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Effect of cryogenic and conventional grinding on the anti-oxidative potential of coriander and turmeric

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Abstract

Spices like coriander and turmeric are one of the commonly used ingredients in Indian food. Furthermore, they are a source of natural antioxidants and can be a potent medicinal source against neurogenic disorders. The present work investigates the impact of cryogenic and conventional grinding on the antioxidant properties of turmeric and coriander. Conventional grinding significantly reduced the antioxidant potential of the spices estimated by DPPH radical scavenging ability, reduction in peroxide radical scavenging capacity and chelation of iron. Furthermore, there was a decrease in total phenolics, flavonoids, reducing power and total antioxidant capacity of the conventionally ground spice samples in relation to the cryogenic samples. To conclude that the spice samples specially coriander loses much of its antioxidant potential which is a measure of the medicinal value by conventional grinding.

Key words : Coriander, Turmeric, Antioxidant, Reducing power, DPPH, Flavonoids.

Introduction

Antioxidants also called as “free radical scavengers” are molecules that neutralize the free radicals, thus preventing them from causing damage. They are widely used in dietary supplements and have been investigated for the prevention of diseases such as coronary heart disease, cancer, aging and altitude sickness. Ascorbic acid, cysteine, flavonoids, phenolic compounds, anthocyanins, α-tocopherol, glutathione etc. are some well-known antioxidants for cardio protective action (Vinson, et. al., 17, 18, Wang, et. al., 20). Various experiments have focused on natural sources of antioxidants and their applications in food systems to cure many human diseases. BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) are the most commonly used supplementary artificial antioxidants in diet but they are failing off due to their instability and suspected action as carcinogens (Namiki, 8). To address this issue, there is a growing interest in the studies of natural healthy (nontoxic) additives as potential antioxidants (Tomaino, et. al., 16).

Herbs and spices are the rich natural source of antioxidants. Most of the antioxidant potential of herbs and spices is due to the redox properties of phenolic moieties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Caragay, 4). Like other herbs and spices, seed spices are good source of antioxidants like vitamin A, vitamin C, vitamin E (http://ndb.nal.usda.gov/ndb/foods/show/2917. 22) and glutathione as well as some enzymes like catalase, superoxide dismutase and various peroxidases Insufficient levels of antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress and this stress play a significant role in many human diseases, including cancers. Many studies were done to analyze the antioxidant properties of Cumin (Thippeswamy and Naidu, 15), Coriander (Wangensteen, et. al., 21), Fenugreek (Bukhari *et al.,* 3), Fennel (Oktay, et. al., 9) and Ajwain (Singh, et. al., 14), but there are limited reports (Saxena *et al.,* 12) available for the effect of grinding temperature on the antioxidant potential of Turmeric and Coriander.

Turmeric (*Curcuma longa*) and Coriander (*Coriandrum sativum*) both are the rich source of antioxidants and are used in powder form in curries. To prepare a fine powder these spices are usually grinded in mixer grinders at home or at large industrial area. Though they are good source of antioxidants in all forms and have great medicinal values but it is necessary to adjudge the most suitable grinding source for these seed spices to retain their maximal medicinal value.

In this study we have estimated the effect of grinding temperature (crushed in liquid nitrogen –L, crushing in mixer grinder- T) on antioxidant potential of coriander and

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turmeric. The antioxidant potential has been evaluated with respect to total antioxidant capacity, flavonoid content, total phenolic content, reducing power determination, estimation of DPPH radical scavenging activity, estimation of H_2O_2 scavenging activity, estimation of Fe (II) chelating activity and inhibition of conjugated diene formation in linoleic acid emulsion.

Materials and methods

Plant Material and sample preparation:

Seeds of Coriander and rhizomes of Turmeric were purchased from the local market of Fayetteville, Arkansas (USA). The experimental material was divided into two sets – one set was grinded to fine powder in liquid nitrogen (100mg each) while the other set was grinded in mixer-grinder and 100mg sample was taken from each and extracted with methanol. Supernatant was collected and stored at -20ºC for further use. The extraction was done in three replicates.

Chemical reagents:

Methanol, folin-Ciocalteu's reagent, sodium carbonate, η-butanol, ethanol, sodium Nitrite, aluminium nitrate, sulphuric acid, sodium phosphate, ammonium molybdate, potassium ferricyanide, trichloroacetic acid, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine and $FeCl_2$ (VWR chemicals, USA)

Estimation of Total Phenolic Content (TPC)

TPC was expressed as tannic acid equivalents. Folin-Ciocalteu reagent was diluted 10 times before use (Vinson, 19) and 500 µl quantity was added with 400 µl of 7.5% (w/v) sodium carbonate (Marinova, et. al., 7) in a 2ml tube and 100µl of methanolic extract of samples was added and allowed to stand for 30 min at room temperature (25ºC). The absorbance was measured at 760 nm.

Estimation of Flavonoid content (FC)

Colorimetric method with few modifications (Jia *et al.,* 6, Basu *et al.,* 2) was used to estimate the flavonoid content of the samples. 10ml methanolic extract was extracted thrice with η-butanol. The residue was re-dissolved in 5ml of 60% (v/v) ethanol and washed twice with 5ml of 30% (v/v) ethanol. Washed samples were filtered. The filtrate was diluted upto 25 ml with 30% (v/v) ethanol. An aliquot of 100 µl of the solution was mixed with 900 µl of 30% (v/v) ethanol and mixed with 60 µl of 5% (w/v) sodium nitrite for 5min. then, 60 µl of 10% (w/v) aluminium nitrate was added. The reaction was stopped after 6min, by adding 2ml of 1M NaOH. The mixture was further diluted to 3ml 30% (v/v) ethanol. The absorbance of the mixture was measured immediately at 510nm. The flavonoid content was calculated and expressed as rutin equivalents (mg g^{-1})

Estimation of Total Antioxidant Capacity (TAC)

The assay is based on the reduction of Mo(VI) to Mo(V) by the methanolic extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH (Prieto 10). Methanolic extract (50µl) was added to tubes containing 1.25ml reagent (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95° for 1.5hr. The reaction mixture was allowed to cool down to room temperature and the absorbance was measured at 695 nm and the results expressed as ascorbic acid equivalents (mg g^{-1}).

Estimation of Reducing Power (RP)

Reducing power of methanolic extracts of samples was estimated by the modified reducing power method (Bukhari, et. al., 3). Methanolic extract (100 µl) of the samples was mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1 %(v/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 ml of 10% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 7500 rpm for 5 min. The upper layer of the solution (2.5 ml) was mixed with 2.5ml DH_2O and 0.5ml of 0.1% (w/v) ferric chloride and then absorbance of the pink color mixture was measured at 700 nm. Increased absorbance of the mixture indicates increased reducing power.

Estimation of DPPH radical scavenging activity

Scavenging of DPPH radical by methanolic extract is the most commonly used procedure to assay antioxidant potential. The free radical scavenging abilities of the samples were measured from the bleaching of the purple-colored methanol solution of 1,1-diphenly-2-picrylhydrazyl (DPPH) (Shimada , et. al., 13). Methanolic extract (100µl) was added to 1ml of 0.004% (v/v) methanolic solution of DPPH. After 30 minutes incubation in dark at room temperature, the absorbance was measured at 517 nm. The percentage inhibition activity was calculated as $\{(A_{Control}-A_{sample})/A_{control}\} \times 100$ where A is the absorbance.

Estimation of H_2O_2 scavenging activity

Methanolic extract (100µl) was added to 750µl of 0.1M phosphate buffer (pH 7.4) and mixed with 150µl of 43mM H_2O_2 solution (prepared in phosphate buffer) for H_2O_2 scavenging activity assay. The absorbance value was recorded twice after 0min and 40min and the concentration of H_2O_2 in the assay mixture was determined using a standard curve. (Ruch, et. al., 11)

Estimation of Fe(II) chelating activity

Measurement of Fe(II) chelating activity was done using reported method (Carter, 5). Methanolic extract (50µl) was added to 100µl of 2mM $FeCl_2$ 200 µl of 5mM ferrozine and mixed properly. After mixing it is kept at 25°C for 10 min. The absorbance was recorded at 562 nm. The % Fe(II) chelating activity was calculated as $\{(A_{Control}-A_{sample})/A_{control}\} \times 100$ where A is the absorbance and expressed as µg EDTA equivalents g^{-1} defatted material

using a standard curve prepared with EDTA.

Statistical analysis

All the analysis was carried out in triplicate, and the experimental results obtained were expressed as means ± Standard Error (n = 3). The statistical significance was evaluated at P = 0.05 by two-sided Student's t-test.

Result and discussion

Analysis of TAC, TPC and TFC

The TPC, TAC and TFC have been studied in coriander and turmeric (Asimi, 1). In our studies we have found that turmeric rhizomes grinded at low temperature (liquid nitrogen) showed maximum antioxidant potential (1.38 mg g^{-1}) where as lowest (0.489 mg g^{-1}) potential was recorded in coriander seeds grounded in a machine. Coriander seeds grounded in LN_2 showed almost double (0.8 mg g^{-1}) total antioxidant capacity when compared with the machine grounded coriander powder (Fig 1A). Total Phenolic content was maximum in turmeric powder (2.2 mg g^{-1}) grinded in LN_2 and minimum in machine grounded coriander powder (0.8 mg g^{-1}) (Fig. 1B). Turmeric when grounded in LN_2 showed maximum flavonoid content (0.02 mg g^{-1}) which was reduced to half when grounded in machine (Fig 1B). Similar pattern was observed for coriander as well (Fig 1C).

Analysis of Free radical scavenging activity

In our study turmeric powder (LN_2) showed maximum of DPPH (82%) which is 1.2 times higher than machine grounded powder (72%) while for coriander ground in liquid nitrogen showed DPPH radical scavenging capacity of 93% which is 2.4 times higher than ground in mixer grinder (Fig 2A).

We have seen that coriander seeds ground in liquid nitrogen and mixer grinder showed H_2O_2 scavenging potential of 32.3% and 2.4% respectively while for turmeric it was 32.2 % and 21.9% respectively. (Fig. 2B).

Analysis of RP and Fe(II) chelating activity

The reducing power is normally associated with the presence of reductones, which shows antioxidant action by breaking the free radical chains by donating a hydrogen atom. A significant reduction in the reducing power was obtained in the extract prepared by grinding in mixer grinder as compared to the one in liquid nitrogen for both coriander and turmeric (1.82 and 1.63 times respectively. In the extracts of coriander and turmeric prepared by liquid nitrogen grinding, turmeric had the highest reducing power (Fig 3A).

Fe(II) chelating activity in the cryogound turmeric and coriander was 81% which was significantly reduced in conventionally ground samples to 24% in both the spices (Fig 3B).

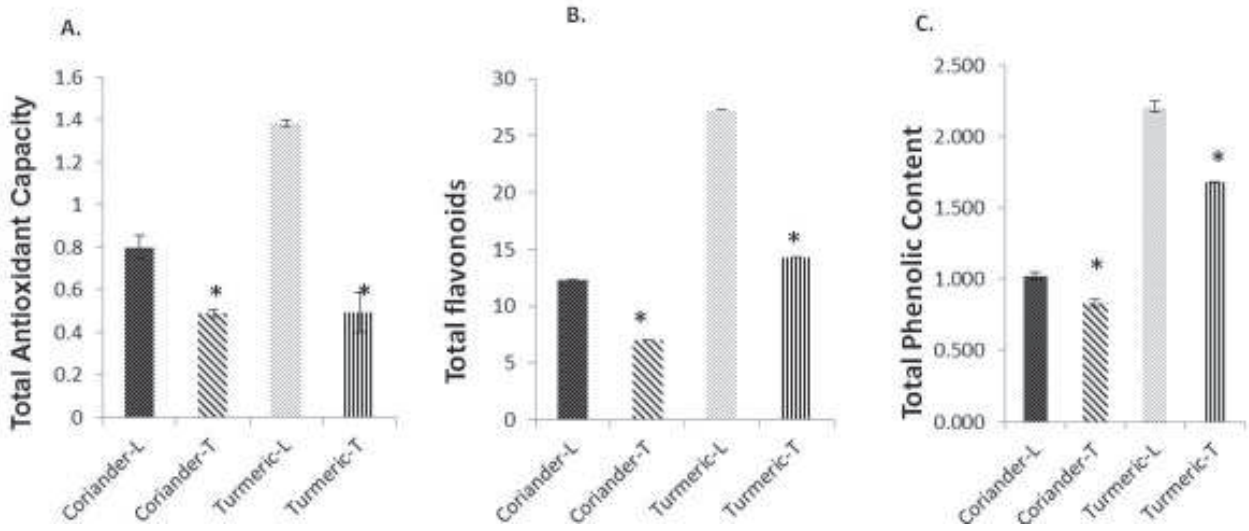

Figure 1: Total Antioxidant capacity (A), total Phenolics content (B) and total Flavonoids (C) in the cryo and conventionally ground samples of coriander and turmeric. Values expressed as ± SE (n=3). * (asterisk) mark above the bar indicates statistically significant decrease at p=0.05. (Unit in mg g^{-1})

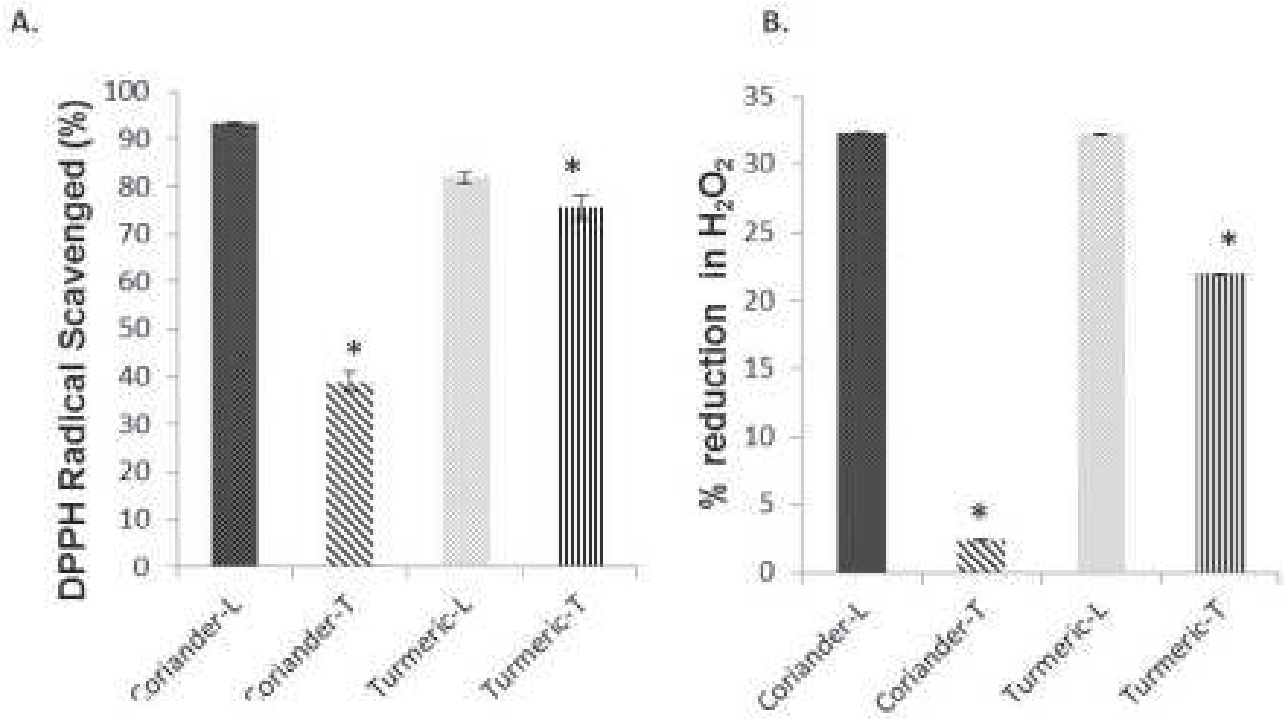

Figure 2: Percentage of DPPH radical scavenging activity (A), reduction in H_2O_2 concentration (B) in the cryo and conventionally ground samples of coriander and turmeric. Values expressed as ± SE (n=3). * (asterisk) mark above the bar indicates statistically significant decrease at p=0.05. (Unit in mg g^{-1})

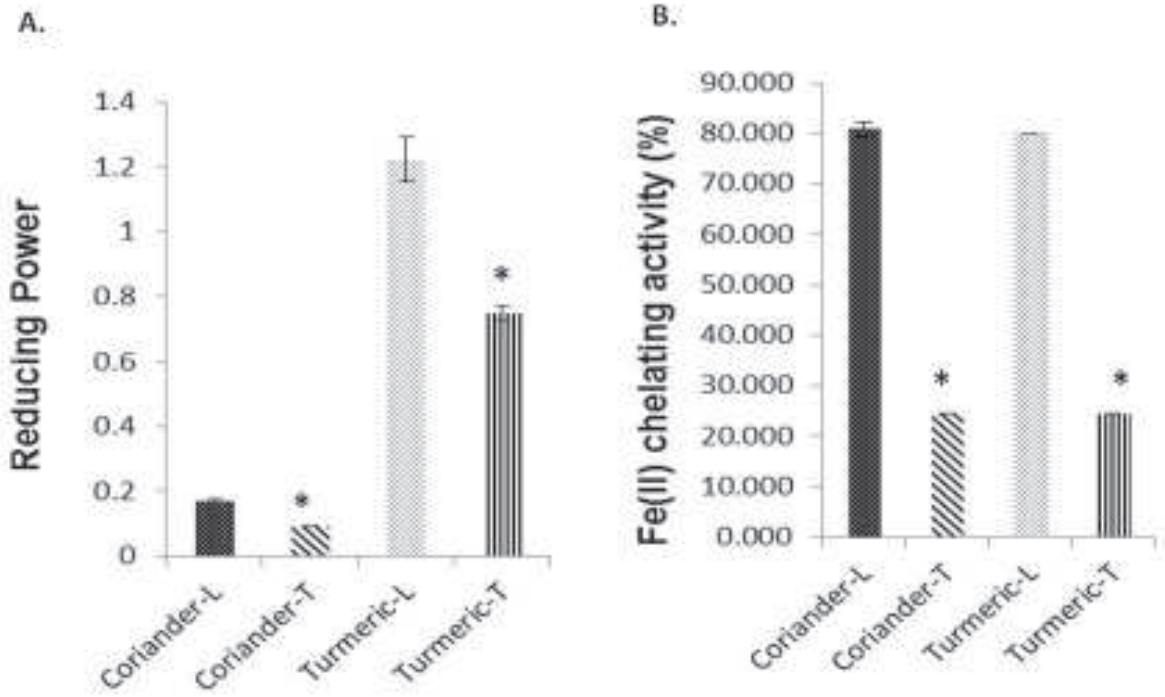

Figure 3: Reducing Power ability (A), Percentage of Fe (II) chelation activity (B) in the cryo and conventionally ground samples of coriander and turmeric. Values expressed as ± SE (n=3). * (asterisk) mark above the bar indicates statistically significant decrease at p=0.05. (Unit in mg g^{-1})

Conclusion

In this study we have investigated the effect of grinding temperature on antioxidant potential of turmeric and coriander. Results demonstrated significant decline in the antioxidant potential in coriander and turmeric both when grounded in a mixer grinder or at large scale as these machines generates heat which causes loss antioxident potential of the spices. Among the four spices powder used in the study, turmeric showed maximum antioxidant potential under both conditions in contrast to coriander.

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