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Effect of cryogenic grinding on oil yield, phenolics and antioxidant properties of ajwain (*Trachyspermum ammi* L.)

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Abstract

Effect of cryogenic grinding on volatile oil, oleoresin content, total phenolics, flavonoid content and antioxidant properties of seed extract of two ajwain (*Trachyspermum ammi* L.) genotypes have been analyzed. Both the genotype showed marked increase in essential oil and oleoresin recovery in cryo ground seeds. Effect of cryo grinding was more in genotype AA 2 as compared to AA 93. Among different solvents hexane and Dimethyl sulfoxide (DMSO) proved better for extraction of phenolic and flavonoid contents from ground seeds. The higher concentration of antioxidant content and DPPH scavenging % suggested high antioxidant activity in cryo ground samples. Total Phenolic Content (TPC) was maximum (109.75 mg GAE ml^{-1} crude seed extract) in methanol crude seed extract of cryo ground samples of genotype AA-2, Whereas in genotype AA 93 maximum TPC (168.00 mg GAE ml^{-1} crude seed extract) was found in DMSO seed extract. Seed extract of both genotypes showed significant increase over non-cryo ground sample. Maximum TFC (797.17 mg QE ml^{-1} crude seed extract) was observed in hexane crude seed extract of AA 2. Total antioxidant content was also more in seed extract of cryo ground samples. However, no significant genotypic variation was observed. Both AA 2 and AA 93 genotype showed maximum antioxidant content (151.61 mg BHT E ml^{-1} crude seed extract) and (145.38 mg BHT E ml^{-1} crude seed extract) in methanol seed extract followed by hexene and DMSO.

Introduction

Spices have been known for ages as effective therapeutic 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 production of 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, flavor compounds, dyes, and agrochemicals. Even today, these metabolites are a major source of new drugs.

Ajwain (*Trachyspermum ammi* L.) is a small, erect, annual, herbaceous plant with branched leafy stems, feather like leaves (2.5 cm long), and 4 - 12 ray flower heads bearing 6 - 16 flowers. Ajwain is a traditional potential herb and is widely used for curing various diseases in humans and animals. (Rathore *et al.,* 8).

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 usually is detrimental to the product and results in significant 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 & Misra, 7), which 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*., 13), 37% for nutmeg, 14% for mace, 17% for cinnamon and 17% for oregano (Andres, 2). 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 & Pahl, 17). The loss of volatile oil can be significantly 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

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generated during the grinding operation. 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. In the above studies, attempts were made to prove that cryogenic grinding of ajwain 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.

Materials and methods

Seeds of two varieties of Ajwain (AA-2 and AA-93) obtained from seed store of ICAR-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

Cryogenic grinding of seeds was done using cryogenic grinder (Spectra Cryogenics, Rajasthan, India) at NRCSS, Ajmer. Feed rate of material was set at 1 kg hr^{-1} with screw speed 3 rpm. Inlet temperature was adjusted to below -30°C and outlet temperature was -5 to 15°C. Product particle size was set on 50 microns. The cryo ground powder was quickly packed in aluminum foil packets using sealing machine and opened at the time of analysis. Non cryogenic grinding was done separately by domestic mixer grinder (Sujata, model Dynamix, 810 W).

Estimation of oleoresin content

Total oil content was extracted using Accelerated Solvent Extraction System (Dionex India Pvt. Ltd.). 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.

Estimation of essential oil content

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

Preparation of seed extracts

Cryogenic and non cryogenically ground seeds (10 gm) of Ajwain was extracted with 100 ml respective solvents (Hexene, Dimethyl sulphoixe, Distilled water and methanol) twice. Supernatant from both extraction were pooled. This supernatent was used for determination of the total phenol and flavonoids concentration, as well as antioxidant activities.

Estimation of total phenols

Total phenol concentrations were determined using a Folin-Ciocalteu assay, as described by Amin *et al.,* (1). An aliquot of 0.1ml from 100000 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.

Estimation of total flavonoids

Total flavonoid concentration was determined using previously reported method by Chang *et al.* (4). One ml of crude seed extract (100000 ppm) 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 flavonoid 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.

Estimation of antioxidant properties

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 (15). 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:

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

Results and discussion

Table 1 showed essential oil and oleoresin percentage of cryo ground and non cryo ground of ajwain genotypes AA 2 and AA 93. Both the genotype showed marked increase in essential oil and oleoresin recovery in cryo ground seeds. Effect of cryo grinding was more in genotype AA 2 as compared to AA 93. 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.,* 13, Saxena *et al.,* 12, Wiestrieich and Schafer, 16; Andres, 1; Russo, 10; Rice, 9; Pesek *et al*. 6; Landwehr and Pahl, 5; Wolf and Pahl, 17). Cryogenic grinding of spices is able to retain more essential and total oil content has been established by studies conducted by Saxena *et. al*., (13) in coriander, Sharma *et. al.,* (14) for cumin, Barnwal *et. al.,* (3) for turmeric, Saxena *et. al.,* (11) for fenugreek. Those spices which have significant proportion of more volatile constituents in their essential oil showed more retention of essential oil.

Table 2 showed total phenolic, flavonoid and antioxidant content in crude seed extract of ajwain genotypes. Total Phenolic Content (TPC) was invariably more in seed extracts of cryo ground samples of both the genotypes. TPC was maximum (109.75 mg GAE ml^{-1} crude seed extract) in methanol crude seed extract of cryo ground samples of genotype AA-2, closely followed by DMSO seed extract (102.89 mg GAE ml^{-1} crude seed extract). Whereas in genotype AA 93 maximum TPC (168.00 mg GAE ml^{-1} crude seed extract) was found in DMSO seed extract followed by methanol seed extract (116.33 mg GAE ml^{-1} crude seed extract). Distilled water seed extract showed minimum Phenolic content being observed 29.80 mg GAE ml^{-1} crude seed extract in AA 2 and 57.92 mg GAE ml^{-1} crude seed extract in AA 93 genotype. Effect of cryo grinding was clearly visible on total flavonoid content in seed extract of ajwain. Seed extract of both genotypes showed significant increase over non-cryo ground sample. Maximum TFC (797.17 mg QE ml^{-1} crude seed extract) was observed in hexane crude seed extract of AA-2 followed by Methanol (589.80 mg QE ml^{-1} crude seed extract), DMSO (304.49 mg QE ml^{-1} crude seed extract) and distilled water (74.27 mg QE ml^{-1} crude seed extract). Similar results were obtained in genotype AA-93 but hexene seed extract was followed by DMSO (737.10 mg QE ml^{-1} crude seed extract), methanol (523.93 mg QE ml^{-1} crude seed extract) and distilled water (75.448 mg QE ml^{-1} crude seed extract). Total antioxidant content was also more in seed extract of cryo ground samples. However, no significant genotypic variation was observed. Both AA 2 and AA 93 genotype showed maximum antioxidant content (151.61mg BHT E ml^{-1} crude seed extract) and (145.38 mg BHT E ml^{-1} crude seed extract) in methanol seed extract followed by hexene and DMSO. Similar results were obtained by Saxena *et. al.,* (13) during the study of the effect of cryogenic grinding on volatile oil, oleoresin

Table 1. Essential oil and oleoresin percentage of cumin genotypes

Variety	Essential oil %			Oleoresin %		
	Cryo ground	Non cryo ground	% Increase in cryo grinding	Cryo ground	Non cryo ground	% Increase in cryo grinding
AA 2	4.845	4.199	15.38	17.29	14.666	17.939
AA 93	4.983	4.485	11.103	23.24	19.87	16.96

Table 2. Total phenolic, flavonoid and antioxidant content in crude seed extract of ajwain genotypes

solvent	Total Phenolic Contents (mg GAE ml^{-1} crude seed extract)				Total Flavonoid Contents (mg QE ml^{-1} crude seed extract)				Total Antioxidant Content (mg BHT E ml^{-1} crude seed extract)			
	AA-2		AA-93		AA-2		AA-93		AA-2		AA-93	
	Cryo	N Cryo	Cryo	N Cryo	Cryo	N Cryo	Cryo	N Cryo	Cryo	N Cryo	Cryo	N Cryo
Hexane	62.92	53.64	101.68	67.60	797.17	536.57	783.33	591.93	134.90	127.85	136.55	126.42
DMSO	102.89	69.78	168.00	93.05	304.39	232.10	737.10	283.25	107.38	77.33	103.7	83.03
DW	29.80	26.37	57.920	46.73	74.27	59.69	75.448	59.40	104.03	62.55	106.63	68.46
methanol	109.75	64.32	116.33	75.51	589.80	358.57	523.03	343.02	151.61	145.38	145.38	133.36

content and anti-oxidant properties of coriander (*Coriandrum sativum* L.) genotypes. In another seed spice, fenugreek Saxena *et. al.,* (11) found more recovery of diosgenin content in fenugreek (*Trigonella foenum- graecum* L) genotypes when ground cryogenic grinding technology.

Cryogenic grinding technique was superior to ambient grinding in terms of retention of volatile oil in seed powder. In present study cryogenic grinding technique has been adopted which successfully proves that cryo-ground seed powder of ajwain provides more flavour and medicinal properties as compare to conventionally ground powder.

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