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Cryogenic grinding technology enhances volatile oil, oleoresin and antioxidant activity of cumin (*Cuminum cyminum* L.)

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Abstract

Effect of cryogenic grinding on volatile oil, oleoresin, total phenolics, flavonoid content and antioxidant properties of seed extract of two cumin (*Cuminum cyminum* L.) genotypes have been analyzed. Cryogenic grinding not only retains the volatiles in both the genotypes but enhanced the recovery also. Non cryogenic or normal grinding at ambient temperature causes 18-19 % loss of volatile oil in both the genotypes. A significant increase (28.28%) in oleoresin percentage was observed when seeds of RZ 209 were ground using cryogenic grinding. This increase was, however less (16.046%) in genotype GC 4. Total phenolic and flavonoid content was also more in cryogenically ground seeds of both the genotypes. Methanol crude seed extract of both the genotypes were evaluated for its antioxidant activity in terms of total antioxidant content and DPPH free radical scavenging %. Antioxidant content was increased in cryo ground seeds by 11.78 and 8.9 mg BHT E/100000 ppm extract in GC 4 and RZ 209 respectively. DPPH free radical scavenging percentage was higher when seeds were ground cryogenic grinding technology up to 25.63 to 23.73% in GC 4 and RZ 209 respectively. The higher concentration of antioxidant content and DPPH scavenging % suggested high antioxidant activity in cryo ground samples.

Key words : Antioxidant activity, cryogenic grinding, cumin, TPC, TFC, scavenging percentage

Introduction

Spices have been known for ages as effective the rapeutic food. The power of spices to impart biological activity is now slowly reemerging as an area of interest for human health. The seed spices constitute an important group of agricultural commodities and play a significant role in our national economy. Historically, India has always been recognized as a land of spices. The states, Rajasthan and Gujarat have together contributed more than 80 per cent of the total seed spices produced in the country. Seed spices produce numerous secondary metabolites or phytochemicals. These are naturally occurring, biologically active chemical compounds in plants, where they act as a natural defense system for host plants and that have historically been used as pharmaceuticals, fragrances, flavour compounds, dyes, and agrochemicals. Even today, these metabolites are a major source new drugs.

Cumin (*Cuminum cyminum* L.) is one of the major seed spices, long associated with man and its use and cultivation of great antiquity. The seeds were used by Romans as an alternative to pepper, or used as a paste to spread over bread and meat. Cumin is mentioned as an essential ingredient of many traditional dishes. It was popular in the middle ages mainly as an ingredient in traditional medicine. In Sanskrit, Cumin is known as *Jira*. Which means "that helps digestion". In Ayurvedic system of medicine, dried Cumin seeds are used for medicinal purposes. It is used internally and sometimes for external application also. It is known for its actions like enhancing appetite, taste perception, digestion, vision, strength, and lactation. It is used to treat diseases like fever, loss of appetite, diarrhea, vomiting, abdominal distension, edema and puerperal disorders (Rathore *et al.*, 11).

Grinding of spices is an age-old technique like grinding of other food materials. The main aim of spice grinding is to obtain smaller particle size with good product quality in terms of flavour and colour. In the normal grinding process, heat is generated when energy is used to fracture a particle into a smaller size. This generated heat is usually detrimental to the product and results in some loss of flavour and quality. The fat in spices generally poses extra problems and is an important consideration in grinding. During grinding, the temperature of the product rises to a level in the range of 42±95°C (Pruthi and Misra, 9), which

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varies with the oil and moisture content of the spices, but spices lose a significant fraction of their volatile oil or flavouring components due to this temperature rise. The losses of volatile oil for different spices have been reported to be in the tune of 40% in coriander (Saxena *et al.,* 16), 37% for nutmeg, 14% for mace, 17% for cinnamon and 17% for oregano (Andres, 2; Pesek *et al*., 8). The loss of volatile oil during grinding of caraway seed has been reported to be 32% with an increase in grinding temperature from 17°C to 45°C (Wolf and Pahl, 22). The loss of volatile oil can significantly be reduced by cryogenic grinding technique using liquid nitrogen that provides the refrigeration needed for pre-cooling the spices and to maintain the desired low temperature by absorbing the heat generated during the grinding operation (Singh and Goswami, 19). The extremely low temperature in the grinder solidifies oils so that the spices become brittle, they crumble easily permitting grinding to a finer and more consistent size. The high quality of ground product would have domestic as well as International market. The earlier work on use of liquid nitrogen for cryogenic grinding of the spices mainly highlights the benefits of cryogenic grinding over the non cryo grinding in ambient condition (Saxena *et al*., 15; Saxena *et al*., 14; Wiestrieich and Schafer, 21; Russo, 13; Rice, 12 and Landwehr and Pahl, 6). In the above studies, attempts were made to prove that cryogenic grinding of cumin is better than non cryo grinding in terms of higher retention of volatile oil, total oil, total phenolic content, total flavonoid content and antioxidant properties of ground powder (Singh and Goswami, 20).

Materials and methods

Seeds of two varieties of cumin (GC-4 and RZ-209) obtained from NRCSS, were cleaned and used for cryogenic grinding and non cryo grinding. Ground powder was used for extraction of volatile oil and total oil as well as total phenolic content, total flavonoid content and antioxidant properties of ground powder.

Grinding of seeds

In the process of cryogenic grinding the material is feed into a feeder hopper and dropped into a conveyor where the material to be processed enters the pre-chilled conveyor. Liquid nitrogen is then sprayed and blended directly onto the material. The material is conveyed via a stainless steel special design auger. The auger not only transports the grinding media, but also mixes it with liquid nitrogen for greater cooling efficiencies. Liquid nitrogen is added until the temperature of the material is reduced to a predetermined set point. This set point is the glass transition temperature of the material. The extremely low temperature in the grinder solidifies oils so that the spices become brittle, they crumble easily permitting grinding to a finer and more consistent size. Finally the brittle material enters an impact (pin) mill where it is ground to a desired particle size. The Cryo grounded powder was quickly packed using sealing machine and only opened at the time of analysis. To obtaining seed powder through non cryo grinding dried seeds (30 gm) was ground separately by domestic mixer grinder.

Total oil content was extracted using Accelerated Solvent Extraction System (Dionex India Pvt. Ltd.). The Accelerated Solvent Extractor is a system can be used with organic solvent, aqueous buffer, water, and small amounts of mineral acids. The system accelerates the traditional extraction process by using solvent at elevated temperatures and pressures. Pressure is maintained in the sample cell to maintain the heated solvent in a liquid state during the extraction. After heating, the extract is rinsed from the sample cell into a collection vessel. Total oil was obtained after evaporating the solvent in rotary evaporator. Thirty gram seed powder was utilized for the estimation of total oil and hexane was used as solvent.

Essential oil from seed powder was estimated using all glass Clevenger apparatus (Clevenger, 5), utilizing 25-30 g samples from each genotype.

Cryogenic and non cryogenically ground seeds (10 gm) of coriander was extracted with 50 ml methanol twice. Supernatant from both extraction were pooled and methanol was evaporated in rotary evaporator. This crude seed extract was used for determination of the total phenol and flavonoids concentration, as well as antioxidant activities.

Total phenol concentrations were determined using a Folin-Ciocalteu assay, as described by Amin *et al.* (1). An aliquot of 0.1ml from 1000 ppm crude methanol extract was taken in a test tube and made the volume 1ml by adding solvent. 3ml of 10% sodium carbonate was added. Previously 10-fold diluted Folin-Ciocalteu reagent was added to the mixture. The mixture was allowed to stand at room temperature for 90 minutes and then absorbance was measured at 710 nm. Gallic acid was used as the standard phenol. The amount of phenolic content was calculated by using the standard curve of Gallic acid prepared having R^2 value ranged from 0.96-0.99 and was expressed as mg Gallic acid equivalents/100000 ppm crude seed extract.

Total flavonoids concentration was determined using previously reported method by Chang *et al.* (3). One ml of crude seed extract was taken in a test tube and 100µl aluminum chloride (1M) solution was added carefully from the side wall of the test tube followed by addition of 100µl potassium acetate. The total volume was made 4 ml by adding 2.8ml of solvent in the test tube. After 30 minute

incubation of reaction mixture at room temperature stable yellow colour was developed. Absorbance was measured at 517 nm. Quercetin was used as the standard flavonoids. The amount of flavonoids was calculated by using the standard curve of quercetin having R^2 value ranged from 0.96-0.99 and was expressed as mg Quercetin Equivalents/100000 ppm crude seed extract.

The antioxidant activity of crude seed extract was evaluated on the basis of its activity in scavenging the stable DPPH (1, 1-Diphenyl-2-picrylhydrazin) radical using the method described by Shimada (18). Crude seed extract was diluted in methanol to give at least 5 different concentrations. An aliquot (1, 1.5, 2, 2.5 ml) of the extract of each concentration was mixed with 1 ml of 1M DPPH solution. The mixture was then homogenized and left to stand for 30 min in the dark. The absorbance was measured at 517 nm against a blank of methanol using a spectrophotometer. DPPH solution plus methanol was used as control and Butyl hydroxyl toluene (BHT) was used as a standard reference synthetic antioxidant with R^2 value ranged from 0.95- 0.99. Results were expressed as mg Butyl hydroxyl toluene (BHT) Equivalent/100000 ppm crude seed extract

The percent scavenging effect was calculated as follows:

$$\text{Scavenging effect}\,(\%) = \frac{A_{517}\ \text{of Control} - A_{517}\ \text{of Extract}}{A_{517}\ \text{of Control}} \times 100$$

Preparation of seed extracts

Cryo grounded seed powder (1 gm) of both the genotypes of cumin was extracted with 10 ml methanol, after filtering the powder was again left in 10 ml methanol for further extraction. Both the supernatant of methanol were pooled together. Crude methanol seed extracted was then obtained by evaporating methanol at room temperature. Extraction in distilled water was done manually in a mortar and pestle. The crude extracts were used for determination of the total phenol, flavonoids concentration and antioxidant activities of seeds.

Results and discussion

Table 1 showed essential oil and oleoresin percentage of cryo ground, non cryo ground and intact seeds of cumin genotypes GC 4 and RZ 209. Essential oil percentage in intact seeds of genotype GC 4 was more (3.99%) as compare to RZ 209 (3.32%). Non cryo grinding causes 18.22% loss of volatile oil in genotype GC 4 and 19.03% in RZ 209. Interestingly, cryogenic grinding not only retains the volatiles in both the genotypes but enhanced the recovery also. The similar results regarding recovery of more essential oil from cryo ground seeds than intact seeds were reported by Saxena *et al.* (15), in coriander. This may be due to the fact that oil bodies in intact seeds became free during grinding process and extraction of oil from oil bodies became easy from powdered samples than intact seeds. Oleoresin percentage was also higher in GC 4 (15.056%) as compared to RZ 209 (11.620%) in non cryo ground samples. A significant increase (28.287%) in oleoresin percentage was observed when seeds of RZ 209 were ground using cryogenic grinding. This increase was, however less (16.046%) in genotype GC 4. Oleoresin include non volatile oil fraction of total oil content. In normal grinding process of cumin, due to high temperature fat is melted and stick on the grinding surfaces. The extremely low temperature in cryogenic grinding solidifies oils so that the spices become brittle, crumble easily permitting grinding to a finer and more consistent size with minimum or no loss of oil during grinding process. Genotype RZ 209 responded well to cryogenic grinding as compare to GC 4. It is well documented that genetic constitution and environmental condition influence the yield and composition of volatile oil produced by medicinal plants (Ramezani *et al.*, 10; Omidbaigi, 7).

Total phenolic and flavonoid content in the extract from both types of samples of each genotype are presented in Table 2. Total phenolic content in non cryogenically ground seeds was ranging from 91.36 mg GAE/100000 ppm extract in GC 4 to 99.58 mg GAE/100000 ppm extract genotype RZ 209. In cryogenically ground seeds TPC was significantly higher in both the genotypes. It was ranging from 110.76 mg GAE/100000 ppm extract in RZ 209 to 113 mg GAE/100000 ppm extract in GC 4. Effect of cryogenic grinding is clearly visible in total phenolic content.

Total flavonoids content in methanol crude seed extract in non cryo ground seeds of GC 4 was 30.30 and in RZ 209 27.30 mg QE/100000 ppm extract. Cryogenic grinding increased the recovery of TFC in both the genotypes being observed 49.05 and 39.60 mg QE/100000 ppm extract in GC 4 and RZ 209 respectively.

Methanol crude seed extract of both the genotypes were evaluated for its antioxidant activity in terms of total antioxidant content and DPPH free radical scavenging % (Table 2). Antioxidant content was at par (7.46 and 6.72 mg BHT E/100000 ppm extract in GC 4 and RZ 209 respectively) in both genotypes when ground non cryogenic grinding technology while increased markedly by 11.78 and 8.9 mg BHT E/100000 ppm extract in GC 4 and RZ 209 respectively. Similarly DPPH free radical scavenging percentage was also at par (19.32 and 18.90% in GC 4 and RZ 209) but increased when seeds were ground cryogenic grinding technology up to 25.63 and 23.73% in GC 4 and RZ 209 respectively. The higher concentration of antioxidant content and DPPH scavenging % suggested high antioxidant activity in cryo ground samples.

It has been recognized that polyphenols and flavonoids

showed antioxidant activity and their effects on human health are well documented (Chu *et al*., 4). Several studies (Shan *et al.,* 17; Wu *et al.,* 24 and Wong *et al.,* 23) conducted on spice and herbs reported that phenolic compounds significantly contributed to their antioxidant properties. In present study we observed a positive correlation between total phenolic and flavonoid content and antioxidant.

Conclusion

From present study it could be concluded that cryogenic grinding technology is superior to non cryogenic grinding for retention of flavour and antioxidant properties of cumin irrespective of the genotype. Further, studies are needed for the isolation and identification of the active compound in the crude seed extract responsible for antioxidant activity.

Table1. Essential oil and oleoresin percentage of cumin genotypes

Variety	Essential oil (%)				Oleoresin (%)		
	Cryo ground	Non cryo ground	Intact seed	% Increase in cryo over non cryo grinding	Cryo ground	Non cryo ground	% Increase in cryo over non cryo grinding
GC 4	4.303	3.263	3.99	31.872	17.472	15.056	16.046
RZ 209	3.706	2.678	3.32	38.386	14.907	11.620	28.287

Table2. TPC, TFC and Antioxidant activity of cumin genotypes

Variety	TPC (mg GAE/100000 ppm extract)		TFC (mg/QE/100000 ppm extract)		Antioxident Content (mg BHT E/100000 ppm extract)		Scavenging percentage (%)	
	Cryo Ground	Non cryo Ground	Cryo Ground	Non cryo Ground	Cryo Ground	Non cryo Ground	Cryo Ground	Non cryo Ground
GC 4	113.94	91.36	49.05	30.30	11.78	7.46	25.63	19.32
RZ 209	110.76	99.58	39.60	27.30	8.90	6.72	23.73	18.90

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