3.2. Estimation of daily intake of hydrophilic antioxidants from vegetables and fruits in Japan

On average, the "typical vegetable" and "typical fruit" consumed in Japan were estimated to contain hydrophilic antioxidants that are equivalent to 6.95 and 12.23 μmol of Trolox per g, respectively. In the recent National Health and Nutrition Survey in Japan (Ministry of Health, Labour and Welfare, Japan), daily intakes of vegetables (including vegetables, potatoes, pulses, and mushrooms) and fruits for adults (≥20 years old) were 427 and 119 g/d, respectively, on the 5-year average (2004–2008). From these data, daily intake of hydrophilic antioxidants was estimated to be 4423 μmol TE/d (2967 μmol TE from vegetables and 1456 μmol TE from fruits). On the other hand, the Japanese government recommends consumption of at least 350 g of vegetables per day (Health Japan 21 (Udagawa et al., 2008)) and 2 servings (ca. 200 g) of fruit per day (Japanese Food Guide Spinning Top (Yoshiike et al., 2007)). Hence, if 350 g/d of "typical vegetable" and 200 g/d of "typical fruit" are consumed, 4879 μmol TE/d of hydrophilic antioxidants (2432 μmol TE from vegetables and 2447 μmol TE from fruits) would be ingested.

In this way, the daily intake of hydrophilic antioxidants from vegetables and fruits was estimated to be 4423 μmol TE/d in Japan based on the data of the National Health and Nutrition Survey. This value is about 10% lower than the value based on the government recommendations. However, it should be emphasized here that this does not mean a deficiency of hydrophilic antioxidants in Japan, because hydrophilic antioxidants are contained in other foods, such as tea beverages and soy foods, that are consumed frequently in Japan. Also, it is unclear whether 4423 μmol TE of hydrophilic antioxidants from vegetables and fruits is "not enough" or "just enough" to maintain health. Further accumulation of experimental data as well as epidemiological studies are required to clarify the appropriate daily AOC intake.

3.3. Relationships of H-ORAC values with polyphenol contents and DPPH radical scavenging capacities of vegetables and fruits

Since polyphenolic compounds contained in vegetables and fruits appeared to be major contributors to H-ORAC, the relationship between H-ORAC values and polyphenol contents was investigated. It was found that H-ORAC values for the vegetables and fruits had a strong positive correlation with polyphenol contents ($r = 0.956$, Fig. 1), suggesting that the antioxidative activities of the vegetables and fruits evaluated result mainly from their contained polyphenolic compounds. Wu et al. (2004a) reported that there was a strong positive correlation between H-ORAC values and polyphenol contents in vegetables and fruits, in accordance with our results, though the correlation was not high in all of the foods analyzed (including vegetables, fruits, nuts, spices and grain-based foods). The averaged values of polyphenolic contents of the vegetables and fruits are shown in Tables 1 and 2 (the full results are shown in Tables S1 and S2 in Supplementary data). The weighted average of polyphenol contents for "typical vegetable" and "typical fruit" were calculated to be 0.43 and 0.75 mg GAE/g, respectively, and the estimated daily intake of polyphenolic compounds would be 272 mg GAE/d (183 mg GAE from vegetables and 89 mg GAE from fruits) in Japan based on the data of the National Health and Nutrition Survey.

The DPPH assay is an in vitro assay widely used to measure AOC for foods (Prior et al., 2005). To evaluate the comparability between the H-ORAC assay and the DPPH assay, the relationship between H-ORAC and DPPH radical scavenging capacities was also investigated. It was found that H-ORAC values for the vegetables and fruits had a positive correlation with DPPH radical scavenging capacities ($r = 0.894$, Fig. 2), but the results of the H-ORAC assay were 1.0–18.2-times higher than the results of the DPPH assay. This large variance in the ratio between two values made it impossible to estimate the H-ORAC values of foods from the results of the DPPH assay (and perhaps other antioxidant assays). The average values of the DPPH assay of the vegetables and fruits are shown in Tables 1 and 2 (the full results are shown in Tables S3 and S4 in Supplementary data). The weighted average of DPPH radical scavenging capacities for "typical vegetable" and "typical fruit" were calculated to be 1.56 and 3.67 μmol TE/g, respectively.

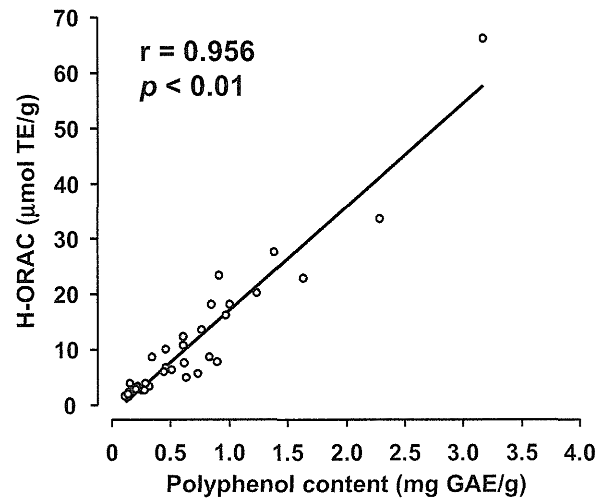

Fig. 1. Relationship between H-ORAC value and polyphenol content of vegetables and fruits commonly consumed in Japan.
Closed circle: vegetable; open circle: fruit; solid line: regression line. The correlation was calculated by using a Pearson product moment correlation coefficient (r).

3.4. Comparison of the results of the present study with results of previous studies

The H-ORAC values obtained in this study were roughly consistent with the H-ORAC values reported by the United States

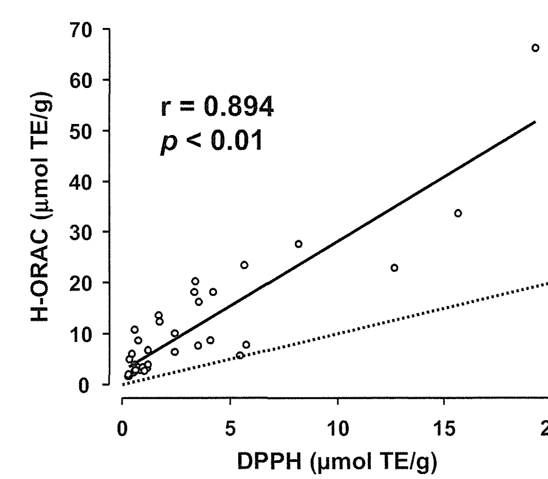

Fig. 2. Relationship between H-ORAC and DPPH values of vegetables and fruits commonly consumed in Japan.
Closed circle: vegetable; open circle: fruit; solid line: regression line; dotted line: $y = x$. The correlation was calculated by using a Pearson product moment correlation coefficient (r).

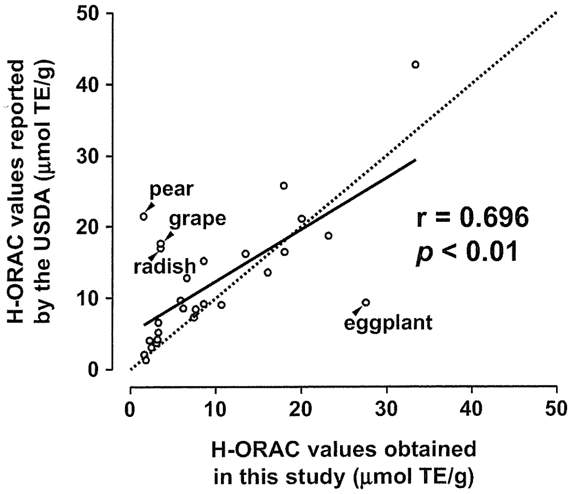

Fig. 3. Comparison of H-ORAC values in this study with those reported by USDA. Closed circle: vegetable; open circle: fruit; solid line: regression line; dotted line: $y = x$. The correlation was calculated by using a Pearson product moment correlation coefficient (r).

Department of Agriculture (USDA) (published on the web: http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/ORAC/ORAC_R2.pdf: accessed December 2011) ($r = 0.696$, Fig. 3). However, some foods, including radish, eggplant, pear and grape, showed large differences in H-ORAC values between Japan and the USA. This may be due to difference in cultivars on the marketplaces. In addition, some foods commonly consumed in Japan, such as Chinese cabbage, Welsh onion, edible burdock, Edamame, shiitake mushroom, East Indian lotus root, bamboo shoot and Japanese persimmon, are not included in the USDA's ORAC database. Of them, edible burdock and East Indian lotus root are rarely eaten as regular dietary components in countries other than Japan, but they appear to be major contributors to daily hydrophilic AOC intake in Japan (Table 1). Hence, a suitable ORAC database for foods consumed in Japan should be established for accurate estimation of AOC intake in the Japanese population.

There have been several reports on daily AOC intake (Chun et al., 2005; Pellegrini et al., 2007; Rautiainen et al., 2008; Wu et al., 2004a; Yang et al., 2011). Wu et al. (2004a) calculated H-ORAC intake from vegetables and fruits in the USA to be 5558 μmol TE/d (2385 μmol TE/d from vegetables and 3174 μmol TE/d from fruits). Rautiainen et al. (2008) estimated the total ORAC (H-ORAC + L-ORAC) intake from vegetables and fruits in Sweden to be 6523 μmol TE/d. These values are generally in accordance with the value estimated in the present study (4423 μmol TE/d). Daily AOC intake was also estimated in other studies (Chun et al., 2005; Pellegrini et al., 2007; Yang et al., 2011), but antioxidant databases used in those study were not based on the ORAC assay. Thus, a direct comparison is impossible because antioxidant capacity of foods differs greatly depending on the assay used.

3.5. Issues and perspectives of this study

In this study, the sum of hydrophilic antioxidants contained in raw vegetables and fruits commonly consumed in Japan was focused, but there are some limitations in the data presented here. (1) Each of the foods also contains a certain amount of lipophilic antioxidants. Thus, the sum of lipophilic antioxidants needs to be measured by the L-ORAC method in a future study. (2) ORAC data for foods other than vegetables and fruits are important. Rautiainen et al. (2008) showed that contributors to the total ORAC intake from all foods in Sweden were fruits (34.8%), vegetables (21.6%), grain products (19.7%), tea (9.5%), chocolate (4.9%), juice (3.9%) and wine (2.5%). Fukushima et al. (2009) suggested that beverages, especially coffee and green tea, contributed greatly to the consumption of polyphenols in the Japanese diet. Furthermore, soy beans and soy bean products were not examined in the present study, but they are consumed frequently in Japan and contain isoflavones, which have potent ORAC activities (Rüfer and Kulling, 2006). (3) The effects of cooking and combinations of ingredients need to be clarified. Yamaguchi et al. (2001) and Jiménez-Monreal et al. (2009) studied changes in AOC of several vegetables during cooking and showed that AOC can change positively or negatively after cooking. Taking the above issues into consideration, a more comprehensive ORAC database is needed for estimation of overall daily intake of antioxidants.

4. Conclusions

The H-ORAC values for 23 vegetables commonly consumed in Japan ranged from 1.63 to 66.07 (average 6.95) μmol TE/g. The H-ORAC values for 13 fruits ranged from 1.58 to 33.47 (average 12.23) μmol TE/g. The daily intake of hydrophilic antioxidants from vegetables and fruits in Japan was estimated to be 4423 μmol TE/d (2967 μmol TE from vegetables and 1456 μmol TE from fruits). This value is generally in accordance with the daily ORAC intakes reported in earlier papers. The H-ORAC values for the vegetables and fruits evaluated had a strong positive correlation with polyphenol contents ($r = 0.956$), suggesting that polyphenolic compounds are major contributors to H-ORAC. The H-ORAC values correlated with DPPH radical scavenging capacities ($r = 0.894$), but the results of the H-ORAC assay were 1.0–18.2-times higher than the results of the DPPH assay. The H-ORAC values obtained here were roughly consistent with those reported by the USDA ($r = 0.696$), but some foods showed largely different H-ORAC values or are not included in the USDA's ORAC database. To elucidate the relationship between daily intake of antioxidants and health, continued research is needed on establishing a more comprehensive AOC database including L-ORAC values, other foods and effects of cooking.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2012.10.006.

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研究論文紹介

日本において一般的に食されている野菜・果物の親水性抗酸化能およびこれらの食品からの親水性抗酸化物質一日摂取量の推算*

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Hydrophilic antioxidant capacities of vegetables and fruits commonly consumed in Japan and estimated average daily intake of hydrophilic antioxidants from these foods

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近年，がん，糖尿病といった生活習慣病，アルツハイマー病など種々の疾病の発症および増悪に活性酸素・フリーラジカルが関与していることが明らかとなってきた．このような活性酸素・フリーラジカルを消去し，健康を維持・増進するため，野菜，果物などの食品に豊富に含まれる抗酸化物質に大きな関心が寄せられている．我々は，通常の食事から複数の抗酸化物質を組み合わせて摂取している．そのため，個々の抗酸化物質について個別に研究するだけでなく，抗酸化物質の総量を反映する食品の抗酸化能（Antioxidant Capacity：AOC）に着目した研究が必要である．現在までに，AOCを指標として用い，抗酸化物質の総摂取量と健康に関する疫学研究が幾つか報告されているが，AOCの高い食事が健康維持に有用であることを示す研究[1)-5)]がある一方，無関係とする研究[6)7)]もある．研究結果が一致しない原因の一つとして，研究に用いた食品の抗酸化能データベースが不適切であり，食品数が限られていたり，名称は同一だが品種が異なる海外の食品の分析結果をそのまま引用しているため，AOC摂取量を正確に推算できていない可能性が考えられる．抗酸化物質摂取の健康影響を疫学研究で明らかにするためには，調査対象者に合致した食品の抗酸化能データベースを構築することが重要である．

今回紹介する論文は，上記の背景を踏まえ，我が国において一般的に食されている野菜・果物に含まれる親水性抗酸化物質の総量を hydrophilic-oxygen radical

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absorbance capacity (H-ORAC) 法を用いて数値化したものである．H-ORAC 法は食品中の親水性抗酸化物質のペルオキシルラジカル消去活性を評価する方法であり，AOC 評価法として広く用いられている[8]．信頼性の高いデータベースを構築するためには，分析法の標準化 (standardization) が重要である．近年，著者らは H-ORAC 法の分析精度向上に成功しており，その妥当性を室間共同試験で確認している (Method Validation, 標準作業手順書に従って，誰がどこで分析しても測定結果が一定の範囲内に収まることを実証)[9]．今回の測定はこの改良 H-ORAC 分析法で行った．

総務省が実施している家計調査[10]には，23 種類の野菜と 13 種類の果物の購入重量が記載されており，その割合は全野菜・果物の購入重量の実に 8 割以上を占める．このことから，家計調査に具体名が示された野菜・果物を日本において一般的に食されているものとし，その H-ORAC 値を測定した (図 1)．その結果，野菜ではなす，ごぼう，れんこんに，果物ではいちご，もも，オレンジに高い抗酸化能が認められた．野菜・果物別に摂食量を考慮して H-ORAC 値を加重平均すると，野菜の H-ORAC 値は平均 6.95 μmol Trolox-equivalent (TE) /g であり，果物のその値は平均 12.23 μmol TE/g であった．加重平均値への各野菜・果物の寄与度を図 2 に示す．寄与度の高い野菜はたまねぎ，ごぼう，なす，果物はりんご，みかん，いちごであった．これらのうち，たまねぎ，みかんなどではそれらの

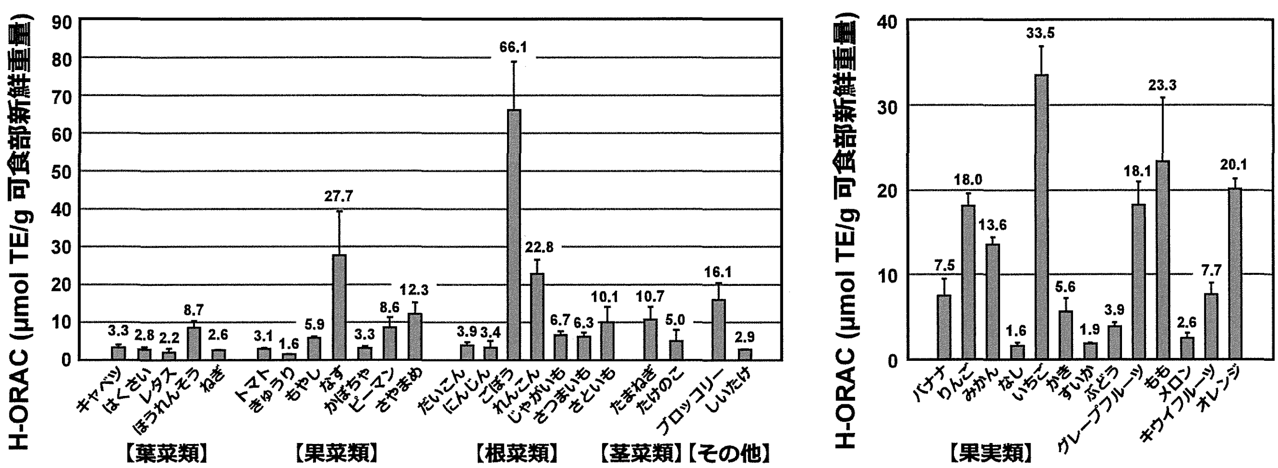

図 1　日本において一般的に食される野菜・果物の親水性抗酸化能
H-ORAC 法を用いて各野菜・果物の抗酸化能を測定した．結果は，可食部 1 g に含まれる親水性抗酸化物質と等しい抗酸化活性を示す Trolox のモル数で表した (μmol Trolox-equivalent (TE) /g)．3 地域で購入した検体の分析結果 (平均 ± 標準偏差) を示した．

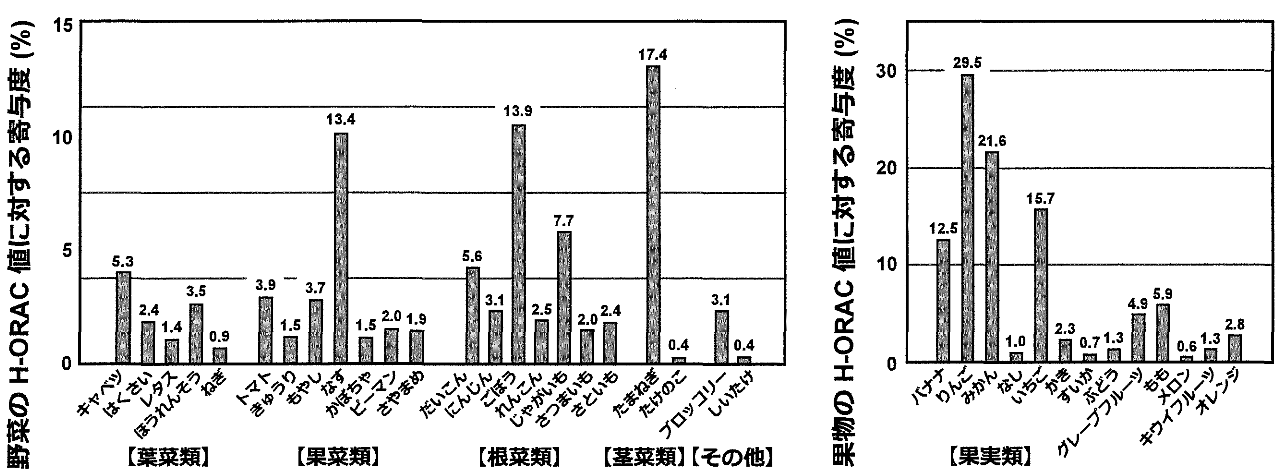

図 2　野菜・果物の平均 H-ORAC 値への各野菜・果物の寄与度

H-ORAC 値は中程度であるが摂食量が多いことが，またごぼう，なすなどではそれらの摂食量は多くないけれど H-ORAC 値が高いことが，寄与度が高い理由であった．

国民健康・栄養調査(2005 ～ 2009 年)[11]によると，野菜(野菜類＋いも類＋豆類＋きのこ類)および果物の一日摂取量はそれぞれ 427 および 119 g である．したがって，野菜・果物からの親水性 AOC 一日摂取量は 4423 μmol TE (野菜から 2967 μmol TE，果物から 1456 μmol TE) であると推算された．なお，この 4423 μmol TE という数字であるが，この量の親水性抗酸化物質が健康の維持・増進に充分なのか，さらなる摂取を必要とするのか，あるいは健康とは無関係なのか，現時点では不明である点に注意する必要がある．抗酸化物質摂取の健康影響を明らかにするためには，食品の抗酸化能データベースをさらに充実させ，高精度の疫学研究を実施することが必須である．

最後に，著者らが食品の ORAC 値に着目して研究している意図を説明したい．食品の ORAC 値については，以前からその生体に及ぼす影響について多くの問題点が指摘されている[12]．著者らも，個々の食品や食品由来成分について，その ORAC 値のみを指標としてその生体に及ぼす影響を論ずるのは不適切だと認識している．しかし，著者らが対象としているのは，単一の食品や抗酸化成分ではなく，日々の食事に含まれる全抗酸化物質であり，疫学的アプローチでその健康に及ぼす影響を明らかにしたいと考えている．疫学研究において，食品の機能性を測るための最も基本的な指標はその重量であろう．野菜・果物は，種類によって当然生体に及ぼす影響が異なるが，それらの総摂取重量が種々の生活習慣病の罹患率と逆相関することを示す疫学研究は数多く，野菜・果物の摂取が推奨される強固なエビデンスとなっている．*In vitro* での食品の ORAC 値は，直接 *In vivo* での抗酸化活性を示すものではないが，野菜・果物の摂取重量と同様に食生活の質を測る指標の一つである．AOC の高い「食生活」が健康に寄与するかを明らかにすることが，著者らの研究意図である．

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