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Thermal Processing Effects on *in vitro* Antioxidant Potential of Fresh and Packaged Turmeric (*Curcuma longa*), Coriander (*Coriandrum Sativum*) and Cumin (*Cuminum Cyminum*)

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ABSTRACT

Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*) are three very important spices and essential flavoring agents in many cuisines, particularly in South-East Asia. The spices are available both as fresh and in commercial packages. The present study deals with the analyses of their *in vitro* antioxidant profile before and after thermal processing in water. The thermal processing resembled methods commonly practiced in India for cooking, viz. pressure cooking and microwave treatment. The assays performed included DPPH radical decolorization assay, reducing power assay and assay for total phenolic contents. It was observed that the antioxidant activity and total phenolic content was different in the two types of spices, and the packaged spices were better than the fresh samples. Antioxidant activity improved in case of all the three spices after thermal processing, probably due to better solublization of the antioxidants in hot water by thermal treatment. Improvement in the total phenolic contents also substantiated the radical scavenging abilities of the spices after aqueous extraction. Among the different extraction procedures, microwave extraction was found to be most effective with respect to radical scavenging abilities.

Keywords: Packaged, *Curcuma longa*, *Coriandrum sativum*, *Cuminum cyminum*, Antioxidant.

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INTRODUCTION

The core of classical nutrition research has been undergoing an important shift in the last few decades where apparently pharmacologically inert food additives have demonstrated rather profound effects in health and diseases. Dietary spices are one of such nutrients that are being identified vital to maintain human health by their antioxidative, chemopreventive, antimutagenic, antiinflammatory, immune modulatory effects on cells and a wide array of beneficial effects on human health via action on gastrointestinal, cardiovascular, respiratory, metabolic, reproductive, neural and other systems[1]. Herbs and spices have been used as food additives for centuries. Spices are important bionutrients both as functional food ingredients and nutritional supplements. They play a role in enhancing the taste and flavour of food. In addition, spices have also been used for treating several disorders due to their potent medicinal properties[2]. Usually, plants contain a number of bioactives which are responsible for their biological effects. Spices and flavoring agents contain volatile essential oils and hydrocarbons which stimulate glandular secretion and may have a weak action on the nervous system[1]. Since humans, unlike other mammals, cannot survive on raw meat and plants, application of aroma and colors to the foods enhances the acceptability of the cuisine which as well has some social values of eating. Spices and their extracts are long known to be used in ancient Mesopotamia, Egypt, India, China and old Greece, where they were appreciated for their specific aroma and various medicinal properties[3]. In modern India, spices are still regarded as important part of the cuisine and cooked. In a variety of methods based on tradition and taste preferences. Among the spices commonly used in Indian cuisines, Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*) are three very important spices and essential flavoring agents. Apart from their culinary uses, they possess excellent pharmacological activities. Turmeric (Haridra in Sanskrit, meaning effective drug for jaundice) is known to be one of the oldest spices (family Zingiberaceae) used in India and is an integral part of Ayurvedic medicine[4]. Rhizomes of Turmeric are known to possess anti hyperlipidemic, anti-diabetic, anti-inflammatory, hepatoprotective, anti-asthmatic and anti-cancer activities. Coriander (family Apiaceae) has been reported to posses many pharmacological activities like antioxidant, anti-diabetic, anti-mutagenic, anti-hyperlipidemic and anti-spasmodic activities[5]. Cumin (family Apiaceae) seeds have been found to possess significant biological activities, such as antibacterial, antifungal, anti-carcinogenic, anti-diabetic, immuno-modulatory, and antioxidant properties[6].

Packaging plays an important role in the industrial development of food ingredients to be used as

functional foods. In recent years, there has been an increasing interest of the food industry in incorporating ingredients with health beneficial properties[7]. Among these ingredients, spices are important due to their flavoring and coloring potential. Transportation of spices to different parts of the world from their source of production demands indemnity of maintenance of integrity of the highly susceptible bioactive principals, i.e. polyphenols. Moreover, spices used in food industries for production of finished materials are generally obtained from a single cultivar to ensure standard quality of the products. Packaging, thus, not only ensures quality of a product, but also maintains the integrity of the active principals for the benefit of the end users, e.g. human race.

For the past few years, extensive works have been done to determine the antioxidant potentials of spices[8,9,10]. In such studies, extraction with solvents like methanol and other aqueous alcohols were a common practice for the determination of bioactives as well as radical scavenging abilities. A few studies were also found where water was used to extract the spices for adjudication of antioxidant profile[11,12]. However, the extraction procedures were mainly percolative and thermal treatments were avoided, probably in order to retain the integrity of the bioactive principals. The present study deals with the *in vitro* antioxidant profile of the three spices before and after thermal processing with water. The design resembled closely with common cooking procedures used in India, i.e. pressure cooking and microwave extraction. To our knowledge, it was one of the very few studies that dealt with human consumable water extractives of foodstuffs for their radical scavenging abilities, and probably the first with the subject spices. In this way, we would be able to know the appropriate cooking methods, which would retain the most effectiveness of these spices for human consumption. The present study reports the achievement of the aim through some common *in vitro* antioxidant assays.

MATERIALS AND METHODS

Chemicals

2,2′-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from Himedia, India. Trichloroacetic acid, ascorbic acid, Folin-Ciocalteau's solution and sodium carbonate were obtained from Merck, India. All other reagents and chemicals used were of analytical grade procured from local sources. Deionized distilled water was used in the entire study.

Selection of samples

Fresh samples of three commonly used spices, namely, Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*) were obtained from local markets in

Barasat, West Bengal, and authenticated by a Botanist. The spices were checked for dirt or any visible damages, which were discarded. The samples were dried at 45°C to a constant weight[11] before pulverization to get powders. Commercial spice powders in packaged form were purchased from grocery shops in Barasat, West Bengal. All the samples were stored in darkness in polyethylene containers at 4°C.

Thermal Processing of the samples

Thermal processing was done following a published method with some minor modifications[13]. Powdered samples were used to apply different thermal stresses. The extractions were done using deionized distilled water with a solid-to-solvent ratio of 1:10 (w/v). About 10 gms of the samples were extracted. Extractive of the samples without heating were also prepared for comparative purpose. The following are the methods of extraction.

Unprocessed sample– The samples in water were macerated in a mechanical blender. Then the mixtures were centrifuged at 6000 rpm for 5 minutes to get a clear supernatant. The supernatant were abbreviated as US and used for further studies.

Pressure cooked sample– The samples in water were subjected to a commercial pressure cooker for 15 minutes. Then the mixtures were centrifuged as above to get a clear supernatant. The supernatant were abbreviated as PC and used for further studies.

Microwave treated sample – The samples in water were subjected to a commercial microwave oven at 160°C for 3 minute. Then the mixtures were centrifuged as above to get a clear supernatant. The supernatant were abbreviated as MO and used for further studies.

DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure[13]. 1 ml DPPH solution (3 mg in 25 ml ethanol) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as IC_{50} of the samples. Gallic acid was used as positive control and comparing with its' IC_{50} and the results were expressed as gallic acid equivalents (mM/gm spices).

Reducing Power assay

The assay was performed using a previously described procedure[14] with minor modifications. Briefly, 0.5 ml of sample solutions was mixed with phosphate buffer (pH 7.4, 2.5 ml) and aqueous potassium ferricyanide solution (2.5 ml). This mixture was kept at 50±2°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% (w/v) trichloroacetic acid was added and

centrifuged at 3000 rpm for 5 min. 2.5 ml of the supernatant was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Gallic acid was used as positive control and comparing with its' IC_{50} and the results were expressed as gallic acid equivalents (mM/gm spices).

Total Phenolic Content assay

The assay was performed using a previously described procedure[15] with minor modifications. Briefly, 0.5 ml of sample was mixed with 1.5 ml Folin-Ciocalteau's solution (1:10 v/v diluted with distilled water) and allowed to stand for 28±2°C for 5 min. Then 2 ml of 7% (w/v) aqueous sodium carbonate solution was added and the mixture were allowed stand for another 90 min and at darkness. The absorbance of the blue color that developed was measured at 725 nm using spectrophotometer (Systronics, Model – 2202). Gallic acid was used to prepare the standard curve (20–100 mg/ml) and the total phenolic concentration in the spice extract was expressed as mg of gallic acid per gram of dry weight of the spices (mM/gm spice).

Statistical analysis

Data were expressed as mean ± standard error of four independent samples. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test for multiple comparisons of the means. The value of $p < 0.05$ was taken as the criterion for statistically significant difference. The analyses were done with the software 'Prism 4.0' (GraphPad Inc., USA).

RESULTS AND DISCUSSIONS

DPPH radical decolorization assay

Reactive oxygen species (ROS) have been implicated in various types of adverse pathophysiological conditions. The main ROS to be considered is superoxide anion (O_2^-), which is predominantly generated in mitochondrial electron transport chain. This deleterious species then produces one more potent noxious species hydrogen peroxide (H_2O_2) by the action of superoxide dismutase (SOD). H_2O_2 in turn produces another harmful species, hydroxyl radical ($OH^{\bullet}$), by a Fenton-type reaction in presence of systemic trace metal cations. Substances which could effectively scavenge free radicals would also be useful in maintaining well being of humans[13]. The determination of antioxidant potential of plant based substances is still being an unresolved problem and not a single assay would be sufficient for the assessment[16]. In the present study, the methods for adjudication of the antioxidant potential of the subject spices were chosen specifically in view of the above proclamation. DPPH assay is based on non-aqueous less

polar medium (i.e. alcohol). The extracts prepared in the present study might contain majority of non-polar antioxidant biomolecules as spices are found to replete with essential oils, carotenoids or flavonoids. The results of the DPPH assay would indicate the extent of liberation of the non-polar principal bioactives of the spices in water. It was observed that the antioxidant potential of the all three spices were more in fresh samples than the packaged sample before any treatment. This might be due to loss of volatile antioxidative bioactives during packaging. However, after thermal processing, the antioxidant activity increased in case of turmeric for both fresh and packaged samples. The improvements were *ca*. 15% (from 240.59 to 277.18 mM/gm dry sample) after pressure cooking and *ca*. 30% (from 240.59 to 313.24 mM/gm dry sample) after microwave treatment for fresh sample. For packaged samples, the improvements were about 2 times (from 141.00 to 291.12 mM/gm dry sample) after pressure cooking and about 2.5 times (from 141.00 to 375.00 mM/gm dry sample) after microwave treatment (Table 1, Figure. 1). Gallo *et al*. (2010)[17] previously had shown that microwave extraction improved antioxidant activity of some spices. One plausible explanation might be the fact that the packaged samples are prepared from spices that are usually collected from a single specific cultivar, which minimizes their class variations. This could be substantiated by the fact that, a steady increment in the DPPH radical scavenging ability was observed in the other two spices in case of the packaged samples (Table 1, Figures. 2 and 3). For fresh samples, the results were inconsistent, probably due to non-homogeneity of the samples procured from local sources.

Table 1: Antioxidant potential of Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*) after processing in different thermal conditions. Results are expressed as gallic acid equivalents (mM/gm dry sample).

Sample	Processing method	Spice type	DPPH assay	Reducing power assay	Total phenolics Content
Turmeric (*Curcuma longa*)	US	Fresh	240.59±4.46	28.41±2.19	0.65±0.06
		Packaged	141.00±3.87	47.65±4.65	2.35±0.50
	PC	Fresh	277.18±6.36	46.11±2.37	4.00±0.26
		Packaged	291.12±5.45	53.88±1.52	4.76±0.23
	MO	Fresh	313.24±6.85	51.41±2.76	1.47±0.15
		Packaged	375.00±5.37	80.88±4.10	2.82±0.27
Coriander (*Coriandrum sativum*)	US	Fresh	2041.59±57.54	134.06±3.59	6.34±0.31
		Packaged	2441.71±93.96	128.82±5.26	6.47±0.25
	PC	Fresh	2784.53±68.62	165.29±3.87	9.53±0.27
		Packaged	2903.65±59.71	205.65±7.50	10.88±0.35
	MO	Fresh	2608.53±43.89	122.94±3.26	6.88±0.08
		Packaged	3364.41±70.85	179.71±3.62	9.65±0.20

Cumin (*Cuminum cyminum*)	US	Fresh	4517.29±62.40	242.71±4.60	8.71±0.27
		Packaged	4498.35±102.98	276.35±5.95	9.22±0.42
	PC	Fresh	4819.35±94.70	154.76±3.71	8.24±0.26
		Packaged	4548.71±60.67	305.35±3.76	12.47±0.47
	MO	Fresh	4010.06±101.46	202.06±6.17	9.94±0.21
		Packaged	5174.35±102.90	353.88±6.60	10.12±0.12

Data are expressed as Mean ± SE (n=4).

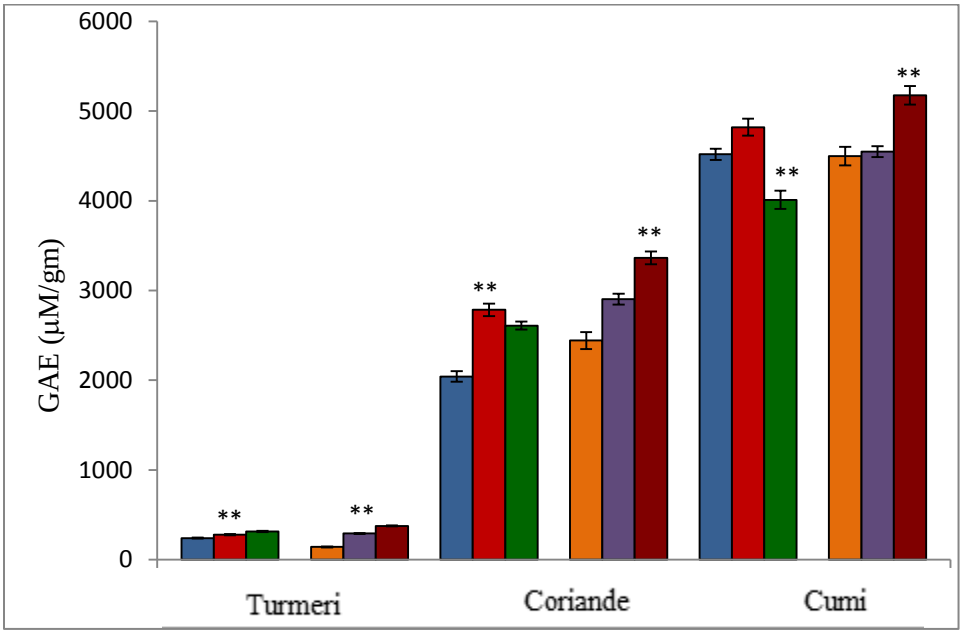

Figure 1: Comparative DPPH radical scavenging activities of Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*), (fresh and packaged) after processing in different thermal conditions. Data are Mean ± SE (n=4); US (Fresh): ; PC (Fresh): ; MO (Fresh): ; US (Packaged): ; PC (Packaged): ; MO (Packaged): .

*** *p*<0.05 and ** *p*<0.01 in comparison with US.**

Reducing Power assay

Reducing power of a sample might serve as a significant reflection of the antioxidant activity *in vitro*. Compounds possessing reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, ultimately minimizing adverse health conditions[18]. The present study indicated that Cumin showed maximum reducing power over the other two spices for both fresh and packaged samples. It was also observed that reducing power of fresh turmeric and cumin are less than the packaged samples. All the three spices exhibited improvement of reducing power after thermal treatment in case of both fresh and packaged samples. In case of packaged turmeric, microwave treatment improved reducing power most (from 47.65 to 80.88 mM/gm dry sample, Table 1, Figure 1). In case of packaged coriander, pressure treatment improved reducing power most (from128.82 to 205.65 mM/gm dry sample,

Table 1, Fig. 2). In case of packaged cumin, microwave treatment improved reducing power most (from 276.35 to 353.88 mM/gm dry sample, Table 1, Figure 3). The results are in accordance with the DPPH radical scavenging assay.

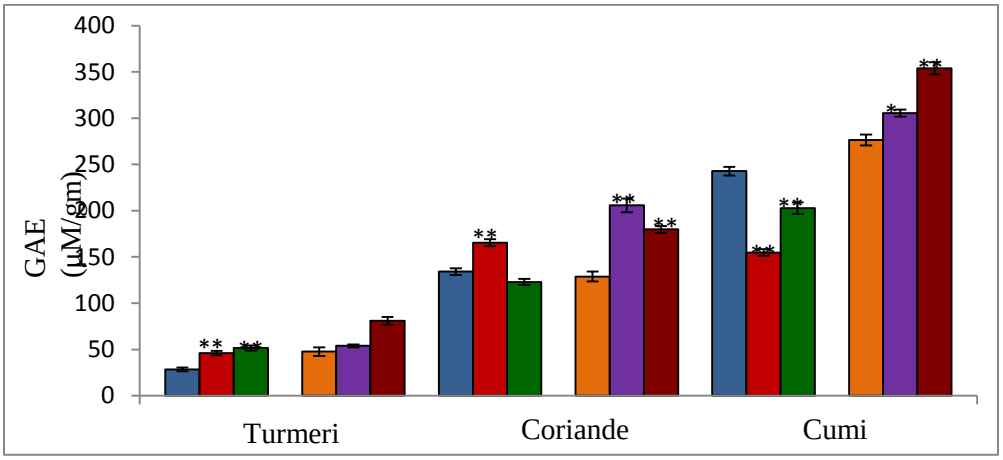

Figure 2: Comparative reducing power of Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*), (fresh and packaged) after processing in different thermal conditions. Data are Mean ± SE (n=4); US (Fresh): ; PC (Fresh): ; MO (Fresh): ; US (Packaged): ; PC (Packaged): ; MO (Packaged): .

*** $p<0.05$ and ** $p<0.01$ in comparison with US.**

Total Phenolic Content assay

Phenols are an important part of the polar fraction of plant based oils. When the seeds or rhizomes of spices are used directly in cuisine without any refinement, the phenolics are partly preserved and these compounds are reportedly responsible for their radical scavenging abilities[19]. Since essential oils are an important constituent of the bioactives of coriander and cumin, phenolics come into play for their antioxidant activities. Turmeric contains curcumin, which is also a phenolic and imparts an essential role in its pharmacological activity. The present study indicated that the thermal processing of the subject spices improved their total phenolic contents. Enhanced phenolic contents of the spices shown after thermal processing in water might be due to enhanced extraction of polyphenols, which might have less solubility in normal water but enhanced solubility in hot water. The result was in accordance with a previous observation of thermal processing of foodstuffs[13]. There was about 6-fold increment in phenolic content (from 0.65 to 4.00 mM/gm dry sample, Table 1, Figure. 1) in the water extractive of fresh turmeric after pressure treatment and about 2-fold increment (from 0.65 to 1.47 mM/gm dry sample, Table 1) in case of microwave treatment. In case of packaged turmeric, only considerable increment was observed upon pressure treatment (from 2.35 to 4.76 mM/gm dry sample, Table

1). In case of coriander, noteworthy improvement was observed in packaged samples and the best method was pressure treatment (from 6.47 to 10.88 mM/gm dry sample, Table 1, Figure 2). In case of cumin also, packaged sample showed greater improvement upon pressure treatment (from 9.22 to 12.47 mM/gm dry sample, Table 1, Figure 3). The bioactives commonly present in the subject spices were reported to be effective against various types of toxic oxidants although not much research have been conducted in this sphere[12]. Phenolic compounds of plants having one or more aromatic rings with one or more hydroxyl groups can potentially quench free radicals by forming resonance-stabilized phenoxyl radicals which play a role in their antioxidant properties[8]. The effectiveness of them against the most harmful ROS after thermal processing that closely resemble cooking methods employed in India, however, was not studied previously. In this context, the present study indicated effect of thermal stress upon antioxidative potential of the three spices upon extraction with water, which would reflect their potential as functional food during human consumption.

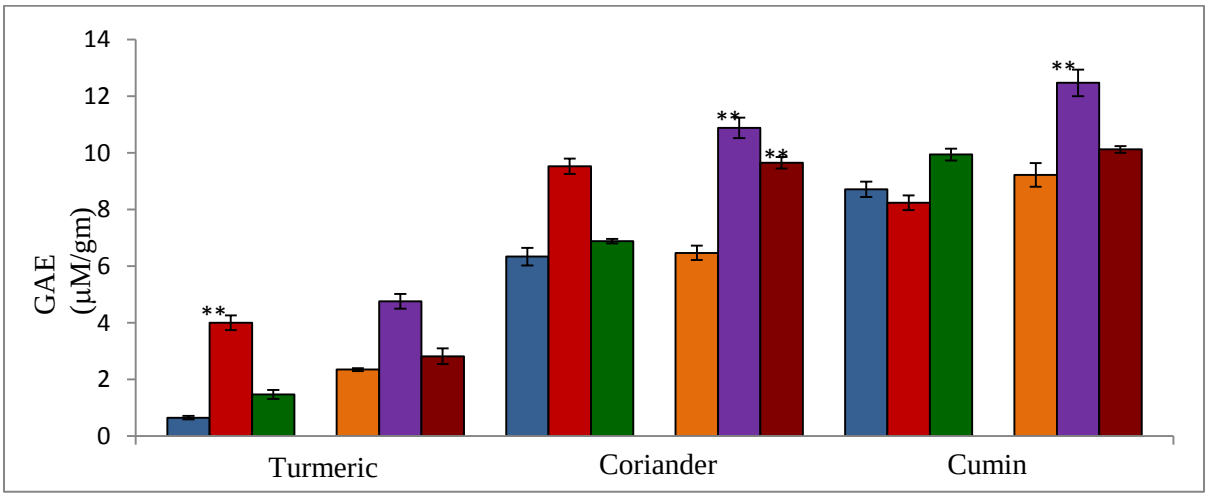

Figure 3: Comparative total polyphenol contents of Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin (*Cuminum cyminum*), (fresh and packaged) after processing in different thermal conditions. Data are Mean ± SE (n=4); US (Fresh): ; PC (Fresh): ; MO (Fresh): ; US (Packaged): ; PC (Packaged): MO (Packaged): . * *p*<0.05 and ** *p*<0.01 in comparison with US.

CONCLUSIONS

The major conclusion arising out of this research was that the antioxidant capacities of the three subject spices could be improved by thermal processing methods that resemble cooking. Enhanced activity of the spices shown after thermal processing in water might be due to enhanced extraction of polyphenols, which might have less solubility in normal water but

enhanced solubility in hot water. There was a strong correlation between the two antioxidant activity assays and the total phenolic contents, which indicated that the antioxidant activities of the spices were mainly due to the polyphenolics extracted in the water by thermal processing. The study also indicated that there were differences in the antioxidant potential of fresh and packaged spices and the packaged spices scored better, probably due to stringent production procedures of food processing industry. The improvements in the antioxidative potential of the spices on heat treatment with water implied their role as functional foods, even after cooking.

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