0.02	0.01	0.01	nd	0.02 $\pm$ 0.03
	Pears, sand pears	3	nd	nd	nd	nd	nd	nd	nd	nd
	Satsuma mandarins	3	4.84 $\pm$ 1.03	nd	0.09 $\pm$ 0.08	0.06 $\pm$ 0.00	1.28 $\pm$ 0.45	0.09 $\pm$ 0.09	nd	1.49 $\pm$ 0.48
	Strawberries	3	0.37	nd	0.05 $\pm$ 0.03	nd	0.00	0.01 $\pm$ 0.00	nd	0.06 $\pm$ 0.02
	Watermelon, red	3	9.22 $\pm$ 5.86	nd	0.06 $\pm$ 0.00	nd	nd	0.72 $\pm$ 0.20	3.49 $\pm$ 0.81	4.26 $\pm$ 0.87
Vegetables										
	Bamboo shoots ^f	3	0.18	nd	0.03	nd	nd	nd	nd	0.03
	Bean sprouts, mung bean sprouts	3	nd	nd	0.03 $\pm$ 0.01	0.00 $\pm$ 0.00	nd	0.01 $\pm$ 0.00	nd	0.04 $\pm$ 0.01
	Broccoli	4	4.41 $\pm$ 2.71	nd	0.83 $\pm$ 0.30	0.02	0.05	0.03 $\pm$ 0.01	0.42 $\pm$ 0.15	1.29 $\pm$ 0.48
	Cabbage	3	0.94 $\pm$ 0.20	nd	0.19 $\pm$ 0.05	0.08 $\pm$ 0.09	nd	0.02 $\pm$ 0.00	0.10 $\pm$ 0.03	0.35 $\pm$ 0.06
	Carrot, orange	3	19.50 $\pm$ 3.78	nd	0.20 $\pm$ 0.03	nd	nd	2.75 $\pm$ 0.98	6.48 $\pm$ 1.05	9.42 $\pm$ 2.03
	Chinese cabbage	3	0.67 $\pm$ 0.05	nd	0.10 $\pm$ 0.03	0.00	nd	0.00	0.04 $\pm$ 0.01	0.14 $\pm$ 0.04
	Cucumber	3	2.34 $\pm$ 0.46	nd	0.87 $\pm$ 0.20	nd	nd	0.01 $\pm$ 0.00	0.29 $\pm$ 0.01	1.17 $\pm$ 0.21
	East Indian lotus root	3	nd	nd	nd	nd	nd	nd	nd	nd
	Edible burdock	3	nd	nd	0.05 $\pm$ 0.03	nd	nd	0.01 $\pm$ 0.01	nd	0.06 $\pm$ 0.02
	Eggplant, Japanese type	3	0.49 $\pm$ 0.15	nd	0.12 $\pm$ 0.03	nd	nd	0.06 $\pm$ 0.01	nd	0.17 $\pm$ 0.05
	Japanese radishes, Daikon	3	nd	nd	nd	nd	nd	nd	nd	nd
	Japanese squash	5	47.43 $\pm$ 18.93	nd	5.36 $\pm$ 2.78	0.41 $\pm$ 0.40	0.11 $\pm$ 0.04	0.18 $\pm$ 0.08	2.84 $\pm$ 1.48	8.90 $\pm$ 3.82
	Kidney beans, "Sayaingen"	3	4.03 $\pm$ 0.52	nd	0.90 $\pm$ 0.37	0.02 $\pm$ 0.00	nd	0.10 $\pm$ 0.04	0.41 $\pm$ 0.12	1.43 $\pm$ 0.54
	Lettuce, head lettuce, crisptype	3	2.02 $\pm$ 1.04	nd	0.29 $\pm$ 0.13	0.00 $\pm$ 0.00	nd	0.03	0.25 $\pm$ 0.11	0.55 $\pm$ 0.23
	Onions	3	nd	nd	nd	nd	nd	nd	nd	nd
	Spinach	3	18.46 $\pm$ 4.24	nd	5.72 $\pm$ 1.93	0.12 $\pm$ 0.06	0.05 $\pm$ 0.04	0.14 $\pm$ 0.03	3.48 $\pm$ 1.07	9.51 $\pm$ 2.96
	Sweet peppers, green	3	2.71 $\pm$ 1.06	nd	0.59 $\pm$ 0.16	0.02 $\pm$ 0.00	nd	0.01 $\pm$ 0.00	0.22 $\pm$ 0.07	0.83 $\pm$ 0.22
	Tomatoes	4	5.01 $\pm$ 1.54	nd	0.14 $\pm$ 0.03	nd	0.02	0.02 $\pm$ 0.02	0.64 $\pm$ 0.14	2.75 $\pm$ 2.09
	Welsh onions, "Nebuka-negi"	3	1.81 $\pm$ 0.79	nd	0.38 $\pm$ 0.14	nd	nd	0.01	0.20 $\pm$ 0.14	0.59 $\pm$ 0.28

^a Data are presented as mean $\pm$ standard deviation of three or more independent samples.^b SOAC values are expressed as micromoles of α -tocopherol equivalents (α -Toc) per gram fresh weight of edible portion ($\mu\text{mol } \alpha\text{-TE/g FW}$).^c Carotenoid content are expressed as milligrams per 100 gram fresh weight of edible portion (mg/100g FW).^d Total carotenoid are sum of each carotenoids.^e Not detected.^f Bamboo shoots, canned in water, were used for the measurement.

Table 4. Daily ORAC and SOAC intakes of Japanese individuals from fruits and vegetables.

Category	Annual purchase weight ratio (%) ^a	Estimated daily intakes (g/day) ^b	H-ORAC intakes (μmol TE/day)	L-ORAC intakes (μmol TE/day)	ORAC intakes		SOAC intakes	
Sample name					(μmol TE/day) ^c	Contribution (%)	(μmol α-TE/day) ^d	Contribution (%)
Fruits								
Apples	5.0%	23.3	389.9	10.7	400.6	12.6%	11.2	0.7%
Bananas	7.3%	24.3	112.6	21.2	133.9	4.2%	11.6	0.7%
Grapefruit, pink	0.6%	2.2	32.1	0.9	33.0	1.0%	13.4	0.8%
Grapes	1.0%	4.6	24.9	0.1	25.0	0.8%	- ^e	-
Japanese persimmons	1.1%	5.7	15.0	0.3	15.3	0.5%	16.8	1.0%
Kiwifruit, green	0.7%	3.2	19.0	1.1	20.1	0.6%	2.6	0.2%
Muskmelon, orange	0.9%	2.6	5.8	0.6	6.4	0.2%	15.6	1.0%
Oranges, Valencia	0.7%	2.2	41.2	0.9	42.1	1.3%	5.5	0.3%
Peaches, white	0.7%	3.1	42.9	1.0	43.9	1.4%	1.0	0.1%
Pears, sand pears	1.6%	7.3	11.9	1.0	12.9	0.4%	-	-
Satsuma mandarins	4.7%	20.7	357.3	6.2	363.5	11.4%	100.3	6.2%
Strawberries	1.0%	5.4	106.6	3.1	109.7	3.5%	2.0	0.1%
Watermelon, red	1.6%	5.3	7.4	1.2	8.6	0.3%	49.0	3.0%
Subtotal	26.6%	110.0	1166.5	48.3	1214.9	38.2%	228.8	14.2%
Vegetables								
Bamboo shoots ^f	0.4%	1.1	11.0	2.0	13.0	0.4%	0.2	0.0%
Bean sprouts, mung bean sprouts	2.7%	16.8	101.7	7.1	108.8	3.4%	-	-
Broccoli	1.5%	4.8	58.5	10.0	68.5	2.2%	21.4	1.3%
Cabbage	7.1%	38.8	166.5	26.7	193.2	6.1%	36.6	2.3%
Carrot, orange	3.4%	21.2	35.0	10.4	45.4	1.4%	414.0	25.7%
Chinese cabbage	3.3%	20.0	50.6	6.1	56.7	1.8%	13.5	0.8%
Cucumber	3.1%	19.7	26.4	9.8	36.2	1.1%	46.0	2.9%
East Indian lotus root	0.5%	2.7	64.8	1.8	66.5	2.1%	-	-
Edible burdock	0.7%	4.2	355.2	3.3	358.6	11.3%	-	-
Eggplant, Japanese type	1.7%	9.7	187.8	4.3	192.1	6.0%	4.8	0.3%
Japanese radishes, Daikon	5.2%	28.4	67.6	7.7	75.3	2.4%	-	-
Japanese squash	1.7%	10.1	41.8	15.9	57.7	1.8%	480.6	29.8%
Kidney beans, "Sayaingen"	0.9%	5.3	25.4	7.2	32.5	1.0%	21.3	1.3%
Lettuce, head lettuce, crisp type	2.4%	14.8	36.3	9.2	45.5	1.4%	29.9	1.9%
Onions	6.5%	38.9	271.1	12.7	283.8	8.9%	-	-
Spinach	1.3%	7.5	122.6	28.2	150.8	4.7%	138.1	8.6%
Sweet peppers, green	1.1%	5.8	45.6	4.3	49.9	1.6%	15.6	1.0%
Tomatoes	4.7%	29.2	93.0	11.2	104.3	3.3%	146.4	9.1%
Welsh onions, "Nebuka-negi"	1.9%	7.3	19.9	4.1	24.0	0.8%	13.3	0.8%
Subtotal	50.2%	286.4	1780.8	182.0	1962.8	61.8%	1381.5	85.8%
Total	76.8%		2947.3	230.4	3177.7	100.0%	1610.3	100.0%
All fresh fruits and vegetables	100.0%							

^a The average of the annual purchase weights per household in the annual report on the Family Income and Expenditure Survey 2012–2016^b Estimated daily intakes were calculated from data of the annual report on the Family Income and Expenditure Survey 2012–2016 and the Standard Tables of Food Composition in Japan 2015, Seventh Revised Edition, and the National Health and Nutrition Survey in Japan 2012–2016. Details are described in Materials and Methods.^c ORAC intakes were calculated from Total-ORAC values and daily intake of each fruit and vegetable.^d SOAC intakes were calculated from SOAC values and daily intake of each fruit and vegetable.^e SOAC value was not detected.^f Bamboo shoots, canned in water, were used for the measurement.

values, which confirmed the previously identified positive correlation between H-ORAC value and total polyphenol content (Isabelle *et al.*, 2010a; Isabelle *et al.*, 2010b; Mikami-Konishide *et al.*, 2013; Takebayashi *et al.*, 2013). However, the correlation between L-ORAC value and total polyphenol content was not significant, which may be due to the marked differences between the extraction solvent composition of the L-ORAC method and that of the total polyphenol analysis.

The SOAC assay method was used to evaluate the singlet oxygen quenching activity of fruits and vegetables. As the SOAC method was recently developed and validated, few reports are yet available regarding the SOAC values of various foods. Aizawa *et al.* (2011) and Iwasaki *et al.* (2015) reported the SOAC values of 3 vegetables and 23 fruits and vegetables, respectively, which suggested that brightly colored vegetables such as spinach, red paprika, and tomatoes had high SOAC values. Also in the present study, the highest SOAC values were observed in brightly colored vegetables such as Japanese squash, carrot, and spinach. Furthermore, it has been reported that carotenoids have high singlet oxygen quenching activity among natural compounds (Aizawa *et al.*, 2011; Di Mascio *et al.*, 1989; Mukai *et al.*, 2012), and that chlorophyll also has relatively high quenching activity against singlet oxygen (Tanielian and Wolff, 1988). Red, orange, and deep green vegetables contain high level of carotenoids and/or chlorophyll; therefore, carotenoids and chlorophyll may represent major contributors of singlet oxygen quenching in these vegetables. As supporting evidence, a positive correlation was confirmed between SOAC values and total carotenoid content.

However, Japanese squash showed relatively higher SOAC values than were expected by their measured carotenoid contents. *Cucurbita*, which includes Japanese squash, contains violaxanthin and neoxanthin (Azevedo-Meleiro and Rodriguez-Amaya 2007; Isabelle *et al.*, 2010b), which were not quantified in this study. Thus, carotenoids not measured in the current study are considered to be the reason for the high SOAC values obtained in Japanese squash. Similar results have been observed in previous reports; red sweet peppers, containing not only the 7 carotenoids measured, but also other carotenoids such as cucurbitaxanthin A and capsorubin (Deli *et al.*, 2001), showed higher singlet oxygen quenching activity than calculated from the 7 carotenoid contents (Aizawa *et al.* 2011). These results suggest that the content measurement of only the 7 carotenoids is insufficient to fully comprehend the actual singlet oxygen quenching activity of foods. However, it is difficult in practice to measure the content of all substances contributing to singlet oxygen quenching; therefore, we suggest that the SOAC method remains useful for evaluation of the relative singlet oxygen quenching activity of food.

Because fruits and vegetables comprise the major sources of antioxidants in our daily diet, the estimated daily intakes of ORAC and SOAC of Japanese individuals from fruits and vegetables were calculated. For this calculation, 13 fruits and

19 vegetables in which purchase weight was surveyed in the annual report on the Family Income and Expenditure Survey in Japan were used. These 32 fruits and vegetables accounted for 76.8 % of the total fruits and vegetables on a weight basis (Table 4). Within the total ORAC intake from fruits and vegetables, the contribution ratios of fruits and vegetables were 38.2 % and 61.8 %, respectively, such that the contribution of vegetables was larger than that of fruits. In individual samples, apples, Satsuma mandarins, and edible burdock had a high contribution toward ORAC intake. It is reported that apples and Satsuma mandarins were the highest contributors among fruits for the Japanese, and that edible burdock represented a characteristic food for H-ORAC intake from vegetables (Takebayashi *et al.*, 2013). In addition, apples and oranges were also reported to contribute highly to H-ORAC intakes from fruits and vegetables in the United States (Wu *et al.*, 2004) and Singapore (Isabelle *et al.*, 2010a; Isabelle *et al.*, 2010b). Takebayashi *et al.* (2013) demonstrated that the H-ORAC intake from fruits, vegetables, potatoes, pulses, and mushrooms of Japanese was 4423 $\mu\text{mol TE/day}$. In the present study, the H-ORAC intake obtained from fruits and vegetables except for potatoes, pulses, and mushrooms was 2947.3 $\mu\text{mol TE/day}$ (Table 4). Potatoes, pulses, and mushrooms were excluded from the calculation of the ORAC intake of fruits and vegetables because these were not included in the daily intake of vegetables on the National Health and Nutrition Survey in Japan. These results indicate that potatoes, pulses, and mushrooms might have a relatively high contribution to the H-ORAC intake of Japanese individuals; it was shown that the contribution ratio of potatoes, sweet potato, taro, and shiitake mushroom account for 12.5 % of that of fruits, vegetables, potatoes, pulses, and mushrooms (Takebayashi *et al.*, 2013). Furthermore, Wu *et al.* (2004) and Rautiainen *et al.* (2008) reported total ORAC (H-ORAC + L-ORAC) intakes in the United States and Sweden of 5724 and 6523 $\mu\text{mol TE/day}$, respectively, which were higher values than those obtained in the present study. In the former report, dried beans and peas (1602.6 $\mu\text{mol TE/day}$), orange juice (454 $\mu\text{mol TE/day}$), apple juice (79 $\mu\text{mol TE/day}$), and lemon juice (25 $\mu\text{mol TE/day}$) were included in the candidates for ORAC intake estimation (Wu *et al.*, 2004). In comparison, in the latter report, fruits and vegetables accounted for only 56.5 % of Total-ORAC intake, and the remainder was accounted for by other foods such as grain products (19.7 %), tea (9.5 %), chocolate (4.9 %), juice (3.9 %), and wine (2.5 %) (Rautiainen *et al.*, 2008). Conversely, the ORAC intakes presented in the present study were calculated from only 13 fruits and 19 vegetables because the intake of other fruits and vegetables was not available from the National Health and Nutrition Survey in Japan. In order to reveal the exact daily ORAC intake, further studies that survey the intake quantity of each food and determine the representative ORAC value through the evaluation of samples obtained from different cultivars, climates, and growing

conditions are necessary.

Regarding SOAC, this is the first report to estimate the SOAC intakes from fruits and vegetables. Unlike ORAC intakes, most SOAC intakes were obtained from vegetables, with the respective contribution ratios of fruits and vegetables in the total SOAC intake being 14.2 % and 85.8 %. In all samples, Japanese squash, carrots, tomatoes and spinach mainly contributed to SOAC intake, together accounting for 73.2 % of total SOAC intake. In fruit samples, Satsuma mandarins contributed the most, although this comprised only 6.2 % of the total SOAC intake. Notably, the correlation between SOAC value and carotenoids content suggests that brightly colored vegetables with high carotenoid content are likely important with respect to SOAC intake. However, no reports are currently available regarding the SOAC values of various foods other than fruits and vegetables. For example, nuts such as almonds are considered to have high SOAC values owing to the richness of vitamin E (tocopherols), which has relatively high singlet oxygen quenching activity. To understand the contribution of each food toward SOAC intake, a more comprehensive SOAC database that includes SOAC data of various foods is required.

In the present study, we revealed not only the ORAC values but also the SOAC values of 32 fruits and vegetables typically consumed in Japan from the same samples. In addition, we estimated the daily ORAC and SOAC intakes of Japanese individuals from fruits and vegetables by using the obtained ORAC and SOAC values combined with the results of a public survey of fruits and vegetables intake in Japan. The results showed that various kinds of fruits and vegetables contribute to ORAC intake, whereas specific fruits and vegetables, i.e., brightly colored fruits and vegetables, contribute to SOAC intake. This is the first report to estimate total antioxidant capacity intake by focusing on the scavenging activity of two representative ROS, peroxy radicals and singlet oxygen; however, the daily ORAC and SOAC intakes presented here are limited because they were calculated from limited samples. The data of total antioxidant activity of foods, including not only radical scavenging but also singlet oxygen quenching activities, should constitute important basic data for clarifying the significance of antioxidant intake for overall health. We consider that the combination of oral intake of antioxidant activity and various bio-markers in epidemiological studies will be conducive toward clarifying the relationship between total antioxidant intake and health.

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