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Effect of low temperature grinding on phytochemicals profile of fenugreek seed powder

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Abstract

The grinding of spices at ambient temperature causes the loss of valuable heat-sensitive constituents. While higher investment and maintenance of cryogenic grinding make it prohibitive for small-scale industries. So, the present study was carried out to check the effects of low-temperature grinding on various phytochemicals of ground powder of fenugreek seeds. Water at ambient temperature, chilled water and coolant were circulated around the grinding chamber to lower the grinding temperature. Additionally, fenugreek seeds were fed at ambient and low temperatures (-10^{0}C). The temperature inside the grinding chamber at the end of grinding for three kg of fenugreek seeds ranged from 91.33^{0}C–53.33^{0}C for different treatments. A fall in grinding temperature resulted in the retention of a higher quantity of total phenol (7.71 mg g^{-1}), total flavonoid (12.08 mg quercetin equivalent/g extract), antioxidant activity (21.29%) and volatile components in powder of fenugreek seeds ground by coolant circulation with low-temperature feed.

Keywords: antioxidant activity, fenugreek seed powder, low-temperature grinding, sotolone, total phenol, flavonoid

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant which belongs to the family Leguminosae (Leela & Shafeekh 2008). Fenugreek seeds look golden-yellow in colour, are small in size, hard and have a four-faced stone-like structure (Meghwal & Goswami 2012). The proximate composition of fenugreek seeds shows 8.84% water, 23% protein, 6.41% fat, 3.4% ash and 58.35% carbohydrate (USDA, 2019). They are best known for their pungent aromatic compounds that impart colour and flavour to food (Meghwal & Goswami 2012; Bano *et al*., 2016). Fenugreek seeds are a rich source of widely-diversified and medicinally important phytochemicals. Fenugreek seeds contain different flavonoids (like luteolin, quercetin, apigenin, isovitexin, orientin and vitexin) of more than 100 mg per 100 g of seeds (Zandi*et al*., 2017; Nair *et al*., 1998). Seeds of

fenugreek contain saponins (like graecunins, fenugrin B, fenugreekine) around 4.8% (Yadav *et al*., 2011; Rao *et al*., 1996). Alkaloids, flavonoids and saponins of fenugreek have pharmacological effects. (Meghwal & Goswami 2012). Fenugreek seeds can act as a potent source of antioxidantas as crude extracts of seeds exhibit antioxidant activity with different solvents, which can be correlated with the polyphenolic components present in the seed extract (Bukhari *et al*., 2008). Fenugreek seeds contain volatile oil in small quantity (Shiva & Malhotra 2008). Of which 3-Hydroxy-4, 5-dimethylf uran-2 (5H)-one also known as sotolone (seasoning-like characteristic aroma) is found to occur present predominantly (character impact flavour compound) (Blank *et al*., 1997).

Ground powder of fenugreek seeds is the most usable form. But during the process of conventional grinding, the temperature rises to the extent of 42-95^{0}C, varying with the moisture and oil content of the spices (Pruthi & Mishra, 1963; Singh & Goswami 1999). A hike in temperature to such an extent causes loss of characteristic aroma releasing volatile oil present in spices. (Besides that, most of the spices contain a significant amount of fat which possesses additional problems while grinding.) Higher temperature developed during ambient grinding also results in deterioration of heat-sensitive medicinally and nutritionally important constituents present in spices. The rise in the grinding temperature can be reduced and the quality of ground spices can be retained by a sophisticated method like cryogenic grinding. But higher cost and continuous maintenance are the limiting factors for it to be adopted by small-scale grinding industries. Another way to reduce the grinding temperature is the circulation of cold liquid or air around the grinding chamber. It has been found that low-temperature ground powder shows better sensory attributes and higher retention of flavour and nutrients in ground powder compared to conventional grinding. Theoretically, low-temperature grinding of fenugreek seeds is better than ambient grinding. However, the available literature is scanty to support the above justification. Hence, in the present study, efforts were made to check the effect of low-temperature grinding on important phytochemicals of fenugreek seed powder.

Materials and methods

Treatment details

Dried raw fenugreek seeds of the variety 'Gujarat-2' were used for the present experiment. The seeds were cleaned carefully using different-sized sieves. After cleaning, the whole lot was mixed thoroughly and packed carefully to protect it from environmental effects. The seeds were ground by eight treatment combinations *viz.,* four grinding treatments and two feed temperatures.

Grinding method

Fenugreek seeds were ground by a hammer mill having a capacity of 25-30 kg h^{-1} (Trimurti Brand, Rajkot, Gujarat) with four different grinding methods, *viz*., grinding without liquid circulation (ambient grinding, the temperature range in the jacket: 33 to 35^{0}C) (L_0), grinding with ambient temperature water circulation (temperature range in the jacket: 31 to 32^{0}C) (L_1), grinding with chilled water circulation (temperature range in the jacket: 2 to 3^{0}C) (L_2) and grinding with coolant circulation (propylene glycol, temperature range -9 to -10^{0}C) (L_3) around the grinding chamber through the prepared jacket.

Feed temperature

Fenugreek seeds were fed at two different feed temperatures, *viz*., ambient temperature (T_0) (ranged 33-35^{0}C) and low temperature (T_1= -10^{0}C).

For all the treatment combinations, sample size (3 kg), sieve size (570 μ), and flow rate of liquid (15 lpm) were kept constant. A probe of a

temperature sensor (Sub-Zero, SZ-7569-P) was fixed precisely inside the grinding chamber to measure the temperature during the grinding operation. The temperature inside the grinding chamber at the end of the grinding operation was noted each time. Digital temperature sensors (Quick Sense, XH-W3001) were used to measure the temperature of the liquid in the tank as well as the liquid entering and leaving the grinding chamber jacket continuously. Sensors were calibrated precisely for different temperature ranges. The probe of the sensors was placed in pipes just before entering and just after leaving liquid from the jacket.

Ground powder of fenugreek seeds obtained by various treatments were packed carefully, stored at room temperature at a dry, dark and hygienic place and opened at the time of analysis only. Self-sealing zip-lock transparent plastic bags were used for packing once the powder got cooled to room temperature.

The different treatments were analysed by estimating different biochemical parameters of powder using standard procedures in triplicates.

Preparation of low-temperature feed

Pre-weighted raw cleaned fenugreek seeds were packed in self-sealing zip-lock transparent plastic bags carefully. An ordinary plastic cover was also used as an additional wrapping. Seeds packed in bags were kept in a freezer at $-10^0C \pm 2^0C$ overnight, the day before grinding. Feed was withdrawn from the freezer at the time of grinding only and once withdrawn, fed through a feed hopper.

Total phenol content

Method suggested by Malick & Singh (1980) was used for the determination of the phenol content of ground powder of fenugreek seeds. Initially, 0.1 g of sample (weighed to the nearest 0.0001 g) was extracted in 10 ml of 80% ethanol. Centrifugation was done and the supernatant was collected carefully. Then 0.1 ml of aliquot was pipetted out into a test tube and was evaporated to dryness. After drying, the residue was dissolved in 1 ml of distilled water. Then 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard was pipetted out into a series of test tubes and the total volume of each was made up to 1 ml with distilled water. 1 ml of distilled water in a separate test tube was set for the blank solution. 0.5 ml of Folin-Ciocalteau reagent was then added to each tube (including blank). After three minutes, 2 ml of 20% Na_2CO_3 solution was added. The solution was then placed in a boiling water bath for one minute after thorough mixing and absorbance was measured at 650 nm using a UV-Visible spectrophotometer (GENESYS 50, Thermo Fisher Scientific, US) once cooled to room temperature. A standard curve of catechol was plotted and per cent total phenol in ground powder was calculated by using the following formula.

$$\text{Total phenol (\%)} = \frac{\text{Graph factor } (\mu g) \times \text{Optical density} \times \text{Total volume (ml)}}{\text{Sample aliquot (ml)} \times \text{Weight of sample (g)} \times 1000}$$

Total flavonoid content

The flavonoid content of ground fenugreek seed powder was estimated with the help of the aluminium chloride colourimetric method as described by Chang *et al*. (2002). Quercetin was used for the formation of the calibration curve. Different diluted quercetin standard solutions (10, 25, 50 and 100 $\mu g\ ml^{-1}$) were prepared in 80% ethanol. The sample was also extracted in ethanol. From each tube, 0.5 ml aliquot was pipetted out in separate test tubes. Then 1.5 ml of 95% ethanol followed by 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate was added to each tube. Total volume in each tube was made up to 5 ml by adding 2.8 ml distilled water. The blank solution was prepared by substituting aluminium chloride solution by the same amount of distilled water. After incubation for 30 minutes at room temperature, colour was read at 415 nm by UV-Visible spectrophotometer (Thermo Scientific,

GENESYS 50). At last, quantity of flavonoid was expressed in mg equivalent of quercetin with the help of following equation.

$$\text{Total flavonoid (mg QE/g)} = \frac{C \times V}{W}$$

where C = Concentration of sample extrapolated from calibration curve (mg ml^{-1})

V = Volume of sample extract (ml)

W = Weight of sample extracted (g)

Antioxidant activity

The antioxidant activity of ground powder of fenugreek seeds was measured by DPPH free radical scavenging method as reported by Chandra Shekhar & Anju (2014). In this method, 0.1 mM of DPPH (2,2- diphenyl-1-picrylhydrazyl) solution was prepared in ethanol by using a volumetric flask, funnel and watch glass. The sample was extracted in ethanol and different concentrations (5, 10, 50, 100, 200 and 500 µg ml^{-1}) were prepared by dilution method. Then 3 ml of each diluted solution was pipetted out in separate test tubes and 1 ml of prepared DPPH solution was added to each. For the control sample, 1 ml of DPPH solution was added to 3 ml of ethanol (without a test sample). While only ethanol was set as a blank solution. The tubes were allowed to stand for 30 minutes at room temperature after shaking vigorously. Then absorbance was measured by UV-Visible spectrophotometer (Thermo Scientific, GENESYS 50) at 517 nm. From the different concentrations, 200 µg ml^{-1} was finalized for the determination of antioxidant activity for all samples. The following formula was used to measure the per cent DPPH scavenging effect.

$$\text{DPPH scavenging (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Volatile metabolites

Volatile components present in fenugreek seed powder were identified using GC-QTOF MS. The process was followed as described by Mebazaa *et al*. (2009). About 10 g of fenugreek seed powder was soaked in 30 ml of methanol agitated and macerated for 48 hours was the extract was filtered using 0.2 µ filter. The analysis was performed in a GC system (Agilent Technologies, 7890 B) with the capillary column DB-5MS [30 m × 0.25 mm ID, 0.25 µm; oven conditions: 50^0C (held for 5 min) then increased at the rate of 2^0C min^{-1} to 100^0C (held for 5 min) and finally 5^0C min^{-1} to 300^0C]. Helium was used as a carrier gas at the constant flow rate of 1 ml min^{-1}. Mass spectra in the electron impact mode (with ionisation energy 70 eV and temperature source 200^0C) were generated. Spectra were collected from m/z 33 to 350 at 0.63 scan sec^{-1}. Mass spectral matches were carried out by comparison of experimental mass spectra with those of the NIST library. The total run time was 77 min.

Statistical analysis

The observed data were subjected to analysis of variance (ANOVA) by using Factorial Completely Randomized Design (FCRD) at 5% level of significance ($p<0.05$) (Sheoran *et al*., 1998).

Results and discussion

Temperature inside the grinding chamber at the end of grinding

The average temperature inside the grinding chamber at the end of grinding for different feed changed with different grinding methods. For ambient temperature feed the temperature inside the grinding chamber at the end of grinding decreased from 91.33 to 55.00^0C when the grinding method varied from without liquid circulation to ambient temperature; water circulation to chilled water circulation and to coolant circulation respectively, while for low temperature feed the corresponding values decreased from 88.00 to 53.33^0C respectively (Table 1). However, analysis of

variance indicated that the feed temperature ($p<0.05$) and grinding method ($p<0.01$) had a significant effect on the temperature inside the grinding chamber at the end of grinding. The interaction effect of the grinding method and feed temperature (L*T) on the temperature inside the grinding chamber at the end of grinding was found non-significant (Table 1).

Total phenol content

The phenol content of turmeric powder obtained through ambient temperature feed increased from 3.78 to 7.50 mg g^{-1} when the grinding method varied from without liquid circulation, ambient temperature water circulation, chilled water circulation to coolant circulation, while for low temperature feed the corresponding valued increased from 4.13 to 7.71 mg g^{-1} (Table 1). Analysis of variance indicated that the feed temperature ($p<0.05$) and grinding method ($p<0.01$) had significant effect on the phenol content. As the temperature inside the grinding chamber decreased, the phenol content of turmeric

Table 1. Variation in temperature inside the grinding chamber at the end of grinding and on various biochemical parameters of ground fenugreek seed powder

Effect		Temperature inside grinding chamber at the end (°C)	Total phenol content (mg g^{-1})	Total flavonoid content (mg QE g^{-1} of extract)	Antioxidant activity (DPPH scavenging %)
Grinding method (L)					
Ambient temperature feed (T_0)	Without liquid circulation (L_0)	91.33	3.78	6.22	7.72
	Ambient temperature water circulation (L_1)	84.67	4.50	7.29	10.08
	Chilled water circulation (L_2)	61.33	6.76	10.55	17.80
	Coolant circulation (L_3)	55.00	7.50	11.74	20.47
Low temp. feed (T_1)	Without liquid circulation (L_0)	88.00	4.13	6.74	8.85
	Ambient temperature water circulation (L_1)	83.67	4.67	7.58	10.70
	Chilled water circulation (L_2)	60.67	6.91	10.79	18.31
	Coolant circulation (L_3)	53.33	7.71	12.08	21.29
CD (0.05)					
L		1.84^{**}	0.30^{**}	0.43^{**}	0.79^{**}
T		1.30^{*}	0.21^{*}	0.31^{*}	0.56^{**}
L x T		2.60^{NS}	0.43^{NS}	0.61^{NS}	1.12^{NS}

NS-Non significant *-Significant at 5% **-Significant at 1%

powder increased. The interaction effect of the grinding method and feed temperature (L*T) on the phenol content was found non-significant at the same level of significance (Table 1). Cosme *et al*. (2020) reported that different food processing operations such as thermal treatments, homogenization, cooking and culinary methods affect the bioavailability of these compounds.

Total flavonoid content

Flavonoids belong to a class of plant secondary metabolites having a polyphenolic structure. They are linked with a broad spectrum of health-promoting effects and are an essential component in a variety of medicinal, nutraceutical and pharmaceutical applications. This is due to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. However, antioxidant activity of flavonoids has generated more attention because of their ability to reduce free radical formation and scavenge free radicals (Panche *et al*., 2016). Flavonoid content in fenugreek powder under ambient temperature feed increased from 6.22 to 11.74 mg QE g^{-1} of extract when the grinding method varied from without liquid circulation, ambient temperature water circulation, chilled water circulation to coolant circulation respectively, while for low temperature feed the corresponding value increased from 6.74 to 12.08 mg QE g^{-1} of extract respectively (Table 1). Analysis of variance indicated that the feed temperature $p<0.05$ and grinding method ($p<0.01$) had significant effect on the flavonoid content. As the temperature inside the grinding decreased, the flavonoid content of fenugreek powder increased. The interaction effect of the grinding method and feed temperature (L*T) on the flavonoid content was found non-significant (Table 1). As flavonoids are the largest group of phenolic compounds (naturally occurring) (Sulaiman & Balachandran 2012), higher temperature leads to degradation of flavonoids and decreased the quantity of total flavonoid in ground powder. The temperature has an effect on the stability of flavonoids and their biological activity. They are more or less sensitive to heat treatment according to their structure (Chaaban *et al*., 2017).

Antioxidant activity

The value for antioxidant activity in fenugreek powder under ambient temperature feed increased from 7.72 to 20.47 DPPH scavenging % when the grinding method varied from without liquid circulation, ambient temperature water circulation, chilled water circulation to coolant circulation respectively, while for low temperature feed the corresponding valued increased from 8.85 to 21.29 DPPH scavenging % respectively (Table 1). Analysis of variance indicated that the feed temperature and grinding method have a significant effect ($p<0.05$) on the antioxidant activity. The interaction effect of the grinding method and feed temperature (L*T) on the antioxidant activity was found non-significant at the same level of significance (Table 1). Since phenols are the largest group of phytochemicals which account for most of the antioxidant activity in plants (Sulaiman & Balachandran 2012), degradation of phenolic compounds at higher temperatures might also cause a decrease in antioxidant activity percentages in ground fenugreek seed powder. In addition to that, Dixit *et al*. (2005) and Bukhari *et al*. (2008) also reported the antioxidant activity to be correlated with polyphenols present in fenugreek seed extract. Besides that, a sudden rise in the graph occurs when moving from grinding method L_1 to L_2. This might also be due to the increase in the temperature difference of grinding chamber which triggered the antioxidant activity percentages.

Volatile metabolites

Volatile components present in the ground powder of fenugreek seeds were identified

Table 2. Compounds found in extract of seed powder of treatment (Without circulation + Ambient temperature feed) and (Coolant circulation + Low temperature feed)

Sr. No.	Name of compound	RT (Min)	
		L_0T_0	L_3T_1
1.	Vitamin E	75.05	75.05
2.	2,6-Difluorobenzoic acid, 4-nitrophenyl ester	43.85	43.85
3.	9,12-Octadecadienoic acid, methyl ester	58.57	58.56
4.	Hexadecanoic acid, methyl ester	55.16	55.15
5.	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	44.35	44.35
6.	2-n-Propylaziridine	21.11	18.89
7.	2-n-Propylaziridine	18.88	21.03
8.	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	54.99	55.0
9.	n-Hexadecanoic acid	55.95	55.96
10.	Epicubenol	47.41	47.40
11.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	65.86	65.87
12.	3-Penten-2-one, 4-methoxy-	45.42	31.34
13.	3-Penten-2-one, 4-methoxy-	-	45.42
14.	Fumaric acid, 2-nitrophenyl cyclohexylmethyl ester	45.09	45.09
15.	Fumaric acid, monoamide, N-(2,5-dimethoxyphenyl)-, undecyl ester	75.30	-
16.	Decanamide, N-(2-hydroxyethyl)-	62.69	62.69
17.	Decanamide, N-(2-hydroxyethyl)-	62.58	62.58
18.	1,8,11-Heptadecatriene, (Z, Z)-	59.36	-
19.	Dodecane, 5-methyl-	50.20	-
20.	(S)-(-)-1-Amino-2-(methoxymethyl)-pyrrolidine	18.89	-
21.	5-Nonadecen-1-ol	59.47	-
22.	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	58.69	-
23.	2,5-Pyrrolidinedione, 1-ethyl-	27.37	-
24.	7-Octylidenebicyclo [4.1.0] heptane	68.69	-
25.	9-Octadecenoic acid	68.74	-
26.	1-Methyl-2-methylene-trans-decalin	73.20	-
27.	Hexazinone	70.66	-
28.	Ethanone, 1-(4-amino-2-methylaminothiazol-5-yl)-	70.74	-
29.	4-Pentenoic acid, 2-(formylamino)-, ethyl ester	73.22	-
30.	beta.-d-Glucopyranoside, methyl 2,3,4-tris-O-(phenylmethyl)-	75.83	-
31.	Pyridine, 2-tridecyl-	73.34	-
32.	Glycine, 2-cyclohexyl-N-(but-3-yn-1-yl) oxycarbonyl-, dodecyl ester	75.67	-
33.	phenol, 4-[[4-[[4-(phenylamino)phenyl] amino] phenyl] amino]-	75.66	-

34.	Phthalic acid, di(6-methylhept-2-yl) ester	-	66.53
35.	Undecane, 3,8-dimethyl-	-	50.20
36.	Tetradecanoic acid	-	51.55
37.	9,12-Octadecadienoyl chloride, (Z, Z)-	-	58.69
38.	Phthalic acid, hept-4-yl isobutyl ester	-	53.87
39.	1,8,11,14-Heptadecatetraene, (Z, Z, Z)-	-	68.84
40.	3-Amino-4,5-dimethyl-2(5H)-furanone	-	27.34
41.	Methyl stearate	-	59.19
42.	2-Pentadecanone, 6,10,14-trimethyl-	-	53.38
43.	1,8,11,14-Heptadecatetraene, (Z, Z, Z)-	-	64.76
44.	Linoelaidic acid	-	59.42
45.	9,12-Octadecadienoic acid, methyl ester	-	64.64
46.	Myo-Inositol, 4-C-methyl-	-	50.11
47.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-	61.65
48.	Carbonic acid, 2-dimethylaminoethyl ethyl ester	-	65.00
49.	Octadecanoic acid, 2,3-dihydroxypropyl ester	-	69.11
50.	cis-3-Methyl-endo-tricyclo [5.2.1.0(2.6)] decane	-	68.71
51.	(1S,4aS,4bS,7S,8aS,10aS)-7-Isopropyl-1,4a-dimethyltetradecahydrophenanthrene	-	73.19
52.	Cyclopropane, 1-ethenyl-2-hexenyl-, [1. alpha.,2.beta.(E)]-(.+/-.)-	-	64.88
53.	delta.- Nonalactone	-	25.50
54.	3,6-Heptanedione	-	49.99
55.	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	-	73.33
56.	Oxazolidine, 2,2-diethyl-3-methyl-	-	11.96
57.	Palmitic acid vinyl ester	-	61.76
58.	Propanoyl chloride, 3-chloro-	-	23.95
59.	Cholest-5-en-3-ol, (3. alpha.)-	-	74.88
60.	Oleic anhydride	-	68.76
61.	Phthalic acid, 2,2,2-trifluoroethyl propyl ester	-	54.66
62.	Thiophene-2-acetic acid, 2-dimethylaminoethyl ester	-	65.13
63.	Lupeol	-	70.59
64.	Nonanedioic acid, bis(2-ethylhexyl) ester	-	70.64
65.	Stigmasta-3,5-diene	-	72.77
66.	Acrylic acid, 4-cyclopropylidenebutyl ester	-	59.51
67.	Propanoic acid, anhydride	-	48.12
68.	Cyclohexyl methylphosphonofluoridate	-	44.24
69.	(7R)-cis-anti-cis-Tricyclo [7.3.0.0(2,6)] dodecan-7-ol	-	70.64
70.	Stigmast-8(14)-en-3. beta. -ol	-	67.44
71.	3H,6H-Thieno[3,4-c] isoxazole, 3a,4-dihydro-6-(1-methylethyl)-	-	73.59

using GC-MS QTOF. The chromatograms obtained in the analysis of the extract of ground powder, obtained through treatment combinations L_0T_0 and L_3T_1 are shown in Table 2. There were 30 compounds present in the extract of treatment L_0T_0 while in the case of treatment L_3T_1, a total of 48 compounds were identified. Out of all the compounds, 13 were found in extracts of both treatments that included-vitamin E; hexadecanoic acid, methyl ester; 2,6-difluorobenzoic acid, 4-nitrophenyl ester; 9,12-octadecadienoic acid, methyl ester; pentanoicacid,5-hydroxy-,2,4-di-t-butylphenyl esters; 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione; n-hexadecanoic acid; epicubenol; 2-n-propylaziridine; hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester; 3-Penten-2-one, 4-methoxy-; fumaric acid; decanamide, N-(2-hydroxyethyl)- and 2-nitrophenylcyclohexylmethyl ester. Thirteen compounds were common to both the extracts and there were 17 compounds which were present in the extract of treatment L_0T_0 but were absent in L_3T_1 treatment. Similarly, there were 35 compounds which were identified in the extract of treatment L3T1 but were not detected in the case of L_0T_0 treatment. More compounds were detected in L_3T_1 compund to L_0T_0. 3-amino-4,5-dimethyl-2 (5H)-furanone the precursor of sotolone was identified in the extract of treatment L_3T_1 (characteristic and dominant impact flavour compound of fenugreek seeds) (Guerra & Yaylayan 2011; Blank *et al*., 1997) which was absent in extract of treatment L_0T_0. Loss of compounds in the case of treatment L_0T_0 might be attributed to the increase in the temperature of the grinding chamber as compared to the treatment involving coolant circulation and low-temperature feed (L_3T_1).

Conclusion

Low-temperature grinding significantly reduced the temperature inside the grinding chamber compared to the ambient grinding method. Lowest temperature inside the grinding chamber was achieved through coolant circulation in the jacket over the grinding chamber with low-temperature feed (-10^0C). This resulted in significantly higher quantity of total phenol, flavonoid and antioxidant activity as well as more volatile components in ground powder of fenugreek seeds. The compound 3-amino-4,5-dimethyl-2(5H)-furanone, the precursor of sotolone was retained in ground powder of fenugreek seeds through low-temperature grinding, which otherwise was lost by ambient grinding. The study will help to retain heat sensitive phytochemicals during grinding of fenugreek seeds.

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