Food Research International 43 (2010) 1017–1020

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India

D. Sreeramulu *, M. Raghunath

Endocrinology and Metabolism Division, National Institute of Nutrition (Indian Council of Medical Research), Jamai – Osmania Post, Tarnaka, Hyderabad, AP 500 064, India

ARTICLE INFO

Article history:
Received 20 October 2009
Accepted 19 January 2010

Keywords:
2,2′-diphenyl-1-picryl hydrazyl (DPPH)
Ferric reducing antioxidant power (FRAP)
Polyphenols
Total phenolic content (TPC)
2,4,6-Tripyridyl-s-triazine (TPTZ)

ABSTRACT

Epidemiological evidence suggests that consumption of vegetables can prevent degenerative diseases caused by oxidative stress. Considering scanty data available on antioxidant activity (AOA) of roots, tubers and vegetables commonly consumed in India, the objective of the present study was to assess their AOA and relate it to their total phenolic content. AOA was assessed in vegetables (n = 19) and roots/tubers (n = 10) by DPPH (2,2′-diphenyl-1-picryl hydrazyl) scavenging activity and FRAP (ferric reducing antioxidant power) methods and the total phenolic content (TPC) using Folin–Ciocalteu reagent. Although AOA as well as TPC showed wide variation among roots, tubers and vegetables studied. AOA (both DPPH and FRAP) was significantly correlated with TPC among all the foods studied, with the correlation coefficient (r) values being 0.76 and 0.85 ($p < 0.01$) respectively with FRAP and DPPH activity among roots and tubers while among the vegetables studied, the corresponding values were 0.85 and 0.79 ($p < 0.01$) respectively. The results suggest that phenolic compounds may be significant contributors to the AOA of the vegetables, roots and tubers studied.

1. Introduction

Excess free radical production underlies the pathogenesis of diseases like atherosclerosis, carcinogenesis, diabetes, cataract and accelerated ageing (Halliwell, Gutteridge, & Cross, 1992; Scalbert, Manach, Remesy, & Morand, 2005). Robust epidemiological evidence suggests the crucial role of diets in preventing chronic degenerative diseases (Van't Veer, Jansen, Klerk, & Kok, 2000). Plant derived phenolic compounds are reported to have multiple biological effects including antioxidant activity (Cao, Booth, Sadowski, & Prior, 1998). It is hypothesized that phytochemicals in plant foods exert health beneficial effects because they combat oxidative stress in body by maintaining a balance between oxidants and antioxidants (Sun, 1990). In recent times natural antioxidants have raised considerable interest among nutritionists, food manufacturers and consumers because of their presumed safety and potential therapeutic value. Indeed, recent research trends indicate a shift towards identifying non nutritional functional foods (Takeoka & Dao, 2003). More than 5000 phytochemicals have been identified in plants and many more remain to be identified (Shahidi & Naczk, 1995). Therefore, food industry is concentrating on plant phenolics since they retard oxidative degradation of biomolecules like lipids, DNA and proteins (Jacobs, Meyer, & Solvoll, 2001).

To derive maximum health benefits, intake of sufficient amounts of phytochemicals from a variety of plant sources such as fruits and vegetables are recommended (Ames, Shagenaga, & Hagen, 1993). Literature is scanty on antioxidant activity and phenolic content of plant foods, specially the vegetables, roots and tubers which are important constituents of Indian diets (Sreeramulu, Vijayakumar Reddy, & Raghunath, 2009; Stratil, Klejdus, & Kuban, 2006). Therefore in the present study we have determined for the first time to the best of our knowledge, the antioxidant activity of roots, tubers and vegetables commonly consumed in India and correlated it with their total phenolic content.

2. Materials and methods

2.1. Chemicals and reagents

2,2′-diphenyl-1-picryl hydrazyl (DPPH), gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ) and ferric chloride were obtained from Sigma Chemical Inc., USA. All other reagents and chemicals used were of analytical grade procured from local sources. Milli Q water was used in the study.

2.2. Sample collection and extraction

Three samples of roots, tubers and vegetables were purchased from each of the three local markets of Hyderabad and Secunderabad. Food samples purchased from three outlets of each local

* Corresponding author. Tel.: +91 40 27197305; fax: +91 40 27019074.
E-mail address: dandesr@yahoo.com (D. Sreeramulu).

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doi:10.1016/j.foodres.2010.01.009

Table 1
Antioxidant activity and phenolic content of roots and tubers.

Sl. no.	Common name	Botanical name	Phenolic content (gallic acid equivalents mg/100 g)	Antioxidant activity (mg/100 g)	
				DPPH (trolox equivalents)	FRAP ($FeSo_4$ equivalents)
1	Beet root (red)	*Beta vulgaris*	169.41 ± 40.19	125.10 ± 13.41	6308.09 ± 916.86
2	Carrot	*Daucus carota*	22.21 ± 5.51	11.06 ± 3.03	256.31 ± 65.72
3	Colacasia	*Colacasia antiquorum*	81.59 ± 21.03	71.03 ± 17.40	3352.40 ± 424.35
4	Onions (big)	*Allium cepa*	64.16 ± 3.69	23.20 ± 2.10	1439.98 ± 350.48
5	Spring onions	*Allium cepa*	73.55 ± 8.68	12.10 ± 3.50	718.91 ± 93.36
6	Potato	*Solanum tuberosum*	38.42 ± 0.62	16.04 ± 8.20	704.73 ± 102.28
7	Radish (white)	*Raphanus sativus*	66.73 ± 18.46	29.02 ± 5.20	1294.36 ± 188.68
8	Sweet potato	*Ipomoes batatas*	53.70 ± 3.44	25.03 ± 4.07	422.56 ± 315.34
9	Tapioca	*Manihot esculenta*	137.55 ± 6.04	51.07 ± 7.10	3002.40 ± 72.17
10	Yam (ordinary)	*Typhonium trilobatum*	54.92 ± 8.15	74.05 ± 10.20	2891.47 ± 310.24
		Range	22.21–169.4	11–125	256–6308

market were pooled and this pooled sample was considered as a single sample of that food from that market. Edible portions of each food sample was extracted in duplicates according to Sing, Chidambara Murthy, and Jayaprakash (2002) and Zielinski and Kozlowska (2000) with slight modifications. Methanol extraction was adopted as per the procedures described by Oki et al. (2002).

Briefly, about 50 g of cleaned, edible portions of sample was ground in a domestic blender and 5 g of the ground sample was extracted for 4 h at room temperature by shaking vigorously with 20 ml of 60% methanol containing 0.1% HCl. The sample suspensions were centrifuged (10,000 *g* for 15 min at 10 °C) and the supernatant was filtered through Whatman #1 filter paper and the filtrate stored at −20 °C till analysis. Analysis was completed with in one month of extraction (Arcan & Yemenicioglu, 2009).

2.3. DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to Yu et al. (2002) and Aoshima, Tsunonue, Koda, and Kiso (2004). This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. Briefly, 100 µl of sample extract or standard was added to 2.9 ml of DPPH reagent (0.1 mM in methanol) and vortexed vigorously. It was incubated in dark for 30 min at room temperature and the discolouration of DPPH was measured against blank at 517 nm. Percentage inhibition of the discolouration of DPPH by the sample extract was expressed as trolox equivalents.

2.4. FRAP assay

Ferric reducing antioxidant power (FRAP) was determined in sample extracts according to Benzie and Strain (1999). This method is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions. In the presence of TPTZ, the Fe^{2+}–TPTZ complex exhibits blue colour which is read at 593 nm. Briefly, 3.0 ml of working FRAP reagent was added to the appropriate concentration of sample extract. After incubation for 6 min at room temperature the absorbance was measured at 593 nm against $FeSO_4$ as standard.

2.5. Determination of total phenolic content

Total soluble phenolic compounds (TPC) were determined in sample extracts using the Folin–Ciocalteu reagent and the values are expressed as equivalents of gallic acid, which is the most commonly used standard in phenolic estimations since gallic acid found to be more stable and pharmacologically active antioxidant. It has also been shown quantitatively to be equivalent to most other phenolics and give consistent and reproducible results (Sing et al., 2002; Singleton & Rossi, 1965).

2.6. Statistical analysis

Data is expressed on fresh weight basis and is presented as mean + SD. Data was subjected to statistical analysis (correlation between AOA and phenolic content of samples) using the SPSS 14.0 statistical package.

3. Results and discussion

Plant foods contain a variety of biologically active, non-nutritive compounds known as phytochemicals, which impart health benefits (e.g. antioxidant activity) beyond basic nutrition. Yet in India, plant foods have received much less attention in terms of quantifying their AOA (Stratil, Klejdus, & Kuban, 2006). As such little data exists on the AOA of plant foods commonly consumed in India let alone their relationship with the phenolic content (Nair, Nagar, & Gupta, 1998; Vijaya Kumar Reddy, Sreeramulu, & Raghunath, 2010). Roots, tubers and vegetables are important constituents of Indian diets (Richfort & Panozzo, 2007). There are around 20 different AOA indices in use and no index by itself is considered sufficient to quantify the AOA of food (Stratil, Klejdus, & Kuban, 2006). As such determination of AOA of plant foods still remains an unresolved issue. Considering that FRAP and DPPH are the most accepted among the AOA indices in use (Huang, Ou, & Prior, 2005; Ozgen, Reese, Tulio, Scheernens, & Miller, 2006), they have been chosen in the present study to determine the AOA. Since abundant literature from other parts of world indicate phenolic compounds to be important antioxidants in plant foods, the TPC was also determined in the present study and correlated with their AOA. Tables 1 and 3 give the AOA and TPC of roots/tubers and vegetables respectively. The correlation between AOA (DDPH and FRAP) and TPC of roots/tubers and vegetables are given in Tables 2 and 4 respectively.

In general, the coefficient of variation in the AOA and TPC of the three samples (pooled) of a given food was less than 10% indicating no significant differences among the food samples purchased from different markets. However, the AOA and TPC showed wide variation among different roots, tubers and vegetables studied.

Table 2
AOA vs. TPC correlation of roots and tubers.

Correlations* (roots and tubers)	*r*	r^2 (%)
TPC vs. DPPH	0.76	57.52
TPC vs. FRAP	0.85	72.90
DPPH vs. FRAP	0.97	95.04 s

Values are mean ± SD.
* $P < 0.01$.

Table 3
Antioxidant content of vegetables.

Sl. no.	Name of the vegetable	Botanical name	Phenolic content (gallic acid equivalents mg/100 g)	Antioxidant activity (mg/100 g)	
				DPPH (trolox equivalents)	FRAP ($FeSo_4$ equivalents)
1	Beans	*Phaseolus coccineus*	129.41 ± 14.93	83.00 ± 11.06	1037.77 ± 195.80
2	Bitter gourd	*Momordica charantia*	139.67 ± 12.20	18.00 ± 2.00	694.72 ± 85.76
3	Bottle gourd	*Lagenaria vulgaris*	50.64 ± 6.72	36.00 ± 3.00	1039.99 ± 173.04
4	Broad beans	*Vicia faba*	188.09 ± 9.40	333.00 ± 14.00	2284.04 ± 262.53
5	Brinjal	*Solanum melongena*	123.77 ± 10.62	150.00 ± 13.00	2099.73 ± 243.02
6	Cabbage (Green)	*Brossica oleracea var. capitata*	85.58 ± 9.18	78.00 ± 21.00	1245.99 ± 224.50
7	Cabbage (Red)	*Brossica oleracea var. capitata*	339.00 ± 19.51	405.00 ± 68.00	10510.62 ± 1426.13
8	Capsicum	*Capsicum annuum var. grossa*	82.30 ± 3.30	96.00 ± 17.00	685.55 ± 117.38
9	Cauliflower	*Brassica oleracea, var. botrytis*	94.84 ± 4.34	66.00 ± 0.00	1346.63 ± 308.86
10	Cluster beans	*Cyamopsis tetragonoloba*	97.92 ± 14.11	102.00 ± 15.00	1150.36 ± 159.99
11	Cucumber	*Cucumis sativus*	31.46 ± 7.17	63.00 ± 13.00	2089.17 ± 485.85
12	Drumstick	*Moringa oleifera*	88.76 ± 9.34	52.00 ± 16.00	480.94 ± 107.11
13	Kovai	*Coccinia cordifolia*	50.39 ± 9.77	78.00 ± 6.00	677.48 ± 157.18
14	Ladies finger (Okra)	*Abelmoschus esculentus*	167.70 ± 39.63	466.00 ± 65.00	3001.28 ± 130.49
15	Mango raw (green)	*Mangifera indica*	130.10 ± 12.30	276.00 ± 45.00	4640.37 ± 396.95
16	Plantain, green	*Musa sapientum*	30.63 ± 1.57	34.00 ± 25.00	718.35 ± 331.03
17	Pumpkin	*Cucurbita maxima*	46.43 ± 12.95	38.00 ± 7.00	243.80 ± 47.89
18	Ridge gourd	*Luffa acutangula*	27.04 ± 6.12	12.00 ± 3.00	246.86 ± 38.09
19	Snake gourd	*Trichosanthes anguina*	29.60 ± 1.60	38.00 ± 6.00	376.41 ± 124.22
		Range	27–339	12–466	243–10,510

Table 4
AOA vs. TPC correlation of vegetables.

Correlation* (vegetables)	*r*	r^2 (%)
TPC vs. DPPH	0.79	62.78
TPC vs. FRAP	0.85	72.02
DPPH vs. FRAP	0.75	55.82

Values are mean ± SD.
* $P < 0.01$.

DPPH radical scavenging activity ranged from 11.06 to 125 mg trolox equivalent/100 g with the highest activity being found in beet root and the least in carrot (Table 1). That DPPH values of only some foods in this study are in agreement with the literature (Kevers et al., 2007) could be due to factors like agronomic, genomic and post harvesting conditions which may affect the chemical composition of plant foods (Imeh & Khokhar, 2002; Kahkonen et al., 1999). In line with the variations observed in the DPPH activity, FRAP activity (256.31–6308.09 mg ferrous sulphate equivalent/100 g) was also the highest in beet root and least in carrot. That the TPC of roots and tubers also showed a wide range (22.21–169 mg gallic acid equivalent/100 g) and beet root had the highest and carrot the least TPC seems to be in agreement with their AOA.

Similar variation was observed in the AOA of nineteen commonly consumed vegetables studied. For example DPPH activity of vegetables ranged from 12.0 to 466 mg TE/100 g, with Okra being the highest and ridge gourd the lowest in DPPH activity. On the other hand FRAP activity ranged from 243 to 10510 mg $FeSO_4$ equivalent/100 g with the highest activity in red cabbage and the lowest activity in pumpkin. Total phenolic content of vegetables ranges from 27 to 339 mg/100 g and here again red cabbage (red) had the highest and ridge gourd the lowest TPC. Our observation that very high values of antioxidant activity and phenolic content were found in intensely coloured vegetables, e.g. beet root and red cabbage is in line with reported data (Stratil, Klejdus, & Kuban, 2006). Further, the TPC content of red cabbage, carrot, okra and onion observed in this study are in the range reported in literature (Nair, Nagar, & Gupta, 1998). While that of other vegetables was somewhat discordant.

In general, there was a good correlation between the TPC and AOA (as assessed by DPPH and FRAP) among the vegetables, roots and tubers studied (Tables 2 and 4). A significant correlation ($p < 0.01$) was observed between TPC and AOA both in roots and tubers (r values being 0.76 and 0.85 respectively with DPPH and FRAP) and other vegetables ($r = 0.79$ and 0.85 with DPPH and FRAP) (Tables 2 and 4). These findings suggest that TPC may be important contributors to the AOA of roots, tubers and vegetables studied here and are in agreement with the literature from other parts of the world (Kevers et al., 2007). However some studies which did not report similar correlation (Mariko, Hassimotto, Genovese, & Lajola, 2005) suggest this lack of correlation could be due to the different AOA parameters determined in those studies and/or different responses of phenolic compounds in different AOA assay systems (Kahkonen et al., 1999). Considering that molecular antioxidant responses of phenolic compounds vary remarkably depending on their chemical structures, the lack of correlation between TPC and AOA in some studies (Marico et al., 2005) could be due to this as well as the fact that total phenolic content estimated by the Folin–Ciocalteu reagent may overestimate TPC because it is known to react with sugars and ascorbic acid present in plant extracts (Matthaus, 2002). Although we did not determine free sugars or ascorbic acid content in the foods reported in the present study, since it is known that roots, tubers and vegetables do not contain significant amounts of free sugars or ascorbic acid, the interference due to these compounds may at best account to <5%, mostly <1% (Stratil, Klejdus, & Kuban, 2006) in the present study. Not withstanding that some of our observations are discordant with some of the available literature, to the best of our knowledge, this is the first study from India reporting non-nutrient antioxidant activity and phenolic content of commonly consumed roots, tubers and vegetables. This data will be useful to nutritionists and consumers to formulate antioxidant rich therapeutic diets. In addition, this data would add valuable new information to the existing knowledge on Indian plant foods.

Acknowledgements

We thank Dr. B. Sesikeran Director National Institute of Nutrition for his encouragement to carryout this study. We thank Mr. B. Giri Babu for the secretarial help in preparing this manuscript.

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