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Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin

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Abstract

Consumers are now increasingly aware of diet-related health problems and therefore, demanding natural ingredients which are expected to be safe and health-promoting. Recently, many studies on health benefits associated with curcumin have been reported. In the present study, an attempt has been made to test individual curcuminoids, such as curcumin, bisdemethoxycurcumin and demethoxycurcumin, for their antioxidant activities by in vitro model systems, such as the phosphomolybdenum and linoleic acid peroxidation methods. Antioxidant capacities of the extracts, as ascorbic acid equivalent (μmol/g) were in the order: curcumin > demethoxycurcumin > bisdemethoxycurcumin. In comparison with butylated hydroxyl toluene (BHT), at 100 ppm, the antioxidant activity, by linoleic acid peroxidation, was found to be highest with curcumin, followed by demethoxycurcumin and bisdemethoxycurcumin. The data obtained by the in vitro models clearly establish the antioxidant potencies of individual curcuminoids. This is the first report on antioxidant activity of individual curcuminoids using the phosphomolybdenum method and linoleic acid peroxidation method.

Keywords: *Curcuma longa*; Turmeric; Antioxidant activity; Curcumin; Demethoxycurcumin; Bisdemethoxycurcumin

1. Introduction

Reactive intermediates in oxidation processes, particularly free radicals, are receiving increased attention in biology, medicine and food chemistry, and as well as in environmental areas (Larson, 1997; Whitehead, Thorpe, & Maxwell, 1992). Radical species are involved in many oxidative chain reactions. A common example of such a process is lipid peroxidation in foods, leading to rancidity. Food additives, such as antioxidants, can be applied to extend the shelf-life of foods and maintain their safety, nutritional quality, functionality and palatability. Antioxidants must be non-toxic, relatively inexpensive, and effective. They should also have a carry-through effect during processing, and should not alter the quality of the end-product (Reische, Lillard, & Eitenmiller, 1998, chap. 16). Currently, food manufacturers/consumers prefer additives labelled as "natural". In recent years, evaluation of antioxidant activity of naturally occurring substances has been our focus of interest (Jayaprakasha, Singh, & Sakariah, 2001; Jayaprakasha, Jagan Mohan Rao, & Sakariah, 2004a; Jayaprakasha, Jena, Negi, & Sakariah, 2002; Jayaprakasha, Selvi, & Sakariah, 2003). However, the use of natural antioxidants is limited by a lack of knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data (Shahidi, Wanasundara, & Amarowicj, 1994).

Several studies in recent years have shown that curcumin has antioxidant, anti-inflammatory, anti-microbial, anti-parasitic, anti-mutagen and anticancer properties

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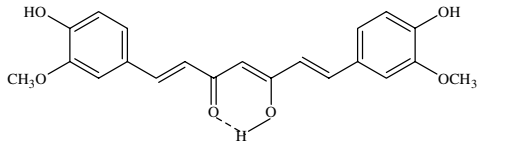
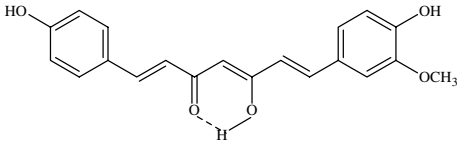
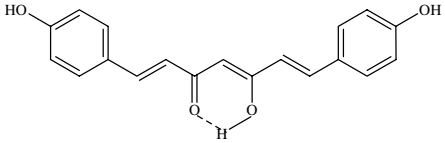
(Khanna, 1999). The potential use of curcumin in the prevention of cancer and in the treatment of infection with human immuno-deficiency virus (HIV) is the subject of intensive laboratory and clinical research (Srimal, 1997). Ahsan, Parveen, Khan, and Hadi (1999) reported the relative antioxidant activities of curcuminoids (Fig. 1) on cleavage of plasmid DNA by the Fe(II)–EDTA system and the generation of singlet oxygen by riboflavin. Recently, the effect of curcuminoids was examined on the proliferation of MCF-7 human breast tumour cells. It was reported that demethoxycurcumin showed the best inhibition of MCF-7 cells, followed by curcumin and bisdemethoxycurcumin (Simon et al., 1998). Pure curcumin, bisdemethoxycurcumin and demethoxycurcumin are not available from commercial sources. Commercial curcumin contains 77% curcumin, 17% demethoxycurcumin and 3% bisdemethoxycurcumin (Ahsan et al., 1999). There is an increasing demand for demethoxycurcumin and bisdemethoxycurcumin, due to the discovery of their new biological activities (Ahsan et al., 1999; Kim, Park, & Kim, 2001; Simon et al., 1998). The pigment curcumin is industrially produced using turmeric oleoresin as the starting material. The mother liquor (approximately 70–80%), after isolation of curcuminoids from oleoresin, has a composition of oil, resin and left-over curcuminoids. The mother liquor is also known as spent turmeric oleoresin (STO)/curcumin removed turmeric oleoresin (CRTO). This material has no commercial value at present (Saju, Venugopal, & Mathew, 1998). Hence, these curcuminoids were isolated from CRTO as described earlier (Jayaprakasha, Jaganmohan Rao, & Sakariah, 2002; Jayaprakasha, Jagan Mohan Rao, & Sakariah, 2004b). In the present study, antioxidant capacities and activities of curcumin, bisdemethoxycurcumin and demethoxycurcumin, using the phosphomolybdenum and linoleic acid peroxidation methods, respectively, have been considered. To our knowledge, this is the first report on antioxidant activity of individual compounds.



Curcumin



Demethoxycurcumin



Bis-demethoxycurcumin

Fig. 1. Structures of curcuminoids.

2. Materials and methods

2.1. Materials

All solvents and other chemicals used were of analytical grade and obtained from Merck (Mumbai, India). Linoleic acid, Tween-40, and butylated hydroxytoluene were obtained from Sigma Chemical Co. (Bangalore, India)

2.2. Isolation and purification of curcuminoids

Curcumin, demethoxycurcumin and bisdemethoxycurcumin were isolated from CRTO and identified as described in our earlier studies (Jayaprakasha et al., 2002; Jayaprakasha et al., 2004b).

2.3. Antioxidant capacity by phosphomolybdenum method

Antioxidant capacities of curcumin, demethoxycurcumin and bisdemethoxycurcumin were evaluated by the method of Prieto, Pineda, and Aguilar (1999). An aliquot of 0.1 ml of curcuminoids (equivalent to 50 and 100 ppm) was combined with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Methanol (0.1 ml) was used, in place of sample solution, for the blank. The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank in a Genesys-5-UV–vis spectrophotometer (Milton Roy, NY, USA). Antioxidant capacities of curcumin, demethoxycurcumin and bisdemethoxycurcumin were expressed as ascorbic acid equivalents (μmol/g of sample).

2.4. Antioxidant activity by linoleic acid peroxidation method

Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin were determined using the thiocyanate method (Jayaprakasha et al., 2001). The linoleic acid emulsion was prepared by homogenizing 0.28 g of linoleic acid, 0.28 g of tween-40 as emulsifier and 50 ml of phosphate buffer (0.2 M, pH 7.0). Curcuminoids were dissolved in MeOH and pipetted (0.5 ml) into different test tubes (equivalent to 100 ppm), then mixed with 2.5 ml of linoleic acid emulsion, 2.5 ml of phosphate buffer (0.2 M, pH 7.0) and

incubated at 37 °C for 168 h. The mixture prepared as above, without test sample, served as control. Aliquots (0.1 ml) were drawn from the incubation mixture at intervals of 24 h and mixed with 5.0 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 20 mM in ferrous chloride in 3.5% hydrochloric acid and allowed to stand at room temperature for 3 min. The colour developed was measured at 500 nm. The degree of linoleic acid peroxidation was calculated at 120 h using the following formula (Pin-Der Duh, 1998). Antioxidant activity = [1 − (increase in absorbance of sample/increase in absorbance of control)] × 100. BHT was used as standard for comparison. All tests and analyses were carried out in triplicate and averaged.

3. Results and discussion

Pure curcuminoids were isolated from CRTO and identified as described in our previous studies (Jayaprakasha et al., 2002; Jayaprakasha et al., 2004b). Total antioxidant capacities of the individual curcuminoids were quantitatively determined by the formation of phosphomolybdenum complex. This method is based on the reduction of Mo(VI)–Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex, which has maximal absorption at 695 nm. The results for antioxidant capacities of curcuminoids were expressed as water-soluble ascorbic acid equivalents (μmol/g of sample). Curcumin, demethoxycurcumin and bisdemethoxycurcumin exhibited various degrees of antioxidant capacity (Fig. 2). The antioxidant capacities of curcuminoids were found to decrease in the order: curcumin > demethoxycurcumin > bisdemethoxycurcumin. Antioxidant capacity of curcumin, demethoxycurcumin and bisdemethoxycurcumin were found to be 3099 ± 66, 2833 ± 25 and 2677 ± 30 μmol/g of ascorbic acid equivalents at 50 ppm concentration, respectively. The active principles in turmeric are a group of phenolic compounds, including curcumin, which is well known for its strong antioxidant activity (Miquel, Bernd, Sempere, Diaz-Alperi, & Ramiraz, 2002). However, in the present investigation, it was found that the other two curcuminoids were also effective antioxidants.

The antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin, in preventing the peroxidation of linoleic acid, as measured by thiocyanate method, are shown in Fig. 3. Absorbance of control increased up to 2.282 at 120 h, and then decreased. This is due to oxidation of linoleic acid, generating linoleic acid hydroperoxides, which leads to many secondary oxidation products (Hua-Ming, Koji, Fumio, & Kiyoshi, 1996). The oxidized products (namely, linoleic acid hydroperoxides) react with ferrous chloride to form ferric chloride, then to ferric thiocyanate (blood-red colour). After the incubation period (120 h), the formation of peroxides is stagnated, due to non-availability of linoleic acid. Also, the intermediate products may be converted to stable end-products. The non-availability of hydroperoxides, results in the retardation of oxidation of ferrous chloride. Hence, the absorbance does not increase. In the presence of curcumin, bisdemethoxycurcumin, demethoxycurcumin and BHT, oxidation of linoleic acid was very slow. Hence, the colour development is slow. The antioxidant activities of the curcumin, demethoxycurcumin and bisdemethoxycurcumin were found to be 81.98%, 81.77% and 73%, respectively, at 120 h. The decreasing order of antioxidant activities of curcuminoids correlates well with the order of antioxidant capacities found by the phosphmolybdenum method.

Curcumin is a major component of food flavouring turmeric, and has been used as a herbal medicine. Curcumin shows a variety of physiological and pharmacological effects, and several studies indicate curcumin to

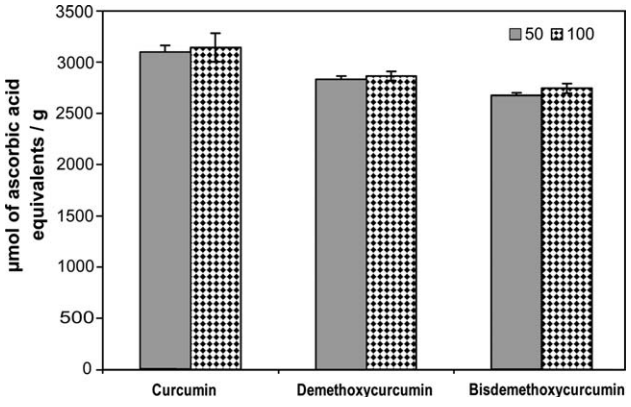


Fig. 2. Antioxidant capacity of curcuminoids by phosphomolybdenum method ▩50, ⊞100.

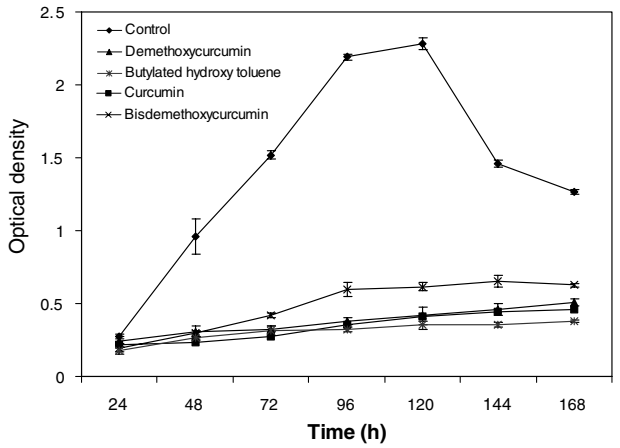


Fig. 3. Antioxidant activities of curcuminoids at 100 μg/ml. -◆- Control, -▲- Demethoxycurcumin, -✻- Butylated hydroxy toluene -■- Curcumin, -×- Bisdemethoxycurcumin.

be anticarcinogenic (Conney et al., 1991) and anti-inflammatory (Huang et al., 1991). Curcumin acts as a superoxide radical scavenger (Reddy & Lokesh, 1994; Ruby, Kuttan, Babu, Rajasekharan, & Kuttsn, 1995) and as a singlet oxygen quencher (Das & Das, 2002). Of the naturally occurring curcuminoids, tetrahydrocurcumin, one of the main metabolites of curcumin, exhibits the most potent antioxidant activity (Osawa, Sugiyama, Inayoshi, & Kawanishi, 1995). Some studies have pointed to the possible involvement of the β-diketone moiety in the antioxidant action of curcumin and its derivatives (Masuda et al., 1999; Schaich, Fisher, & King, 1994). A recent report (Jovanovic, Steenken, Boone, & Simic, 1999) describes the H-atom donation from the β-diketone moiety to a lipid alkyl or a lipid peroxyl radical as a potentially more important antioxidant action of curcumin.

Ahsan et al. (1999) reported that curcumin, bisdemethoxycurcumin and demethoxycurcumin are able to degrade DNA in the presence of Cu(II), the order of activity being curcumin > demethoxycurcumin > bisdemethoxycurcumin. Curcuminoids are capable of inhibiting damage to super coiled plasmid DNA by hydroxyl radicals. Kim et al. (2001) reported the radical-scavenging activities of curcumin, bisdemethoxycurcumin and demethoxycurcumin. It was concluded that, bisdemethoxycurcumin and demethoxycurcumin are good in trapping the DPPH radical as efficiently as a well-known strong antioxidant, i.e., curcumin. The concentrations of these two components are high in spent turmeric oleoresin compared to commercial turmeric oleoresin. The results of the present study indicated that the spent turmeric oleoresin could be used for the isolation of the other two curcuminoids, which could be used as potential antioxidants.

It is known that the structure of curcumin is very similar to diarylheptanoids. Researchers attributed the anti-inflammatory activity of curcumin and its derivatives to the hydroxyl and phenol groups in the molecule and these groups are also essential for the inhibition of prostaglandins PG synthetase and leucotrienes synthesis (LT) (Iwakami, Shibuya, Tseng, Hanaoka, & Sankawa, 1986; Kiuchi, Shibuya, & Sankawa, 1982). Claeson et al. (1996) suggested that the anti-inflammatory action is associated with the β-dicarbonylic system, which has conjugated double bonds (dienes). This system seems to be responsible, not only for anti-inflammatory power, but also to antiparasitic activity (Araujo et al., 1999). The presence of a diene ketone system provides a lipophilicity to the compounds and thus probably better skin penetration. Structure–activity relationship studies suggest that a hydroxy group at the para-position is most critical for the expression of biological activity (Kim & Kim, 2001).

Numerous biological effects demonstrated by curcumin indicate that turmeric, in the diet, could be considered as a nutraceutical or functional food ingredient. The pharamacological safety of curcumin is shown by the non-toxic consumption of up to 100 mg/day in humans and up to 6 g/day in rats (Commandeur & Vermeulen, 1996). Acceptable daily intake (ADI) for curcumin is 1–3 mg/kg body weight, based on the no observed effect (NOE) level of 250–320 mg/kg body weight per day in the multi generation study in rats and the application of a safety factor of 100 (WHO Series, 2004). Ireson et al. (2001) studied the biotransformation of curcumin and hexahydrocurcuminol as the major metabolites of curcumin. The minor metabolites are dihydrocurcumin, tetrahydrocurcumin, hexahydrocurcurcumin, and hexahydrocurcuminol. These studies suggest that the bioavailability of curcumin is greatest in the colon. Because the gastrointestinal tract seems to be exposed more predominantly to unmetabolised curcumin than in any other tissue, their results support the clinical evaluation of curcumin a colorectal cancer chemopreventive agent.

4. Conclusion

The antioxidant activities and antioxidant capacities of curcumin, dimethoxycurcumin and bisdemthoxycurcumin have been studied with in vitro model systems. The results of the present work have established that demethoxycurcumin and bisdemethoxycurcumin from CRTO/STO are also good antioxidants, along with curcumin. It may be suggested that these three compounds could be used in food systems to increase the shelf-life. Further work is required to study mode of their different antioxidant mechanisms and, also, in vivo studies are needed for better understanding these mechanisms of activity.

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