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Abstract. Sunflower (*Helianthus annuus* L.) pollen is a chemically rich but underexplored matrix containing bioactive lipids with nutraceutical and pharmaceutical relevance. Lipid fractions were isolated from hand-collected pollen samples across three distinct agroclimatic zones in Moldova via mini-Soxhlet extraction. Subsequent alkaline saponification and acid methylation enabled GC-MS characterization of fatty acid methyl esters (FAMEs) and unsaponifiable constituents. The mean lipid yield (9.23% w/w) exhibited regional variability, with the highest value in the sample from Visoca (9.90%), likely due to climatic modulation of lipid biosynthesis. Chromatographic profiling resolved >40 constituents, including: Polyunsaturated fatty acid esters (e.g., methyl linolenate, a ω -3 precursor); diterpenoid derivatives (methyl labdatrien-19-oate); oxygenated sterols (lanostan-3-one); lipophilic vitamins (α -tocopherol, retinol derivatives). Chemometric analysis revealed stress-induced shifts in metabolite distribution, with heat/water deficits favouring terpenoid accumulation. The lipidome's structural diversity-spanning hydrocarbons, esters, and ketones-suggests multifunctional bioactivity (antioxidant, anti-inflammatory). These findings position sunflower pollen as a sustainable source of phytochemical precursors for functional ingredients. Further studies should address structure-activity relationships, stabilization strategies, and green extraction optimization.

Keywords: lipid fraction, gas chromatography-mass spectrometry (GC-MS), phytosterol, terpenoid, nutraceutical value, pollen.

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops cultivated worldwide, mainly used for the production of edible oil from seeds. However, the pollen of this plant represents a valuable yet unexplored source of bioactive compounds with potential applications in the nutritional, pharmaceutical and cosmetic industries [1-3]. Pollen is a complex biological matrix, rich in proteins, essential amino acids, carbohydrates, lipids, vitamins, enzymes, flavonoids and other secondary metabolites [4,5]. Specifically, sunflower pollen is recognized as an important nutritional resource for larvae and young bees, providing lipids, vitamins, and minerals.

High-performance liquid chromatography (HPLC) analysis of three types of sunflower pollen (fresh, bee-harvested, and stored), collected from South Africa, revealed the presence of 18 amino acids, including 10 essential amino acids, among them the most abundant being leucine,

histidine and lysine, as well as non-essential amino acids - glutamic acid, proline and alanine [6]. Although lipids typically represent only 5–10% of the total pollen mass, they include essential fatty acids (*omega*-3 and *omega*-6), sterols, phospholipids and fat-soluble vitamins (A, D, E and K), which may contribute to the antioxidant, anti-inflammatory and immunomodulatory activity of the product [3,7,8].

The fatty acid profile of sunflower pollen varies depending on the geographical origin. For instance, Farag, R.S. *et al.* reported that in sunflower pollen collected by bees in Egypt, *myristic acid* constituted about 47% of the total fatty acid content, while lauric acid was found to be the lowest [9]. Contrary, French researchers determined a high content of lauric acid in sunflower pollen, followed by palmitic and α -linolenic acid [6]. In the current literature, data regarding the chemical composition of oils extracted from pollen are limited, with most

studies focused on the analysis of lipids from *Helianthus annuus* L. seeds. A more comprehensive chemical investigation of *H. annuus* L. pollen was carried out by Schulz, S. *et al.* [10], using raw material collected from the Quebec region, Canada. The extraction was performed with hexane using ultrasonic irradiation or the Soxhlet extraction methods. Gas chromatography–mass spectrometry (GS-MS) analysis of the resulting extracts revealed the presence of several classes of compounds, including triterpenes, β -diketones, hydroxyketones, 1,3-alkanediols, alkanes, carboxylic acids, esters, and β -dioxoalkanoic acids. The main component has been found to be helianyl octanoate, which is a seco-triterpenoid.

The methods of oil extraction from pollen usually involve the use of organic solvents in/and Soxhlet type extractors or ultrasound-assisted extractions, followed by saponification and derivatization to allow identification of components by gas chromatography coupled to mass spectrometry [11,12].

The aim of this study was to perform a comprehensive characterization of the lipid fraction extracted from *Helianthus annuus* L. pollen collected under different climatic conditions in the Republic of Moldova in order to evaluate its chemical composition, functional potential, and environmental susceptibility. By combining Soxhlet-based extraction with GC-MS profiling and quantitative analysis, this study aimed to: (i) determine the lipid yield and free fatty acid content in samples collected from different agroclimatic zones, (ii) identify and classify the major, medium, and minor lipid derivatives with known or potential bioactivity, and (iii) evaluate the influence of heat and water stress on lipid biosynthesis and metabolite variability. According to literature data, sunflower fields can produce up to 80 kg of pollen per hectare, highlighting the potential of sunflower pollen as a valuable by-product of

sunflower cultivation [13]. This investigation contributes to the valorisation of sunflower pollen as a new and sustainable source of functional lipids with potential applications in nutraceutical, pharmaceutical, and cosmetic industries and supports the development of biogeographical traceability markers for pollen-derived products.

Experimental

Materials and methods

Fresh biological material (ligulate and tubular flowers) was collected in 2022 from different agroclimatic regions at the experimental fields of the State Commission for Crops Variety Testing of the Republic of Moldova: Visoca (P1), Pelinia (P2) and Bacioi (P3). The pollen was collected manually from the same sunflower hybrid cultivated in different agroclimatic zone in the first decade of July. The samples were dried and stored in hermetically sealed containers at room temperature, protected from light and humidity. The pollen used in the research was obtained by sieving from the tubular flowers collected separately for each sample (Table 1, Figure 1(a-c)).

Across all experimental fields, identical agricultural practices and cultivation technologies were applied, with the only variable being the climatic conditions. Thus, these regions were characterized by distinct climatic patterns (Table 2) during the period of microsporogenesis (May, June, July) and pollen grain formation.

Meteorological data for the year 2022 were obtained from the databases of the State Hydrometeorological Service [14].

Table 1
Amount of sunflower pollen samples collected for analysis.

Sample code	P1	P2	P3
Weight (g)	7.93	5.75	16.96

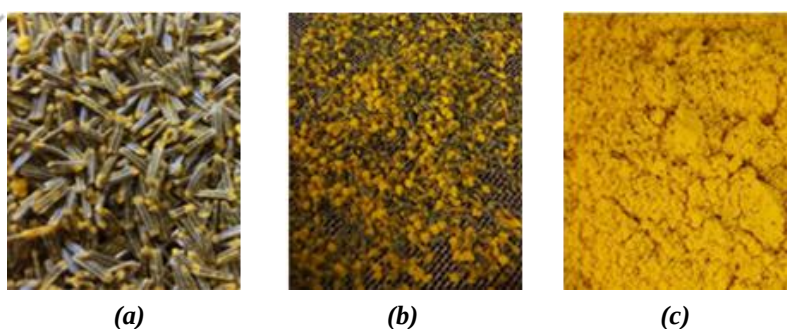


Figure 1. The steps of the process of obtaining pollen samples for study: tubular flowers of sunflower (source of pollen) (a), pollen sifting and separation from tubular flowers (b) and fresh pollen samples prepared for analysis (c).

In the Republic of Moldova, the year 2022 was characterized by a high temperature regime and significant precipitation deficit during spring-summer period. Therefore, the studied regions showed significant differences in temperature and humidity during pollen formation (microsporogenesis). Visoca was characterized by more moderate temperatures and the highest rainfall in June (71 mm), suggesting relatively favourable growth conditions. Pelinia exhibited a similar temperature regime, but with lower precipitation levels. In contrast, Bacioi recorded the highest temperatures (23.6°C in July), along with a significant precipitation peak of 83 mm (Table 2).

Extraction of lipid fraction

Mini-Soxhlet type extractors were used to extract lipids from pollen. A fixed mass from each sample (5.0 g) of dried pollen was placed in filter paper cartridges, then thirty mL of petroleum ether was added to the extraction flask, and extraction was performed under reflux for 1.5 hours. The extracts were concentrated to dry, at 30°C under reduced pressure using a rotary evaporator and the obtained lipid fractions were weighed and stored at 4°C until further analysis.

Saponification of extracted lipids

The lipid samples individually were subjected to saponification reaction to separate the free fatty acid's fractions. Excess of methanolic KOH solution was added to each sample and the mixtures were refluxed for 30 min. After cooling, distilled water and 2M HCl solution were added until to a pH < 7 and the acid fractions were extracted with diethyl ether ((C₂H₅)₂O), then after drying and filtration organic extracts were evaporated to dryness under reduced pressure.

Methylation of the free fatty acid's fractions

For derivatization of the free fatty acids, the obtained acid fractions were dissolved in anhydrous diethyl ether and treated with diazomethane (CH₂N₂, 1M in diethyl ether) until a persistent yellow coloration was obtained. Then, the ester mixtures were evaporated to

dryness under reduced pressure using a rotary evaporator, weighed and prepared for GC-MS analysis.

Methyl esters GC-MS analysis

Qualitative and quantitative analysis of the methyl esters samples was performed by gas chromatography Agilent 7890A (GC) system coupled to mass spectrometry 5975C (MS) detector and equipped with an HP-5M calibrated capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The separation was operated under the following conditions: oven temperature program: from 70°C, held for 2 min, up to 200°C at a rate of 5°C/min for 20 min., then up to 300°C in 5 min. Injector and detector temperatures, 250°C; carrier gas, helium at a flow rate of 1.1 mL/min; split ratio, 1:50. The samples for GC-MS analysis were prepared using a standard procedure, which requires the complete dissolution of 20 mg of methyl esters in 1.5 mL of diethyl ether. Compound identification was performed by comparing retention times and mass spectra with those in the NIST library.

Results and discussions

The experimental analysis of the lipid fraction extracted from *Helianthus annuus* L. pollen revealed valuable insights regarding both the yield and the phytochemical profile of the fatty acid components and associated bioactive compounds.

Lipid content and their yield of extraction

As presented in the Table 3, the average lipid content extracted from the sunflower pollen samples was 9.23%, aligning with values reported in previous studies [11,15], and indicating a relatively high lipid concentration for this type of biological material. Among the analysed samples (P1 to P3), the lipid content ranged between 8.40% and 9.90%, confirming the potential of sunflower pollen as a sustainable source of bioactive lipids suitable for nutraceutical applications.

Table 2

Climatic conditions in sunflower pollen collection regions, 2022.

Regions	May		June		July	
	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)
Visoca	15.8	7.0	19.4	71.0	22.0	6.0
Pelinia	15.4	10.0	19.9	17.0	21.5	19.0
Bacioi	16.8	21.0	22.3	7.0	23.6	83.0

Sample P1 collected from Visoca region showed the highest lipid content, suggesting a richer chemical composition of the pollen harvested in that agroclimatic zone (Table 3). According to the literature, the lipid content in sunflower pollen generally ranges between 5% and 12%, depending on the geographical area, soil and climatic conditions, plant maturity and extraction method. For example, Campos, M.G. *et al.* [11] showed that lipid content in pollen collected from different regions varied between 5.0% and 13.5%.

The subsequent saponification and methylation steps allowed the separation and derivatization of fatty acids into their methyl ester forms.

Table 3

The amount and content of lipids extracted from sunflower pollen samples.

Sample code	P1 (Visoca)	P2 (Pelinia)	P3 (Bacioi)
Weight (g)	0.49	0.47	0.42
Fat content (%)	9.90	9.40	8.40
Average (%)	9.23		

Acid fraction extracted from pollen lipids

The amount of free fatty acids is comparable between samples, indicating a significant proportion of saponifiable fraction in the extracted oil. In the present study the mass of the acid fractions obtained after saponification varied slightly across the different samples (Table 4), ranging between 0.40 g and 0.46 g. The acidic fraction represented a high proportion (~95–99%) of the total lipid mass, suggesting a lipid profile rich in fatty acids, including free fatty acids, which is a typical feature of unprocessed oils or oils obtained from biological raw materials.

Literature indicates that pollen lipids are composed primarily of free fatty acids, mono-, di-, and triacylglycerides, phospholipids, and sterols [16]. Fatty acids are a key component of the lipid fraction in pollen, existing both in free form and more commonly as esters, predominantly triacylglycerols [17]. In sunflower pollen, Conte, G. *et al.* [18] reported that 70–80% of the total lipid content consists of free fatty acids, while in willow and chestnut pollen the acidic fraction can account for up to 90% of total lipids. The current study supports these findings, a very high content of acid fraction (almost equal to the total mass

of the lipids) being established, which confirm the fact that sunflower pollen is a rich source of free fatty acids.

Regarding climatic conditions, sample P1 from Visoca, with a more moderate thermal regime and more precipitation (Table 2), showed the highest lipid content and the highest mass of the acid fraction (0.46) (Table 4). P3 from Bacioi, a region with high temperatures and a more variable water regime, registered the lowest lipid content, suggesting a negative influence of heat stress on pollen lipid biosynthesis. Fatty acid methyl esters were synthesized with yields ranging from 87% to 92% (Table 4). While the methylation efficiency was satisfactory, further optimization of the chemical process could enhance conversion yields.

GC-MS analysis

The GC-MS profile revealed a rich chemical spectrum comprising more than 40 identified compounds, among which methylated fatty acids, terpene derivatives, sterols and lipid-soluble vitamins were distinguished (Table 5).

Notably, terpenic acids such as methyl labda-8(20),12,14-trien-19-oate (up to 21.37%), methyl kaurenoate (up to 5.13 and 13.49%), and methyl podocarpenoate (2.26%) derivatives represented substantial fractions. These diterpenoid compounds are recognized for antimicrobial, anti-inflammatory, and antioxidant properties, suggesting additional pharmaceutical potential of the pollen-derived oil.

Compounds such as vitamin A (up to 2.90%), vitamin E (up to 0.57%), and sterolic compounds like lanostan-3-one (up to 11.08%) were also detected, contributing to the antioxidant and bioactive profile of the lipid fraction. These findings suggest the nutraceutical and functional value of this type of lipids. Phytosterols such as lanostan-3-one also contribute to the hypocholesterolemic potential of the product [19].

The dominant compounds that are identified in all extracted lipid samples up to an average of 4.5 % are included in Figure 2.

Table 4

Mass of acid fractions and methyl esters obtained from sunflower pollen.

Sample code	Acid fraction (g)	Methyl esters (g)
P1 (Visoca)	0.46	0.42
P2 (Pelinia)	0.45	0.43
P3 (Bacioi)	0.40	0.39

Table 5

Chemical composition of sunflower pollen oil identified by GC-MS.

No.	RT* (min)	Component	Content (%)		
			P1 (Visoca)	P2 (Pelinia)	P3 (Bacioi)
1.	6.46	Methyl hexanoate	0.17	-	0.16
2.	6.66	α -Pinene	0.16	-	-
3.	9.09	Acetophenone	0.29	-	0.30
4.	9.50	Linalool	0.63	0.48	-
5.	9.79	Methyl octanoate	1.41	1.38	0.82
6.	10.15	(Z)-Verbenol	0.28	0.29	-
7.	10.53	3-Pinanone	0.17	0.13	-
8.	11.35	Linalyl acetate	0.64	0.49	0.96
9.	11.77	Undecanone	0.31	0.19	0.27
10.	12.06	Methyl decanoate	0.37	0.31	0.29
11.	13.83	Methyl dodecanoate	2.63	2.21	4.18
12.	15.22	2-Pentadecanone	0.65	0.60	0.71
13.	15.40	Methyl myristate	1.37	1.13	1.47
14.	16.11	Methyl pentadecanoate	-	-	0.19
15.	16.67	2-Heptadecanone	0.35	-	0.39
16.	16.82	Methyl palmitate	3.96	5.14	5.82
17.	17.56	Methyl 3-methoxy tetradecanoate	-	-	1.02
18.	17.98	α -Methyl linolenate	9.53	10.59	12.14
19.	18.09	Methyl octadecanoate	-	1.53	-
20.	18.29	Dimethyl dodecanedioate	0.32	0.22	0.21
21.	18.51	Cycloheptyl decyl adipate	0.27	0.25	-
22.	18.70	Methyl podocarp-8(14)-en-18-oate	2.26	1.46	2.19
23.	19.06	Methyl labda-8(20),12,14-trien-19-oate	17.49	21.37	11.90
24.	19.15	Methyl (13(Z)-eicosenoate	6.42	7.05	7.51
25.	19.38	Methyl 4 β -kaur-16-en-18-oate	13.49	9.00	7.65
26.	19.57	Methyl 14-oxononadec-10-enoate	1.70	2.16	2.14
27.	19.59	Methyl arachidonate	1.71	-	0.51
28.	19.65	Methyl 11(Z),14(Z)-octadecadienoate	4.09	3.86	5.69
29.	19.83	Vinyl stearate	-	0.23	1.01
30.	20.18	Hexacosane	1.49	0.98	0.65
31.	20.33	Methyl docosanoate	-	-	0.26
32.	20.59	Methyl steviol	1.18	0.65	1.22
33.	20.66	Methyl 4 α -13-hydro-kaur-16-en-18-oate	5.13	3.36	1.92
34.	21.20	Heptacosane	1.15	2.29	1.72
35.	21.68	2,5-Heptadecadione	1.34	2.17	1.41
36.	22.13	Vitamin A	2.46	2.90	1.16
37.	23.05	Octacosane	1.25	0.86	0.71
38.	23.43	Vitamin E	0.57	0.51	0.56
39.	23.78	Etil iso-alocolate	0.91	1.64	1.24
40.	24.51	Lanostan-3-one	6.06	9.23	11.08
41.	24.83	Allyl stearate	0.82	1.28	1.01

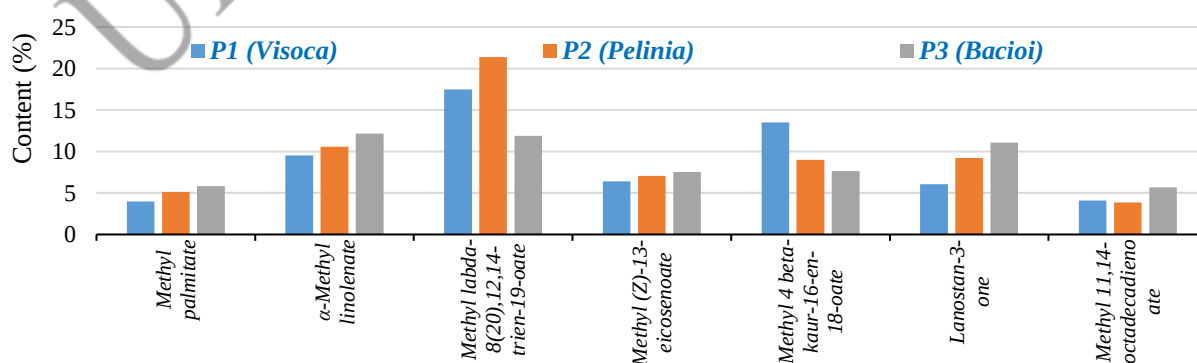


Figure 2. Dominant compounds identified by GC-MS analysis.

Methyl palmitate, which ranges from 3.96% (P1) to 5.82% (P3), contributes significantly to the structural lipid components, especially phospholipids and glycolipids, involved in cell membrane architecture. Its presence indicates the potential of sunflower pollen as a source of precursors for the biosynthesis of biological membranes, influencing their fluidity, rigidity and membrane stability. Methyl linolenate (9.53-12.14%), an omega-3 fatty acid, is known for its anti-inflammatory and cardioprotective effects. Methyl 11(Z),14(Z)-octadecadienoate (up to 5.69%) and methyl 13-(Z)-eicosenoate (up to 7.51%) reflect the major presence of unsaturated fatty acids in the lipid composition of sunflower pollen.

Methyl palmitate, α -methyl linolenate and lanostan-3-one were present in all samples, with the highest values found in P3 (Bacloi), possibly reflecting phytochemical adaptations to water or heat stress, leading to enhanced accumulation of specific secondary metabolites, despite a lower total lipid content of this sample. These dominant compounds contribute to the high degree of unsaturation of the lipid fraction, which is associated with significant biological benefits, such as maintenance of cell membrane integrity, reduction of inflammation and cardiovascular protection. The high content of these acids underlines the potential of pollen lipids as a source of functional and bioactive compounds with nutritional and therapeutic applications. They confer antioxidant, anti-inflammatory properties and pharmacological potential to the oil, in agreement with other previous studies on sunflower pollen [20-22]. In this context, the valorisation of pollen as a raw material can contribute to the diversification of sources of functional lipids and to the promotion of a circular

economy in the agricultural sector. Previous studies have demonstrated that pollen is a rich source of diverse fatty acids, particularly unsaturated fatty acids. Thus, in investigation including maize hybrids, a total of 28 fatty acids were quantified, with palmitic and heneicosanoic acid being the most abundant saturated fatty acids. Among monounsaturated fatty acids, oleic, elaidic, and 10(Z)-heptadecenoic acid were predominant, while linoleic and 11(Z),14(Z)-eicosadienoic acid represented the most abundant polyunsaturated fatty acids. Moreover, the composition of fatty acids was shown to be significantly influenced by the type of maize hybrid analysed [23].

The chemical composition of sunflower pollen is remarkably complex and diverse, comprising a wide range of volatile and semi-volatile compounds with significant biological roles. Based on their average content percentages, these compounds can be classified into three main categories (Table 6):

- *major compounds* (> 6%), represent more than 50% of the total identified content;
- *medium-abundance compounds* (2–6%), contribute to the functional structure of the oil and may influence its olfactory, taste, and pharmacological properties;
- *minor compounds* (< 2%, approximately 30) present in small quantities.

This stratification supports the phytochemical complexity and potential multifunctionality of sunflower pollen oil. Major compounds primarily contribute to biological activity, while medium and minor compounds influence aroma, oxidative stability, and may have synergistic roles.

Table 6

Dominant and medium-abundance compounds in sunflower pollen oil

Dominant compounds	Content (%)	Medium-abundance compounds	Content (%)
Methyl labda-8(20),12,14-trien-19-oate	16.92	Methyl palmitate	4.97
α -Methyl linolenate	10.75	Methyl 11(Z),14(Z)-octadecadienoate	4.55
Methyl 4 β -kaur-16-en-18-oate	10.05	Methyl 4 α -13-hydroxy-kaur-16-en-18-oate	3.47
Lanostan-3-one	8.79	Methyl dodecanoate	3.01
Methyl 13(Z)-eicosenoate	6.99	Vitamin A	2.17

Table 7

Highly and low-variable compounds in sunflower pollen oil.

Stable compounds	Mean	Std. Dev.	Variable compounds	Mean	Std. Dev.
3-Pinanone	0.15	0.03	Methyl labda-8(20),12,14-trien-19-oate	16.92	4.76
Cycloheptyl decyl adipate	0.26	0.01	Methyl 4 β -kaur-16-en-18-oate	10.05	3.06
Acetophenone	0.30	0.007	Lanostan-3-one	8.79	2.54
Methyl hexanoate	0.165	0.007	Methyl 4 α -13-hydroxy-kaur-16-en-18-oate	3.47	1.61
(Z)-Verbenol	0.285	0.007	α -Methyl linolenate	10.75	1.31

Some compounds, such as 3-pinanone and acetophenone, showed low standard deviations across samples, indicating a stable presence and potential use as chemical markers for sunflower pollen oil. In contrast, methyl labda-8(20),12,14-trien-19-oate and lanostan-3-one showed high variability, likely influenced by environmental conditions or genetic background (Table 7).

Geographical trends were clearly reflected in the distribution of certain compounds. For example, compounds such as methyl octanoate, methyl decanoate, dimethyl dodecanedioate, and hexacosane were more abundant in northern samples and decreased toward central regions. In contrast, compounds like α -methyl linolenate and lanostan-3-one peaked in southern locations (e.g., Bacioi).

Some compounds were detected only in one or two samples (methyl octadecanoate - only in P2, vinyl stearate – P2 and P3, methyl 3-methoxy tetradecanoate - only in P3). Such occurrences might reflect natural variability, chemical instability, or differences in extraction efficiency.

This study demonstrates the value of chemical profiling in sunflower pollen oil. The identification of stable and variable compounds, along with their correlation to geographic origin, underlines the potential for traceability, authenticity assessment, and functional evaluation. Further research involving a broader range of samples and conditions would strengthen the understanding of these bioactive matrices.

Conclusions

This study presents a comprehensive physico-chemical characterization of the lipid fraction extracted from *Helianthus annuus* L. pollen, highlighting its chemical heterogeneity, molecular functionality and susceptibility to abiotic stressors such as temperature and humidity. The average lipid yield of 9.23% (w/w) is within the expected range for sunflower pollen and supports its viability as an alternative lipid source for nutraceutical formulations, lipophilic drug carriers and therapeutic emulsions.

Sample P1 (Visoca), collected under more moderate thermic and hydric regimes, exhibited the highest total lipid content, including increased levels of free fatty acids (FFA), monoacylglycerols, and triglycerides, indicating enhanced lipid biosynthesis pathways under optimal environmental conditions. In contrast,

sample P3 (Bacioi), collected under high temperature and irregular rainfall, exhibited significantly lower lipid concentrations, suggesting a climate-driven modulation of lipid metabolism and oxidative degradation of unsaturated lipids.

The gas chromatography-mass spectrometry (GC-MS) profile revealed a diverse molecular spectrum comprising over 40 identified compounds, including methyl esters of polyunsaturated fatty acids (PUFAs) such as methyl linolenate (C18:3) and methyl palmitate (C16:0), oxygenated diterpenes (e.g., methyl labda-8(20),12,14-trien-19-oate, methyl kaurenate), phytosterolic ketones (e.g., lanostan-3-one) and lipophilic micronutrients such as retinol (vitamin A) and tocopherol isomers (vitamin E). These bioactive lipids exhibit radical-scavenging activity, cyclooxygenase (COX) pathway inhibition, and membrane stabilization, contributing to their antioxidant, anti-inflammatory, and immunomodulatory potential.

Quantitative stratification of the compounds based on relative abundance, dominant (>6%), medium (2–6%), and trace (<2%), supports the multicomponent functionality of *H. annuus* pollen oil, with implications for synergistic biological activity.

These findings position sunflower pollen as a renewable and sustainable feedstock for the production of bioactive lipid formulations, with promising applications across pharmaceutical lipidomics, cosmeceuticals, and functional foods. Future research should focus on the *in vitro* and *in vivo* evaluation of individual lipid fractions, kinetic stability studies, bioaccessibility and biotransformation, and the optimization of extraction and purification processes using green chemistry approaches for industrial scalability.

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