



# A STUDY ON ANALYSIS OF FATTY ACID PROFILE IN MUSTARD OIL IN INDIA

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**Abstract:** Mustard seeds are world wide important for its use as edible oil and industrial purposes. They contain both saturated and unsaturated fatty acids. The MUFA and PUFA present in mustard oils play a significant role in cholesterol levels on consumption. The presence of long chain fatty acid especially erucic acid is an important factor for accumulation of cholesterol leading to Cardio vascular diseases. The percentage of fatty acids present varies within the species. The objective of the study was analysis for the fatty acid profile in mustard oil in India. The fatty acids were analysed by Gas chromatograph- Agilent make-model-7890B with the method from FSSAI lab manual oils and fats- No-FSSAI 02.019:2021. The percentage of Saturated Fatty Acids (SFA) varied from 6.48 to 25.49, Mono Unsaturated Fatty Acids (MUFA) from 47.29 to 64.99 and Poly Unsaturated Fatty Acids (MUFA) from 19.72 to 39.7. The percentage of Methyl erucate (C22:1) varied from 12.69 to 46.63 from the 26 nos. of analysed samples.

**Key words:** Mustard oil, C22:1-Methyl erucate, SFA, MUFA, PUFA

## 1. INTRODUCTION:

Mustard seeds are known with the genus Brassica and widely cultivated for oils for edible and industrial use. The Fatty acids present in the mustard seeds may vary from species to species. The mustard oil is obtained by pressing the seeds to have a characteristic bright and pungent oil. The oil is commonly used for cooking and to add a hot and spicy flavor to food [1]. Brassica species contain saturated and unsaturated fatty acids. Saturated fatty acids technically contain single bond between the chain along their fatty acid chains, whereas Mono Unsaturated Fatty acids are essentially fat molecules that contain one (mono) unsaturated carbon and Poly unsaturated fatty acids contain two or more unsaturated carbon atoms.

The saturated fatty acids (SFAs) includes Palmitic acid whereas, the unsaturated fatty acids are erucic acid and oleic acid (mono unsaturated Fatty acids) or polyunsaturated fatty acids (PUFAs) like linolenic and linoleic acid. The availability of these fatty acids indicates the nutritional quality of the edible oils [2]. Fats intake by food should contain saturated, monounsaturated and polyunsaturated fatty acids. Saturated acid should cover <10% of the available energy from food, polyunsaturated acids n-6, 4 – 8% on average, about 5% of MUFA[3]. Mustard seed oils are characterized by significant Quantity of long-chain MUFA, mainly erucic acid (C22:1), which is known for antinutritional property[4]. and are absent in any commercial oil[5]. Higher erucic acid in cooking oil hampers the myocardial conductance in humans and leads to increased blood cholesterol levels [6,7]. Several genotypes of mustard oils are higher levels of erucic acid, which can be utilized only for industrial purposes. PUFAs are the precursors of long chain fatty acids which involves in synthesis of hormone prostaglandins. The essential fatty acid Linoleic acid is not synthesized in human body and to be obtained from diet alone. It has been reported high linoleic acid levels in edible oil reduces blood cholesterol and prevents atherosclerosis [8].

The objective of the study involved in the analysis of fatty acid composition of mustard oil available in the market all over India.

## 2. MATERIALS AND METHODS:

### 2.1. Sample Collection

A total of 26 samples of mustard oil were collected from various regions of India.

### 2.2. APPARATUS AND REAGENTS

The apparatus and reagents included adequate nos. of 24/29 joint 100ml conical flasks, Air condensers, Centrifuge tubes of 50 ml capacity, water bath, weighing balance, centrifuge, vials, micropipette – 5ml capacity and Gas chromatograph- Agilent make- model-7890B. FAME 37 mix standard CRM, Methanolic HPLC grade, sodium hydroxide, methanolic boron Tri fluoride, Heptane.

### 2.3. METHOD:

100mg of mustard oil was weighed separately in the concerned labelled 100ml conical flask. 10 ml of 0.5 N Methanolic sodium hydroxide was added and the conical flask was fit with the air condenser. The setup was placed with the water bath for 45 minutes. The samples are saponified. 10ml of 14% methanolic BF<sub>3</sub> was added after cooling the above saponified samples. Then each samples was placed in fuming chamber for 30 minutes to volatile the BFW. Then the solution is transferred to the 50mL Centrifuge tube. The Conical flask was rinsed with 10-15 mL of Saturated NaCl Solution and added the same in the centrifuge tube. 5ml of heptane was added in the centrifuge tube. The centrifuge tubes were vortexed for a minutes and then Centrifuged at 5000 rpm for 5 minutes. Upper Hexane layer of the centrifuged samples filtered through 0.45d filter paper and transferred to the 1.5 mL vial. The labelled sample filed vials kept on Auto sampler of GC. The individual fatty acids present in the samples were read with peaks obtained in GC under the following instrumental conditions.

### 2.4 INSTRUMENTAL CONDITION

The following conditions were adopted at **Central Agmark Laboratory, Nagpur** in GC during the analysis of Fatty acid profile in mustard oil samples

**Table-1**  
**INSTRUMENTAL CONDITIONS**

S.NO.	DESCRIPTION	DETAILS
1	Oven temperature	240°C
2	Initial temperature	60 C
3	Intial temperature Hold time	1 minute
4	Ramp rate	4°C /minute
5	final temperature	220°C
6	final temperature Hold time	20 minutes
7	Carrier gas	nitrogen
8	Flow rate	1ml per minute
9	Average Velocity	26.511 cm/Sec
10	Column	DB-225 ( dimensions- 30m x 250µm x 0.25 µm).
11	Injection Volume	1µL (Auto sampler)
12	Septum purge flow	3 mL/min with a total flow of 34 mL/Min
13	Mode	Split
14	Split ratio	30:1 at the flow of 30mL/Minute in the Split less, single Tapper, wool, ultra Liner (Agilent 5190-2293:900µL).
15	Detector	FID
16	Heater temperature	300°C
17	Hydrogen flow rate	40mL/Min
18	Air flow rate	400 mL/Min
19	Makeup flow	25ml/min

### 2.5 CALCULATION

The percentage of each fatty acid for the 26 nos. of mustard oil samples were calculated as per the area obtained in the chromatogram by the formula given below.

$$\% \text{ of Fatty acid Component} = \frac{A_i}{\sum A} \times 100$$

$A_i$  = Area of desired fatty acid

$A$  = Total area of Fatty acid Component

### 3 METHOD VALIDATION:

The method validation for this study on Fatty Acid profile was carried out using FAME standard with reference to C22:0 and C22:1n9 standards. Table -2 shows the method validation of fatty acid profile with the FAME mix.

**TABLE-2 METHOD VALIDATION OF FATTY ACID PROFILE WITH FAME MIX**

S.No.	Description	Acceptance Criteria	Observation
1	<b>A. C22:0 standard</b>		
	Linearity Range	From calibration curve	0.9-66.87
	Regression	NLT 0.99	0.9998
	LOD(ug/ml)	From calibration curve	1.36
	LOQ(ug/ml)		4.12
	<b>B. C22:1n9 standard</b>		
	Linearity Range	From calibration curve	41.53-2734.56
	Regression	NLT 0.99	0.9998
	LOD(ug/ml)	From calibration curve	61.21
	LOQ(ug/ml)		185.49
2	Precision(%RSD)	NMT 20%	
	C22:0		6.85
	C22:1n9		1.71
3	Recovery	70%-120%	
	Spike-A		73.04
	Spike-B		83.98
	Spike-C		85.59
	Spike-D		85.27
	Spike-E		86.08

### 3. Results and Discussion

Table 3 represents the percentage of SFA, MUFA, PUFA and methyl erucate in mustard oil samples.

**TABLE- 3 PERCENTAGE OF SFA, MUFA, PUFA & METHYL ERUCATE IN MUSTARD OIL SAMPLES**

Sl. No	Code No	SFA	MUFA	PUFA	% of Methyl Erucate
1	FSP-420	7.07	63.87	29.06	45.55
2	FSP-421	7.2	64.44	28.37	45.15
3	FSP-422	6.48	64.96	28.56	44.43
4	FSP-444	7.23	64.82	27.95	45.35
5	FSP-445	10.44	50.68	38.88	13.61
6	FSP-446	10.16	50.14	39.7	12.69
7	FSP-447	7.18	62.86	29.96	43.38
8	FSP-448	6.76	64.26	28.98	44.03
9	FSP-589	6.92	63.31	29.77	46.3
10	FSP-590	7.2	64.48	28.31	46.63
11	FSP-610	7.12	64.73	28.15	44.23
12	FSP-611	7.09	64.69	28.22	43.94
13	FSP-612	7.21	64.21	28.58	44.71
14	FSP-613	18.34	47.29	34.37	14.8
15	FSP-617	7.2	64.35	28.45	44.72
16	FSP-618	25.9	54.37	19.72	32.17
17	FSP-620	11.51	55.43	33.06	28.92
18	FSP-644	7.26	64.34	28.4	43.86
19	FSP-645	9.04	61.95	29.01	40.39
20	FSP-646	7.83	63.09	29.08	42.34
21	FSP-647	8.48	61.85	29.67	43.98
22	FSP-648	7.23	64.99	27.79	45.61
23	FSP-649	7.45	63.58	28.97	45.83
24	FSP-754	9.68	62.43	27.89	43.05
25	FSP-755	7.36	63.53	29.11	46.38
26	FSP-756	7.49	63.71	28.8	45.07

The observation from the study conducted at Central Agmark Laboratory, Nagpur was the percentage of SFA varied from 6.48 to 25.49. It is also observed that SFA was between 5- 10% in 21 samples accounting 80.77% of the analysed

samples. MUFA of the analysed samples ranged between 47.29 to 64.99%. 21 samples (80.77 per cent ) had MUFA values between 61-70%. and PUFA values ranged from 19.72 to 39.7% of the analysed samples. 80.77 % of the samples (21 samples) had PUFA values between 21-30%.

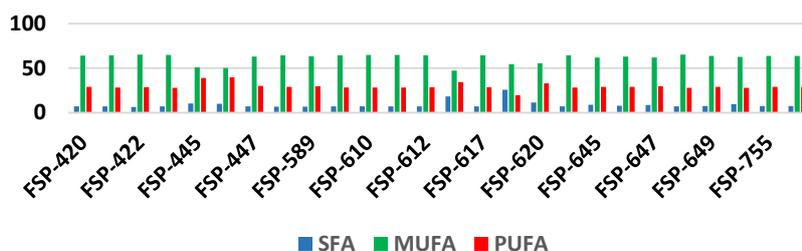
The oil samples collected over India as mustard oil. The mustard oil available is generally addressed as mustard oil/Rape seed oil in India and extracted from the Brassica species. A large number of species and sub-species of oilseed are cultivated in India under the name rapeseed/mustard seed, including Rai, Torah, Brown Sarson, Yellow Sarson, Swedi, Rape/Karan Rai and Taramira. Some of these are hybrid and some locally grown seed varieties[9].The Food standards safety authority of India had specified the percentage of Methyl erucate in mustard oil and rape seed oil as 40.0-58.0 and >2.0-60.0 respectively[10]. The percentage of Methyl erucate (C22:1) varied from 12.69 to 46.63. The percentage of methyl erucate ranged from 40.39 to 46.63 per cent for 21 nos. and 12.69 to 32.17 for 5 nos. The results obtained were comparable with the results of *Brassica rapa* and *B. oleracea* had the highest level of erucic acid 41 %and 46%, respectively[11]. Similarly a study conducted in Poland during 2020 in 2 cultivars of white mustard ‘ Borowska’ and ‘Bamberka’ indicated the mean C22:1 was 22.22±0.09 and 3.80±0.02 for the species respectively[12].

The study conducted at Turkey for 57 mustard seed genotype during 2018 revealed that the average of methyl erucate was 31.53 (31 cultivars of *B. juncea*), 37.56 (6 cultivars of *B. rapa*), 9.54 (2 cultivars of *B. napus*), 10.20( 6 cultivars of *B. nigra*), 12.36 (10 cultivars of *B. arvensis*), 8.65 ( 2 cultivars of *B. alba*). The study also revealed that BJ-A20-India of *Brassica juncea* has maximum percent of total polyunsaturated fatty acid; BA7-Turkey, Şereflikoçhisar of *Brassica arvensis* was selected as promising genotype in terms of saturated and monounsaturated fatty acid contents[13].

The Methyl erucate content in *Brassica* species varied from 0.80- 49.40% in *B. juncea* varieties, 10.04- 34.96% in *B. napus* varieties and 43.77- 49.99% in *B. rapa* varieties . The PUFAs i.e. linoleic and linolenic acids should be present in lower levels in the cooking oil. The amount of linoleic and linolenic acids varied between 11.00- 45.30%, 11.10- 26.72%; 18.57- 26.93%, 9.99- 17.23% and 14.08- 18.18%, 9.82- 26.66% in *B. juncea*, *B. napus* and *B. rapa* genotypes, respectively [4].

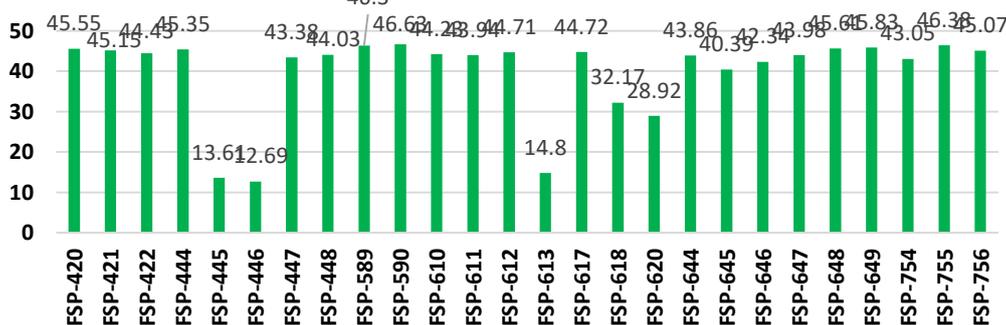
**GRAPH-1**

**FATTY ACID PROFILE OF THE MUSTARD OIL SAMPLES**

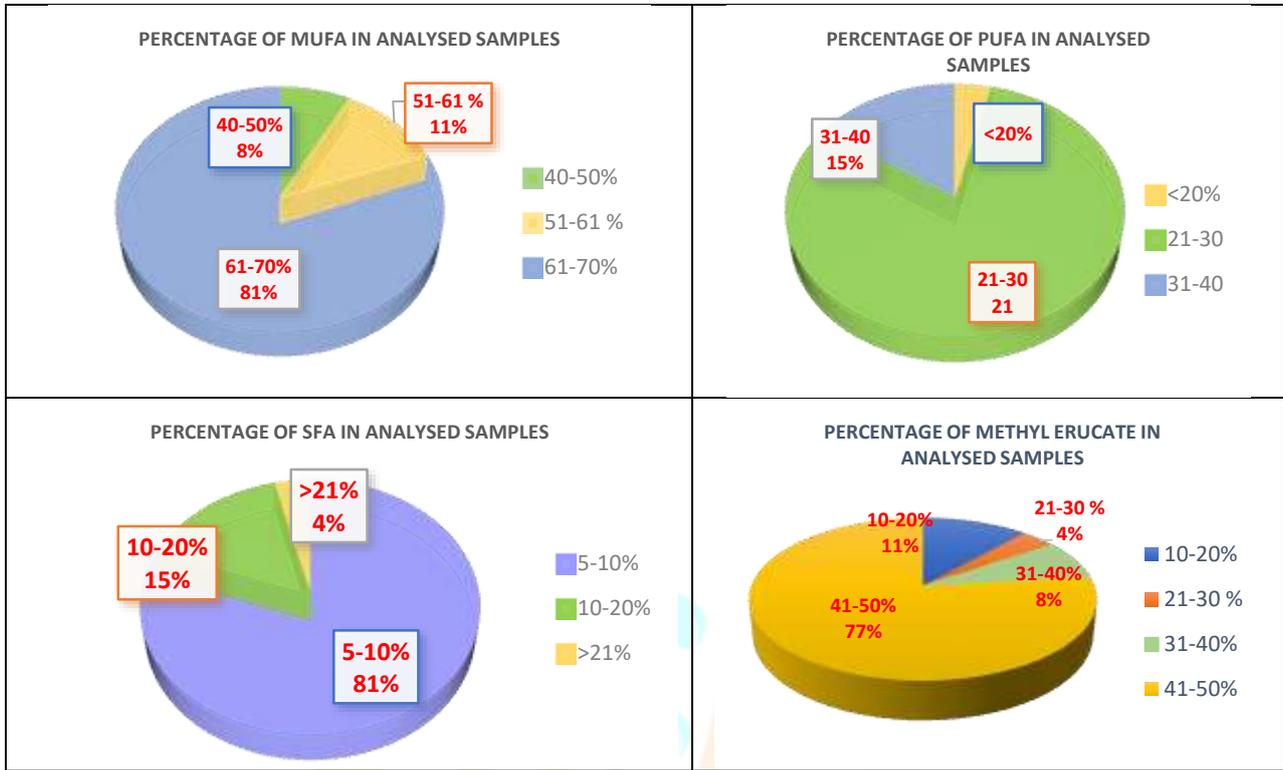


**GRAPH-2**

**PERCENTAGE OF METHYL EURACATE IN THE MUSTARD OIL SAMPLES**



**Graph-3 Percentile range of Fatty acids in the analysed mustard oil samples**



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#### 4. Conclusion:

The sample received for the study at Central Agmark Laboratory, Nagpur were from various regions of India. The Food standards safety authority of India had specified the percentage of Methyl erucate in mustard oil and rape seed oil as 40.0-58.0 and >2.0-60.0 respectively. The percentage of methyl erucate ranged from 40.39 to 46.63 per cent for 21 nos. and 12.69 to 32.17 for 5 nos. . The increase in methyl erucate along with other samples as directly proportional to MUFA content of the analysed vegetable oils. 21 Nos. of mustard oils had MUFA content more than 61.5 per cent.

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