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Effect of temperature on the antioxidant activity of fresh turmeric rhizome (*Curcuma longa* L.)

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Abstract

Spices are important sources of natural antioxidants and used in alternate systems of Indian and Chinese medicine for ages. The study compares the antioxidant potential of 23 accessions of fresh turmeric, in hot and cold extracts of water and ethanol, by 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity and total antioxidant potential by phosphomolybdenum method. DPPH radical scavenging activity was positively correlated with the curcumin content in hot and cold ethanol extracts. Ethanolic extracts had three times higher DPPH radical scavenging activity (84%) compared to water extracts (28%). Curcumin content (which ranged between 0.39 and 5.83%) was positively correlated with total antioxidant potential in hot ethanol ($r = 0.3$; $p = 0.05$) and cold water extracts ($r = 0.31$; $p = 0.05$); the cold extracts of water and ethanol had twice the values of hot extracts. The turmeric accessions with high DPPH radical scavenging activity and total antioxidant activity, with a correlation to their curcumin content is indicated. The study indicates that antioxidant potential is reduced when turmeric is subjected to heat.

Keywords: Curcumin, DPPH, ethanol extract, phosphomolybdenum, turmeric processing

Introduction

Food rich in antioxidants play an essential role in the prevention of cardiovascular diseases (Gerber *et al*., 2002; Kris-Etherton *et al*., 2002) and neuro-degenerative disorders including Parkinson's and Alzheimer's disease (Di Matteo and Esposito, 2003); it is therefore important to increase the antioxidant intake in the diet. In order to prevent the auto-oxidation in food stuffs, antioxidation is an extremely significant activity which can be used as a preventive against a number of diseases (Aruoma, 1994; Basaga, 1990; Halliwell and Chirico, 1993). Antioxidants are usually polyphenol compounds (Helle and Grete, 1995; Yen *et al*., 2003) which are found in all plants (Aruoma *et al*., 1997; Kim *et al*., 1997). Phenolic compounds have been known to act as antioxidants not only because of their ability to donate electrons but also because of their stable radical intermediates, which can effectively prevent the oxidation at cellular and physiological level (Cuvrelier *et al*., 1992).

Spices are common food adjuncts that impart flavour, aroma and colour to foods; their therapeutic value is also being increasingly accepted (Srinivasan, 2005a). Turmeric has long been known as an important culinary spice in addition to its use in traditional medicine for wound healing, inflammation and stomach acidity (Suresh Kumar *et al*., 2006). This plant contains medicinally active chemicals with over 500 distinct activities in animal systems from over 100 distinct secondary metabolites. Much work has been carried out on the antioxidant activity of compounds derived from turmeric rhizomes (Duke, 2004). Curcumin in turmeric rhizome is a potent quencher of singlet O_2 species (Das and Das, 2002).

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Drying fresh turmeric reduced the ability of extracts to scavenge DPPH radical (Cousins *et al*., 2007). The decreased availability of the spice active principles in their original forms when spices are heat processed, as in domestic cooking, was reported by Srinivasan (2005b).

The Indian Institute of Spices Research, Calicut, Kerala, India, has the world's largest germplasm collection of spices, including turmeric. These include accessions that differ markedly in their quality attributes. The objective of the present study was to assess the antioxidant activities of water and ethanol extracts at both room temperature and 60 °C, by the DPPH radical scavenging activity and the total antioxidant potential by the phosphomolybdenum method in 23 selected accessions of turmeric rhizomes differing in their curcumin content and to correlate the antioxidant potential with the total curcumin content of the rhizomes.

Materials and Methods

Plant material

Fresh rhizomes of 23 accessions of turmeric (*Curcuma longa* L.) Indigenous Collection (IC) Numbers 348779 (Suguna), 348964 (TCR-132), 349211 (Alleppey Supreme), 349078 (Prathibha), 348823 (Sudarsana), 349021, 349054, 349086, 349101, 349003, 349107, 348802, 348985, 349056, 348806, 349001, 349096, 348999, 349059, 349098, 349073, 349006 and 349028 were planted at the Indian Institute of Spices Research (IISR), Calicut, Kerala, in June 2008 and harvested in January 2009.

Preparation of extracts

The turmeric rhizomes (500 mg) from the above listed accessions were homogenized using a mortar and pestle in (a) 10 ml of water at room temperature, (b) 10 ml of water at 60 °C, (c) 10 ml of ethanol at room temperature and (d) 10 ml of ethanol at 60 °C. These extracts were centrifuged at 10,000 rpm (10,700 x g) for 20 min, and the supernatant was used to quantify the antioxidant potential *in vitro*.

Each value is a mean of two replicates. ANOVA was done using the MStatC package, and the correlation analysis using SPSS software. DPPH values were subjected to arcsine transformation for ANOVA.

Estimation of curcumin (ASTA, 1968)

Turmeric rhizome (0.1 g dry weight) was refluxed for 2.5 hours in 30 ml of 95 per cent ethanol. The extract was cooled and filtered through cotton and made up to 100 ml with alcohol using a volumetric flask. Further, 2 ml of the filtered extract was diluted to 25 ml with alcohol. The absorbance of the extract and standard curcumin solution (0.0025 g/l) was measured at 425 nm against an alcohol blank in a Shimadzu UV-Vis-160A spectrophotometer.

Antioxidant assays *in vitro*

Antioxidant potential of the ethanol extract was analysed using DPPH (Braca *et al*., 2001). Total antioxidants were estimated by phosphomolybdenum method (Prieto *et al*., 1999). The total antioxidant capacity was expressed as ascorbic acid equivalents (µmol/g sample).

Results and Discussion

The total curcumin content in 23 accessions of turmeric ranged from a low of 0.39 per cent in IC No. 349101 to a high of 5.83 per cent in the variety Prathibha (IC No. 349078). In this study, accessions with total curcumin values above 2 per cent are considered high curcumin accessions, while those below 2 per cent are low curcumin accessions. Accordingly, the accessions IC Nos. 349078, 348823, 349211, 349001, 349054, 349098, 349021, 349028, 349086 and 349006 (in descending order) are high curcumin containing accessions. In IC Nos. 349056, 349059, 348999, 348964, 349073, 349076, 348779, 348802, 348806, 348985, 349003, 349107 and 349101 recorded curcumin content below 2 per cent.

Table 1 shows the antioxidant potential of 23 different turmeric accessions by the DPPH radical scavenging assay. The mean values revealed that IC Nos. 349086, 348985, 348802 and 348964 showed high antioxidant activity in all the extracts, whereas IC Nos. 349056, 349003, 349107, 348806 and 348779 showed lesser antioxidant potential (expressed in per cent over control of DPPH free radical scavenging activity). DPPH radical scavenging activity was positively correlated with

Table 1. DPPH radical scavenging ability of fresh turmeric rhizomes

Sl. No.	Turmeric IC No.	DPPH radical scavenging activity (% over control)				
		Cold water	Hot water	Cold ethanol	Hot ethanol	Mean
1	349078	25.55 (30.36)	21.47 (27.60)	93.21 (74.90)	82.07 (64.95)	55.57 (49.45)^{e}
2	348823	43.75 (41.41)	14.13 (22.08)	89.41 (71.02)	81.80 (64.75)	57.20 (49.81)^{e}
3	349211	39.68 (39.04)	20.11 (26.64)	94.02 (75.85)	81.52 (64.54)	58.83 (51.52)^{d}
4	349001	43.75 (41.41)	25.00 (30.00)	88.32 (70.02)	82.38 (65.18)	59.86 (51.65)^{d}
5	349054	31.80 (34.32)	11.69 (19.99)	94.02 (75.85)	87.50 (69.30)	56.25 (49.86)^{e}
6	349098	22.83 (28.54)	15.22 (22.96)	96.74 (79.75)	84.24 (66.62)	54.76 (49.46)^{e}
7	349021	24.73 (29.82)	20.39 (26.84)	92.35 (73.94)	84.79 (67.05)	55.56 (49.41)^{e}
8	349028	31.79 (34.32)	23.37 (28.91)	85.87 (67.92)	81.53 (64.55)	55.64 (48.92)^{f}
9	349086	45.65 (42.50)	27.45 (31.60)	89.95 (71.52)	87.78 (69.55)	62.70 (53.79)^{a}
10	349006	36.96 (37.44)	25.55 (30.36)	79.35 (62.97)	75.54 (60.36)	54.35 (47.78)^{g}
11	349056	25.00 (30.00)	16.31 (23.82)	82.34 (65.15)	57.34 (49.22)	45.25 (42.05)^{k}
12	349059	39.13 (38.72)	14.13 (22.08)	91.35 (72.90)	81.52 (64.54)	56.53 (49.56)^{e}
13	348999	42.39 (40.62)	23.37 (28.91)	84.79 (67.05)	71.20 (57.55)	55.44 (48.53)^{f}
14	348964	44.30 (41.72)	34.24 (35.81)	85.88 (67.88)	82.61 (65.35)	61.76 (52.69)^{bc}
15	349073	29.08 (32.63)	23.64 (29.09)	86.41 (68.37)	76.36 (60.91)	53.87 (47.75)^{g}
16	349096	27.72 (31.77)	19.02 (25.86)	92.94 (74.60)	83.69 (66.19)	55.84 (49.60)^{e}
17	348779	44.30 (41.73)	31.53 (34.16)	85.87 (67.92)	51.90 (46.09)	53.40 (47.47)^{gh}
18	348802	36.69 (37.28)	29.62 (32.97)	93.48 (75.21)	82.07 (64.95)	60.46 (52.60)^{c}
19	348806	32.61 (34.82)	17.93 (25.05)	83.15 (65.76)	78.80 (62.58)	53.12 (47.05)^{h}
20	348985	43.75 (41.41)	35.60 (36.63)	88.04 (69.77)	81.52 (64.54)	62.23 (53.09)^{b}
21	349003	14.95 (22.74)	13.04 (21.17)	84.78 (67.04)	80.16 (63.55)	48.23 (43.63)^{j}
22	349107	22.01 (27.98)	14.40 (22.30)	87.22 (69.08)	83.43 (65.98)	51.76 (46.33)^{i}
23	349101	30.71 (33.65)	29.62 (32.97)	86.69 (68.63)	68.48 (55.85)	53.87 (47.77)^{g}
Total mean		33.87 (35.40)^{c}	22.03 (27.73)^{d}	88.53 (70.57)^{a}	78.62 (62.79)^{b}	

L.S.D @ 5% for accessions = 0.007 (0.480); extracts = 0.001 (0.199); (Figures in parentheses are arcsine transformed values)

the curcumin content of the accessions in the case of hot ethanol (r = 0.33; p = 0.05) and cold ethanol (r = 0.41; p = 0.01) extracts, whereas the water extracts were not significantly correlated.

There was a significant variation between hot and cold extracts of water and ethanol (Figs. 1 and 2). Ethanolic extracts had three times higher DPPH radical scavenging activity (84%) compared to water extracts (28%). The mean per cent increase in DPPH free radical scavenging activity ranged from 45.24 (IC No. 349056) to 62.70 (IC No. 349086) over control (ethanol extracted).

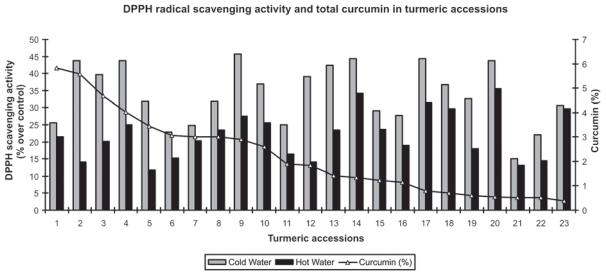

Fig. 1. DPPH radical scavenging activity and total curcumin content in cold and hot water extracts of 23 turmeric accessions

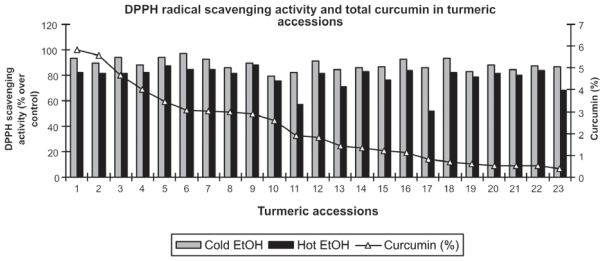

Fig. 2. DPPH radical scavenging activity and total curcumin content in cold and hot ethanol extracts of 23 turmeric accessions

The total antioxidant capacity of 23 different turmeric accessions as measured by the phosphomolybdenum method is given in Table 2. Higher antioxidant activity was present in IC Nos. 349096, 349098, 348779, 348806 and 349021 in all the extracts while lower values were observed in IC Nos. 349056, 348985, 349107, 349073 and 349086 (expressed in µm of ascorbic acid equivalents/g of sample). A significant variation existed between the hot and cold extracts of water and ethanol. The total antioxidant capacity of the accessions varied between 1.146 (IC No. 349056) to 1.696. (IC No. 349096) (Figs. 3 and 4). Curcumin

Table 2. Total antioxidant capacity in fresh turmeric rhizome as determined by the phosphomolybdenum method

Sl. No.	Turmeric IC No.	Antioxidant activity (Ascorbic acid equivalents/g sample) Cold water	Hot water	Cold ethanol	Hot ethanol	Mean
1	349078	1.214	0.657	2.650	1.666	1.547 efgh
2	348823	1.223	0.702	2.777	1.612	1.579 cdef
3	349211	1.204	0.656	2.635	1.891	1.596 cde
4	349001	1.486	0.825	2.858	1.078	1.561 defg
5	349054	1.199	0.929	2.045	1.828	1.500 h
6	349098	1.075	0.557	3.024	1.989	1.661 ab
7	349021	1.233	0.690	2.745	1.748	1.604 bcde
8	349028	1.301	0.709	2.344	1.390	1.436 ij
9	349086	1.194	0.650	2.340	1.180	1.341 k
10	349006	1.278	0.702	2.667	1.623	1.568 defg
11	349056	0.809	0.495	2.236	1.045	1.146 m
12	349059	0.920	0.471	2.943	1.767	1.525 fgh
13	348999	1.138	0.542	2.672	1.344	1.424 j
14	348964	1.118	0.660	2.476	1.885	1.535 fgh
15	349073	1.123	0.492	2.333	1.375	1.331 k
16	349096	1.224	0.583	3.130	1.848	1.697 a
17	348779	1.174	0.959	3.013	1.395	1.635 bc
18	348802	1.496	0.709	2.518	1.324	1.512 gh
19	348806	1.022	0.551	3.044	1.829	1.611 bcd
20	348985	1.199	0.630	2.103	0.925	1.214 l
21	349003	0.919	0.634	2.986	1.432	1.493 hi
22	349107	1.069	0.555	2.518	1.088	1.307 k
23	349101	1.104	0.880	2.725	1.339	1.512 gh
Total mean		1.162[c]	0.662[d]	2.643[a]	1.504[b]	

L.S.D @ 5% for accessions = 0.061; extracts = 0.093

content was positively correlated with antioxidant potential in hot ethanol (r = 0.3; p = 0.05) and cold water (r = 0.31; p = 0.05) extracts.

There was a marked reduction (almost 50 per cent) in the antioxidant potential in hot extracts (water and ethanol) when compared to cold treatments, almost half. The mean value of the accessions extracted in cold water was 1.16 µmole/g, while in hot water extracts it was 0.66 µmole/g sample; extracts of cold ethanol showed 2.64 µmole/g sample, and hot ethanol recorded 1.50 µmole/g.

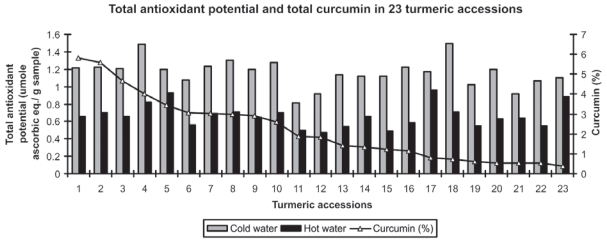

Fig. 3. Total antioxidant potential as measured by the phosphomolybdenum method and total curcumin of cold and hot water extracts in 23 turmeric accessions

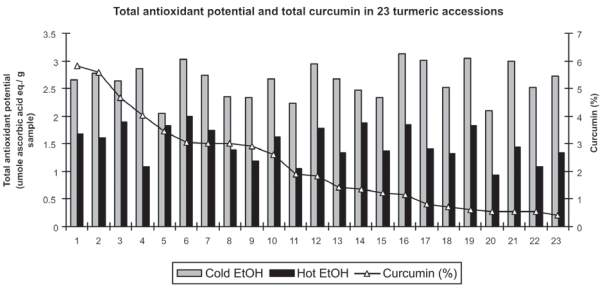

Fig. 4. Total antioxidant potential as measured by the phosphomolybdenum method and total curcumin of cold and hot ethanol extracts in 23 turmeric accessions

The colouring pigment in turmeric, the curcuminoids, is well known for their antioxidant activity (Das and Das, 2002). However, they are readily decomposed on exposure to bright light (Schieffer, 2002), high temperatures and oxidative conditions (Buescher and Yang, 2000). Considerable decrease in the concentration of the bioactive principle, curcumin, has been observed during the heating process of turmeric (Suresh *et al*., 2009). They have characterized three major molecules among the several degradation compounds of curcumin *viz*. ferulic acid, vanillin and vanillic acid. The diketone bridge in the curcumin molecule is vulnerable to heat. In addition, formation of vanillic acid and vanillin indicated that the molecule is sensitive to heat at the first carbon atom of the alkyl chain which connects the two phenyl moieties (Suresh *et al*., 2009).

In addition to the curcuminoids, other compounds possessing antioxidant properties in turmeric include γ-terpinene, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, campestrol, camphene, dehydrocurdione, eugenol, *p*-coumaric acid, protocatechuic acid, stigmasterol, syringic acid, turmerin, turmeronol-a, turmeronol-b and vanillic acid (Duke, 2004). The volatile components are easily lost during the heat processing of turmeric rhizomes.

Selvam *et al.*, (1995) isolated "turmeric antioxidant protein" that was stable at high temperatures, which explains the antioxidant activity displayed by the hot extracts, even though lower than the cold extracts. Srinivas *et al.*, (1992) had characterized a water soluble, heat stable, 5-kDa non-cyclic peptide, containing 40 amino acids – turmerin – from turmeric, which was found to be an efficient antioxidant/ DNA protectant/ antimutagen. Turmerin

forms 0.1% of the dry weight of turmeric, which contains three residues of methionine which are partly responsible for the observed antioxidant activity.

Thus, the present study gives demonstrable proof of the antioxidant property of turmeric rhizomes, and that heating reduces both the DPPH radical scavenging activity and total antioxidant capacity of fresh turmeric rhizomes since the cold extracts were more efficient than the hot extracts.

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