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Effect of cryogenic grinding on recovery of diosgenin content in fenugreek (*Trigonella foenum- graecum* L.) genotypes

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ABSTRACT

Fenugreek produces diosgenin as a steroidal sapogenin, belonging to triterpene group and has great significance for pharmaceutical industry due to its oestrogenic effect on the mammary gland. Diosgenin is generally used as starting material for partial synthesis of oral contraceptives, sex harmones and other steroids. To date, diosgenin and related steroidal saponins were commercially obtained from the various Dioscorea species, however, it is crucial to discover new and alternate source of these compounds due to decreasing plant resources as well as increasing demand. Fenugreek is a potent source of diosgenin. Fenugreek seeds contain 0.1 to 1.5% diosgenin. There are various studies on analysis of diosgenin content in fenugreek seeds, leaves and other plant parts but no studies have been made on effect of grinding technology on recovery of diosgenin content in different genotypes of fenugreek. Present study was undertaken to analyze the effect of cryogenic grinding on recovery of diosgenin content from fenugreek seeds. Three genotypes of fenugreek namely AM 1, RMT 305 and RMT 1 were taken and seeds were ground to fine powder using conventional grinder and cryogenic grinder. Total sapogenin percentage was ranging from minimum of 9.35% in genotype RMT 1 to a maximum of 10.78 % in genotype RMT 305 in cryo ground seeds while in non cryo ground seeds showed minimum 6.61% in RMT 1 to a maximum of 8.10% in AM 1. Diosgenin percentage was significantly more in all three genotypes. In non cryo seeds it was ranging from 1.3 to 1.5% while increased significantly in cryo ground samples and ranging from 2.1 to 2.5%. Cryogenic grinding technology was found superior in recovery of more diosgenin content from fenugreek seeds.

Key words : Cryogenic grinding, diosgenin, fenugreek and sapogenin

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum)* is an annual herb from leguminosae family. This native crop is extending from Iran to northern India (Petropoulos, 15). Fenugreek leaves and seeds have been used extensively for preparing extracts and powders in medicinal performance (Basch et al., 3); Nithya and Rmchandramurthy, 13), in some region of Asia, the young plant are performed as potherbs and the seeds for herbal medicine usages. The Species name "*foenum-graecum"* means "Greek hay" indicating its use as a forage crop in the past (Petropoulos, 15). Fenugreek is a rich source of steroidal sepogenine. Previous studies reported the anti-diabetic (Broca et al., 6), Srinivasan and Karun devi 19); Khan and Anderson, 9), Anti-bacterial (Bonjar, 5), Antioxidant (Semalty et al., 18) hypocholesterolaemic (Suboh et al., 20) anti-cancer (Devasena and Menon, 7), thyroxin induced hyperglycaemia (Tahiliani and kar, 21) properties of fenugreek seeds. Fenugreek plays an important role in the control of cholesterol metabolism (Bahram et al., 2).

Fenugreek produces diosgenin as a steroidal sapogenin, belonging to triterpene group and has great significance for pharmaceutical industry due to its oestrogenic effect on the mammary gland (Oncina *et al*., 14). It also plays an important role in the control of cholesterol metabolism, variation in the lipoxygenase activity of human ethyroleukemia cells and is responsible for morphological and biochemical changes in megakaryocyte cells (Beneytout *et al*.; 4 Nappez *et al*., 12). Furtehermore, diosgenine was found to be the most effective cell death inducer. Diosgenin is generally used as starting material for partial synthesis of oral contraceptives, sex harmones and other steroids (Zenk, 24). The partial synthesis of steroids from plant-based precursors has been a boon because of the increasing demand for corticosteroids, contraceptives, sex hormones and anabolic steroids since about 1980 (Hall and Walker, 8). To date, diosgenin and related steroidal saponins were commercially obtained from the various Dioscorea species; however, it is crucial to discover new and alternate source of these compounds due to decreasing plant resources as well as increasing demand (Savikin-Foduloic *et al*., 17). Fenugreek is a potent source of diosgenin. There are

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various studies on analysis of diosgenin content in fenugreek seeds, leaves and other plant parts but no studies have been made on effect of grinding technology on recovery of diosgenin content in different genotypes of fenugreek. Present study was undertaken to analyze the effect of cryogenic grinding on recovery of diosgenin content from fenugreek seeds.

MATERIAL AND METHODS

Grinding of seeds:

Three varieties of fenugreek namely AM 1, RMT-1 and RMT-305 was obtained from seed store of NRCSS, Ajmer. The seeds were cleaned and used for grinding. Cryogenic grinding of seeds was done using cryogenic grinder (Hoso-Kava Alpine, Germany) model Fine Impact Mill 100UPZ at Central Institute for Post Harvest Engineering and Technology, Ludhiana. Feed rate of material was set at 1 kg/hr with screw speed 3 rpm. The speed of pin mill was set at 10,000 rpm. Inlet temperature was adjusted to below -50 and outlet temperature was -5 to 15ºC. Product particle size was set on 50 microns. 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. Computer controls the entire process. The Cryo ground powder was quickly packed in aluminum foil packets using sealing machine and opened at the time of analysis. For obtaining seed powder through conventional grinding dried seeds (30 gm) was ground separately by domestic mixer grinder (Sujata, model Dynamix, 810 W) and packed in sealed polythene bags.

Oil Extraction (De fatting):

Total oil from seed powder was extracted using Accelerated Solvent Extraction System (Dionex India 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. Oleoresin was obtained after evaporating the solvent in rotary evaporator. Thirty gram seed powder was utilized for extraction of oil and hexane was used as solvent.

Sapogenin extraction:

The defatted cake (residue after oil extraction) was kept under the hood overnight. The next day 100 ml methanol was added to the cake and the mixture was shacked overnight on a rotary shaker at 50 rpm. This mixture was centrifuged at 4000 rpm for 5 minutes. The centrifugation process was repeated three times and supernatant were pooled. Methanol was evaporated in a hood to obtained yellow crystal powder of crude sapogenins.

Diosgenin Determination:

Diosgenin was determined as per the methods described by Baccau et al. (1977 (1) and Uematsu et al. (2000) (22), with some modification. Standard diosgenin and p-Anisaldehyde were procured from sigma-aldrich, USA. All other chemicals were of analytical grade. The diosgenin level was determined by measuring absorbance at 430 nm, based on the colour reaction with anisaldehyde, sulfuric acid and ethyl acetate. Briefly, two colour developing reagent solutions were prepared: (A) 0.5 ml p-Anisaldehyde (99%) and 99.5 ml ethyl acetate and (B) 50 ml concentrated sulfuric acid and 50 ml ethyl acetate. A 1000 ppm solution of crude saponins was prepared in methanol. 100 ìl of this solution was placed in another glass tube. The methanol was evaporated under reduced pressure. This residue was dissolved in 2 ml of ethyl acetate; 1 ml each of reagents A and B were added to the tube and stirred. The test tube was placed in a water bath maintained at 60^0C for 10 minutes to develop colour and then allowed to cool down for 10 min. in 25^0C. The absorbance of the developed colour was measured with a spectrophotometer (Labindia) at 430 nm. Ethyl acetate was used as a control for the measurement of absorbance. As a reagent blank, 2 ml ethyl acetate was placed in a tube and assayed in similar manner. For the calibration curve, 10-80 ppm standard diosgenin in 2 ml ethyl acetate was used (Figure 2). Each sample was repeated thrice and the average was taken. The amount of diosgenin was calculated by using the standard curve of diosgenin prepared with methanol having R^2 value ranged from 0.96-0.99.

RESULTS AND DISCUSSION

In the normal grinding process, heat is generated when energy is used to fracture a particle into a smaller size, that generate heat causes temperature rise in the grinder to the extent of 95ºC which is responsible for a

loss of volatile oil in the tune of about 30% and also produces dark coloured powder. Many important constituents may be deformed and their quality may be affected badly.

The quality of ground powder may be maintained by cryogenic grinding technique using liquid nitrogen that provides the refrigeration needed to pre-cool the spices and maintain the desired low temperature by absorbing the heat 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. (Landwehr and Pahl 10); Wolf and Pahl 23); Li et al. 11).

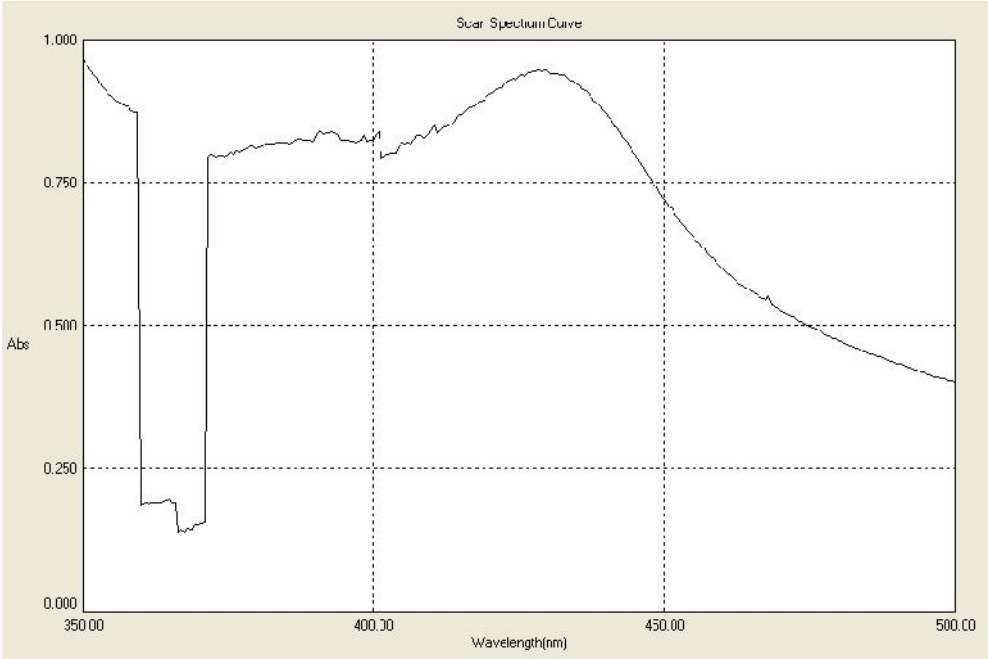

Figure 1: Absorption spectrum of diosgenin

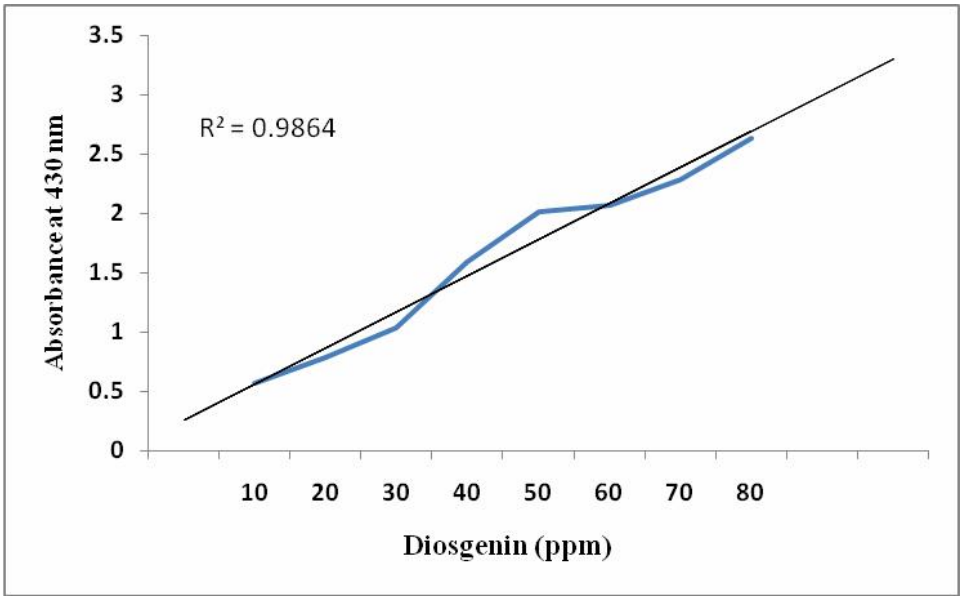

Figure 2: Calibration curve for the determination of diosgenin.

Table 1. Effect of cryogenic grinding on total sapogenin content (%), diosgenin content (%) and Oil % in three genotypes of fenugreek

Genotype	Total sapogenin (%)		Total diosgenin %	
	Cryo	Non-Cryo	Cryo	Non-Cryo
AM-1	10.023	8.103	2.113	1.393
RMT-305	10.780	7.683	2.317	1.327
RMT-1	9.350	6.610	2.557	1.577
	SEm±	CD at 5%	SEm±	CD at 5%
Grinding Technology (A)	0.075	0.226	0.025	0.075
Variety (B)	0.091	0.277	0.014	0.043
AxB	0.129	0.391	0.017	0.053
CV	2.968		2.672	

Table 1 showed the genotypic variation and the effect of grinding techniques on total sapogenin content (%) and diosgenin content (%) in fenugreek. Effect of genotype and grinding technology was found significant in both the parameters studied. Total sapogenin percentage was ranging from minimum of 9.35% in genotype RMT 1 to a maximum of 10.78 % in genotype RMT 305 in cryo ground seeds while in non cryo ground seeds showed minimum 6.61% in RMT 1 to a maximum of 8.103% in AM 1. Diosgenin percentage was significantly more in all three genotypes. In non cryo seeds it was ranging from 1.3 to 1.5% while increased significantly in cryo ground samples and ranging from 2.1 to 2.5%.

In a previous study Saxena *et al.* (16) estimated antioxidant activity, phenolic and flavonoid content of cryo and conventionally ground seeds of coriander (*Coriandrum sativum L.) and fenugreek (Trigonella foenum-graecum L.*). Oleoresin content was significantly high in cryogenically ground samples. Total phenolic and flavonoids content was also high in both the genotypes of coriander and fenugreek. Methanol crude seed extract of all genotypes were evaluated for its antioxidant activity in terms of total antioxidant content and DPPH free radical scavenging %. DPPH scavenging % was invariably more in cryo ground seeds in all the genotypes. Higher concentration of antioxidant content and DPPH scavenging % suggested high antioxidant activity in cryo grinded samples. It could be concluded that cryogenic grinding technology is able to retain flavour and medicinal properties of coriander and fenugreek irrespective of the genotype and can be used to recover higher amount of diosgenin from fenugreek for commercial use.

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