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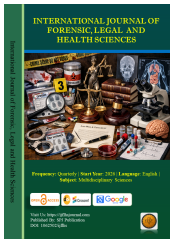

Original Research

Detection and Characterisation of Adulteration in Commercial Edible Oils Using Physicochemical Parameters and Gas Chromatography-Mass Spectrometry (GC-MS)

[1]Ravi Kumar, [2]Ashish Kumar*

[1]Assistant Professor, Department of Forensic Sciences, Teerthanker Mahaveer University, Moradabad (UP), India
[2]M. Sc. Scholar, Department of Forensic Sciences, Teerthanker Mahaveer University, Moradabad (UP), India

***Corresponding Author:** Ashish Kumar, Department of Forensic Sciences, TMU Moradabad, India.

Email: ashishkumar3800@gmail.com

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ABSTRACT

Edible oil adulteration is a significant food safety issue that compromises nutritional quality, product authenticity, and consumer health. This study investigated the quality of ten commercially and locally available edible oils using a combination of physicochemical analysis and gas chromatography–mass spectrometry (GC–MS). Free fatty acid content, acid value, moisture content, relative density, refractive index, and saponification value were determined and compared with standard reference ranges. Several samples, particularly local refined, peanut, yellow-seed, olive, and sunflower oils, exhibited values exceeding acceptable limits, indicating possible adulteration or quality deterioration. GC–MS analysis of commercial mustard and refined oils identified their characteristic fatty acid profiles while also detecting glycidyl palmitate, a processing-related contaminant, and linoleyl palmitate, suggesting compositional alterations. The findings demonstrate that integrating routine physicochemical testing with GC–MS provides a reliable and effective approach for detecting adulteration, assessing edible oil quality, and supporting food safety surveillance and regulatory quality control.

Keywords: Edible oils, Adulteration, Physicochemical analysis, GC–MS.

INTRODUCTION

Edible oils are an essential part of the human diet, providing a major source of energy, essential fatty acids, and fat-soluble vitamins required for growth, brain development, and the proper functioning of various physiological processes. Due to their high nutritional value and extensive daily consumption, edible oils represent one of the most economically significant food products worldwide. Their high market demand and price differences among oil varieties make them particularly vulnerable to adulteration, posing serious concerns for both food quality and public health. [1] Edible oil adulteration refers to any practice that intentionally or unintentionally reduces the purity, quality, or safety of an oil. Common fraudulent practices include blending premium oils with less expensive edible oils, mixing them with non-edible oils, or introducing harmful contaminants to increase profit margins. Such practices not only deceive consumers but also compromise nutritional quality and may expose individuals to hazardous substances. [2-7] The risk of adulteration has increased with the expansion of global food supply chains. Edible oils often pass through multiple stages of processing, transportation, storage, and distribution before reaching consumers, creating numerous opportunities for contamination and fraudulent substitution. In India, several investigations have reported adulteration in loose and unbranded edible oils, where substances such as mineral oil, karanja oil, castor oil, and synthetic colouring agents have been detected. Continuous

consumption of adulterated oils has been associated with adverse health effects, including liver damage, cardiovascular disorders, and severe toxic conditions such as epidemic dropsy caused by argemone oil contamination. [8] Adulterants found in edible oils can generally be classified into four major categories. Intentional adulterants are deliberately added for financial gain and include cheaper edible oils, mineral oils, and artificial colouring agents. Accidental adulterants arise during cultivation, processing, transportation, or storage and may include pesticide residues, microbial contaminants, or degradation products. Natural adulterants consist of toxic or anti-nutritional compounds that are inherently present in certain plant materials, whereas metallic contaminants such as arsenic, lead, and mercury may enter oils through environmental pollution or contact with processing equipment. [9]

Reliable identification of adulteration is therefore essential to ensure product quality and consumer safety. A variety of analytical techniques have been developed for edible oil authentication, including Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy, electronic nose systems, and chromatographic methods. [10] Among these, gas chromatography–mass spectrometry (GC–MS) is regarded as one of the most accurate analytical tools because it enables precise identification and quantification of fatty acid methyl esters (FAMEs) and can detect foreign compounds, contaminants, and chemical alterations with high sensitivity and specificity. Despite its excellent analytical performance, GC–MS requires sophisticated instrumentation, skilled personnel, and relatively long analysis times, making it less suitable for routine large-scale screening. [11-14] To overcome these limitations, the present study employed a stepwise analytical approach. Rapid and cost-effective physicochemical tests were first used to screen edible oil samples for potential quality deviations. Samples showing notable variations were subsequently analysed using GC–MS to confirm their authenticity and identify possible adulterants or contaminants. The specific objectives of this study were to: (i) evaluate the physicochemical quality parameters of ten commercially available edible oils and compare them with established reference standards; (ii) determine the fatty acid composition of selected branded mustard and refined oils using GC–MS; and (iii) assess the potential health risks and regulatory implications associated with the identified compounds. [15-17]

MATERIALS AND METHODS

Sample Collection: A total of ten edible oil samples were included in this study. These comprised commercial mustard oil (Sample A), refined oil (Sample B), sesame oil (Sample C), locally produced refined oil (Sample D), locally produced mustard oil (Sample E), peanut oil (Sample F), coconut oil (Sample G), olive oil (Sample H), sunflower oil (Sample I), and yellow-seed oil (Sample J). Commercially branded oils were purchased from supermarkets and retail stores in Moradabad, Uttar Pradesh, India, while loose oil samples were collected directly from local oil mills in the surrounding area. After collection, all samples were transferred to amber-coloured glass bottles and stored at room temperature away from direct light until further analysis. Each experiment was carried out in triplicate to ensure the reliability and reproducibility of the results.

Physicochemical Characterisation

Free Fatty Acid (FFA): Content. The free fatty acid content was determined using the standard titration method. Approximately 1–10 g of oil was dissolved in 25 mL of hot neutralized ethanol, followed by the addition of a few drops of phenolphthalein indicator. The mixture was titrated with 0.1 N potassium hydroxide (KOH) until a faint pink colour persisted for at least 15 seconds. The FFA content was expressed as the percentage of oleic acid equivalent.

Acid Value: The acid value was measured by dissolving a known quantity of oil in freshly neutralized hot ethanol containing phenolphthalein. The solution was then titrated with standardized alkali until a stable pink endpoint was obtained. The acid value was calculated according to the standard analytical procedure.

Moisture Content: Moisture content was estimated using the oven-drying method. One gram of each oil sample was placed in a pre-weighed crucible and dried in a hot-air oven. After drying, the crucible was cooled in a desiccator for 15 minutes before weighing. The heating and weighing cycle was repeated at 30-minute intervals until a constant weight was achieved. Moisture percentage was calculated based on the loss in sample weight.

Saponification Value: For determination of the saponification value, approximately 0.2–0.5 g of oil was refluxed with 10 mL of 0.5 N ethanolic potassium hydroxide in a water bath maintained at 80–85°C for 30 minutes. After cooling, the excess alkali was titrated with 0.5 N hydrochloric acid using phenolphthalein as the indicator. A reagent blank was analysed simultaneously under identical conditions.

Refractive Index: The refractive index of each oil sample was measured using an Abbe refractometer at a controlled temperature. Before each measurement, the prism surfaces were thoroughly cleaned with ethanol and acetone to prevent contamination. The observed values were compared with standard refractive index ranges reported for authentic edible oils.

Relative Density: Relative density was determined using a calibrated pycnometer following the gravimetric method. Measurements were corrected for buoyancy and calculated with reference to the density of water at 27.6°C. The equations used for density calculations are provided in the Supplementary Material.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis: Commercial mustard oil and commercial refined oil were selected for detailed molecular characterization using gas chromatography–mass spectrometry (GC–MS).

One millilitre of each oil sample was mixed with 1 mL of 5% sulfuric acid prepared in methanol to convert triglycerides into fatty acid methyl esters (FAMEs). The reaction mixture was vortexed for approximately 2 minutes and heated in a water bath at 100°C for 1.5–2 hours to complete the transesterification process. After cooling, an equal volume of *n*-hexane was added, and the mixture was vortexed again for 2 minutes. Once phase separation occurred, the upper hexane layer containing the FAMEs was carefully collected into clean sample vials for GC–MS analysis (16). Individual compounds were identified by comparing their mass spectra with standard spectral library databases, and their relative abundance was estimated from chromatographic peak areas.

Statistical Analysis: All experimental measurements were performed in triplicate, and the results are presented as mean values. Statistical differences among physicochemical parameters and fatty acid compositions of the oil samples were assessed using one-way analysis of variance (ANOVA). A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Physicochemical Characteristics of Edible Oil Samples: The physicochemical properties of the ten edible oil samples were evaluated and compared with the recommended reference standards (Table 1).

Table 1. Accepted reference ranges of physicochemical indices for the studied edible oils.

Edible oil	FFA (%)	Acid value	Moisture (%)	Rel. density	Refractive index	Saponification
Mustard oil	< 1.5	≤ 2.99	< 1	0.910	1.456–1.465	186–198
Refined oil	< 1.0	≤ 1.99	< 1	0.909–0.917 †	1.466–1.470	189–195
Local mustard oil	2–3	3.98–5.97	< 1	~0.910	~1.465 †	180–185
Local refined oil	2–2.5	3.98–4.98	< 1	~0.910 †	~1.465 †	180–185
Sesame oil	< 1.5	≤ 2.99	< 1	0.920	1.465–1.469	187–195
Olive oil	≤ 1.0	< 1.99	< 1	0.913–0.916	1.468–1.471	184–196
Coconut oil	≤ 2.0	< 3.98	< 1	0.908–0.921	1.448–1.449	248–264
Yellow-seed oil	< 2.5	< 4.98	< 1	0.905	1.467–1.470	185–195
Sunflower oil	< 1.0	≤ 1.99	< 1	0.918–0.923	1.467–1.469	188–194
Peanut oil	< 3.0	< 5.97	< 1	0.912–0.920	1.460–1.465	187–196

FFA, free fatty acid (% as oleic); acid value in mg KOH/g; saponification value in mg KOH/g. † Values marked require verification against the current FSSAI/BIS/Codex editions before submission (see Notes).

The analysed parameters included free fatty acid (FFA) content, acid value, moisture content, relative density, refractive index, and saponification value. The measured values for all samples are presented in Table 2.

Table 2. Experimentally obtained physicochemical values (mean of triplicate determinations).

Edible oil	FFA (%)	Acid value	Moisture (%)	Rel. density	Refractive index	Saponification
Mustard oil	1.51	3.00	0.65	0.899	1.458	219.0
Refined oil	1.01	2.01	0.32	0.919	1.469	248.8
Local mustard oil	1.43	2.85	0.11	0.847	1.467	218.5
Local refined oil	2.99	5.95	0.19	0.892	1.470	318.7
Sesame oil	0.45	0.90	1.50	0.892	1.470	257.7
Olive oil	3.50	6.97	0.98	0.915	1.435	238.5
Coconut oil	3.10	6.17	0.76	0.917	1.405	245.6
Yellow-seed oil	4.56	9.07	0.56	0.854	1.498	305.0
Sunflower oil	2.80	5.57	0.95	0.920	1.505	244.8
Peanut oil	5.60	11.14	2.23	0.995	1.498	210.0

Bold deviations from Table 1 (acid value, saponification, moisture) are discussed in Section 4. Units as in Table 1.

Significant variations were observed among the investigated oils. The acid value, an important indicator of hydrolytic deterioration and oil quality, exceeded the recommended limits in several samples. Local refined oil (5.95 mg KOH/g), olive oil (6.97 mg KOH/g), coconut oil (6.17 mg KOH/g), sunflower oil (5.57 mg KOH/g), yellow-seed oil (9.07 mg KOH/g), and peanut oil (11.14 mg KOH/g) exhibited acid values substantially higher than the acceptable limits. Among these, peanut oil recorded the

highest acid value, suggesting extensive hydrolysis of triglycerides or possible blending with inferior-quality oil. In contrast, commercial mustard oil, commercial refined oil, local mustard oil, and sesame oil showed acid values that were either within or close to the recommended range. A similar trend was observed for free fatty acid content (Figure 1).

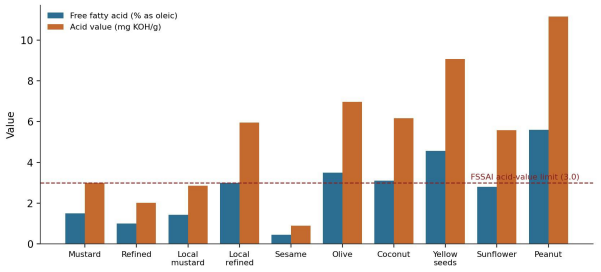

Figure 1. Free fatty acid content and acid value of the ten edible oils. The dashed line marks the FSSAI acid-value limit (3.0 mg KOH/g).

Peanut oil contained the highest FFA concentration (5.60%), followed by yellow-seed oil (4.56%), olive oil (3.50%), coconut oil (3.10%), local refined oil (2.99%), and sunflower oil (2.80%). Elevated FFA levels indicate progressive lipid hydrolysis during processing or storage and may also suggest adulteration or poor handling practices. Sesame oil exhibited the lowest FFA content (0.45%), reflecting comparatively better quality. Moisture content remained below the recommended limit of 1% in most samples. However, sesame oil (1.50%) and peanut oil (2.23%) contained higher moisture levels than the permissible range. Increased moisture accelerates hydrolytic and oxidative deterioration, thereby reducing the storage stability and shelf life of edible oils. Considerable variation was also observed in the saponification values of the analysed oils (Figure 2).

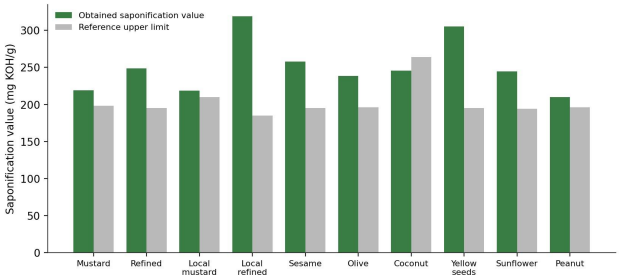

Figure 2. Obtained saponification values compared with the upper limit of the accepted reference range for each oil.

Most samples exhibited values exceeding the expected reference range. The highest saponification value was recorded for local refined oil (318.7 mg KOH/g), followed by yellow-seed oil (305.0 mg KOH/g) and sesame oil (257.7 mg KOH/g). Elevated saponification values generally indicate the presence of fatty acids with shorter average carbon chain lengths and may reflect blending with oils rich in medium-chain triglycerides, such as coconut or palm kernel oil. Conversely, peanut oil exhibited the lowest saponification value (210.0 mg KOH/g) among the analysed samples. Relative density measurements were generally consistent with standard values for most oils. However, local mustard oil (0.847) and yellow-seed oil (0.854) exhibited comparatively lower densities, whereas peanut oil showed an unusually high relative density (0.995), suggesting possible compositional differences or adulteration. The refractive index values of most oil samples were within or close to the expected range. Nevertheless, coconut oil showed an unusually low refractive index (1.405), while sunflower oil (1.505) and yellow-seed oil (1.498) exhibited values higher than the reported reference limits. Since refractive index reflects the degree of unsaturation and molecular composition of lipids, these deviations may indicate changes in fatty acid composition or the presence of adulterants.

GC–MS Characterization of Commercial Mustard Oil (Sample A): The chemical composition of commercial mustard oil was investigated using gas chromatography–mass spectrometry (GC–MS), and the identified compounds are summarized in Table 3.

Table 3. Compounds identified by GC–MS in branded mustard oil (sample A).

Retention time (min)	Compound	Relative abundance (%) ‡
5.60	Solketal methyl ether	2.7
9.70	Octanoic acid (C8:0)	0.2
11.79	Imidodicarbonic diamide	0.3
13.40	Decanoic acid (C10:0)	0.2
16.64	Dodecanoic acid (C12:0)	0.4
17.76	Acetic acid	0.2
19.56	Methyl tetradecanoate (C14:0)	5.2
31.33	Eicosenoic acid (C20:1)	64.9
39.21	Oleic acid (C18:1)	49.2
41.82	Octadecenoic acid (C18:1)	21.7
46.97	Silane, diethyl-	2.2

The chromatographic profile was dominated by long-chain monounsaturated fatty acids, which are characteristic constituents of mustard oil. Eicosenoic acid (C20:1) was identified as the predominant compound with a relative abundance of 64.9%, followed by oleic acid (49.2%) and octadecenoic acid (21.7%). The predominance of these unsaturated fatty acids is consistent with the characteristic lipid composition of authentic mustard oil. In addition to the major fatty acids, trace amounts of saturated medium-chain fatty acids, including octanoic acid (0.2%), decanoic acid (0.2%), dodecanoic acid (0.4%), and methyl tetradecanoate (5.2%), were detected. These compounds are generally present at very low concentrations in pure mustard oil, and their detection may indicate blending with

oils containing higher proportions of medium-chain fatty acids. Several non-lipid compounds, including solketal methyl ether (2.7%), imidodicarbonic diamide (0.3%), acetic acid (0.2%), and a diethyl silane derivative (2.2%), were also identified. These compounds may originate from processing, packaging materials, analytical derivatization, or environmental contamination rather than being natural constituents of mustard oil. Overall, the GC–MS profile confirmed that unsaturated fatty acids constituted the major lipid fraction of the mustard oil sample. However, the presence of medium-chain fatty acids and other non-native compounds suggests the possibility of partial adulteration or contamination during processing.

GC–MS Characterization of Commercial Refined Oil (Sample B): Compounds identified in commercial refined oil by GC–MS are listed in Table 4.

Table 4. Compounds identified by GC–MS in branded refined oil (sample B).

Retention time (min)	Compound	Relative abundance (%) ‡
19.60	Methyl tetradecanoate (C14:0)	2.6
22.88	Hexadecanoic acid / palmitic (C16:0)	20.9
28.10	6-Octadecenoic acid (C18:1)	30.3
30.94	Glycidyl palmitate	16.6
38.39	Tricosanoic acid (C23:0)	1.0
39.15	Oleic acid (C18:1)	9.4
42.37	Ethanol	17.6
46.79	Lauric acid (C12:0)	2.2
50.82	Linoleyl palmitate (wax ester)	29.8
57.41	Luperol	3.2

‡ Relative peak-area percentage, as reported.

The chromatographic profile revealed that palmitic acid (hexadecanoic acid; 20.9%), 6-octadecenoic acid (30.3%), and linoleyl palmitate (29.8%) were the predominant constituents of the sample. Oleic acid was also detected with a relative abundance of 9.4%, indicating that both saturated and unsaturated fatty acids contributed substantially to the overall lipid composition. One of the most notable findings was the detection of glycidyl palmitate (16.6%), a glycidyl ester commonly formed during high-temperature refining and deodorization of vegetable oils. Glycidyl esters are recognized as process-induced contaminants because they can generate glycidol during digestion, a compound regarded as potentially genotoxic. The relatively high abundance of glycidyl palmitate suggests that the oil may have undergone intensive thermal processing during refining. Another significant observation was the presence of linoleyl palmitate, a wax ester that is not typically reported as a major constituent of conventional refined edible oils. Its relatively high abundance may indicate blending with other lipid sources or compositional changes resulting from industrial processing. Minor quantities of methyl tetradecanoate (2.6%), lauric acid (2.2%), tricosanoic acid (1.0%), ethanol (17.6%), and lupeol (3.2%) were also detected. Ethanol is most likely associated with sample preparation, whereas lupeol represents a naturally occurring triterpenoid reported in several plant-derived oils. Collectively, the GC–MS analysis demonstrated that although the refined oil contained the expected major fatty acids, the detection of glycidyl palmitate and the unusually high concentration of linoleyl palmitate indicates significant processing-related chemical changes and raises concerns regarding product authenticity and quality.

DISCUSSION

The present study employed a two-tier analytical approach involving conventional physicochemical analyses followed by GC–MS characterization to evaluate the quality and authenticity of commercially available edible oils. This approach enabled rapid screening of oil quality while providing molecular evidence for potential adulteration and processing-related changes. The physicochemical evaluation revealed considerable variation among the analysed edible oils. Acid value and free fatty acid (FFA) content are widely recognized indicators of hydrolytic degradation and overall oil quality. Elevated acid values observed in local refined oil, olive oil, coconut oil, sunflower oil, yellow-seed oil, and particularly peanut oil indicate extensive triglyceride hydrolysis resulting from prolonged storage, improper handling, repeated thermal exposure, or possible adulteration with lower-quality oils. [18] Similarly, the increased FFA levels in these samples support the occurrence of lipid degradation, as free fatty acids accumulate during hydrolysis and accelerate oxidative deterioration. Oils with high FFA values generally exhibit reduced nutritional quality, shorter shelf life, and inferior sensory characteristics. Moisture content is another critical quality parameter influencing the stability of edible oils. [19]

Although most samples contained moisture within the recommended limits, sesame and peanut oils showed comparatively higher moisture levels. The presence of excess moisture promotes hydrolytic rancidity by facilitating triglyceride breakdown and enhances susceptibility to microbial growth during storage. Consequently, oils with elevated moisture content are expected to exhibit faster quality deterioration and reduced storage stability. The unusually high saponification values observed in several samples, particularly local refined oil and yellow-seed oil, suggest the presence of fatty acids with relatively shorter average chain lengths than expected for authentic oils. Such deviations may result from blending with oils naturally rich in medium-chain triglycerides, including coconut or palm kernel oil. Likewise, abnormal relative density and refractive index

values recorded for several samples indicate alterations in lipid composition. Since refractive index is closely associated with the degree of unsaturation and molecular structure of fatty acids, deviations from standard values may reflect compositional modification, oxidation, or adulteration. GC–MS analysis provided detailed molecular evidence supporting the physicochemical observations. The fatty acid composition of commercial mustard oil was characterized by the predominance of eicosenoic acid and oleic acid, which agrees with the characteristic lipid profile reported for authentic mustard oil. However, the detection of trace quantities of octanoic, decanoic, dodecanoic, and tetradecanoic acid derivatives suggests the possible presence of medium-chain fatty acid-containing oils. Although these compounds occurred at low abundance, their presence may indicate partial blending during production or contamination introduced during processing. The refined oil exhibited a more complex chemical profile. Palmitic acid, oleic acid, and 6-octadecenoic acid represented the major fatty acid constituents, reflecting the expected composition of refined vegetable oils. However, the detection of glycidyl palmitate is of particular significance. Glycidyl esters are generated during high-temperature refining and deodorization processes and are considered undesirable process contaminants because they can release glycidol, a compound classified as potentially genotoxic and carcinogenic. Their occurrence therefore reflects not only extensive thermal processing but also raises concerns regarding food safety and regulatory compliance.

Another noteworthy finding was the relatively high abundance of linoleyl palmitate, a wax ester that is not commonly reported as a major constituent of conventional refined edible oils. The occurrence of this compound may indicate blending with alternative lipid sources or compositional changes induced during industrial refining. Although additional confirmatory analyses would be required to establish the exact origin of this compound, its presence highlights the importance of chromatographic techniques for identifying chemical constituents that cannot be detected through routine physicochemical tests alone. Overall, the findings demonstrate that physicochemical analyses serve as effective preliminary screening tools for detecting abnormal quality characteristics, whereas GC–MS provides detailed molecular confirmation of compositional changes, processing-derived contaminants, and possible adulteration. The complementary use of both analytical approaches offers a comprehensive strategy for quality assurance of edible oils and may support regulatory agencies in monitoring market products to ensure consumer safety.

CONCLUSION

This study demonstrated that the combined application of physicochemical analysis and GC–MS is an effective approach for evaluating the quality and authenticity of edible oils. Several oil samples exhibited elevated acid values, free fatty acid contents, abnormal saponification values, and deviations in refractive index, indicating deterioration, improper processing, or possible adulteration. GC–MS further confirmed characteristic fatty acid profiles while identifying processing-related contaminants such as glycidyl palmitate and unusual lipid constituents that may reflect compositional alterations or blending. These findings highlight the importance of integrating routine quality assessment with advanced chromatographic techniques for reliable edible oil authentication. Continuous regulatory surveillance and stringent quality control measures are essential to minimize adulteration, improve product quality, and safeguard public health.

DECLARATIONS

Ethics Approval and Consent to Participate: This study was conducted using commercially available edible oil samples purchased from local markets. The research did not involve human participants, animals, clinical specimens, or personal data. Therefore, ethical approval and informed consent were not required.

Consent for Publication: Not applicable.

Availability of Data and Materials: All data generated or analysed during this study are presented in this manuscript. Additional information can be obtained from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no competing interests related to this study.

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Author Contributions: All authors contributed to the study design, sample collection, laboratory experiments, data analysis, interpretation of the findings, manuscript writing, and approval of the final version of the manuscript.

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