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The determination of vitamin C, total phenol and antioxidant activity of some commonly cooking spices crops used in West Bengal

Manas Denre

Department of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India.

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In the present study, vitamin C, total phenols and antioxidant activity of some common cooking spices crops used in West Bengal were analyzed. Ten (10) spices (onion, chilli, garlic, ginger, turmeric, mustard seed, cumin seed, clove, cardamom and cassia leaf) were selected in order to determine the concentration of ascorbic acid (AA), total phenol (TP) and antioxidant activity as DPPH radical scavenging activity (DPPHRAC). The results obtained show that the values of different variables varied from 5.55 to 0.08 mg g^{-1} (AA), 21.55 to 7.67 GAE mg g^{-1} (TP) and 0.18 to 5.99 IC_{50} value: mg ml^{-1} (DPPHRAC), respectively. There were negative correlations between TPC and IC_{50} value of DPPHRAC.

Key words: Vitamin C, total phenols, antioxidant, spices crops.

INTRODUCTION

Food provides not only essential nutrients needed for life but also other bioactive compounds for health promotion and disease prevention. Thus, consumers demand for natural foods with antioxidant property, which enhances health and food preservation. There is a great demand for antioxidants that are derived from natural resources. Also, there is increased demand for natural dietary products which produce antioxidants in the body (Barlow, 1990; Rice-Evans et al., 1997).

Antioxidants prevent oxidative damage caused by free radicals; they interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Shahid and Wanasundara, 1992; Buyukokuroglu et al., 2001). One of the paradoxes of life on this planet is that the molecule that sustains aerobic life, oxygen, is not only fundamentally essential for energy metabolism and respiration, but also has implication in many diseases and degenerative conditions (Marx, 1985). A common element in such diverse human disorders such as ageing, arthritis, cancer, Lou Gehrig's disease and many others is the involvement of partially reduced form of oxygen. Oxygen can accept single electron to form unstable derivatives referred to as Reactive Oxygen Species (ROS) or Active Oxygen Species (AOS) produced during various metabolic cellular processes. ROS include free

E-mail: sanama06020803@sify.com.

radicals such as uperoxide radical anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), various peroxyl radicals ($ROO^{\bullet}$) and non-free radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) (Halliwell, 1995; Odukoya et al., 2005). Normal range for the production of ROS helps in the regulation of cell proliferation, intercellular signalization, phagocytosis and also synthesis of biologically active compounds. But hyper production of ROS develops oxidative stress which causes oxidative damage to various bimolecular including proteins, lipids, lipoproteins and DNA (Farber, 1994). This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as cardiovascular diseases, diabetes, tumors, rheumatoid arthritis, epilepsy, mutagenesis, carcinogenesis, arterio-sclerosis, Alzheimer's disease, tissue injury (Halliwell and Gutteridge, 1989; Alho and Leinonen, 1999).

Spices are various parts (bud, fruit, seed, bark, rhizome, leaf and bulb) of plants used as a flavoring or seasoning, although many can also be used as herbal medicine. The term 'spice' originated from the Latin word 'species', meaning of specific kind. A closely related term, 'herb' is used to distinguish plant parts having the same functions. Spices have many functions in food. Primarily they are used for flavoring food products; in addition they are also used for preservation of food and for providing nutritional and health benefits (Nazeeem, 1995).

The two terms may be used for the same plants, that is, '*spices*' and '*herb*'. They have been investigated in recent scientific developments throughout the world, one due to their flavor, color, nutritional, health, or preservative effects and another due to their potent antioxidant activities. As per literature survey, so many researchers have reported that chilli, onion, garlic, ginger, turmeric, mustard seed, cumin seed, clove, cardamom and cassia leaf are used as common spices cum herb crops from ancient times.

Apart from providing basic nutrition, these crops are well known for their health benefits; antioxidant constituents and antioxidant activities have been reported in chilli (Bhattacharya et al., 2010; Biswas et al., 2011; Denre et al., 2013a, 2014), onion (Benkeblia, 2005; Denre, et al., 2011), ginger (Hinneburg et al., 2006; Kim et al., 2007; Kota et al., 2008; Suhaj and Horvathova, 2007), garlic (Agarwal, 1996; Banerjee et al., 2003; Denre et al., 2013b) turmeric (Kaur and Kapoor, 2002; Tangkanakul et al., 2009), mustard seed (Dande and Manchala, 2011; Dubie et al., 2013), cumin seed (Dua et al., 2012; Nadeem and Riaz, 2012), clove (Lee and Shibamoto, 2001; Suhaj and Horvathova, 2007; Devi et al., 2012), cardamom (Devi et al., 2012) and cassia leaf (Yong et al., 2012). Foods rich in antioxidants play a role in preventing cardiovascular diseases, cancers (Garber et al., 2002), neurodegenerative diseases (Di-Matteo and Esposito, 2003), inflammation and problems caused by cell and cutaneous aging (Ames et al., 1993). The aim of the present work was to study the determination of vitamin C, total phenols and antioxidant activity of some commonly cooking used spices crops, which may be added in its right (proper value) while cooking.

MATERIALS AND METHODS

The 10 spices crops [Chilli (*Capsicum annuum* L.), onion (*Allium capa* L.), garlic (*Allium sativum* L.), Ginger (*Zingiber officinale* L.), turmeric (*Curcuma Longa* L.), mustard seed (*Brassica Juncea*), cumin seed (*cuminus cyminum*), clove (*Syzygium aromaticum* L.), cardamom (*Elattaria cardamomum*), cassia leaf (*Cinnamomum cassia* L.)] were collected from the market (Shyampur market, Uluberia, Howrah, West Bengal, India) for the experiment. All the collected samples were washed, air dried in shade and then samples were oven dried at 40°C for 96 h until constant weight was gained. These dried materials were prepared for chemical analyses by grinding into a fine powder using an electric grinder. The powder of each sample was stored at three replications in an air-tight cellophane bag as stock sample in a refrigerator (4°C) until further analysis. All the analytical work was performed at Agricultural Biochemistry laboratory, Department of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India.

Determination of ascorbic acid content (AAC)

Ascorbic acid was determined by the 2, 6-dichlorophenol indophenol (DCPIP) titration procedures based on the method of Casanas et al. (2002). The 0.1 g powdered sample was extracted with 20 ml of 4% oxalic acid. Then the material was centrifuged at 10,000 rpm for 30 min. 10 ml of sample's aliquot and 10 of ml of 4% oxalic acid were taken in a conical flask and titrated against 2,6-dichlorophenol indophenol (DCPIP) dye (V_2) until the appearance of a faint pink colour that persisted for a few minutes. Another 5 ml of 100 ppm solution of ascorbic acid and 10 ml of 4% oxalic acid were taken and also titrated against DCPIP dye (V_1). The ascorbic acid content (mg g^{-1}) was determined using the following formula:

$$\text{Amount of ascorbic acid (mg g}^{-1}) = \frac{0.5 \times V_2 \ 20}{V_1 \times 10 \times 0.1}$$

V_1 = dye consumed by 0.5 mg ascorbic acid; V_2 = dye consumed by 10 ml of test solution.

Determination of total phenols content (TPC)

The total phenols content in spices crops were determined by the Folin-Ciocalteau reagent (Vinson et al., 1998) using gallic acid as standards. The 0.1 g powdered samples was extracted with 15 ml of 1.2 M HCl in 50% aqueous methanol and heated at 85°C for 2 h to measure the conjugated plus unconjugated ('total') phenols. After cooling the extracted material was centrifuged at 10,000 rpm for 30 min. The supernatant was decanted off in a beaker and evaporated to dryness. The crude extract was diluted to 25 ml with distilled water. For estimation of the total phenols content 0.5 ml of sample's aliquot, 2.5 ml of distilled water and 0.5 ml Folin-Ciocalteau reagent were pipetted out in a test tube. After 3 min, 2 ml of 10% sodium carbonate was added. Then the test tubes were kept on water bath and maintained at 60-70°C for 5 min. The solution was cooled and the absorbance was read at 650 nm. The total phenols content was measured (mg g^{-1}) using a calibration curve against gallic acid equivalent.

Table 1. Concentration of vitamin C, total phenols and antioxidant activity of some commonly cooking spices crops used in West Bengal.

Spices crop	Scientific name	Part used	AAC^n (mg g^{-1})	TPC (mg g^{-1})	DPPH (IC_{50} value: mg ml^{-1})
Onion	*Allium capa* L.	Bulb	0.62±0.09	7.67±0.55	2.28±0.08
Chilli	*Capsicum annuum* L.	Pod	5.55±0.55	8.30±0.67	1.26±0.15
Garlic	*Allium sativum* L.	Bulb	0.37±0.08	14.30±0.69	1.12±0.03
Ginger	*Zingiber officinale* L.	Rhizome	0.48±0.03	15.52±0.96	1.11±0.02
Turmeric	*Curcuma Longa* L.	Rhizome	0.75±0.06	13.00±0.51	5.99±0.68
Mustard	*Brassica Juncea*	Seed	0.08±0.03	13.79±0.79	0.66±0.05
Cumin	*cuminus cyminum*	Seed	0.09±0.02	20.32±1.32	2.40±0.07
Clove	*Syzygium aromaticum* L.	Bud	0.14±0.05	21.55±0.67	0.26±0.03
Cardamom	*Elattaria cardamomum*	Pod	0.27±0.06	16.00±1.00	0.18±0.05
Cassia	*Cinnamomum cassia* L.	Leaf	0.76±0.12	10.51±0.51	1.09±0.09

Data are mean ± standard deviation values.

Determination of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPHRAC)

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was performed based on the method of Braca et al. (2001). In order to determine the DPPH radical scavenging activity present in spices crops, 0.5 g powdered samples was extracted with 10 ml of distilled water. Extracted sample was centrifuged at 10,000 rpm for 15 min. Each 6.4 ml reaction mixture containing 0.1-0.4 ml of sample's aliquot, 0.1-0.4 ml of distilled water, and 6 ml of 0.004% DPPH were taken in tubes and shaken by hand. They were kept for 30 min at room temperature in dark place. Absorbance of the reaction mixture was read at 517 nm against the blank. The reaction mixture lacking sample developed the most intense colour. The colour decreased with increasing volume of extract added. The ability to scavenge the DPPH radical was calculated using the following equation: DPPH radical scavenging activity (% inhibition) = $[(A_0 - A_e)/A_0] \times 100$, where A_e was the absorbance of sample and A_0 was the absorbance of the control. The % inhibition value was plotted against concentration of the sample extract. From the graph, value of the sample extract producing 50% inhibition (IC_{50} value) was calculated.

Statistical analysis

Data were analyzed statistically by using Daniel's XL Toolbox 6.52 software for analysis of variance and correlation.

RESULTS AND DISCUSSION

Ascorbic acid

Ascorbic acid is one of the most powerful antioxidants (Smirnoff, 1996; Noctor and Foyer, 1998; Arrigoni and de Tullio, 2000; Horemans et al., 2000b; Smirnoff, 2000) that scavenge harmful free radicals and other ROS; it also regenerates other antioxidants like tocopherol to its functional state. In the present study (Table 1), the wide remarkable variation in ascorbic acid concentration was shown among the spices crops that varied from 5.55 to 0.08 mg g^{-1}; and ranking from high to low concentration was chilli>cassia leaf>turmeric>onion>ginger>garlic>cardamom>clove>cumin seed>mustard seed, respectively. The recommended daily allowance of ascorbic acid is 60 mg in USA. However, Australia and United Kingdom recommend 30 to 40 mg; Russia recommends 100 mg. Higher intake of ascorbic acid has been linked to lower risk of cardiovascular diseases (Simon et al., 1988) and several types of cancers (Howe et al., 1990) by forming N-nitroso compounds in stomach and by stimulating the immune system (Byers and Perry, 1992). Vitamin C deficiency exacerbates atherogenesis in animal models. Risk of oesophageal, pancreatic and lung cancer also appears to be lower in people that take ample vitamin C (Wargovich, 2000).

Total phenols

Phenolics are diverse secondary metabolites that are abundant in plant tissues (Grace and Logan, 2000). Antioxidant properties of phenols arise from their high reactivity as hydrogen or electron donors, from the ability of the phenol derived radical to stabilize and delocalize the unpaired electron (Chain breaking function) and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modifying the lipid picking order and decreasing the fluidity of the membranes (Arora et al., 2000). These changes could sterically hinder diffusion of free radicals and restrict peroxidation reaction. In the present experiment (Table 1), the total phenols contents ranged from 21.55 to 7.67 GAE mg g^{-1} with decreasing order: clove>cumin seed>cardamom>ginger>garlic>mustard seed>turmeric>cassia leaf>chilli>onion, respectively. The health benefit of phenolics is linked primarily to their antioxidant potential. Phenolics are effective antioxidants because the radicals products of these molecules are resonance stabilized and thus relatively stable. To

Table 2. Correlation matrix among variables.

Variable	AAC	TPC
AAC		
TPC	-0.541	
DPPHRAC (IC_{50} value)	0.005	-0.194

overcome the potential hazard from oxidative damage in the body, consumption of a diet rich in antioxidant phenolics including flavonoids and phenolic acids is considered the first line of defense against highly reactive toxicants. Scalbert and Williammson (2000) estimated that the total human intake of phenolics is about 1 g day^{-1} which consists of two- thirds flavonoids and one- third phenolic acids.

DPPHRAC

DPPH analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity (Zhou and Yu, 2004). It is a stable free radical because of its spare electron delocalization over the whole molecule. In the present experiment (Table 1), it is revealed that the DPPH radicals scavenging activity ranges from 0.18 to 5.99 mg ml^{-1} following the downward activity order: Cardamom>clove>mustard seed>cassia leaf>ginger>garlic>chilli>onion>turmeric, respectively. The smaller the IC_{50} value the greater the radical scavenging activity and reducing ability of the extracts.

Correlation among variables

Table 2 did not show any significant correlation among the variables, but apparently there were negative correlations between AAC with TPC and TPC with IC_{50} values of DPPHRAC. Interestingly, the negative correlation of TPC with IC_{50} value of DPPHRAC indicated that the higher total phenol content of extracts resulted in lower IC_{50} value of DPPHRAC. That means the higher total phenol content of extracts resulted in higher antioxidant (DPPH radical scavenging activity) activity, because the lower IC_{50} value indicated the higher DPPH radical scavenging activity in extract of spices crops. Maizura et al. (2011) reported that the higher total phenolic content of extracts resulted in higher antioxidant (DPPH radical scavenging activity) activity in extracts of kesum, ginger and turmeric.

Conclusion

Based on the mean and ANOVA results, there is a wide variation among variables in the ten selected cooking species crops. The maximum values of ascorbic acid, total phenol and DPPH radical activity were exhibited as 5.55 mg g^{-1} (chilli), 21.55 mg g^{-1} (clove) and 0.18 IC_{50} value: mg ml^{-1} (Cardamom), respectively. Thus, it is crucial to determine not only their antioxidant capacity but also their characterization potential in promoting health.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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