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Human Health and Chromatographic Analysis of Untreated and Treated Masturd (*Brassica Campestris*) Oil with Respect to Its Chemical Composition

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ABSTRACT: This study is focused to highlight the role of chemical composition of edible oil on its quality and oxidative stability. The oil of Indian mustard (*Brassica Campestris*) is quite popular in Indian kitchens as cooking medium, frying oil,, pickle oil, hair oil and also for number of different medicinal applications. The chemical composition obtained by gas chromatographic studies suggests that mustard oil has high percentage of Mono Unsaturated Fatty Acids (MUFA) and lower percentage of Poly Unsaturated Fatty Acids (PUFA) which makes it less prone to lipid oxidation and keep it intact from high deteriorations. The result of peroxide values which were determined before and after thermal treatments and longer storage in glass and iron metal container. The results also supports that natural mustard oil extracted by classical methods has good resistance lipid oxidation and reversion & rancidity due to the presence of high MUFA and natural antioxidants and may be a good and rich source of energy for human beings .

KEY WORDS: Anti oxidants, Brassica, Gas chromatography, Mustard, MUFA, Oxidative stability.

I. INTRODUCTION

Edible oils are the most important ingredient of food especially in Indian sub-continent. There are large varieties of edible oils being used nowadays which are extracted from various types of sources. The indigenous oils such as mustard, sesame, groundnut etc. are available easily and extracted generally from oilseeds by classical cold pressing methods as these oil seeds are rich in oil content. The other oils like soybean, sunflower, safflower etc available in the market are extracted by industrial solvent extraction methods [1].

All the edible oils and fats contains mixture of long chain saturated and unsaturated fatty acids in the form of glyceroids along with some other components such as Lignin, Pigments, Sterols and some other nutraceuticals [1-3]. A certain oil has an almost fix percentage of various fatty acids which is called fatty acid content (FAC) of the oil. Unsaturated fatty acids have different number of double bonds varying from oil to oil. So the level and number of unsaturated sites are also varying from oil to oil. Some oils are rich of Mono Unsaturated Fatty Acids (MUFA) and some are of Poly Unsaturated Fatty Acids (PUFA) [2, 4]. The oil composition can plays an important role in the shelf life, nutraceutical content, oxidative stability and hence the oil quality [3-4].

Due to the presence of unsaturation, oils are prone to oxidation as the oxygen radical readily reacts with the double bond and makes peroxides and hydro peroxides. Those peroxides and hydroperoxides then undergo many transformations and form various undesirable products [5]. Those products leads to appearance of unwanted odor, bad taste and hence, deterioration of food product prepared by using those oils[6-7]. The shelf life of oils is also greatly affected by the oxidation [7]. It is not just about bad taste; these products are a threat to human health too. These can be responsible for many diseases as they might lead to formation of toxic compounds [7-9]. Apart from these oxidation products there are some other components also present in oils and fats which can be responsible for several complications in our body which can leads to some Cardiovascular diseases [9-10].

The Gas chromatographic analysis provides the information about the compositional information such as MUFA and PUFA content of the oil. The composition can address the different behavior of oils when they are subjected to

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oxidation. This study explains the oxidative stability of Indian Mustard on the basis of its compositional information obtained by gas chromatography.

II. RELATED WORK

The seed oil of *Brassica compestris* is the most versatile member of oleiferous brassicas. Apart from cooking, this particular oil has its multipurpose applications in Indian house hold as condiment for skin infections. It is also used with table salt for massage to address mouth gum problems. Acording to Shenoliker, there are no evidences found for cardiac lipidosis or long term lesions due to high erucic acid diet in man [11].
Oil from fresh, healthy and dry seeds of *Brassica compestris* contains various intact glucosinolates such as 3-Butyle isothiocynate, 4-Pentenyl isothiocynate, 4-Methyl thiobutyl isothiocynate glucosinolate etc [12]. Also, the oilseeds contain sinapine which enhances the taste of food and non toxic in nature and phytic acid which can chelate to the metal like zinc and can reduce their free availability [12]. The total tocopherol content in mustard species having high erucic acid is observed about 790 mg kg^{-1} into which the α-Tocopherol is 268 mg kg^{-1} & γ- Tocopherol is 426 mg kg^{-1} The tocopherol content works as an efficient antioxidant [1].

III. METHODOLOGY

Mustard oil was collected from Indian mustard (*Brassica campestris)* oilseed by using cold pressing technique called 'Ghani' which does not alter the nutraceutical values of the oil. The chemicals such as methanol, petroleum ether, chloroform, and acetic acid used for methylation and peroxide value were purchased from Merck and are of AR grade. Sodium thiosulphate, cupric sulphate, starch, potassium iodide and potassium hydroxide were purchased from Fisher scientific.

A. Preparation of samples

1000 mL quantity of oil was taken in iron frying container for thermal treatments and treated as follows:

I. 100 mL of untreated oil was taken apart and named as Mu1
II. 100 mL of untreated oil was stored in colored glass bottle before any treatment. This sample was termed as 'untreated stored' and named as Mu2 and kept for making the sample for long storage. (Three months)
III. Slow heating started with gradually increasing temperature up to 200ºC.
IV. 5 batches of 10 potato fries were fried for 1 minute with the gap of 15 minutes to each fry between the temperature ranges of 200-190ºC. A portion of 100mL of the oil was taken out, cooled and placed in a colored glass bottle. This sample is termed as '1h treated' sample Mt3
V. After step IV, the leftover oil was kept on continues slow heating at around 180ºC and on completion of every 1 h, one batch of fries were fried on 200ºC and repeated three times. A sample then collected as the previous manner and termed as '4h treated' Mt4
VI. Remaining oil was used again as the same procedure mentioned in step III on next day i.e. 24 hours later. Collected and termed as '(4+1) h treated'Mt5
VII. After step VI, the oil left in container was kept in the iron container for 10 days without any heating and collected in colored glass bottle termed as 'treated with iron container' Mt6.

B. Preparation of fatty acid methyl ester (FAME)

1 gm of mustard oil sample taken into a round bottom flask and 4 mL quantity of methanol was added to it and refluxed it around 65-80 ºC for 60-90 minutes with continuous stirring on magnetic hot plate. The reaction mixture then taken into a separating funnel and allowed to separate the methylated oil and methanol and collected in glass vial.

Gas Chromatography

Gas chromatographic analysis of Fatty Acid Methyl Esters (FAME) conditions are as follows: Shimadzu GC2014 with Headspace injector (FID), column: Carbowax 20M polyethylene glycol column, Program: Oven temperature 150 ºC (hold time 5 min) increased it up to 190 ºC by increasing rate of 8º C min^{-1} (hold for zero min), then increased up to 220 ºC by the rate of 2º C min^{-1} (hold time 10 min). Injection temperature was 270 ºC and detector temperature was 270 ºC. Detector: Flame ionisation detector was used.

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C. Peroxide value

Peroxide value (PV) of samples was determined according to the IUPAC titrimetric (acetic acid chloroform) method. Briefly, 5 g oil, 30 mL of glacial acetic acid-chloroform mixture (3:2v/v) and 0.5 mL saturated KI were added and kept in dark for 1 minute. The solution was then titrated against standard sodium thiosulphate solution (previously standardized cupric sulphate) using fresh starch indicator [13].

IV. EXPERIMENTAL RESULTS

Table 1. Fatty acid composition of mustard oil measured as per our sample

Fatty Acid	Percentage
SFA (Palmitic & Stearic)	5.60
Oleic C18:1(MUFA)	19.80
Linoleic C18:2 (PUFA)	10.83
Linolenic C18:3(PUFA)	10.86
Ecosenoic C20:1(MUFA)	10.90
Erucic C22:1(MUFA)	27.09
Above C22	14.92

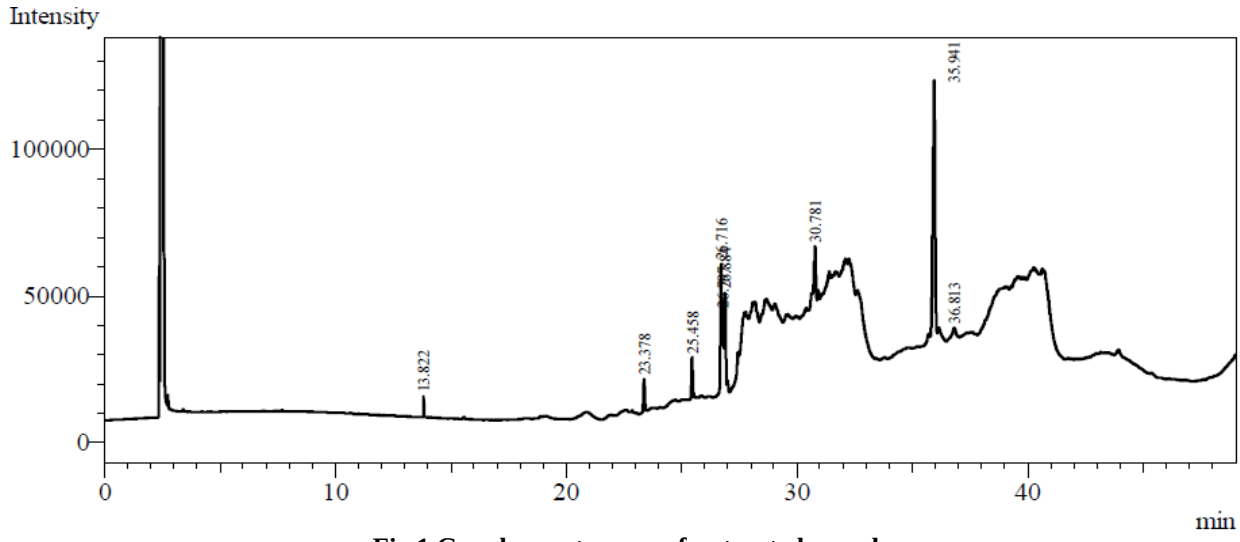

Fig.1 Gas chromatogram of untreated sample

The gas chromatography results shown in Table 1 and Figure 1 indicate high amount of MUFA (~57.79%) and SFA (~5.6%) as compared to PUFA (21.69%) in the seed oil. There are some essential fatty acids such as α-linolenic ω-3 is found to be 10.86% and ω-6 linoleic is 10.83%. This is well documented in scientific and ancient ayurveda literatures as well that essential fatty acids and glucosinolates should be taken by diet which can be accomplished by mustard oil with medicinal benefits [14-16]. The studies on composition of the oil also reveal the information about the fibers,

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nitrogenous compounds, and isothiocynates specially allyl isothiocynates and benzyl isothiocynates which are found to be potent inhibitors against carcinogens [16-18].

Table 2: Peroxide values of differentially treated mustard oil in miliequalent kg^{-1}

Sample Treatment	(Untreated) M_{u1}	(Treated 1h) M_{t3}	(Treated 4h) M_{t4}	(Treated 4h+1h) M_{t5}	(Treated with iron container) M_{t6}	(Untreated stored in colored glass container) M_{u2}
PV [meq kg^{-1}]	6.4	7.6	10.8	18.5	42.8	7.0

Table 2 shows the variation in the peroxide values of the samples of oil. The peroxide value is an indicator of oxidative stability of oils. Low peroxide value shows the resistance of oil towards rancidity and reversion and high value indicates an attraction towards oxidation. The peroxide value of untreated mustard oil samples is found to be 6.4 mili equalent kg^{-1}. After 1 hour treatment of heating and frying, it increases slightly by the magnitude of 1.2 units only and reached to 7.6 meq kg^{-1}. Upon treatment of 4 hours continuously, the PV rises by 3.2 units and reached upto 10.8 meq kg^{-1}. The increase occured in PV is not too high and also it is not exponential. After 4 hours of frying and heating, it is still near 10 meq kg^{-1}. This reflects the oxidative stability of mustard oil against thermal abuses. This stability can be attributed by the content of MUFA and natural anti oxidants. After a period of 24 hours, a treatment of 1 hour increases its PV by a magnitude of 7.7 and reached to 18.5Meq kg^{-1}. The deterioration is slowly increasing in short span of time after thermal treatments given to the mustard oil. Further, the PV of the sample treated highest (4+1hr) and then kept in iron container for 10 days is found to be 42.8 meq kg^{-1} and shows high deterioration during this period suggesting that the metal iron also plays a significant role towards denaturing of the oil during longer storage in iron container with open exposure to light and air with time.

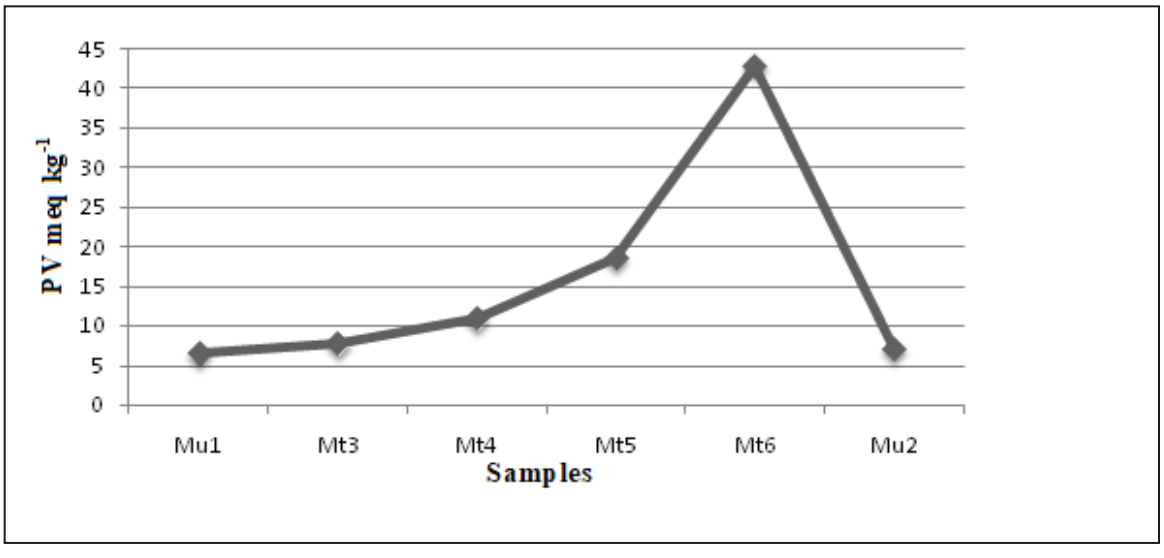

Figure 2. Trends in PV of seed oil of B. Campestris upon differet treatments

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The PV of untreated long stored oil in colored glass bottle was found to be 7.0 meq kg-1 and the trend is easily observable by Figure 2. This is again a proof of the more oxidative stability of the mustard oil. This is the main reason to select the mustard oil for cooking, frying and to make pickles in India from long back.

V. CONCLUSION AND FUTURE WORK

Mustard oil extracted with traditional method is natural seed oil with high MUFA content and natural antioxidants. The seed oil of Indian mustard contains various nutraceuticals with many medicinal properties. Peroxide value indicates the high resistance of mustard oil against the thermal abuses similar to the treatments given in Indian kitchens during traditional cooking, frying and pickle making. This native Indian oil is a good choice to be the cooking oil considering its oxidative stability and beneficial components present in it. This is also recommended by our ancestors as a rich and healthy source of energy from hundreds of years

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