Analysis of Heavy metals and Physico-Chemical Properties of *Sesamum indicum* Seed Oil from Arid Zone of Rajasthan

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Abstract
In the current study, we reported the concentration of heavy metals (ppm), physicochemical properties and fatty acid composition of Sesamum indicum seed oil from arid zone in Rajasthan. MP-AES is used for the analysis of heavy metals from the seed oil of Sesamum indicum. The heavy metals are found in seed oil in following order: Zn (7.92), Fe (1.14), Mn (0.74), Pb (0.07), Ni (0.05) and Cr (0.01) in ppm respectively. Sesamum indicum oil was tested for its physicochemical properties. The proximate analysis revealed 46.00% oil content.

HPLC was used to determine the fatty acid composition of the oil sample. Saturated, monounsaturated and polyunsaturated fatty acids were recorded for 15.52%, 42.86% and 41.32% of the total amount of fatty acids respectively. Linoleic acid (42.38%) was the most abundant fatty acid followed by oleic acid (41.20%) and palmitic acid (9.90%). Stearic acid (5.62%), linolenic acid (0.48%) and palmitoleic acid (0.12%) were present in small amounts.

Keywords: *Sesamum Indicum* seed oil**,** Heavy metals, Fatty acids, Physico-chemical properties.

Introduction

Sesamum indicum is a family of Pedaliaceae belonging to the Sesamum genus. It is an annual crop that grows to a height of 0.305 to 1.0058 meters. The seeds are small, ranging in length from 3 to 4 mm, width from 2 mm and thickness 1 mm. The seeds are oval and flattened in shape (Fig. 1).[9] The plant is found in the Rajasthan (India) areas of Pali (Maximum), Jodhpur, Churu, Nagaur, Sawai Madhopur, Bharatpur, Dholpur, Bikaner and Ajmer. Uttar Pradesh (maximum), Gujarat, Maharashtra andhra Pradesh, Tamil Nadu, Karnataka, Madhya Pradesh and Sunderbans of West Bengal are other areas where it can be found.[11] Sesame seeds and oil have been used in pharmaceuticals, cosmetics, perfumery, soaps, paints and insecticides as well as for human consumption. Sesame is still a significant oil seed crop in many parts of the world. The majority of sesame seeds is used to extract cooking oil.[14]

The fatty acid composition of vegetable oil determines its suitability for a specific use such as nutritional, industrial, or pharmaceutical applications. The ancient oilseed crop *Sesamum indicum* contains 45–60% oil and high protein content of 22% in its seeds. When the seed-bearing capsules reach maturity, they dehisce. Because plant growth is initially indeterminate, it is possible to see different developmental stages of capsules in a plant. Plants can flower as long as the weather permits.[1] It is a good radiation protector (Sun, UV light and wind) because of this it is used in a variety of cosmetics as well as in baby and children's skincare. Sesame oil has been reported to be used as a natural antioxidant and vitamin E also found in it.[2] Fats and oils are vital components of human diets, as they provide energy, fat-soluble vitamins and essential fatty acids.

Despite its high content of unsaturated fatty acids, sesame oil has a higher oxidative stability due to the presence of lignans like sesamolin, sesamin, sesamol and tocopherols.[3,5,8] Sesame oil has been shown to have anti-inflammatory, antihypertensive effects and anti-cancer properties.[16] Because of their high affinity for oxygen, antioxidants protect cells from oxidative damage, preventing cancer, aging-related disorders and cardiovascular disease.[4] Although, sesame oil oxidation stability and oil quality are affected by a variety of factors including sesame seed growth conditions (soil, environment and genotype), seed treatments, oil extraction methods, processing conditions and trace element presence. Due to the development of industries and increasing environmental pollution in the last decades, food contamination by heavy metals has become an important issue.[3,7]

The micro-elements such as Zn, Cu, Cd and Fe etc. are only needed in trace amounts in human body. Otherwise, their deficiency causes diseases and their overabundance causes toxicity to human life by disrupting organ and central nervous system function.[10] For example, Anemia is caused by an iron deficiency which affects more than half of pregnant women and at least one-third of children under the age of five. Lead is a well-known metal toxin that causes a variety of fatal diseases including kidney, blood and neurological system dysfunction. Because of the high toxicity and great solubility in soil and water, Zn has been considered an extremely significant pollutant, even in small amounts, affecting all forms of life.[6,15] Given the rising trend of vegetable oil consumption, particularly sesame oil, it is critical to understand sesame oil's properties as well as its effects on human health. The goal of this study was to determine the levels of both essential (Zn, Mn and Fe) and non-essential metals (Cr, Ni and Pb), physicochemical properties and the fatty acid composition in sesame oil.

Figure 1: Picture of *Sesamum indicum* plant and seeds

Material and Methods

Sampling: *Sesamum indicum* seeds were collected from western Rajasthan India, arid region. Damaged seeds were discarded. Seeds were cleaned, de-shelled and dried for 40 minutes at the temperature of 95-100°C. The seeds were ground in a grinder for oil extraction.

Oil extraction: The oil was extracted from the seeds using the solvent extraction method. Oil was extracted for 7-8 hours in a Soxhlet apparatus with petroleum ether (60-80°C). The obtained oil was kept cool (in the refrigerator) until further analysis. The standard American Oil Chemist Society (AOCS) methods were used to determine the analytical values of seed and seed oil. Methyl esters of oil were prepared using direct analytical TLC test, 2,4DNP TLC test, Halphen test, picric acid TLC test and alkaline picrate test performed for indication of any unusual fatty acid.[12]

Reagents: All reagents used were of the analytical grade. All dilutions were performed with double deionized water before use. All plasticware and glassware were cleaned by chromic acid and then washed them with distilled water. The standard operating solutions of heavy metals were made by mixing of standard stock solution of 1000 µg/L (Pb, Mn, Zn, Cr, Fe and Ni).

Digestion of seed oil: 1 gram seed oil was taken in a beaker and minimum amount of conc. HNO_3 was added to digestion of seed oil. For cold digestion, the solution was kept at room temperature for 24 hours before being heated at 50°C for 4 hours. To digest all the organic matter, the leftovers were boiled in HCl and HNO_3 solution (1:5). The solution was then cooled, filtered and made up to a final volume of 200 ml with deionized water.[13]

Preparation of mixed fatty acid: Hydrolysis of oil and fats yielded the fatty acid mixture. To begin, 1 gm of oil sample was placed in an oven dried round bottom flask and saponified with 1-2 ml of 1N standard alcoholic NaOH solution and 10 ml alcohol as solvent, then refluxed at 60-70^{o}C for 2 hours while TLC was monitored. Both saponified and unsaponified matter were present in the final mixture. 30 mL double distilled water was added to the mixture to dilute it further. The saponified matter was removed using a separating funnel by repeated washings with diethyl ether. The unsaponified matter in the upper organic (ether) layer was collected in a separate beaker. Diethyl ether is collected after evaporation with a rotatory evaporator. Dilute hydrochloric acid was used to acidify the lower aqueous solution which contained fatty acid salts (HCl-6N).

Fatty acids were extracted from this mixture by repeatedly washing it with diethyl ether. The lower aqueous layer was discarded and the upper combined ether extract, which contained a fatty acid mixture, was collected in an oven dried flask. These mixed fatty acids (MFA'S) were washed with double distilled water and dried over Na_2SO_4. Clean and pure MFA'S were collected after the excess ether was evaporated. TLC was used to monitor the entire procedure. HPLC was used to examine the methyl esters that were obtained. TLC plates were made by coating a glass plate with about 0.025 mm of layer silica gel. The mobile phase was a 70:29:1 mixture of petroleum ether, diethyl ether and acetic acid and the spot was visible in the iodine chamber.

For quantitative analysis, the fatty acid mixture was further derivated into esters and quantified in HPLC. Mixed fatty acids (MFA'S) were refluxed in a round bottom flask with excess methanol (1:6) on a water bath at 100^{o}C for approx. 1-2 hours using 1% H_2SO_4 as catalyst for the preparation of fatty acid methyl ester (FAME). After complete trans-esterification (as monitored by a TLC plate), the assembly was removed and the flask was cooled at room temperature to evaporate excess solvent and cooled over an ice bath before adding 30 mL double distilled water. Diethyl ether was used to extract the stirred wall and fatty acid methyl ester (FAME). The lower aqueous layer was discarded and the combined ether extracted in the upper layer was collected in a dried flask. Anhydrous FAME was obtained, after the solvent was evaporated and dried over Na_2SO_4, which was collected and stored at low temperature for further analysis.

Results and Discussion

Heavy metals analysis: Microwave Plasma Atomic Emission Spectroscopy (MP-AES) with nitrogen as the source gas was used to analyse zinc (Zn), lead (Pb), iron

(Fe), manganese (Mn), chromium (Cr) and nickel (Ni) in oil samples. Heavy metal concentrations in the seed oil ranged from 0.01 to 7.92 ppm (Table 1 and Fig. 2). The seeds of *Sesamum Indicum* can be used as a rich source of zinc for humans and plants, according to a large amount of analysis (Zn). *Sesamum Indicum* zinc and iron concentrations, on the other hand, are higher than the recommended limits for humans, plants and animals.

High concentrations of these metals, on the other hand, indicate that the plant has the ability to uptake and accumulate these toxic metals and thus can be used to treat highly contaminated soil in industrial and urban areas. As a result, *Sesamum Indicum* can be recommended as a bio indicator for determining the levels of pollution in urban areas. The presence of other metals, on the other hand, can be attributed to metal uptake from the soil and metal translocation within the plants. MP-AES eliminates the need for a gas source by delivering a plasma release using microwave energy and nitrogen sourced from a gas chamber or expelled from the surrounding air.

Determination of physico-chemical parameters: The physicochemical properties of *Sesamum indicum* seed oil are shown in table 2, it was discovered that the test results were within the acceptable range. At room temperature (25°C), extracted sesame oil is pale yellowish in colour with a nutty flavour and viscous texture. The iodine value was 105 g/100 g, putting it in the non-drying oil category and indicates that it could be used in a topical formulation. The saponification value of oil is low 187 mg/g indicating that it could be used to make soap. The free fatty acid concentration in this oil is 0.73 mg KOH/g and indicates that it is stable.

The low degree of oxidation in the oil was demonstrated by a peroxide value of 7.20 meq KOH/g, which could be attributed to the oil's nutty flavour. Freshness and edibility are indicated by the acid value of 0.62 mg KOH/g of oil. *Sesamum indicum* seed oil's low acid and free fatty acid levels suggest that it would have a long shelf life. The pH value is 5.60 and the specific gravity is 1.27. The oil content was found to be 46 % (Table 2).

Determination of fatty acid composition: The High Performance Liquid Chromatographic analysis of the total lipids indicates that the amount of linoleic acid was the highest (42.38 %), oleic acid was the second most abundant unsaturated acid (41.20 %), palmitoleic acid (0.12 %) present in minor amounts and saturated acids such as palmitic acid (9.90 %) and stearic acid (5.62 %) (Table 3 and Figure 3).

Table 1
Heavy metal content of *Sesamum indicum* seeds oil

S.N.	Heavy Metal	Concentration in ppm
1	Cr	0.01
2	Pb	0.07
3	Mn	0.74
4	Zn	7.92
5	Ni	0.05
6	Fe	1.14

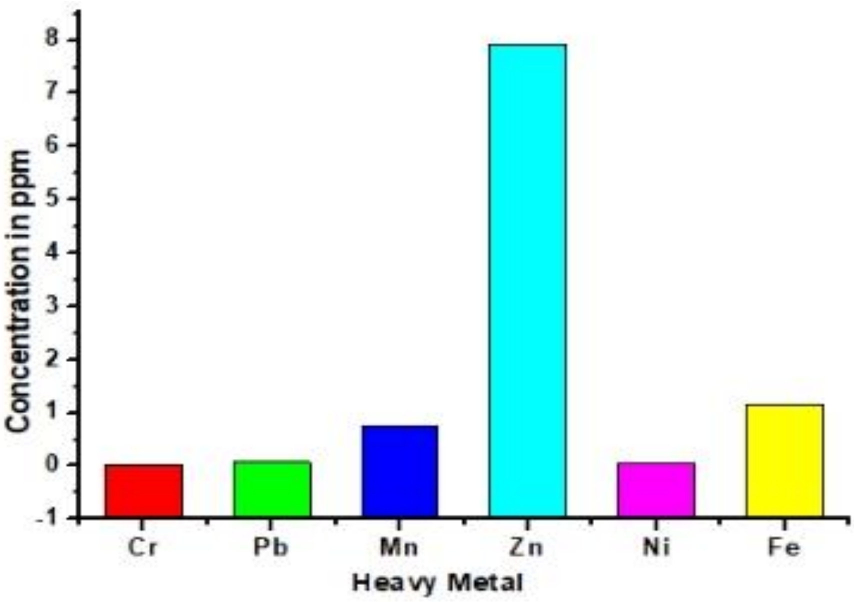

Figure 2: Plot showing comparison between Heavy metal Content

Table 2
Physico-Chemical properties

S.N.	Characteristics	Values
1	Oil Content %	46
2	Iodine Value (g I_2/100g)	105
3	Acid Value (mg KOH/g)	0.62
4	Free Fatty acid	0.73
5	Peroxide Value (Meq KOH/g)	7.20
6	Saponification Value (mg KOH/g)	187
7	Specific Gravity (g/cm^3)	1.27
8	Cyanide Test	Negative
9	pH	5.60
10	Appearance	Light Yellowish-pale yellowish
11	Odour	Nutty flavour

Table 3
Fatty acid composition of the seed oil of *Sesamum indicum*

S.N.	Fatty acid	% Composition
1	Linoleic acid (C 18:2)	42.38
2	Oleic acid (C 18:1)	41.20
3	Palmitic acid (C 16:0)	9.90
4	Stearic acid (C 18:0)	5.62
5	Linolenic acid (C 18:3)	0.48
6	Palmitoleic acid (C 16:1)	0.12

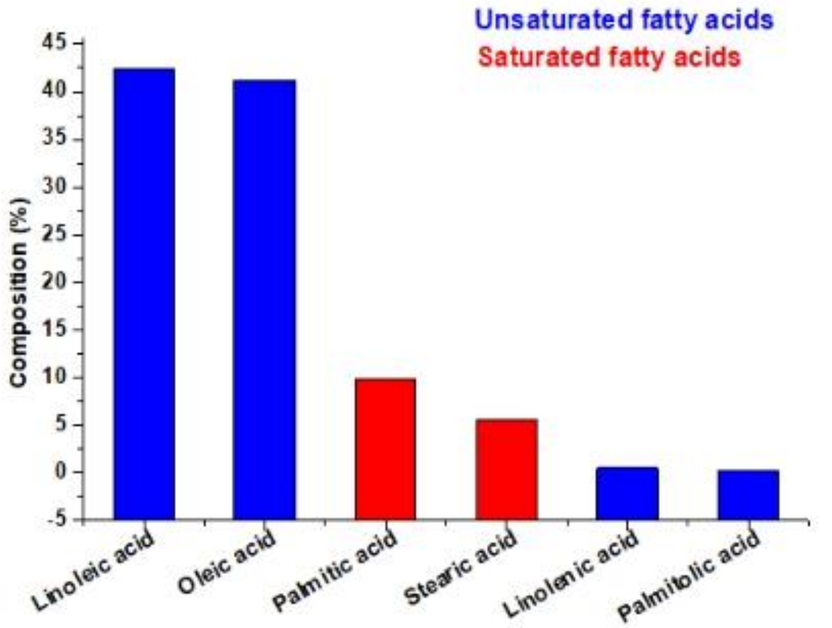

Figure 3: Fatty acid composition of the oil from seed of *Sesamum indicum*

Conclusion

In this study, we concluded that the *Sesamum indicum* seed oil has a high level of contamination of metals like zinc (7.92 ppm) and iron (1.14 ppm) indicating that this plant can uptake toxic metals from the soil as well as through foliar absorption. Furthermore, the *Sesamum indicum* seed oil contains high percentage of unsaturated fatty acids (84.18 %) making it useful for cooking and industrial purposes (in place of coconut oil). *Sesamum Indicum* oil was tested for its physicochemical properties. The proximate analysis revealed 46.00% oil content in *Sesamum indicum* seed oil.

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References

1. Borchani C., Besbes S., Ch Blecker and Attia H., Chemical Characteristics and Oxidative Stability of Sesame Seed, Sesame Paste and Olive Oils, *J. Agr. Sci. Tech.,* **12**, 585-596 **(2010)**

2. Gebrekidan A. and Desta A.A., Assessment on the levels of selected essential and non-essential metals in sesame seeds (Sesamum indicum L.) collected from Sheraro Town, Northwest Tigray, Ethiopia, *Bull. Chem. Soc. Ethiop*., **33(2),** 191-202 **(2019)**

3. Ghazani S.M., García-Llatas G. and Marangoni A.G., Minor constituents in canola oil processed by traditional and minimal refining methods, *J. Am. Oil Chem. Soc.,* **90**, 743–756 **(2013)**

4. Hashempour-Baltork F., Torbati M., Azadmard-Damirchi S. and Savage G.P., Quality properties of sesame and olive oils incorporated with flaxseed oil, *Adv. Pharm. Bull.,* **7(1)**, 97 **(2017)**

5. Kheirati R.S., Akrami M.F., Moshtaghi B.H., Pourramezani F., Jambarsang S., Kiani H. and Khalili S.E., The chemical composition and heavy metal content of sesame oil produced by different methods: A risk assessment study, *Food Sci. Nutr.,* **9**, 2886–2893 **(2021)**

6. Kocak S., Tokusoglu O. and Aycan S., Some heavy metals and trace essential elements. Detection in canned vegetable foodstuffs by differential pulse polarography, *Electronic J. Environ. Agric. Food Chem.,* **4**, 871-878 **(2005)**

7. Lee S.W., Jeung M.K., Park M.H., Lee S.Y. and Lee J., Effects of roasting conditions of sesame seeds on the oxidative stability of pressed oil during thermal oxidation, *Food Chem.,* **118**, 681–685 **(2010)**

8. Mondal N., Bhat K.V. and Srivastava P.S., Variation in fatty acid composition in Indian germplasm of sesame, *J. Am. Oil Chem. Soc.,* **87(11)**, 1263-1269 **(2010)**

9. Putnam A.R., Kaminski C.V., Hanson E.A., Oplinger E.S., Oelke E.E. and Doll J.D., Sesame production, United States Department of Agriculture, Farmer's, Bulletin No. 2119 **(2003)**

10. Ramezani M. and Rezaei Gorgani M., Physicochemical Properties of Heavy Metals and Aflatoxin Levels in Sesame Oil: A Review Study, *J. Nutrition Fasting Health*, **6(1)**, 45-51 **(2018)**

11. Sesame seed production in 2018, Crops/World Regions/Production Quantity from pick lists", UN Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) 2020 **(2020)**

12. Shahid M. and Arora A.K., Analysis of heavy metals and physico-chemical properties of Ricinus communis seed oil from arid zone of Rajasthan, *Int. J. Appl. Sci. Eng.,* **6(3)**, 2515-2519 **(2018)**

13. Shreshtha S., Omprakash, Praveen K. and Kumar A.A., A potential source of Omega-6 and Omega-9 in Cucumis melo Seed Oil from Arid Zone of Rajasthan, *Res. J. Chem. Environ.,* **25(6)**, 158-162 **(2021)**

14. Uzun B., Arslan C. and Furat S., Variation in fatty acid compositions, oil content and oil yield in germplasm collection of Sesame (Sesamum indicum L.), *J. Am. Oil Chem. Soc.,* **85**, 1135–1142 **(2008)**

15. Vinas P., Pardo-Martinez M. and Hernandez-Cordoba M., Rapid determination of selenium, lead and cadmium in baby food samples using electro thermal atomic absorption spectrometry and slurry atomization, *Anal. Chim. Acta,* **412**, 121-130 **(2000)**

16. Wichitsranoi J., Weerapreeyakul N., Boonsiri P., Settasatian C., Settasatian N., Komanasin N., Sirijaichingkul S., Teerajetgul Y., Rangkadilok N. and Leelayuwat N., Antihypertensive and antioxidant effects of Dietary black sesame meal in pre-hypertensive Humans, *Nutr. J.,* **10(1)**, 1-7 **(2011)**.

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