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Physico-chemical investigations of Mustard seed (*Brassica juncea L*)

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Abstract- The objective of the study was to determine the physicochemical characterization and fatty acids composition. Oils are the most important lipids found in nature. They are one of the three major 'food factor' needed for human body, the other two being proteins and carbohydrates. Oils are widely distributed in foods and are of great nutritional value. Physicochemical properties like moisture content, saponification value, acid value, iodine value, peroxide value, specific gravity, color, USM, RI, mineral oil, argemone, castor of mustard oils were studied to evaluate the compositional quality of oils and also to investigate the effect on the use of same oil for repeated frying as it ultimately changes the physicochemical, nutritional and sensory properties of the oil. Fatty acid analysis of the mustard oils was carried out using a Gas Chromatograph equipped with a Flame Ionization Detector (FID) and stainless steel packed column. The analysis of fatty acids showed 3.364 % of saturated and 29.275 % of polyunsaturated acids, with 14.505 % of linoleic (C18:2), 11.987 % of -linolenic (C18:3) and 48.586 % of erucic acid (C22:1) and the highest percentage of long chain mono and polyunsaturated fatty acids (96.636%).

***Keywords*:** Mustard seed (*Brassica juncea L),*Physico-chemical, fatty acid profile

I. INTRODUCTION

Brassica juncea L is an important Rabi season oilseed crop. Belongs to family Cruciferae and genus Brassica [1]. Mustard seed is the world's second leading source of vegetable oil, after soybean. It is also the second most leading source of protein meal in the world after soybean. It is mainly grown in northern part of India, Rajasthan is the largest producing state followed by Uttar Pradesh [2].

Brassica juncea is an economically important plant that has been well-known in India for centuries for its medicinal and nutritive values [3]. Food preparation of Indian mustard leaves is helpful in lowering the cost for diabetic patients suffering with comorbid anxiety disorders [4]. Oils are being used in various medicinal formulations for years due to their non-toxic effects and pharmaceutical preparations like capsules, creams, emulsions, fragrances, flavors, intramuscular injections, nasal sprays, ointments, plasters, and in a number of cosmetics [5].

II. MATERIAL AND METHODS

Seed samples (5 kg) of species, *Brassica juncea L* were purchased from the local market of Kairwara, Alwar. The seed samples were further identified and authenticated by agriculture supervisor.

Extraction of mustard oil

The soxhlet techniques of extraction of mustard oil were employed using soxhlet extraction method (hot process).
30 g of the powdered mustard seed samples were packed into a thimble, and extracted thrice with 100 ml each n-hexane by reflux using soxhlet extractor for 10 hour. The solvent partitioning of crude mustard extract was carried out with n-Hexane.

Determination of moisture [6]

Moisture content of mustard sample was determined by a hot air oven. Empty aluminum moisture dish was weighted (W1) and 10 g sample was taken in a moisture dish and weighted (W2). The sample was spread evenly and placed without lid in oven and dried samples one hour at 105 °C temperature. The dishes were transferred to desiccators to cool. Aluminum dish was weighed after cooling (W3).

Saponification value [6]

At first, 2 g oil was taken in 250 ml round bottom flask and 25 ml, 0.5 N alcoholic potassium hydroxide solution added in same flask. The flask was fitted with a long air condenser and heated solution at reflux temperature about 30 minutes. Finally, the flask was cooled and added 1 ml of 1 percent phenolphthalein solution and titrate the excess of the alkali against standard 0.5 N HCl. At the same time and under similar conditions carry out a blank titration without fat (25 ml, 0.5 N same alcoholic KOH solution was taken in another

round bottom flask and heated in a similar way and titrated, against 0.5 N acid).

Iodine value [6]
5 g of oil was taken into 200 ml a glass stoppard bottle. 5 ml of CCl_4 was added to dissolve this oil after 25 ml of Wij's solution was added and to allow it at least 1 hour in a dark place. Then 5 ml of 10% potassium iodide solution and 50 ml water were added to each bottle and titrated against 0.1N Na2S2O3 solution using starch solution as the indicator, near the end point of titration the color of solution becomes pale yellow. Blue color disappears which indicates the end point. At the same time and under similar conditions carry out a blank titration without oil.

Peroxide Value [7]
The peroxid value is a measure of the concentration of substances that oxidize potassium iodide to iodine. 5 g weight of oil samples is dissolved in acetic acid then chloroform and saturated KI mixture are added to the sample and the amount of iodine liberated from KI by the oxidative action of peroxides present in the oil is determined by titration with 0.1 N sodium thiosulphate using starch solution as an indicator. Titration was also performed for blanks.

Acid Value [7]
About 5 g of oils were taken in 250 ml conical flask. Then 25 ml of neutral ethyl alcohol was added to it and then boiled on water bath. Phenolphthalein indicator solution (1-2 drop) was added then the mixture while hot was titrated against with standard potassium hydroxide solution with shaking. The end point was noted to the first pink colour which persist for 30 seconds.

Determination of Specific Gravity, Refractive Index, Colour, Unsaponifiable Matter, Test For Presence of Mineral ,argemone and castor oil.

The test for presence of mineral, argemone and castor oil, specific gravity, refractive index, colour, unsaponifiable matter, iodine value was determined by the manual of methods of analysis of food, fssai, 2015. [8].

Chromatography
Nuckon – 5700 gas chromatograph equipped with a flame-ionization detector (FID) and glass column, digital controls, separate independent proportional temperature controllers for oven, detector, Injector, Detector temperature 390 ^{0} C in 10 C steps set by thumbwheel switches, oven temperature 399 ^{0} C in 1 C step set by thumbwheel switches. Temperature programming: - microprocessor based programmer with rate 0.1 ^{0} C – 29.9 ^{0} C per minute with initial time hold up to 99 minute. Dual channel data station, 2 detectors can be operated simultaneously. Fame column packed s.s column of 2 m length packed with 2% sp-2300and 3% sp -2310 on chromosorb w HP-100/120 mesh. Use Nitrogen gas cylinder -*60 psi. Hydrogen gas cylinder -20 psi. Zero air gas cylinder -20 psi.*

Preparation of fatty acid methyl esters (FAMEs):
Take 2 ml oil sample in a graduated cylinder with stopper. to which 10 ml n-Heptane have been added .Mix the content well ,add 3 ml of alcoholic potassium hydroxide and remaining HPLC grade water for making a 25 ml approx. Mixture and keep the mixture for 2 hrs approx with closing it with stopper for making a volatile mixture. 1 µl fatty acids methyl ester was injected, peaks were recorded for their respective RT (Retention time), areas by data processor unit of the gas chromatography.

III. RESULT AND DISCUSSION

The physicochemical tests (refractive index value, specific gravity, iodine value, saponification value, acid value, peroxide value, moisture content, colour, unsaponifiable matter, test for presence mineral, argemone, castor) of Mustard oils are studied to evaluate the compositional quality of oils (table -1).
Mousavi et al., (2012) were used these parameters to monitor the quality of edible oil [9].

Table 1 Physicochemical characteristies of mustard seed oil

Parameter	Result
Moisture Content	0.15 ± 0.03
Acid Value	0.85 ± 0.01
Specific Gravity	0.910
Refractive Index	1.4652
Colour	35.5 Unit
Saponification Value	170.22 ± 0.13
Unsaponifiable Matter	0.56 ± 0.01
Iodine Value	109.14 ± 0.03
Test For Presence of Mineral Oil	Negative
Test for presence of Argemone Oil	Negative
Test for presence of Castor Oil	Negative
Peroxide value	2.84 ± 0.03

Acid value is an important indicator of vegetable oil quality. AV is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1.0 g of oil. Adulteration is important factor which may affect quality of food articles. Adulteration may be defined as substitution of genuine raw material with inferior or toxic materials. In number of cases, adulteration of castor, mineral and *Argemone mexicana* seed oil in edible oils has been reported as cause of epidemic dropsy.

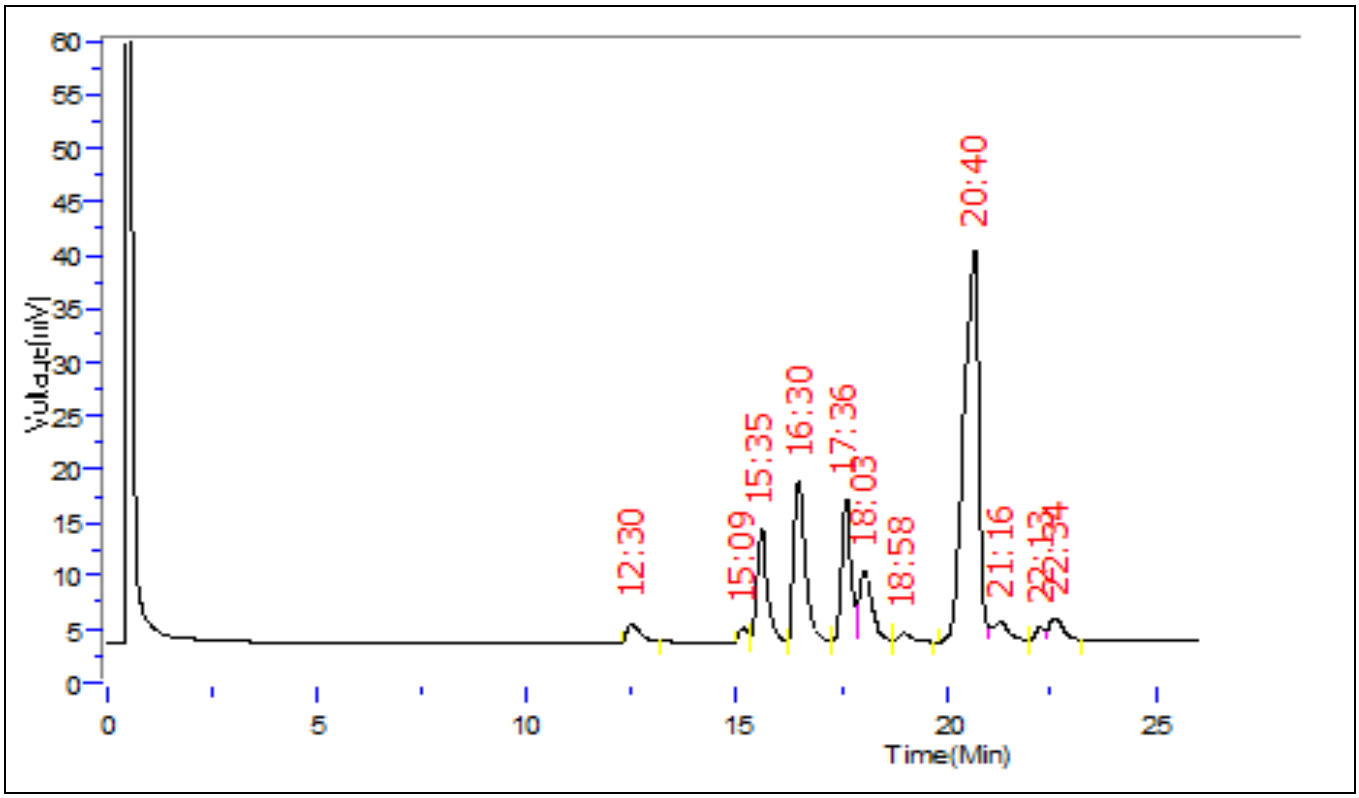

Figure: 1 Gas chromatogram of mustard oil. Description of peaks is as follows: (12:30 Palmitic acid), (15:9 Stearic acid), (15:36 Oleic acid), (16:30 Linoleic acid), (17:36) Linolenic acid), (18:30 Ecosenoic acid), (18:59 Eicosedenoic acid), (20:41 Erucic acid).

Table: 2 presenting the different parameters of the fatty oils of the seeds of *Brassica juncea L*

Peak No.	RT (Min)	Area (mV-Sec)	Height (mVolt)	RF	Amount (ML)	Amount%	Component Name
1	12:30	33.170	1.697	1.000	33.170	1.802	Palmitic
2	15:9	12.307	1.105	1.000	12.307	0.669	Steric
3	15:36	175.445	10.011	1.000	175.445	9.533	Oleic
4	16:30	266.943	14.840	1.000	266.943	14.505	Linoleic
5	17:36	220.591	13.225	1.000	220.591	11.987	Linolenic
6	18:3	128.373	6.488	1.000	128.373	6.976	Eicosenoic
7	18:59	12.434	0.653	1.000	12.434	0.676	Eicosedenoic
8	20:41	894.137	36.455	1.000	894.137	48.586	Erucic
9	21:16	38.776	1.738	1.000	38.776	2.107	Docosedenoic
10	22:14	16.434	1.125	1.000	16.434	0.893	Lignoceric
11	22:35	41.702	2.063	1.000	41.702	2.266	Tetracosenoic

Table 3. The content of SFA, MUFA, PUFA (% w/w). (SFA- Saturated fatty acid, MUFA- Monounsaturated fatty acid, PUFA- Polyunsaturated fatty acids

SFA	MUFA	PUFA	MUFA+ PUFA
3.364	67.361	29.275	96.636

The major fatty acids in mustard oil are palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) and erucic acid. Fatty acids are both important dietary sources of fuel for animals and they are important structural components for cells. Mustard oil has high levels of both alpha-linolenic acid and erucic acid. Erucic acid appears to have toxic effects on the heart at high enough doses. Oleic acid is present in all foods that contain fat and is easily produced by the fatty acid synthetic pathways present in normal human hepatic cells. It constitutes 15% of the fatty acids in erythrocyte membranes. Oleic acid is the principal lipid of a

class that makes LDLs resistant to oxidation and thus a diet rich in this fatty acid reduces foam cell accumulation rates and thereby lowers the chances of atherosclerosis. Many plants produce this polyunsaturated fatty acid, but because of the small amounts of fresh vegetables consumed, it is one of the least abundant of the essential fatty acids in most diets. It is found in flax, hemp, rape (canola) seed, soybean, walnut oils, and dark green leaves and must be supplied by such foods [10].

IV. CONCLUSION

In conclusion, based on the oil positive response of the physicochemical analysis, suggested that Brassica juncea L seed has potential for use alternative source of industrial oil for shampoo, soap making and pharmaceutical creams, capsules, emulsions, fragrances, flavors, intramuscular injections, nasal sprays, ointments, plasters, and in a number of cosmetics. In addition they are also used in large quantities as raw material for bio-resources. The fatty acid profile of Brassica juncea L seed oils were determined while oieic and linoleic are most prominent fatty acids in these oils, they also contain significant amounts of saturated fatty acids. The fatty acid profile and chemical analysis will also contribute to enrich knowledge for further advanced research in the field of phytochemical.

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