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RESEARCH PAPER

Chemical and Mineral Composition of the Mono-floral Pollen of Honeybees (*Apis mellifera*) in Ethiopia

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Abstract

This investigation aimed to investigate the chemical and mineral composition of pollen collected from Oromia, Ethiopia. The moisture level of analyzed pollen ranged from 10.3 ± 1.3% (*Sesame indicum*) to 17.3 ± 0.5% (*Eucalyptus globules*), the ash concentration ranged from 1.7 ± 0.3% (*G. scabra*) to 3.0 ± 0.5% (*Brassica carinata*), the protein content ranged from 16.3 ± 0.5% (*G. scabra*) to 24.9 ± 5.6% (*Eucalyptus globules*), the total dietary fiber ranged from 1.4 ± 0.7% (*E.* globules) to 2.6 ± 0.85% (*B. carinata*) and crude carbohydrate ranged from 54.1 ± 5.2 % (*E. globules*) to 69.1 ± 1.0% (*G. scabra*). Potassium and magnesium were the most prevalent minerals in bee-collected pollen samples. *B. carinata* pollen has the greatest calcium (Ca) (2321.3 ± 608.78 mg/kg) and magnesium (Mg) (1024.8 ± 19.9 mg/kg) concentrations compared to others. *E. globules* pollen had the greatest levels of potassium (K) (10596.9 ± 1610.1 mg/kg) and sodium (Na) (380.9 ± 95.9 mg/kg). Lead, a toxic element, was not detected in pollen samples from the study's site. Because of its botanical and geographic origins, bee pollen has a diverse nutritional composition. Results indicated that pollen is a useful food supplement for human nutrition due to its greater concentrations of essential components.

Introduction

Bee pollen is one of the products of bees and is frequently referred to as the most complete food. Bee pollen contains at least 200 biologically active compounds that may have therapeutic benefits (Kurek-Górecka et al., 2020; Thakur and Nanda, 2020). Pollen contains about 2 - 60% of proteins, 13 - 55% of carbohydrates, and 1 - 20% of lipids (Ares et al., 2022). Moreover, bee pollen comprises various minerals such as sodium, potassium, magnesium, calcium, phosphorus, iron, copper, and zinc that support different physiological activities in honeybees as well as in humans (Zhang et al., 2022). Apart from its nutritional value, bee pollen is composed of considerable amounts of polyphenolic compounds, primarily flavonoids, which may act as strong antioxidants (Thakur & Nanda, 2020). They possess diverse biological properties such as antioxidant, anti-aging, anti-carcinogen, anti-inflammatory, cardioprotective, and improved endothelial function (Matuszewska et al., 2021).

Because it includes substances that are beneficial to human health, bee pollen is categorized as a functional food. This means that it can be added to food products to boost their nutritional content and bioactive content. Additionally, bee pollen can improve an animal's development, reproduction, and immunity; it is suggested as a feeding supplement for livestock (Al-Kahtani et al., 2021). Therefore, bee pollen has gained increasing research attention worldwide (Thakur & Nanda, 2020). However, the nutritional composition of pollen varies widely depending on floral types; geographic origin, climate, and soil type (Morais et al., 2011).

Promoting bee-collected pollen as a dietary supplement for the enhancement of human health requires identifying the most important pollen source plants and evaluating the quality of their pollen, particularly in light of the growing interest in bee-collected pollen as a nutritional and api-therapeutic substance. Ethiopia is equipped with a high population density of *Apis mellifera* and a diverse range of flora, which provide ideal conditions for year-round pollen harvesting (Gratzer et al., 2021). As a result, the country has enormous potential for producing large amounts of high-quality pollen. Concerning Ethiopia's central highlands, cultivated crops, associated weeds, and a high density of honey bee colonies all contribute to an impressive amount of pollen collection that can be

turned into food supplements for both domestic and foreign markets. Thus, describing the nutritional characteristics encourages the use of pollen as a dietary supplement both domestically and abroad. Although bee pollen is a rich source of bioactive and nutritious substances, and Ethiopia has significant potential for high-quality pollen production, little research has been conducted on the characteristics of monofloral pollen. Therefore, this study is intended to examine the chemical and mineral properties of pollen collected from various floral sources in Oromia for further application in food or health.

Material and Methods

Sampling of the pollen

The pollen samples were collected from the central parts of Ethiopia representing highlands, midland, and lowland areas. The specific areas were: Holeta (9°03’26.19” N, 38°33’22.45” E, altitude 2370 m), Menagesha Forest (38°34’30” 8°57’0” N, 38°31’30”, altitude 2924 m), Gedo (9°00’59.12” N, 37°26’58.19” E, altitude 2515 m) and Bako (9°06’59.23” N, 37°03’23.02” E, altitude 1670 m). In each apiary, twelve colonies of *Apis mellifera* honey bees were equalized to ensure that they were at the same strength for pollen collection. The colonies were arranged in 3 frames of equal strength (honey, pollen, sealed brood), and a 1:1 sugar syrup was used for feeding (Oztokmak et al., 2023). The colony strength of each selected colony was estimated by observing the number of frames covered with bees, honey, brood, and pollen. The colony entrances were equipped with 16% efficient pollen traps, which removed pollen loads from workers' corbicula over 24 hours. Pollen traps were harvested twice a week.

Preparation of sample

The pollen loads were cleaned to remove impurities, weighed on a balance for the total weight and moisture, and classified using a set of sieves with different-sized meshes (0.5, 1.0, 2.0, and greater than 2.0 mm). Pollen of the same botanical origin was then isolated from the pooled samples based on visual appearance and color. Further confirmation of the botanical origin and monoflorality was achieved through melissopalynological analysis. The grinding process typically begins with drying the bee pollen to reduce moisture content. Bee pollen was then dried until the mass stabilized (humidity of 9 - 12%). Once dried, the pollen is weighed and placed into the grinder. After grinding, the pollen must be mixed thoroughly to achieve homogeneity using a vortex mixer. The mixing duration should be sufficient to ensure that all particles are evenly distributed, typically around 5-10 minutes.

Following that, the samples were then kept in the dark at -20 °C until laboratory analysis for 1 to 2 months (Ghosh et al., 2020).

Identification of botanical origin of the pollen loads

Pollen load samples (2 g) were mixed with water to disaggregate the pollen grains from the surrounding material, facilitating their separation and analysis. They were rinsed, centrifuged, and mounted in glycerin jelly after being properly homogenized, following the protocol outlined by Louveaux et al. (1978). Pollen sediments were placed on microscope slides with glycerin jelly. Types of pollen were determined by comparing them to slides from the Holeta Bee Research Center's pollen reference collection (Figure 1). According to Ares et al. (2022), the predominant taxon in the composition of the sample that was collected is well-defined if it is more than 80%. When these criteria are not met, the pollen is classified as multifloral. (Escuredo et al., 2012).

Characterization of pollen chemical composition

Moisture content

The moisture content of pollen was examined using AOAC procedures (AOAC, 1995) in oven (BioBase).

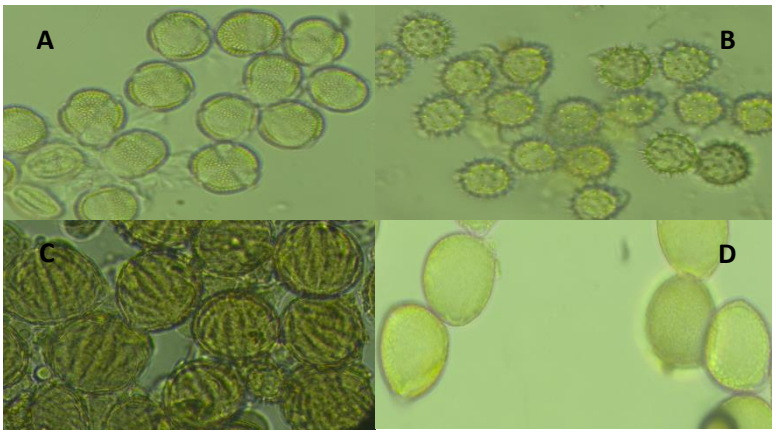

Figure 1: Photographs of pollen types analyzed. A, *Brassica carinata*, B, *Guizotia scabra*, C, *Sesamum indicum,* D, *Trifolium* spp.

The crucibles' weight was recorded using an analytical balance (W_1), and in every dry crucible (W_2), 5 g of pollen samples were measured and then dried for 3 hours at 105°C in an oven. After 3 hours, the crucibles were taken out of the oven and allowed to cool in desiccators. Once they had cooled, the weight of the sample and the crucibles was recorded (W_3). After the sample-containing crucibles were taken out of the oven to dry, their weight was recorded until it was constant. At last, the moisture content was estimated after the last constant measurement was obtained.

$$\text{Moisture } \% = \left(\frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}}\right) \times 100$$

Where;
W_1= Weight of crucible
W_2=Weight of the sample with crucible
W_3 = Final weight with crucible

Ash content determination

The ash content was assessed in the Muffle furnace (BioBase JKKZ.5.12GJ, Shandong, China) following Thiex et al. (2012) protocol. About 2.5 g of pollen samples were measured into each crucible. The samples were burned on a hot plate under a fume hood until the smoke stopped, and then they were placed in a Muffle furnace and heated to 550°C for 5 hours. The weight of the crucibles was measured after they were cooled in a desiccator. The total amount of ash was determined following the formula from AOAC (2000).

$$\text{Ash(wet basis)}\% = \frac{\text{M}_{\text{Ash}}}{\text{M}_{\text{Wet}}} \times 100$$

Crude protein determination

For characterizing the total protein content, 1 g of the samples was digested in a macro Kjeldahl flask containing 4 g of the catalytic mixture (1:3 $CuSO_4$ and K_2SO_4) and 20 mL of concentrated H_2SO_4 (95 - 97%) in the presence of a catalyst (potassium sulfate, copper sulfate) until the solution turned clear and blue-green in color. The resulting ammonia was collected in a boric acid solution and distilled, after which it was neutralized with 90 mL (30%) NaOH. To determine the total protein, the nitrogen values were multiplied by a conversion factor of 6.25 (Roulston et al., 2000).

$$\text{Nitrogen}\% = \frac{\text{V HCl} \times \text{N HCl} \times 14.0 \times 100}{1000 \times \text{Wo}}$$

$$\text{Protein}\% = 6.25 \times \text{Nitrogen}\%$$

Where;
V = Amount of HCl (in milliliters) used to reach the titration's endpoint
N = Molarity (M) of the HCl
Wo = Weight of the sample based on dry matter
14 = Molecular weight of atomic nitrogen
6.25 = Conversion factor

Total dietary fiber determination

For determining the fiber content, 2 g of milled pollen, defatted in petroleum ether, were heat digested with a solution of H_2SO_4 0.113 M, and subsequently, with a solution of NaOH 0.313 M, for 30 minutes in each digestion. After neutralizing the residue with hot water, washing was performed with 20 mL of ethanol and 10 mL of ethyl ether. The residue was then incinerated at 550°C in an oven and the fibers were quantified by gravimetry (AOAC, 2000).

Carbohydrate concentration of the pollen

The carbohydrate content of bee pollen was determined by calculation using different methods (AOAC, 2000).

$$\text{Total Carbohydrate (\%)} = 100 - \%\,(\text{Moiture} + \text{Protein} + \text{Total dietary fiber} + \text{Ash})$$

Since it is not feasible to incorporate crude fat content data, it is omitted from the formula of carbohydrate calculations. Although bee pollen is supposed to have a very low-fat content (less than 5%), the formula used to calculate the carbohydrate content lumps fat content in the proximate composition into the carbohydrate fraction, which causes the carbohydrate content to be overestimated.

Mineral analysis of pollen

The mineral content of pollen samples was evaluated by flame atomic absorption spectrometer and flame photometer instruments (Elico, CL-378, India). By dilution of stock solutions (1000 mg/L) of each element, standard solutions of trace elements have been prepared for calibration. Fresh serial dilutions of every element under examination had been prepared. To reduce the impact of the organic matrix, sample digestion with microwave assistance was done before analysis. Briefly, 0.5 g of the pollen sample was digested with 2 mL H_2O_2 (30% v/v) and 4 mL HNO_3 (65% v/v). A blank control was digested similarly. The initial 500 W was used for the digesting program, which ramped up for one minute and was maintained for four minutes. The second phase started at 1000 W, increased for 5 minutes, and then had a 5-minute hold period. The third phase started at 1400 W of power and ramped up for 5 minutes, with a 10-minute pause time in between dilution steps (up to a ten-fold dilution) was carried out before analysis. Ca, Mg, and Pb studies were carried out using flame atomic absorption spectroscopy (AAS). To analyze Na and K, flame atomic emission spectroscopy was used. The calibration standards were prepared by diluting the standards with a 2% solution of nitric acid in ultrapure water. Calibration curves were constructed by plotting the signal on the y-axis (analyte peak area) against the analyte concentration on the x-axis. Quantification of mineral elements (Ca, K, Mg, Na,) was carried out using a calibration curve that covered a range of concentrations from 10 to 150 mg/L (10, 25, 50, 75, and 150 mg/L). The graphs obtained in all the

calibration curves were straight lines, with the coefficient of the determination values (R^2) higher than 0.99. Using the appropriate fuel and oxidant combination, each trace mineral element was measured at its wavelength using cathode lamps (AOAC, 1990).

Data Analysis

The obtained data are presented as mean values ± standard error (SE), calculated from measurements taken in triplicate. Data analysis was performed using SPSS software (version 20), and mean differences were assessed with Tukey's multiple range test at a significance level of $P < 0.05$.

Results and Discussion

Chemical composition

Bee pollen collected by honey bees is considered a healthy food with a wide range of nutritional and therapeutic properties. The results of the analysis of pollen from different plant types are presented in Table 1.

Moisture content

The current study found that the moisture content of *Eucalyptus globules* (17.3 ± 0.5%) pollen samples was significantly different ($P < 0.05$) from others, whereas *Sesamum indicum* pollen has the lowest (10.3 ± 1.3%). The moisture content detected in this study is higher than that of Romanian pollen samples, which ranges from 3.0 to 11.9% (Oroian et al., 2022). However, these findings were lower than those of an earlier study conducted in Ethiopia by Addi et al. (2017), who observed a moisture content of 19.3 - 25.0% of bee-collected pollen in Southwest Ethiopia. Based on pollen composition and the standardization of the analytical method, these results showed that the moisture content of the current study was within the permissible ranges of 20 - 30% moisture (Campos et al., 2008). The study found that the moisture content of dried pollen should range between 6 to 8% to maintain the quality and stability of pollen (Campos et al., 2008). Fresh pollen has a higher nutritious and biological value than dried pollen collected by bees. Its high water content also makes it a perfect culture medium for microorganisms. The excessive moisture content detected in some pollen samples could be related to insufficient pollen drying or storage conditions. Bee-collected pollen should be harvested daily and stored in a nitrogen atmosphere to preserve its high quality until consumption (Campos et al., 2010).

Ash content

B. carinata pollen had significantly ($P < 0.05$) higher ash content (3.0 ± 0.5%) than other pollen types (Table 1). From the current study finding, the lowest ash content was recorded for *Guizotia scabra* pollen samples (1.7 ± 0.3%). These results are consistent with findings by Almeida-Muradian et al. (2005), who reported that pollen collected by bees in Romania had an ash content of 2.2%, and Oroian et al. (2022), who documented ash concentrations ranging from 2.29% to 4.02%. However, Addi et al. (2017) discovered a lower ash concentration in pollen from forests in Southwest Ethiopia for *Guizotia* spp. (1.4 ± 0.1%) and *Planatago lanceolatum* (1.3 ± 0.3%). In the case of *Brassica* spp. Yang et al. (2013) also reported a similar ash percentage, 3.53 ± 0.10%. In China, bee pollen from *Brassica* spp. is commonly used as a natural dietary supplement and as a herbal remedy that increases the body's resilience to illnesses, such as cancer (Wu et al., 2007). According to Velásquez et al. (2017), pollen collected by bees from *Brassica* spp. exhibited antibacterial activity against harmful pathogens. Almeida-Muradian et al. (2005) suggested a 2-6% ash percentage for dry pollen pellets internationally. According to Herbert and Shimanuki (1978), pollen pellets usually incorporate 2 to 4% ash by dry weight. Ash content is a quality indicator that is dependent on the kind of soil, the plant's ability to accumulate minerals, and its botanical origin (Carpes et al., 2009).

Protein content

The usefulness of bee pollen obtained from different floral origins for bees and humans can be assessed based on its protein, amino acid, and fatty acid compositions. The current study found that protein content varied significantly among pollen samples, ranging from 16.3 ± 0.5 g/100 g (*G. scabra*) to 24.9 ± 5.6 g/100 g (*E. globules*). The protein content of *Eucalyptus*

Table 1: Proximate composition of pollen (average ± SE)

Pollen type	Proximate composition				
	Moisture content (%)	Ash content (%)	Protein (%)	Crude fiber (%)	Carbohydrate (%)
Sesamum indicum	**10.3±1.3^{b}**	2.2 ±0.1^{bc}	21.4±0.3^{ab}	2.2±0.4^{a}	64.0±1.5^{b}
Guizotia scabra	10.5±1.2^{b}	**1.7 ±0.3^{c}**	**16.3±0.5^{c}**	2.4±1.3^{a}	**69.1±1.0^{a}**
***Plantago* spp.**	12.5±2.0^{b}	2.6 ±0.1^{ab}	19.7±1.4^{bc}	1.4±0.7^{b}	63.9±0.7^{b}
Eucalyptus globules	**17.3± 0.5^{a}**	2.3±0.6^{bc}	**24.9±5.6^{a}**	**1.40±0.7^{b}**	**54.1±5.2^{c}**
Brassica carinata	12.4± 2.8^{b}	**3.0±0.5^{a}**	19.4± 0.5^{bc}	**2.6±0.9^{a}**	64.7±0.9^{b}

The lowest and highest values of the corresponding parameters are indicated by bold numbers. There is no significant difference ($P < 0.05$) between means with the same letters in the same column

globules was found significantly different ($P < 0.05$) from other pollen types. The results align with the findings published by Addi et al. (2017), who reported 15.0 - 27.1 g/100 g. The protein content of monofloral bee pollen varies significantly between countries despite coming from distinct botanical sources. According to Taha et al. (2019), the protein content of *Brassica* pollen has a nutritious value of 18.9% in Saudi Arabia and (27.27 ± 0.72) in China. Spulber et al. (2020) revealed that *Brassica* spp pollen is an important source of protein, even though the highest protein content was recorded for *E. globules* in the current study. Furthermore, Alshallash et al. (2023) observed a greater protein content of 30% for *Eucalyptus* spp. The results of the current study are also in line with the guidelines provided by Bogdanov (2004) and Campos et al. (2008), who set the protein content of bee-collected pollen dry weight at 10 to 40 g/100 g. Proteins were the second most abundant component in pollen. The amount of crude protein in pollen varies greatly depending on the type of plant the pollen comes from. The primary feature that determines the quality of honey bee-collected pollen is its protein content (Somerville, 2001).

E. globules pollen is regarded as a superfood with a variety of nutritional and medicinal benefits. Flavonoids, alkaloids, tannins, and propanoids are just a few of the phytochemical compounds that can be found in abundance in the leaves, stems, and roots of *E. globulus* (Dixit et al., 2012). Alshallash et al. (2023) observed the highest DPPH scavenging activity, while Araújo et al. (2017) reported a significant inhibitory effect of pollen from *Eucalyptus* spp. *Eucalyptus* spp. has been used as an antibiotic, to support liver and renal function, or just to give the body an extra boost of vitamins and nutrients (Campos et al., 2021). According to the current findings, the protein content of *S. indicum* and *E. globules* pollen is categorized as average. According to Hernández-Monzón et al. (2019), sesame (*Sesamum indicum* L.) is regarded as a superfood because it includes approximately 15 essential amino acids, 80% polyunsaturated fats, and minerals like bioavailable calcium, iron, and zinc that aid in the digestion of carbohydrates, proteins, and fats. Protein levels in pollen samples vary greatly, which could be influenced by floral type, geographical location, and condition of storage (Negrao & Orsi, 2018). Proteins are highly valued in the food industry not only for their role in producing amino acids essential for human growth and nutrition but also for their numerous functional properties in food systems and their ability to enhance nutritional value.

Total dietary fiber

B. carinata pollen had significantly ($P<0.05$) the highest value of total dietary fiber (2.6 ± 0.9%) than *Plantago* spp (1.4 ± 0.7) and *E. globules* (1.40 ± 0.66%). The results presented here agree with the findings made by Kostic et al. (2015), who found an average total dietary fiber of 2.7 ± 1.2 % in Serbia, and Bogdanov (2016) found that the total dietary fiber of pollen ranged between 0.3 and 20%. Our findings are further confirmed also by Hassan's (2011) study, which found a total dietary fiber content of 1.37% in the palm pollen grain. Furthermore, the total dietary fiber in the current investigation fits within the range recommended by Campos et al. (2008) (1 - 13%). The variance in crude fiber composition could be attributed to different plant species. Starch and insoluble polysaccharides such as cellulose, pectin, cellulose, and sporopollenin make up crude fiber (Bogdanov, 2016). The primary components of bee pollen are cellulose and callose, which make it a useful source of fiber for food. Dietary fiber, also referred to as roughage, is the portion of food that stays intact in the stomach and small intestine and adds no nutritional value to the food but is vital to human health.

Carbohydrate content

Carbohydrate content ranged from 54.1 ± 5.2 (*E. globules*) to 69.1 ± 1.0 g/100 g (*G. scabra).* In the present study, the carbohydrate content of *G. scabra* was significantly different ($P < 0.05$) from other pollen types. A similar conclusion was reached by Spulber et al. (2020), who noted that *Plantago lanceolata* has the highest concentration of macronutrients (carbohydrates). The values observed in the current study are closely comparable to those reported by Nogueira et al. (2012) (67.8 - 73.2 g/100 g) and Yang et al. (2013) (59.4 - 75.7 g/100 g). However, these results were greater than Campos et al. (2008), who reported 13 to 55%. Thakur and Nanda, (2020) summarized in their review that pollen consists of 54.2% (18.5-84.3%) carbohydrates on average. Carbohydrates in pollen vary

Table 2: The mineral content (average ± SE) of pollen

Pollen type	Mineral content			
	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)
Sesamum indicum	339.2±1.1^{b}	7231.4±310.3^{bc}	234.1 ±3.7^{cd}	152.4±6.4^{b}
Guizotia scabra	1998.3±1123.1^{ab}	4470.0±1196.8^{cd}	555.0±421.0^{bcd}	139.04±98.8^{b}
***Plantago* spp.**	577.4±5.0^{b}	8277.6±57.5^{ab}	199.1±0.5^{d}	81.6±2.1^{b}
***Trifolium* spp.**	2202.4±869.4^{a}	4180.3±421.3^{d}	1227.1±12.6a	123.3±19.1^{b}
Brassica carinata	2321.3±608.8^{a}	7396.3±3336.8^{bc}	1024.8±19.9^{ab}	280.7±133.2^{ab}
Eucalyptus globules	2123.5±404.3^{ab}	10596.9±1610.1^{a}	863.4±242.4^{abc}	380.9 ±95.9^{a}

There is no significant difference ($P < 0.05$) between means that have the same letters in the same column

based on the botanical and geographic origins. The main carbohydrates included are fructose, glucose, and sucrose (more than 90% of the total carbohydrate content) (Bertoncelj et al., 2018). Pollen is primarily composed of carbohydrates, which are an essential source of energy and nutrition.

Mineral content

Table 2 illustrates the mineral content of pollen collected by honey bees. In this study, the calcium (Ca) concentration of pollen obtained from honey bees ranged from 339.2 ± 1.1 mg/kg (*S. indicum*) to 2321.3 ± 608.8 mg/kg (*B. carinata*). In comparison to this finding, Addi et al. (2017) found a lower calcium concentration (160 to 435 mg/kg) in pollen collected from several plant species. The calcium concentrations of *G. scabra*, *Trifolium* spp, and *P. lanceolata* are 379.0 ± 0.8 mg/kg, 232.1 ± 51.0 mg/kg, and 214.5 ± 5.2 mg/kg, respectively, according to Addi et al. (2017). However, the calcium content observed in the current study was higher than the values reported by Asmae et al. (2021), which ranged from 2.2 ± 1.0 mg/kg to 22.7 ± 2.6 mg/kg in Moroccan monofloral pollen samples. Furthermore, the Ca amount found is within the recognized ranges of 200 - 3000 mg/kg based on the composition of pollen and standardization of analytical methods (Campos et al., 2008). Ca is involved in both root development and plant physiological processes (Amadou et al., 2022). It is also responsible for the water balance in extracellular and intracellular media and the depolarization of cellular membranes.

In all samples, potassium (K) was the most prevalent element. The maximum potassium concentration (10596.9 ± 1610.1 mg/kg) was found in *E. globules* pollen, whereas *Trifolium* spp pollen had the lowest (4180.3 ± 421.3 mg/kg). At $P < 0.05$, the K concentration of *E. globules* pollen is significantly different from other pollens. In a similar study, Addi et al. (2017) revealed that the concentration of K in pollen samples from several plant species ranged from 0.9 to 592.3 mg/kg. Furthermore, the K concentration in the current investigation was within the acceptable ranges of 4000 - 20000 mg/kg based on the composition of pollen and analytical method standardization (Campos et al., 2008). Asmae et al. (2021) also found a similar average of K in Moroccan monofloral bee pollen, ranging from 485.4 ± 9.3 to 4594.3 ± 18.3 mg/kg. In plant nutrition, potassium is a crucial element involved in several processes, including enzyme activation, photosynthesis, and water absorption. In addition, potassium is essential for nerve impulse transmission, protein synthesis, lipid metabolism, muscle contraction, and maintaining fluid and electrolyte balance in animals and humans.

Trifolium spp pollen contains a significantly greater concentration of magnesium (Mg) (1227.1 ± 12.6 mg/kg) than other pollen species, whereas *Plantago* spp pollen contains less Mg (199.1 ± 0.5) mg/kg. The Mg concentration range in this study agrees with the findings of Aldgini et al. (2019), who reported Mg content of 641.4 to 1575.2 mg/kg. In a related study conducted in Romania, the amounts of magnesium ranged from 702 to 965 mg/kg (Harmanescu et al., 2007). The magnesium concentration of pollen collected by bees ranged from 68.7 ± 5.3 to 793.4 ± 13.6 mg/kg, according to comparable data found by Asmae et al. (2021). The current study's Mg concentration was similarly within the acceptable ranges of 200 - 3000 ppm based on the composition of pollen and analytical method standardization (Campos et al., 2008). Magnesium (Mg) is an essential element for human as well as plant physiology. It is present in many enzymes and involved in the structure of proteins, lipids, and carbohydrates. According to Graikou et al. (2011), bee pollen from *Trifolium* spp is high in phenolic acids and flavonoids. This mixture has been shown to stimulate cellular antioxidant systems by other natural products and to exhibit the observed free radical scavenging activity on HFL-1 human fetal lung embryonic fibroblasts.

Except for *B. carinata*, the sodium (Na) concentration in *E. globules* (380.89 ± 95.85 mg/kg) is statistically significantly higher than that of other pollen types. These results agree with those of Addi et al. (2017), who discovered that bee pollen collected from several plant species had a Na concentration ranging from 4.8 - 610 mg/kg. The same authors previously reported a Na concentration of *G. scabra* (405.8 ± 0.3 mg/kg), which is comparable to the current study. The results of this investigation aligned with those of Asmae et al. (2021), who showed that sodium levels in Moroccan bee-collected pollen samples ranged from 91.9 ± 0.6 mg/kg to 397.2 ± 4.1 mg/kg. Because bee pollen is a significant source of minerals, it has great nutritional value and is used extensively in Ethiopian food.

Hemolymph osmotic pressure and intracellular and intercellular fluids are regulated by a mixture of calcium (Ca), phosphorus (P), and magnesium (Mg) (Matuszewska et al., 2021). The composition and mineral variation in pollen can be influenced by factors such as soil type, climate, geographical origin, and botanical species, as plants accumulate varying amounts of mineral salts (Liolios et al., 2019; Taha, 2019). Other parameters, such as the season of collection (Taha et al., 2019) and pollen load storage (Human & Nicolson, 2006), influenced the mineral content.

Lead (Pb) was not found in all pollen types sampled from the study area. Asmae et al. (2021) also reported that Moroccan monofloral bee pollen was free of lead (Pb). The lead level of the pollen collected by bees cannot exceed 50 µg/100 g, hence these values are within allowable limits for pollen quality (Campos et al., 2008). Lead is a toxic metal that poses a serious risk to human health. This component is considered to be one of the main causes of pollution in the environment.

Because bee pollen is a naturally occurring source of nutrients, consuming it is highly recommended as a

dietary supplement. However, because it may contain heavy metals, pesticides, bacterial and fungal toxins, and allergic reactions, there are some risks involved (Dinkov & Stratev, 2016). According to Mauriello et al. (2017), lactic acid bacteria, yeasts, molds, total viable count, and Enterobacteriaceae which thrive at moderate temperatures may cause microbial deterioration in bee pollen due to unsanitary manufacturing and storage circumstances. Additionally, individual pollen grains are collected from wind-pollinated weeds and trees as well as insect-pollinated plants, which may cause allergic reactions due to accidental intake of airborne pollens. This is why allergic reactions, including anaphylaxis, are typically immediate IgE-mediated hypersensitivity responses observed after pollen intake (Choi et al., 2015). According to McNamara and Pien (2019), consuming pollen can reduce the threshold for mast cell degranulation during exercise because it increases gastrointestinal permeability or osmotic effects. As a result, people who consume pollen can experience exercise-induced anaphylaxis. It is advised that these people skip exercise for 4-6 hours after consuming pollen. Considering these factors, it can be concluded that the main causes of bee pollen allergies include its combination with airborne allergens, fungi reacting to allergic compounds, pesticide contamination, and exercise following ingestion.

Conclusion

The Chemical and mineral contents of monofloral pollen obtained from the Oromia region were evaluated in this study. The best bee-collected pollen for animal or human food supplements can be found with the help of the periodic compilation of pollen nutrition data. According to the data we obtained, the average moisture content, ash content, total dietary fiber, protein, and carbohydrate concentrations of pollen samples are 12.3± 2.8%, 2.2 ± 0.6%, 2.1 ± 1.0%, 19.6 ± 3.6%, and 64.1 ± 5.5 %, respectively. *E. globules* pollen has the maximum moisture and protein content, but the smallest total dietary fiber and carbohydrate content. *G. scabra* has the highest carbohydrate content, but the lowest protein and moisture level. Regarding the mineral composition, potassium, and magnesium were found to be prominent in all bee-collected pollen samples, while lead was not traceable in any of them. It is concluded that because pollen has a nutritional composition that meets human needs, it can be used to supplement food for humans. These data can be utilized to guide healthcare recommendations and consumer decisions in addition to assisting beekeepers in producing pollen from bees. The certification and standardization of bee-collected pollen produced in Ethiopia will be a future endeavor to improve bee pollen knowledge to promote its application in the food industry.

Ethical Statement

There are no ethical issues with the publication of this article.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Teferi Damto: investigation, methodology, data curation, formal analysis, writing-original draft, and writing-review and editing. Meseret Gemeda: conceptualization, data curation, investigation, methodology, and writing-review and editing. Dheressa Kebaba: investigation data curation, methodology, formal analysis, and writing review, and editing.

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