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Effect of cryogenic grinding on phenolic compounds and antioxidant properties of fenugreek (*Trigonella foenum-graecum* L.) seed extract

S N Saxena*, P Saxena, S S Rathore, L K Sharma, R Saxena & P Barnwal[1]

ICAR-National Research Centre on Seed Spices
Tabiji, Ajmer-305 206, Rajasthan.
**E-mail: shail.nrcss@gmail.com*

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Abstract

Effect of cryogenic grinding on oil content, total phenolics, flavonoid content and anti-oxidant properties of seed extract of three fenugreek (*Trigonella foenum-graecum*) genotypes AM 1, RMt 1 and RMt 305 have been analyzed. Oil content was 37.36% more in cryogenically ground sample of genotype AM 1. Yield of methanol crude seed extract was 76.75% more in cryo ground sample of genotype AM 1. Total phenolic content (TPC) was also high in cryo ground samples of all three genotypes. It ranged from a minimum of 75.72 mg in RMt 1 to a maximum of 94.03 mg gallic acid equivalent (GAE) g^{-1} crude seed extract in genotype AM 1. Similarly total flavanoid content (TFC) was also increased in all cryogenically ground samples and ranged from 17.75 mg in RMt 305 to 26.37mg quercetin equivalents g^{-1} crude seed extract in genotype AM 1. Methanol crude seed extract of all genotypes were evaluated for its antioxidant activity in terms of total antioxidant content (AOC), 1, 1-Diphenyl-2-picrylhydrazin (DPPH) free radical scavenging % and EC_{50} value. The amount of total antioxidant content in cryo ground seeds was significantly high in all genotypes ranging from 9.32 mg in genotype RMt 1 to 11.08 mg butyl hydroxyl toluene (BHT) Equivalent g^{-1} crude seed extract in RMt 305. DPPH scavenging % was invariably more in cryo ground seeds in all three genotypes. Higher concentration of antioxidant content and DPPH scavenging % suggested high antioxidant activity in cryo ground samples.

Keywords: antioxidant, cryogenic grinding, fenugreek, flavonoid, oil, phenolic content

The grinding of spices is done to obtain smaller particle size with good product quality in terms of flavour and aroma. In the normal grinding process, temperature rises in the grinder to the extent of 95°C which is responsible for a loss of volatile oil to the tune of about 30% and produces dark coloured powder which is of inferior quality. The loss of volatile can be significantly reduced 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. This advantage of cryogenic grinding has more significance in case of seed spices since these crops are known

[1]Dairy Engineering Division, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana.

for their flavour and medicinal properties. A laboratory model cryogenic grinding system was developed and some basic studies on the grinding characteristics of cumin and clove under cryogenic and ambient conditions have been conducted and reported by Singh & Goswami (1999a), (Singh & Goswami 1999b; Singh & Goswami 2000). Effect of cryogenic grinding on sapogenins and diosgenin content in cryo ground fenugreek seeds have also been reported by Saxena *et al.* (2013). The present investigation was carried out at Basic Science Unit at ICAR-National Research Centre on Seed Spices (NRCSS), Ajmer to find out the effect of cryogenic grinding on phenolic, flavonoid compounds and antioxidant properties of fenugreek (*Trigonella foenum-graecum*) seed extract.

In present investigation comparative analysis of cryogenic and non cryogenically ground fenugreek seeds of three genotypes have been made in terms of difference in oil content and its Fatty Acids Methyl Esters, total phenolic and flavonoid content and its antioxidant properties in crude seed extract of each genotype. The aim of this work is to find out genetic variation in retention of medicinally important compounds and antioxidant properties as affected by cryogenic grinding technology.

Seeds of three genotypes of fenugreek namely AM 1, RMt 1 and RMt 305 were obtained from seed store of ICAR-NRCSS, Ajmer. Cryogenic grinding of seeds was done using cryogenic grinder (Spectra Cryogenics, Kota, Rajasthan). In the process of cryogenic grinding the material is fed into a feeder hopper and dropped into a pre-chilled conveyor. Liquid nitrogen is sprayed and blended directly onto the material. Liquid nitrogen is added until the temperature of the material is reduced to a predetermined set point. The brittle material enters an impact (pin) mill where it is ground to a desired particle size. 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). Ground powder was used for oil extraction and preparation of seed extracts.

Oil content from 25 g of ground seeds was extracted using Accelerated Solvent Extraction System (Dionex India Pvt. Ltd.) using hexane as solvent. Methanol crude seed extract of cryo and non cryogenically ground seeds (10 g) of fenugreek was extracted with 50 mL methanol twice. Supernatant from both extraction were pooled and methanol was evaporated in rotary evaporator. This crude methanol seed extract was used for determination of total phenolic and flavonoid content and antioxidant activity.

Total phenol concentration was determined using a Folin-Ciocalteu assay, as described by Amin *et al.* (2006). The amount of phenolic content was calculated by using the standard curve of gallic acid having R^2 value ranged from 0.96-0.99 and was expressed as mg Gallic Acid Equivalents (GAE) g^{-1} crude seed extract.

Total flavonoid concentration was determined by using previously reported method by Chang *et al.* (2002). 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 (QE) g^{-1} crude seed extract.

The antioxidant activity of crude seed extract was evaluated on the basis of its activity in scavenging the stable DPPH radical using the method described by Shimada (1992). Results were expressed as mg Butyl hydroxyl toluene (BHT) Equivalent/g crude seed extract. The EC_{50} value for each sample defined as the concentration of the test sample leading to 50% reduction of the DPPH concentration was calculated from the non linear regression curve of the test extract. Experimental data were analyzed using Microsoft Excel (Microsoft Inc.). Each observation was replicated three times and data were analyzed using Randomized Block Design. Significance of differences between samples was analyzed using analysis of variance (ANOVA). Pearson's correlation was used to determine the correlation of data between antioxidant content and total phenolic and flavonoids content.

Table 1 showed the oil content and total yield of methanol crude seed extract of cryogenically and non cryogenically ground seeds of three genotypes of fenugreek. Total oil content was minimum (3.94%) in genotype AM 1 and maximum (4.8%) in genotype RMt 305, when seeds were ground by normal grinding. The same seeds when ground using cryogenic grinding technology, a significant increase was observed in all three genotypes. This increase was maximum (59.6%) in AM 1 followed by RMt 305 (24.0%) and RMt 1 (22.4%). In normal ground seeds the yield of crude seed extract was ranging from a minimum of 0.59 g in RMt 1 to a maximum 0.78 g in RMt 305 genotype. There is enough variation in yield of extracts in three genotypes. Yield was invariably high in cryo ground samples of all three genotypes. Total yield of methanol seed extract in cryo ground samples was ranging from a minimum of 0.93 g in AM 1 to the maximum of 1.08 g in RMt 305. Maximum increase in yield of crude extract was observed in genotype AM 1 (76.3%) followed by RMt 1 (38.8%) and RMt 305 (38.5%). It is well documented that genetic constitution and environmental condition influence the yield and composition of oil produced by medicinal plants (Ramezani *et al.* 2009; Omidbaigi 2007). The reason of obtaining higher oil content in cryogenically ground seed is that in normal grinding process of fenugreek, 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. Higher quantities of methanol crude seed extract in cryogenically ground seeds may be the effect of temperature on constituents of fenugreek during grinding.

Different organic solvents have different polarity and therefore have different nature to extract the compounds. Methanol and Ethanol are best known solvents for non fatty compounds while Hexane and Dichloromethan are used to extract lipids and oils from plant

Table 1. Effect of cryo grinding on recovery of oleoresin in fenugreek genotypes

Genotypes	Oil content (%)			Crude methanol seed extract (g)		
	Cryo	Normal	% increase	Cryo	Normal	% increase
AM 1	6.29	3.94	59.6	1.04	0.59	76.3
RMt 1	5.63	4.6	22.4	1.08	0.78	38.5
RMt 305	5.95	4.8	24	0.93	0.67	38.8
Mean	5.95	4.44	34	1.02	0.68	50
SD (±)	0.33	0.45		0.07	0.09	

Table 2. Total phenolic and flavonoid content in methanol crude extract of normal and cryogenically ground seeds of fenugreek genotypes

Genotype	Total phenolic content (mg GAE g^{-1} seed extract)			Total flavonoid content (mg QE g^{-1} seed extract)		
	Cryo	Normal	% increase	Cryo	Normal	% increase
AM 1	94.03	72.99	28.8	26.37	16.44	60.4
RMt 1	75.72	67.37	12.4	18.89	13.63	38.6
RMt 305	83.54	71.02	17.6	17.75	12.56	41.3
Mean	84.43	70.46	19.8	21.00	14.21	47.8
SEm (±)	0.07	0.08		0.01	0.02	
CD (P<0.05)	0.30	0.34		0.04	0.07	
CV (%)	0.16	0.21		18.89	13.63	

Table 3. Antioxidant content, DPPH scavenging % and EC_{50} value in methanol crude extract of normal and cryogenically ground seeds of fenugreek genotypes

Genotypes	Antioxidant content (mg BHT E g^{-1} crude seed extract)			DPPH Scavenging %			EC_{50}		
	Cryo	Normal	% increase	Cryo	Normal	% increase	Cryo	Normal	% increase
RMt 1	9.32	3.29	183.3	74.86	27.22	175	6.229	6.051	2.9
RMt 305	11.08	8.95	23.8	88.70	71.92	23.3	6.250	6.223	0.4
AM 1	10.00	8.43	18.6	80.20	67.82	18.3	6.238	6.215	0.4
Mean	10.14	6.89	47.2	81.25	55.65	46	6.23865	6.163	1.2
SEm (±)	0.006	0.02		0.04	0.08		0.00001	0.031	
CD (P<0.05)	0.02	0.08		0.17	0.35		0.00004	0.123	
CV (%)	0.09	0.56		0.09	0.27		0.00030	0.879	

samples. Tangkanakul *et al.* (2009); Souri *et al.* (2007); Parichat & Artiwan (2007) used methanol extract for total phenol content measurement in fenugreek and other plant species while Kaur & Kapoor (2002) used ethanol extract for measurement of antioxidant activity and total phenol content of some Asian vegetables. In present study methanol has been used as extraction solvent.

The data on total phenolic and flavonoid content (TPC and TFC) in methanol crude extract of three fenugreek genotypes is presented in Table 2. All three genotypes differ significantly in TPC when ground cryogenically or by normal grinding. TPC in normal ground seeds ranged from a minimum of 67.37 mg GAE g^{-1} crude extract in RMt 1 genotype to a maximum of 72.99 mg in AM 1. In cryogenically ground samples, TPC was significantly higher in all three genotype. It ranged from a minimum of 75.72 mg in RMt 1 to a maximum of 94.03 mg GAE g^{-1} in genotype AM 1 followed by genotype RMt 305 (83.544 mg). Maximum increase in TPC was observed in genotype AM 1 (28.8%) followed by RMt 305 (17.6%) and RMt 1 (12.4%).

There is significant genotypic variation in TFC in cryo and normal ground seeds. TFC in normal ground seeds was minimum 12.56 mg in genotype RMt 305 and maximum 16.44 mg QE g^{-1} in AM 1 genotype. All cryogenically ground samples showed significant increase in TFC in all three genotypes, which was minimum (17.75 mg) in RMt 305 and maximum 26.37 mg QE g^{-1} in AM 1 genotype. Maximum increase in TFC was observed in genotype AM 1 (60.4%) followed by RMt 305 (41.3%) and RMt 1 (38.6%).

Methanol crude seed extract of all genotypes were evaluated for its antioxidant activity in terms of total antioxidant content, DPPH free radical scavenging percentage and EC_{50} value. The data are presented in Table 3. All genotypes significantly differed in antioxidant content whether ground normally or by cryogenic grinding technology. Total antioxidant content in normal ground seeds was minimum (3.295 mg) in genotype RMt 1 and maximum (8.952 mg) in RMt 305, closely followed by AM 1 (8.431 mg gm^{-1} BHT E g^{-1} crude seed extract). The amount of total antioxidant content in cryo ground seeds was significantly high in all three genotype which was ranging from a minimum of 9.32 mg in genotype RMt 1 to a maximum of 11.08 mg in RMt 305 closely followed by AM 1 (10.005 mg gm^{-1} BHT E g^{-1} crude seed extract). Similar trend was also observed in DPPH % scavenging in all three genotypes. However, EC_{50} value showed very less variation between genotypes. Both cryo ground and normal ground samples showed at par value of EC_{50} ranging from 6.0 to 6.25 (Table 3). EC_{50} value showed the required concentration of BHT equivalent compounds to scavenge 50% of DPPH free radicals and estimated on the basis

of total antioxidant content in one gram extract and its ability to scavenge DPPH free radicals in the test solution. Significant genotypic variation in total antioxidant content and scavenging percentage indicated the different quantity of seed powder required to get sufficient antioxidant properties. 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.* 2000). Several studies (Shan *et al.* 2005; Wu *et al.* 2006; Wong *et al.* 2006) conducted on spice and herbs reported that phenolic compounds significantly contributed to their antioxidant properties. In present study, a non significant positive correlation between total phenolic content with antioxidant properties was observed. Contrary to above reports no correlation was observed between total flavonoid content and antioxidant properties.

This can be explained on the basis of high anti-oxidant activity of some individual phenolic units, which may act as efficient anti-oxidants rather than contributing to high total phenolics. The scavenging action of various phenolic compounds is closely connected with their spatial conformation. Similar results have been reported by Chu *et al.* (2000) in vegetables like white cabbage and crown daisy, which despite having low phenolic contents had moderate anti-oxidant activity. They attribute this to the presence of some other phytochemicals such as phenolic acid, ascorbic acid, tocopherol and pigments, which also contribute to total anti-oxidant activity.

From these findings, it is concluded that cryogenic grinding of fenugreek resulted in retention of 60% more total oil, 28% more TPC, 60% more TFC and 180% more antioxidant activity of ground powder than normal grinding.

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