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► **To cite this version:**

Lounès Haroune, Sabrina Saibi, Isabelle Rabbat, Yann Loranger, Mathilde Tissier. Multidimensional assessment of nutritional composition, contaminants and biological properties of bee pollen. *Applied Food Research*, 2025, 5 (2), pp.101223. <10.1016/j.afres.2025.101223>. <hal-05369754>

HAL Id: hal-05369754

<https://hal.science/hal-05369754v1>

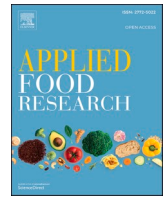
Submitted on 18 Nov 2025

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Multidimensional assessment of nutritional composition, contaminants and biological properties of bee pollen

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ARTICLE INFO

Keywords:

Bee pollen
Nutritional content
Mass spectrometry
Cytotoxicity
Contaminants

ABSTRACT

Honey-bee collected pollen is renowned for its nutritional richness and potential health benefits, but compositional variations and contamination are significant challenges. This research investigates the organic and inorganic content, morphological aspects, and anticancer properties of 18 different pollen samples from Canada (Québec). Results revealed variability in macronutrients (carbohydrates: 39.1 to 57.0 g 100g⁻¹, proteins: 14.3 to 23.5 g 100g⁻¹, and lipids: 3.58 to 35.4 g 100g⁻¹) and micronutrients, including vitamin B and C (up to 1052 µg g⁻¹) and antioxidants. However, pollen was found to contain pesticide residues (diazinon, thiamethoxam and glyphosate at up to 32.1 ng g⁻¹) and heavy metals (lead and arsenic at up to 3.86 mg kg⁻¹), indicating the need for environmental monitoring, including regular assessment of pollen contamination and implementation of mitigation strategies to reduce exposure. Cytotoxicity assays showed promising anticancer effects against HeLa cells, with up to 40 % cell growth inhibition with an IC₅₀ at 247 µg mL⁻¹ was observed in cells treated with pollen extract compared to untreated cells. Future research should focus on profiling bioactive compounds and their bioavailability while establishing standardized characterization methods and a centralized database for accurate nutritional and safety assessments. This study provides novel insights into the composition of pollen, its biological effects and contamination levels, illustrating its nutritional and therapeutic potential.

1. Introduction

Bee pollen, a complex substance produced by honeybees, is often regarded as a nutritionally complete food. It is composed of plant pollen collected by bees, which is then combined with nectar and bee secretions to form nutrient-dense granules. Pollen is essential for bee nutrition and is recognized for its potential in health benefits in humans (Thakur & Nanda, 2020; Sattler et al., 2015).

Rich in proteins, carbohydrates, lipids, vitamins, and minerals, bee pollen also contains bioactive compounds like phenols and flavonoids, which contribute to its antioxidant properties. The diverse nutritional profile and biological activity of bee pollen have attracted interest in nutrition, functional food, and potential medicinal applications (Végh et al., 2021). Known for its antimicrobial, anti-inflammatory, and energy-boosting properties, bee pollen is often described as a superfood

(Didaras et al., 2020; Pascoal et al., 2014). Recent studies suggest its bioactive compounds may offer therapeutic potential against various cancers, including breast and prostate cancer (Premratanachai & Chanchao, 2014; Hanafy et al., 2023). Therefore, a multidimensional exploration of pollen properties could significantly enhance our understanding of its therapeutic potential.

However, the chemical composition and the morphological characteristics of pollen vary widely depending on the plant species and environmental conditions, complicating its characterization (Thakur & Nanda, 2020). Despite growing interest, comprehensive characterization of bee pollen from different botanical sources remains limited in research (Lau et al., 2022).

Studies have primarily focused on quantifying nutrients like proteins, lipids, and carbohydrates using conventional methods such as colorimetric assays or chromatography techniques. Carbohydrates, a

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<https://doi.org/10.1016/j.afres.2025.101223>

Received 6 November 2024; Received in revised form 27 June 2025; Accepted 30 July 2025

Available online 31 July 2025

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major component of bee pollen, include numerous simple sugars and fibres, with glucose and fructose predominant (da Silva et al., 2014). Colorimetric methods, such as phenol-sulfuric acid assay, are commonly used to determine total digestible carbohydrates (Lau et al., 2022; Hassan, 2011). Factors influencing carbohydrate content include the incorporation of nectar during pellet formation, botanical species and geographic location (Thakur & Nanda, 2020). Total carbohydrates vary significantly, ranging from 18.5 % to 82.8 % globally (Thakur & Nanda, 2020; De-Melo et al., 2018).

Proteins, the second most abundant component, are measured using various methods, including colorimetric approaches (Bradford, bicinchoninic acid and Lowry assay) or nitrogen content measurements (Dumas and Kjeldahl assays) (Lau et al., 2022; Asmae et al., 2021). Variations are observed across different countries and botanical species. For example, *Brassica spp.* pollen varied from 19.6 % in India to 27.3 % in China, while *Zea mays* ranged from 14.8 % protein content in Greece to 21.3 % in Egypt (De-Melo et al., 2018; Alshallash et al., 2023; Yang et al., 2013; Liolios et al., 2015).

Lipids, including essential fatty acids like Omega-3 and Omega-6, are crucial for both bee and human nutrition. Total lipids measured by gravimetric or colorimetric methods ranged from 1 % to 13 %, depending on botanical species and location (Thakur & Nanda, 2020; Campos et al., 2008). Beyond macronutrients, bee pollen is a source of essential micronutrients, including minerals and vitamins. The presence of phosphorus, potassium, sodium, calcium, iron, zinc, magnesium, manganese, and selenium underscore its nutritional value (Thakur & Nanda, 2020). Elemental analysis is typically performed using nitric acid digestion followed by inductive-coupled plasma spectrometry (ICP-MS) analysis (Asmae et al., 2021; Mayda et al., 2020). Vitamins found in pollen include β carotene, vitamin C and B-complex vitamins (Thiamine, Riboflavin, niacin (Niacin), Pantothenic acid, Pyridoxin, and Folic acid). Vitamin levels are generally determined by UV or fluorescence detectors. (Sattler et al., 2015; Bayram, 2021). While few studies have conducted comprehensive analyses of the vitamin composition of bee pollen, high levels of B-complex vitamins have been reported in Brazilian samples (de Arruda et al., 2013a; de Arruda et al., 2013b). The antioxidant properties of bee pollen are attributed to vitamins, flavonoids and phenolic compounds, assessed through various methods, such as colorimetric assay and LC-MS analysis (Mayda et al., 2020; Harif Fadzilah et al., 2017; Ilie et al., 2022).

However, despite the nutritional value of bee pollen, potential contaminants like pesticides and heavy metals require thorough investigation to ensure safety for both bees and human consumers. Pesticide residues can originate from the application before blooming, contaminated bees, or environmental sources like water and soil (Murcia-Morales et al., 2022). Studies have shown pesticide persistence in pollen, including fungicides and insecticides like neonicotinoids, with concentrations ranging from 6.10 ng g⁻¹ to 4530 ng g⁻¹ (Friedle et al., 2021).

The present study characterizes bee pollen samples from various botanical sources to determine their multidimensional nutritional profiles, including proteins, lipids, vitamins, minerals, bioactive compounds (e.g., vitamins, antioxidants, total phenol, total flavonoid), potential contaminants (pesticides, pharmaceutical compounds, and heavy metals), and cytotoxicity on HeLa cancer cell lines. Pollen grain morphology was also analyzed using microscopy. Microscopy techniques will provide a detailed examination of pollen grain morphology, offering more insight into structural adaptations facilitating the ecological role of pollen and interaction with pollinators (Hanafy et al., 2023). In addition to nutritional properties and morphology, the current study investigates the potential therapeutic (i.e., cytotoxic in cancer cells) properties of bee pollen extracts on HeLa cancer cell lines (Hanafy et al., 2023). The multidimensional approach implemented in the current study explores and enhances our understanding of bee pollen safety and potential health benefits, with implications for its use in functional foods, dietary supplements, and novel therapeutic applications. Such

multidimensional analyses of pollen samples can also benefit wild bee conservation (e.g., bumble bees, solitary bees) for which information on nutritional requirements and nutrient availability beyond macronutrients are strongly lacking (Vaudo et al., 2024; Lau et al., 2023). Implementing this multidimensional characterization of pollen could improve our understanding of the nutritional landscapes of these essential pollinators (Vaudo et al., 2024; Lau et al., 2023).

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used in this work were of analytical grade. Water (H₂O), methanol (MeOH), ethyl acetate (EtOAc) (Optima® grade for LC/MS) and formic acid (FA) were purchased from Fisher Scientific (Ottawa, ON, Canada). The standards used in this work were of analytical grade with a 98 % or higher purity. Vitamins such as pyridoxine (B6), thiamine (B1), nicotinamide (B3), niacin (B3), riboflavin (B2), folic acid (B9), cyanocobalamin (B12), retinol (A) and ascorbic acid (C) were purchased from Sigma Aldrich (Oakville, ON).

2.2. Bee pollen

Sample collection: Pollen samples characterized in this study were provided by Happy-Culture Inc. (18 samples). These 18 monofloral pollen samples were collected by honey-bees (*Apis mellifera*) in southern Québec (in the surroundings of Roxton Falls, 45.56667 –72.51666) in 2019, 2021 and 2022. Pollen was collected from the hives using pollen traps. Sampling was systematically conducted between 5:00 and 8:00 pm, except on extremely hot and humid days to limit variation of quality between samples. Upon collection, pollen samples were placed in light-proof jars at –20°C for a few weeks, before being transferred to –80°C to preserve pollen integrity until analysis.

Sample observation: The botanical origin of all samples was identified by observing a subsample under the microscope (magnification x1000). Pollen samples from the identified plant was collected and also observed under magnification x1000 to confirm the identification. The identification considers the color of the pollen pellets collected by the bees, the day of harvest in the year (flowering time), the size of the grains and their appearance under the microscope following a palynology guide (Girard, 2014).

Sample comparison: Two pollen samples were ordered from Plant Products (commercial mix no 17222, wildflower pollen, Plant Products Canada; see Table S1). These commercial mix pollen are commonly used by beekeepers, farmers or scientists to feed honeybees and bumblebees. The pollen nutrients characterization was conducted on these 18-bee pollen samples from different botanical origins. All validation experiments were performed in triplicate ($n = 3$). Detailed information about the bee pollen samples is summarized in the supplementary information Table S1.

2.3. Physicochemical characterization

Ash content (%) was determined according to the AOAC official methods 942.05 (AOAC, 2016). Briefly, 1 g of each bee pollen sample was accurately weighed into a pre-dried and pre-weighted porcelain crucible. The samples were incinerated in a muffle furnace at 550 °C for 5 h (until a constant weight was achieved). The crucibles were cooled in a desiccator and weighted for ash content determination.

Moisture content (%) was determined using the oven-drying methods according to AOAC Official Method 925.09 (AOAC 2016). Briefly, 1 g of each sample was accurately weighed into a pre-dried and pre-weighted aluminum dishes. The samples were dried at 105 °C for 3 h (until a constant weight was achieved). The dishes were cooled in a desiccator and weighted for moisture content determination.

pH measurement was performed according to Feas et al., 2012 (Feas

et al., 2012). Briefly, an aqueous solution of bee pollen was prepared by suspending 10 mg of samples in 500 μL of milliQ water ($\geq 18.6 \text{ m}\Omega$). The mixture was sonicated for 10 min to ensure complete dispersion. The pH was then measured using a calibrated pH meter (Accumet, AE150).

2.4. Macronutrient determination

2.4.1. Total carbohydrates

The measurement of total carbohydrate content was conducted using the phenolic-sulfuric acid method with a kit assay (MAK104, Sigma Aldrich). For each sample, 50 mg of bee pollen was homogenized in 200 μL of ice-cold assay buffer (MAK104A) provided in the KIT Assay. The mixture was centrifuged at $13,000 \times g$ for 15 min at 4°C , and the supernatant was collected. The total carbohydrate determination was performed in 96-well plates by combining 30 μL of sample extracts with 150 μL of concentrated sulfuric acid. The plate was protected from light and incubated for 15 min at 90°C in the dark. After incubation, 30 μL of developer reagent (MAK104B) was added, and the plate was mixed. Glucose was used as the standard reference, and the absorbance was measured at 490 nm. Results are expressed as $\text{g } 100 \text{ g}^{-1}$ of dry matter.

2.4.2. Total proteins

Proteins were extracted from 20 mg of pollen using 0.5 mL of 0.1 M NaOH, followed by heating for 5 min at 90°C before analysis (Lau et al., 2022; Westreich & Tobin, 2021). The total protein concentration was determined using a Bradford colorimetric assay (QCPR-500, Fisher Scientific). A 10 μL of protein extract was added to a 96-well plate and mixed with 200 μL of working reagent provided by the KIT assay. The absorbance was measured at 595 nm and bovine serum albumin (BSA) was used as the standard reference. Results are expressed as $\text{g } 100 \text{ g}^{-1}$ of dry matter.

2.4.3. Total lipids

The total lipids were determined using two methods: the Folch method and the Loveridge assay. In the Folch method (Lau et al., 2022; Folch et al., 1957), lipid extraction was performed by placing 100 mg of bee pollen into 750 μL of Dichloromethane: Methanol solvent mixture at a ratio of 2:1 (V: V). Each sample was sonicated for 10 min and agitated for 1 min before being centrifuged at 4500 rpm for 10 min. The resulting supernatant, containing the lipid extract, was then evaporated to dryness and the residual dry lipids were weighed. The Loveridge assay is based on gravimetric measurement, comparing the sample weight before and after lipid extraction using the Folch method (Lau et al., 2022). Results are expressed as $\text{g } 100 \text{ g}^{-1}$ of dry matter.

The macronutrient methods were validated using a reference material (commercial bee pollen). The amounts found using these methods compared to the reference materials are presented in supplementary information Table S2.

2.5. Micronutrient determination

2.5.1. Vitamins

8 vitamins were analyzed in bee pollen, including B-complex vitamins (the two forms of vitamin B3 were characterized), ascorbic acid (vitamin C) and retinol (vitamin A). Two extractions were performed, an acidic extraction for the water-soluble vitamins (B-complex vitamins and ascorbic acid) and an organic extraction for the fat-soluble vitamin (retinol). For the acidic extraction of B-complex vitamins and ascorbic acid, which are water-soluble, 50 mg of bee pollen was extracted with a 5 % metaphosphoric acid solution. For the organic extraction of fat-soluble vitamins (Retinol), acetonitrile (MS grade) was used. To prevent vitamin oxidation, 100 mM tris(2-carboxyethyl) phosphine (TCEP) was added to each extract. The samples were mixed and placed in an ultrasound bath for 15 min. After sonication, the samples were centrifuged at 4500 rpm for 10 min at 4°C . The extract was filtered before analysis. All the sample preparation was performed under reduced light

exposure due to the sensitivity of vitamins such as ascorbic acid and retinol.

Vitamin analyses were performed using an Acquity UPLC Xevo TQ mass spectrometer (Waters Corporation, Milford, MA, USA). LC-MS was selected for vitamin analysis due to its high sensitivity, specificity and ability to quantify multiple vitamins in complex matrices such as pollen. Unlike other methods, it does not require derivatization, can analyze both water and lipid-soluble vitamins, and provides precise molecular quantification. Furthermore, its low detection limits allow the measurement of trace amounts of vitamins that may be undetectable using other methods like UV-VIS assays. These advantages make LC-MS the most reliable and efficient method for analyzing multiple vitamins in complex samples. Accuracy, precision, limits of detection (LOD) and limit of quantification (LOQ) are summarized in supplementary information Table S3. Results are expressed as $\mu\text{g } \text{g}^{-1}$ of dry matter.

2.5.2. Macroelements, trace element and heavy metals

24 elements were assessed in the elemental analysis of bee pollen, including 5 macroelements [Calcium (Ca), Sodium (Na), Phosphor (P), Potassium (K) and Magnesium (Mg)], 10 trace elements [Iron (Fe), Manganese (Mn), Zinc (Zn), Molybdenum (Mo), Barium (Ba), Bore (B), Titanium (Ti), Aluminium (Al), Cupper (Cu) and Nickel (Ni)] and 9 heavy metals [Arsenic (As), Cadmium (Cd), Cobalt (Co), Chrome (Cr), Lead (Pb), Antimony (Sb), Selenium (Se), Vanadium (V) and Silver (Ag)]. The analysis was performed using inductively coupled plasma atomic emission spectroscopy (ICP-OES) after digesting 250 mg of bee pollen in 5 mL of trace metal-grade nitric acid. Results are expressed as $\text{mg } \text{kg}^{-1}$ of dry matter.

2.6. Bioactive molecule

100 mg of each bee pollen was suspended in 1 mL ethanol (1:10; w + v). The samples were mixed thoroughly and placed in an ultrasound bath for 60 min at 40°C . Following sonication, the samples were centrifuged at 4500 rpm for 10 min at 4°C . The supernatant was carefully collected for the determination of bioactive compounds.

2.6.1. Total flavonoids

The total flavonoid content was determined using a method adapted from Fadzilah et al. (2017) and Mayda et al. (2020). Briefly, 50 μL of the ethanolic extract pollen was mixed with 50 μL of 2 % aluminum chloride (AlCl_3) solution. After a 10-minute incubation at room temperature, the absorbance was measured at 510 nm. Quercetin (QE) was used as the standard reference and results are expressed as Quercetin equivalent ($\text{mgQE } \text{g}^{-1}$).

2.6.2. Total phenols

The total phenolic content was determined using a method adapted from Fadzilah et al. (2017) and Mayda et al. (2020). The Folin-Ciocalteu method was applied using gallic acid (GA) as the reference standard. The assay was performed in a 96-well plate by adding 50 μL of samples, 50 μL of Folin-Ciocalteu reagent, and 100 μL of sodium hydroxide solution. After a 30-minute incubation at room temperature, the absorbance was monitored at 760 nm. Results are expressed as gallic acid equivalent ($\text{mgGA } \text{g}^{-1}$).

2.6.3. Antioxidant capacity

The antioxidant capacity was determined using the ABTS assays (2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) according to Mayda et al. (2020). To prepare the reagent, 7 mL of ABTS solution was reacted with 2.45 mM potassium persulfate and left in the dark overnight. After incubation, the ABTS solution was diluted to achieve an absorbance of 0.70 at 734 nm. 100 μL of pollen extract was mixed with 100 μL of ABTS solution and 400 μL of methanol. The mixture was incubated in a closed tube at room temperature for 10 min before measuring the absorbance at 734 nm. Trolox (a water-soluble vitamin E derivative) was used as the

reference and results are expressed as Trolox (TR) equivalent (mgTR g⁻¹).

2.7. Organic contaminants

The analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) adapted from Haroune et al. (2015). This analysis assessed 65 pesticides (including fungicides, insecticides, and herbicides), 10 polar pesticides (e.g. glyphosate) and 17 pharmaceutical compounds (table S4). For pesticide and pharmaceutical compounds analysis, 100 mg of bee pollen was extracted with ethyl acetate containing 0.1 % formic acid. The samples were mixed and placed in an ultrasound bath for 30 min at 40 °C. After sonication, the samples were centrifuged at 4500 rpm for 10 min at 4 °C. The supernatant was collected and evaporated to dryness. The dry residue was reconstituted in a mixture of water: methanol and formic acid (50:50:0.2 %) and the extract was filtered using a 0.2 µm PTFE syringe filter before the analysis. For polar pesticides, a second extraction was performed. 100 mg of pollen was extracted with acidified water containing 15 mM EDTA to prevent compound chelation. After 30 min of vortex agitation, an equal volume of methanol was added and mixed for another 30 min. The sample was then centrifuged, and the supernatant was filtered through a 0.2 µm RC syringe filter before analysis. Accuracy, precision, limits of detection (LOD) and limit of quantification (LOQ) are summarized in supplementary information Table S4. Results are expressed as ng g⁻¹ of dry matter.

2.8. Cytotoxicity assays

The human cervical adenocarcinoma (HeLa) cells were maintained in EMEM (Eagle's Minimum Essential Medium) supplemented with 10 % FBS and 1 % penicillin-streptomycin. The cancer cell was obtained from ATCC (Manassas, Virginia) and was cultured at 37 °C in a humidified atmosphere containing 5 % CO₂. Bee pollen extract was prepared by dissolving pollen grain in DMSO solution before adding it to cell culture.

Cell viability was determined using the microculture tetrazolium technique (MTT). Confluent cells in a 96-well plate were treated with either control (containing the same amount of DMSO) or bee pollen extract at different concentrations (20 µg mL⁻¹ and 200 µg mL⁻¹) for 48 h. The cells were then subjected to the MTT assays using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solutions. After a 4-hour incubation period, an HCl-isopropanol solution was added to each well, and the plate was incubated in the dark overnight. After incubation, absorbance was measured at 570 nm. The purple solution in the well-plate indicates viable cells, while a colourless solution indicates dead cells. Cell viability was calculated by the ratio of the absorbance of the sample treated with bee pollen extract in DMSO to the control treated with the same amount of DMSO. Results are expressed as a percentage of cell viability. Statistical analysis of the data was performed using one-way ANOVA at a significance level of $p \leq 0.01$.

2.9. Optical and scanning electron microscopy

Pollen pellets were suspended in 1 mL of water to dissociate the grain. After agitation, 10 µL of this suspension was transferred into the microscope slide for the optical microscopy observation. For the Scanning Electron Microscopy (SEM), the pellet pollen was suspended in 1 mL ethanol before the coating step with Au/Pd.

3. Results and discussion

3.1. Physicochemical characterization

The pH values of pollen samples ranged from 4.12 for pollen mix 1 to 6.45 for willow 2021 (Table 1), suggesting an acidic nature typical of bee pollen samples (from pH 4.0 to 5.0, (Asmae et al., 2021)). This

acidity contributes to the stability and preservation of bioactive compounds while inhibiting microbial growth. However, environmental stressors, such as elevated CO₂ levels, can intensify acidification, potentially altering the bioavailability of nutrients. Such changes may disrupt metabolic pathways, lead to protein misfolding, and reduce the functionality of bioactive molecules (Duarte et al., 2020). These disruptions may not only affect the biochemical composition and quality of bee pollen but could also impact the health and metabolic processes of bees by influencing their gut microbiota and overall physiological balance (Hoover et al., 2012). The observed pH variability could also potentially be influenced by environmental factors, such as storage conditions. Prolonged storage or exposure to fluctuating temperature and humidity levels may lead to chemical changes, including oxidation or microbial activity, that can alter the pH. These factors, along with the inherent properties of the samples, may explain the pH variability in pollen samples. However, in our study, each sample was carefully conditioned to ensure proper sealing, avoid thaw cycles and maintain stability. Overall, the observed variation in pH between our samples could mostly be attributed to environmental differences in plants' surroundings, including variations in CO₂ levels, soil physicochemical properties, and pedoclimatic conditions. Furthermore, we evaluated critical parameters before and after 1 year of storage at -80 °C, with no significant changes observed.

The Ash content (%) of the analyzed bee pollen samples is presented in Table 1, ranging from 3.30 % in Goldenrod to 10.2 % for wild blackberries and organic Maize. This range is consistent with previously reported values in the literature (Asmae et al., 2021), reflecting the mineral composition influenced by botanical origin and environmental factors.

The moisture content (%) varied between 9.20 % for organic maize and 24 % for non-organic maize (Table 1), which falls within the acceptable range for quality bee pollen, typically 10–27 % (Asmae et al., 2021). Adequate moisture levels are crucial for maintaining pollen quality and preventing microbial growth during storage (Liolios et al., 2024).

The range in ash and moisture level could also be influenced by several factors beyond botanical origin, including environmental conditions (soil type, climate, altitudes), as well as potential contamination from dust or debris during processing. Additionally, the maturity of the plant material may contribute to these differences. These factors, combined with botanical origin, likely explain the observed variability (Thakur & Nanda, 2020).

Table 1
Physicochemical characterization of pollen samples.

Pollen	Ash (%)	Moisture (%)	pH
Goldenrod	3.30	18.0	4.28
Red Clover	6.17	19.0	4.68
Pussy willow 2021	4.30	16.2	6.45
Pussy willow 2022	4.30	16.2	5.41
Dandelion	8.60	17.0	5.70
Sugar Maple	8.51	14.5	6.05
Wild blackberry	10.2	15.5	5.91
Organic Maize	10.2	9.20	4.75
Non-organic Maize	5.26	24.0	4.44
Common jewelweed ^a	–	–	5.59
Sumac	7.07	14.7	6.30
Hawthorn	3.67	14.8	6.69
Pollen Mix 1	4.31	14.1	4.12
Pollen Mix 2	2.27	14.6	4.37
Knapweed	1.28	15.2	5.43
Buckwheat	2.70	17.8	5.44
Red Maple 2021	6.00	13.8	6.25
Red Maple 2022	7.29	15.0	5.23
Horse Chestnut ^a	–	–	5.93
Poppy ^a	–	–	5.87

^a The ash and moisture content of Common jewelweed, Horse Chestnut and Poppy pollen were not determined due to the low available quantity.

Overall, the physicochemical properties of bee pollen samples provide valuable information about their quality, stability, and potential applications. Ash content reflects mineral composition, moisture level indicates storage stability, and pH influences the bioavailability of nutrients and overall product stability. Understanding these environmental stressors is crucial for evaluating the nutritional and functional properties of pollen under changing ecological conditions.

3.2. Macronutrient composition

This section presents the macronutrient composition of bee pollen, analyzed in terms of total carbohydrates, total proteins and total lipids.

3.2.1. Total carbohydrates

Total carbohydrates in the analyzed bee pollen samples ranged from 39.1 to 57.0 g 100 g⁻¹. Sumac pollen had the highest carbohydrate content (57.0 g 100 g⁻¹) and Buckwheat pollen had the lowest (39.1 g 100 g⁻¹). This range aligns with previously reported values (13 to 80 g 100 g⁻¹) (Thakur & Nanda, 2020; Alshallash et al., 2023; Campos et al., 2008). Table 2 summarizes the carbohydrate content of the different pollen types analyzed in this study. Interestingly, the samples showed a higher average carbohydrate content (47.0 g 100 g⁻¹) compared to those reported in Mediterranean regions (31.6 g 100 g⁻¹) (Bakour et al., 2022), possibly due to differences in floral sources and environmental conditions. Moreover, the carbohydrate content in monofloral pollen shows significant variation across different countries, highlighting the influence of geographical factors. For instance, maize (*Zea mays*) pollen in this study showed a lower carbohydrate content (45.1 g 100 g⁻¹) compared to maize pollen from China (75.5 g 100 g⁻¹) and Thailand (64.4 g 100 g⁻¹) (Yang et al., 2013; Chantarudee et al., 2012). These disparities underscore the importance of considering the location of the pollen during the characterization. Factors such as climate, soil conditions, variety, method of collection and local flora likely contribute to these differences, suggesting that localized studies are crucial for accurate nutritional assessment.

When compared to other common carbohydrate sources, the carbohydrate level in bee pollen is comparable to whole grains (40 to 80 g 100

Table 2
Macronutrient composition of pollen samples (g 100 g⁻¹)^a.

Pollen	Total carbohydrates	Total proteins	Total Lipids	
			Folch	Loveridge
Goldenrod	43.8	14.3	13.1	35.4
Red Clover	45.8	20.9	4.96	12.7
Willow 2021	46.3	18.6	4.95	4.95
Willow 2022	50.4	19.9	4.95	15.1
Dandelion	51.0	14.5	11.5	23.2
Sugar Maple	51.5	22.3	5.10	26.1
Wild blackberry	44.0	21.8	6.04	20.7
Organic Maize	37.7	16.2	8.23	23.2
Non-organic Maize	45.1	14.3	6.20	20.6
Common jewelweed ^a	47.9	16.5	5.00	23.7
Sumac	57.0	17.1	4.88	21.9
Hawthorn	45.9	19.2	9.89	12.1
Pollen Mix 1	51.9	21.8	10.5	13.1
Pollen Mix 2	44.1	18.9	6.30	21.2
Knapweed	50.5	23.5	15.0	10.0
Buckwheat	39.1	16.8	2.77	16.7
Red Maple 2021	48.6	19.9	3.58	16.6
Red Maple 2022	40.2	23.2	4.73	21.4
Chestnut ^b	–	–	–	–
Poppy ^b	–	–	–	–

^a Results are expressed on a dry basis, except for Common jewelweed pollen for which the results are expressed as fresh mass since the quantity of pollen available was too low to determine the moisture content of this pollen sample.

^b The quantity of Chestnut and Poppy pollens was insufficient to determine their macronutrient content.

g⁻¹) (Seal et al., 2021) and higher than most nuts (10 to 30 g 100 g⁻¹) (Agrawal et al., 2015), positioning it as a potentially valuable dietary carbohydrate source. The relatively high carbohydrate content, particularly in samples like Sumac pollen (57.0 g 100 g⁻¹), clearly suggests bee pollen as an energy-rich food source. However, the nutritional impact depends on the specific types of carbohydrates present, which we did not differentiate in this study. However, previous research highlighted that glucose and fructose are the primary sugars in bee pollen along with various dietary fibres (da Silva et al., 2014).

A limitation of this study is the lack of differentiation between soluble and insoluble fibres, as well as the absence of a detailed sugar profile. Future research should use chromatography techniques for comprehensive sugar and fibre characterization, providing a holistic understanding of nutritional value. Standardizing glycemic index measurements across bee pollen types could clarify their relevance for both human diets and bee nutrition.

3.2.2. Proteins

The protein content in the analyzed bee pollen samples ranged from 14.3 to 23.5 g 100 g⁻¹. Knapweed (*Centaurea* spp.) pollen showed the highest protein content (23.5 g 100 g⁻¹), and Goldenrod (*Solidago* spp.) pollen the lowest (14.3 g 100 g⁻¹) (Table 2). This range is consistent with previously reported values of 7.0 to 40.0 g 100 g⁻¹ (Alshallash et al., 2023; Taha et al., 2019), indicating that the 18 pollen samples characterized in this study represent typical bee pollen protein content. When examining the protein content in monofloral pollen such as maize pollen, the same variability as found with carbohydrate content was not observed (Section 3.1.1). The pollen protein content measured in maize (16.2 g 100 g⁻¹ in this study) was consistent with those reported globally (17.9, 19.1, and 16.7 g 100 g⁻¹ in China, Thailand and France respectively) (Yang et al., 2013; Chantarudee et al., 2012; Odoux et al., 2012). This consistency suggests that bee protein synthesis in maize pollen may be less influenced by geographical factors than carbohydrates. However, the observed variation among different pollen types (Table 2) highlights the importance of pollen botanical sources in determining nutritional composition, as previously reported (Vaudo et al., 2024).

Pollen is the primary protein and essential amino acid source for bee growth and development (Blüthgen & Klein, 2011; Stephen et al., 2024). In bees, pollen protein levels can significantly impact queen fertility, colony health and productivity, potentially supporting more resilient colonies with access to high-protein pollen sources (Stephen et al., 2024). From a human nutrition perspective, this protein level is comparable to some legumes (20 to 25 g 100 g⁻¹) and higher than most cereals (8 to 15 g 100 g⁻¹) (Seal et al., 2021; Erbersdobler et al., 2018), positioning bee pollen as a potentially valuable protein source, especially for plant-based diets.

The variation in protein content (Table 2) also suggests potential applications in beekeeping practices, such as informing supplementary feeding strategies during periods of pollen scarcity. For human nutrition, high-protein varieties like red or sugar maple pollen could be attractive ingredients for protein-enriched food or dietary supplements, although potential allergenicity must be considered.

This study determined total protein content using spectrophotometry, which offers rapid analysis with minimal use of hazardous chemicals compared to traditional methods like Kjeldahl (AOAC, 1997). While efficient and widely used, these approaches measure only total protein content without providing information on individual amino acids or protein quality. Understanding the amino acid profile is crucial for assessing essential amino acid content and identifying limiting amino acids. For example, tryptophan, an essential amino acid often limited in human diets, plays a crucial role in protein synthesis and overall health (Thakur & Nanda, 2020). Interestingly, studies showed a wide variation in tryptophan content among different pollen types, ranging from 0.70 to 14.8 g 100 g⁻¹ for Sunflower (*Helianthus annuus* L.) and *Camellia japonica* L. pollen respectively (Yang et al., 2013). This significant

variability highlights the potential of bee pollen as a valuable source of essential amino acids in specific pollen types.

Future research should address these limitations through amino acid analysis profiling using liquid chromatography to identify potential limiting amino acids for bee nutrition. Protein characterization via electrophoresis or size exclusion chromatography could separate and characterize different protein fractions, while proteomic analysis using LC-MS/MS would provide more insight into the identity and diversity of the protein present. Additionally, *in vitro* protein digestibility assays could assess the bioavailability of pollen protein for both bees and humans. Such studies would offer deeper insight into their nutritional value. Efforts to standardize protein characterization methods across studies would facilitate comparing pollen types and geographical regions.

3.2.3. Lipids

The total lipid content was quantified using both the Folch and the Loveridge methods (see Section 2.4.3) to evaluate how extraction methodology may influence lipid level and data interpretation. This comparison is particularly relevant for studies aiming to compare lipid content across samples or assess nutritional properties. Results showed considerable variation between samples and between methods within the same samples, as previously reported (Lau et al., 2022). The lipid content ranged from 4.95 to 35.4 g 100 g⁻¹ using the Loveridge method and from 2.77 to 13.1 g 100 g⁻¹ using the Folch method (Table 2). This range is consistent with the level reported in the literature, which is from 0.66 to 25 g 100 g⁻¹ (Alshallash et al., 2023; Mayda et al., 2020; Prdun et al., 2021), indicating that these samples represent typical bee pollen lipid content (Table 2). In our study, the lipid content of maize was measured at 6.20 g 100 g⁻¹ for organic maize and 8.23 g 100 g⁻¹ for conventional maize, using the Folch method, a standard technique for lipid extraction. This lipid level is comparable to that found in maize pollen from Thailand with 7.0 g 100 g⁻¹ (Chantarudee et al., 2012).

Among samples, Goldenrod pollen showed the highest lipid content, with 35.4 g 100 g⁻¹ using the Loveridge method and 13.1 g 100 g⁻¹ using the Folch method. In contrast, Willow 2021 had the lowest at 4.95 g 100 g⁻¹ using Loveridge and Buckwheat pollen had the lowest at 2.77 g 100 g⁻¹ using Folch. Interestingly, the Loveridge method consistently yielded significantly higher values than the Folch method for all tested samples (Table 2).

The observed differences between the two methods can be attributed to several factors. The Folch method involves a solvent evaporation step, during which volatile lipids may be lost, potentially leading to lower total lipid levels. In contrast, the Loveridge method measures the total mass difference before and after extraction and does not rely on solvent evaporation. This method may include a broader range of lipid classes such as triglycerides, and sterols and potentially some non-lipid compounds insoluble in the Folch solvent system, contributing to the higher values observed (Table 2). While both methods provide valuable data, researchers should be aware of such differences when interpreting and comparing lipid content.

The variation in lipid content among different pollen types (Table 2) suggests that floral sources and environmental conditions play a significant role in the lipid composition of bee pollen. For instance, while Willow 2021 remained consistent across methods (4–5 g 100 g⁻¹, Table 2), Willow 2022 showed a significant difference (15 g 100 g⁻¹) using the Loveridge method. This difference highlights the complexity of bee pollen composition through the years and the challenges in accurately quantifying these components.

Compared to other similar or equivalent foods, bee pollen lipid content is relatively moderate. For example, nuts (almonds, walnuts) typically have higher lipid content (50–60 g 100 g⁻¹) and seeds (chia, flaxseed) have around 30 g 100 g⁻¹. In contrast, grains (oats and quinoa) generally have lower lipid content (5–10 g 100 g⁻¹) similar to the lower range observed in bee pollen.

Lipids are essential for bee nutrition, providing crucial energy and

essential fatty acids necessary for development, survival, and overall colony health (Stephen et al., 2024). The protein-to-lipid (P:L) ratio of pollen has also been shown to be critical for wild bee communities (Vaudo et al., 2024). For human nutrition, the lipid content in bee pollen contributes to its caloric value and offers essential fatty acids that are beneficial for health, including omega-3 and omega-6 fatty acids (Stephen et al., 2024). Studies have reported high levels of these essential polyunsaturated fatty acids from 8.07 to 44.1 % for Omega 3 and 1.77 to 38.3 % for omega 6 (Thakur & Nanda, 2020). Understanding the fatty profile of different pollen types can help in tailoring nutritional strategies to meet specific health needs.

While this study focused on total lipids, future research could benefit from a more detailed analysis of the specific lipid fraction extracted by each method. Techniques such as thin-layer chromatography or gas chromatography-mass spectrometry could be used to characterize the lipid profiles more comprehensively. Additionally, Alharbi et al. (2024), present a rapid and efficient method for lipid profiling of vegetable oils to detect olive oil adulteration using NMR spectroscopy (Alharbi et al., 2024). Their results highlight the potential of NMR for applications beyond food authentication. This approach could also be applied to other lipid-rich natural products, such as bee pollen, which is abundant in lipids. By combining the complementary lipid profiles of vegetable oils and bee pollen, advanced oil-based self-nano emulsifying drug delivery systems (SNEDDS) could be developed. These systems hold significant potential to enhance the bioavailability of bioactive compounds in bee pollen, opening new avenues for pharmaceutical and nutraceutical applications.

3.3. Micronutrient composition

This section examines the micronutrient composition of bee pollen, focusing on its vitamin B, C and A content, and conducting an elemental analysis.

3.3.1. Vitamins

The analysis of bee pollen samples revealed a diverse and variable vitamin profile, likely influenced by the botanical origin and environmental conditions during pollen development. Table 3 presents the vitamin content across different pollen types analyzed.

For instance, Pyridoxine (vitamin B6) levels ranged from 0.2 µg g⁻¹ in Red Maple (*Acer rubrum*) 2022 to 11.7 µg g⁻¹ in Red Clover (*Trifolium pratense*) pollen. Similarly, Thiamine (vitamin B1) levels varied significantly from 0.6 µg g⁻¹ in Sumac to 25.1 µg g⁻¹ in Red Maple 2021. Goldenrod pollen showed the highest Nicotinamide (vitamin B3) content at 164 µg g⁻¹, contrasting with Hawthorn pollen at 8.22 µg g⁻¹. Riboflavin (vitamin B2) was most abundant in Maize pollen (8.84 µg g⁻¹) and less abundant in knapweed pollen (0.73 µg g⁻¹), whereas many pollen showed a similar result for this vitamin, ranging from 1.75 to 2.56 µg g⁻¹ as for Red Clover, Willow 2022, Red Maple 2021 and Sumac (Table 3). Niacin (vitamin B3) ranged from 3.23 µg g⁻¹ in Red Maple 2021 to 30.8 µg g⁻¹ in Red Clover. Acid ascorbic (vitamin C) showed significant variability, from 6.42 µg g⁻¹ in Red Clover to an impressive 1052 µg g⁻¹ in Willow 2021 (Table 3).

While some bee pollen samples (e.g., Willow 2021 at 1052 µg g⁻¹) showed high vitamin C content, this is still lower than some berries (e.g., acerola at about >1600 µg g⁻¹).

The studied pollen samples exhibited a high concentration of B-complex vitamins, aligning with results reported in the literature (de Arruda et al., 2013a; de Arruda et al., 2013b). This underscores the rich content of water-soluble vitamins in pollen. The content of B-complex vitamins found in our pollen samples is comparable to previous studies on pollen from Brazil (de Arruda et al., 2013b). For instance, the thiamine (B1) level observed in Willow 2021 and 2022 is similar to what has been recorded in Willow in France by Pollenergie (7 µg g⁻¹ and 10 µg g⁻¹, respectively), and to values between 6.6 and 10 µg g⁻¹ recorded by Arruda et al. 2013 (de Arruda et al., 2013b), while Red Maple in 2021

Table 3
Vitamins content in pollen sample ($\mu\text{g g}^{-1}$)^a.

Pollen	Thiamine (B1)	Riboflavine (B2)	Nicotinamide (B3)	Niacine (B3)	Pyridoxine (B6)	Folic Acid (B9)	Cobalamine (B12)	Retinol (A)	Ascorbic Acid (C)
Goldenrod	14.4	7.14	164	16.9	0.58	1.58	3.63	21.0	9.17
Red Clover	12.1	2.52	105	30.8	11.7	0.46	nd	0.39	6.42
Willow 2021	7.04	0.86	27.1	28.9	2.32	1.00	nd	nd	1052
Willow 2022	7.90	2.12	49.5	22.0	1.23	0.53	nd	nd	155
Dandelion	23.3	0.85	10.7	4.92	1.74	3.15	nd	nd	31.7
Sugar Maple	7.20	1.34	16.7	5.20	2.25	0.66	nd	nd	172
Wild blackberry	9.76	1.93	60.9	8.11	4.93	0.27	nd	nd	61.4
Organic Maize	10.7	8.84	25.3	17.5	2.72	0.42	nd	nd	53.1
Non-organic Maize	8.94	10.5	34.5	18.6	2.05	nd	nd	2.37	31.8
Common jewelweed ^a	4.95	13.3	127	30.1	2.97	0.30	nd	nd	7.44
Sumac	0.69	2.56	16.2	5.72	1.69	0.49	nd	nd	129
Hawthorn	0.87	0.78	8.22	28.7	2.61	0.96	nd	1.39	639
Pollen Mix 1	12.0	9.48	83.7	37.4	1.15	1.60	6.23	15.5	1.36
Pollen Mix 2	9.21	4.28	63.4	18.8	4.03	2.04	nd	nd	4.30
Knapweed	18.7	0.73	12.1	7.34	2.02	nd	nd	nd	21.2
Buckwheat	13.9	5.88	66.1	15.0	2.53	0.74	nd	1.40	11.7
Red Maple 2021	25.1	1.75	39.4	3.23	0.73	0.14	nd	nd	355
Red Maple 2022	22.8	1.42	54.8	6.33	0.23	0.14	nd	nd	31.7
Chestnut ^a	9.17	1.47	47.8	10.2	6.32	0.56	nd	nd	549
Poppy ^a	16.2	1.54	65.8	8.92	4.36	0.48	nd	nd	197

^a Results are expressed as dry mass, except for Common jewelweed, Chestnut and Poppy pollens for which the results are expressed as fresh mass due to the low quantity of pollen available, which did not allow to determine their moisture content.

contains three times this amount ($25.1 \mu\text{g g}^{-1}$ in Red Maple 2021). However, the absence of cobalamin (vitamin B12) in most samples aligns with the understanding that vitamin B12 is primarily found in animal products (Lee et al., 2015). The limited presence of folic acid (vitamin B9) and retinol (vitamin A) indicates a limited role of bee pollen as a reliable source of these vitamins, consistent with earlier studies (Thakur & Nanda, 2020; Bayram, 2021).

Despite these results being within the range observed in the literature, the potential degradation of reactive vitamins like A and C during storage or collection cannot be excluded. While pollen is generally not a reliable source of vitamin B12 (Thakur & Nanda, 2020), two samples presented detectable levels of B12, suggesting that specific pollen types might possess inherent nutritional value (El Ghouizi et al., 2023). However, we cannot exclude that the presence of B12 could result from a transfer from bee saliva or contact with water sources in the environment. In our study, Vitamin B12 was quantified using a targeted LC-MS/MS method optimized for cyanocobalamin, a commonly used and analytically stable form of cobalamin. This approach allowed for reliable and reproducible quantification under our experimental conditions. However, since the analysis focused specifically on cyanocobalamin, the presence of other naturally occurring isoforms such as methylcobalamin, adenosylcobalamin, or hydroxocobalamin was not assessed. Future work using form-specific detection would help clarify the contribution of bioactive B12 forms in bee pollen samples.

The variability observed in vitamin content across different pollen types underscores the importance of pollen source diversity for bee nutrition and potential human applications. Future research could investigate the effects of environmental biotic and abiotic factors affecting vitamin synthesis in pollen, investigating storage conditions and collection methods to enhance vitamin stability and conducting bioavailability studies to determine the actual nutritional impact of these vitamins for both bees and humans.

3.3.2. Elemental composition of bee pollen

The elemental analysis of bee pollen samples showed substantial variability in mineral content across different types (Table 4), reflecting the diverse floral origins and environmental conditions of the collected samples. This section discusses the results related to macrominerals, trace elements and heavy metals in bee pollen.

3.3.2.1. Macrominerals. Calcium (Ca) content varied significantly across samples, ranging from 595 mg kg^{-1} in non-organic maize to 2328 mg kg^{-1} in Red Maple 2021 (Table 4). Potassium (K) levels were consistently high, with values between 654 and 2717 mg kg^{-1} . Willow 2021 showed the highest potassium content (2717 mg kg^{-1}), while Dandelion (*Taraxacum officinale* L.) pollen had the lowest (654 mg kg^{-1}). This highlights bee pollen as a valuable source of potassium. In contrast with the high amount of Ca and K found in the pollen sample, Sodium (Na) levels were significantly low (Table 4) ranging from 13 mg kg^{-1} (Red Maple 2022) to 98 mg kg^{-1} (Dandelion), which could be advantageous for individuals seeking to manage sodium intake. This range is also significantly lower than some previously reported studies. Indeed, Brazilian pollen studies report a sodium level of around 230 mg kg^{-1} , while Turkish pollen reached an average of 5772 mg kg^{-1} (Kalaycıoğlu et al., 2017; Morgano et al., 2012).

The magnesium (Mg) content ranged from 156 to 4397 mg kg^{-1} , demonstrating significant variability among pollen types. As for Ca content, the Red Maple 2021 showed the highest Mg level (4397 mg kg^{-1}) compared to Dandelion (156 mg kg^{-1}). These values are within the range of previous reports, which range from 321 – 3000 mg kg^{-1} (Thakur & Nanda, 2020). Interestingly, the maize pollen in this study (782 mg kg^{-1}) showed a similar result to maize pollen from China (815 mg kg^{-1}) (Yang et al., 2013). Phosphor (P) levels ranged from 1621 to 8479 mg kg^{-1} , with Willow 2021 having the highest and Dandelion the lowest amount (Table 4). The P amount found in this study is similar to those found in Australian and Chinese pollen (Yang et al., 2013; Somerville & Nicol, 2002).

3.3.2.2. Trace elements. Iron (Fe) (27.5 to 153 mg kg^{-1}) and Zinc (Zn) (25.5 to 109 mg kg^{-1}) were present in significant amounts (Table 4). A significant difference in Fe and Zn content was observed between pollen collected in 2021 and 2022 from Willow and Red Maple. Specifically, the 2021 samples showed lower levels of these trace elements compared to 2022, suggesting that the timing of pollen collection can significantly affect trace elemental composition. Other trace elements, such as Manganese (3.38 to 496 mg kg^{-1}), Copper (2.64 to 23.0 mg kg^{-1}) and Nickel (0.19 to 3.21 mg kg^{-1}) were also detected in significant amounts. Globally, the trace elements level found in pollen samples aligns with previous reports (e.g., 11 – 317 , 28 – 76 , 0.53 – 5.41 mg kg^{-1} for Fe, Zn and Ni, respectively) (Yang et al., 2013). The variability in micronutrient

Table 4
Macroelements, trace elements and heavy metals content of pollen samples (mg kg⁻¹)^a.

Pollen	Macroelements										Trace elements										Heavy metals									
	Ca	Na	P	K	Mg	Fe	Mn	Zn	Mo	Ba	B	Ti	Al	Cu	Ni	As	Cd	Co	Cr	Pb	Sb	Se	V	Ag						
Goldenrod	1622	60.5	2376	1147	348	32.0	8.80	41.0	nd	nd	49.2	nd	12.2	17.7	1.56	3.19	1.13	1.07	0.24	1.17	2.92	1.68	nd							
Red Clover	1521	26.8	6372	2635	888	109	29.2	58.5	0.51	nd	13.0	nd	1.17	11.8	1.87	0.93	0.92	1.13	nd	1.25	2.21	4.17	nd							
Willow 2021	2202	39.0	8479	2717	1189	68.3	32.1	84.1	nd	1.34	15.2	nd	22.4	6.03	2.89	0.34	1.99	1.24	nd	1.22	2.12	5.72	nd							
Willow 2022	2022	26.8	7839	2502	1195	153	26.7	109	nd	1.43	13.4	2.02	96.0	5.73	0.38	0.91	1.77	1.13	nd	nd	1.94	6.02	nd							
Dandelion	1847	98.0	1621	654	156	25.5	3.88	32.8	nd	nd	99.4	nd	6.66	5.01	0.60	1.42	0.99	0.82	nd	0.33	1.80	0.80	nd							
Sugar Maple	1056	31.0	6109	2047	1101	62.8	51.7	58.1	nd	nd	23.0	nd	7.89	15.2	0.87	0.49	1.09	0.90	nd	nd	2.21	5.60	nd							
Wild blackberry	1777	38.6	5160	1942	1036	104	496	59.4	nd	7.79	18.7	nd	13.9	7.72	3.21	1.11	1.22	1.29	0.21	1.91	1.66	5.13	nd							
Organic Maize	857	25.3	4103	1772	782	33.8	16.1	107	nd	nd	22.3	nd	8.86	4.80	0.84	0.59	1.08	1.16	nd	1.20	1.66	3.76	nd							
Non-organic Maize	595	30.4	4359	1758	906	27.5	16.2	83.1	nd	nd	12.6	nd	1.40	2.64	0.19	0.84	1.00	1.04	0.25	1.08	1.93	4.33	nd							
Common jewelweed ^a	1266	37.6	5200	1843	849	56.7	13.3	43.2	0.44	nd	10.3	nd	nd	11.2	0.10	1.71	1.32	1.50	nd	1.54	2.28	4.19	nd							
Sumac	1591	25.0	6952	2386	2393	74.3	23.0	43.0	nd	0.18	10.4	nd	4.16	13.1	0.25	0.48	0.75	0.87	nd	0.70	1.55	9.81	nd							
Hawthorn	1367	48.8	5252	2439	1020	61.6	28.7	43.6	nd	5.03	35.7	nd	19.4	7.53	2.38	0.79	1.30	0.98	nd	0.99	1.81	4.86	nd							
Pollen Mix 1	1975	38.0	2974	1129	475	40.8	8.54	42.2	nd	nd	53.6	nd	15.9	10.2	0.46	1.20	0.98	0.94	0.19	1.06	2.03	2.35	nd							
Pollen Mix 2	1653	42.7	5363	2013	1076	85.3	49.7	46.9	0.19	1.13	29.0	nd	17.5	8.40	1.90	0.91	0.92	1.10	nd	1.25	2.21	4.94	nd							
Knapweed	2031	38.0	3620	1112	504	30.8	8.75	51.5	nd	nd	70.3	nd	3.74	23.0	1.29	0.93	1.03	0.97	nd	0.75	2.28	2.35	nd							
Buckwheat	1846	59.0	6580	2694	843	63.1	34.0	54.9	nd	2.18	71.3	nd	9.76	4.89	0.29	1.27	0.98	1.66	nd	0.95	2.13	4.20	nd							
Red Maple 2021	2328	36.5	7654	1981	4397	31.2	3.38	25.5	nd	2.01	32.9	nd	11.1	nd	nd	3.86	2.26	0.74	nd	1.54	1.32	3.54	nd							
Red Maple 2022	1716	13.0	6149	2247	720	115	222	56.8	nd	2.11	22.9	0.69	49.1	17.5	0.55	0.93	1.02	1.21	0.47	0.62	2.13	3.73	nd							
Chestnut ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
Poppy ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							

^a Results are expressed as dry mass, except for the Common jewelweed for which the results are expressed as fresh mass since the quantity of pollen available was too low to determine the moisture content.

^b The amount of available Chestnut and Poppy pollen was insufficient to perform the elemental analysis. nd: not detected.

levels has significant implications for human dietary supplementation, as it indicates that the nutritional composition of the source material may not always be consistent. This inconsistency could affect the efficacy and reliability of supplementation, underscoring the importance of rigorous quality control and standardization to ensure consistent nutrient content. Without addressing this variability, there is a risk of under- or over-supplementation, potentially impacting health outcomes.

3.3.2.3. Heavy metals. The presence of potentially toxic elements such as Arsenic (0.34 to 3.86 mg kg⁻¹), Cadmium (0.75 to 2.26 mg kg⁻¹), and Lead (up to 0.47 mg kg⁻¹) were detected across all samples (Table 4). Other heavy metals such as Chromium, Antimony, and Vanadium were also detected in trace amounts (Table 4). While detected levels were generally low, their presence highlights the importance of environmental contaminant surveillance in bee pollen production to ensure safety for both bees and human consumers. It is worth mentioning that Silver (Ag) and Cobalt (Co) were not detected in all samples (Table 4).

The variability in mineral content across different pollen types is influenced by factors such as soil composition, floral types, environmental conditions, and possibly beekeeping practices. The high potassium and magnesium levels highlight the potential of bee pollen as a valuable dietary supporting metabolic and cardiovascular health (Dinicolantonio et al., 2018). The low sodium content is advantageous for individuals managing sodium intake. Although the levels of heavy metals detected in this study are generally low, they remain below those reported in other studies, where As levels range from 2.19 to 14.7 mg kg⁻¹ and Pb levels range from 0.05 to 1.35 mg kg⁻¹ (Yang et al., 2013), ongoing monitoring is essential to ensure safety for both bees and human consumers. While the concentrations obtained in this study are significantly lower than those reported in other studies, no specific MRLs are available for bee pollen. However, the range of heavy metals levels detected in this study in Pb (0.19–0.47 mg kg⁻¹), Cd (0.75–2.26 mg kg⁻¹), and As (0.34–3.86 mg kg⁻¹) exceeds the general permissible limits established for food products (e.g., Pb: 0.010–0.50 mg kg⁻¹ and is extended to 1 mg kg⁻¹ in canned food), Cd (0.003–0.50 mg kg⁻¹), and As (0.01–0.50 mg kg⁻¹) (FAO, 1995; WHO, 2011). These results suggest potential health risks, particularly with prolonged consumption, and highlight the need for monitoring heavy metal levels in bee pollen to ensure consumer safety. Even if the detected concentrations were relatively low, they could be a concern for children or pregnant women. However, it is noteworthy to mention that pollen consumption rarely exceeds 15 g day⁻¹, which significantly reduces exposure and associated risks. Therefore, recommendations should consider this quantity when assessing risks and benefits. For instance, while pollen is generally considered safe, guidelines could suggest not exceeding 10–15 g day⁻¹.

These results emphasize the need for future research into the environmental and botanical factors influencing mineral uptake in pollen. Understanding these factors could lead to strategies for enhancing beneficial mineral content while mitigating heavy metal contamination. Future studies should also explore the bioavailability of these minerals in bees and humans to better understand their impacts.

3.4. Bioactive compounds

This section explores the bioactive compounds present in bee pollen, focusing on their total antioxidant properties, including flavonoids and phenolic compounds.

Total antioxidants: The total antioxidant capacity measured as Trolox equivalents (mgTR g⁻¹), varied significantly among samples. Buckwheat showed the lowest activity at 1.25 mgTR g⁻¹, while Red Maple 2022 reached 18.6 mgTR g⁻¹ (Table 5). Interestingly, Willow 2021 and 2022 presented similar antioxidant levels (12.8 and 13.8 mgTR g⁻¹ respectively) suggesting a significant influence of botanical origin on antioxidant content.

Flavonoids: Flavonoid content expressed as Quercetin equivalent

Table 5
Bioactive compounds content of pollen samples (mg g⁻¹)^a.

Pollen	Total antioxidant mgTR/g	Total flavonoids mgQE/g	Total phenols mgGA/g
Goldenrod	5.15	8.57	5.41
Red Clover	11.1	8.88	4.10
Willow 2021	12.8	26.7	11.3
Willow 2022	13.8	21.0	8.77
Dandelion	5.42	26.4	6.77
Sugar Maple	16.6	16.7	8.89
Wild blackberry	7.52	7.88	5.99
Organic Maize	2.71	3.77	1.74
Non-organic Maize	3.08	2.17	0.75
Common jewelweed ^d	12.9	3.49	3.40
Sumac	10.7	14.8	5.00
Hawthorn	4.72	3.29	5.29
Pollen Mix 1	1.93	7.02	2.75
Pollen Mix 2	9.19	12.2	4.68
Knapweed	2.64	2.57	1.26
Buckwheat	1.25	2.56	0.54
Red Maple 2021	13.7	23.5	5.13
Red Maple 2022	18.6	19.9	5.22
Chestnut ^b	–	–	–
Poppy ^b	–	–	–

^a Results are expressed as dry mass, except for Common jewelweed pollen for which the results are expressed as fresh mass since the quantity of pollen available was too low to determine the moisture content.

^b The amount of available Chestnut and Poppy pollen was insufficient to assess the bioactive compounds content.

(mgQE g⁻¹) also varied considerably, from 2.17 in non-organic Maize to 26.7 mgQE g⁻¹ in Willow 2021. Both Willow and Red Maple maintained consistent flavonoid levels around 20 mgQE g⁻¹ (Table 5) across 2021 and 2022, indicating stability within these botanical sources.

Phenolic compounds: Total phenolic content measured in Gallic Acid equivalents (mgGA g⁻¹), ranged from 0.54 in Buckwheat to 11.3 mgGA g⁻¹ in Willow 2021 (Table 5). Willow 2021 pollen showed the highest phenolic content (11.3 mgGA g⁻¹), with minimal variation between years for both Willow (11.3 and 8.77 mgGA g⁻¹ in 2021 and 2022, respectively). The same trend was observed for Red Maple across 2021 and 2022, with a consistent total phenolic content of 5.13 mgGA g⁻¹ and 5.22 mgGA g⁻¹ respectively (Table 5). This suggests a strong potential influence of botanical origin on phenolic composition.

Overall, the obtained results align with those reported in the literature. Globally, total phenolic content ranged from 0.035–135 mgGA g⁻¹ (Harif Fadzilah et al., 2017; Ilie et al., 2022), total flavonoids content ranged from 0.02–31.8 mgQE g⁻¹ (Harif Fadzilah et al., 2017) and total antioxidant capacity ranged from 0.12–15.1 mgTR g⁻¹ (Ilie et al., 2022). A recent study reports high total phenol and total flavonoids content in sunflower pollen, reaching up to 3816 mgGA kg⁻¹ and 865 mgQE kg⁻¹ respectively (Prinsen et al., 2016). The significant variability in bioactive compounds across different bee pollen types highlights the influence of botanical diversity. The high levels of antioxidants, flavonoids and phenolic compounds in specific pollen types support their use as functional food ingredients. The antioxidant properties, comparable to those of certain berries (e.g., blueberries, which contain 9–24 mgTR g⁻¹), can neutralize free radicals and reduce oxidative stress (Chantarudee et al., 2012). It is worth mentioning that total antioxidant properties may include flavonoids, vitamins (such as C and E mainly) and other phenolic/polyphenolic compounds.

Regarding flavonoid content, bee pollen with concentrations up to 26.7 mgQE g⁻¹ surpasses many common nuts like almonds and walnuts, typically below 10 mgQE g⁻¹ (Milbury et al., 2006). Phenolic compounds reaching up to 11.3 mgGA g⁻¹ are less than those found in green tea, a beverage known for its phenolic content (Khokhar & Magnusdottir, 2002).

The presence of flavonoids and phenolic compounds extends beyond their antioxidant properties, offering, anti-inflammatory or antiviral effects (Didaras et al., 2020; Pascoal et al., 2014; Ilie et al., 2022). While

total antioxidant capacity provides a broad overview of oxidative stress protection, measuring antioxidant capacity, flavonoids and phenols separately allows for a nuanced understanding of health benefits and potential applications. The synergistic effects of different compounds could enhance health benefits. The variability in bioactive compound levels has important implications for human dietary supplementation. This variability indicates that the nutritional composition of the source material may fluctuate, potentially affecting the efficacy and reliability of supplementation. Our results underscore the importance of implementing rigorous quality control measures and standardization protocols to ensure consistent micronutrient levels in dietary supplements.

This study focused on total content, without profiling specific antioxidant properties. Future research should investigate detailed profiles and consider potential degradation during storage and processing. Exploring stability and optimizing storage conditions could enhance the understanding and application of bioactive compounds of bee pollen. Including phenolic profiles such as flavonoids, phenolic acids, and polyphenols like tannins and lignans could provide better insight into pollen properties. Recent advancements in food metabolomics have significantly enhanced the understanding of food composition, quality, and safety. Emwas et al. (2021) provide a comprehensive review of metabolomics approaches, particularly emphasizing the role of NMR spectroscopy and mass spectrometry in profiling food components and detecting contaminants (Emwas et al., 2021). The integration of metabolomics into future research could address challenges in compound identification, bioavailability, synergy and standardization, ultimately contributing to the development of a centralized database for accurate nutritional and safety assessments. Additionally, recent advances in NMR spectroscopy, such as lipid signal suppression, enable better detection of low-abundant metabolites by reducing interference from dominant signals (Singh et al., 2024). Given the high lipid content in some bee pollen samples, this method could enhance future analysis of minor bioactive compounds, improving sensitivity for a more comprehensive nutritional and therapeutic profiling.

3.5. Contaminants

The analysis of organic contaminants in bee pollen samples revealed varying levels of several pesticides, including atrazine, diazinon, glyphosate, metolachlor, thiamethoxam, and trifloxystrobin. The presence of these contaminants was not uniform across all samples, indicating potential variability in environmental exposure and agricultural practices. It is worth mentioning that except for commercial pollen mix, pollen samples were collected in non-agricultural areas or organic cropping areas.

Atrazine was detected in several samples, with concentrations ranging from 0.15 ng g⁻¹ in Hawthorn to 0.63 ng g⁻¹ in Wild blackberry (*Rubus spp.*) (Table 6). The presence of this herbicide, even at a relatively low-level raises concerns due to its persistence in the environment and potential endocrine-disrupting effects (Huang et al., 2015; Fellows et al., 2022). Metolachlor was found in Sumac at 0.77 ng g⁻¹ and Hawthorn at 0.19 ng g⁻¹. As a commonly used herbicide, its presence suggests exposure to agricultural spray drift (Graham et al., 2021). Glyphosate residues were detected in bee pollen samples, with levels of 7.13 ng g⁻¹ for wild blackberry (*Rubus spp.*), 6.25 ng g⁻¹ for Sumac and 32.1 ng g⁻¹ for commercial pollen mix 2 (Table 6). This suggests that bees are collecting pollen from areas where glyphosate is applied. Glyphosate exposure can negatively affect the health of bees, potentially impacting their navigation, foraging behaviour, and immune system (Weidenmüller et al.). Diazinon was only detected in the Willow 2021 sample at a concentration of 1.76 ng g⁻¹ (Table 6). This insecticide is known for its acute toxicity to bees with a LD₅₀ for bees at 0.22 µg/bee (U. EPA Office of Pesticide Programs). This highlights the risk of contamination from nearby agricultural activities. Trifloxystrobin was identified in Red Clover at 0.22 ng g⁻¹. This fungicide, while less toxic for bees, still presents environmental concerns due to its persistence and

Table 6
Pesticide's concentration in pollen samples (ng g⁻¹).

Pollen	Atrazine	Diazinon	Glyphosate	Metolachlor	Thiamethoxam	Trifloxystrobin
Goldenrod	nd	nd	nd	nd	1.45	nd
Red Clover	nd	nd	nd	nd	nd	0.22
Willow 2021	nd	1.76	nd	nd	nd	nd
Willow 2022	nd	nd	nd	nd	nd	nd
Dandelion	nd	nd	nd	nd	nd	nd
Sugar Maple	nd	nd	nd	nd	nd	nd
Wild blackberry	0.63	nd	7.13	nd	nd	nd
Organic Maize	nd	nd	nd	nd	nd	nd
Non-organic Maize	nd	nd	nd	nd	nd	nd
Common jewelweed	0.55	nd	nd	nd	nd	nd
Sumac	0.25	nd	6.25	0.77	nd	nd
Hawthorn	0.15	nd	nd	0.19	nd	nd
Pollen Mix 1	nd	nd	nd	nd	nd	nd
Pollen Mix 2	nd	nd	32.1	nd	nd	nd
Knapweed	nd	nd	nd	nd	nd	nd
Buckwheat	0.29	nd	7.60	nd	nd	nd
Red Maple 2021	nd	nd	nd	nd	nd	nd
Red Maple 2022	nd	nd	nd	nd	nd	nd
Chestnut*	–	–	–	–	–	–
Poppy*	–	–	–	–	–	–

* The quantity of available Chestnut and Poppy pollen was insufficient to perform pesticides analysis. nd: not detected.

potential to affect ecosystems (Schaad et al., 2023). Thiamethoxam was detected in Goldenrod only, at 1.45 ng g⁻¹ (Table 6). As a neonicotinoid, it poses a significant risk to pollinator health, especially wild bees (Shahmohamadloo et al., 2024), contributing to declining bee populations. The results obtained from the pollen samples showed less contamination than to those reported in the literature. For example, trifloxystrobin levels in bee pollen from Europe were significantly higher than those of North America, and both were higher than those found in this study (e.g., 710 ng g⁻¹ in Europe; 22.4 ng g⁻¹ in North America; 0.22 ng g⁻¹ in this study) (Friedle et al., 2021; Mullin et al., 2010).

The presence of organic contaminants in bee pollen samples underscores the impact of agricultural practices on pollinator health and ecosystem integrity. The variability in contaminant levels across different pollen types suggests different exposures based on geographic position, local agricultural practices, and the proximity of pollen collection sites to the treated area. The detection of glyphosate and atrazine is associated with significant environmental and health concerns and underscores the need to monitor pesticide contamination. Moreover, the presence of neonicotinoids like thiamethoxam in pollen samples is particularly concerning given their role in pollinator decline. It is important to mention that this study presents data that are not systemic (recorded only throughout southern Quebec) but samples coming from sustainable beekeeping practices. As the hives are not placed in an intensive agricultural environment, these results do not represent the entire area. Instead, they reflect the low contamination of these samples due to the sampling type.

Additionally, while there may not be universally established Maximal Residue Limits (MRLs), especially for diazinon in bee pollen, the regulation of pesticide residues in bee products is an evolving field. Currently, there are more comprehensive guidelines for honey than other bee products like pollen. However, a previous health risk assessment of pesticide-contaminated pollen conducted by Li et al. (2023) concluded that, in most cases, the risk to both bees and humans was negligible, even when >30 active substances were detected in pollen samples, with concentrations ranging from non-detectable levels to 785 ng g⁻¹.

Overall, this study highlights the need for more comprehensive and systematic sampling across diverse agricultural environments to gain a more representative understanding of pesticide contamination in bee pollen. It also underscores the importance of developing specific MRLs for a wider range of bee pollen products for better protection of bee populations and human consumers.

Further studies should focus on the temporal and spatial distribution

of these contaminants to better understand their sources and pathways. Additionally, research into the synergistic effects of multiple contaminants on bee health could provide deeper insight into risk for pollinators.

3.6. Cytotoxicity

The cytotoxicity assays conducted on various bee pollen sample extracts revealed differential effects on cervical cancer (HeLa) cell viability at concentrations of 20 µg mL⁻¹ and 200 µg mL⁻¹. The results indicate a spectrum of cytotoxic activities, with certain pollen types demonstrating significant inhibition of cancer cell growth.

At low concentrations (20 µg mL⁻¹), most pollen samples exhibited negligible effects on HeLa cell viability, suggesting minimal potential at lower doses ($p \geq 0.05$; Fig. 1). At higher concentrations (200 µg mL⁻¹), several pollen samples showed significant cytotoxic effects for cancer cells ($p \leq 0.01$; Fig. 1). The batch pollen Mix 1 and knapweed demonstrated significant inhibition of cancer cell growth, with 40.5 and 36.5 % inhibition percentages, respectively (Fig. 1 and Table 7). These pollen extracts were followed by Goldenrod and organic and non-organic Maize, with 22.7 and 24.5 % inhibition rates, respectively (Fig. 1 and Table 7). Pollen extract from Red maple 2021 and Red clover showed a similar inhibition rate of 21.7 % (Fig. 1 and Table 7).

The IC50 value (Table 7), indicating the concentration required to inhibit 50 % of cell viability, was calculated for the most potent samples. The pollen Mix 1 (composed mainly of *Asteraceae* pollen) exhibited an IC50 of 247 µg mL⁻¹, and knapweed had an IC50 of 274 µg mL⁻¹, highlighting their potential as sources of bioactive compounds with potential anticancer properties. The anticancer potential of bee pollen was demonstrated by Hanafy et al. (2023) where Clover bee pollen extract from Egypt showed cytotoxicity against lung cancer cells, with an IC50 of 17.6 µg mL⁻¹ after 48 h exposure (Hanafy et al., 2023).

The significant cytotoxicity activity observed in certain pollen may be attributed to specific flavonoids, phenolic acids, and other phytochemicals known for their anticancer properties (Premratanachai & Chanchao, 2014). These compounds may act synergistically to induce apoptosis or cell proliferation inhibition. These results suggest that bee pollen, particularly from specific botanical sources could be explored as a natural adjunct in cancer therapy. Identifying and isolating active compounds responsible for the observed cytotoxic effects on cancer cells could lead to developing novel anticancer agents.

While the in vitro cytotoxicity assays provide valuable insights, further studies are required to elucidate the mechanisms underlying the anticancer properties of bee pollen. Additionally, in vivo studies and

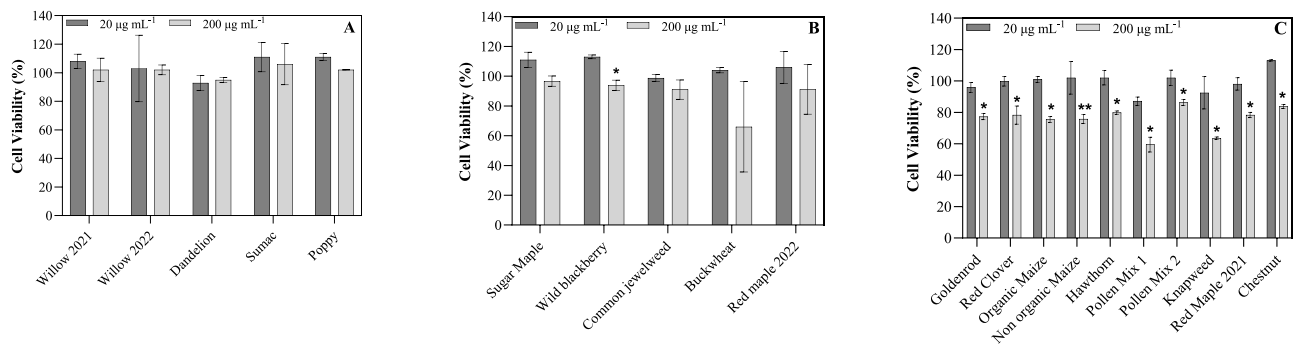


Fig. 1. Histograms for the cell viability results on HeLa cancer cells after 48 h incubation with the dark grey square for the concentration of 20 $\mu\text{g mL}^{-1}$ and light grey square for the concentration of 200 $\mu\text{g mL}^{-1}$ of bee pollen extract. A: bee pollen extract with no effect on HeLa cells cancer. B: bee pollen extract with a medium effect on HeLa cells cancer. C: bee pollen extract with visible effect on HeLa cells cancer (Table 6). *, $p < 0.001$, **, $p < 0.01$.

Table 7
HeLa cells growth inhibition percentage and IC50 for pollen samples exceeding a cell growth inhibition of 16 %.

Pollen	Cells growth inhibition (%)	IC50 ($\mu\text{g mL}^{-1}$)
Goldenrod	22.7	441
Red Clover	21.7	460
Organic Maize	24.5	407
Non-organic Maize	24.2	414
Hawthorn	20.2	496
Pollen Mix 1	40.5	247
Pollen Mix 2	13.7	731
Knapweed	36.5	274
Red Maple 2021	21.7	462
Poppy	16.2	616

clinical trials could assess the efficacy and safety of bee pollen or pollen-derived compounds in cancer treatment. Future research into specific compounds and mechanisms involved could provide innovative approaches to cancer therapy.

3.7. Microscopic analysis

The microscopic analysis of bee pollen samples using both optical and electronic microscopy techniques provided detailed insights into the morphological characteristics and surface structures of the pollen grain while confirming the monofloral characteristic of most pollen samples, except for the two commercials mix of wildflower pollen (Fig. 2 and Supplementary information Figure S1).

Optical microscopy showed the diverse morphological features of pollen grains from different botanical origins (Sup Info Figure S1), with

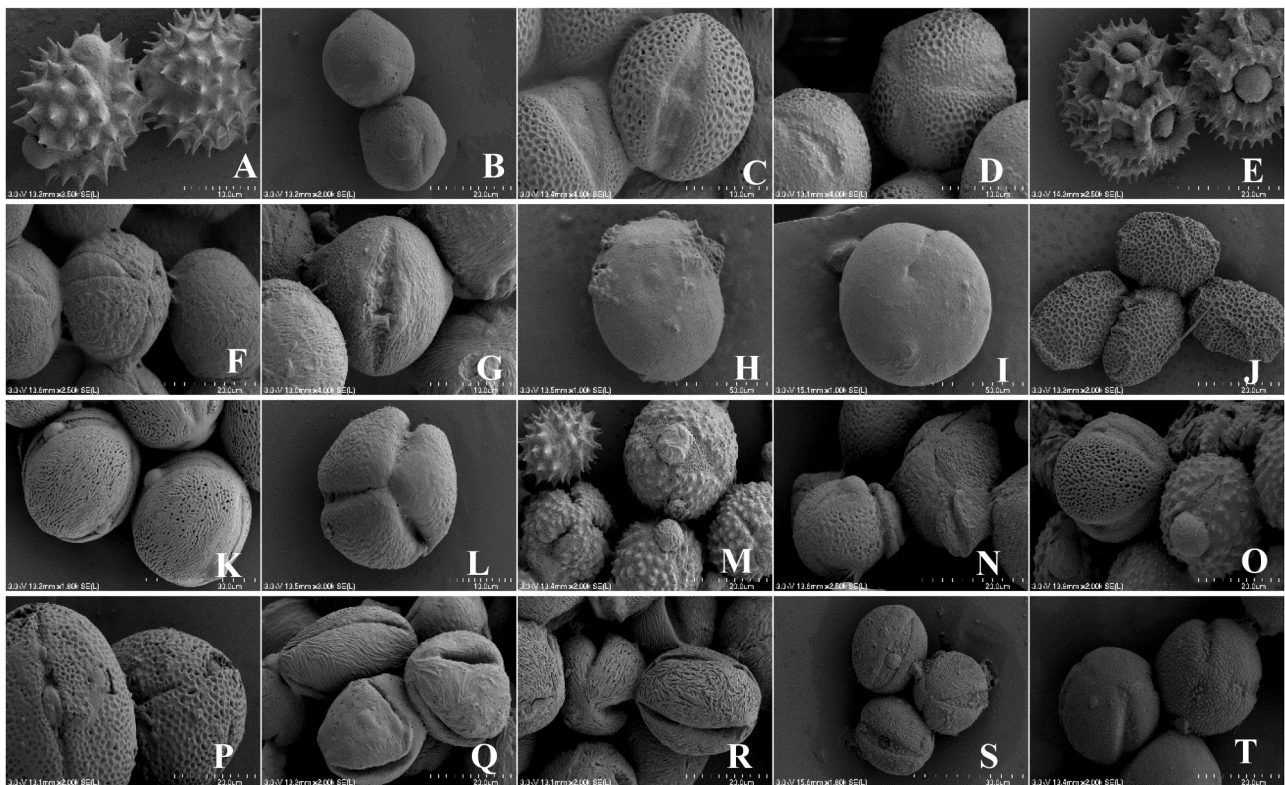


Fig. 2. Bee pollen morphology and surface texture (examined using Scanning Electron Microscope, SEM) from diverse botanical origin. [A] Goldenrod, [B] Red Clover, [C] Willow 2021, [D] Willow 2022, [E] Dandelion, [F] Sugar Maple, [G] Wild blackberry, [H] Organic Maize, [I] Non-organic Maize, [J] Common jewelweed, [K] Sumac, [L] Hawthorn, [M] Pollen Mix 1, [N] Pollen Mix 2, [O] Knapweed, [P] Buckwheat, [Q] Red Maple 2021, [R] Red Maple 2022, [S] Horse Chestnut, [T] Poppy. Other pictures (10 pictures/pollen) are available in a repository (see data availability statement in the manuscript).

Asteraceae pollen characterized by their well-known spiny pollen grains, compared to the spherical and smooth pollen grains of Clover and Maize (Vega & Dematteis, 2011; Tellería et al., 2023). Studying the morphology of bee pollen for bee health and conservation, as the spiny pollen of some *Asteraceae* like sunflower is known to express substantial benefits for bumble bees in fighting gut pathogens and for honey bees against Varroa (Figuroa et al., 2023; Palmer-Young et al., 2023; Fowler et al., 2022).

The morphology and surface texture using Scanning Electron Microscopy (SEM) provided high-resolution images of the pollen surfaces, revealing intricate details not visible under optical microscopy (Fig. 2). The SEM analysis highlighted the presence of microstructures such as pores and furrows, which are critical for pollen identification and classification. For instance, SEM images of Dandelion pollen revealed a dense exine that was constituted by many cavities and covered with long pointed spines, possibly enhancing its adhesion properties (Tellería, 2017) (Fig. 2). In contrast, maize has a thicker exine that is fully covered with tectum. Whereas pollen such as hawthorn and sugar maples exhibit a tetrahedral shape and a reticulate surface (Fig. 2).

These microscopic analyses of bee pollen samples showed the morphological diversity and complexity inherent in pollen grains from different botanical sources (Tellería et al., 2023). These results have significant implications for understanding pollen-pollinator interactions and the ecological roles of various pollen types (Figuroa et al., 2023), especially when combined with pollen nutritional profile (Vaudo et al., 2024). The ability to accurately identify and classify pollen grains has many applications such as in forensic science and environmental monitoring (Serrudo González et al., 2024).

4. Conclusion

This study provides a comprehensive and multidimensional analysis of bee pollen nutrient composition, highlighting its potential as a functional food ingredient. Bee pollen is identified as a rich source of carbohydrates, proteins, and lipids, with nutrient levels comparable to whole grains and legumes, thus offering significant applications in both human nutrition and bee health. B-complex vitamins and antioxidants further underscore the value of adding pollen to plant-based diets or its use as a functional food. However, detecting organic and inorganic contaminants, including pesticides and heavy metals, emphasizes the critical need for environmental monitoring to ensure safety for both bees and human consumers. Contaminant levels were particularly low in pollen characterized in this study. However, these samples were collected in non-agricultural areas or organic cropping (except for non-organic corn) and may not represent Southern Québec. Nonetheless, the collection sites were within distance of contamination from nearby agricultural fields, which could explain the potential pesticide drift exposure of some samples, despite not being collected directly in agricultural areas. This further warrants the need to monitor bee pollen before its commercialization as human food, while supporting the need for larger monitoring (including in more intensive agricultural areas). Despite these concerns, the potential health benefits, such as anticancer properties against cervical cancer cell lines, warrant further exploration.

Regarding pollinator conservation, the results of our study could inform management practices aimed at restoring pollinator habitats and ensuring diversified nutritional landscapes to support reproduction, survival and health (Lau et al., 2023). Pesticide exposure and malnutrition are recognized as two major drivers of pollinator decline (Parreño et al., 2022), and current conservation efforts focus on reducing pesticide exposure while promoting diversified food resources in agricultural landscapes. However, limited knowledge of pollen nutrient content continues to hinder such efforts. By providing a multidimensional assessment of pollen from both crops and native plants, we aim to provide valuable data to support the establishment of nutritious, pollinator-friendly habitats with minimized risks of contaminant exposure.

Future research should focus on the detailed profiling of bioactive compounds and their bioavailability to better understand their specific health impacts. In particular, exploring amino acid and lipid subclasses may provide insight into protein quality and lipid-related bioactivity, respectively. Advanced techniques can also improve our understanding of the glycemic impact and protein digestibility. Additionally, characterizing flavonoids and phenolic compounds could identify specific molecules responsible for health benefits and offer new avenues for therapeutic applications. To maximize the potential of bee pollen, future studies should integrate total content analysis with detailed molecular profiling. While this study focused on total lipids and total carbohydrates, a complete profile could provide deeper insights into the overall composition and potential health benefits of bee pollen lipids and carbohydrates. This dual approach will refine our understanding of the health impacts of bee pollens and their application, enhancing their therapeutic potential. Finally, standardizing pollen characterization methods and establishing a centralized database for bee pollen analysis will facilitate the identification of trends and anomalies, ensuring comprehensive nutritional and safety assessments.

Funding

This research was supported by a Liber Ero fellowship and a K2A research fund from the Quebec Center for Biodiversity Science (QCBS), awarded to Mathilde Tissier in 2021 and 2023, respectively.

Ethical statement

This research project does not involve any studies with human participant or animal as test subjects. Therefore, formal ethics approval from my institutional ethics committee was not required.

CRediT authorship contribution statement

Lounès Haroune: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Sabrina Saibi:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Isabelle Rabbat:** Writing – review & editing, Validation, Resources. **Yann Loranger:** Writing – review & editing, Validation, Resources. **Mathilde L. Tissier:** Writing – review & editing, Validation, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Sarah MacKell and Parker Smale from WPC for providing the commercial mix pollen samples.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2025.101223](https://doi.org/10.1016/j.afres.2025.101223).

Data availability

All data on pollen nutrients, pollen contaminants and pollen images (SEM) will be publicly available on the Canadian Federated Research Data Repository (FRDR-DFDR). A dynamic database, updated over time with new pollen characterization, will be submitted for publication, as per FAIR principles.

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