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Evaluation of volatile compounds and fatty acid methyl ester (Fame) through gas Chromatograhy in cumin seeds (*Cuminum cyminum*)

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Abstract
Volatile Oils extracted from cumin seeds rich in bioactive components such as cuminal, β-pinene and γ-terpinene and dl-limonene. Oleic and linoleic acids were the most unsaturated fatty acids in fixed *c*. oil, while palmitic and stearic acids were the most saturated fatty acids. Oils tested through Fatty Acid Methyl Ester were rich in natural antioxidants included α-tochopherol, β-carotene and poly phenols. Fatty acid composition has varied immensely, due to the conversion of PUFA and MUFA into saturated fatty acids after roasting as well as frying. Hence, the health benefits of cumin seeds are well known such as powerful external or internal antiseptic, analgesic, anti-inflammatory, haemolytic, or anti enzymatic action, sedative, stimulants and stomachics.

Keywords: fatty acid methyl ester, volatile compounds, bioactive compounds, medicinal properties

Introduction
Cuminum cyminum L. is an annual plant of the family umbelliferae. In India cumin is commonly known as 'jeera' or 'jira'. Cumin is herbaceous annual plant, with a slender branched stem 30-90cm tall. The leaves are 5-10cm long, pinnate or bipinnate, thread-like leaflets. The flowers are small, white or pink, and borne in compound umbels. The fruit a cremocarp, often separated into mericarps, brown with light coloured ridges ellipsoidal, elongated, about 4-6 mm long, 2mm wide, tapering at ends and slightly compressed laterally, mericarps with 5 longitudinal hairy primary ridges from base to apex, alternating with 4 secondary ridges which are flatter and bear conspicuous emergences, seeds orthospermous, odour umbelliferous characteristic, taste, richly spicy. For years, the physical properties of agricultural products have been of interest to many reasearchers. They have reported physical and mechanical properties of seeds, nuts, kernels, and fruits such as arigo seeds (Davies, 2010) [1]. Cumin fruit are similar to fennel in appearance, but are smaller and darker in colour. Cumin is native from the East Mediterranean to East India, cultivated almost throughout India. In India Rajasthan and Gujrat states is main cultivator. The medicinal component of the plant is cumin oil extracted from the ripe fruits through Soxhlet method for 16 hr (IS: 1797:1985) [18]. Cumin is used as a carminative for stomach disorders, diarrhoea, and spasmolytic and for medicinal uses (Khare CP. 2004) [19]. The present study reports physicochemical characterization of essential oils was carried out according to the method described by Association of Official Analytical Chemists (AOAC; 1997). The oil of cumin is especially used as an antioxidant and flavor. Cuminaldehyde has been found as the characteristic constituent of the cumin seed (Lee HS, 2005) [15] and employed advantageously in many types of flavouring preparations particularly in curries and culinary preparations of oriental type and flavouring component such as cuminaldehyde 18.3% and perillaldehyde 8.17%, in addition to the terpenic hydrocarbons, whereas Egyptian cumin oil contains 39.2% cuminaldehyde (Srinivas, 1986). It is also used to an extent in soap perfumery and in flavouring beverages. Essential oil of *Cuminum cyminum* L. is more or less powerful external or internal antiseptic, analgesic, anti-inflammatory, haemolytic, or anti enzymatic action, sedative, stimulants and stomachics (Gruenwald, *et al*. 2004) [7]. Components may have antioxidant, anticancer, antibacterial, and larvicidal effects (Iacobellis, *et at*. 2005) [17]. Cumin may lower blood sugar, reduce seizures, strengthen bones, and treat the eye. Cumin is generally recognized as safe for human consumption as a spice and flavoring agent. The antibacterial activity of cumin Eos could attribute to high level of antimicrobial properties (Neda *et al*. 2013).
The chemical composition of cumin oil from different countries includes: α-pinene (0.5%), Myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), p-mentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β-bisabolene (0.9%), β-pinene (13.0%), P-cymene

(8.5%), β-phellandrene (0.3%), D-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β-farnesene (1.1%) together with much smaller quantities of α-phellandrene, α-terpinene, cis and trans sabinene, Myrtenol, α-terpineol and phellandral.

The major components of cumin oil include: The major compounds include cumin aldehyde (p-isopropyl benzaldehyde, 25 to 35%), terpinene (29.5%), α- and β-pinene (21%), ρ-cymene (8.5%), ρ-mentha-1, 3-dien-7-al (5.6%), cuminyl alcohol (2.8%) and β-farnesene (1.1%). Perilla aldehyde, cumin alcohol, dipentene, and β-phellandrene are also present in cumin. Limonene, eugenol, α-and β- pinenes and some other minor constituents have been found in cumin oil (Gohari. R.A *et al*, 2011) [4]. The main components in the EOS (Essential oils) of green cumin are ɣ-terpinene, ɣ-terpinene-7-al and β-pinene.

In fact, medicinal usage of cumin seeds has also been immensely widespread in diverse ethnomedical system form Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia and North America, where these have remained as an integral part of their folk medicines (Zargary A 2001). Cumin paste is used to treat boils by its topical application. It is used as a stimulant and an antispasmodic and said to relieve, anemia, and diarrhoea. It can help with flatulence, indigestion, nausea, morning sickness, and atonic dyspepsia.

The plants are annual or biennial herbs and cultivated in different parts of Pakistan for the recovery of their essential oil (Nasir & Ali, 1972) [14]. *Cuminum cyminum* seeds are largely used as a condiment or spice in curries and pickles etc. Seeds have cooling affect and therefore form an ingredient of most prescriptions for gonorhoea, chronic diarrhoea and dyspepsia; externally they are applied in the form of poultice to allay pain and irritation of worms in the abdomen. *Cuminum cyminum L*. Positive effects on reductions of cholesterol, phospholipids, free fatty acids, and triglycerides in plasma and tissue (Dhandapani *et al*. 2002; Malini *et al*. 1987) [16] Seeds reduced to powder, mixed with honey, salts and butter are applied to scropian bites (Al-Yahya & Colpharm, 1986; Johnson & Nam 1998, Kartikar & Basu, 1984) [9, 11]. Studies have been carried out on the essential oils (Chopra, 1970) but no work has been done on fixed oil of this species. The present work reports on the fatty acid composition of neutral and polar lipids of *Cuminum cyminum* L.

Materials and Methods

All the chemicals, reagents and solvents used for determination of physicochemical parameters of essential oils were of analytical grade.

Identification of active constituents in raw, roast and fried cumin seeds

Raw cumin

Raw seeds of *Cuminum cyminum L*. (150g) were dried in an oven at 105 ºC and then ground into fine powder.

Roasted cumin

Roast cumin is prepared by heating the cumin seeds conventionally up to a temperature of 130-135 ^{0}C. Color change takes place due to the heat and the moisture and volatile oil content is reduced.

Fried cumin

Cumin seeds are added to the hot frying pan having edible oil at a higher temperature but not boiling.

1. Extraction of fat from *Cuminum cyminum*

In this study, Soxhlet method is used to extract volatile oil from *Cuminum cyminum*, by weighing cumin sample of 5 gm in a thimble of filter paper. Take 250ml round bottom flask (R.B.F) and tare it. Put the thimble in the siphon tube by adding 200ml petroleum ether to the R.B.F.

Later, set the Soxhlet assembly on the heating mantle at 20 ^{0}C for 16 hrs (Fig. 1.). Regularly check the petroleum ether in the RBF and condenser is cool or not. Remove the assembly from heating mantle after 16 hrs. Evaporate the solvent (petroleum ether) completely on water bath. Put RBF in oven at 105 ^{0}C for 4-5 hrs. Take out and put it in desiccator. Weigh the RBF. Repeat these operations until mass difference between two successive readings obtained is less than 0.001 gm.
(Mohammad H. Eikani, 2007) [21].

Calculation= Fat (%) = Final wt. - empty wt. X 100/sample wt.

2. Extraction of volatile oil from *Cuminum cyminum*

Present study, determines extraction of volatile oil from *Cuminum cyminum*, by Clevenger assembly. Weighing cumin sample (20-40 gm) in 100ml R.B.F. Rinse the Clevenger assembly with chronic acid, add 400ml distilled water (approx.) to cover the sample and glass beads to the Flask (Fig. 2.). Heat the flask for 4-6 hrs on heating mental. Measure the final volume of volatile oil collected on Clevenger assembly.

Calculation= Volatile oil content (%)= Measured vol.x100/sample wt.

Fig 1: Fixed oil extraction by Soxhlet

Fig 2: Cumin seeds after roasting.

Fatty acid methyl ester (FAME) characterization by AOCS method

This process of fatty acid methyl ester (FAME) analysis consists of esterification of lipids, and of injection, separation, identification and quantitation of the FAMEs. In order for the required accuracy and precision to be attained, each of these steps has to be optimized.

GC-Programming

Injector temperature: 260 ºC Column used: HP-88, 100 meter for FID for FAME (fatty acid methyl esters) characterization. Mobile phase: Helium Detector temperature: 260 ºC

1-Evaluation of FAME from cumin seeds

In the preparation of FAME, weighing of 0.05 gm of fat has been collected from cumin seed in 15ml test tube. For saponification addition of 200µl of 2N Methanolic KOH, after that put the sample at 50 ºC in water bath for 10 minutes with continuous shaking at regular interval of time. Cool it for 10 minutes and by adding 1ml of 5% Methanolic HCL for complete digestion keeping the sample at 70 ºC. In water bath for 10-15 minutes with continuous shaking at regular interval of time. Cooling the sample and add 2ml petroleum ether to collect the FAME. Transfer upper layer of petroleum ether to Gas Chromatography vials and inject on Flame Ionization Detector for the analysis of FAME. Transfer upper layer to G.C vials 10 minutes with shaking (Table 1).

Table 1: Standard of 37 fatty acids

1	Butyric acid	SFA
2	Caproic acid	SFA
3	Caprylic acid	SFA
4	Capric acid	SFA
5	Undecanoic acid	SFA
6	Lauric acid	SFA
7	Tridecanoic acid	SFA
8	Myristic acid	SFA
9	Myristoleic acid	MUFA
10	Pentadecanoic acid	SFA
11	Cis-10- Pentadecanoic acid	SFA
12	Palmitic acid	MUFA
13	Palmitoleic acid	SUFA
14	Heptadecanoic acid	MUFA
15	Cis-10- Heptadecanoic acid	SFA
16	Stearic acid	Trans
17	Elaidic acid	MUFA
18	Oleic acid	Trans
19	Linolelaidic acid	PUFA (-6)
20	Linoleic acid	SFA
21	Arachidic acid	PUFA (-6)
22	Gamma Linolenic acid	MUFA
23	Cis-11-Eicosenoic acid	PUFA
24	Linolenic acid	SFA
25	Heneicosanoic acid	PUFA
26	Cis-11,14-Eicosadienoic acid	PUFA
27	Behanic acid	SFA
28	Cis-8,11,14-Eicosatrienoic acid	PUFA
29	Erucic acid	MUFA
30	Cis-11,14,17-Eicosatrienoic acid	PUFA
31	Arachidonic acid	PUFA (-6)
32	Tricosanoic acid	SFA
33	Cis-13,16-Docosadienoic acid	PUFA
34	Lignoceric acid	SFA
35	Cis-5,8,11,14,17-Eicosapentaenoic acid	PUFA (-3)
36	Nervonic acid	MUFA
37	Cis-4,7,10,13,16,19-Docosahexaenoic acid	PUFA (-3)

Results & Discussion: The fatty acid composition of roasted cumin seeds composed of 8.83% saturated fatty acids, 7.14% MUFA, 1.97% PUFA and no trans fatty acids were found and total fat content was 17.94%. A significant amount of reduction in MUFA and PUFA content was observed due to processing of cumin seeds.

The fatty acid composition of mustard oil used for frying cumin seeds had 59.67% saturated fatty acids, 11.69% MUFA, 28.56% PUFA and 0.08% Trans fatty acids were found and total fat content was 100% (Table 2).

Due to frying of cumin seeds in mustard oil, the fatty acid profile changed to 12.60% saturated fatty acids, 14.35% MUFA content and 10.36% PUFA and the total fat content was 37.31%.

Similarly total volatile oil content of raw cumin was 4.99% which consists of 4.79% saturated fatty acid, 0.10% MUFA, 0.11% PUFA and no trans fatty acids were found.

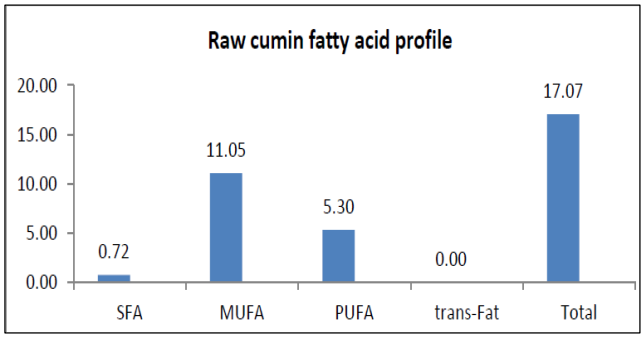
The total volatile oil content of roast cumin was 1.99% and comprised of 1.95% saturated fatty acid, 0.02% MUFA, 0.02% PUFA and no trans fatty acids were found.

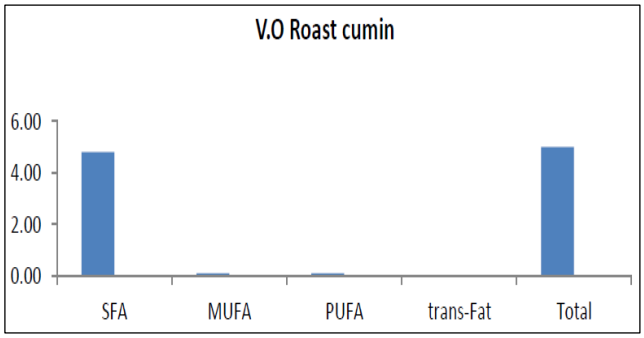
The total volatile oil content of fried cumin was 3.99% which comprised of 3.88% saturated fatty acid, 0.05% MUFA, 0.06% PUFA and no Trans fatty acids were found.

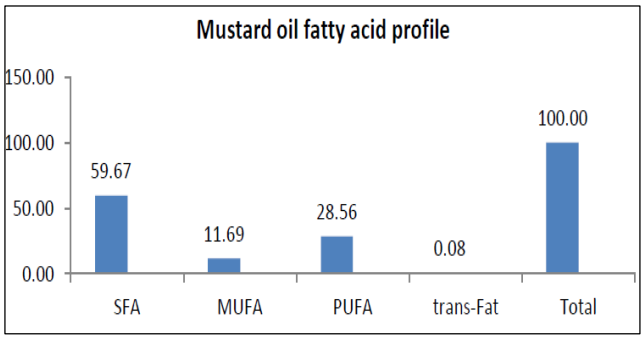
Fame (Fatty-Acid Methyl Ester)

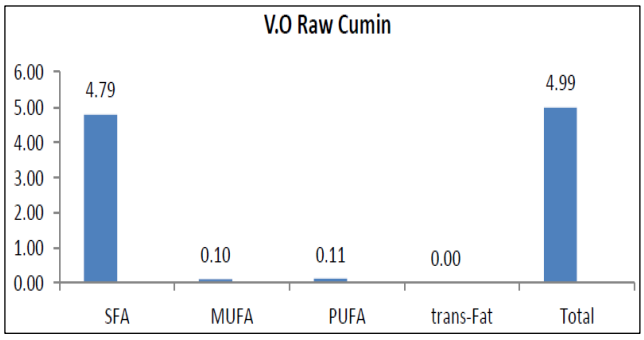
Table 2: Analysis of Fame

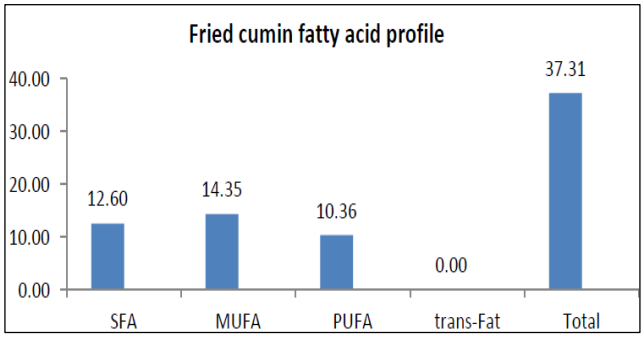
Sample Name	Total Fat %	Total Fatty Acids	Total Fatty Acids %	Calculated Fatty Acid %
Raw Cumin	17.07	SFA	4.24	0.72
	17.07	MUFA	64.72	11.05
	17.07	PUFA	31.04	5.30
	17.07	Trans-FAT	0.00	0.00
	17.07	Total	100.00	17.07
Fried Cumin	37.31	SFA	33.77	12.60
	37.31	MUFA	38.46	14.35
	37.31	PUFA	27.77	10.36
	37.31	Trans-Fat	0.00	0.00
	37.31	Total	100.00	37.31
Roast Cumin	17. 94	SFA	49.21	8.83
	17. 94	MUFA	39.79	7.14
	17. 94	PUFA	11.00	1. 7
	17. 94	Trans-Fat	0.00	0.00
	17. 94	Total	100.00	17.94
Volatile oil roast cumin	4.99	SFA	95.90	4.79
	4.99	MUFA	1.98	0.10
	4.99	PUFA	2.12	0.11
	4.99	Trans-Fat	0.00	0.00
	4.99	Total	100.00	4.99
Volatile oil roast cumin	1.99	SFA	98.01	1.95
	1.99	MUFA	0.99	0.02
	1.99	PUFA	1.00	0.02
	1.99	Trans-Fat	0.00	0.00
	1.99	Total	100.00	1.99
Volatile oil fried cumin	3.99	SFA	97.18	3.88
	3.99	MUFA	1.24	0.05
	3.99	PUFA	1.58	0.06
	3.99	Trans-Fat	0.00	0.00
	3.99	Total	100.00	3.99

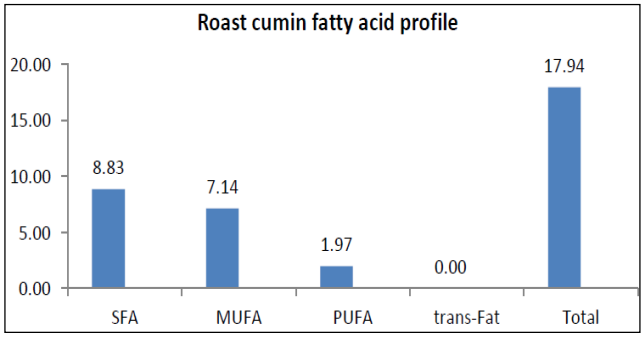


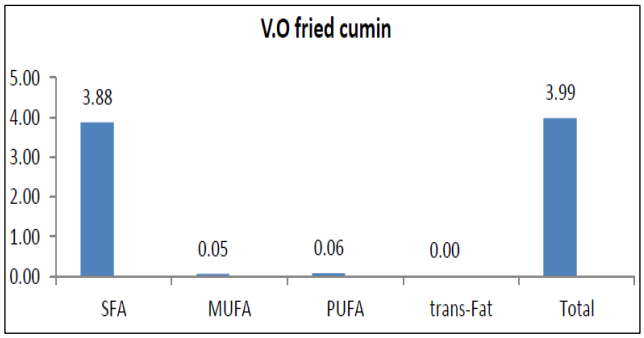












Conclusion

Fatty acid composition has varied immensely due to the conversion of PUFA and MUFA into saturated fatty acids after roasting as well as frying. Hence, the health benefits that cumin seeds are well known for does not remain with it after its processing and it may lead to adverse effects on human health, leading to increased cardiovascular diseases and other heart diseases and several health problems. So, it is better to consume cumin seeds in least processed form.

This study was carried out to extract volatile oils from cumin seeds, and fixed oils. The extracted volatile and fixed oils were evaluated physically and chemically using standard methods. Cuminaldehyde has been found as the characteristic constituent of the cumin seed. The unsaturated fatty acids mainly oleic (C18:1), linoleic (C18:1), linolenic (C18:3) and saturated fatty acids are myristic (C14:0), palmitic (C16:0) and stearic (C18:2). The total phenolic content of methanolic extracts of different cumin weight ranges from 4.1 to 53.6 mg/g dry weight. The major compounds include cumin aldehyde (p-isopropylbenzaldehyde, 25 to 35%), terpinene (29.5%), α- and β-pinene (21%), ρ-cymene (8.5%), ρ-mentha-1,3-dien-7-al (5.6%), cuminyl alcohol (2.8%) and β-farnesene (1.1%). Perilla aldehyde, cumin alcohol, dipentene, and β-phellandrene are also present in cumin. Limonene, eugenol, α- and β- pinenes and some other minor constituents have been found in cumin oil. EOs also containing phenolic compounds, e.g. thymol, carvacrol, ɣ-terpinene and p-cymene, are widely reported to possess high levels of antibacterial activity.

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