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Antioxidant phytochemicals and curcuminodes content in different genotypes of turmeric (*Curcuma longa*)

S K SANWAL[1], B C DEKA[2] and MARCIN KOZAK[3]

Indian Institute of Vegetable Research, P B 01, P O Jakhini (Shahanshahpur), Varanasi, Uttar Pradesh 221 305

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ABSTRACT

Fresh rhizomes of 21 genotypes of turmeric, including commercial cultivars and experimental genotypes, were assayed for curcuminodes content and antioxidant activity for two consecutive years. Curcumin was the most abundant curcuminode in all the genotypes, which occurred at concentrations ranging from 1.3 % to 4.5 % (with mean of 2.8 % ± standard deviation of 0.45 %), whereas dimethoxycurcumin and bismethoxycurcumin occurred in lower concentration: dimethoxycurcumin ranged from 0.48 to 2.40 % (1.50 ± 0.12 %) while bismethoxycurcumin from 0.43 to 3.70 % (1.37 ± 0.11%). Genotype RCT-2 had the highest contents of curcuminodes and the highest antioxidant activity. Correlation of total phenolics with different antioxidant assay was linear and strong. Curcumin and demethoxycurcumin also had a strong positive correlation with both the antioxidant assay.

Key words: Antioxidants, Bismethoxycurcumin, Curcumin, Dimethoxycurcumin, Phenol, Turmeric

Turmeric (*Curcuma longa* L.) is a perennial herb thought to have originated in India or Southeast Asia, from where it was introduced to other parts of the world (Braga *et al*. 2003). The rhizome of turmeric has a rich history in India as spice, food preservative, and colouring agent and has been used for centuries in the Ayurvedic system of medicine (Chattopadhyay *et al*. 2004). Long before the time of cheaper synthetic food preservatives and colouring agents, spices like turmeric played a key role as food additive (Majeed *et al.* 1995). Its use as a remedy for hypercholesterolemia, arthritis, indigestion and liver problems has been known since long (Srimal 1997). The continuing research indicates that turmeric and its active principle curcumin have unique antioxidant, antimutagenic, antitumorigenic, and anticarcinogenic, antiinflammatory, antiarthritic, anti-microbial, and hypocholesterolemic properties, as reviewed elsewhere (Majeed *et al*. 1995, Miquel *et al*. 2002). Kim *et al*. (2001) also found that curcumin, demethoxycurcumin and bisdemethoxycurcumin are powerful antioxidants and anti-inflammatory compounds.

Antioxidant compounds in food play important roles as health protecting factors. Spices have received increased attention as source of many effective antioxidants. Population-wise average dietary intake of common spices has been estimated as 0.5 g/person per day in Europe and 0.4 g/person per day in the United States (American Spice Trade Association). In contrast, on the Indian subcontinent, turmeric consumption alone has been estimated as 1.5 g/person per day (Sharma *et al*. 2001). Processes that prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, or prevent mutations are important mechanisms in cancer prevention (Gordon 1996). Turmeric (*Curcuma longa*) has been identified in several studies as a plant with high antioxidant content (Kaur and Kapoor 2002, Thaikert and Paisooksantivatana 2009).

The present study was thus undertaken to explore the relationship between antioxidant phytochemicals and curcuminodes content and possibilities of identifying a genotype with high higher curcumin and antioxidant activity.

MATERIALS AND METHODS

Twenty-one genotypes of turmeric grown in different parts of India were selected. Some of them are released varieties while others are local genotypes that have good potential in terms of yield and colouring. These genotypes were grown in the experimental field of ICAR Research Complex for North Eastern Hill Region, Barapani, Meghalaya, India (latitude 26º N and 92º E, with an elevation of 950 m above mean sea level) in the month of April during 2007 and 2008. The soil was a sandy loam, acidic (pH 5.6),

[1]Senior Scientist (e mail: satishsanwal@rediffmail.com), [2]Principal Scientist (e mail: bcdeka@gmail.com), ICAR (RC) for NEH Region, Umiam, Meghalaya; [3]Assistant Professor (e mail: nyggus@gmail.com), Department of Experimental Design and Bioinformatics, Warsaw University of Life Sciences, Nowoursynowska 159, Warsaw 02-776, Poland

having an organic carbon content of 1.8%, with 275, 13.50 and 196 kg/ha available N, P and K respectively. Rhizomes were harvested at the end of January. After harvesting, fresh rhizomes were washed and cut into pieces. Four samples from one plant were prepared for each genotype. Take 5 g sample in a centrifuge tube and add 10 ml of 99% methanol and centrifuged for 10 minutes at 5000 rpm. The supernatant was collected and filtered.

The HPLC analysis of the extracts was performed on a Shimadzu system equipped with a C18 column. Methanolic stock solutions of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were prepared separately at a concentration of 0.5 mg/mL. Turmeric powder samples (1.0 g each) were extracted with 50 mL of methanol for 2 hr. One milliliter of this solution was transferred to a 10 mL volumetric flask, and the volume was adjusted to 10 mL with methanol. The elution was carried out with gradient solvent systems with a flow rate of 1.0 mL/min at ambient temperature. The mobile phase consisted of methanol (A), 2% acetic acid (B), and acetonitrile (C). The sample volume was 20 μL. Curcuminoid concentrations were calculated on the basis of linear calibration functions and with regard to the dilution factor.

Total phenolics were determined using the Folin-Ciocalteau reagent (Singleton and Rossi 1965). The absorbance measured at 650 nm in a Bausch and Lomb spectronic-21 UVD spectrometer after 60 min using catechol as a standard. The results were expressed as mg catechol/ 100g of fresh weight material.

Total antioxidant potential for sample was determined using a ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996) as a measure of "antioxidant power" while the antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was conducted as described by Taga *et al.* (1984) with some modifications.

Multiple linear regression was employed to study the influence of curcumin, demethoxycurcumin and bismethoxy-curcumin contents on FRAP and BCO assays (Quinn and Keough 2002). Akaike Information Criterion based stepwise model selection was employed to fit the final models. Owing to correlation among predictors, simple regression was also employed to study the bivariate relationships between FRAP/ BCO assay and curcumin, demethoxycurcumin and bismethoxycurcumin contents. The analyses were performed in R (R Development Core Team 2010).

RESULTS AND DISCUSSION

Total phenolics

Total phenolic content varied from 316.5 mg catechol/ 100 g fresh weight in RCT 2 to 182.0 mg in VIK 145, with the mean of 258.5 mg (Table 1).

Quantification of curcuminodes

The mean concentrations of curcumin, dimethoxycur-

Table 1 Antioxidant activities of different genotypes of turmeric (mean data of 2 years)

Genotype	Phenol (mg/100g)	BCO assay (%)	FRAP (microgram/ gram)
Lakadong	292	88.50	4.96
PCT 18	244	83.70	4.55
Kuchipudi	302	88.60	5.01
VIK 145	182.00	76.45	4.36
VIK 17	198.50	78.20	4.48
CLI 370	226	80.20	4.65
Prabha	230	79.45	4.50
Madhukam	264	85.35	4.76
Sugandam	298.40	89.10	4.98
CLI Jyoti	284	87.55	4.80
Suvarna	256	86.90	4.72
Suguna	288	87.05	4.80
Sudarsana	238	82.80	4.70
Prathiba	242	81.65	4.64
Roma	275	87.90	4.86
BSR 1	268	87.30	4.86
BSR 2	210	79.25	4.48
Rasmi	264	84.35	4.78
Suroma	266	85.25	4.84
RCT 2	316.50	90.80	5.16
Megha Turmeric 1	284	87.30	4.82

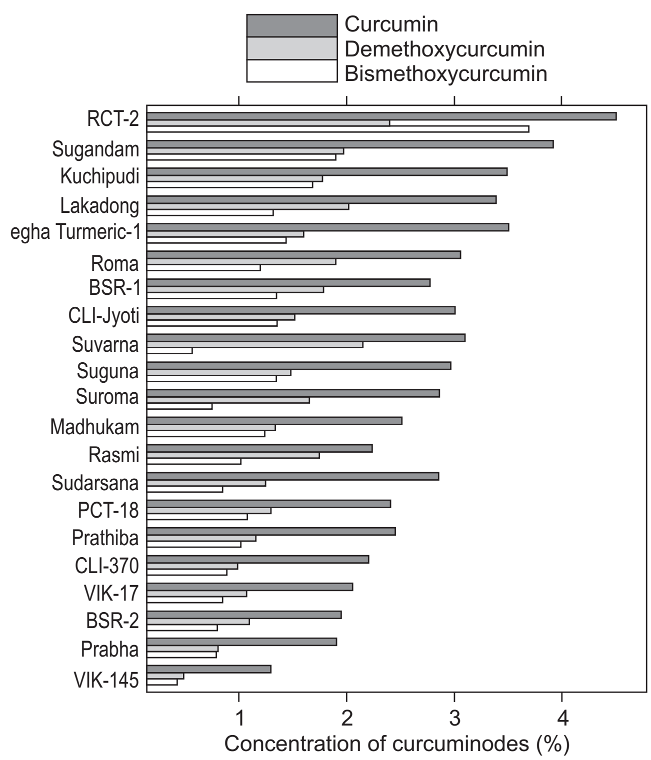

Fig 1 Concentration of curcumin, demethoxycurcumin and bismethoxycurcumin in the tumeric genotypes studied

cumin, and bismethoxycurcumin were calculated from the peak areas obtained at 430 nm. Significant variation was recorded amongst most of the genotypes except Sugana and Suvarna (Fig 1). Curcumin was the most abundant curcuminodes in all the genotypes, which occurred at concentrations ranging from 1.3% to 4.5% (mean of 2.8 ± standard deviation of 0.45%), whereas dimethoxycurcumin and bismethoxycurcumin occurred in lower concentrations: dimethoxycurcumin ranged from 0.48 to 2.40% (1.50 ± 0.12%) while bismethoxycurcumin from 0.43 to 3.70% (1.37 ± 0.11%) (Fig 1). The genotype RCT 2 had the highest total curcuminodes content (10.6%).

In all the genotypes, curcumin was the most abundant curcuminode, which is in accordance with the literature (Thaikert and Paisooksantivatana 2009). In the present study, the mean content of total curcuminodes of some of the genotypes was lower than that reported in other investigations. This may be due to the effect of environmental factors such as climate, soil characteristics etc. The mean ratio of curcumin, dimethoxycurcumin, and bismethoxycurcumin varied from genotype to genotype (Fig 1).

Antioxidant activities

It is difficult to estimate the antioxidant activity of an individual phytochemical dietary compound. Thus, determination of the total antioxidant activity (AOX) allows a more realistic evaluation of potential health effects of foods. So, the total antioxidant activity in turmeric extract was determined by two antioxidant activity assays, namely FRAP and BCO assays (Table 1). All the extracts demonstrated varying degrees of efficacy within each antioxidant assay. Antioxidant activity as determined by FRAP method ranged from 4.36 to 5.16 mmol Trolox/g. These values are consistently higher than those for other potential spices and vegetables, such as ginger, coriander, garlic, green chillies (Kaur and Kapoor 2002), carrots, cauliflower, white onions (Ou *et al*. 2002), pea, snap beans and broccoli (Chu *et al*. 2002). Antioxidant activity as measured by BCO assay method showed oxidation of linoleic acid ranging from 76.45 to 90.8% inhibition in the emulsion. The genotype RCT 2 showed the highest antioxidant activity.

Relationship between phenolic content and antioxidant activity

Generally, extracts with higher phenolic content also had higher antioxidant activity (AOX) as reflected in all the assay systems. Correlation of total phenolics with different antioxidant assay was linear and strong. The determination coefficient ranged from 0.901 in FRAP (Fig 2B) to R^2 = 0.938 in BCO (Fig 2A).

Strong correlation of phenolics with AOX indicates that phenolics are key contributors to AOX in turmeric. Kurilich *et al*. (2002) also reported that hydrophilic extracts contributed to 80-95% of the antioxidant activity in the ORAC assay. Earlier several studies have been performed to study the relationship between the phenolic structure and antioxidant activity (Faure *et al*. 1990, Cuvelier *et al*. 1992), but no relationship has been elucidated because of the many different evaluation systems used to test for anti-oxidant activity. Recently, Kaur *et al*. (2007) have demonstrated a strong linear relationship between total phenolic and antioxidant activity. However, Gazzani *et al*. (1998), Heinonen *et al*. (1998) and Kahkonen *et al*. (1999) did not detect any correlation between total phenolic content and antioxidant activity of the plant extracts. According to them, different phenolic compounds have different responses in the Folin-Ciocalteau method. Similarly, the molecular anti-oxidant response of phenolics in methyl linoleate varies remarkably depending on their chemical structure (Statue-Gracia *et al*. 1997).

Relationship between curcuminodes content and antioxidant activity

In multiple regression analysis, for both FRAP and BCO assays, only curcumin and demethoxycurcumin content were

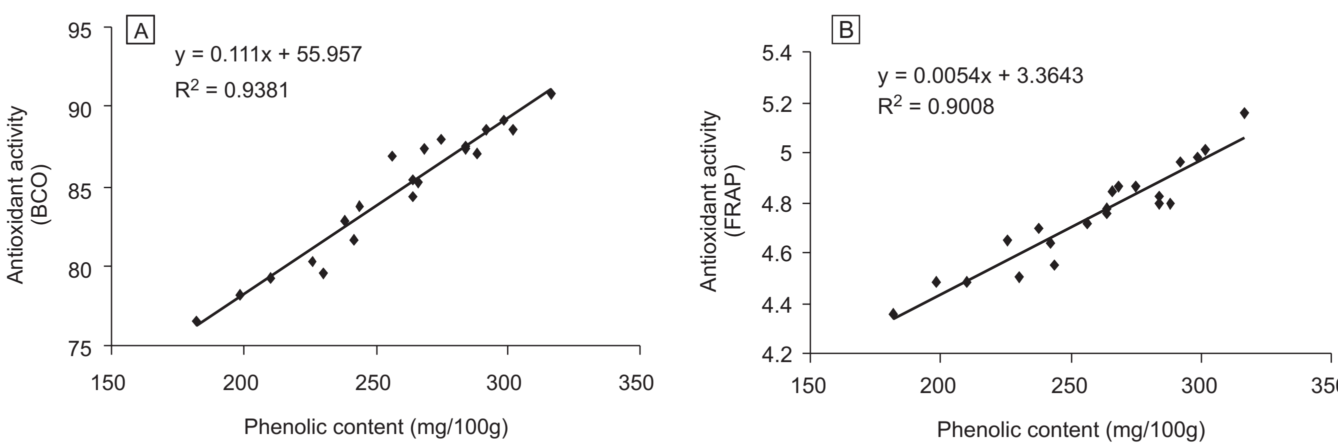

Fig 2 Scatter plots illustrating the correlation between phenolic content and antioxidant activity (BCO) (A) and phenolic content and antioxidant activity (FRAP) (B)

included in the final model; the models had similar determination coefficients:

FRAP = 4.07 + 0.169 curcumin + 0.141 demethoxycurcumin (R^2 = 87.6%, R^2_{adj} = 86.2%)

BCO = 71.0 + 2.64 curcumin + 4.20 demethoxycurcumin (R^2 = 89.0%, R^2_{adj} = 87.8%)

This does not indicate, however, that bismethoxycurcumin contents affects neither FRAP nor BCO, but rather that it is too strongly correlated with the other curcuminants to be included in these models. This is why we decided to

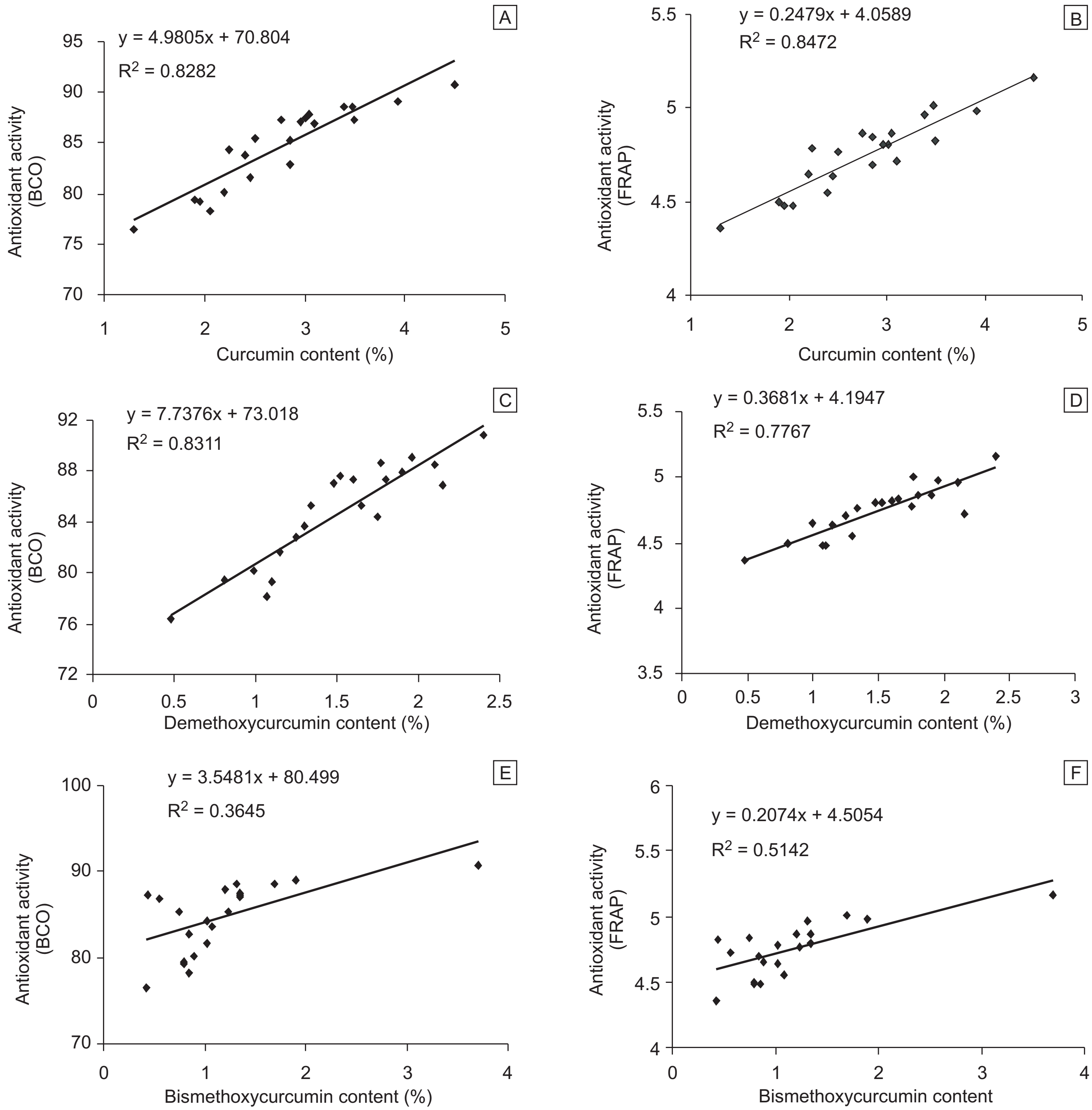

Fig 3 Scatter plots illustrating the correlation between curcumin content and antioxidant activity (BCO) (A), curcumin content and antioxidant activity (FRAP) (B), demethoxycurcumin content and antioxidant activity (BCO) (C), demethoxycurcumin content and antioxidant activity (FRAP) (D), bismethoxycurcumin content and antioxidant activity (BCO) (E), bismethoxycurcumin content and antioxidant activity (FRAP) (F).

analyze the data with simple regression, as was done above and in Fig 3.

Correlation of curcuminodes with different antioxidant assays was strong to moderate, depending upon the type of curcuminodes (Fig 3). Curcumin (Fig 3 A & B) and demethoxycurcumin (Fig 3 C & D) had a strong positive correlation with both antioxidant assays while its moderate correlation was observed with bisdimethoxycurcumin (Fig 3 E & F). Reddy and Lokesh (1992) reported that high anti-oxidant activity in turmeric was attributed to the active principal curcumin. Curcumin (diferuloylmethane), the active ingredient of the spice turmeric (*Curcuma longa*), is a strong antioxidant (Sharma *et al*. 2001) and reportedly several times more potent than vitamin E as a free radical scavenger (Zhao *et al*. 1989). Kapoor and Priyadarsini (2001) also reported the strong correlation between curcumin and antioxidant activity. However, Kim *et al*. (2001) reported the strong antioxidant activity of demethoxycurcumin and bisdemethoxycurcumin to be as efficient as the well-known strong antioxidant curcumin.

Curcumin was the most abundant curcuminode in all the genotypes and had a strong positive correlation with both the antioxidant assay. The genotype RCT 2 had the highest contents of curcuminodes and the highest antioxidant activity.

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