

Analysis of Fixed Oils of Few Wonder Seeds

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Abstract—The physicochemical parameters of the fixed oil content of selected wonder seeds were determined. Oil was extracted from flaxseeds, pumpkin seeds, chia seeds, watermelon seeds, dill seeds, pomegranate seeds, sesame seeds and kalonji (black cumin) seeds. The oil samples were extracted using Soxhlet extractor by hexane. The oil samples were analysed for their specific gravity, refractive indices, colour, acid value, saponification value and iodine values. The fatty oils were then converted into their fatty acid methyl esters (FAMES) and analysed over GC with reference to standard fatty oils for identification. The fixed oil content present in sesame seeds was found to be 27.48% while dill seeds had 1.49% oil content. The acid value obtained was maximum i.e., 84.983 mg KOH/g for sesame oil indicating high free fatty acids content. The high iodine values for chia oil (155.59 mg/g), flax seed oil (180.83 mg/g) and pomegranate seeds (172.58 mg/g) show their drying oil characteristics. The saponification values varied from 253 – 172.56 mg KOH/g for the oil samples. GC of the oil samples showed the presence of high percentage of methyl esters of unsaturated fatty acids namely oleic, linoleic and linolenic acids in the oil samples. Few samples also showed presence of myristic, palmitic and palmitoleic acid methyl esters. These seeds rich in ω -3 fatty acids have astounding amount of minerals and nutrients like niacin, folic acid, protein, fibre, iron and zinc acting as small natural capsules.

1. INTRODUCTION

It is well understood in today's market that good health comes from good diet. One of the most important nutrients for good health is lipids. Seed oils constitute a significant part of our diet as they are the greatest source of some important essential nutrients such as tocopherols, fatty acids and phytosterols. Health conscious individuals consume oilseeds and oils which are rich in constituents like omega-3 fatty acids, tocopherols and sterols. Among the lipid components the most important ones are linolenic acid- omega-3 fatty acids, antioxidants and sterols as they reduce the risk of heart diseases [1,2]. Flax, pumpkin, chia, watermelon, dill, pomegranate, sesame and black cumin seeds have been used for analysis as their specialty oils have high content of linolenic acid.

Flax seeds (*Linum usitatissimum*) have been used for fibre and oil is being used in painting and linoleum industry. Flaxseeds were also used as bakery ingredient but only for decorative purpose and in negligible amount. But nowadays research is being done on the beneficiary properties of flaxseeds especially in edible oil [3].

Pumpkin seeds (*Cucurbitapepo*) were earlier used simply for eating and that too in small amount or for garnishing in salads. Researches have shown that pumpkin seed oil has unique properties which are beneficial against breast and prostate cancer. It has also shown antibacterial properties against gram positive and gram negative bacteria. The kernels of pumpkin seeds are rich source of MUFA (monounsaturated fatty acids) such as **oleic acid (18:1)**. MUFA is helpful in reducing bad LDL-cholesterol and increasing good HDL-cholesterol thereby reducing the risk of cholesterol [4].

Chia seeds (*Salvia hispanica*) belongs to mint family native to southern Mexico. Chia disappeared as crop and was rediscovered in 20th century because of being a unique source of linolenic acid [5]. Chia seeds are rich source of Omega-3 and Omega-6 fatty acids which are helpful in development of central nervous system and cell membranes. They are rich source of alpha linolenic acid. Watermelon seeds (*Citrullus lanatus*) comprise of monounsaturated fats, polyunsaturated fats and omega-

6 fatty acids. The mono- and polyunsaturated fatty acids help in controlling blood cholesterol and omega-6 fatty acids reduce the blood pressure. Pomegranate seeds (*Punicagranatum*) lowers blood pressure as well as cholesterol thereby protecting heart against various diseases. Pomegranate seed oil contains puniceic acid (65.3%), palmitic acid (4.8%), stearic acid (2.3%), oleic acid (6.3%) and linoleic acid (6.6%) [6].

Dill seeds (*Anethum graveolens*) contains 2 types of healing component. These include: monoterpenes- carvone, limonene and anethofuran, flavonoids- kaempferol and vicenin. Dill oil is chemoprotective in nature as it contains enzyme glutathione-S-transferase which helps to neutralize carcinogens. Volatile oil of dill seeds have shown antibacterial properties. Dill seeds contains many volatile oils such as- D-carvone, dillapiol, DHC, eugenol, limonene, terpinene and myristicin. Dill seed oil is anti-spasmodic, carminative, appetizer, digestive, disinfectant, galactagogue (helps in breast milk secretion) and have sedative properties.[7]

Kalonji seeds (*Nigella sativa*) contains Thymoquinone and Nigellone which are the main ingredients found in Kalonji oil.

Thymoquinone is an antioxidant, anti-inflammatory, has a choleric effect and good for fat metabolism, detoxification and its bronchodilating effect is protective against asthma attacks. Nigellone is also bronchodilatory which has an antispasmodic and warming effect. This is another reason why black seed/Kalonji oil can help treat respiratory disorders such as asthma and whooping cough. [8]

2. MATERIALS AND METHODS

2.1 Materials

Flaxseed, chia seed, kalonji and sesame seeds were purchased from local market in Delhi, India. Watermelon seeds and pumpkin seeds were purchased from PUSA institute in Delhi, India. Pomegranate seeds and dill seeds were purchased online from Ebay.com.

2.2 Extraction of fixed oil

Seeds were grounded in a food processor and 50g seeds were homogenized with 250ml n-hexane, following soxhlet extraction method. After the completion of extraction of fixed oil in n-hexane it was concentrated using rotatory evaporator. The fixed oil obtained was stored in a pre-weighed flask at room temperature.

2.3 Trans esterification

Fixed oils obtained from seeds were converted to Fatty Acid Methyl Esters (FAMES) for analysis of fatty acid composition of various seed oil. 0.15g mixture of oil sample was weighed in a pre-weighed tube and then was dissolved in 2ml of n-hexane and 1ml of 2M KOH. The tube was capped and vigorously shaken for 30 sec and was heated for half an hour in water bath at 70°C. The tubes were then cooled and then 1M HCl was added and gently stirred. After phase separation 1ml hexane was added. The upper phase containing FAMES was transferred into analysis vial using syringes and 1µL of FAME was injected in GC [9].

The GC used was RASTECH model-2806. Carrier gas used was H₂ gas with flow rate 10psi. Initial column temperature was kept at 200°C. Temperature was maintained for 5min and increased at rate of 10°C/min at final temperature of 270°C and held for 5 min. Fatty acids were identified by comparison of the retention data with authentic standards.

2.4 Refractive Index

Refractive index was measured according to AOAC method [10]. Abbe's refractometer was used to measure the refractive index. Liquid sample was filtered to remove impurities. Stream of water was circulated through instrument and temperature was adjusted as per requirement. Prism was cleaned and dried. A few drops of sample were placed on the prism and prism was closed allowing to stand for 1-2 min. Instrument and lighting were adjusted to obtain distinct readings and refractive index was determined.

2.5 Specific Gravity

Specific gravity of oil is basically the ratio of mass of the oil to the mass of the reference substance of equal volume. The procedure was conducted according to AOAC method [11].

$$\text{Specific gravity} = (W_3 - W_2) / W_1$$

W₃: weight of empty container with oil

W₂: weight of empty container

W₁: weight of equal volume of water

2.6 Acid Value

Acid value is basically the amount of KOH required in order to neutralize one gram of chemical substance. The acid number gives information about the number of carboxylic acid group present in oil that is free fatty acids. Here the sample is dissolved in fat solvent and is titrated against standardized KOH using phenolphthalein as indicator [12].

$$\text{Acid value} = (56.1 \times (V_s - V_b) \times N) / W$$

V_s = volume of KOH used for sample

V_b = volume of KOH used for blank

N = normality of KOH

W = weight of oil sample used

2.7 Saponification Value

Saponification value is amount of KOH (in mg) required to saponify 1g of fat. It gives information about average molecular weight of all the fatty acids present within the fat [13].

Formula used is:

$$\text{Saponification value} = (56.1 \times (V_b - V_s) \times N) / W$$

V_s = volume of KOH used for sample

V_b = volume of KOH used for blank

N = normality of KOH

W = weight of oil sample used

2.8 Iodine Value

Iodine value is basically the amount of iodine compound consumed per 100g of sample. Iodine value is basically used in case of oils and fats to determine the amount of unsaturation present within the oil sample. Greater the number of double bonds present in fat or oil, greater will be the iodine value [14].

$$\text{Iodine value} = ((V_b - V_s) \times 1.269) / W_s$$

3. RESULTS AND DISCUSSION

3.1 Percentage of Fixed Oil

The percentage of oil obtained was found to be highest in case of sesame seeds with the value being 27.48%. After sesame

the second in line for high content of oil was found to be flaxseed with percentage oil content of 17.79%. The lowest percentage of oil was obtained from dill seeds with a percentage of 1.49%.

Table 1: % Fixed oil content in seed samples

S. No	Sample	Weight of grounded seeds (g)	Weight of oil obtained (g)	Percentage of oil obtained from sample
1	Flax seed	50.039	8.902	17.79%
2	Pumpkin seed	50.296	7.017	13.95%
3	Chia seed	51.031	4.513	8.84%
4	Watermelon seed	47.096	7.268	15.43%
5	Dill seed	53.059	0.792	1.49%
6	Pomegranate seed	50.204	6.498	12.94%
7	Kalonji seeds	50.124	3.562	7.65%
8	Sesame seeds	50.460	10.602	27.48%

Table 2: Specific Gravity, Acid, Saponification, Iodine Values, Refractive Indices of Oil Samples

S. No.	Sample	Specific Gravity	Acid Value (mg KOH/g)	Saponification Value (mg KOH/g)	Iodine Value (mg/g)	Refractive Index
1	Flax seed	0.724	6.545	243.1	180.8325	1.48
2	Pumpkin seed	0.584	12.518	247.5	82.9176	1.47
3	Chia seed	0.65	7.381	242.1	155.59	1.481
4	Watermelon seed	0.7	4.999	252	132	1.475
5	Dill seed	0.946	13.331	176.7	124.59	1.49
6	Pomegranate seed	0.939	5.461	243.3	172.584	1.498
7	Kalonji seeds	0.935	0.34	172.56	112.32	1.473
8	Sesame seeds	0.923	84.983	253	92.97	1.469

Flax, Pumpkin, Chia, Watermelon, Pomegranate and Sesame oil showed high saponification index. The iodine value was highest for flax seed oil indicating high value of unsaturation and least in pumpkin seed oil showing less amount of unsaturation present.

3.7 Analysis of FAMES on GC

In the GC profile of flax seeds, the peaks at RT 8.01, 9.98 and 10.19 are corresponding to Palmitic, Linoleic and Linolenic acid respectively obtained on comparison with standards.

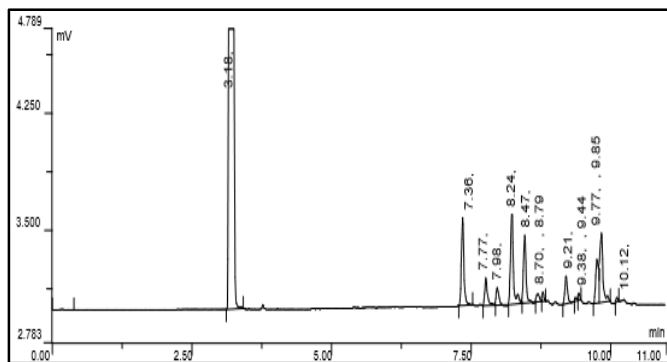


Fig. 1: GC profile of standards

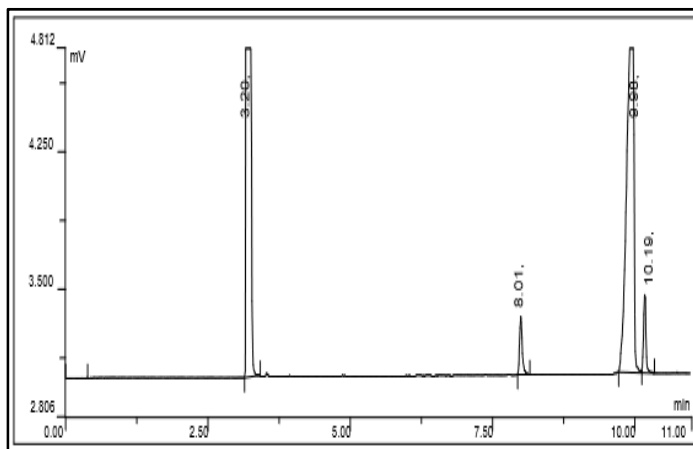


Fig. 2: GC profile of Flax seed oil

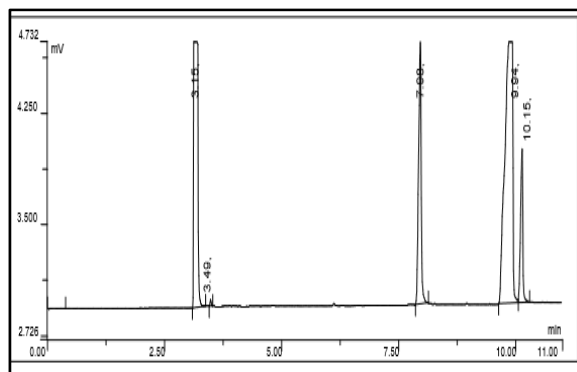


Fig. 3: GC Profile of Pumpkin seed oil

Pumpkin seed was found to contain Myristic acid, Palmitic acid, Linoleic acid and Linolenic acid corresponding to peaks obtained at RT 3.49, 7.98, 9.94 and 10.15 respectively.

Watermelon seed was found to contain Myristic acid, Palmitic acid, Linoleic acid and Linolenic acid corresponding to peaks obtained at RT 3.56, 8.03, 9.97 and 10.24 respectively.

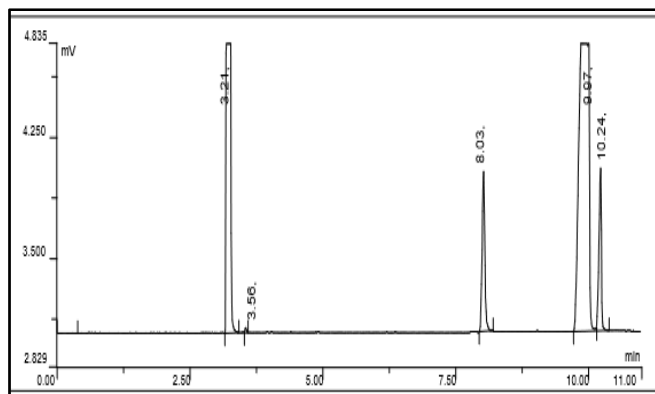


Fig. 4: GC profile of Watermelon seed oil

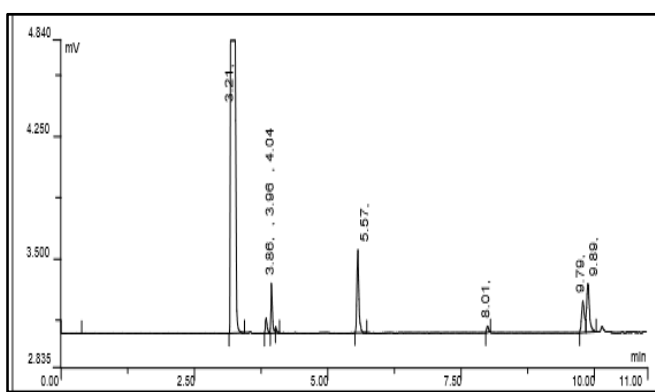


Fig. 5: GC profile of Dill Seed Oil

Dill seed was found to contain Myristic acid, pentadecanoic acid, Palmitic acid, Linoleic acid, Stearic acid, Hexadecadienoic acid and Linolenic acid corresponding to peaks obtained at RT 3.86, 3.96, 4.04, 5.57, 8.01, 9.79 and 9.89 respectively.

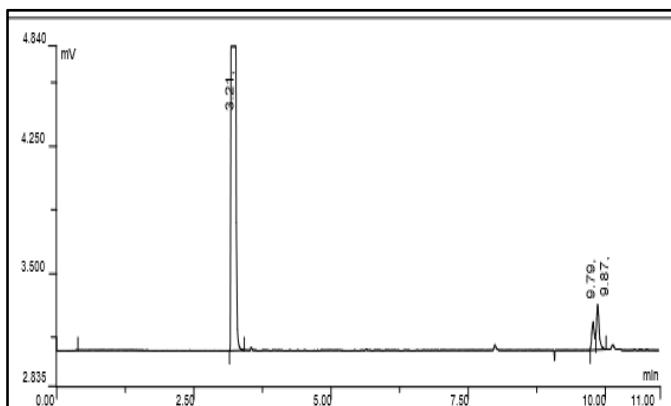


Fig. 6: GC Profile of Sesame oil

Sesame seed was found to contain Stearic and Oleic acid corresponding to peaks obtained at RT 9.79 and 9.87 respectively.

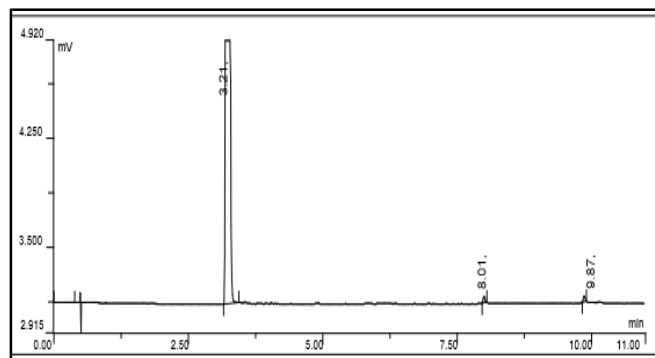


Fig. 7: GC profile of Kalonji seed oil

Kalonji seed was found to contain Palmitic acid and Oleic acid corresponding to peaks obtained at RT 8.01 and 9.87 respectively.

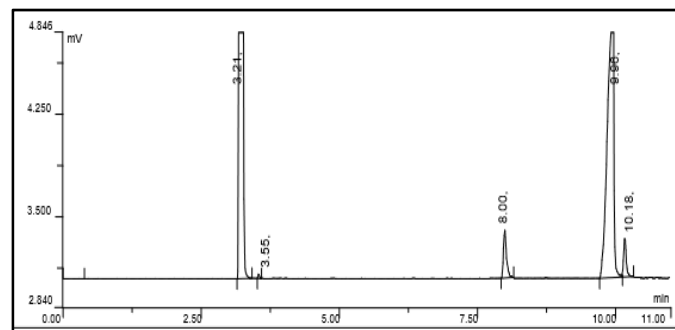


Fig. 8: GC Profile of Chia seed oil

Chia seed was found to contain Myristic acid, Palmitic acid, Linoleic acid and Linolenic acid corresponding to peaks obtained at RT 3.55, 8.00, 9.96 and 10.18 respectively.

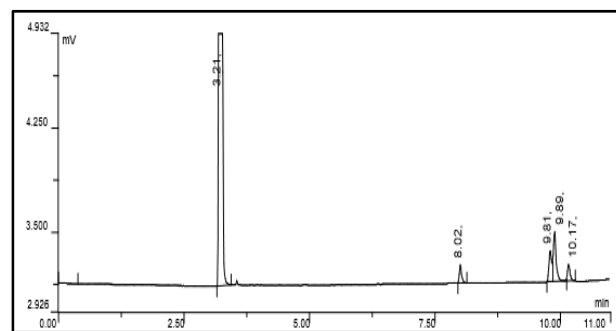


Fig. 9: GC profile of Pomegranate seed oil

Pomegranate seed was found to contain Palmitic acid, Stearic acid, Oleic acid, Linoleic acid, Punicic acid and Linolenic acid corresponding to peaks obtained at RT 8.02, 9.81, 9.89, 10.17, 11.53 and 11.66 respectively.

The percentage of alpha-linolenic acid (omega-3 fatty acid) was found to be maximum in case of watermelon seeds followed by pumpkin seeds, flaxseed and dill seed

respectively. Linolenic acid is an omega-3 fatty acid found in plants. Other omega-3 fatty acids include EPA (eicosapentanoic acid) and DHA (docosahexanoic acid). Our body can change alpha-linolenic acid to EPA and DHA. These omega-3 fatty acids—EPA and DHA—have shown to reduce inflammation and chronic diseases such as heart disease and arthritis. They are also beneficial for brain health and development as well as normal growth and development [15].

Oleic acid which is an important omega-9 fatty acid was found in sesame seed, pomegranate seed oil and kalonji seed. Oleic acid is considered as good cholesterol and lowers the level of LDL (low density lipoprotein) which ultimately lowers the bad cholesterol and systemic blood pressure [16].

Palmitic acid was found to be present in moderate concentration in almost all the seed oils except sesame seed oil [17].

Linoleic acid is omega-6 fatty acid which is important to human health. Conjugated linoleic acid is a potent antioxidant, anti-carcinogen and anti-catabolite as well as powerful immune system enhancer. Flax seed was found to be a rich source of linoleic acid followed by pumpkin seed and watermelon seed. Pomegranate seed oil is the one of the only plant sources that contain punicic acid (18:3 fatty acid, 9Z,11E, 13Z-Octadeca-9,11,13-trienoic acid) a compound closely related to conjugated linoleic acid (CLA) [18].

Punicic acid is omega-5 fatty acid. Omega-5 is a very powerful antioxidant. It is a high energy molecule that interferes with the production of inflammatory prostaglandin and leukotrienes that cause disease. It also helps reduce cell damage and regulates glucose transport. Pomegranate seed oil contains 70% of punicic acid [19].

4. CONCLUSION

Results of the present study revealed that flax, watermelon, pumpkin and dill seed lipids are excellent sources of ALA, which is a precursor of the long chain PUFA metabolically formed in humans.

It was also observed that sesame, kalonji and pomegranate seed oil are a great source of oleic acid which helps to reduce the levels of bad cholesterol in human body.

One of the most important fatty acids detected in case of pomegranate seed oil was punicic acid, an omega-5 fatty acid. The presence of these unsaturated fats was supplemented by their high iodine value.

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