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Comparative Physiochemical Analysis of Kachi Ghani, Solvent Oil and refined Oil Extracted from *Brassica nigra*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Brassica nigra or black mustard is traditionally used in various states of India from the ages. It is being commercially used in the form of kachi ghani, solvent oil (non-edible), and refined oil. Due to the antioxidant and antibacterial properties of kachi ghani mustard oil, it is considered to be of better quality. On the other hand, literatures also state that refined oil is better due to the purification processes. There is ambiguity regarding quality aspects of these three oil fractions. Therefore, the present study was planned for the comparative analysis the quality and physiochemical characterization of kachi ghani, solvent and refined oil. Commercially free fatty acids value, acid value, color, presence of argemone, pungency of oil and content of various monounsaturated fatty acids, saturated fatty acids and polyunsaturated fatty acids is determined to check quality of commercial grade edible oils. In consequence, it was observed that kachi ghani consists of less free fatty acids (0.37±0.02%), less acid value (0.74±0.04mg/g) and high pungency (0.279%) as compared to solvent and refined oil. Kachi ghani embrace more natural color (32.0 units) in comparison to solvent oil which was way darker and refined oil which was very light. The percentage of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids was spotted to be 6.44%, 64.360% and 28.64% respectively in kachi ghani oil. The study shows that saturated fatty acids such as palmitic C-16, stearic C-18, behanic C-22 and lignoceric C-24

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were present in kachi ghani. In solvent oil, palmitic C-16, stearic C-18, behanic C-22, and lignoceric C-24 were found whereas only palmitic acid was present in refined oil. High content of monounsaturated fatty acids were found in oil. Eicosenoic C-20:1, Oleic C-18:1, Eurcic C 22:1 and Nervonic C-24:1 unsaturated fatty acid was found in all the three fractions of oil.

Keywords: Brassica nigra; cold pressed oil (kachi ghani); antioxidants; solvent oil; refined oil; free fatty acids; physiochemical analysis.

1. INTRODUCTION

Brassica nigra is the member of the Brassica genus, Family: Brassicaceae which is grown worldwide in temperate regions [1]. Commonly called as black mustard, *B. nigra* is one of the most commonly used oil seeds in northern and eastern states of India [1]. Its oil is used traditionally for the treatment of epilepsy, toothache, snakebite, pneumonia, and bronchitis and nerve stimulant. The presence of phytochemicals such as alkaloids, flavanoids, sinapine, myrosin, sinigrin, glycosides indicates strong antioxidant activity which is related with the low risk of numerous cancers [2]. Mustard oil has been considered very unique oil amidst of all fatty oils due to its unique characteristic pungency, which is due to the presence of allyl isothiocyanate. Mustard oil carries out very unique monounsaturated fatty acids like erucic acid. The existence of considerable amount of unsaturated fatty acids and fewer amounts of saturated fatty acids manifest mustard oil as good oil for heart and lower the risk of heart diseases [3]. Mustard oil also has glucosinolate which has many antibacterial, antifungal, and many anticarcenogenic properties which narrate the oil to be used for medicinal properties [4].

Commercially three different types of oils 1) kachi ghani 2) solvent oil and 3) refined oil are available in market that is extracted using three different methods. Kachi ghani mustard oil is extracted using 'cold press' extraction process in which seeds are crushed at low temperature to maintain the natural properties, essential oil and antioxidants in the oil. The essential oil in kachi ghani acts as a preservative and expresses antioxidants and antibacterial properties [5]. The other method for extracting the oil from the cake (pressed seed after cold pressed procedure) is performed by using the solvent like hexane and recovering the oil with distillation, the resulting solution known as miscella. The solvent recovery is done by evaporation and condensation [6]. The solvent oil obtained from the solvent extraction plant consist of impurities, which must be removed from the crude oil to make it edible oil which is more stable and palatable against rancidity while storage. The process of removing all impurities and purifying the crude oil is known as refining. The oil obtained is called as refined oil which is edible oil [7]. Although kachi ghani and refined oil is widely used, there is a paucity of literature studies depicting the physiochemical differences between kachi ghani, solvent and refined oil. Commercially free fatty acids value, acid value, color, presence of argemone, pungency of oil and content of various monounsaturated fatty acids, saturated fatty acids and polyunsaturated fatty acids is determined to check quality of commercial grade edible oils. So, the present study was planned with the objective to select the best fraction of oil by analyzing the quality parameters of three fractions of mustard oil.

2. MATERIALS AND METHODOLOGY

2.1 Sample Collection

B. nigra seeds were obtained from 'Shree GRG Oil Mill', Sriganangar, Rajasthan and cleaned to make it free from extraneous matter. The moisture content of the seeds was reduced to 5.46%. Cleaned seeds were stored in cloth bags at 25◦C and used for the extraction of oil using different methods as given below:

2.1.1 Extraction of oil through Kachi Ghani

10-15 Kg of seeds were added to each Kohlu (Kachi ghani Plant), where these were added in kohlu machines, and then in expellers where the oil, called as kachi ghani oil, was extracted. Then the extracted oil was collected and stored.

2.1.2 Extraction of solvent oil

Mustard cake (approx.10 Kg) the byproduct of the mustard oil extraction process- was transferred into the solvent plant to extract more oil from it through cracking and distillation, using hexane as a solvent and miceller oil was produced which after modification was stored in a tanker where it is called solvent oil.

2.1.3 Extraction of refined oil

The solvent oil (10L) was sent to the refinery plant for purification by removing the chemicals, color and smell from it with the help of various techniques like de-gumming, neutralization, bleaching and filtration, and after final polish filtration, the refined oil was taken from the plant. Three fractions of the oil so obtained were used for studying various physiochemical parameters as described below:

2.2 Determination of Free Fatty Acid (FFA)

The free fatty acid test was directed with the method of AOCS [8] in which 5g of oil sample (kachi ghani, Solvent and refined oil), after adding 50ml of neutralized ethanol, was heated till mixing of the contents, and titrated against 0.1 N sodium hydroxide, using phenolphthalein as an indicator. The p*henolphthalein indicator* gives a bright pink colour in the pH range of 10-12 and hence it is used as an acid-base indicator. The calculations were performed with the formula:

$$\text{FFA} = \frac{\text{Normality of NaOH} \times \text{ burette reading} \times 28.2}{\text{Weight of the oil in grams}}$$

where, normality of NaOH is 0.1N

2.3 Determination of Acid Value

The free fatty acid test was directed with the method of Aocs [8] in which 5g of oil sample (kachi ghani, solvent and refined oil), after adding 50ml of neutralized ethanol, was heated till mixing of the contents, and titrated against 0.1 N sodium hydroxide, using phenolphthalein as an indicator. The calculations were performed with the formula:

$$\text{Acid Value} = \frac{\text{Normality of NaOH} \times \text{ burette reading} \times 56.1}{\text{Weight of the oil in grams}}$$

where, normality of NaOH is 0.1N

2.4 Color Determination

Colors of the oil samples were measured with Tintometer Lovibond (Model F). The oil sample (2 ml) was added into ¼ inch Cuvette, kept in the tintometer and the color of the oil sample was matched with the standard color shown in the instrument. The color of the oil sample was determined by using the formula:

Color determination of oil sample =Y+5(R)

where, Y is for yellow color and R is for red color

2.5 Determination of Pungency of Oil

For the determination pungency, 5ml of the sample oil was taken in neck round bottom flask and 25ml of ethanol was added, followed by the addition of 10ml of ammonia. On the other hand, in long neck florence/boiling flask, 25ml of 10% silver nitrate and 10% ammonium sulphate were added. Round bottom flask was attached with boiler for steam and florence/boiling flask was attached to another end of the distillation apparatus. Distilled solution (250 ml) was collected in florence/boiling flask and it was kept in heating mantle at 40^0C temperature for 1 hour and then kept for cooling as well as sedimentation for 1 hour. Then filtration was done with filter paper and filtrate was collected. The filtrate (100ml) was taken and 5ml of nitric acid was added with ammonium ferrous sulphate as an indicator and titrated against 0.1 N sodium thiosulphate to the end point. The percentage of pungency was determined by using the formula:

$$\text{Pungency in \%} = \frac{9.915 \times \text{Blank reading} - \text{Burette reading} \times \text{ Normality of NH4SCN } \times 100}{\text{Weight of the sample oil}}$$

where, normality of NH_4SCN is 0.1N

2.6 Determination of Presence of Argemone

The presence of argemone was tested with the help of thin layer chromatography. Oil sample (10ml) was taken in a test tube with 10ml of diethyl ether and 5ml of conc. hydrochloric acid (5mL). The mixture was continuously shaken to remove the gas, and then it was kept in water bath for 10 minutes till green and yellow portions developed. The layers were separated by using separating funnel. Green portion was collected in a beaker for further testing. The green colored solution was kept on a hot plate to dry. Chloroform (9ml) and acetone (1ml) were mixed and from that mixture 1 ml was

added in the green dried solid, and mixed well. The solvent preparation was done by adding 60ml of n-heptane and 40ml of acetone. TLC sheet (aluminum sheet with silica G layer) was taken and it was cut into the pieces of dimensions 14 cm x 5cm. The margin was marked 1cm from the bottom of the sheet. TLC plate was kept in solvent, covered it and kept in the refrigerator for half an hour. Then the TLC plate was dried normal and observed in UV chamber.

2.7 Determination of Fatty Acid Composition by using Gas Chromatography

CS-5770 gas chromatograph was used for the detection of fatty acids present in the oil sample with flame ionization detector at temperature of 240 ^{o}C. The instrument was preheated to 130^{o}C and acetone was added in injector to wash the column whereas sample was prepared by taking 50 µL of oil sample with 1µL of FAME solution and 2µL of hexane. The solution was centrifuged for 5 minutes at rpm and sample was injected in the injector and allowed to run for 30 minutes. After that a graph was plotted between concentration (y-axis) and retention time (x-axis).

3. RESULTS AND DISCUSSION

The total amount of oil was extracted from the *B.nigra* seeds was 40-41%. Initially, during the cold press procedure, the majority of the oil was taken from the cold press plant. The kachi ghani mustard oil extracted from the cold press procedure seed was approximately 28-30%. When the cake was taken from the kachi ghani plant and transferred into solvent plant, the amount oil which was extracted from solvent plant varied from 12-15%. The oils extracted using solvent can be further purified to obtain refined oil. The oil extracted by cold press represents the highest proportion of the oil. In the literature, Sharif et al [9] did the comparative analysis of three varieties of specific mustard genotypes which were BARI Sarisha-15, BARI Sarisha-16, and BARI Sarisha-17 in which it was observed that BARI Sarisha-17 contain more oil (41.98%), than BARI Sarisha-16 (40.95%) and BARI Sarisha-15 (41.85%). In another study, Verma et al [10] did the oil extraction from *B. juneca* seed variety by using the solvent petroleum ether in which this was observed that *B. juneca* PDZ1 contain more oil 37.01±0.28% than *B.juneca* PM21 25.15±0.35%. Another extraction was done with solvent hexane in which *B.juneca* PDZ1 showed more oil percentage 35.18±7.71% than in variety of *B.juneca* PM21 the oil content was 35.04±8.19%.

The FFA value is considered as an important qualitative parameter in the food industry. More the free fatty acid value less is the quality of the oil. This is the measure of hydrolytic rancidity of fats and oil. The percentage oleic acid commonly expresses FFA of oil. Increased FFA in the oil can cause health hazards and also decrease in the smoke point of oil. Table. 1 indicates FFA content was observed to be 0.37%, 0.79% and 1.35% in kachi ghani, solvent oil and refined oil respectively. Chakraborty et al [4] examined the quality of mustard oil in which the value of FFA was ranging from 0.40-1.28%. Uddin et al [11] performed the free fatty acid tests on different palm oil (*Elaeis guineensis*), in which results were observed in crude palm oil (CPO) 1.90, in degummed palm oil (DPO) 1.00, and in degummed bleached palm oil (DBPO) the FFA was 0.90. In another study, Kumar et al [12] studied individual and blended oils in which the FFA value ranged from 0.18% (soybean oil) to 0.19% (mustard oil) in individual oils while the FFA value ranged from 0.14- 0.18% in blended oils (Mixed oils). In the present study, it has been observed that kachi ghani oil contains less FFA in comparison to refined oil and solvent oil.

Acid value is known to be the number of milligrams of potassium hydroxide (KOH) required to neutralize the fatty acid in one gram of sample. Acid value is always twice the amount of FFA and is independent of molecular weight [13]. Acid value is considered to be the crucial parameter in terms of oil quality. The high acid value of the sample leads to deterioration of oil, which results in less nutritive value of oil. The results indicate the acid value of kachi Ghani, solvent oil and refined oil was 0.74 mg/g, 2.7 mg/g, 1.58 mg/g respectively (Table 1). These may be due to differences in the extraction process. Chakraborty et al [4] determined the acid value in mustard oil sample varieties of *B. nigra*, *B. juneca* and *B. hirta*, in which the value ranged from 0.90- 2.55 mg/g. Aleena et al [13] performed the study on sunflower oil, in which the oil was heated at 180^{o} C for 6 hours and acid value was found to be 0.67 mg/g. In another study, Pardeshi et al [14] did the test on mustard oil in which the acid value was checked before and after frying in which the value ranged from 2.98-5.12 mg/g before frying and after frying the value ranged from 7.98-11.94 mg/g. In

comparison of kachi ghani, solvent and refined oil it was observed that kachi ghani has less acid value which means kachi ghani can be considered to be of good quality oil in comparison of solvent and refined oil.

The natural dark color of mustard oil is due to the presence of carotenoids and other pigments. These polymers provide red color to the oil and mixture of aldehydes and peroxide provides the yellow color to the mustard oil. Besides this, the aroma, color and taste of the fatty oil are also affected by lipase enzyme by the breakage of ester bond. Table.1 shows the color intensity of the kachi ghani as 32.0 Units, refined oil as 5 Units and solvent oil as 48.5 units. In comparison, kachi ghani has natural dark color but solvent oil contain darker color than kachi ghani due to the presence of solvents and refined in too light in color due to the purification of oil. In consequence, kachi ghani is of good quality because there is no addition of chemicals in the oil and is not processed. So, by looking at the color the quality of the oil can be determined.

The pungency of the oil is the pungent smell and aroma of the oil which tingle the throat and buzzes the nose. An enzyme myronsinase is released when the mustard seeds are cold pressed. As mustard seeds also contain glucosinolate also known as sinigrin, combines with myronsinase and produce AITC (Allyl isothiocyanate), which is responsible for pungent taste of mustard oil. The quality of the oil can be also observed with the pungency of the oil which gives oil a taste and makes the oil pharmacologically healthy [15]. More the pungency of the oil more the oil is good in quality. Table.1 depicts the kachi ghani value of pungency 0.279% and solvent oil 0.031%. Masud [16] detected the allyl isothiocyanate value in different brands of mustard oil and the results were ranging from 0.12% to 0.27%. Chakraborty et al [4] checked the amount of allyl isothiocyanate in mustard oil sample varieties in which the value ranged from 0.12% to 0.33%. The result shows that kachi ghani oil contains more allyl isothiocyanate than solvent oil which depicts that kachi ghani has more health benefits.

Argemone maxicana is a species of poppy found majorly in Mexico and various parts of the world. Argemone seeds contain pale yellow color non-edible oil known to be as argemone oil which consists of various alkaloids sanguinarine and dihydrosanguinarine. Consumption of argemone seed with oil is toxic as this can cause oxidative stress and death of RBCs (Red blood cells) by the formation of met-hemoglobin by changing pyridine nucleotide(s) and glutathione redox potential. The argemone seeds have the resemblance of *B. nigra* which results in the adulteration in the mustard seeds and making the mustard oil poisonous. To determine the presence of argemone oil in the sample this layer chromatography technique has been used. After testing of oil, it was observed that there is no presence of argemone oil in kachi ghani oil, solvent oil and refined oil as there was no orange/yellow color was observed under UV light (Table no.1). The comparative analysis was performed by Khansili and Rattu [17] on mustard oil and canola oil for argemone presence, the results shown were negative in both the oils which depicts that both oils were free of argemone. Chakraborty et al [4] checked the presence of argemone oil in different mustard varieties like *B.nigra*, *B. juneca* and *B. hirta*, in which it was observed that there is no presence of argemone oil in all samples. The argemone test was negative for kachi ghani, refined and solvent oil samples, which depicts that the oil is of good quality with no argemone adulteration (Table 1).

Fatty acids present in the mustard oil are crucial in determining its quality. Mustard oil contains many essential fatty acids such as linolenic acid, which helps to maintain the cholesterol level of the human body. Oleic acid is considered to be the major unsaturated fatty acids playing an important role in human nutrition. Mustard oil contains monounsaturated fatty acids, saturated fatty acids and polyunsaturated fatty acids but the concentration of monounsaturated fatty acids is usually more. Fig. 1depicts that the saturated fatty acids were found in kachi ghani were palmitic C-16 (2.89%), stearic C-18(1.18%), behanic C-22(1.18%) and lignoceric C-24 (1.13%). In solvent oil, saturated fatty acids were palmitic C-16 (3.69%), stearic C-18 (1.17%), behanic C-22(0.79%), and lignoceric C-24 (0.98%) and in refined oil only one saturated fatty acid was observed (palmitic C-16, 3.76%) other saturated fatty acids were absent.

The monounsaturated fatty acids were found to be more in quantity, as in kachi ghani Eicosenoic C-20:1(6.97%), Oleic C-18:1(10.36%), Eurcic C 22:1(45.09%), Nervonic C-24:1(1.92%). In solvent oil, monounsaturated fatty acids present

were eicosenoic C-20:1(7.71%), oleic C-18:1(12.19%), eurcic C-22:1(40.46%), nervonic C-24:1(1.75%) and in refined oil eicosenoic C-20:1(11.05%), oleic C-18:1(15.04%), eurcic C 22:1(48.30%) but nervonic acid was absent (Fig 2, 3).

Table 1. Physicochemical analysis of different fractions of oil

S. No	Parameters	Kachi Ghani Oil	Solvent Oil	Refined Oil
1.	FFA %	0.37±0.02	1.35±0.03	0.79±0.005
2.	Acid Value (mg/g)	0.74±0.04	2.7±0.06	1.58±0.01
3.	Color	32.0 Units	48.5 Units	5 Units
4.	Pungency%	0.279%	0.031%	-
5.	Argemone oil	Absent	Absent	Absent

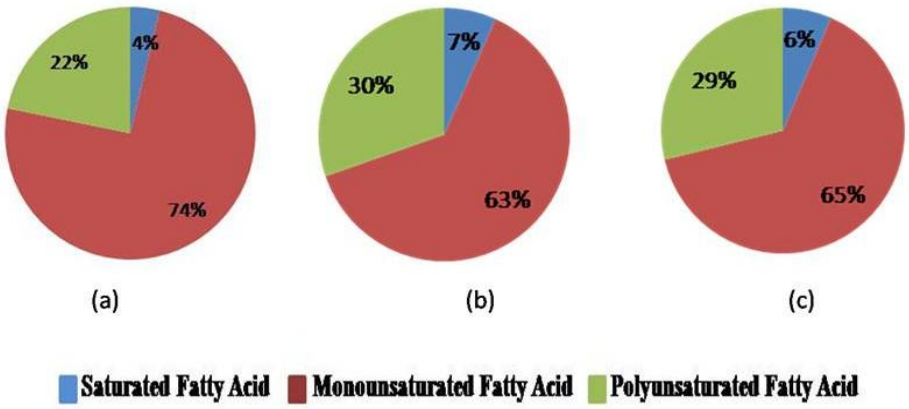

Fig. 1. Percentages of fatty acids present in oil (a) Refined oil (b) Solvent oil (c) Kachi ghani

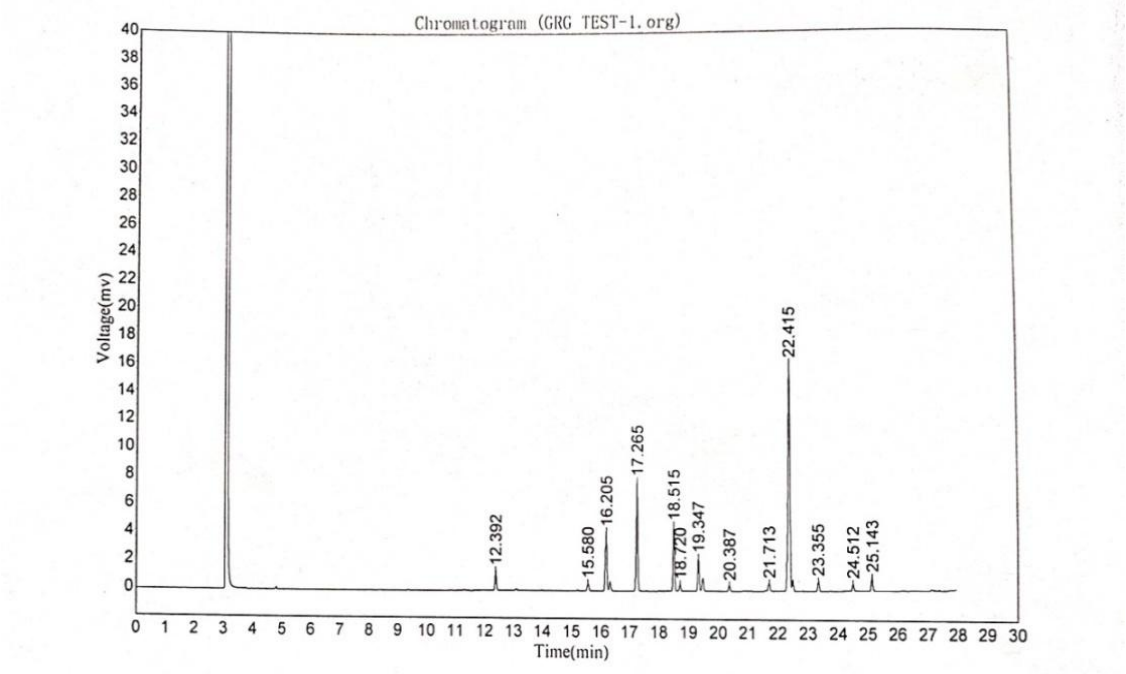

Fig. 2. Chromatogram of kachi ghani (*Brassica nigra*)

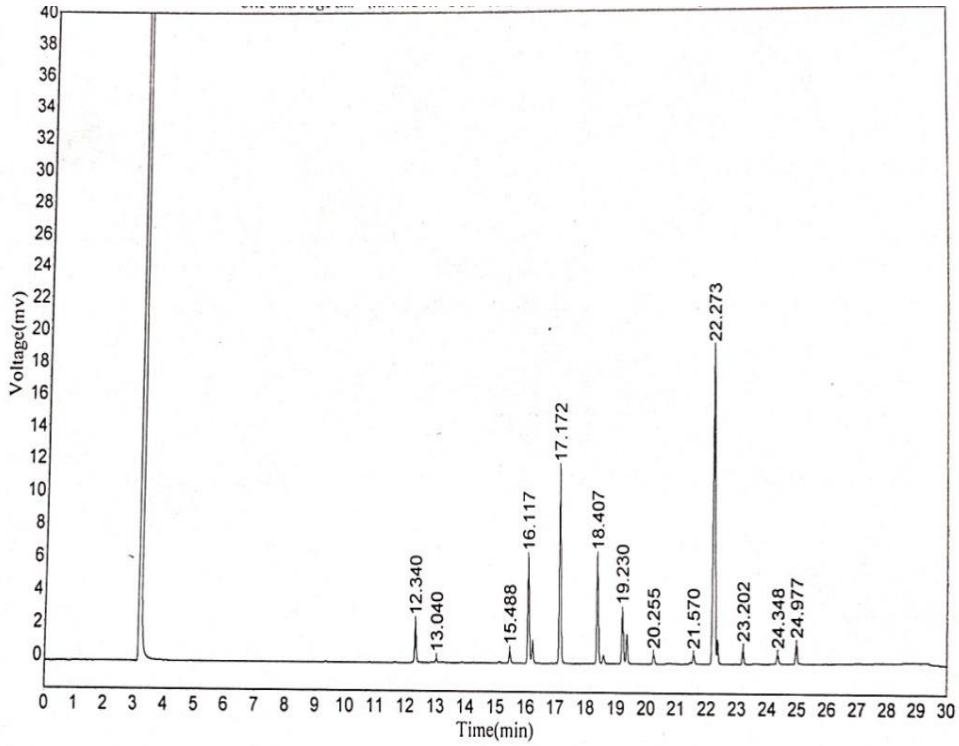

Fig. 3. Chromatogram of Solvent oil (*Brassica nigra*)

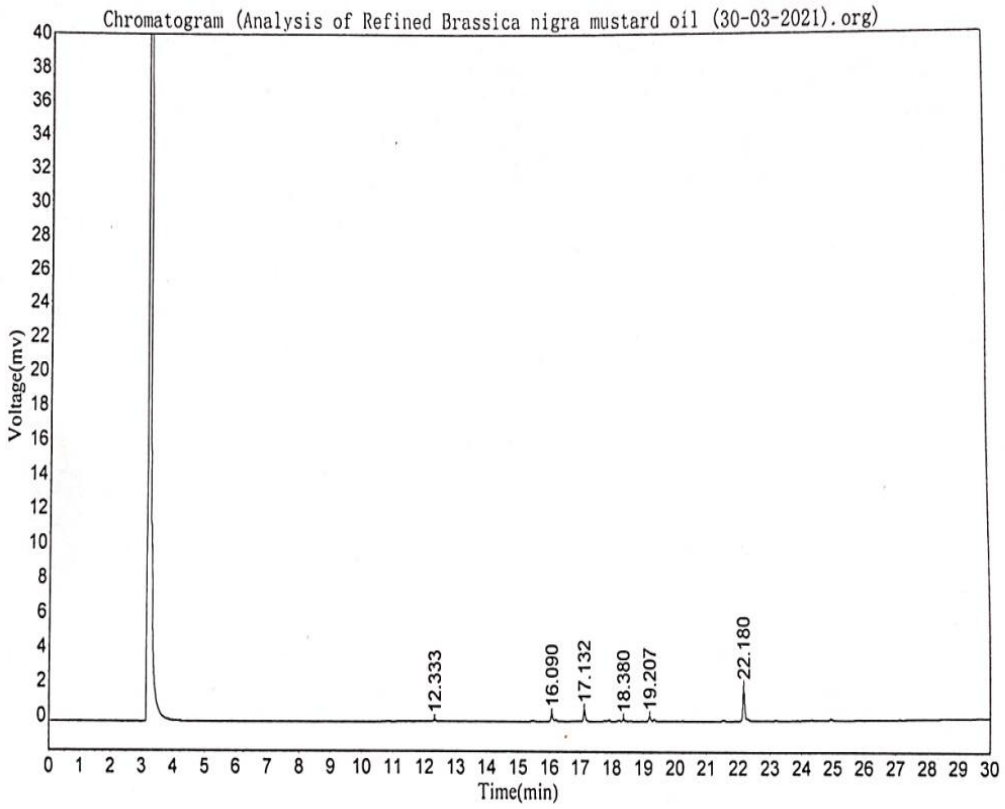

Fig. 4. Chromatogram of Refined oil (*Brassica nigra*)

Table 2. Concentration of fatty acid in oil samples determined by gas chromatography

Fatty acid	Kachi Ghani	Solvent oil	Refined oil
Saturated Fatty Acid			
Palmitic C-16	2.89	3.69	3.76
Stearic C-18	1.18	1.17	-
Behanic C-22	1.18	0.79	-
Lignoceric C-24	1.13	0.98	-
Monounsaturated Fatty acid			
Eicosenoic C-20:1	6.97	7.71	11.05
Oleic C-18:1	10.36	12.19	15.04
Eurcic C 22:1	45.09	40.46	48.30
Nervonic C-24:1	1.92	1.75	-
Polyunsaturated Fatty acid			
Linoleic C 18:2	16.33	17.88	18.07
Linolenic C 18:3	9.63	10.63	3.75
Arachedeic C-20	1.00	-	-
Docosadienoic acid C-22	1.43	1.42	-

The polyunsaturated fatty acids present in kachi Ghani were linoleic C 18:2(16.33%), linolenic C 18:3(9.63%), arachedeic C-20 (1.00%), docosadienoic acid C-22(1.43%), in solvent the polyunsaturated acids were linoleic C 18:2(16.33%) linolenic C 18:3(9.63%), docosadienoic acid C-22(1.43%) but arachedeic was absent in solvent oil. In refined oil linoleic C 18:2(18.07%), linolenic C 18:3(3.75%) were present and other polyunsaturated fatty acids were absent (Fig. 4). In mustard oil sample of kachi ghani, the saturated fatty acids were found to be 6.44%, the monounsaturated fatty acids were 64.36% and polyunsaturated fatty acids were found to be 28.64%. Similarly, in solvent oil, 6.73% saturated fatty acids, 62.93% monounsaturated fatty acids were and 30.33% polyunsaturated fatty acids were found. In refined oil, 3.76% saturated fatty acids, 74.40% monounsaturated fatty acids and 21.82% polyunsaturated fatty acids were found (Table 2). The content of monounsaturated fatty acids is more in all three fractions of oils, thus indicating the better quality of oil because unsaturated fatty acids are healthy.

Sharafi et al [18] conducted a study in which fatty acids of Brassica species were analysed in which unsaturated fatty acids were linoleic (12.66-19.92%), linolenic (6.84-20.22%), oleic (10.08-61.83%) and erucic (1.16-46.19%), and saturated fatty acids of stearic (0.79-2.36%) and palmitic (2.63-4.52%). The recent study was performed by Kayacetin et al [19] in which gas chromatography was performed on 57 mustard genotypes belonging to Brassica species of *B. juneca* (31 genotypes), *B. rapa* (6 genotypes), *B. napus* (2 genotypes), *B. nigra* (6 genotypes), *B.arvensis* (10 genotypes), *B.alba* (2 genotypes) were collected in which the evaluation of unsaturated fatty acid composition of Brassica genotypes was majorly determined in Erucic acid (C22:1; 20.63-47.87%), oleic acid (C18:1; 7.42-24.54%) and linolenic acid (C18:2; 9.61-25.11%) respectively. In comparison, it was found that the monounsaturated fatty acids were more in all the samples especially in kachi ghani than saturated and polyunsaturated fatty acids. The more fatty acids were present in kachi ghani because the oil was extracted in natural manner and all the components were secured in oil. In comparison, some fatty acids were missing in solvent oil due to the treatment with solvents like hexane and most of the saturated and polyunsaturated fatty acids were absent in refined oil due to the purification and refining treatments. In consequence, kachi ghani consists of more nutrition and considered as more healthy oil in comparison of solvent oil and refined oil.

4. CONCLUSION

Mustard oil is considered to be the healthiest oil which contains many nutrients. The oil from the seed was extracted by cold pressed (kachi ghani oil) and solvent extraction method. The solvent oil (non-edible oil) was purified to obtain refined oil (edible oil). The present study was planned with the objective to select the best fraction of oil by analyzing the quality parameters of three fractions of mustard oil. Through this research it has been concluded that kachi ghani is of better quality compared to solvent and refined oil. It contains less FFA, less acid value and more color and more pungency that makes it commercially more viable option. It contains

more monounsaturated fatty acids in comparison of solvent and refined oil offering better health benefits. Most of the literature studies only describes the quality aspects of refined oil. None of the study describe the comparative analysis of three fractions of oil. Various other studies need to be conducted for comparative analysis of antibacterial or antioxidant potential of these three oil fractions. Precision technology may be used to determine various other volatile and non-volatile components present in these three fractions to better conclude the study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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