

Chemical Profiling of Roasted Mustard Oils of Khokana, Nepal

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Authors' contributions

This work was carried out in collaboration among all authors. Author SD did the bench work. Authors SD and SK designed the study and prepared the manuscript. Authors SD and PS analyzed the GC-MS of all samples. Author AA supervised the whole research. All authors read and approved the final manuscript.

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ABSTRACT

Background: Khokana, commonly known as “the living museum” of Nepal is famous for “the roasted mustard oil”. People have been using oil for a long time ago and it is trusted that roasted mustard oil has many health benefits. Detail chemical profiling of roasted mustard oil of Khokana has not been reported yet.

Objectives: Detail chemical profiling of roasted mustard oil and chemical variations in different seeds available for roasting.

Methods: Three different roasted mustard oils (Nepali, Indian, and other origins seeds) were taken for chemical profiling of oil. The GC/MS of all samples was analyzed by the gas chromatography-mass spectrometer Shimadzu GCMS-QP2010 Ultra.

Results: The GC/MS of all samples were carried out and the GC-MS analysis revealed that Nepali (brown seed) and other origins (yellow seed) sample showed erucic acid as a major compound with almost 40-50%. Nepali oil showed *gamma*-tocopherol (<1%) which is a potent antioxidant. Whereas Indian mustard (black seed) oil showed cis-oleic acid as a major compound with 50-60% and Erucic acid was below 1% in Indian seed oil.

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1. INTRODUCTION

Khokana, a traditional Newar town 8 kilometers south of Kathmandu commonly known as “the living museum” of Nepal, carries its own historic and cultural values. It was nominated for the world heritage list by UNESCO in 1996, what made Khokana the living heritage is “the roasted mustard oil”. Roasted mustard oil is widely used for newborn infants, dietary, blood pressure, and cholesterol control [1]. There is no history which tells when Khokana began to produce roasted mustard oil, but the village is very popular for the item. From birth to death and other religious rituals, people use mustard oil dregs to purify and qualify for the functions [2]. The oil mill is like a real-life museum on the identity of the Newar civilization, its culture, tradition, and its ethnic identity and uniqueness [3]. Mustard oil is fatty vegetable oil and is dark yellow and tastes slightly pungent. Being one of the edible oils, it is widely used for cooking as it is beneficial for the heart and also for a therapeutic massage [4].

There are approximately 40 different varieties of mustard plants, but the three principal ones which also vary in color and most commonly used are black mustard (*Brassica nigra* L.), Oriental, brown or Indian mustard (*B. juncea* L.), and white/yellow mustard (*Sinapis alba/B. hirta*), all belonging to the Brassicaceae family (formerly called Cruciferae) [5]. Canada is currently the largest producer of mustard seeds, but seeds can also come from other countries including the United States, Hungary, Great Britain, and India [6]. Oils are used as cooking oils and ingredients in a variety of foods [7]. Mustard oil is naturally dark colored oil. Carotenoids and chlorophyll are the main color pigments in vegetable oils. Oxidation of these compounds or reactions of oxidized triglycerides with carotenoids could cause oil darkening. Another factor is the lipase enzyme which affects the taste, color, and aroma of the fatty oils by breaking the ester bond. The oil is consumed after extraction from mustard seeds, generally without any further processing. Mustard oil is unique amongst all the fatty oils for its pungency due to the presence of allyl isothiocyanate [8]. The roasting of the seeds may cause the formation of volatile compounds such as pyrazines, which are responsible for the pleasant roasting flavor [9].

Roasted mustard oil is a very common and popular oil in Nepal [10]. Mustard oil contains a

high amount of selenium and magnesium, which gives it anti-inflammatory properties. Mustard is not just edible oil also an important medicine in the indigenous Ayurveda system of healthcare. It is used for therapeutic massages, muscular and joint problems. Oil with garlic and turmeric is used for rheumatism and joint pains. Mustard oil is also used as a mosquito repellent [11]. Rapeseed oil is a major source of vegetable oil in the world. Its production is estimated at 27.71 million metric tons, the third just behind palm and soybean oil, supporting the 2018 official data of the United States Department of Agriculture [12]. It contains around 1.5–6% palmitic acid, around 0.5–3.1% stearic acid, around 28% oleic acid, around 11–23% linoleic acid, and around 5–13% linolenic acid [13]. However, neither there is any research has reported on the chemical analysis of roasted mustard oil of Khokana nor its composition variation. So, in the present study, the detail chemical composition of roasted mustard oil and chemical variations in different seeds available for roasting are reported.

2. MATERIALS AND METHODS

2.1 Sample Collection

Three different types of mustard oils were collected from local Mills of Khokana (50 mL each) and named as A, B, and C. Sample A is a brown Mustard seed from local places of Nepal (Khokana, Sankhu.). Sample B is a black mustard seed imported from India and Sample C is yellow mustard that is imported from Canada, Russia, Australia, etc. for comparative study. Separate oil samples of 3 frequently available mustard oils were collected to perform GC-MS and find chemical variations.

2.2 Processing of Mustard Oil

First, the mustard seeds are shifted in a large grinder/Crusher. Next, the mustard flour is slowly roasted in a large pan over an open hearth. After the mustard flour is poured and a small quantity of water in a pouch between two horizontal beams. Next, the workers push the heavy wooden beams toward each other to crush the roasted mustard flour. Finally, they gather the oil that seeps out in a large bowl under the pouch.

2.3 Analytical Condition for GC/MS

GC/MS analysis was performed on the gas chromatography-mass spectrometer Shimadzu GCMS- QP2010 Ultra under the following condition: GC-MS using an Agilent 6890 GC with an Agilent 5973 mass selective detector (MSD), operated in the EI mode (electron energy = 70 eV), with scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was a ZB5ms fused silica capillary with a (5% phenyl)-polydimethylsiloxane stationary phase, a film thickness of 0.25 μm , a length of 60 m, and an internal diameter of 0.25 mm. The carrier gas was helium (80 psi) with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. The injector temperature was 260°C, and the detector temperature was 280°C. The GC oven temperature was program was as used as follows: hold a 40°C initial temperature for 10 min; followed by an increase in temperature at 3°C/min to 200°C, and then increased at 2°/min to 260°C. A 5% w/v solution of each sample in CH_2Cl_2 was prepared, and 1 μL of the sample was injected using a 30:1 split ratio.

Identification of compounds was based on the retention indices determined by reference to a homologous series of n-alkanes and by comparison of the mass spectral fragmentation patterns with those reported in NIST 14, NIST 17, FFNSC (1,2,2,3), Wiley 10, and PRABODH libraries. The percentages of each component were determined as raw percentages based on total ion current without standardization [14,15].

2.4 Derivatization of Oil for GC/MS

0.5 N methanolic NaOH solution was prepared (0.20 g) of NaOH was weighed in a clean and dry 250 mL Erlenmeyer flask and added 10 mL methanol. The sodium methoxide mixture was dissolved by heating a gently in flame hood. It was labeled and parafilm until future use. 0.15 g of oil was weighed in a clean and dry Erlenmeyer Flask and added 4 ml of sodium methoxide solution. The mixture was swirled and added to a hot water bath set at 210°C for 10 min. It was then taken off from the water bath and added boron trifluoride methanol solution and allowed to boil for two min. The solution was then transferred to a 10 mL Volumetric Flask and then added enough brine (saturated sodium chloride) solution to fill the volumetric flask. It was then kept in 2 mL vial then added a glass insert to sample vials. Finally, four drops of top

layer oil were pipetted out, added DCM, and shaken well then, the sample was run using 110 min GC-MS [16].

3. RESULTS AND DISCUSSION

3.1 Chemical Profiling of Oil

Three types of roasted Mustard oil showed different chemical compositions. Sample A which is locally available brown mustard seed oil showed Erucic acid 46.97%, following Linoleic acid 12.75%, cis-Oleic acid 11.99%, and cis-11-eicosenoic acid 7.09%. whereas. Sample B which is imported black mustard seed from India showed cis-Oleic acid 61.62% following Linoleic acid 21.24%, and Palmitic acid 4.94%. Sample C which is yellow mustard seed showed Erucic acid 53.45% following cis-Oleic acid 18.53% and Linoleic acid 9.71%.

The below table shows, altogether 43 compounds are identified from 3 different oil samples. Sample A has Erucic acid 47.96%, Linoleic acid 12.75%, cis-Oleic acid 11.99%, cis-11-eicosenoic acid 7.09%, Linolenic acid 3.54% as major compounds. Sample A also contains Cholesterol 0.62% and gamma - Tocopherol (0.07%) which is known as an antioxidant. Sample B contains Linoleic acid 21.24%, cis-Oleic acid 61.62%, Palmitic acid 4.94% as major compounds but contrary to sample A, it contains only 0.02% Erucic acid. Whereas Sample C shows Erucic acid 53.45%, Linoleic acid 9.71%, cis-Oleic acid 18.53%, cis-11-eicosenoic acid 5.03% as major compounds.

The GC/MS analysis showed that mustard oil has a special fatty acid composition, it contains about 20–28% Oleic acid, 10–12% Linoleic, 9.0–9.5% Linolenic acid, and 30–40% Erucic acid. The high Erucic acid content of mustard seed could be reduced by breeding, some low Erucic acid content genotypes are in cultivation in several countries. These traditional mustard varieties contain 22–60% of Erucic acid and are rich in glucosinolates [17]. In Nepal, High Erucic Mustard (HEM) seed is generally roasted before oil extraction for its typical flavor. In most parts of the world, high erucic traditional varieties have been replaced by new varieties, developed by different breeding practices, containing low Erucic acid (<2%) [11]. Therefore, the consumption of HEM seed oil is very limited, only in some specific areas of the world, particularly in Nepal and India [18]. Roasting of mustard seed and rapeseed are known to produce a potent radical scavenger [17].

Table 1. Chemical composition of roasted mustard oil of Khokana

SN	Compounds	RI	RT	Roasted Mustard Oil		
				A	B	C
1	Myristic acid	1770	45.241	0.02	0.05	0.02
2	trans-7-Methyl hexadecenoate	1803	48.23	-	0.01	-
3	Pentadecanoic acid	1869	48.792	-	0.02	-
4	cis-Hexadecatrienal	1886	51.073	0.01	0.06	0.01
5	trans-Hexadecantriennial	1891	51.24	0.02	0.08	0.03
6	cis-7-Hexadecenoic acid	1899	51.336	-	0.04	0.02
7	Palmitoleic acid	1953	51.495	0.09	0.2	0.08
8	Palmitic acid	1970	52.269	1.76	4.94	1.92
9	cis-10-Heptadecenoic acid	2073	54.697	0.02	0.14	0.01
10	Heptadecanoic acid	2086	55.47	0.02	0.09	0.02
11	Linoleic acid	2140	57.816	12.75	21.24	9.71
12	Linolenic acid	2143	57.945	3.54	-	-
13	cis-Oleic acid	2098	58.081	11.99	61.62	18.53
14	trans-Oleic acid	2104	58.139	1.09	2.98	1.1
15	Stearic acid	2158	58.654	1.72	2.29	1.28
16	Oxacyclononadec-6-en-2-one	2167	59.315	-	0.02	-
17	1-Docosene	2194	60.778	-	0.08	-
18	10-Nonadecenoic acid	2256	60.864	0.01	0.06	-
19	cis-11,14-Eicosadienoic acid	2288	63.546	0.96	1.5	0.25
20	cis-11-eicosenoic acid	2294	63.823	7.09	0.04	5.03
21	cis-13-Eicosenoic acid	2305	63.913	-	-	1.09
22	Arachidic acid	2359	64.462	1.62	0.73	1.05
23	4,8,12,16-Tetramethylheptadecan-4-olide	2364	64.993	0.11	-	-
24	1-Tetracosene	2390	66.59	-	-	0.04
25	Heneicosanoic acid	2428	67.163	0.04	0.02	0.04
26	cis-13,16-Docasadienoic acid	2492	69.038	0.27	-	-
27	1-Pentacosene	2496	69.21	-	0.69	-
28	Erucic acid	2524	69.698	46.97	0.02	53.45
29	Behenic acid	2569	69.982	2.72	0.55	1.64
30	Tricosanoic acid	2632	72.355	0.13	-	0.1
31	1-Heptacosene	2684	74.296	-	0.35	2.67
32	cis-15-Tetracosenoic acid	2709	74.308	4.71	-	0.1
33	Lignoceric acid	2760	74.84	1.75	0.36	0.96
34	Squalene	2819	76.782	-	-	0.06
35	Pentacosanoic acid	2822	77.139	0.05	0.02	0.03
36	Nonacosane	2898	78.719	0.02	0.03	-
37	Hexacosanoic acid	2940	79.427	0.1	-	0.04
38	gamma -Tocopherol	3074	82.041	0.07	-	-
39	Brassicasterol	3156	84.635	-	0.16	0.14
40	Cholesterol	3175	85.935	0.16	0.62	0.15
41	Angelicin	3197	87.709	0.18	0.88	0.43
42	Fucosterol	3305	88.126	-	0.06	-
43	Stigmast-4-en-3-one	3435	90.203	0.01	-	-

RT*=Retention Time, RI*=Retention Index, A*= Roasted brown mustard seed oil, B*=Roasted black mustard seed oil, C*=Roasted yellow mustard seed oil

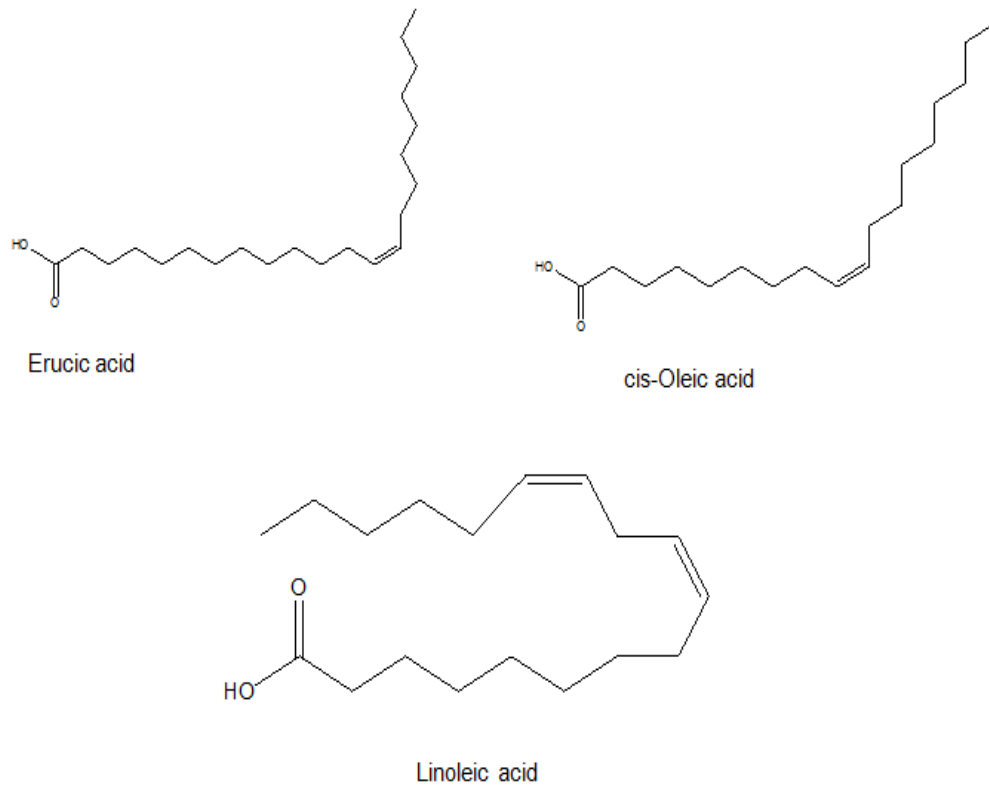


Fig. 1. Some major compounds of roasted mustard oil

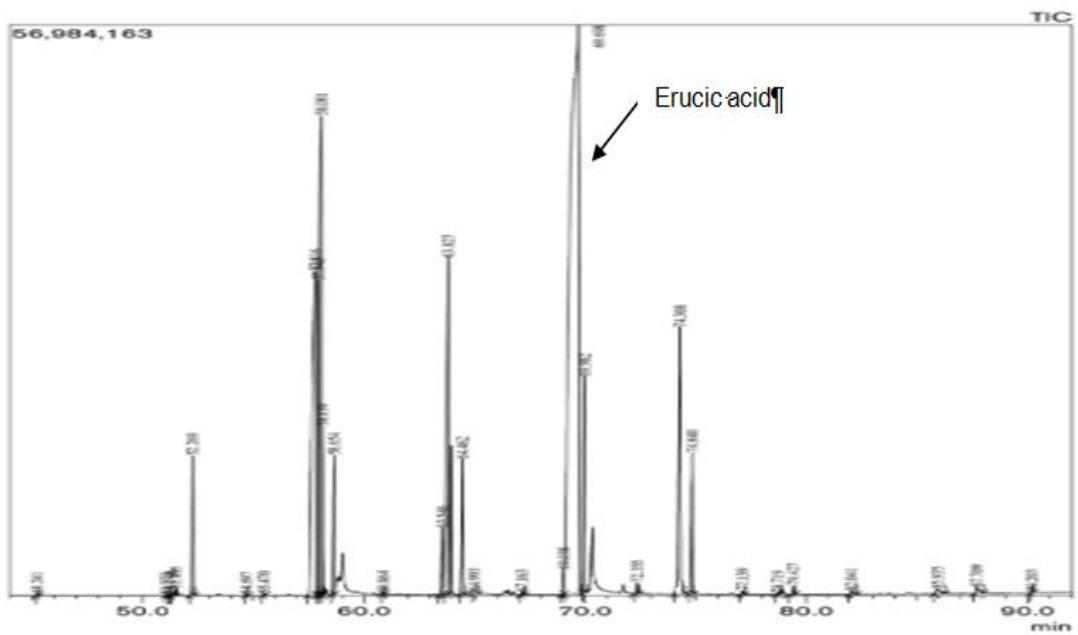


Fig. 2. GC-MS spectrum of sample A (roasted brown mustard oil)

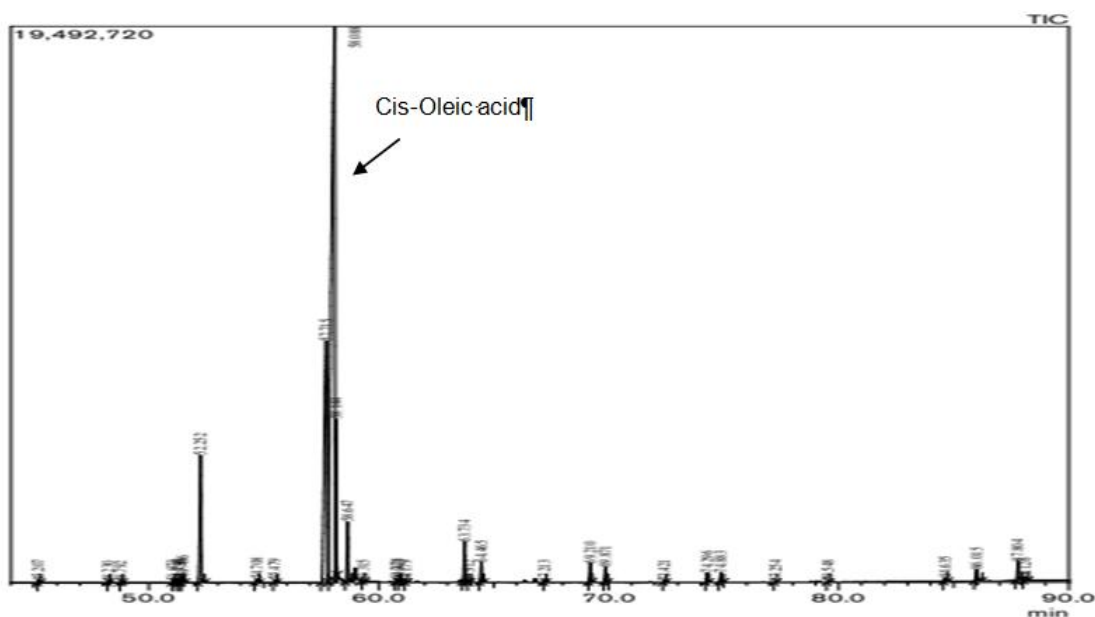


Fig. 3. GC-MS spectrum of sample B (roasted black mustard oil)

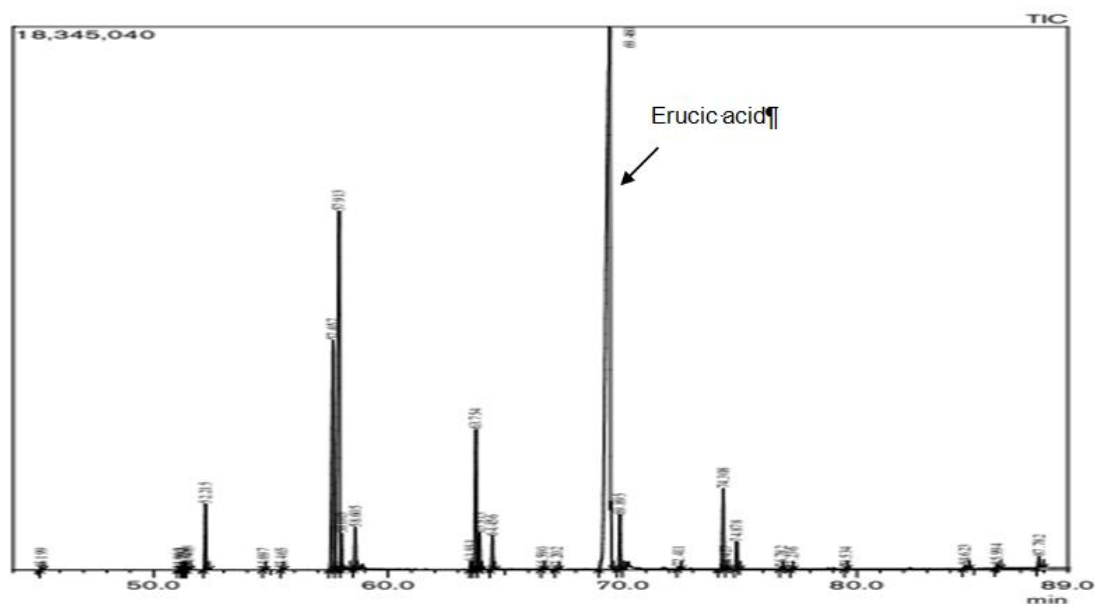


Fig. 4. GC-MS spectrum of sample C (roasted yellow mustard oil)

4. CONCLUSION

The chemical composition of roasted mustard oil of Khokana was determined by GC/MS which has not been reported yet. Sample A from Nepal, roasted brown mustard oil showed Erucic acid as a major compound with other minor constituents. Sample B, whose seeds were imported from India, contains low Erucic acid i.e

less than 1%, and cis-Oleic acid as a major compound with almost 50-60% whereas, Sample C, which is roasted yellow mustard oil from various nations showed Erucic acid as the major compound from the traditional procedure of roasting. So, the present study provides the fatty acid profile of all available mustard seed oils and variation within each fatty acid, which will be useful in evaluating fatty acid intake of the

population. It further suggests that roasted mustard oil of Khokana contains omega-3, omega-6, and omega-9 fatty acids which are essential constituents and have a crucial role in our health.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

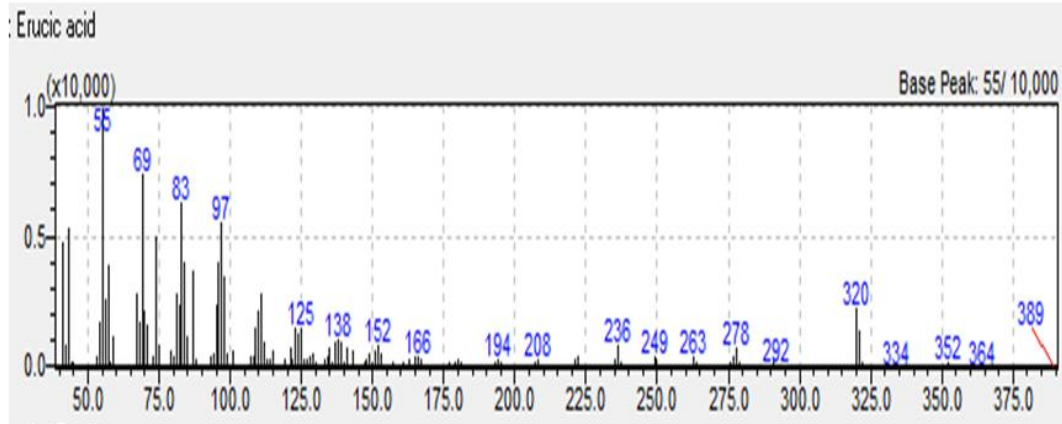
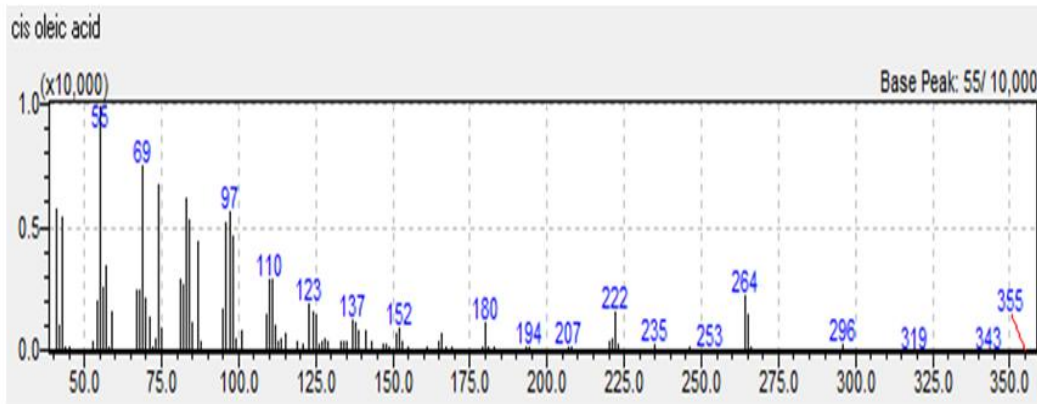
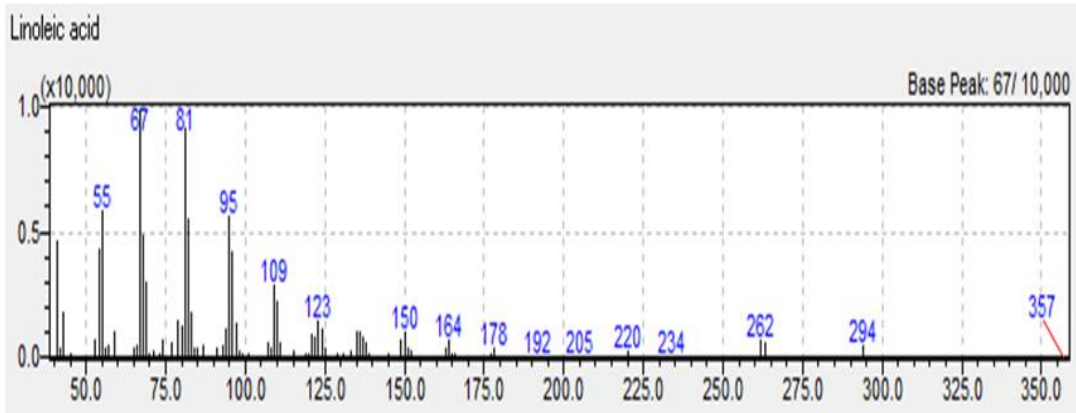
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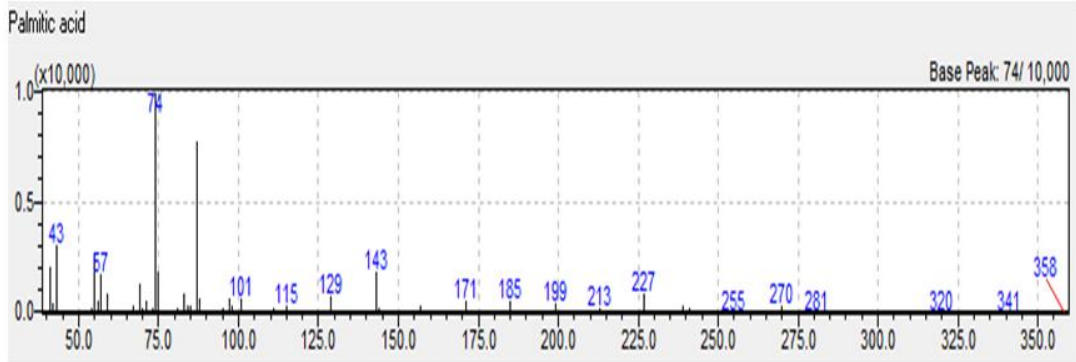
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APPENDIX

Chromatogram of Some Major components of Roasted Mustard Oil





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