



## **Analysis of Heavy metals in seed oil of *Solanum lycopersicum* from Western region of Rajasthan**

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### **ABSTRACT**

*The tomato (*Solanum lycopersicum*) is the most important vegetable crop worldwide due to its edible seeds. The tomato plant belongs to the Solanaceae family which fruits are used by a human being and is an important source of food, salad, spice, medicine, etc. The demand for oil and oil-based production is increasing day by day due to the increase in population and improvement in the standard of living under the impact of development planning. Sample tomato seeds were collected from the western zone of Rajasthan, India. Oil extracted by using Soxhlet apparatus and heavy metals were detected by AAS (Atomic Absorption Spectroscopy) that show tomato seed oil contains heavy metals like (Fe, Cr, Zn, Cu, Cd, Ni, Pd, Co). The physicochemical properties of *Solanum lycopersicum* seed oil has specific gravity (1.10) at 25°C, Iodine Value (162.5 g I<sub>2</sub>/100g), Saponification Value (150.8 mg/g KOH), Un-saponification value (3.05 % w/w), Refractive Index (1.01) and Acid value (3.15). The heavy metal concentration found in tomato seed oil was in the following order: Cd (28.9), Fe (13.35), Zn (8.03), Cu (1.87), Pb (1.61), Ni (1.48), Cr (1.37). The dominant saturated acids were palmitic acid (14.41%) and stearic acid (5.27%), while Omega-6 or linoleic acid (41.47%) and oleic acid (33.42%) unsaturated fatty acids were determined by GC-FID.*

**Keywords:** *Tomato seed oil, AAS, Heavy metals, fatty acids, linoleic acid Omega-6.*

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### **INTRODUCTION:**

The tomato plant (*Solanum lycopersicum*), is the most important larger vegetable crop worldwide due to its edible seeds and belongs to the Solanaceae family. The tomato plant is an herbaceous plant. It is a perennial and annual plant. It has a weak and woody stem often vines over other plants. The tomato (*Solanum lycopersicum*) is a worldwide plant that is mostly used in Phytochemistry. Oilseed crops take on great significance or value in agriculture fields [1]. Although oil seeds crops are an important source of edible oil and food, they are also known as promising substituents for regular diesel fuel as they are renewable and environmentally friendly [2]. The leading five oil seeds producing countries are the USA, China, Brazil, Argentina, and India [3]. The tomato plant has highly rich nutritional value in their products like Juices, purees, ketchup, sauce, paste, etc. But after the extraction of these products the solid waste parts which are mainly seeds create an ecological problem [4]. This could be due to industrial wastewater, sewage sludge applications, fertilizers, and watering practices in agricultural lands [5, 6]. Several unit operations like drying, grinding, squeezing, and filtrating were used to obtain oil from tomato seeds [7]. Seeds of tomatoes have in composition essential fatty acids (EFA's), vitamins (A, D, E, and K) phytosterols, and other components with an important role in nutrients that play an important role in human health and diet [8].

Tomato plants growing in western region have been shown medicinally and nutritionally important. Although all these oil-seeds crops are grown in loamy soils, which are saline/ sodic or contaminated with heavy metals including non-essential cadmium (Cd) and lead (Pb) and essential Copper (Cu), Zinc (Zn), and Manganese (Mn), etc. Soiled heavy metals contamination not only reduces the growth and yield of oilseed crops but also enters the food chain and causes human health hazards [9]. Higher concentrations of heavy metals reduce the speed of uptake of essential nutrients by plants causing oxidative stress, which reduces plant biomass and grain yield [10]. Heavy metal concentrations varied among the plants, which reflect the differences in their uptake capabilities and their further translocation to the edible portion of the plants [11].

Heavy metals, which are considered as “the group of metals and metalloids with an atomic density greater than 4g/cm<sup>3</sup>, or 5 times or more, greater than water. Heavy metals are also called trace elements due to their presence in trace (10 mg/kg) or in ultra-trace (1~g kg<sup>-1</sup> or 1g/kg) quantities in the environmental metrics [12]. Thus, some metals can be toxic at very low concentrations (e.g., the maximum residue limits for Cd and Pb in many foods are 0.05 and 0.1mg/kg) respectively [13, 14]. The importance of heavy metals and their toxicological effect on human health and nutrition have been extensively studied in the last few years. Some elements i.e., Cu, Zn, and Fe can act as nutrients and are important for health, while some metals such as Ni, Pb, Cd, As, and Hg are more generally regarded as toxic. Toxic heavy metals are regarded as serving inorganic pollutants due to their toxic effects on life [15].

Tomato is an excellent source of many nutrients and secondary metabolites that are important for human health, including minerals, matter, and vitamins. Lycopene, flavonoids, organic acids, phenolic and chlorophyll [16, 17]. Additional tomato fruits contain several anti-oxidants such as vitamin C, Carotenoids; phenolic of nutritive and toxic elements in tomato samples depends on the growing condition and the utilization of pesticides and fertilization [18]. Seeds of the tomato plants are a rich source of various kinds of lipidic compounds including fatty acids, tocopherols, triacylglycerol, phospholipids, sphingolipids, and sterols. Fats and oil have outstanding importance not only in nutrition and pharmacy but also in the field of renewable resources. The tomato seeds are a source of vegetable oil, which contain approx. 30% oil [19]. This oil showed excellent quality for edible vegetable oil and bioactive compounds [20].

For the heavy metals detection of tomato seed oil samples, AAS (Atomic Absorption Spectroscopy) is used. This technique was used as one of the most common instrumental methods for analyzing metals and some metalloids. These techniques are also widely used for metal determination in food samples. The most commercially available instrument of this type has been designed for liquid samples and requires solid samples to be dissolved prior to analysis [19]. Atomic Absorption Spectroscopy determines the presence of metals in liquid samples. Metals that can be analyzed include Fe, Cu, Al, Pb, Ca, Zn, Cd, etc. Metalloids like antimony (Sb), Arsenic (As), Selenium (Se), and Tellurium (Te) are now routinely analyzed by hydride generation Atomic Absorption Spectroscopy [21, 22]. Fe is essential metals for all plants in the biological process like Photosynthesis, chloroplast development and chlorophyll biosynthesis and it is a major constituent of the cell redox system. Although the Fe<sup>+2</sup> excess can use free radical production that impairs cellular structure irreversibly and damages membrane, DNA and Protein [23, 24]. The higher value of iodine is measured by the amount of unsaturation in fats and oil [25]. Density and viscosity parameters show that tomato seed oil can be used as fuel after trans-esterification or not to damage the engine [26]. The higher value of saponification shows the capacity of forming soap of oil. The high Iodine value shows that the tomato seed oil has a high content of unsaturation in fatty acid.

The peroxide value (PV) is a measure of the extent of oxidation of a fat or oil that indicates the quantity of an oxidized substance. Oxidation of fatty acid might be causing the formation of hydroperoxide. Ash content is important to determine the concentration of heavy metals and expresses to determine the degree of impurity [27].

The high content of polyunsaturated fatty acids (PUFAs) makes it high-quality nutritional oil. Due to this high PUFAs content, it exhibits many types of properties like inhibition of cardiovascular disease, reduction of cholesterol in serum, dilation of blood vessels, and regulation of autonomic nerves [28]. Two types of PUFA Linoleic and Linolenic are important with regards to the health, disease, and stability of tomato seeds oil [29].

## **MATERIAL AND METHODS: -**

### **1. Sampling and Oil extraction: -**

Tomato plant seeds were collected in Rajasthan, India, in the western zone of Jodhpur (shown in figure - 1). Seeds were washed several times in water and dried in the open air. After the clean and dried seeds sample was crushed in motor and pistil. The oil from crushed seeds was extracted with petroleum ether (boiling range between 60-80°C) using the Soxhlet's procedure for 6-8 hours.

### **2. Seed oil content determination: -**

10 grams of seed flour was mixed with solvent and packed in Soxhlet apparatus for extraction of oils. The solvent was evaporated with the help of a rotary evaporator and thus obtained oil was dried in the oven at 105°C in over 20 minutes for removing solvent traces remaining in oil. The mass of oil was measured and percentage of oil was calculated using following formula:

$$\% \text{Oil} = (P/M) \times 100$$

here,

P = mass of oil and M = mass of plant seed flour used.

Determination of oil content in plants is important because it predicts the profitability of given plants as potential sources of oil. High oil content in plant seeds implies that processing them for oil would be economical [30].



**Figure1: - Map of study zone - Jodhpur**

**3. Reagents:** - All the reagents were of analytical grade.  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HClO}_4$ ,  $\text{H}_2\text{O}_2$ ,  $\text{HCl}$ , and  $\text{HF}$ . All the glassware was rinsed with distilled water and was cleaned by soaking in dil.  $\text{HNO}_3$ . All the standard solution for calibration of heavy metals was prepared by diluting a stock solution of  $1000\mu\text{g/L}$  (Pb, Cd, Zn, and Fe & Ni).

**4. Preparation of Standard for Metals:** -

The entire standards were of analytical grade. The standard for analysis of metals is prepared by dissolving 1gm of metals (Cd, Cu, Fe, Pb, Ni, Zn, etc.) dissolve in a minimum quantity of aqua-regia (1:3)  $\text{HCl}:\text{HNO}_3$ , make-up to 1liter in volumetric flask by adding double distilled water.

**5. Digestion of seed oil:** -

For the seed oil samples analysis, 1g oil sample was taken in a beaker containing 5 ml concentrated nitric acid. Beaker was placed on a hot plate to evaporate the sample to near dryness. After cooling another 5 ml of concentrated nitric acid was added, the beaker was covered with a watch glass and returned to the hot plate. Heating was continued until digestion was completed. 1-2 ml concentrated nitric acid was added and the beaker was warmed to dissolve the residue. Watch glass and sides of the beaker were washed with deionised water and the sample was filtered and adjusted to volume of 100 ml.

**Preparation of Fatty Acid Methyl Esters (FAMES):** -

FAME was obtained by transesterification with Sodium methylate in methanol. For the preparation of fatty acid methyl esters, fatty acids were refluxed in a round bottom flask with excess (1:6) on a water bath ( $100^\circ\text{C}$ ) for approx. 1-2 hours using 1%  $\text{H}_2\text{SO}_4$  as a catalyst. Flask was cooled at room temperature to evaporate the excess solvent and cooled over ice-bath and 30ml double distilled water was added in it, stirred well and FAMES were extracted with diethyl ether. The Organic layer was separated and dried over anhydrous  $\text{Na}_2\text{SO}_4$  [31].

## RESULTS AND DISCUSSION: -

### Physicochemical Characteristics of *Solanum lycopersicum* Oil:

To evaluate the suitability of tomato oil for a given purpose, it is necessary to determine their physicochemical characteristics and FA compositions. *Solanum lycopersicum* oils vary in their physicochemical properties such as specific gravity, melting point, saponification value, percentage of unsaponifiable value, acidic value, and FA composition. The tomato seed oil yield is 30.5 by a dry weight [32]. The seed oil obtained from tomato was yellow-reddish in liquid at room temperature and has a pleasant odour [33, 34]. The Physicochemical properties of Seed oil of *Solanum lycopersicum* were obtained by using the method described by AOC'S as given in table 1.

**Table 1: - Physicochemical Properties of *S. lycopersicum* seed oil.**

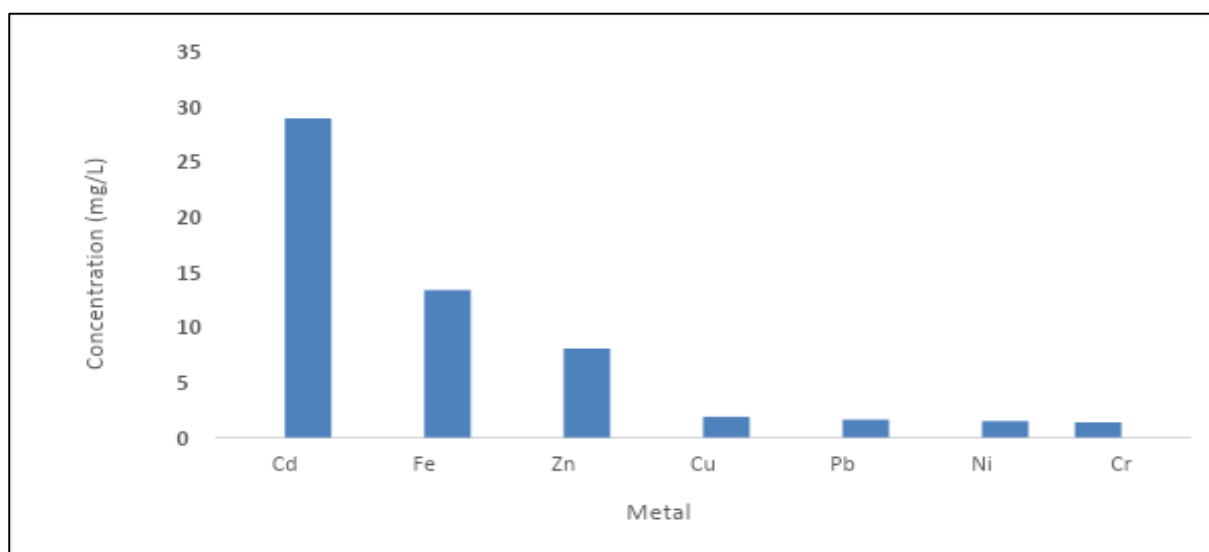
S. No	Characteristics of seed oil	Value
1.	Oil content (% by w)	30.7
2.	Moisture content (% by w)	73.5
3.	Protein content (% by w)	8.94
4.	Refractive index ( $40^\circ\text{C}$ )	1.01
5.	Saponification value (mg/g KOH)	150.8
6.	Un-saponification value (%w/w)	3.05
7.	Acid Value	3.15
8.	Iodine value ( $\text{g I}_2/100\text{g}$ )	162.5

### Heavy metals concentration analysis:

Quantitative estimation of heavy metals components was carried out using atomic absorption spectroscopy. In the atomic absorption spectrometer, the absorbance is linearly related to the concentration of metal in the sample. So, the determination of concentration of metals in plant seed oils by atomic absorption spectrophotometer. The wavelength dial was adjusted based on the nature of the metal present in the sample and to be analyzed. The desired wavelengths for various metals are given below –

**Table 2: Wave lengths for various metals uses in AAS**

S.No	Element	Wave length of main resonance line $\lambda$ (nm)	Type of Flame	Absorbance [mg/L1%]
1	Cd	228.8	Air Acetylene mixture	25
2	Fe	248.3	Air Acetylene mixture	100
3	Ni	232.0	Air Acetylene mixture	100
4	Pb	283.3	Air Acetylene mixture	500
5	Zn	213.9	Air Acetylene mixture	15
6	Cu	324.8	Air Acetylene mixture	15



**Figure 2: Graphical representation of concentration of heavy metals detected in seed oil of *S. lycopersicum***

Heavy metals analysis in the tomato seed oil show the presence of Cd (28.9 mg/L), Fe (13.35 mg/L), Zn (8.03 mg/L) are present in high amount whereas Cu (1.87 mg/L), Pb (1.61 mg/L), Ni (1.48 mg/L) and Cr (1.37 mg/L) are present in trace amount.

**Table 3: - Concentration of heavy metals detected in seed oil of *S. lycopersicum***

S.No.	Metal	Concentration(mg/L)
1	Cd	28.9
2	Fe	13.35
3	Zn	8.03
4	Cu	1.87
5	Pb	1.61
6	Ni	1.48
7	Cr	1.37

### 2. GC-FID Analysis: -

fatty acids were observed in tomato seeds oil in which unsaturated fatty acids are Linoleic acid (C18:2), Oleic acid (C18:1), Linolenic acid (C18:3) while saturated fatty acids like Palmitic acid (C16:0), Stearic acid (C18:0), Arachidic acid (20:0), Capric acid (10:0), and Caprylic acid (8:0) that are present in small amount were observed. The tomato seed oil contains more unsaturated fatty acids, especially Linoleic and Oleic acid, which is determined by using GC-FID spectroscopy. Figure 3 shows that Palmitic acid was the major saturated fatty acid (14.41%) and to be more saturated fatty acid followed by Stearic acid (5.27%). The polyunsaturated fatty acids are 76.10% where Linoleic acid (41.47%) was the major unsaturated

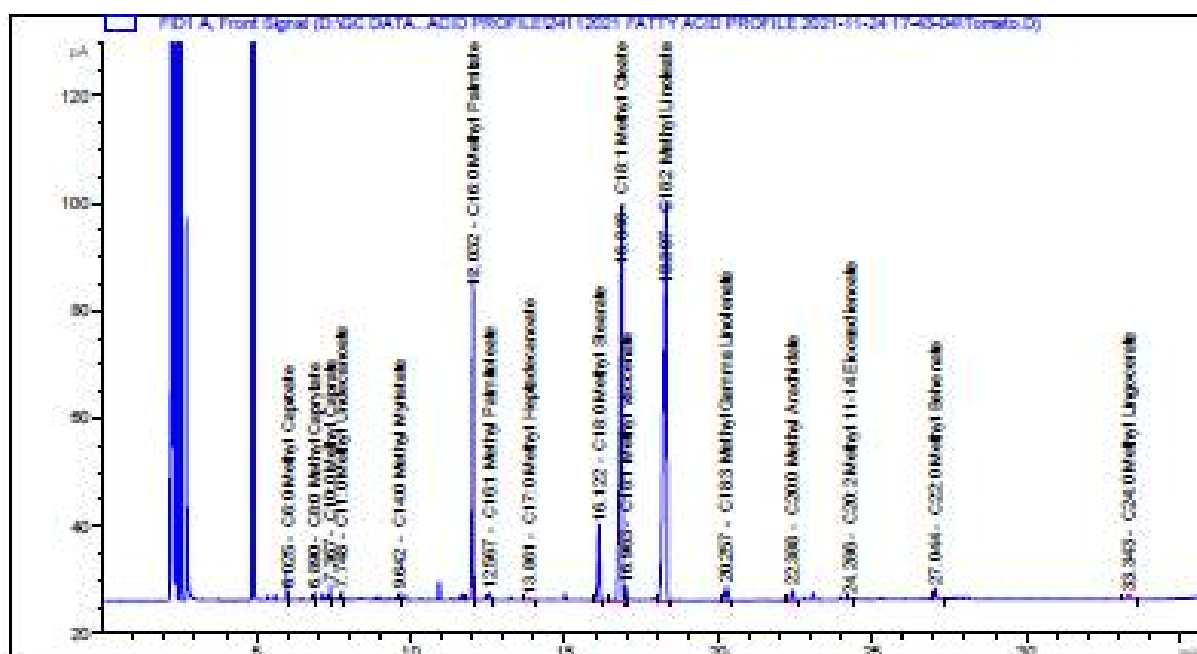
fatty acid followed by Oleic acid (33.42%), Linolenic acid (1.21%). Table 4 shows the fatty acid composition of *solanum lycopersicum* seeds oil.

**Table 4: Fatty acid content in *Solanum lycopersicum* seeds.**

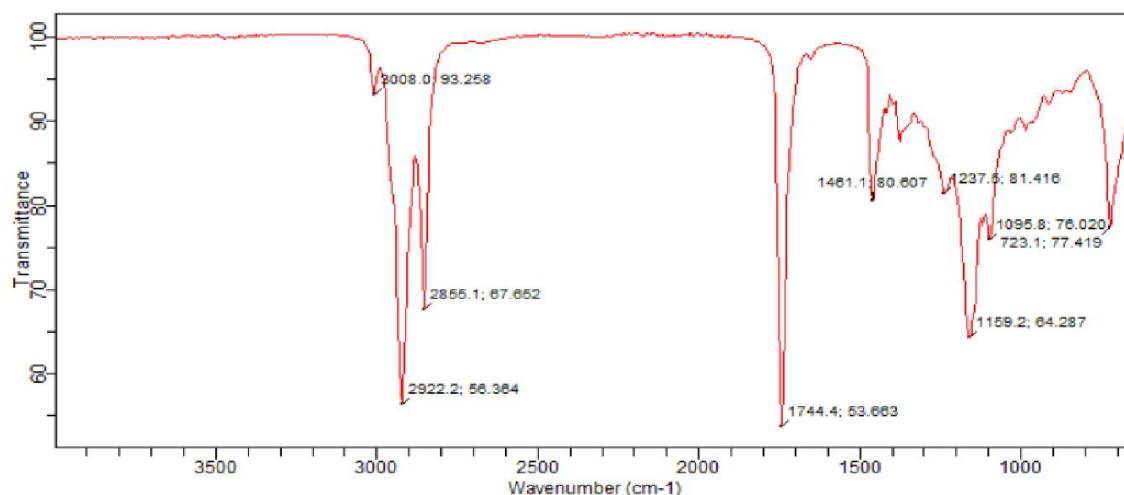
Fatty Acid	Obtained % by weight
Linoleic acid (18:2)	41.47
Oleic acid (18:1)	33.42
Palmitic acid (16:0)	14.41
Stearic acid (18:0)	5.27
Linolenic acid (18:3)	1.21
Arachidic acid (20:0)	0.73
Capric acid (10:0)	0.21
Caprylic acid (8:0)	0.09

**Table 5: Functional group and modes of Vibration in *Solanum lycopersicum*.**

Assignment	Frequency (cm-1)	Functional group vibration
1	3008	C-H stretching vibration of the cis -double bond
2	2922 and 2856	Asymmetric and symmetric stretching vibration of methylene (-CH <sub>2</sub> ) band
3	1740	Carbonyl (C=O) functional group
4	1488 and 1461	Bending vibration of CH <sub>3</sub> and CH <sub>2</sub> aliphatic groups
5	1364	Symmetric bending vibration of CH <sub>3</sub>
6	1244 and 1196	Vibration of stretching mode from C-O group in esters
7	1017	-CH bending and -CH deformation vibration of fatty acid
8	723	Overlapping of methylene (-CH <sub>2</sub> ) rocking vibration and to the out of plane vibration of cis- disubstitued olefins



**Figure 3: - Gas Chromatography of FAME of *Solanum lycopersicum***



**Figure 4: - FTIR spectra of *Solanum lycopersicum***

### CONCLUSION:

During the present investigation, the value of iron (Fe) was found much higher, which is significant due to the iron-rich soil of the area. Excessive concentration of iron replaces other vital minerals such as zinc, copper manganese, and many others in hundreds or even thousands of enzyme binding sites. The concentration of Cd metals is higher than the limits of AAS range limits. Cadmium is a toxic metal that causes different kinds of hazards including cell death or an increase in cell proliferation. Cadmium causes neurological disorders such as learning disabilities and hyperactivity in children. The concentration of heavy metals in seed oil was found quite near or above the permissible limit. Higher concentrations of heavy metals in seed oils represent a high level of contamination of the site where plants have grown thus the plants act as bio-indicators of pollution. The tomato seed oil contains high concentrations of Cd, Zn, & Fe heavy metals. Whereas Ni, Cu, and Pb metals are found in trace amounts. Metals' present behaviour in soils and plant uptake is dependent on the nature of the metals, soil physicochemical properties and plant species. In order to solve the aim of investigation, seed oil is digested and analyzed by atomic absorption spectroscopy (AAS).

The present work shows that tomato seed wastes were a potential source of edible oil. It contains a healthy mixture of saturated and unsaturated fatty acids. Tomato seed oil results showed that it is a good source of omega-6 (linoleic acid C18:2) fatty acid and Omega-9 (oleic acid C18:1) fatty acid. So, the tomato seed oil can be used as a dietary supplement in EFA deficient diets, it prevents heart and cardiovascular diseases.

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