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RESEARCH ARTICLE

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Thermal Processing Effects on *In vitro* Antioxidant Potential of fresh and packaged Black Pepper (*Piper nigrum*) and Indian Red Chili (*Capsicum annuum*)

[1]Moumita Shib, [1]Pallabi Saha, [2]Tapan Kumar Pal and [1*]Sauryya Bhattacharyya

[1]Department of Food & Nutrition, Ramakrishna Vivekananda Mission Sarada Ma Girls' College, Barasat, Kolkata, India
[2]Department of Biotechnology, Bengal Institute of Technology, On Basanti Highway, Hadia, Kolkata India
[*]Corresponding email: sauryya.b@gmail.com

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ABSTRACT

Black pepper (Piper nigrum) and Indian red chili pepper (Capsicum annuum) are two 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 was designed to analyze 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 activities and total phenolic content was different in the packaged or fresh spices, and the packaged spices were better than the fresh ones in general. Antioxidant activity improved in case of the two spices after thermal processing, probably due to better solublization of the antioxidants in hot water after 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 antioxidative capacities.

Keywords: Antioxidant, Black pepper, *Piper nigrum*, Red chili pepper, *Capsicum annuum*

INTRODUCTION

The classical nutrition research has been undergoing an important change in the last few decades where apparently pharmacologically inert food additives have demonstrated 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 long been used by mankind as food additives. Spices are important both as functional food ingredients and nutritional supplements. They play a role in enhancing the taste and flavour of food. In addition, spices like peppers and chilies have also been used for treating several disorders as they have potent medicinal properties [2].

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 in the form of spices 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, black pepper (*Piper nigrum*) and red chili pepper (*Capsicum annuum*) are two very important spices and essential flavoring agents. Apart from their culinary uses, they possess excellent pharmacological activities. Black pepper (family – Piperaceae) is known to possess antimicrobial, anti-inflammatory, antioxidant and anticancer activities [4]. There are reports that black pepper has digestive stimulating activity. It improves appetite, cures cold, cough, diseases of the throat, intermittent fever, colic dysentery, worms and piles. Red chilies (family – Solanaceae) contain Vitamin A, Vitamin C and capsaicin which are good antioxidants and anti-inflammatory agents, which can also boost immune system. They are good radical scavengers and also act as detoxifier by removing the waste products from our body and increase the blood supply to the tissues [5].

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 [6]. In today's society, the activities of packing and keeping ingredients are further related with technical considerations, which include safety, shelf life, convenience, appearance, cost of raw materials, transportation costs, handling, law, manufacturing and equipment. However, the concept of packaging also includes art, graphic, marketing and psychology aspects as well [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, a number of studies have been published to determine the antioxidant potentials of peppers [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 peppers for adjudication of antioxidant profile.[11,12] However, standard extraction procedures were followed in such reports, probably in order to retain the integrity of the bioactive principals. The present study deals with the *in vitro* antioxidant profile of the two 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. Analytical grades of 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 two commonly used spices, namely, Black pepper (*Piper nigrum*) and Indian red chili (*Capsicum annuum*) 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^{o}C to a constant weight before pulverization to get powders [13]. Commercial spice powders in packaged form were purchased from grocery shops in Barasat, West Bengal. Batch number and date of packaging was noted. 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 [14]. 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^{o}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 [14]. 1 ml DPPH solution (3 mg DPPH powder 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. Ascorbic acid was used as positive control and comparing with its' IC_{50} and the results were expressed as ascorbic acid equivalents (mM/gm spices).

Reducing Power assay

The assay was performed using a previously described procedure with minor modifications [15]. 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±2ºC 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 in a Systronics spectrophotometer (model – 2202). 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 (µM/gm spices).

Total Phenolic Content assay

The assay was performed using a previously described procedure with minor modifications [16]. 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 µg/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 (µM/gm spice).

Statistical analysis

Data were expressed as mean ± standard deviation of four independent samples. Data were analyzed by Student's *t*-test using the software 'Prism 4.0' (GraphPad Inc., USA).

RESULTS

DPPH radical decolorization assay

It was observed that DPPH neutralization potential of black pepper was less in fresh samples than the packaged sample at room temperature (Table 1). The activity for the fresh samples increased after the thermal treatments and it was maximum after microwave treatment (Fig. 1). This finding was in consonance with a previous study, which showed that microwave extraction improved antioxidant activity of some spices [17]. However, no increment of DPPH radical scavenging potential after pressure cooking was observed for packaged black peppers and after microwaving, the activity reduced drastically (from 3.10 to 1.54 mM/gm dry sample, Table 1). In case of red chili, DPPH scavenging potential was more in fresh samples than the packaged sample at room temperature (Table 2). The antioxidant potential of fresh sample reduced drastically after thermal pressure cooking (Fig. 1), but deteriorated drastically on pressure cooking (from 3.07 to 1.15 mM/gm dry sample in case of fresh samples, and from 2.03 to 0.91 mM/gm dry sample in case of packaged samples, Table 2). Changes during microwave treatment were not prominent (Fig. 1).

Reducing Power assay

Reducing power of a sample provides a significant reflection of the antioxidant activity *in vitro*. Compounds possessing reducing power are usually electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, ultimately minimizing adverse health conditions [18]. Reducing power assay indicates presence of electron donors in an extract which might serve as good antioxidant. Fresh Black pepper showed improvement of reducing power after thermal treatment and the improvement was maximum after microwave

treatment (from 139.88 µM/gm dry sample to 241.29 µM/gm dry sample, Table 1). The effect was not prominent in case of packaged samples (Fig. 2). However, in case of both the spices, packaged samples showed better reducing power than the fresh samples at normal conditions (Tables 1 and 2). The results are in accordance with the DPPH radical scavenging assay.

Table 1: Antioxidant potential of Black pepper (*Piper nigrum*) after processing in different thermal conditions. Results for DPPH assay are expressed as ascorbic acid equivalents (mM/gm dry sample) and as gallic acid equivalents (µM/gm dry sample) for the rest assays

Processing method	Category	DPPH assay	Reducing power assay	Total phenolics Content
US	Fresh	1.82±0.07	139.88±12.96	5.71±0.82
	Packaged	3.10±0.09	241.29±9.15	6.76±0.26
PC	Fresh	2.82±0.05	160.24±9.55	10.71±0.95
	Packaged	3.25±0.08	235.65±8.41	11.24±0.38
MO	Fresh	3.03±0.06	172.35±10.69	9.24±0.79
	Packaged	1.54±0.06	241.29±8.74	10.35±0.29

Data are expressed as Mean ±SD (n=4), US: Untreated sample, PC: Pressure cooked sample, MO: Microwave cooked sample

Table 2: Antioxidant potential of Indian red chili (*Capsicum annuum*) after processing in different thermal conditions. Results for DPPH assay are expressed as ascorbic acid equivalents (mM/gm dry sample) and as gallic acid equivalents (µM/gm dry sample) for the rest assays

Processing method	Category	DPPH assay	Reducing power assay	Total phenolics Content
US	Fresh	3.07±0.06	108.24±9.39	6.88±0.07
	Packaged	2.03±0.06	140.12±10.21	6.76±0.10
PC	Fresh	1.15±0.09	88.00±10.50	9.47±0.39
	Packaged	0.91±0.05	101.24±5.84	8.06±0.40
MO	Fresh	3.41±0.02	116.00±3.90	8.06±0.74
	Packaged	1.81±0.02	122.00±9.54	7.24±0.61

Data are expressed as Mean ±SD (n=4), US: Untreated sample, PC: Pressure cooked sample, MO: Microwave cooked sample

Fig. 1: Comparison of DPPH radical scavenging assay of fresh and packaged Black pepper (BP) and Red chili (RC) after processing in different thermal conditions. Results are expressed as ascorbic acid equivalents (mM/gm dry sample). [US: Untreated sample, PC: Pressure cooked sample, MO: Microwave cooked sample].

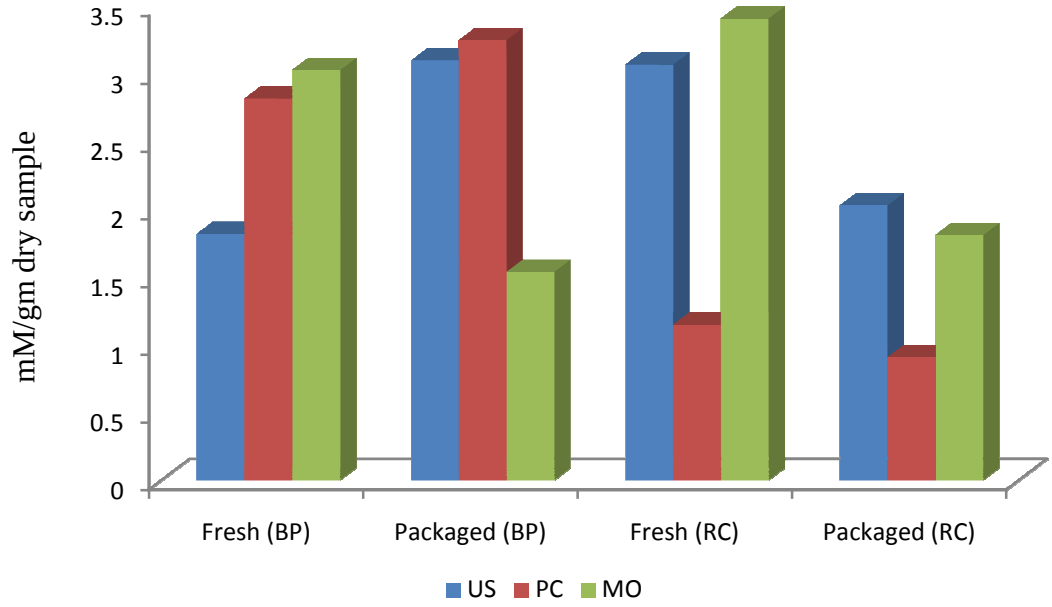


Fig. 2: Comparison of reducing power assay of fresh and packaged Black pepper (BP) and Red chili (RC) after processing in different thermal conditions. Results are expressed as gallic acid equivalents (µM/gm dry sample). [US: Untreated sample, PC: Pressure cooked sample, MO: Microwave cooked sample].

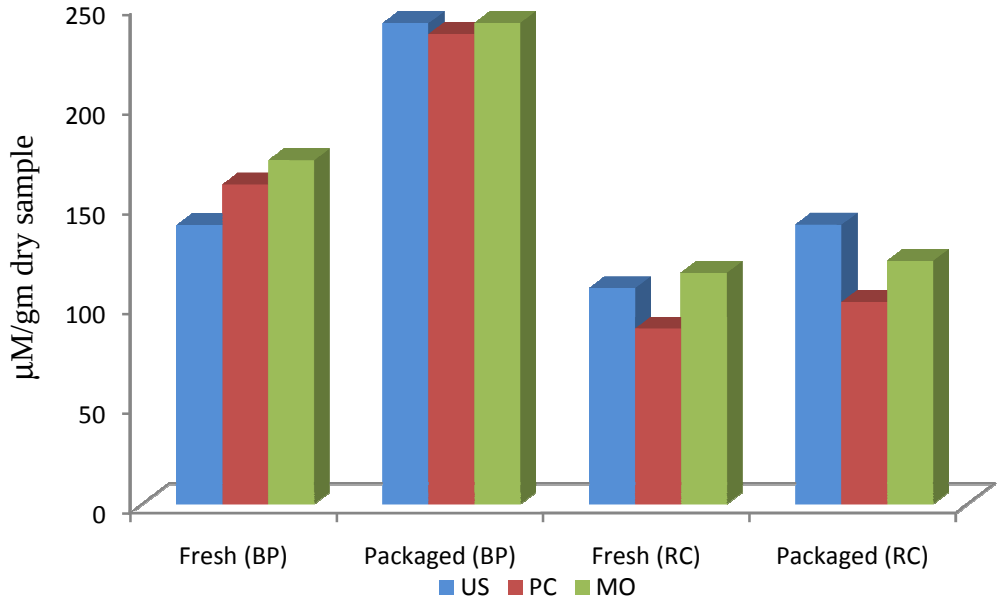
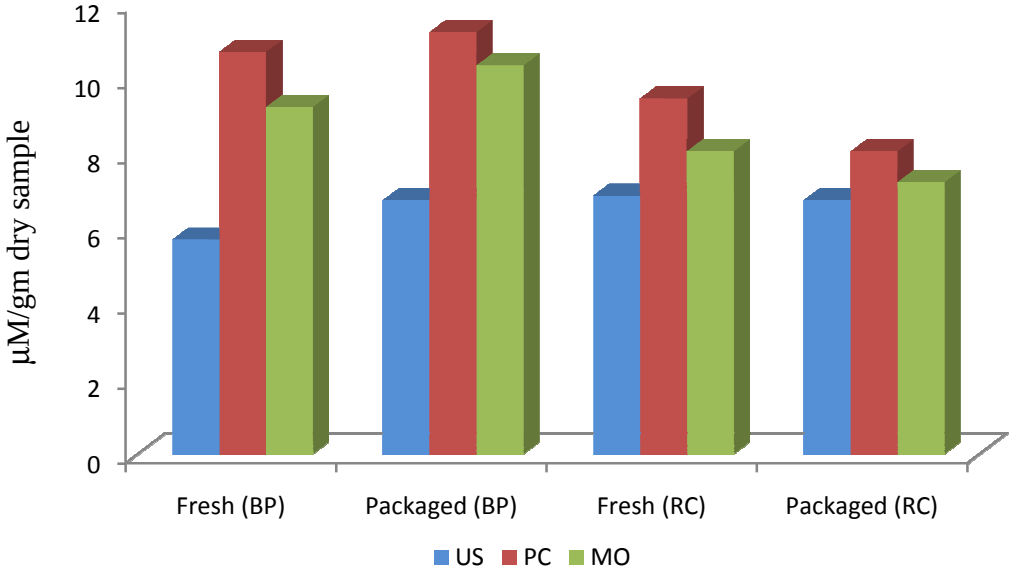


Fig. 3: Comparison of total phenolic content of fresh and packaged Black pepper (BP) and Red chili (RC) after processing in different thermal conditions. Results are expressed as gallic acid equivalents (µM/gm dry sample). [US: Untreated sample, PC: Pressure cooked sample, MO: Microwave cooked sample].



Total Phenolic Content assay

The present study indicated that the thermal processing of the subject spices improved their total phenolic contents (Fig. 3). The result was in accordance with a previous observation of thermal processing of foodstuffs [14]. There was about 2-fold increment in phenolic content (from 5.71 to 10.71 µM/gm dry sample, Table 1) in the water extractive of fresh Black pepper after pressure treatment and about 1.5-fold increment (from 5.71 to 9.24 µM/gm dry sample, Table 1) in case of microwave treatment. In case of packaged Black pepper, the improvement was similar and pressure cooking gave the best result (Fig. 3). In case of red chili, little improvement was observed after thermal treatment and the best method was found to be pressure treatment. Noteworthy improvement after pressure treatment was shown by fresh sample (from 6.88 to 9.47 µM/gm dry sample, Table 2).

DISCUSSION

Reactive oxygen species (ROS) have been implicated in various types of adverse pathophysiological conditions. The main ROS to be considered is superoxide anion ($O_2^{\bullet-}$), 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. Cooked foodstuffs that would effectively scavenge free radicals would also be useful in maintaining well being of humans [14].

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 [19]. 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 greater extent of liberation of the non-polar principal bioactives of the spices in water. On the other hand, reducing power assay implicated effectiveness of the spices in aqueous (i.e. polar) medium. This can be correlated directly with improvement in phenolic contents of the spices shown after thermal processing in water. The observed enhanced antioxidant profile in some treatment regimens as well as greater extraction of polyphenols might be due to their enhanced solubility in hot water which otherwise have less solubility in water at room temperature.

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 [20]. 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 subject spices on extraction with water, which would render their potential as functional food during human consumption.

CONCLUSION

The major conclusion arising out of this research was that the antioxidant capacities of the two 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 followed in the 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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