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Variability in the fatty acid composition of *Robinia pseudoacacia* L. seed oils

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Abstract:

The aim of this investigation is the determination of the fatty acid composition of oils extracted from *Robinia pseudoacacia* seeds. Seeds were harvested from two Tunisian sites; Tunis and Ain Drahem. Oils were extracted using Soxhlet apparatus. After methylation, fatty acids were analyzed using gas chromatography. Results showed significant differences between the two studied oils. The main fatty acids were; palmitic, oleic, linoleic and linolenic acids. For the two studied oils, linoleic acid was recognized as the major fatty acid with respective rates of 48.22 % for oil from Tunis and 58.46 % for oil from Ain Drahem. *R. pseudoacacia* seed oil represents a promising source of nutritionally valuable unsaturated fatty acids, including essential omega-3 and omega-6 fatty acids.

1. Introduction

Robinia pseudoacacia L., commonly known as black locust, is a fast-growing deciduous tree from the *Fabaceae* family. Native to the southeastern United States, it has become widely naturalized and cultivated in many temperate regions across the globe. The species is easily recognized by its pinnately compound leaves and paired, unbranched spines resembling rose thorns (Vítková *et al.*, 2017; Cierjacks *et al.*, 2013). Due to its adaptability, tolerance to poor soils, and nitrogen-fixing capabilities, R. pseudoacacia has been extensively used in forestry, agroforestry, landscaping, erosion control, and land reclamation (Keresztesi, 1988; Cierjacks *et al.*, 2013; Vítková *et al.*, 2020).

Today, it is considered one of the most widely planted woody species in the world and the second most commonly introduced broadleaved tree in Europe after *Quercus rubra*, mainly for wood and biomass production (MCPFE, 2007). However, it is also ranked among the 40 most invasive woody angiosperms worldwide, due to its aggressive spread and impact on native biodiversity and ecosystem processes (Richardson & Rejmánek, 2011; Vítková *et al.*, 2017).

In addition to its silvicultural importance, *R. pseudoacacia* holds considerable value as a melliferous species. Its pale yellow to greenish-yellow flowers produces a distinctive honey characterized by a high fructose content, low crystallization rate, and fruity, floral aroma (Keresztesi, 1977; Farkas & Zajácz, 2007; Bogdanov *et al.,* 2004). *Robinia* honey is recognized for its antioxidant activity and phenolic content, making it valuable not only as a food product but also for therapeutic purposes (Kasper-Szel *et al.,* 2003; Lukasiewicz *et al.,* 2015; Khalifa *et al.,* 2020).

Photo 1: *R. pseudoacacia* plant and seeds

The essential oils derived from *R. pseudoacacia* flowers have also been characterized, revealing a composition rich in monoterpenes and sesquiterpenes with potential antimicrobial properties (Rahmonov, 2009; Božović *et al.,* 2018). Furthermore, various studies have examined the chemical composition of the plant's litter leaves, bark, and branches and its influence on soil fertility, nitrogen cycling, and ecosystem functioning (Dziadowiec, 1990; Kharin *et al.,* 2001; Read, 2002; Radtke *et al.,* 2013).

Despite the growing body of research on the ecological and biochemical aspects of *R. pseudoacacia*, little is known about the lipid profile of its seeds, especially their fatty acid composition. Seed oils are important sources of bioactive lipids with nutritional, pharmaceutical, and industrial applications (Gunstone, 2011; Shahidi & Ambigaipalan, 2018). In plants, the fatty acid composition can be significantly influenced by environmental factors such as temperature, altitude, and soil type (Jukić Špika *et al,* 2021; Haddou *et al.,* 2023; Kadda *et al.,* 2024; Imran *et al.,* 2024; Merimi *et al.,* 2025; Kadda *et al.,* 2026).

More than 3900 articles were published in Scopus on *Robinia pseudoacacia* from 1949 to 2025. It's an interesting indicator to know the most published countries and authors via a bibliometric analysis which becomes actually widely realized for various topics. In other words, Bibliometric analysis is a quantitative statistical method used to map and analyze academic literature to identify trends, influential authors and research structures within a specific field. It involves defining a research question, collecting data (usually from Scopus, Web of Science or PubMed), cleaning up data and using tools such as VOSviewer or Bibliometrix R for visualization and analysis. (Pan, 2018; Mouloudi *et al.,* 2023 ; Laita *et al.,* 2024; Öztürk *et al.,* 2024; Hammouti *et al.,* 2025; Salghi *et al.,* 2025 ; Prinsloo, 2026).

In this context, the present study aims to investigate, for the first time, the fatty acid composition of oils extracted from *Robinia pseudoacacia* seeds, comparing samples collected from two ecologically distinct regions in Tunisia. The objective is to assess the variability in lipid content and composition, and to explore the potential of this species as a novel source of bioactive seed oil.

2. Methodology

2.1. Plant material

Mature seeds of *Robinia pseudoacacia* L. were collected from wild populations at two distinct locations in Tunisia: Tunis, situated in the northeastern region, and Ain Draham, located in the northwestern part of the country. The botanical identification of the species was carried out by Professor Abdelhamid Khaldi, a researcher at the National Research Institute of Rural Engineering, Water and Forests (INRGREF). Species confirmation was based on morphological characteristics and comparison with reference specimens housed in a local herbarium. Voucher specimens were deposited in the INRGREF Herbarium under the accession number RP-2024 to ensure future traceability and reference. Following collection, the seeds were air-dried, ground into a fine powder using a laboratory mill, and stored at 4°C in airtight containers until further analysis.

2.2. Oil extraction and yield

The extraction of seed oil was carried out using a Soxhlet apparatus under a continuous reflux cycle for 3 hours. n-Hexane was used as the solvent due to its high efficiency in extracting non-polar lipids. The oil yield was calculated using the following formula:

$$\text{Yield (\%)}=(mo/mp)\times 100$$

Where:
mo = mass of the extracted oil (g)
mp = mass of the dry plant material used for extraction (g)
The extracted oils were collected, filtered, and stored in sealed amber glass vials at 4°C until further analysis.

2.3. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared from the extracted seed oils following the method described by Morrison and Smith (1964), with slight modifications. Briefly, 100 mg of oil sample was mixed with 3 mL of 14% boron trifluoride in methanol (BF_3–MeOH) and heated at 50°C for 30 minutes to ensure complete transesterification. After cooling, 30 mL of n-hexane were added to the mixture to extract the methyl esters. The resulting solution was then washed twice with saturated sodium chloride (NaCl) solution to remove residual methanol and impurities. After each wash, the aqueous phase was discarded, and the upper hexane layer containing the FAMEs was collected for further analysis.
FAMEs were analyzed using gas chromatography (GC) equipped with a flame ionization detector (FID) (Hewlett Packard, Palo Alto, CA, USA). Separation was performed on a polar capillary column (HP-INNOWax; 30 m × 0.25 mm i.d., 0.25 µm film thickness), which consists of a polyethylene glycol (PEG) stationary phase known for its high polarity and thermal stability. Prior to injection, heptadecanoic acid (C17:0) was added to each sample as an internal standard for quantification. One microliter (1 µL) of each FAME sample was injected in split mode. The oven temperature was programmed to rise from 180°C to 250°C at a rate of 10°C/min. The injector and

detector temperatures were set at 220°C and 280°C, respectively. Individual fatty acids were identified by comparing their retention times with those of known standards. The relative content (%) of each fatty acid was determined by integrating the peak areas using HP ChemStation software.

2.4. Statistical analysis

All data were expressed as mean ± standard deviation (SD) of at least three replicates. Statistical analyses were performed using the General Linear Model (GLM) procedure of SAS software version 9.0. Differences among means were evaluated using the least significant difference (LSD) test at a significance level of $p < 0.05$. The GLM was used to assess the effect of geographic origin on oil yield and fatty acid composition, accounting for the variability between locations.

3. Results and Discussion

The bibliometric analysis conducted on "Robinia pseudoacacia" using Scopus gathers more than 3900 articles from 1949 to 2025; and 3732 articles in the period (1990-2025). An increase of number pf articles is observed recently reaching over 280 articles (**Figure 1**). The majority of publications (>98%) are articles, conferences papers or review as shown in **Figure 2left**, and that of **Figure 2right** indicates the wide studied domains *are* Agricultural and Biological Sciences (2501 articles *i.e. (39.4%)) and* Environmental Science (1654 articles *i.e. (26.0%)*). *The most published author is the Chinese* Han, Xinhui Northwest A&F University, Yangling *contributed by 52 articles on "Robinia pseudoacacia*", *and* reachin***g*** a total of 8700 citations for 182 articles and an H-index of 54 **(Figure 3).** We noticed that among the best ten authors, 9 researchers are from China. The tenth one is Rédei, K. (Debreceni Egyetem), from Hungary. This result clearly demonstrates China's dominance in several research areas, and in particular, work on this natural plant. **Figure 4** indicates that China publishes 1730 articles compared to the US only 333 articles.

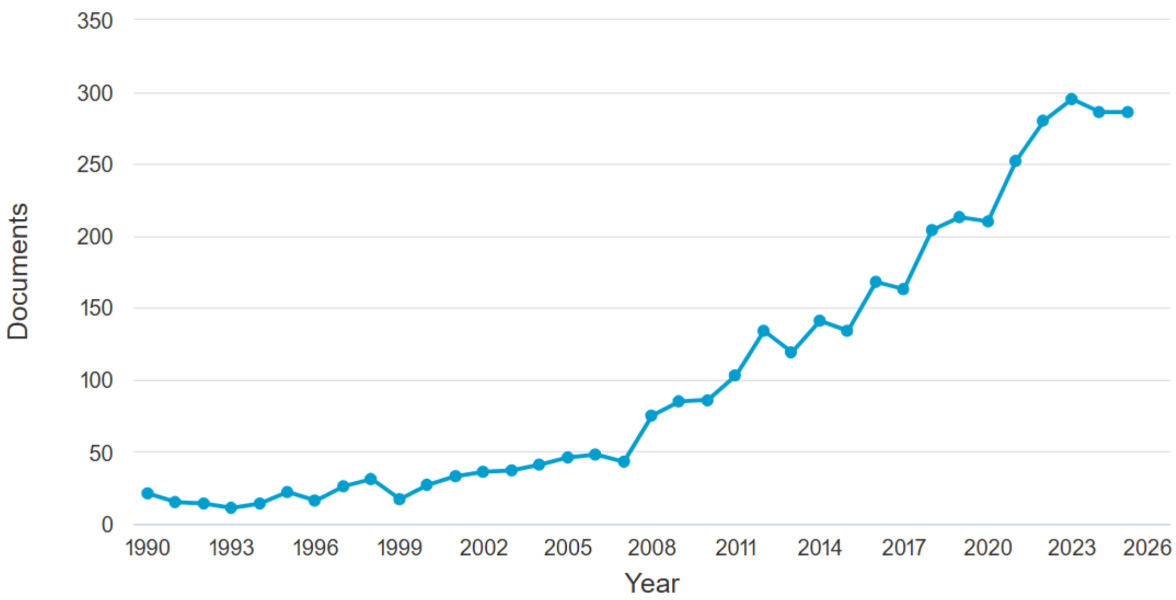

Figure 1. Evolution of number of articles on "*Robinia pseudoacacia*" over time

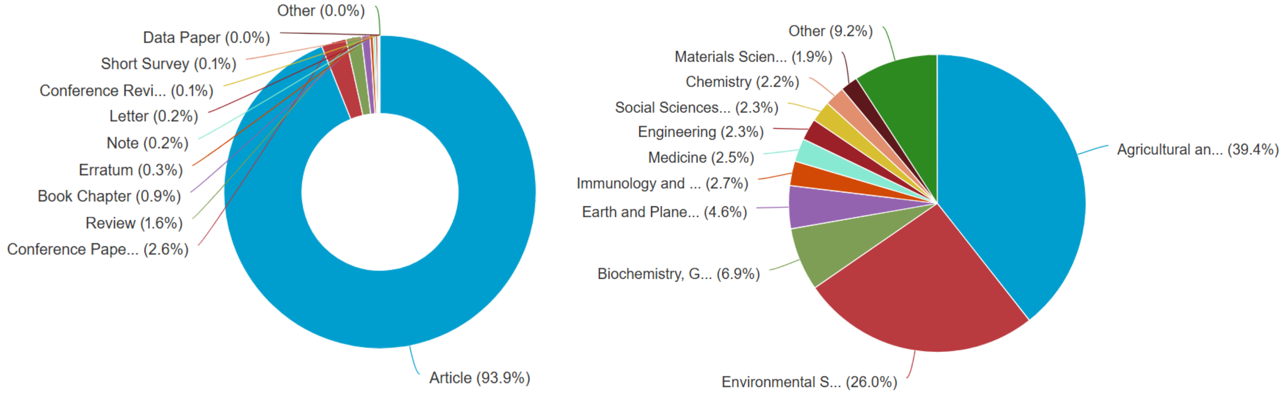

Figure 2. Distribution of number of articles on "*Robinia pseudoacacia*" by **left)** type and **right)** domain

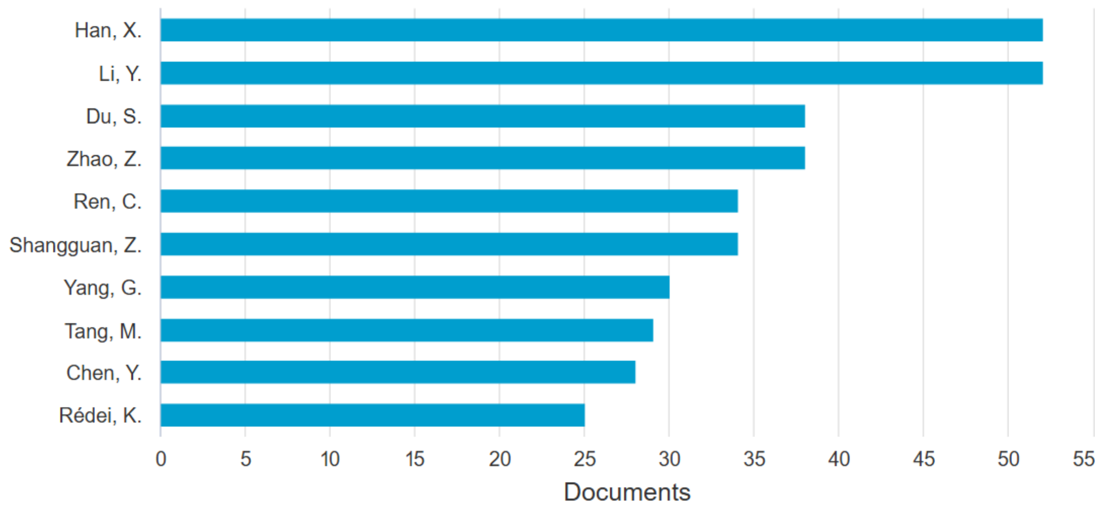

Figure 3. Top ten published authors on "*Robinia pseudoacacia*" in (1990-2025) period.

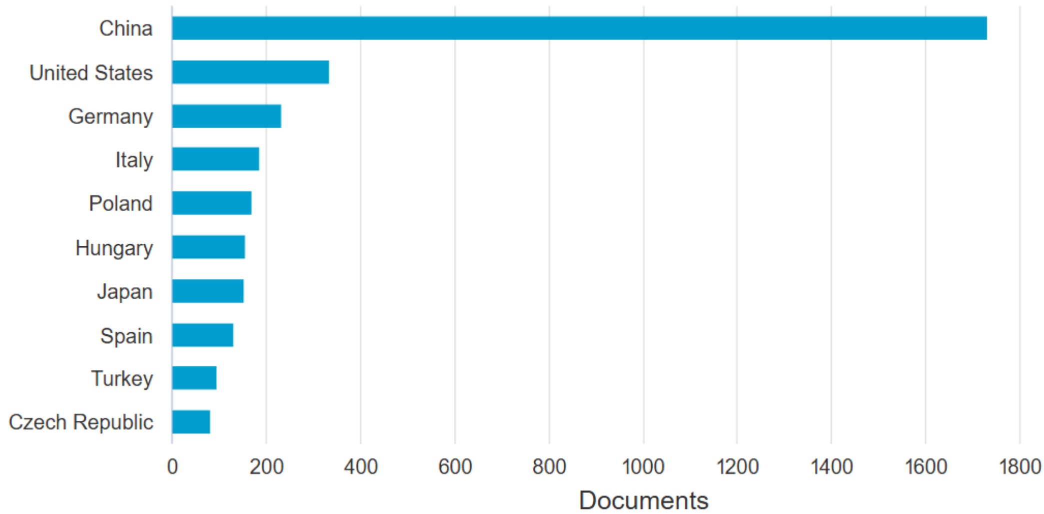

Figure 4. Top ten published countries on "*Robinia pseudoacacia*" in (1990-2025) period.

The oil yield of *Robinia pseudoacacia* seed samples showed a significant difference ($p < 0.05$) between the two studied locations (**Table 1**). Seeds collected from the Tunis site exhibited the highest oil yield (4.89%), whereas those from Ain Draham showed a lower yield (3.00%).

Table 1. Oil yield (%) of *R. pseudoacacia* seeds from two Tunisian locations

Site	Oil yield (%)
Tunis	4.89±0.1
Ain Drahem	3±0.12

Fatty acid analysis revealed the presence of 25 different fatty acids in the seed oils (**Table 2**). The predominant fatty acids were palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2, ω-6), and linolenic acid (C18:3, ω-3) (**Figure 1**).

Table 2. Fatty acid composition of *R. pseudoacacia* seed oils (%)

Fatty acid	Common Name	Retention Time (min)	Kovats Index (RI)	Tunis	Ain Drahem
C12:0	Lauric Acid	9.2	860	0.20 ± 0.01	0.24 ± 0.01
C14:0	Myristic Acid	10.5	925	0.17 ± 0.02	1.80 ± 0.05
C14:1	Myristoleic Acid	12.1	938	0.08 ± 0.01	0.03 ± 0.01
C16:0	Palmitic Acid	14.7	1160	6.62 ± 0.15	12.46 ± 0.20
C16:1	Palmitoleic Acid	16.1	1165	0.28 ± 0.02	0.40 ± 0.03
C17:0	Margaric Acid	17.2	1225	0.06 ± 0.01	1.46 ± 0.04
C17:1	Heptadecanoic Acid	18.0	1235	0.22 ± 0.02	0.75 ± 0.04
C18:0	Stearic Acid	20.0	1260	2.63 ± 0.10	1.70 ± 0.08
C18:1	Oleic Acid	23.5	1315	23.65 ± 0.50	13.50 ± 0.40
C18:2	Linoleic Acid	25.3	1310	48.22 ± 0.70	58.46 ± 0.90
C20:0	Arachidic Acid	29.5	1440	0.16 ± 0.01	0.11 ± 0.01
C18:3	Alpha-Linolenic Acid	30.5	1445	13.82 ± 0.30	1.30 ± 0.05
C20:1	Eicosenoic Acid	33.0	1450	0.76 ± 0.03	2.31 ± 0.06
C21:0	Heneicosanoic Acid	35.0	1500	0.05 ± 0.01	0.14 ± 0.01
C20:2	Eicosadienoic Acid	36.5	1480	0.91 ± 0.03	0.25 ± 0.02
C22:0	Behenic Acid	38.5	1560	0.06 ± 0.01	0.16 ± 0.01
C20:3	Eicosatrienoic Acid	40.0	1520	0.42 ± 0.02	1.96 ± 0.05
C22:1	Erucic Acid	41.5	1575	0.27 ± 0.02	1.36 ± 0.04
C20:4	Arachidonic Acid	43.5	1610	0.55 ± 0.02	1.22 ± 0.03
C22:2	Docosadienoic Acid	45.0	1600	0.32 ± 0.01	0.11 ± 0.01
C24:0	Lignoceric Acid	47.0	1680	0.46 ± 0.02	0.14 ± 0.01
C24:1	Nervonic Acid	49.0	1685	0.07 ± 0.01	0.23 ± 0.02
∑ Unsaturated fatty acids	-	-	-	89.58 ± 0.80	81.79 ± 0.90
∑ Saturated fatty acids	-	-	-	10.42 ± 0.80	18.21 ± 0.90

Linoleic acid was the most abundant fatty acid in both samples, accounting for 48.22% in Tunis oil and 58.46% in Ain Draham oil. Tunis oil contained significantly higher levels of oleic acid (23.65%) and linolenic acid (13.82%) compared to Ain Draham oil (13.50% and 1.30%, respectively).

Conversely, Ain Draham oil showed higher levels of linoleic acid (58.46%) and palmitic acid (12.46%) than Tunis oil (48.22% and 6.62%, respectively). The total unsaturated fatty acid content was higher in Tunis oil (89.58%) compared to Ain Draham oil (81.79%), whereas saturated fatty acids were lower (10.42% vs. 18.21%). The significant variation in oil yield between the two locations suggests a strong influence of environmental and edaphic factors on oil biosynthesis and accumulation in *R. pseudoacacia* seeds. Differences in climatic conditions such as temperature, rainfall, and altitude, as well as soil composition, may explain the higher oil yield observed in Tunis compared to Ain Draham. Similar findings have been reported by Sakar et al. (2022) and Nguy et al. (2022), highlighting the role of growing conditions in determining oil yield and quality.

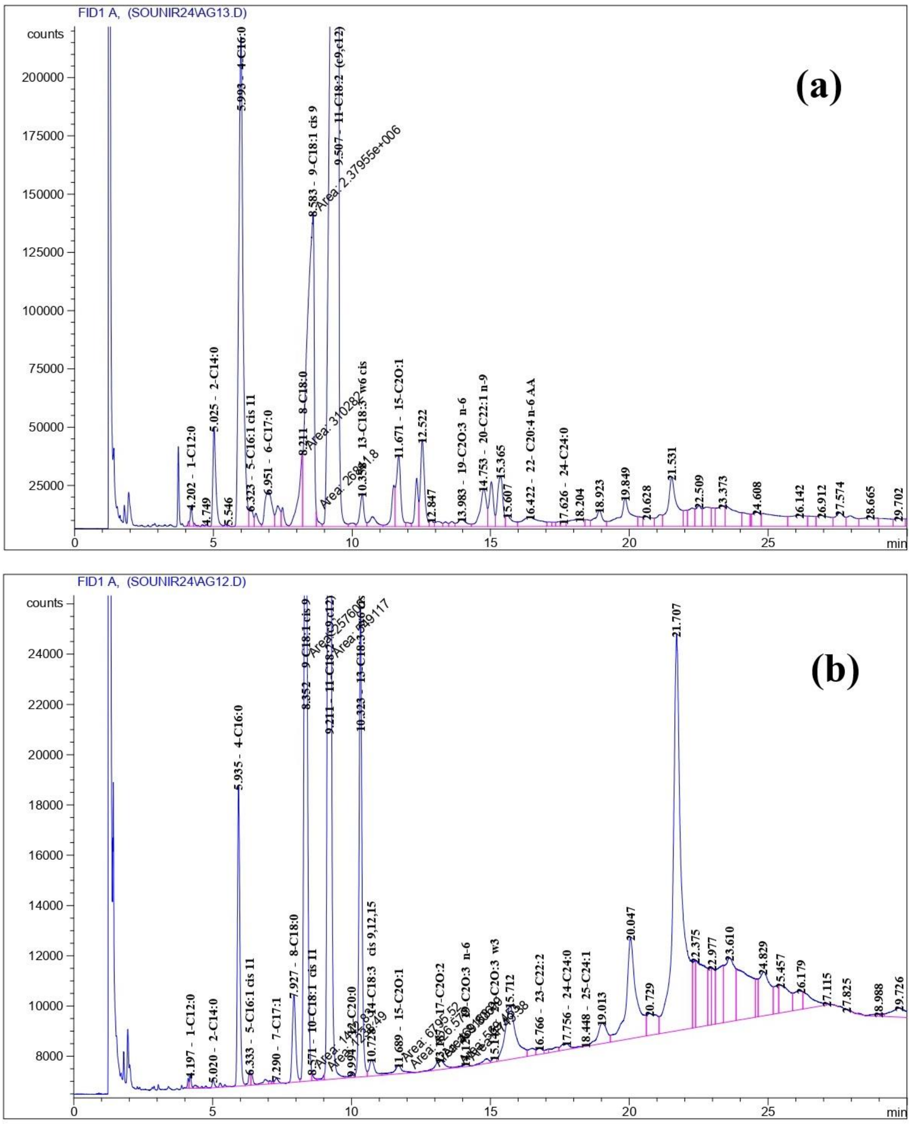

Figure 1. Fatty acid composition of *Robinia pseudoacacia* seed oils from two Tunisian locations; (a) Seed oil from Ain Draham; (b) Seed oil from Tunis

The fatty acid profile indicates a dominance of linoleic acid, an essential omega-6 fatty acid known for its important physiological roles. Linoleic acid contributes to membrane structure and is involved in cardiovascular health, immune function, and inflammation regulation (Ramsden et al., 2012; Behrman et al., 1971). Previous studies have also associated its intake with reduced risks of atherosclerosis (Das, 2021), hypercholesterolemia (Salem et al., 1996; Ramsden et al., 2021), and chronic inflammatory diseases (Pizzino et al., 2017; Suzuki et al., 2020). The higher proportions of oleic and linolenic acids in Tunis oil indicate a more favorable nutritional profile, particularly due to the higher unsaturated-to-saturated fatty acid ratio. This characteristic is often associated with improved cardioprotective and anti-inflammatory properties. Environmental factors are known to influence fatty acid composition, particularly the degree of unsaturation in plant oils. Temperature and precipitation have been reported to significantly affect lipid metabolism (Canvin, 1965; Hou et al., 2019; He et al., 2020). Additionally, soil fertility and geographical origin can lead to variations in lipid profiles, as demonstrated by Gifty et al. (2018). These factors likely contributed to the observed differences between the two studied sites.

Conclusion

In conclusion, *R. pseudoacacia* seed oil, especially from the Tunis site, represents a promising source of nutritionally valuable unsaturated fatty acids, including essential omega-3 and omega-6 fatty acids. Its high content of beneficial lipids suggests potential applications not only in nutrition, but also in functional foods, cosmetics, and pharmaceuticals, given the well-established links between unsaturated fatty acid intake and the prevention of chronic diseases such as cardiovascular disorders, Crohn's disease, hypertension, arthritis, lupus, autism, and depression.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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