trans-Anethole Based Detection of Adulteration of Fennel (Foeniculum Vulgare Mill.) Seeds in Cumin (Cuminum Cyminum L.) Seeds Using GC & GC-MS

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Abstract—**The adulteration of low grade fennel seeds (*Foeniculum vulgare* Mill.) in cumin seeds (Cuminum cyminum L.) were reported in certain instances in India. The present study was undertaken for the detection of adulteration of fennel seeds in cumin seeds with the help of *trans*-anethole which is reported to be absent in the essential oil of cumin seeds but reported as a major marker compound of the fennel seeds essential oil. *trans*-Anethole was detected in the samples prepared by mixing fennel seeds in cumin seeds with the help of GC and GC-MS. An adulteration range of 1-50% was studied and the adulteration level upto 5% was detected. The major compounds identified in the essential oil of cumin seeds were γ-terpin-7-al (22.9%), γ-terpinene (22.6%), β-pinene (22.2%) and cuminaldehyde (13.1%) whereas the major compounds identified in the essential oil of fennel seeds were trans- anethole (50.4%), methylchavicol (22.4%), limonene (11.4%) and fenchone (11.1%).**

***Keywords-* *Cuminum cyminum* L.; *Foeniculum vulgare* Mill.; essential oil; *trans*-anethole.**

I. INTRODUCTION

Spice exporters, manufacturers, food safety and regulatory agencies have been actively concerned with the adulteration/contamination in the spices. The major concern in seed spices viz cumin, fennel, aniseed, black cumin etc. is the presence of other similar seeds. Cumin (Cuminum cyminum L.), an important commercial seeds spice, belongs to the family Apiacea and is used as a spice and a seasoning in food. India is the largest producer of cumin worldwide [1] (Sowbhagiya et al. 2011), Rajasthan and Gujrat are the major cumin producing states in India [2] (Pagaria and Jain 2012). Adulteration of low grade fennel (Foeniculum vulgare Mill.) seeds in cumin is one of the problems in cumin trade and a few instances were reported in India where adulterated cumin seeds were seized, raw marble dust applied as a thin layer to 'C' grade and smaller size fennel seeds using a binder. Artificial color has been used to give this coated fennel cumin like look, these adulterated cumin seeds are hazardous to human health [3,4] (Times of India 2012; Times of India 2001). In powdered from it is difficult to identify the adulteration of fennel seeds in cumin.

The chemical compositions of fennel seeds essential oil reported trans-anethole as one of the major marker compound [5,6]. The principal constituents from volatile oil from fruits are 50–60% trans-anethole and 15–20% fenchone [7]. Review of the essential oil chemical compositions of cumin (Cuminum cyminum L.) seeds suggests the absence of trans-anethole, the major constituents of cumin are cuminaldehyde, ρ-cymene, γ-terpinene, safranal, *o*-cymene, limonene and β-pinene [1,8,9,10,11,12]. The essential oil chemical compositions of cumin and fennel are quite different; this difference may be utilized to find mixing of fennel seeds in cumin. The present study is to detect the level of adulteration of fennel seeds in cumin seeds and/or cumin powder with the help of trans-anethole the major marker compound of the fennel seeds.

II. MATERIALS AND METHODS

A. Plant Materials

Dried cumin seeds and fennel seeds were collected from different markets of Delhi, India in December 2013.

B. Samples Preparation and essential oil extraction

The cumin seeds and fennel seeds were physically inspected for the presence of other seeds and then samples were prepared by mixing fennel seeds in cumin (Table 1) followed by grinding it in a blender. 100 g of each of the samples was subjected to hydrodistillation for 2h in a Clevenger type apparatus. The essential oils obtained were separated from water and dried over anhydrous Na_2SO_4.

C. GC-FID Analysis

The essential oils were analyzed by using Shimadzu gas chromatograph (Model Shimadzu GC-2010) fitted with Rtx-5MS fused silica capillary column (30 m × 0.32 mm internal diameter, film thickness 0.25 µm). The oven temperature was programmed from 50°C to 150°C at 3°C/min, then held isothermal at 150°C for 10 min and finally raised to 250°C at 10°C/min using N_2 as carrier. The injection temperature was 250°C, detector temperature 260°C and the injection volume 0.6 µL, (using a 1:4 solution of the oil in n-hexane), and the split ratio 100:1.

D. GC-MS Analysis

The GC-MS analysis of the essential oils were conducted on a Perkin Elmer GC Clarus 680 fitted with ELITE-5MS fused silica capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm) and interfaced with Perkin Elmer Clarus 600 T mass spectrometer. The oven temperature was programmed from 50°C to 150°C at 3°C/min, then held isothermal at 150°C for 10 min and finally raised to 250°C at 10°C/min using helium as carrier gas at 1.0 mL/min. The Injector, Ion source and MS transfer line temperatures were 290°C, 230°C and 250°C respectively, the injection volume 1µL (1:4 solution of oil in n-Hexane), and the split ratio 20:1. MS were taken at 70 eV with a mass range of m/z 40-400 amu.

E. Identification of the Marker Compounds

Identification was made on the basis of Linear Retention Index (LRI, determined with reference to a homologous series of n-alkanes (C_8-C_{20}, Fluka) under identical experimental conditions, co-injection with standards (Sigma), MS Library search (NIST), by comparison with MS literature data [8].

III. Results and discussion

The results obtained by GC and GC-MS analysis of essential oils of fennel seeds (Fig. 1) and cumin seeds (Fig. 2) are presented in (Table 2). As a result of GC and GC-MS analysis of the essential oils γ-terpin-7-al (22.9%), γ-terpinene (22.6%), β-pinene (22.2%) and cuminaldehyde (13.1%) were the major compounds identified in cumin seeds, whereas *trans*- anethole (50.4%), methyl chavicol (22.4%), limonene (11.4%) and fenchone (11.1%) were the major compounds in fennel seeds. Complete absence of *trans*-anethole was seen in the essential oil of cumin seeds. *trans*-Anethole in the essential oils of the mixture of cumin and fennel seeds was studied in the range of 1-50% as shown in Table 1. Mixture of 1% and 2.5% fennel seeds in cumin seeds showed the absence of *trans*-anethole. 5-50% showed the presence of *trans*-anethole which clearly indicates the detectable level of adulteration of fennel seeds in the cumin seeds. The cumin (*Cuminium cyminum* L.) seeds essential oil has unique chemical composition with complete absence of *trans*-anethole, thus presence of foreign compound *trans*-anethole suggests the adulteration of fennel seeds as per the experimental model. The study showed the presence of *trans*-anethole in the adulteration range from 5-50%. The detection of *trans*- anethole in the essential oil of cumin seed samples showed the adulteration of fennel seeds and upto 5% adulteration of fennel seeds in cumin seeds were detected (fig. 3). The detection of the *trans*-anethole in the essential oil of cumin seeds or cumin powder indicates the presence of low grade fennel seeds.

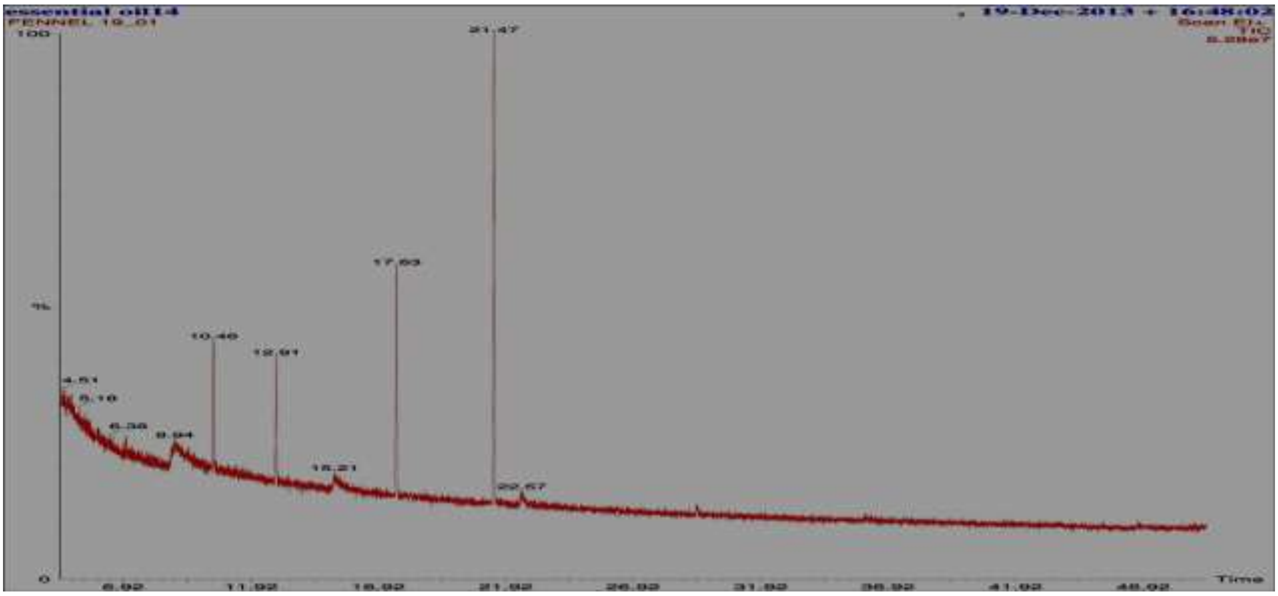


Figure 1. GC-MS chromatogram of fennel seeds essential oil

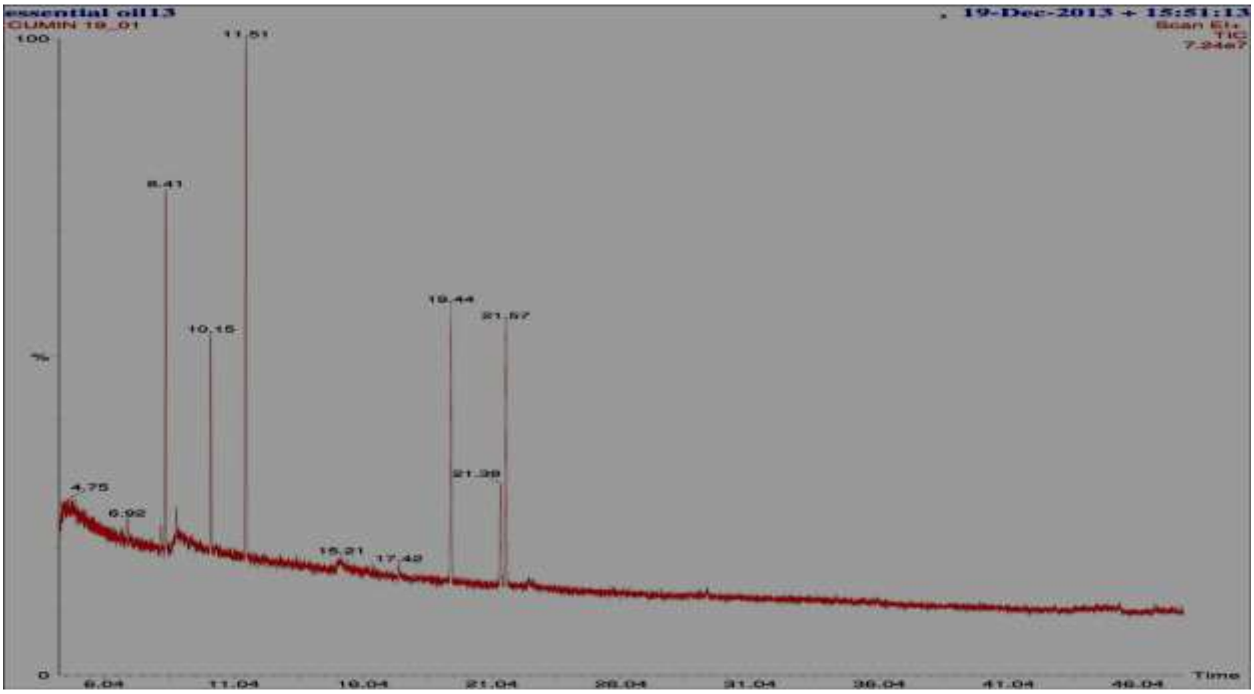
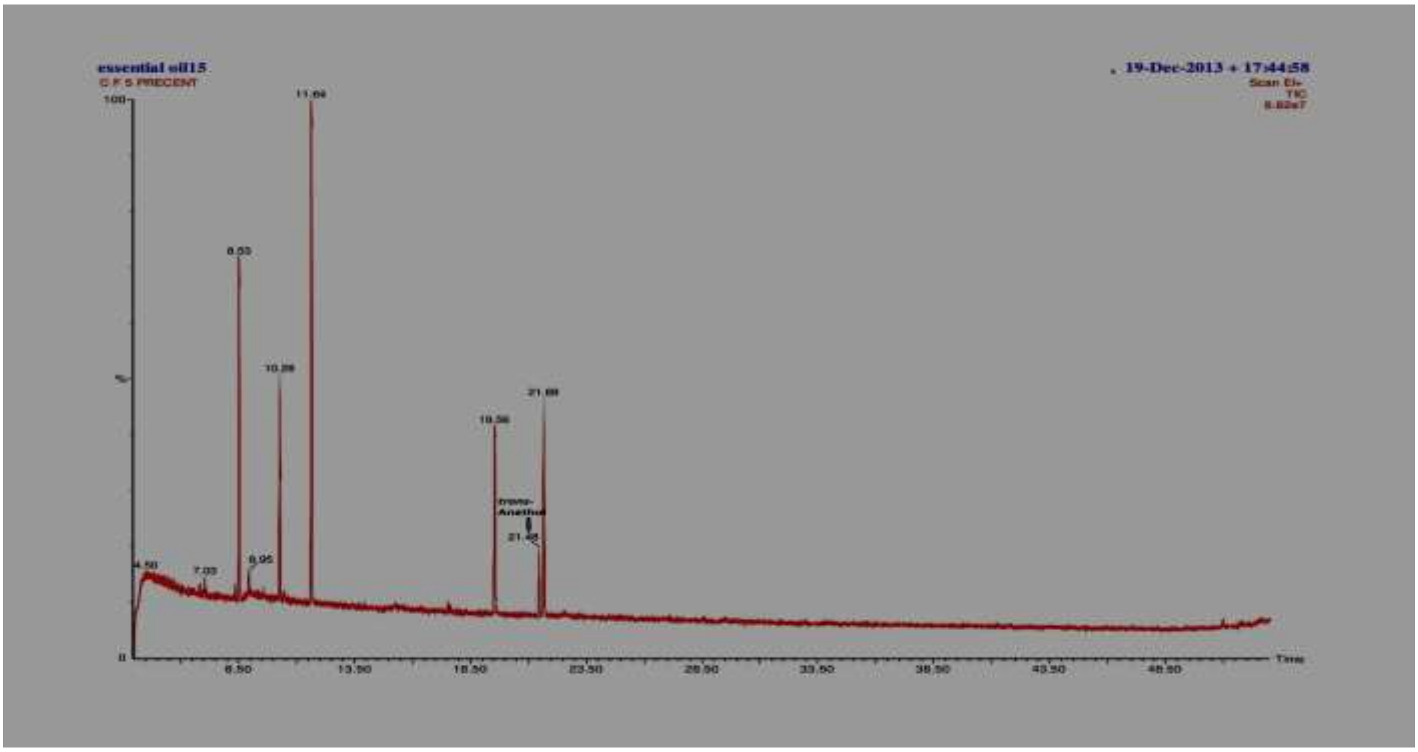


Figure 2. GC-MS chromatogram of cumin seeds essential oil



GC-MS chromatograom showing 5% adulteration fennel seeds in cumin

Table 1. Detection of *trans*-anethole in the cumin (*Cuminum cyminum* L) seeds and fennel (*Foeniculum vulgare* Mill.) seeds mixture.

S. No.	**Sample code**	**Cumin (g)**	**Fennel (g)**	**Percentage of adulteration (%)**	**Detection of *trans*-Anethole***
1	C	100	0	0	Not detected
2	F	0	100	100	Detected
3	CF 50	50	50	50	Detected
4	CF 20	80	20	20	Detected
5	CF 15	85	15	15	Detected
6	CF 10	90	10	10	Detected
7	CF 5	95	5	5	Detected
8	CF 2.5	97.5	2.5	2.5	Not detected
9	CF 1	99	1	1	Not detected

Note **trans*-anethole was detected with the help of LRI on Rtx-5 & ELITE 5MS capillary column, co-injection with authentic compound and GC-MS, C-cumin seeds, F-fennel seeds, CF- cumin seeds and fennel seeds mixture, the detections were carried out in triplicate.

Table 2. Percentage composition of the essential oils of seeds of *Cuminum cyminum* L. and *Foeniculum vulgare* Mill.

S.No.	Compounds	LRI	%FID *F. vulgare*	%FID *C. cyminum*	*Identification
1.	*α*-Thujene	926	-	0.3	a, b
2.	*α*-Pinene	933	1.1	1.1	a,b
3.	***β*-Pinene**	**980**	t	**22.2**	a,b
4.	Myrecene	993	-	1.2	a,b
5.	***p*-Cymene**	**1026**	-	**9.6**	a,b
6.	***γ*-Terpinene**	**1063**	-	**22.6**	a,b
7.	**Cuminaldehyde**	**1246**	-	**13.1**	a,b
8.	*α*-Terpin-7-al	1289	-	4.6	a,b
9	***γ*-Terpin-7-al**	**1301**	-	**22.9**	a,b
10	**Limonene**	**1030**	**11.4**	-	a,b
11	**Fenchone**	**1091**	**11.1**	-	a,b
12	**Methyl chavicol**	**1206**	**22.4**	-	a,b
13	***trans*- anethole**	**1297**	**50.4**	-	a,b
14	*o*-Cymene	1254	t	-	a,b
15	Eugenol	1377	t	t	a,b
18	*γ*-Terpinene	1064	t	t	a,b
21	Camphor	1154	-	t	a,b
22	*p* -Anisaldehyde	1947	-	t	a,b
	Total Identified		**96.4 %**	**97.6%**	

Note: * Mode of Identification: a, linear retention index (LRI based on homologous serious of n-alkanes C_8-C_{20}), co-injection with standards/peak enrichment with known oil constituents; b, Gas Chromatography-Mass Spectrometry (GC-MS); t, trace (<0.1%); (-), Not detected; compounds >5.0% are represented in bold face.

IV. CONCLUSION

The adulteration of low grade fennel seeds in cumin seeds is harmful to cumin trade and human health because the malpractice of mixing is carried with the help of foreign materials such as marble dust, artificial color etc [3,4]. The cumin seeds or cumin powder should have pure cumin seeds or pure powdered cumin seeds respectively. The essential oil compositions of spices are unique for a particular spice unless chemotypes are not present, thus the presence of any foreign compound present in the essential oils of a particular spice may be a hint towards adulteration/contamination. The study showed complete absence of *trans*-anethole in cumin seeds, thus detection of *trans*-anethole in the essential oil composition of cumin seeds is indication towards the presence of adulteration/contamination in this study adulteration with low grade fennel seeds in which *trans*-anethole is a major marker compound. This study may be helpful for the spice industry to detect the adulteration of fennel seeds in cumin seeds more specific to cumin powder adulterated with low grade fennel seeds where physical and other chemical analysis have their own limitations. Further development of this method is required for the quantification of *trans*-anethole in the cumin-fennel mixture. Essential oil compositions of spices may be a future tool for predicting adulteration/contamination, use of documented essential oil profiles of spices and study on chemotypes may be carried out for justification of volatile compounds in the spice essential oils and their uniqueness.

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