International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:9, No:7, 2015

Antioxidant Properties, Ascorbic Acid and Total Carotenoid Values of Sweet and Hot Red Pepper Paste: A Traditional Food in Turkish Diet

Kubra Sayin, Derya Arslan

***Abstract*—**Red pepper (*Capsicum annum* L.) has long been recognized as a good source of antioxidants, being rich in ascorbic acid and other phytochemicals. In Turkish cuisine red pepper is sometimes consumed raw in salads and baked as a garnish, but its most wide consumption type is red pepper paste. The processing of red pepper into pepper paste includes various thermal treatment steps such as heating and pasteurizing. There are reports demonstrating an enhancement or reduction in antioxidant activity of vegetables after thermal treatment. So this study was conducted to investigate the total phenolic, ascorbic acid and total carotenoids as well as free radical scavenging activity of raw red pepper and various red pepper pastes obtainable on the market. The samples were analyzed for radical-scavenging activity (RSA) and total polyphenol (TP) content using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteu methods, respectively. Total carotenoids and ascorbic acid contents were determined spectrophotometrically. Results suggest that hot pepper paste contained significantly ($P<0.05$) higher concentrations of TP than sweet pepper paste. However there is no significant ($P>0.05$) difference in RSA, ascorbic acid and total carotenoids content between sweet and hot red pepper paste products. It is concluded that the red pepper paste, that has a wide range of consumption in Turkish cuisine, presents a good dose of phenolic compounds and antioxidant capacity and it should be regarded as a functional food.

***Keywords*—**Antioxidant properties, Red pepper paste, Total carotenoids, Total phenolic content.

I. Introduction

RED pepper and red pepper based products possesses a high nutritional value, due to its content of different types of bioactive compounds: vitamins (C and E), carotenoids and phenolic compounds [1], [2]. Carotenoids, that gives the red coloure of pepper, are predominantly pro-vitamin A (α- and β-carotene and β-cryptoxanthin) and xanthophylls and oxygenated carotenoids such as capsanthin, capsorubin and cryptocapsin [3]. Carotenoids are shown to be effective free radical scavengers [4]. Epidemiological studies demonstrating that an increased consumption of a diet rich in carotenoids is associated with a reduced risk for different kinds of cancer [5], [6]. Apart from carotenoids, red pepper fruit is rich in phenolic compounds such as phenolic acids, hydroxycinnamates, flavonoids and flavones [7].

K. S. is with the Nutrition and Dietetics Department, University of Necmettin Erbakan, Konya, Turkey (corresponding author to provide phone: 0530 3459574; e-mail: kubrakucur@hotmail.com)
D. A. is with the Department of Food Engineering, Division of Food Sciences, University of Necmettin Erbakan, Konya, Turkey (e-mail: dears@konya.edu.tr).

In Turkish cuisine red pepper is sometimes consumed raw in salads and baked as a garnish, but its most wide consumption type is red pepper paste. It is the second most popular vegetable paste after tomato that used in cooking. There are a number of investigations demonstrating the health preventing effects of red pepper paste in the products of different countries. For example, Korean fermented red pepper paste (Kochujang) reduced body fat gain, adipocytes size, serum lipid levels, leptin secretion, tumor necrosis factor-α mRNA levels and lipid accumulation in 3T3-L1 adipocytes [8] and showed anti-obesity effects at rats [9].

Red pepper paste is processed in a similar way to tomato paste. Production mainly consist of these steps; separation of seeds and stem, washing, breaking and concentration. İn traditional techniques, the red pepper is concentrated under the sun in climatic conditions [10]. Insufficiency of amount of traditional pepper paste and consumer requests towards more hygienic products necessitates the industrial production of this traditional product [11]. Industrial production steps are; separation of seeds and stem, washing, breaking, first heating for inactivating the enzymes, evaporation under vacuum and low temperature and pasteurization [11]. So the processing of fresh red pepper into pepper paste includes various thermal treatment steps. The antioxidant activity of red pepper end products often changes due to the processing steps is used. Different cooking methods have different effects on the antioxidant properties of colored peppers. In general, limited data are available towards the total amount of the antioxidant components and antioxidant activity of red pepper paste. So we need to identify the beneficial health effects of this widely consumed product. Accordingly this study was conducted to investigate the total phenolic, ascorbic acid and total carotenoids as well as free radical scavenging activity of raw red pepper and various red pepper pastes obtainable on the market.

II. Materials and Methods

A. Chemical Reagents and Samples

Acetic acid, methanol, hydrochloric acid, sodium hydroxide, metaphosphoric acid, petroleum ether and acetone were purchased from Merck (Darmstadt, Germany). Folin–Ciocalteu's phenol reagent was purchased from Sigma–Aldrich (St. Louis, MO, USA). Deionized water was used throughout the experiment. Pepper paste samples were collected from commercial markets in Konya, Turkey.

B. Extraction of Samples for DPPH Radical-Scavenging Activity (RSA) and Total Polyphenol Content (TP)

Pepper paste samples (3 mg) were extracted with 30 ml of methanol solution (90% methanol containing 0.5% acetic acid). The solution was vortexed for 5 min, followed by centrifugation at 3000 rpm for 10 min. The extraction was repeated three times. The supernatant was collected for the analysis.

C. Determination of Total Phenolic Content

Total phenolic (TP) content was measured using the Folin–Ciocalteu colorimetric method described previously [12]. 0.2 ml of Sample extracts prepared for total phenolic content were mixed with 4.8 ml of distilled water and 0.5 ml of 1:3 diluted Folin–Ciocalteu reagent added and then incubated at room temperature for 30 min. Following the addition of 1 ml of 35% sodium carbonate to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue color was measured at 765 nm with a spectrophotometer. Quantification was done with respect to the standard curve of Gallic acid. All determinations were performed in triplicate (n = 3).

D. Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined according to [13]. This method is based on the ability of the antioxidant to scavenge the DPPH caution radical. Briefly, 100 ml of sample extract or standard was added to 0.9 ml buffer (3.0276 g trisHCI in water) and 2 ml of DPPH reagent (0.0394 g in methanol) and vortexed vigorously. It was incubated in dark for 30 min at room temperature and the discolorations of DPPH were measured against blank at 517 nm. Percentage inhibition of the discolouration of DPPH by the sample extract was expressed as trolox equivalents
The DPPH radical-scavenging activity was calculated according to the following equations:

$$\% \text{ scavenging activity} = (C_{control} - C_{extract}) / C_{control} * 100$$

E. Determination of Total Carotenoid Content

Red pepper paste samples (5 g) were ground and extracted with a mixture of acetone and petroleum ether (1:1, v/v) repeatedly using a mortar and pestle until a colorless residue was obtained. The upper phase was collected and combined with crude extracts after being washed for several times with water. The extracts were made up to a known volume with petroleum ether. Total carotenoid content was determined by recording the absorbance at 450 nm with a spectrophotometer [1]. The content of carotenoids was expressed in mg equivalent β-carotene per 100 ml fresh weight.

F. Determination of Ascorbic Acid Content

The paste (200 mg) was extracted with 10 ml of 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2.6-dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020 – 0.12 mg/ml). The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as mg of ascorbic acid/g of extract [14].

G. Statistical Analysis

The data are presented as the mean of three determinations and standard deviation. Analysis of variance was used to determine significance of differences between hot and sweet red pepper paste and raw red pepper using SPSS (Version 16.0).

III. Results and Discussion

A. Analysis of Total Phenol Content and Antioxidant Capacity of Red Pepper Paste

The content of TP evaluated by Folin–Ciocalteu assay is shown in Table I. TP content ranged from 0.64 ± 0.017 mg/g to 1.98 ± 0.025 mg/g in sweet pepper paste, from 0.66 ± 0.034 mg/g to 2.74 ± 0.073 mg/g in hot pepper paste. The results showed that hot pepper paste contained significantly ($P<0.05$) higher concentrations of TP than sweet pepper paste. This variation may have been caused by differences in water soluble dry matter content (^{o}Brix) and in the kind of phenolics. This TP content diversity may be caused the variation of types of phenolic compounds. The major phenolic compounds of sweet pepper consists of 5 types hydroxycinamic acids and 23 types flavonoids [7]. In addition hot red pepper contains capsaicinoids, such as capsaicin and dihydrocapsaicin which have important health effects [15]. Although, the effects of processing on the antioxidant activity and total phenols of sweet red pepper paste have not been investigated, the effects of paste-making processes on tomato fruit have been widely studied in the literature [16]-[18]. Some studies have reported that industrial processing of tomatoes may cause to increase the levels of some basal antioxidants, but other studies have reported that some labile antioxidants may be damaged as a result of processes [18]-[20]. We can say that thermal processing of red pepper into paste involved a number of heating stages which could be disrupt the cellular matrix of pepper and increase the bioavailability of TP and antioxidant capacity. Reference [21] reported that after cooking TP was reduced in red pepper but the decline was not statistically significant ($P> 0.05$). In contrast our results showed an increase in TP in pepper paste compared to fresh red pepper. This can be explained by the changes in specific polyphenols during the paste production process.

Total antioxidant activity is an important parameter in establishing the health functionality of a vegetable product and there are many methods for its measurement [22]. In the present study the antioxidant activity measured through the evaluation of the free radical-scavenging effect on DPPH radicals (Fig. 1). DPPH radical scavenging activity ranged from 76% to 90% (% inhibition as compared to control) in hot red pepper paste, and from 44% to 89% in sweet red pepper paste. Although the difference was not significant hot pepper paste showed a higher antioxidant activity than sweet pepper paste. Therefore we suggest that significant difference of TF of two pastes directly affected the antioxidant capacity of paste samples. Various factors such as cultivars, seasons, and pre and post-harvest conditions may affect the chemical composition of plant foods [23]. For example phenolics were accumulated along the developmental stages of pepper fruit, so the fully colored red peppers showed significantly higher phenolic contents and antioxidant capacity than the green fruits [2].

TABLE I
TOTAL PHENOLICS, ASCORBIC ACID AND TOTAL CAROTENOID CONTENTS IN DIFFERENT RED PEPPER PASTE SAMPLES

	Total phenolics (mg GAE/g)	Ascorbic acid (mg/100ml)	Total Carotenoids (mg/100ml)
Hot pepper paste			
A	2.21 ± 0.053a	320 ± 15.0a	5.82 ± 0.44a
B	2.68 ± 0.018b	296 ± 9.2b	5.22 ± 0.32b
C	2.27 ± 0.013a	280 ± 11.5c	4.66 ± 0.28c
D	2.74 ± 0.073b	292 ± 18.2b	4.52 ± 0.31c
E	2.44 ± 0.139a	271 ± 11.8c	5.64 ± 0.42a
F	2.70 ± 0.065b	378 ± 15.6d	4.30 ± 0.25c
G	1.88 ± 0.051c	278 ± 10.5c	4.29 ± 0.34c
H	1.66 ± 0.034c	155 ± 8.8e	2.98 ± 0.17d
Sweet pepper paste			
A	1.71 ± 0.015c	288 ± 14.4c	5.49 ± 0.51a
B	1.93 ± 0.007c	157 ± 6.0e	3.05 ± 0.28d
C	1.98 ± 0.025c	301 ± 12.6b	5.27 ± 0.42b
D	1.36 ± 0.012d	143 ± 8.5e	2.87 ± 0.19d
E	0.95 ± 0.03e	119 ± 10.2f	2.71 ± 0.11d
F	0.64 ± 0.017f	175 ± 8.0e	3.65 ± 0.21e
Raw red pepper	1.43 ±0.085d	194 ± 9.2g	3.14 ± 0.19d

[a]Values are means ± SD, n = 3. Means within a column with different letters are significantly different at $P<0.05$.

B. Ascorbic Acid Content

Ascorbic acid content was similar in both hot pepper paste and sweet pepper paste (Table I). In the hot pepper paste total ascorbic acid content ranged from 156.67 ± 5.68 mg/100ml to 322.67 ± 5.51 mg/100ml, in the sweet pepper paste it ranged from 118.33 ± 3.05 mg/100ml to 300.33 ± 2.29 mg/100ml. There is no significant difference between two red pepper pastes. In addition to carotenoids and phenolic compounds red pepper is recognized as an excellent source of ascorbic acid. The mean value of ascorbic acid content found for fresh red pepper (194 mg/100g) is accorded with described in the literature [23], [24].

In the literature, thermal processing of different vegetable products causes degradation of ascorbic acid. For instance, [25] found up to 90% of vitamin C reduction after pasteurization of tomato purée. In other studies, around half of the vitamin C was lost during thermal processing of tomatoes [21], [26]. Loss of vitamin C in different tomato products increased with heating time and number of processing steps [27]. Studies have also reported an increase in the level of ascorbic acid during pepper ripening [3], [7], [28]. Therefore we suggest that high ascorbic acid content of red pepper pastes based on the over-maturation of peppers used in paste production.

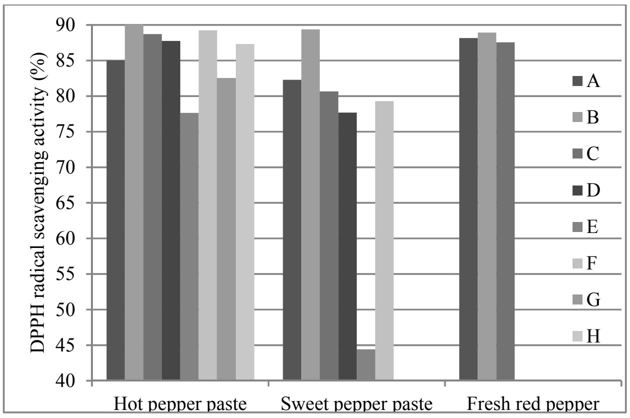

Fig. 1 DPPH radical scavenging activity of red pepper paste samples

C. Total Carotenoid Content

In the hot red pepper paste tested total carotenoid content ranged from 5.82 mg/100ml to 2.98 mg/100ml, in the sweet red pepper paste it ranged from 5.49 mg/100ml to 2.71 mg/100ml (Table I). No significant difference ($P>0.05$) was found between hot pepper paste, sweet pepper paste and fresh red pepper in total carotenoid content. The mean value of total carotenoid content found for fresh red pepper is 3.14 mg/100g. Pepper is a good source of carotenoids. Different reports showed that carotenoids content in pepper vary with cultivars, maturity and processing conditions [4], [28]-[30]. In the study of Zhuang et al. [4] the carotenoid contents in nine peppers ranged from 85.32 lg/g to 1414.78 lg/g and red peppers showed a significantly higher carotenoid content than green peppers.

Reference [31] argues that food processing includes thermal treatment and homogenization has an enhancing effect on carotenoid content. Reference [32] shows that tomato paste production process is enhanced the carotenoid availability. In general microwave heating and short time cooking without water did not affect the total carotenoid content of red pepper, but boiling with much water and longtime has a reducing effect on total carotenoid content [21]. On the contrary, [33] reported that the processing conditions like domestic freezing and boiling do not negatively affect the bioaccessibility of carotenoids from peppers but freezing decreases the content of carotenoids particularly β-carotene and β-cryptoxanthin. The same cooking procedure might affect the carotenoids of various vegetables differently [34].

Several studies have reported that components of foods can react and interact with each other and can change the nutritive value of the food [35]. Various processes that red pepper has been exposed while becoming paste could lead new interactions between compounds and formation of new compounds with different antioxidant capacities.

IV. CONCLUSIONS

This study has confirmed that red pepper paste contains significant amounts of ascorbic acid, carotenoids and phenolics, and that they have high levels of antioxidant activity. Processing red pepper in different ways tends to increase the nutrients compared to the raw pepper. Further studies are required to fully understand the role that fresh and processed red pepper have on the diet and their role in promoting health and preventing human disease.

ACKNOWLEDGMENT

This project was supported by a grant from the Scientific Research Projects (BAP) Coordinating Office of Necmettin Erbakan University, Turkey.

REFERENCES

[1] M. Serrano, P. J. Zapata, S. Castillo, F. Guillén, D. Martínez-Romero, D. Valero, "Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments," Food Chem, vol. 118, pp. 497–503, 2010.

[2] Y. Zhuang, L. Chen, L. Sun, J. Cao, "Bioactive characteristics and antioxidant activities of nine peppers," Journal of functıonal foods, vol. 4, pp. 331–338, 2012.

[3] L. A. Howard, A. D. Wong, A. K. Perry, B. P. Klein, "B-carotene and ascorbic acid retention in fresh and processed vegetables," Journal of Food Science, vol. 64, pp. 929–936, 1999.

[4] H. Matsufuji, H. Nakamuro, M. Chino, T. Mitsuharo," Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*)," J Agr Food Chem, vol. 46, pp. 3462–3472, 1998

[5] S.T. Omaye, N.I. Krinsky, V.E. Kagan, S.T. Mayne, D.C. Liebler, W.R. Bidlack, "β-Carotene: friend or foe?" Fundam. Appl. Toxicol., vol. 40, pp. 163–174, 1997.

[6] G.S. Omenn, G.E. Goodman, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens, B. Valanis, J.H. Williams, S. Barnhart, S. Hammar, "Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease," N. Engl. J. Med., vol. 334, pp. 1150–1155, 1996.

[7] A. Marín, F. Ferreres, F. A. Tomás-Barberán, M. I. Gil, "Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.)," J Agr Food Chem, vol. 52, pp. 3861–3869, 2004.

[8] I.S. Ahn, M.S. Do, S.O. Kim, H.S. Jung, Y.I. Kim, H.J. Kim, K.Y. Park, "Antiobesity effect of Kochujang (Korean fermented red pepper paste) extract in 3T3-L1 adipocytes," J Med Food, vol.9(1), pp. 15-21, Spring 2006.

[9] L. H. Kim, Y. Lim, "Anti-Obesity Effect of Commercial Kochujang and Fermented Wheat Grain Products in Sprague-Dawley Rats," The Korean J Food and Nut, Vol. 27, pp.641-649, 2014.

[10] H. Bozkurt, O. Erkmen, "Effects of salt, starter culture and production techniques on the quality of hot pepper paste," J Food Eng, vol. 69, pp. 473–479, 2005.

[11] H. Kuleaşan, M. Okur, "Industrial production of traditional red pepper paste and prevention of spoilage during storage," J Food, Agric Environ, vol.10, pp. 241-246, 2012.

[12] A. Wojdyło, J. Oszmian´ski, R. Czemerys, "Antioxidant activity and phenolic compounds in 32 selected herbs," Food Chem, vol.105, 940–949, 2007.

[13] L. Yu, S. Haley, J. Perret, M. Harris, J. Wilson, M. Qian, "Free radical scavenging properties of wheat extracts," J Agr Food Chem, vol. 50, pp.1619–1624, 2002.

[14] M. Buret, "Tomato sampling for quality assessment," Milano: IVTPA 1991

[15] D. Hornero-Mendez, J. Costa-Garcia, M.I. Minguez Mosquera, "Characterization of carotenoid high-producing *Capcicum annum* cultivars selected for paprica production," J Agr Food Chem, vol. 50, pp.5711–5716, 2002.

[16] G. R. Takeoka, L. Dao, S. Flessa, D. M. Gillespie, W. T. Jewell, B. Huebner, et al. "Processing effects on lycopene content and antioxidant activity of tomatoes," J Agr Food Chem, vol. 49, pp. 3713–3717, 2001.

[17] R. Re, P. M. Bramley and C. Rice-Evans, "Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety," Free Radical Res, vol. 36, pp. 803–810, 2002.

[18] A. A. Abushita, H. G. Daood, P. A. Biacs, "Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors," J Agr Food Chem, 48, 2075–2081. 2000.

[19] V. Dewanto, X. Wu, K. K. Adom, R. H. Liu, "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity," J Agr Food Chem, vol. 50, pp. 3010–3014, 2002.

[20] E. Sahlina, G.P. Savagea, C.E. Listerc, "Investigation of the antioxidant properties of tomatoes after processing," J Food Compos Anal, vol. 17, pp. 635–647, 2004.

[21] A. M. Chuah, Y. C. Lee, T. Yamaguchi, H. Takamura, L. J. Yin, T. Matoba, "Effect of cooking on the antioxidant properties of coloured peppers," Food Chem, vol. 111, pp. 20–28, 2008.

[22] C. Kaur and H. C. Kapoor, “Antioxidants in fruits and vegetables – the millennium’s health," Int J Food Sci Tech, vol. 36, pp. 703–725, 2001.

[23] N. Deepaa, C. Kaura, B. Singhb, H.C. Kapoorc, "Antioxidant activity in some red sweet pepper cultivars," J Food Compos Anal, vol. 19, pp. 572–578, 2006.

[24] M.A. Murcia, A.M. Jiménez-Monreal, L. García-Diz, M. Carmona, L. Maggi, M. Martínez-Tomé, "Antioxidant activity of minimally processed (in modified atmospheres), dehydrated and ready-to-eat vegetables," Food Chem Toxicol, vol. 47, pp. 2103–2110, 2009.

[25] D. Perez-Conesa, J. Garcia-Alonso, V. Garcia-Valverde, M. D. Iniesta, K. Jacob, L. M. Sanchez-Siles, et al. "Changes in bioactive compounds and antioxidant activity during homogenization and thermal processing of tomato puree," Innov Food Sci Emerg, vol. 10(2), pp. 179–188, 2009.

[26] E. Capanoglu, J. Beekwilder, D. Boyacioglu, R. Hall and R. De Vos, "Changes in antioxidant and metabolite profiles during production of tomato paste," J Agr Food Chem, vol. 56(3), pp. 964–973, 2008.

[27] S. Gahler, K. Otto, V. Böhm, "Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products," J Agr Food Chem, 51(27), 7962–7968, 2003.

[28] A.H. Simmone, E.H Simmone, R.R. Eitenmiller, H.A. Mill, N.R. Green, "Ascorbic acid and provitamin A content in some unusually coloured bell peppers," J Food Compos Anal, vol. 10, pp. 299–311, 1997.

[29] H.G. Daood, H. Vinkler, F. Markus, E.A. Hebshi, P.A. Biacs, "Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors," Food Chem, vol. 55, pp. 365–372, 1996.

[30] F. Conforti, G. A. Statti, F. Menichini, "Chemical and biological variability of hot pepper fruits (*Capsicum annuum* var. *acuminatum* L.)," Food Chem, vol. 102, pp.1096–1104, 2007.

[31] M. L. Nguyen, S. J. Schwartz, "Lycopene: Chemical and biological properties. Developing neutraceuticals for the new millenium," Food Technol, vol. 53, pp. 38-45, 1999.

[32] K. H. Van Het Hof, C. E. West, J. A. Weststrate, J. G. Hautvast, "Dietary factors that affect the bioavailability of carotenoids," J Nutr, vol. 130, pp. 503-510, 2000.

[33] A. Pugliese, M. R. Loizzo, R. Tundis, Y. O’Callaghan, K. G. F. Menichini, N. O’Brien, "The effect of domestic processing on the content and bioaccessibility of carotenoids from chili peppers (*Capsicum* species)," Food chem, vol. 05, pp. 046, 2013.

[34] F. J. Kao, Y. S. Chiu, W. D. Chiang, "Effect of water cooking on antioxidant capacity of carotenoid-rich vegetables in Taiwan," J Food Drug Anal, vol. 22, pp. 202-209, 2014.

[35] J. Takebayashi, T. Oki, J. Watanabe, K. Yamasaki, J. Chen, M. Sato-Furukawa, M. Tsubota-Utsugi, K. Taku, K. Goto, T. Matsumoto, Y. Ishimi, "Hydrophilic antioxidant capacities of vegetables and fruits commonly consumed in Japan and estimated average daily intake of hydrophilic antioxidants from these foods," J Food Compos Anal, vol. 29, pp. 25–31, 2013.