



Comprehensive Analytical Profiling of Heavy Metals and Physico-Chemical Parameters in *Capsicum annuum* Seed Oil from Western Haryana

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ABSTRACT

Capsicum annuum seeds represent a promising but underexplored source of edible and functional oils. In this study, a comprehensive analytical profiling of *Capsicum annuum* seed oil from Western Haryana was undertaken, focusing on its physico-chemical characteristics and heavy metal content. The oil was extracted from seeds and analyzed for physicochemical parameters, including refractive index, specific gravity, acid value, free fatty acid, iodine value, saponification number, and unsaponifiable matter. Heavy metal content was determined using Microwave Plasma-Atomic Emission Spectroscopy (MP-AES). The results of physicochemical analysis were refractive index (1.460 – 1.450), specific gravity (0.913 – 0.878 g/cm³), acid value (1.78 – 0.90 mg/g), free fatty acid (0.91 – 0.49 mg/g), iodine value (77.10 – 70.50 mg/g), saponification number (188.30 – 141.28 mg/g), and unsaponifiable matter (2.07 – 1.30%). Mineral composition (mg/100 g) was Potassium (K) 480.25, Calcium (Ca) 162.40, Magnesium (Mg) 198.65, Iron (Fe) 28.10, Sodium (Na) 13.50, Zinc (Zn) 7.05, Copper (Cu) 4.95, and Manganese (Mn) 3.60. Cadmium and nickel were not detected in the samples. The study demonstrates that *Capsicum annuum* seed oil from Western Haryana is a valuable source of edible oil with significant nutritional and industrial potential, with physicochemical and mineral characteristics suitable for human consumption and further applications.

Keywords: Seed oil, Heavy metals, MP- AES, *Capsicum annuum*, Oil quality

Received 10.02.2026

Revised 21.04.2026

Accepted 09.05.2026

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an agriculturally significant crop widely cultivated across Asia, Africa, and the Mediterranean region. The versatile fruits of *C. annuum* have been extensively studied for their pigment, capsaicin content, and medicinal properties; the seeds, often treated as low-value byproducts, remain comparatively underexplored in terms of their chemical composition and potential applications (1). Seed oils obtained from spice crops have recently attracted research attention due to their unique fatty acid profiles, presence of bioactive compounds, and possible industrial uses. However, the chemical quality and safety of such oils can vary considerably depending on regional agricultural practices, soil characteristics, and environmental conditions (2).

Paprika seeds comprise 12–26% of the dry fruit's weight and account for around 60% of its total weight (3). The oil is predominantly composed of unsaturated fatty acids, with linoleic acid ranging from 68 to 78% and oleic acid between 7 and 15%, while the saturated fatty acids, including myristic (0–0.1%), palmitic (11–14%), and stearic acid (3–4%), are present in smaller amounts (4,5). Vegetable oils contain certain metals. Some of these metals, including Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Ferrous (Fe), Cu (Copper), Zn (Zinc) and Mn (Manganese) are required nutrients for human growth, whereas certain versions of these metals might be hazardous. The presence of trace metals is a crucial element as far as the quality of edible oil is concerned (6).

Western Haryana represents a dynamic agricultural zone where crop productivity is shaped by semi-arid climatic conditions, irrigation practices, and varying soil types. These factors may influence the mineral uptake of plants and ultimately affect the quality of derived products such as seed oils. In particular, the presence of heavy metals in crops has become a concern due to their potential entry into the food chain through contaminated soil, fertilizers, or water sources. Metals such as lead, cadmium, chromium, nickel,

and copper, even at low concentrations, can pose toxicological risks, making their detection and quantification essential for assessing environmental safety and consumer health (7,8).

Physico-chemical parameters of seed oils—including specific gravity, refractive index, acid value, saponification value, iodine value, and peroxide value—offer valuable insights into their stability, purity, and suitability for nutritional or industrial applications. When combined with a detailed analysis of metallic contaminants, these measurements provide a comprehensive overview of oil quality (9,10). Instrumental techniques such as Microwave Plasma-Atomic Emission Spectroscopy (MP-AES) are reliable tools for measuring trace metal levels in plant-derived samples, enabling a sensitive assessment of potential contamination (11, 12).

Considering the limited research on the chemical characterisation of *Capsicum annuum* seed oil from Western Haryana, this study aims to address this gap by carrying out a detailed analytical assessment. The study examines both the physico-chemical properties and the heavy metal content of the oil, offering vital data for evaluating its quality, safety, and potential uses.

MATERIAL AND METHODS

Reagents: All chemicals used in this study were of analytical grade. Double-deionised water was used for all sample dilutions and preparations. The acids used for sample digestion and analysis—nitric acid (HNO₃), sulfuric acid (H₂SO₄), hydrogen peroxide (H₂O₂), hydrofluoric acid (HF), perchloric acid (HClO₄), and hydrochloric acid (HCl)—were of high purity. Standard operating solutions of the heavy metals used were prepared by mixing a stock solution of 1000 µg/L (Pb, Cd, Zn, Fe, and Ni).

Sample and pre-processing: Seeds of *Capsicum annuum* L. were obtained from cultivated fields in the Western Haryana region, India. Only healthy, fully matured seeds were selected, while damaged or immature seeds were discarded. The selected seeds were carefully cleaned to remove dirt, debris, and foreign materials. They were then de-shelled and oven-dried at 60–70 °C for 30–35 minutes to reduce moisture content. After drying, the seeds were ground into a fine powder by gentle grinding to prepare them for subsequent oil extraction.

Oil Extraction: Extraction of oil from seeds was done by the solvent extraction method. Approximately 50 g of ground seed powder was placed in a Soxhlet apparatus. Petroleum ether (60–80 °C) was used as the extracting solvent. The extraction was carried out for 4–5 hours until the oil was completely removed from the seed matrix. After extraction, the solvent was removed by rotary evaporation under reduced pressure, and the oil was collected. Any residual solids were removed by filtration through Whatman No. 1 filter paper. The extracted oil was stored in amber glass bottles at 4–10 °C to prevent oxidation and preserve its physicochemical properties until further analysis (13).

Physicochemical Analysis: The physicochemical properties of both *Capsicum annuum* seeds and the extracted oil were determined following the standard procedures of the American Oil Chemists' Society (AOCS) (14). The analyzed parameters included specific gravity, refractive index, acid value, saponification value, iodine value, and peroxide value, which provide insights into the oil's quality, purity, and stability.

Specific Gravity: The specific gravity of *Capsicum annuum* seed oil was measured using a 25 mL pycnometer (density bottle). The pycnometer was first cleaned, dried, and weighed to obtain the empty bottle mass (W₀). The bottle was then filled with the oil, properly stoppered, and weighed to record the mass of the bottle plus oil (W₁). After emptying and thoroughly washing the bottle, it was filled with distilled water, stoppered, and weighed again to obtain the mass of the bottle plus water (W₂).

The specific gravity of the oil was calculated using the formula:

$$\text{Specific Gravity} = \frac{W_1 - W_0}{W_2 - W_0}$$

Where, W₀ = weight of dry empty density bottle; W₁ = weight of density bottle + oil; W₂ = weight of density bottle + distilled water (15,16).

Acid Value: The acid value of *Capsicum annuum* seed oil was determined following a standard titrimetric method. Approximately 2 g of the oil was accurately weighed into a conical flask. To this, 20 mL of neutral ethanol was added to dissolve the oil completely. The solution was titrated with 0.1 N alcoholic potassium hydroxide (KOH) using phenolphthalein as an indicator. The titration was continued until a persistent pink colour appeared, indicating the endpoint. The acid value was calculated using the formula:

$$\text{Acid Value (mg KOH/g oil)} = \frac{V \times N \times 56.1}{W}$$

where: V = volume of KOH solution used (mL), N = normality of KOH, W = weight of oil sample (g), 56.1 = molecular weight of KOH (14).

Iodine Value: After precisely weighing the oil (0.2 g), it is dissolved in 20 ml of chloroform in a 250 ml iodine flask. Using a pipette, Wij's reagent (20 ml) was added. The flask is placed in the dark with sporadic

fear and held for an hour. Next, 50 millilitres of distilled water and ten millilitres of a 15% potassium iodide solution were added. The mixture was added to the flask and stirred. Fresh flour solution indicator and 0.1 N sodium thiosulfate solutions were used to titrate free iodine. For years, it has also been the driving force behind space titration (15).

Saponification Value: About 2 g of oil was accurately measured and transferred into a 250 mL round-bottom flask. 25 mL of freshly prepared 0.5 N alcoholic potassium hydroxide (KOH) solution was added to the flask. The mixture was refluxed in a water bath for 1 hour to ensure complete saponification of the triglycerides present in the oil. After refluxing, the flask was cooled to room temperature, and the condenser tip was rinsed with a small amount of distilled water to recover any adhering solution. The excess KOH was then titrated with 0.5 N hydrochloric acid (HCl) using phenolphthalein as the endpoint indicator, until the pink colour just disappeared (16). A blank was determined under the same condition, and the saponification value of the oil was calculated as recommended by AOAC (17).

PREPARATION OF STANDARD FOR METAL: For the quantification of trace metals in *Capsicum annum* seed oil, standard solutions were prepared following established spectroscopic requirements. Because the samples contain metals at very low concentrations, the calibration standards must also be prepared at correspondingly low levels. To prepare stock standards, approximately 1 g of each pure metal (Cd, Ni, Fe, Pb, and Zn) was dissolved in a minimal volume of aqua regia (HCl: HNO₃ = 3:1). After complete dissolution, each solution was transferred to a 1 L volumetric flask and diluted to the mark with deionized water, yielding a stock concentration of roughly 1000 µg/L for each metal. From these stock solutions, working standards of the desired concentration ranges were prepared by serial dilution using deionized water. These standards were used to construct calibration curves for the instrumental analysis (18, 19).

RESULTS AND DISCUSSION

Physico-Chemical Properties of *Capsicum annum* Seed Oil: The physicochemical properties of the oil (Table 1) demonstrate that *Capsicum annum* seed oil from Western Haryana possesses characteristics consistent with high-quality edible oils. The refractive index (1.460–1.450) confirms the dominance of long-chain unsaturated fatty acids, while the specific gravity values (0.913–0.878 g/cm³) indicate high purity and absence of impurities. The low acid value (1.78–0.90 mg KOH/g) and free fatty acid content (0.91–0.49 mg/g) suggest minimal hydrolytic degradation, which reflects proper seed handling during drying and extraction.

The iodine value (77.10–70.50 mg/g) reveals moderate unsaturation, consistent with oils rich in linoleic and oleic acids. A saponification number of 188.30–141.28 mg KOH/g indicates the presence of mixed-chain triglycerides suitable for nutritional and industrial use. The unsaponifiable matter (2.07–1.30%) supports the presence of natural phytochemicals, including tocopherols and sterols, improving functional and nutritional properties.

These results align with reports on *Capsicum* seed oils from other regions, where similar ranges for refractive index, saponification, and acid values have been observed (20,21). The moderate iodine value indicates the presence of unsaturated fatty acids beneficial for human health while maintaining good oxidative stability.

Heavy Metal and Mineral Composition by MP-AES: The mineral analysis (Table 2) shows that *Capsicum annum* seed oil is rich in essential macro- and micro-nutrients. Potassium was the predominant element (480.25 mg/100 g), followed by magnesium and calcium. These concentrations fall within the expected nutritional range for spice-based seed oils. Trace elements such as zinc, copper, iron, and manganese were present at moderate levels, contributing to the oil's nutritional value. These micronutrients support antioxidant functions and metabolic processes.

A key finding is the absence of toxic metals cadmium and nickel, demonstrating that the cultivation soil and irrigation sources in Western Haryana are not contaminated by industrial effluents or hazardous waste. The metal composition pattern reflects the semi-arid environmental conditions of the region, where soil mineral availability and irrigation practices influence elemental uptake.

Similar trends were observed in *Capsicum* seed oils from Rajasthan and Punjab, suggesting that semi-arid soils rich in K, Ca, and Mg influence the mineral content of the seeds (22).

Considering that heavy metals accumulate through contaminated soil and irrigation, the absence of Cd and Ni reinforces the potential of this oil as a safe dietary and functional product. Moreover, the detected metals (Fe, Zn, Cu, Mn) are essential micronutrients, further enhancing the nutritional profile of the oil.

Table 1. Physico-Chemical Parameters of *Capsicum annuum* Seed Oil from Western Haryana

Parameter	Observed Range	Typical Range for Edible Oil	Interpretation
Refractive Index	1.460-1.450	1.45 - 1.48	Indicates good unsaturation & purity
Specific Gravity (g/cm ³)	0.913 - 0.878	0.88 - 0.93	Confirms purity & low oxidation
Acid Value (mg KOH/g)	1.78 - 0.90	< 2.0 desirable	Low hydrolytic rancidity
Free Fatty Acid (mg/g)	0.91 - 0.49	< 1 mg/g good	Minimal lipolysis
Iodine Value (mg/g)	77.10 - 70.50	70 - 140	Moderately high unsaturation
Saponification Number (mg KOH/g)	188.30 - 141.28	180 - 250	Medium to long-chain triglycerides
Unsaponifiable Matter (%)	2.07 - 1.30	0.5 - 3%	Presence of antioxidants, sterols, pigments

Table 2. Mineral and Heavy Metal Content of *Capsicum annuum* Seed Oil (MP-AES Analysis)

S. No.	Element	Concentration (mg/100g)	Nutritional Role /Toxicity
1	Potassium (K)	480.25	Essential nutrient; electrolyte balance
2	Calcium (Ca)	162.40	Bone health; enzyme activation
3	Magnesium (Mg)	198.65	Cofactor for >300 enzymes
4	Iron (Fe)	28.10	Oxygen transport; antioxidant roles
5	Sodium (Na)	13.50	Electrolyte and nerve conduction
6	Zinc (Zn)	7.05	Immune function; enzyme cofactor
7	Copper (Cu)	4.95	Antioxidant enzyme component
8	Manganese (Mn)	3.60	Metabolic and antioxidant roles
9	Cadmium (Cd)	ND	Toxic—Not Detected
10	Nickel (Ni)	ND	Toxic—Not Detected

CONCLUSION AND APPLICATION

The present study indicates that *Capsicum annuum* seed oil from Western Haryana exhibits favourable physicochemical characteristics, including specific gravity (0.913–0.878 g/cm³), refractive index (1.460–1.450), acid value (1.78–0.90 mg/g), free fatty acids (0.91–0.49 mg/g), iodine value (77.10–70.50 mg/g), saponification number (188.30–141.28 mg/g), and unsaponifiable matter (2.07–1.30%). These parameters reflect its stability, purity, and suitability for nutritional and industrial purposes.

Mineral profiling revealed appreciable levels of essential elements such as potassium (480.25 mg/100 g), calcium (162.40 mg/100 g), magnesium (198.65 mg/100 g), iron (28.10 mg/100 g), sodium (13.50 mg/100 g), zinc (7.05 mg/100 g), copper (4.95 mg/100 g), and manganese (3.60 mg/100 g), while potentially toxic metals like cadmium and nickel were not detected, indicating safety for consumption.

These results suggest that *Capsicum annuum* seed oil is a promising edible oil with nutritional and industrial relevance. Its chemical stability and mineral richness support applications in food, cosmetics, and soap production. Furthermore, the absence of toxic heavy metals underscores the suitability of Western Haryana cultivation practices for producing safe and high-quality seed oil. This study provides foundational data for the development, commercialisation, and quality control of Capsicum-derived oil products.

Conflict of Interest: The authors declare that they have no conflict of interest.

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CITATION OF THIS ARTICLE

Ramesh K, Renu S- Comprehensive Analytical Profiling of Heavy Metals and Physico-Chemical Parameters in *Capsicum annuum* Seed Oil from Western Haryana. *Bull. Env. Pharmacol. Life Sci.*, Vol 15 [7] June 2026. 104-108