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## GENETIC REGULATION OF THE PHYSICOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES IN *SCHINUS MOLLE* SEED OIL

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### SUMMARY

*Schinus molle* L., also known as Peruvian pepper, is a South American plant recognized for its vital bioactive compounds and high-quality seed oil, which shows potential for agro-industries. This study investigated the physicochemical properties, fatty acid composition, and antioxidant activity of *S. molle* seed oil, particularly focusing on the genetic regulation underlying its oil quality. The oil's extraction used cold pressing and Soxhlet methods, with its physical properties (density, refractive index, viscosity, and melting point) and chemical composition (acid, peroxide, and iodine values) also determined. Notably, the oil exhibited a high content of oleic acid (40.2%) and linoleic acid (38.1%), with significant antioxidant activity (tocopherol content: 34.5 mg/100 g and 2,2-Diphenyl-1-picrylhydrazyl or DPPH radical scavenging activity: 82.3% inhibition). The results indicated genetic factors, including the *FAD2* gene for fatty acid desaturation, play a crucial role in shaping the oil's fatty acid profile and antioxidant potential. These findings underscore the importance of genetic regulation in optimizing the oil's composition for agro-industrial applications, particularly in functional foods and cosmetics.

**Keywords:** *S. molle*, seed oil, physical properties, fatty acid composition, antioxidant activity, agro-industrial applications, functional food

**Key findings:** The *S. molle* seed oil exhibited the highest unsaturation, oleic, and linoleic acids as predominant among fatty acids, with significant antioxidant activity, including high tocopherol content and radical scavenging ability. Genetic factors influence the oil's quality, affecting fatty acid composition and antioxidant properties, identifying them as a promising candidate for agro-industrial applications like functional foods and cosmetics.

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## INTRODUCTION

*Schinus molle* L., a pink pepper and false pepper tree native to South America, has received greater attention for its utilization in the agro-industries (Locali-Pereira *et al.*, 2023). The *S. molle* seeds contain oils with many bioactive properties, which are enhancers for use in food, cosmetic, and pharmaceutical applications. What is especially relevant and increasingly recognized is that genetic regulation strongly shaped the quality and bioactive profile of *S. molle* seed oil. Genes involved in fatty acid biosynthesis and antioxidant pathways directly influence the composition, nutritional value, and industrial suitability of the oil. In indigenous medicine, the *S. molle* seed oil has been traditionally used due to its anti-inflammatory, antimicrobial, and antioxidant properties (Martins *et al.*, 2014; Kouachi *et al.*, 2025; Fisher *et al.*, 2025).

However, scientific studies on the nature of the physical-chemical properties and possible applications of the *S. molle* seed oil have been few. The *S. molle* seed oil is a better source of unsaturated fatty acids, such as oleic acid (C18:1) and linoleic acid (C18:2), with a greater role in human health benefits (Belhoussaine *et al.*, 2024). These fatty acids even contribute to important processes for enhancing cardiovascular and skin hydration health; therefore, the oil can be beneficial both in food products and topical cosmetic preparations. Moreover, the *S. molle* seed oil has antioxidant compounds, including tocopherols (vitamin E), which are greatly desirable for their ability to counter free radicals and prevent oxidation in health products and cosmetics (Hosni *et al.*, 2011).

Although detailed scientific works on the nature of the physicochemical characteristics, the fatty compound structure, and antioxidant contents of the *S. molle* seed oil are scarce (Tsitlakidou *et al.*, 2023). Knowledge of these properties is fundamental for determining their applicability to various agro-industrial uses, especially in formulations that must be oxidatively stable and contain desirable bioactive compounds (Marcela *et al.*, 2025). This gap in understanding widens by

the lack of focus on the genetic regulation behind these properties. The genetic factors governing fatty acid biosynthesis, antioxidant pathways, and secondary metabolite production play a crucial role in shaping the composition and quality of the oil. Understanding the genetic basis of these properties is essential for optimizing the oil for industrial applications. The concerned study aimed to determine the physicochemical properties, fatty acid profile, and antioxidant potential of *S. molle* seed oil for establishing its capability as a high-value raw material for various industrial applications. Specifically, this research evaluated significant features including density, refractive index, viscosity, and melting point; the oil's acid and peroxide, as well as iodine values. Additionally, the oil's fatty acid composition and antioxidant potential determination employed standard procedures, such as gas chromatography (GC) and DPPH radical-scavenging assays. This research work presented crucial findings about the properties of *S. molle* seed oil and its promising use as a sustainable ingredient in the agro-industry. The complete picture of *S. molle* seed oil appears in this investigation, as well as contributes to agro-industry research with useful data, laying the ground for its commercial application in numerous industries.

## MATERIALS AND METHODS

### Materials

Collected fresh mature seeds of *S. molle* came from trees growing in the Oil and Fat Technology Laboratory-FOCAM of the Professional School of Agroindustrial Engineering, National University of Huancavelica, at the peak of harvesting season (late summer to early autumn). The seeds bore cleaning and careful washing to get rid of any alien growth of plants. After cleaning, seeds sustained air-drying at an ambient temperature (25 °C) until reducing the moisture content to below 10% (Fu *et al.*, 2025). Then, the *S. molle* seeds reached further drying before being sealed into containers at room temperature until oil extraction.

## Chemicals and reagents

During the study, the use of various chemicals and reagents eased the process of extraction, purification, and analysis of *S. molle* seed oil (Mohammed *et al.*, 2016). The primary solvent used for oil extraction was hexane (99%), a non-polar solvent effective in dissolving lipids. After extraction, employing ethanol (99%) degummed and cleaned the oil, removing impurities such as phospholipids and other polar compounds. Additionally, acetone (99%), as used in the purification process, eliminated residual contaminants and enhanced the oil's clarity. For acid value determination, the treatment of sodium hydroxide (NaOH) aided in the reaction with free fatty acids to evaluate the quality of the oil and magnitude of hydrolysis. Potassium hydroxide (KOH) application served both in saponification and iodine value tests, as it increased the breaking down of triglycerides into soap and glycerol in the former and helped determine the level of unsaturation in the latter. Tocopherol (vitamin E) standard served as a reference compound in the measurement of antioxidant activity with the possible quantification of antioxidant content and data comparison of tested seed oil samples.

## Solvents

Applying chloroform and methanol in combination during the preparation of fatty acid methyl esters (FAMES) for GC analysis facilitated the efficient extraction and transesterification of fatty acids from the *S. molle* seed oil. Sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) employment in the titration process determined the peroxide value, which measured the extent of primary oxidation in the oil samples (Semb, 2012). Iodine monochloride (ICI) utilization in the iodine value determination, reacting with the double bonds in unsaturated fatty acids, quantified the degree of unsaturation found in the oil.

## Oil extraction

Two extraction techniques used in extracting the oil from the *S. molle* seeds comprised a

hydraulic press applied for cold processing of oil from the dried seeds. Cold pressing is a mechanical extraction method involving no heat, thereby preserving the natural composition of the oil, including sensitive bioactive compounds—tocopherols and polyunsaturated fatty acids. This method provides oil that closely resembles the naturally occurring composition of the seeds, making it ideal for evaluating the oil's quality in its most natural form. The seeds reached powdered form before packing them under the press. The resulting oil underwent passing through a fine mesh to remove any residual seed particles therein. The oil's extraction also used a Soxhlet extractor in hexane as a solvent to efficiently extract oil from seeds, ensuring higher yields and a more complete range of lipid components. This method is applicable when higher yields are necessary or to extract both volatile and non-volatile components of the oil, allowing for a broader analysis of its chemical composition. The ground seeds were in a thimble and sucked out for six hours at 60 °C. After extraction, the solvent underwent drying with the help of an aerator under reduced pressure at 40 °C. Later on, the oil poured into amber bottles prevented degradation.

## Characterization of oil

### *Physical properties*

The physical properties of the *S. molle* seed oil entailed evaluation to determine the basic behavior and implementation for different applications. Detecting density at 25 °C utilized a pycnometer with the ASTM D4052 protocol; a drop of oil into the sample holder enabled directly reading the refractive index from the instrument. Viscosity values for experimental detection engaged a Brookfield viscometer at 25 °C. In establishing the melting point, the study used a differential scanning calorimeter (DSC) by approximately pouring 10 mg of oil into the sample pan and carrying out the experiment in a nitrogen atmosphere with a heating rate of 10 °C min<sup>-1</sup>. The recorded melting point was the temperature associated with the phase transition. The observed

melting point of  $-17.5\text{ }^{\circ}\text{C}$  ( $\pm 0.5$ ) indicates that the oil is highly unsaturated, which is typical of oils with a high proportion of polyunsaturated fatty acids. A lower melting point is particularly advantageous for industrial applications. In food processing, oils with lower melting points achieved easier incorporation into formulations, offering better fluidity and smoother textures, especially in products like spreads, dressings, and baked goods. Similarly, in the cosmetics industry, a lower melting point ensures that the oil remains in a liquid state at ambient temperatures, enhancing its stability and ease of application in creams, lotions, and topical formulations. Therefore, the relatively low melting point of *S. molle* seed oil makes it an ideal candidate for both the food and cosmetic industries, where ease of use and stability are critical. The recorded melting point emerged as the temperature associated with the phase transition.

### **Chemical properties**

A set of parameters earlier identified helped determine the chemical composition of the *S. molle* seed oil. Acid value measurement as 5 g of oil dissolved in 50 mL of ethanol reached titration with standard 0.1 N potassium hydroxide (KOH) and phenolphthalein as indicators. Acid value (case of free fatty acids) calculation was according to the following formula:

$$\text{Acid value} = V \times N \times 56.1 \backslash W$$

Where V is the volume of KOH used (mL), N is the normality of KOH, and W is the weight of oil in grams. Determining peroxide value, an indicator of primary oxidation products, followed the ISO 3960. Using a solvent system of acetic acid and chloroform (5 grams of oil per 100 ml) and titrating it with standardized sodium thiosulfate solution (using 0.01 N solution) obtained the results expressed in milliequivalents of active oxygen per kilogram of oil. For the saponification value, 5 g of oil succeeded in refluxing for 30 min with an excess of alcoholic KOH. The excess unreacted

alkali gained titration with 0.1 N hydrochloric acid, with the saponification value used to calculate the average molecular weight for the triglycerides.

### **Fatty acid composition**

Determining the species and the amounts of fatty acids continued in detecting the composition by fatty acid components in the oil. This proceeded via transesterification of the FAMES, performing methanol and sulfuric acid in the presence of the catalyst. The resulting FAMES underwent gas chromatographical analysis on a GC (GC-2010) of Shimadzu model with a BPX70 capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ), with a flame ionization detector (FID). The GC-temperature program, as initiated at 150  $^{\circ}\text{C}$  for 5 min, continued with an increase to 250  $^{\circ}\text{C}$  at the rate of 5  $^{\circ}\text{C}/\text{min}$ , and a final hold at 250  $^{\circ}\text{C}$  for 10 min.

### **Antioxidant properties**

The study chose two main analyses to observe the antioxidant capacity of the oil. Tocopherol (vitamin E) quantification used HPLC with a fluorescence detector. The oil, dissolved in a proper solvent, entailed analysis by using a C18 reverse-phase column. The excitation wavelength at 295 nm and emission wavelength at 330 nm progressed, permitting sensitive assay of tocopherols. In addition, the DPPH radical scavenging activity assay for the oil was successful in determining the capability of the oil to neutralize free radicals. The process comprised a milliliter of oil diluted with an equivalent volume of 1 mM DPPH solution made in methanol. The 30-min incubation of the mixture in the dark at room temperature took place. Absorbance measurement of the samples reading was at 517 nm, with the antioxidant activity given as inhibition (%) using the following formula:

$$\text{Inhibition (\%)} = (A_0 - A_1) \backslash A_0 \times 100$$

Where  $A_0$  is the absorbance of the control, and  $A_1$  is the absorbance of the sample.

## Statistical analysis

All the experiments were in three replicates ( $n = 3$ ), with the results expressed in mean values with the standard deviation. The study used the Shapiro-Wilk test to determine whether the data conformed to normal distribution. Where applicable, one-way analysis of variance (ANOVA) employment determined differences among the means for various samples of *S. molle* seed oil. With highly pronounced mean differences, pairwise comparison under Tukey's honest significant difference (HSD) test occurred. Similarly, Levene's test entailed examining the differences of variances in different groups. The results were significant if the  $p$ -value was less than 0.05. In addition to univariate statistical tests, multivariate analysis ensued to examine the relationships between multiple variables simultaneously. This approach allows for a more comprehensive understanding of how different physicochemical properties, fatty acid composition, and antioxidant activity display interrelations. Multivariate techniques, such as principal component analysis (PCA) or cluster analysis, help identify patterns and underlying structures in the data that might not be apparent through univariate analysis alone. By considering multiple factors together, multivariate analysis provides meaningful insights into the complex interactions between genetic regulation and environmental conditions and how these factors collectively influence the oil's quality. This approach was particularly useful for assessing the overall variation in oil composition and identifying key factors that contribute to its quality.

## RESULTS

The systematic checking of physical properties of the *S. molle* seed oil samples had the results summarized in mean values, standard deviations, and reported analysis. The seed oil density's determination appeared to be  $0.92 \text{ g/cm}^3$  with a standard deviation of  $3 \pm 0.0$  when measured in a pycnometer according to ASTM D4052 protocol. Such value was within

the range typical for vegetable oils, with a relatively low molecular packing and a regularity of structural composition. Refractive index detection at  $25 \text{ }^\circ\text{C}$  used an Abbe Refractometer, with a mean value of 1.465 and a standard deviation of  $\pm 0.001$  (Table 1). The said value represents the optical clarity and molecular structure of the oil, and when higher, it tends to indicate greater unsaturation. The statistical homogeneity of the measurements was proven by the Levene's test, which demonstrated nonsignificant differences among the oil samples in refractive index ( $p > 0.05$ ), thus confirming homogeneity of this optical character. Finding the viscosity of the oil was via Brookfield viscometer, with a value of 28.5 cP and a standard deviation of  $\pm 1.2$ . The melting point's acquisition was by differential scanning calorimetry (DSC), with a value of  $-17.5 \text{ }^\circ\text{C}$  ( $\pm 0.5$ ) (Table 1).

The chemical characterization of the oil revealed key quality indicators based on standardized analytical methods. The acid value was 1.56 mg KOH/g, with a standard deviation of  $\pm 0.03$ , indicating a low level of free fatty acids and a suggested minimal hydrolytic degradation. This parameter showed the significant differences among the groups based on a one-way analysis of variance ( $p = 0.045$ ), reflecting slight variations in oil acidity (Table 2). The peroxide value averaged 5.2 meq  $\text{O}_2/\text{kg}$ , with a deviation of  $\pm 0.15$ , indicating the presence of primary oxidation products within acceptable limits for edible oils. The Tukey's HSD test revealed a significant difference ( $p = 0.032$ ), suggesting some variability in oxidative stability. The iodine value emerged to be 88.4 g  $\text{I}_2/100 \text{ g}$ , with a small standard deviation ( $\pm 0.3$ ), reflecting a moderate degree of unsaturation.

The fatty acid composition of the seed oil, determined by gas chromatography, revealed an oil profile rich in unsaturated fatty acids. Palmitic acid (C16:0) appeared with a mean concentration of 18.5% and a standard deviation of  $\pm 0.2\%$ , showing nonsignificant differences among the oil samples as per one-way ANOVA ( $p > 0.05$ ). Notably, the oil exhibited a high content of oleic acid (40.2%) and linoleic acid (38.1%), both of which are

**Table 1.** Physical properties of *Schinus molle* seed oil.

Properties	Mean values	Standard deviation	Units	Methods used	Statistical test
Density	0.92	±0.003	g/cm <sup>3</sup>	Pycnometer (ASTM D4052)	One-Way ANOVA (p > 0.05)
Refractive index	1.465	±0.001	-	Abbe Refractometer (25 °C)	Levene's Test (p > 0.05)
Viscosity	28.5	±1.2	cP	Brookfield Viscometer	One-Way ANOVA (p = 0.012)
Melting point	-17.5	±0.5	°C	Differential Scanning Calorimetry (DSC)	Shapiro-Wilk Test (p = 0.046)**

**Table 2.** Chemical properties of *Schinus molle* seed oil.

Properties	Mean values	Standard deviation	Units	Methods used	Statistical test
Acid value	1.56	±0.03	mg KOH/g	Titration (ASTM D974)	One-Way ANOVA (p = 0.045)
Peroxide value	5.2	±0.15	meq O <sub>2</sub> /kg	ISO 3960 Method	Tukey's HSD (p = 0.032)*
Iodine value	88.4	±0.3	g I <sub>2</sub> /100 g	Wijs Method (ISO 3961)	Levene's Test (p = 0.083)
Saponification value	190	±1.5	mg KOH/g	ASTM D5558 Method	One-Way ANOVA (p > 0.05)

known for their health-promoting properties. Oleic acid, a monounsaturated fatty acid, has recognizable cardiovascular benefits, including reducing bad cholesterol (LDL) levels and promoting heart health. Linoleic acid, an essential polyunsaturated fatty acid, contributes to improved skin hydration and has anti-inflammatory properties, making it beneficial for both internal health and topical cosmetic applications. These unsaturated fatty acids incurred genetic regulations, with enzymes like FAD2 playing a role in the conversion of oleic acid to linoleic acid. Variability in the expression of such genes can lead to different fatty acid profiles, influencing the oil's quality and suitability for various industrial applications. The high levels of oleic and linoleic acids make *S. molle* seed oil an excellent choice for functional foods, cosmetic formulations, and pharmaceutical products. These fatty acids are crucial for maintaining healthy skin, supporting heart health, and contributing to the oil's stability and nutritional value.

Stearic acid (C18:0), a saturated fatty acid, manifested at a lower concentration (2.5% ± 0.1%) and showed significantly (p = 0.027) varied values across the oil samples, suggesting slight fluctuations in the saturated fraction. The monounsaturated oleic acid

(C18:1) was the most abundant fatty acid (40.2% ± 0.5%), with nonsignificant differences across the groups based on Tukey's HSD test (p > 0.05). Linoleic acid (C18:2), a polyunsaturated fatty acid, with a percentage of 38.1% ± 0.3% of the total fatty acids, revealed significant variability across oil samples (p < 0.05), which could influence oxidative stability and nutritional quality (Table 3).

The antioxidant potential of the *S. molle* seed oil reached evaluation through two primary assays: tocopherol content and DPPH radical scavenging activity. The tocopherol (vitamin E) content quantification used high-performance liquid chromatography (HPLC) with fluorescence detection, which occurred to be 34.5 mg/100 g, with a standard deviation of ±1.2. The said value indicates a relatively high level of natural antioxidants in the oil, contributing to its oxidative stability and nutritional benefits. Analysis using the Student's *t*-test showed a significant difference (p = 0.045), suggesting variability in tocopherol concentration across different oil samples. The DPPH radical scavenging activity, which measures the oil's ability to neutralize free radicals, resulted in 82.3% inhibition, with a standard deviation of ±0.9 (Table 4). The high DPPH percentage indicates considerable

**Table 3.** Fatty acid composition of *Schinus molle* seed oil.

Fatty Acids	Mean values	Standard deviation	Units	Methods used	Statistical test
Palmitic acid (C16:0)	18.5	±0.2	%	Gas chromatography	One-Way ANOVA ( $p > 0.05$ )
Stearic acid (C18:0)	2.5	±0.1	%	Gas chromatography	One-Way ANOVA ( $p = 0.027$ )*
Oleic acid (C18:1)	40.2	±0.5	%	Gas chromatography	Tukey's HSD ( $p > 0.05$ )
Linoleic acid (C18:2)	38.1	±0.3	%	Gas chromatography	Tukey's HSD ( $p < 0.05$ )*
Arachidic acid (C20:0)	0.7	±0.1	%	Gas chromatography	One-Way ANOVA ( $p > 0.05$ )

**Table 4.** Antioxidant properties of *Schinus molle* seed oil.

Antioxidant properties	Mean values	Standard deviation	Units	Methods used	Statistical test
Tocopherol (vitamin E) content	34.5	±1.2	mg/100 g	HPLC (Fluorescence Detection)	Student's t-test ( $p = 0.045$ )
DPPH radical scavenging activity	82.3	±0.9	% Inhibition	DPPH Radical Scavenging Assay	One-Way ANOVA ( $p = 0.001$ )**

antioxidant activity, correlating well with the presence of tocopherols and possibly other minor antioxidant compounds in the seed oil.

The comparative analysis of the *S. molle* seed oil with commonly used edible oils, such as olive, soybean, and sunflower oils, revealed similarities and differences in physicochemical properties. The density of *S. molle* seed oil (0.92 g/cm<sup>3</sup>) matched that of soybean and sunflower oils and was slightly higher than olive oil (0.91 g/cm<sup>3</sup>) (Table 5). Its refractive index (1.465) was marginally lower than the other oils, suggesting slightly less unsaturation. In terms of viscosity, *S. molle* seed oil (28.5 cP) exhibited a lower flow resistance than olive, soybean, and sunflower oils, indicating a lighter consistency. Its melting point was notably lower at -17.5 °C, which implies a higher proportion of unsaturated fatty acids and a more liquid state at lower temperatures. The acid value of *S. molle* seed oil (1.56 mg KOH/g) was higher than all other oils, particularly sunflower oil (0.3 mg KOH/g), signifying a greater free fatty acid content and potential need for refining. The iodine value (88.4 g I<sub>2</sub>/100 g) of *S. molle* seed oil was moderate to higher than olive oil; however, it was significantly lower than soybean and sunflower oils, reflecting a balanced level of unsaturation.

Beyond the physicochemical properties, genetic regulation plays a crucial role in shaping the fatty acid composition of these oils. In particular, the *FAD2* gene, responsible for converting oleic acid to linoleic acid, has been evidently influencing the unsaturation levels in oils. The higher oleic acid content in *S. molle* seed oil may be partially due to the genetic regulation of fatty acid desaturase enzymes, like *FAD2*, and their expression in different plant species. In contrast, oils like sunflower oil, which is more abundant in linoleic acid, could acquire influences from variations in the *FAD2* gene expression that favor linoleic acid biosynthesis over oleic acid production. Genetic variation in these fatty acid desaturase genes contributes to the differences in oil composition between *S. molle* and other commonly used edible oils. Thus, understanding the genetic regulation of fatty acid biosynthesis can help optimize oil quality for specific industrial uses, such as in food, cosmetics, and pharmaceuticals.

Variations in the expression of *FAD2* and other biosynthetic genes could explain the observed differences in the oil's iodine and saponification values, which reflect the degree of unsaturation and molecular weight of the fatty acids. Notably, the tocopherol (vitamin E) content, earlier quantified at 34.5 mg/100 g,

**Table 5.** Comparison of physical-chemical properties of *Schinus molle* seed oil with other vegetable oils.

Properties	<i>Schinus molle</i> seed oil	Olive oil	Soybean oil	Sunflower oil
Density	0.92±0.003	0.91±0.002	0.92±0.003	0.92±0.002
Refractive index	1.465±0.001	1.467±0.001	1.467±0.001	1.468±0.001
Viscosity	28.5±0.5	34.8±1.5	34.0±1.3	32.0±1.0
Melting point	-17.5±0.5	-6.0±0.2	-10.0±0.3	-12.0±0.4
Acid value	1.56±0.03	0.8±0.05	1.5±0.07	0.3±0.02
Peroxide value	5.2±0.15	3.8±0.1	4.5±0.12	3.2±0.08
Iodine value	88.4±0.3	81.0±0.5	130.0±1.0	120.0±1.2
Saponification value	190±1.5	190±0.5	190±1.0	188±0.8

**Table 6.** Genetic correlation with physicochemical and antioxidant properties of *Schinus molle* seed oil.

Properties	Genetic correlation	Possible genetic factors
Fatty acid composition	Genetic factors influence the desaturation of fatty acids, affecting the ratio of oleic and linoleic acids	FAD <sub>2</sub> (oleic acid to linoleic acid desaturase)
Iodine value	The iodine value correlates with the degree of unsaturation, which is controlled by genes involved in fatty acid biosynthesis	FAD <sub>2</sub> , SAD (stearoyl-ACP desaturase)
Saponification value	The saponification value, reflecting the molecular weight of fatty acids, is influenced by genetic regulation of lipid biosynthesis	KAS (3-Ketoacyl-ACP synthase)
Tocopherol (vitamin E) content	Genetic variability in tocopherol biosynthesis contributes to the antioxidant properties of the oil	VTE4 (tocopherol synthesis gene), HPPD (hydroxyphenylpyruvate dioxygenase)
DPPH radical scavenging activity	Genetic variation in phenolic acid biosynthesis pathways influences antioxidant activity	PAL (phenylalanine ammonia-lyase), cinnamate-4-hydroxylase (C <sub>4</sub> H)

plays a critical role in the oil's antioxidant activity. This antioxidant potential could have a direct link to genetic factors, particularly the *VTE4* gene, which is responsible for regulating tocopherol synthesis. Genetic variability in the *VTE4* gene may contribute to the observed differences in tocopherol content, highlighting the genetic regulation of antioxidant properties in *S. molle* seed oil (Table 6). The genetic factors influencing the enzymatic pathway for tocopherol synthesis, such as the *VTE4* gene, may account for the observed tocopherol content (34.5 mg/100 g) as an important contributor to the antioxidant activity of the *S. molle* seed oil (Table 6). Moreover, the oil's antioxidant potential, measured through DPPH radical scavenging activity (82.3% inhibition), can refer to the genetic diversity in the synthesis of secondary metabolites.

## DISCUSSION

The presented research investigates the properties and antioxidant features of *S. molle* seed oil, and these findings suggest it has essential uses in the food, cosmetics, and pharmaceutical industries. The oil's vital qualities are being highly unsaturated, showing antioxidant activity, being chemically stable, and being a better alternative than olive, sunflower, and soybean oils (Lokesh *et al.*, 2019; Muttagi and Joshi, 2020). The high oleic and linoleic acid content in *S. molle* seed oil matches the quality of recognized healthy oils. Therefore, it has become a proposal for unsaturated fatty acids to be especially valuable, with oleic acid acknowledged for its benefits to heart health and prevention of chronic diseases (Shahidi *et al.*, 2019). The oil

with linoleic acid makes it more nutritious, and this study agrees with past research carried out on sunflower and soybean oils (Zhang *et al.*, 2025).

Given its considerable levels of essential fatty acids, *S. molle* seed oil is an excellent component for heart-healthy foods. According to Masiero *et al.* (2021), the presence of high tocopherol (34.5 mg/100 g) and significant radical scavenging qualities are good evidence that the product is highly resistant to oxidation and is safe for therapeutic and food uses. In this research, the differences in tocopherol concentration agree with other studies, which also revealed how the oils are made, where the seeds come from, and the considerable factors that can alter the level of antioxidants in oils (Silva *et al.*, 2010). The tests suggested that *S. molle* seed oil has antioxidant effects, and its properties are like the oils of olive and sunflower (Lokesh *et al.*, 2019).

The data analysis showed significant differences existed in viscosity, acid value, peroxide value, and composition of fatty acids among the different *S. molle* seed oil samples used in the study. Its viscosity (28.5 cP) makes *S. molle* seed oil well suited for use requiring a light oil, unlike oils with a higher viscosity, viz., olive oil (34.8 cP). These results were greatly analogous to the earlier studies, showing that oils with lower viscosities work better in some food processing applications (Suri *et al.*, 2023). The oil melts at a very low temperature (-17.5 °C), as a result of its various double bonds, and the said feature was also evident in sunflower oil and other polyunsaturated oils that melt easily (Shahidi *et al.*, 2019). The *S. molle* seed oil, having an acid value of 1.56 mg KOH/g, showed free fatty acids, which probably emerged because of hydrolysis. However, the said level was higher than that observed in sunflower (0.3 mg KOH/g) and olive (0.8 mg KOH/g) oils (Silva *et al.*, 2019). Though the acid and peroxide values of the *S. molle* seed oil mean it could require refinement to be fit for food, it still passes for safe use in cosmetics and pharmaceuticals (Masiero *et al.*, 2021).

By comparing it with olive, sunflower, and soybean vegetable oils, the *S. molle* seed

oil showed the similarities and differences among their physicochemical characteristics. The *S. molle* seed oil (0.92 g/cm<sup>3</sup>) has a similar density to soybean and sunflower oils; however, it has just a little higher value than the olive oil density (0.91 g/cm<sup>3</sup>). These findings match with those reported by Ye *et al.* (2024), who mentioned different vegetable oils tend to have a density in this area, with minor variations based on the oil's ingredients. The *S. molle* seed oil has a refractive index of 1.465, which suggests its structure may be a little less unsaturated than other vegetable oils. Shahidi *et al.* (2019) reported the refractive index of vegetable oils can tell us how much unsaturated fats the oil contains.

According to research, the different chemical compounds in *S. molle* seed oil appeared to be shaped mostly by genes. Therefore, it can theoretically mean that higher levels of oleic and linoleic acids found in this study could have resulted from the action of desaturase enzymes, mainly FAD2, changing oleic acid to linoleic acid. Ye *et al.* (2024) reported specific genes regulate how many fatty acids are in oils; hence, selective breeding could help make the oil healthier. Besides, genes taking part in making tocopherol, such as VTE4, also tend to help decide the oil's antioxidant properties (Li *et al.*, 2025). The quality of *Schinus molle* seed oil gained influences from both genetic regulation and environmental factors. Meanwhile, genes like FAD2 and VTE4 regulate fatty acid composition and antioxidant activity, and environmental conditions such as temperature, soil quality, water availability, and agronomic practices play a crucial role. For example, warmer climates can enhance the expression of FAD2, leading to higher unsaturated fatty acid content, while nutrient stress or drought can increase tocopherol production through VTE4, boosting antioxidant activity. These environmental factors, in combination with genetic regulation, affect oil yield, quality, and bioactive content. Understanding these interactions can help optimize cultivation practices for targeted industrial applications, such as in functional foods, cosmetics, and pharmaceuticals.

Like other vegetable oils, the assay findings in DPPH obviously demonstrated genetic effects on the antioxidant activity (She *et al.*, 2024). Genetic regulation of phenolic acids by the *PAL* and *C4H* genes may be responsible for the plant's antioxidant properties (Lokesh *et al.*, 2019). Further examination of the main genes and their connection to oil quality could make it easier to develop promising cultivars of *S. molle*. Any improvement made may enlarge the role of palm oil in the food industry, especially by reducing unpleasant compounds, like free fatty acids, and enhancing the oil's stability.

### Limitations and future directions

Although this study provides valuable insights into the genetic regulation of *Schinus molle* seed oil's properties, several limitations require addressing. Genetic variability within the *S. molle* species could influence the results, as the oil's composition may differ based on factors such as plant variety, cultivation environment, and genetic diversity. The study primarily focused on a few samples, which may not fully represent the breadth of genetic variation present in the species. Additionally, the role of epigenetic factors and gene-environment interactions remains an area for further exploration. Future studies involving a broader range of cultivars and environmental conditions, as well as advanced genomic techniques, could provide in-depth insights into the genetic mechanisms regulating oil quality and its implications for industrial applications.

### CONCLUSIONS

This study demonstrates the promising potential of *S. molle* seed oil, highlighting its favorable physicochemical properties, high unsaturated fatty acid content, and considerable antioxidant activity. The *S. molle* seed oil composition, particularly the high levels of oleic and linoleic acids, supports its nutritional benefits and its advantages as a viable candidate for the food, cosmetics, and pharmaceutical industries. Variability in antioxidant content emphasizes the role of

extraction methods and genetic factors, which can reach optimization through further research. With its nutritional profile and industrial potential, the *S. molle* seed oil could serve as a sustainable alternative to traditional vegetable oils.

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