JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(2), 173–183
https://doi.org/10.12911/22998993/156611
ISSN 2299–8993, License CC-BY 4.0

Received: 2022.11.02
Accepted: 2022.12.12
Published: 2023.01.01

Bee Pollen as a Functional Product – Chemical Constituents and Nutritional Properties

Khalid S. Alshallash[1*], Gomaa Abolaban[2], Sam M. Elhamamsy[3], Ayman Zaghlool[4], Ali Nasr[4], Ashraf Nagib[4], Ahmed F. Abd El-Hakim[3], Abdullah A. Zahra[2], Ashraf E. Hamdy[5*], Ibrahim M. Taha[4*]

[1] College of Science and Humanities - Huraymila, Imam Mohammed Ibn Saud Islamic University (IM SIU), P.O. Box 5701, Riyadh 11432, Saudi Arabia

[2] Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Cairo, 11884, Egypt

[3] Department of Biochemistry, Faculty of Agriculture, Al-Azhar University, Cairo, 11884, Egypt

[4] Department of Food Science and Technology, Faculty of Agriculture, Al-Azhar University, Cairo 11884, Egypt

[5] Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Cairo 11884, Egypt

* Corresponding author's e-mail: sam79e@azhar.edu.eg; ashrafezat@azhar.edu.eg; ibrahimtaha164@azhar.edu.eg

ABSTRACT

Nutritional supplements play a role in promoting human protein synthesis, fitness recovery, and mental health protection. Pollen is considered a natural food with excellent nutritional value. The use of bee-gathered pollen as a nutritional supplement is now widespread around the world, and it is appreciated for its healing qualities. However, depending on the floral species and the region of origin, pollen has a wide range of nutritional value. It was found that clover pollen had the highest protein content (31.4 g/100 g DM), while the lowest content was observed in maize pollen (21.3%). Eucalyptus pollen had the highest lipid amount (9.49 g/100 g DM), while clover pollen had the lowest content (7.46 g/100 g). Phytochemical analysis showed that eucalyptus pollen occupied the highest total antioxidant activity (67.02%), followed by clover (58.25%) then maize (52.18%), whereas clover pollen had the highest phenolic content (1165 mg GAE/100 gm), compared with the other pollen varieties (949.4 to 1073 mg-GAE/100 gm). The contents of branched-chain amino acids were 29, 33.3, and 38.4 mg/g for maize, eucalyptus, and clover respectively, representing 17.87, 18.44, and 16.53% of total amino acids. The contents of total essential amino acids (EAAs) were 61.8, 73.0, and 83.5 mg/g for maize, eucalyptus, and clover pollen, respectively. Only eucalyptus pollen ultimately met the minimum requirements of EAAs for adults. In contrast, the other two types of pollen contained at least 3–4 limiting amounts of the essential amino acids leucine, lysine, phenylalanine, and valine. In conclusion, pollen is a good, affordable source of nutrients that can be utilized as beneficial dietary supplements for human health.

Keywords: eucalyptus; clover; maize; phenolic content; amino acid; pollen.

INTRODUCTION

In the anthers of angiosperm stamens, pollen, a male floral gametophyte, is a significant source of chemicals with potential health advantages (Sadeq et al., 2021). It has a high protein content and a reliable supply of bioactive substances that are typically used to identify the nutritional value of pollen (Mayda et al., 2020). Hence, it is consumed by humans as a nutritional supplement and is recognized as a superior functional food ingredient (Kostić et al., 2020). As reported by Food and Agriculture Organization (FAO), pollen may contain more than 16 amino acids (Taha et al., 2019).

Pollen has advantages for health and immunity; hence it can be described as anti-inflammatory, anticarcinogenic, antioxidant, antimicrobial, antiallergenic, antiulcer, hepatoprotective, and chemo-preventive properties, pollen has been suggested as a natural nutraceutical. Pollen has recently been discovered to modulate the gut microbiota to support gut health (Kalaycıoğlu et al., 2017; Wu et al., 2019).

Consumers who are concerned about their health these days prefer to eat value-added goods that allow them to replace traditional food ingredients with components that have a high nutritional value in order to enrich the already existing processed goods. The human diet should include energy and other essential nutrients in amounts that fulfil criteria for physical and mental development and health (Kieliszek et al., 2018). Pollen can be supplemented in a healthy diet, offering a significant daily intake of nutrients because of its superior nutrient profile. Even elderly persons can consume it as a stand-alone diet or supplement, because of its nutritionally balanced composition and low-calorie count (Campos et al., 2021).

Branched-chain amino acids (BCAA) leucine, isoleucine, and valine which make up 33% of human muscle protein, are also important for protecting mental health and promoting protein synthesis (Günalan et al., 2022). According to the situation, the effects of BCAA, which are necessary amino acids, on metabolic health, have been described as both negative and beneficial (Bishop et al., 2022). Only outside food can be used to obtain BCAA because the body cannot, or can only do so in very little amounts. Leucine is one of the branched-chain amino acids and makes up a significant fraction of the amino acids in proteins. It is also the principal by-product of protein catabolism. The Office of Dietary Supplements at the National Institutes of Health advises taking 10–20 grams of BCAA a day, either as supplements or in the form of protein that is eaten (National Institutes of Health Office of Dietary Supplements, 2017).

More attention is paid to the production of pollen in most countries, since it has beneficial effects on the economy, society, and environment. The top producing countries of pollen are Australia, Argentina, Brazil, China, Spain, and Vietnam (Lorini et al., 2020). The main composition of pollen has drawn the attention of researchers across a variety of disciplines, including plant physiology, biochemistry, nutrition, food biotechnology, and even material science. More than 200 different compounds have been identified in pollen, demonstrating the wide variety in its chemical composition (Komosinska-Vassev et al., 2015).

The main goal of this study was to see if certain types of pollen collected in Egypt might be used as nutritional supplements. For this reason, the chemical composition, which comprises carbohydrates, proteins, lipids, fiber, and ash, was determined. The total phenolic content and antioxidant potential of pollen from maize, clover, and eucalyptus was also checked. In addition, the nutritional value of pollen samples was demonstrated by using chemical scores for amino acids, specifically branched-chain amino acids “BCAA”, following the Food and Agriculture Organization’s recommendations for human nutritional needs of pollen samples from eucalyptus, clover, and maize. Finally, the ability of various pollen types to give vital nutrients for individuals’ daily needs was examined.

MATERIALS AND METHODS

Materials

Chemicals

The chemicals and solvents were high purity. Folin-Ciocalteu solution, Gallic acid, and all chemicals were obtained from El-Nasr Pharmaceutical Chemicals Company, Cairo.

Pollen samples

As shown in Table 1, three pollen varieties were selected. Eucalyptus pollen was gathered from Nasr City, Cairo Governorate, Egypt, while maize and Egyptian clover pollen were collected from Bassioun Center, Gharbia Governorate, Egypt. For collecting pollen, Italian and Carniolan hybrids were used in the present study. During the collecting phase, a typical pollen trap was placed to the hive entrance. Fresh pollen loads were stored in paper bags and traps were regularly emptied. Following that, pellets underwent

Table 1. Plant sources of trapped pollen collected during seasons of 2021

No	Common name	Family	Scientific name	Collecting period
1	Maize	*Poaceae*	*Zea mays*	Jul 15 to Aug 5
2	Egyptian clover	*Fabaceae*	*Trifolium alexandrinum*	Jun 10 to Jul 22
3	Eucalyptus	*Myrtaceae*	*Eucalyptus* sp.	Aug 30 to Oct 26

cleaning and color-based separation. The collected pollens were kept in polyethylene bags at -20 °C until further tests.

Analytical methods

Proximate analysis

Proximate analysis (moisture, crude proteins, crude fats, crude fiber, and ash contents) of pollen was conducted using the standard methods of reference (Official methods of analysis of the association, 2016).

The total carbohydrates were calculated by means of the difference method using the formula:

$$\text{Total carbohydrates (\%)} = 100 - - \% \text{ (moisture + protein + fat + ash)} \tag{1}$$

Energy values

The energy value was calculated as the following equation (cal/100g-1).

$$\text{Energy (kcal)} = 4 \times (\text{g proteins} + + \text{g carbohydrates}) + 9 \times (\text{g lipids}) \tag{2}$$

Determination of phytochemical contents

Preparation of pollen extraction

The extraction was performed following the steps specified in the reference (Keskin & Özkök, 2020). Using a mechanical shaker (150 rpm) for 12 hours at room temperature, three grams of each pollen sample were added to 20 mL of ethanol absolute. At 3000 rpm, the extract was centrifuged for 15 minutes. A final amount of 30 mL of ethanol absolute was then added after filtering. The extracts were utilized to determine the antioxidant capability and total phenol content.

Total phenolic content (TPC)

Folin-Ciocalteu colorimetric method was applied for evaluating the total phenolic content (TPC) of samples (Amarasinghe et al., 2021), where 2.5 mL (10 %) Folin-Ciocalteu reagent, was added to 0.5 mL extract. The mixture was settled for 8 min. in the absence of light, then 2 mL of Na_2CO_3 (7.5%) was added and allowed to stand at room temperature for 60 min in the dark. Absorbance was read at 765 nm using a UV-VIS spectrophotometer (Cary 50 Bio Varian, Australia). The results were calculated as mg Gallic acid (GA)/100 g of dry matter (DM).

Antioxidant activity

The antioxidant compounds of pollen samples was evaluated by the DPPH radical-scavenging method according to reference (Sakooei-Vayghan et al., 2020), the DPPH radical-scavenging technique was used to assess the antioxidant components in pollen samples. To 2 mL of extract, 0.16 mM DPPH solution was first added. After being vortexed, the reaction mixture was incubated for 30 min. in the dark. To quantify the absorbance, a UV-visible spectrophotometer was used at 517 nm (Thermo Electron Corporation, Beverly, MA, USA).

This equation was used to compute the % inhibition of the radical scavenging activity:

$$\text{DPPH Inhibition (\%)} = ((\text{AC} - \text{AS})/\text{AC}) \times 100 \tag{3}$$

where: AS – absorbance of the sample extract;
AC – absorbance of control sample.

Amino acids

An automatic amino acid analyzer was used to determine the content of amino acids (AAA 400 INGOS Ltd. Czech Republic). About 100 mg of pollen samples were hydrolyzed in 10 mL of 6 M HCl in a sealed tube at 110 °C for 24 h. After hydrolysis, the acid was evaporated at 80 °C in a vacuum evaporator under reduced pressure. The HCl-free residue was dissolved in 2 mL of loading buffer (0.2 M, pH 2.2) to inject into the apparatus (Block et al., 2016).

Amino acid score

To calculate amino acid scores (%) the observed value of an essential amino acid (g/100g of protein) was compared to the reference pattern as provided by FAO, 2017 according to the following formula:

$$\text{CS} = \text{AP} \times 100 \ / \ \text{AR} \tag{4}$$

where: P – refers to amino acid in tested protein;
R – refers to amino acid requirements for adult humans.

Analytical statistics

Every measurement was taken three times using the Duncan's Multiple Range test. Using the SPSS statistics package application, the data was statistically analysed (SPSS software version 21). The data were analyzed utilizing quantitative approaches using a one-way ANOVA to compare any significant differences between the means.

RESULTS

Nutritional value of pollen samples

Table 2 provides an overview of the chemical composition of the pollen samples under investigation. The results illustrated significant differences between the chemical contents of the three tested pollen. The highest moisture content was observed in the pollen of clover (26.12%), followed by maize (23.21%), while the least value of moisture was detected in eucalyptus pollen (19.85%) with significant differences (Figure 1).

Pollen's protein concentration is seen as a clear indicator of how nutrient-dense it is. Proteins are the largest component in pollen after carbohydrates. It is highly ($p < 0.05$) varying in pollen collected from diverse plant sources (Figure 2). The results showed that the protein contents of pollen from maize, eucalyptus, and clover were, respectively, 21.30, 21.46, and 31.40%. These values are following the studies reported by Bakour et al., 2019; Kieliszek et al., 2018; Mayda et al., 2020 the protein content is around 14–37% in both bee products of their dry weight. As mentioned before, variability between pollens is strongly dependent on the plant source (Mayda et al., 2020).

Lipids are an important macronutrient that forms the nutritional composition for pollen samples, and it varies depending on the plant origin (Khalifa et al., 2020; Kieliszek et al., 2018). The

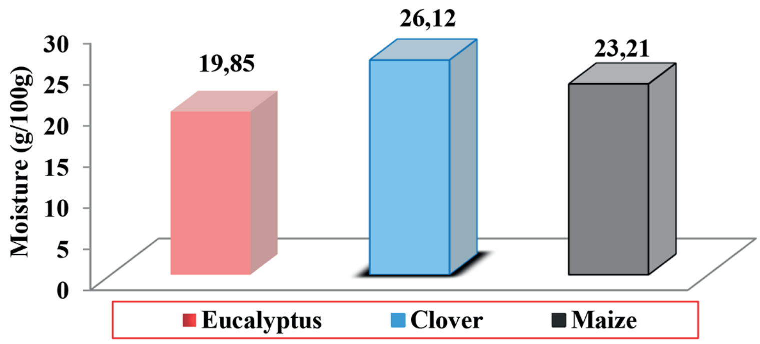

Figure 1. Moisture content of bee pollen samples

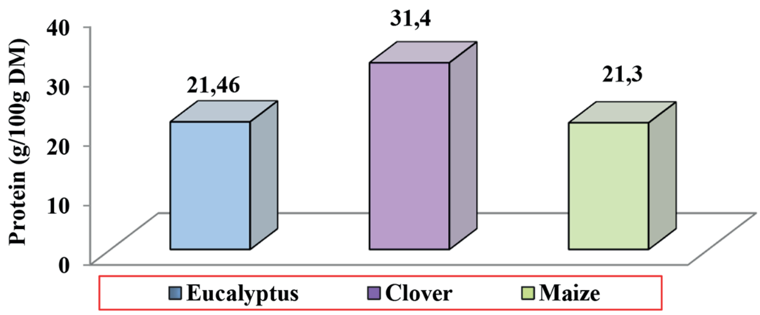

Figure 2. Protein content of bee pollen samples

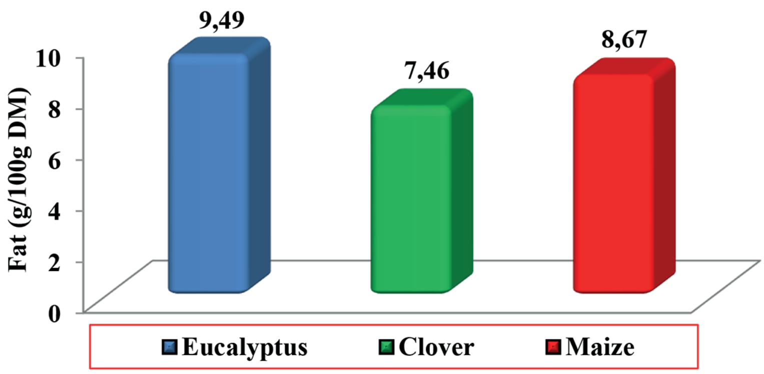

Figure 3. Fat content of bee pollen samples

results in Figure 3, presented the crude lipid content percentages of pollen. The highest lipid content (9.94%) was found in the pollen of eucalyptus, followed by maize (8.67%), while the least value was recorded in clover pollen at 7.46%. These results are supported by the findings of Kieliszek et al., 2018; Thakur & Nanda, 2020a, 2020b; Wu et al., 2019 who found that pollen contains approximately fat 1–13%. The richness of the eucalyptus pollen seems evident, compared to the others. The minimum and maximum values of the pollen samples differed significantly.

Moreover, from Figure 4, the average ash contents for the three tested pollen types were determined as 3.54, 5.33, and 5.55%, respectively for maize, clover, and eucalyptus. These values are within the range revealed by Kieliszek et al., 2018. All pollen samples of different plant sources contain a moderate amount of ash. Our results were higher than that of Almeida-Muradian et al., 2005 who found that the ash content of pollens had an average of (2.2%).

Over the plant sources, pollen of clover contained the highest ($p < 0.05$) fiber (6.78%) followed by eucalyptus (5.51%), while the lowest fiber was found in maize pollen 5.14% (Figure 5).

The majority of pollen's dry weight, or approximately two-thirds of it, is made up of

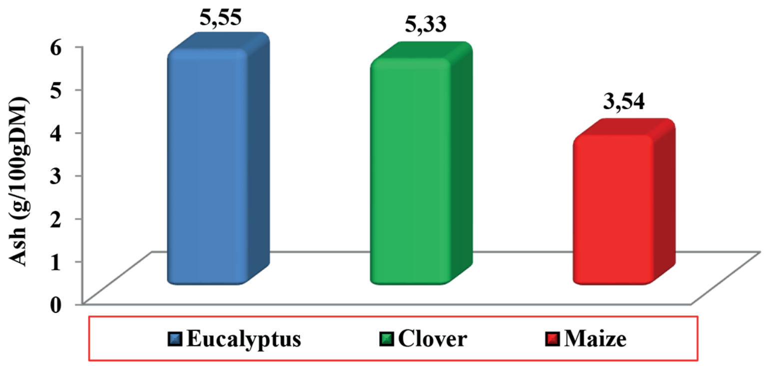

Figure 4. Ash content of bee pollen samples

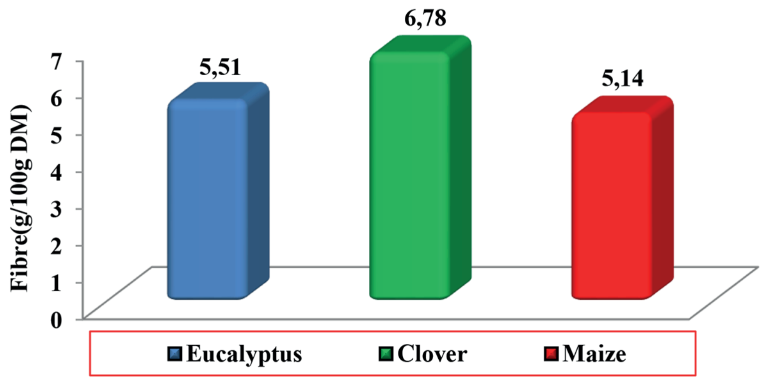

Figure 5. Fiber content of bee pollen samples

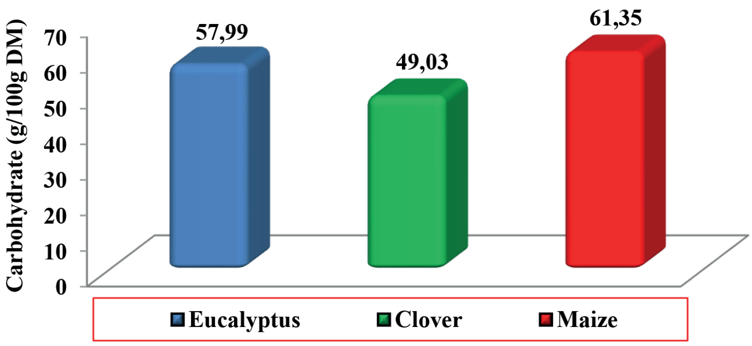

Figure 6. Carbohydrate content of bee pollen samples

Table 2. shows the nutritional requirements and pollen composition

Main components	Pollen sample	g/100g FM	g/100g DM	Average RDI g/day	% RDI for 25 g DM of bee pollen	Average % RDI for 25 g DM of bee pollen
Moisture	Eucalyptus	19.85 b ± 1.00	-	-	-	-
	Clover	26.12 a ± 2.20	-	-	-	-
	Maize	23.21 a ± 1.10	-	-	-	-
Protein	Eucalyptus	17.20	21.46 b ± 0.80	50	21.46	24.72
	Clover	23.20	31.40 a ± 1.00		31.40	
	Maize	16.36	21.30 b ± 0.75		21.30	
Fat	Eucalyptus	7.97	9.49 a ± 0.50	80	5.93	5.33
	Clover	5.51	7.46 b ± 0.40		4.66	
	Maize	6.66	8.67 a ± 0.45		5.41	
Fibre	Eucalyptus	4.42	5.51 b ± 0.40	30	9.18	9.68
	Clover	5.01	6.78 a ± 0.30		11.30	
	Maize	3.95	5.14 b ± 0.25		8.56	
Carbohydrates*	Eucalyptus	58.50	63.50 a ± 2.00	320	9.85	9.65
	Clover	41.23	55.81b ± 1.80		8.72	
	Maize	51.05	66.49 a ± 1.50		10.38	
Ash	Eucalyptus	4.54	5.55 a ± 0.30	-	-	-
	Clover	3.94	5.33 a ± 0.45	-	-	-
	Maize	2.72	3.54 b ± 0.25	-	-	-
Total energy	Eucalyptus	374.53	425.25 a ± 18.00	-	-	-
	Clover	307.31	415.98 a ± 15.00	-	-	-
	Maize	329.94	429.19 a ± 12.00	-	-	-

Note: The means values for each parameter that begin with a different letter differ considerably ($p < 0.05$). Carbohydrates* (including fiber). FM: fresh mater, DM: dry mater, RDI: recommended daily intake.

carbohydrates (Li et al., 2018). The carbohydrates in pollen around the world vary greatly, ranging from 18.50 to 82.80% (Thakur & Nanda, 2020b). Total carbohydrates content was 49.03, 57.99 and 61.35% for pollen of clover, eucalyptus, and maize respectively (Figure 6), with a statistically significant ($p < 0.05$) difference between them (Table 2).

Moreover, as reported in Table 2, FAO, 2011 recommends that adults (>18 years), for an individual of 75 kg consume, 50 g/day of protein, 80 g/day of fat, 30 g/day of fiber and 320 g/day of carbohydrates including fiber. For these individuals, a 25 g of eucalyptus pollen (the equivalent of one soupspoon) can provide up to 8.6, 2.49, 3.68 and 3.95% of the recommended daily intake of protein, fat, fiber, and carbohydrates, while it can provide up to 11.6, 1.72, 4.18, and 3.22%, respectively, for pollen of clover. Meanwhile, it is provided 8.18, 2.08, 3.29, and 3.99% of daily requirement intake for adult humans form the main components of maize pollen, respectively.

Figure 7 shows that the fresh pollen samples had an energy value ranging from 307.31 to 342.29 kcal. The energy has been primarily attributed to its higher sugar content which is easily digestible (White, 1980). The enzymes break down polysaccharides into the necessary energy sources monosaccharaides and disaccharides.

Polyphenols have great importance, because they help the human body to fight against diseases. Determining the functions of foods or dietary supplements used as antioxidants has grown crucial, especially in recent years as the prevalence of several diseases such as cancer has increased (Gercek et al., 2021). Since phenolic chemicals have been acknowledged as potentially helpful taxonomic identifiers, there have been more investigations on their characterisation in pollen (Mosić et al., 2019). Depending on the geographic origin, time of harvest, and plant source, the phenolic content of pollen varies considerably (Soares de Arruda et al., 2020). The results in Figure 1 showed the phytochemical measurements (total phenolic content, antioxidant capacity) of

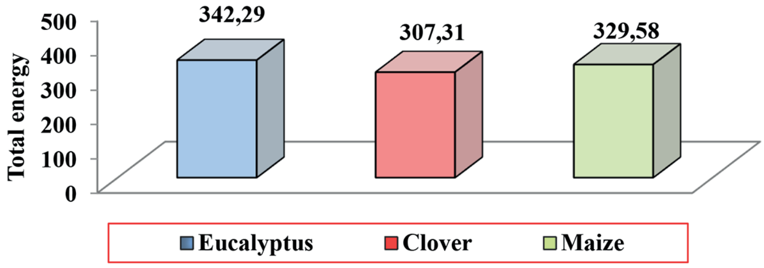

Figure 7. Total energy of bee pollen samples

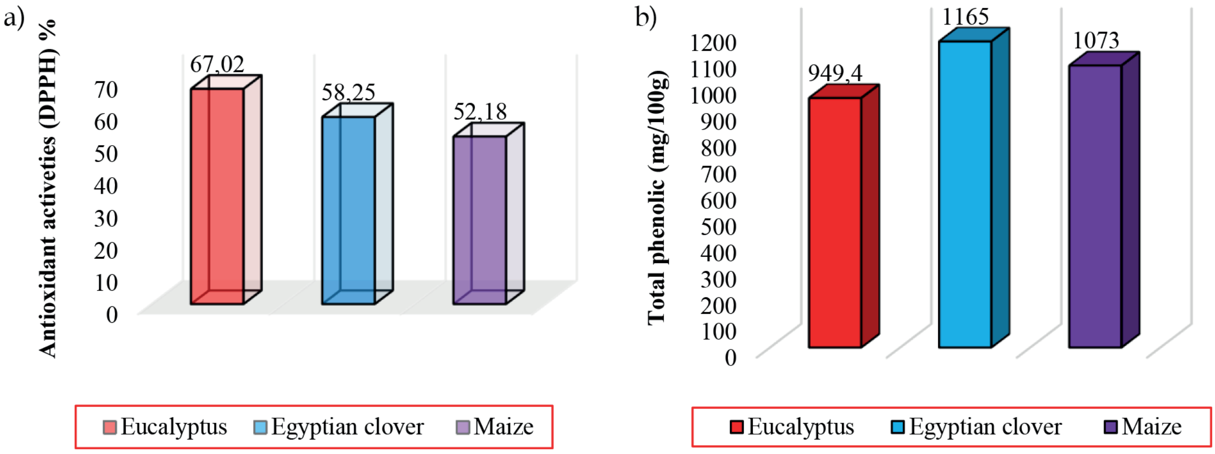

Figure 8. Total phenolic contents (mg GAE/100 g) and antioxidant activities (%) of pollen samples

three types of pollen collected by honeybee colonies. The tested pollen contains considerable amounts of phenolic compounds.

Total phenolic ranged from 949.4 to 1165 mg/100 g of the pollen samples. The largest amount was detected in the pollen of clover (1165 mg/100 g), followed by maize pollen (1073 mg/100 g), while the lowest value was recorded for the eucalyptus sample (949.4 mg/100 g), with statistically significant differences ($p < 0.05$) between all pollen types as shown in Figure 8. These findings were similar to those obtained by Barbieri et al., 2020 who showed that the total phenolic content ranged between 578 to 2015 mg/100g of pollen, while the values were lower than those obtained by Domenci et al., 2017; Feas et al., 2012 where they were ranged from 1290 to 2475 mg/100g. Meanwhile, the values found in the presented paper were higher than those obtained by Bujang et al. (2021), and Žilić et al. (2014), they ranged from 741.94 to 843.38 mg/100g.

The DPPH free radical scavenging activity is one of the most widely used assays for assessing pollen antioxidant properties. Figure 8 shows the ascorbic acid equivalents and depicts the antioxidant capabilities of the pollen under consideration. Eucalyptus pollen exhibited the highest DPPH scavenging activity (67.02%), whereas maize pollen had the lowest percentage (52.18%), with significant variations. According to the findings, the high phenolic content may be responsible for the free radical scavenging effect. Pascoal et al. (2014) showed an association between pollen's total phenolic content and antioxidant activity, which supported the conclusions of this study.

Amino acid profile

The content of individual amino acid in the eucalyptus, Egyptian clover, and maize pollen were investigated and are presented in Table 3. From the Table, at least 17 amino acids, including nine essential amino acids (EAAs) and eight non-essential amino acids (NEAAs) were determined. Tryptophan was not determined, presumably due to the limitation of the acid hydrolysis process.

Amongst essential amino acids, leucine shows the highest quantities, ranging from 11.7 to 16.9 mg/g DM and making up 7.21–8.19% of total amino acids. Valine (9.7–11.7 mg/g), lysine (9.2–11.7 mg/g). Moreover, moderate amounts of other essential amino acids were observed in pollen samples. Among non-essential amino acids, glutamic and aspartic acid were found predominant

(23.5 and 20.1 mg/g DM) and made up 13.02 and 11.3% of total amino acids in eucalyptus pollen. In clover pollen, proline followed by glutamic and aspartic acid present 16.7, 11.02, and 10.25 % of total amino acids (39.4, 25.6 to 23.8 mg/g DM). Additionally, in maize pollen, proline followed by aspartic then glutamic acid present 12.33, 11.16, and 10.36%, respectively, of total amino acids (20, 18.1, and 16.8 mg/g DM). Meanwhile, moderate amounts of other nonessential amino acids were observed in both pollen samples.

Leucine, isoleucine, and valine are branched-chain amino acids (BCAA) that play a role in stimulating protein synthesis and constitute 33% of muscle protein in humans, recovery processes from exercise, and protecting mental health. From Table 3, BCAA was 29, 33.3, and 38.4 mg/g of maize, eucalyptus, and clover pollen, respectively, it is present at 17.87, 18.44, and 16.53% of total amino acids.

For total amino acid contents, pollen showed variations as presented in Table 4. The average total amino acid amount was 191.6 g/100g DM. The highest values of total amino acids (232.2 mg/g DM) were obtained from clover pollen, followed by pollen of eucalyptus (180.5 mg/g DM). Meanwhile, the lowest values (162.1 mg/g DM) were recorded for maize pollen.

Based on the total measured amino acids, the amounts of total essential amino acids (EAAs) in

Table 3. Amino acid composition (mg/g DM and % of individual amino acid from total amino acids) of pollen samples

Amino acid	Eucalyptus		Clover		Maize		** Minimal levels (g/100 g protein)
	mg/g	%	mg/g	%	mg/g	%	
Leucine*	14.8	8.19	16.9	7.27	11.7	7.21	5.9
Isoleucine*	8.3	4.60	9.8	4.22	7.6	4.68	3.0
Valine*	10.2	5.65	11.7	5.04	9.7	5.98	3.9
Threonine*	9.8	5.42	11.5	4.95	8.8	5.42	2.3
Phenylalanine*	9.5	5.26	12.0	5.16	7.8	4.81	3.8
Histidine*	4.9	2.71	5.3	2.28	3.4	2.09	1.5
Lysine*	11.6	6.42	11.7	5.04	9.2	5.67	4.5
Methionine*	3.9	2.16	4.6	1.98	3.6	2.22	1.6
Arginine	11.5	6.37	10.8	4.65	8.2	5.05	-
Proline	9.9	5.48	39.4	16.97	20.0	12.33	-
Tyrosine	7.6	4.21	9.2	3.96	5.3	3.26	-
Serine	9.2	5.09	12.4	5.34	8.7	5.36	-
Glutamic	23.5	13.02	25.6	11.02	16.8	10.36	-
Glycine	9.4	5.20	10.9	4.96	8.7	5.36	-
Alanine	11.4	6.31	13.0	5.60	11.0	6.78	-
Cysteine	4.9	2.71	3.6	1.55	3.5	2.15	-
Aspartic	20.1	11.13	23.8	10.25	18.1	11.16	-
***BCAA mg/g	33.3	18.44	38.4	16.53	29.0	17.87	10-20 g/day

Note: *represents the essential amino acid for humans, **minimal levels of essential amino acids required by adult humans (FAO, 2007). BCAA: branched-chain amino acids (leucine, isoleucine, and valine), ***dietary supplements were recommended by BCAA by 10–20 grams per day.

Table 4. Total amino acids, essential amino acids, and non-essential amino acids composition of different pollen samples

Botanical origin	TAs* (mg/g)	TEAs (mg/g)	TEAs** %	TNEAs (mg/g)	TNEAs*** %
Eucalyptus	180.5	73.0	40.45	107.5	59.55
Egyptian clover	232.2	83.5	35.97	148.7	64.03
Maize	162.1	61.8	38.13	100.3	61.87
Average	191.6	82.93	38.19	108.66	61.81

Note: *total amino acids, **TEAs % of total essential amino acids/total amino acids, ***TNEAs % of total non-essential amino acids/total amino acids.

Table 5. Amino acid score of pollens compared to the minimum requirements of adult humans

Amino acid	Eucalyptus	Egyptian clover	Maize
Leucine	116.77	91.18	93.05
Isoleucine	128.66	104.0	118.66
Threonine	198.26	159.13	154.78
Phenylalanine	116.31	100.52	96.31
Histidine	152.0	112.0	106.0
Lysine	120.0	82.66	95.77
Valine	121.79	95.38	116.66
Methionine	113.12	91.25	105.62
Average	133.36	104.51	110.85

maize, eucalyptus, and clover pollen were 61.8, 73, and 83.5 mg/g respectively, for maize, eucalyptus, and clover pollen. The eucalyptus pollen sample had the highest ratio of total essential amino acids to total amino acids (40.45%) while the lowest ratio (35.97%) was discovered in clover pollen. The calculated values exceed FAO reference protein by 33.9% (FAO, 1991). The nutritive value of proteins for any biological function is limited by the relative proportions comprising EAAs. Adult humans may benefit nutritionally from high quantities of EAAs. These findings support the notion that pollen is a "healthy" source of superior protein.

An indication for determining the effectiveness of proteins and amino acids needed for various demographic groups is amino acid scoring. It is based on the idea that body cells cannot synthesise proteins unless they are provided with the necessary amino acids through nutrition. In light of the amount of essential amino acids contained in food material, the amino acids score reflects the protein quality, and amino acids present in low concentrations are referred to as limiting amino acids (FAO, 2013). Therefore, concerning the minimal amino acid requirements of humans FAO/WHO/UNU (FAO, 2007) the chemical scores of the EAAs of pollen can be calculated, reflecting the nutritional value of pollen for humans Table 5. According to the chemical scores of essential amino acids, only the essential amino acid content of the pollen eucalyptus met the requirements of adult humans (Table 5). The pollen of maize had three limiting amino acids (leucine, lysine, and phenylalanine). In addition, four limiting amino acids were found in the clover pollen (lysine, leucine, methionine, and valine). The lowest values for chemical scores indicate that there were fewer amino acids present than was necessary.

CONCLUSIONS

In this research, three pollen varieties, eucalyptus (*Eucalyptus sp.*), Egyptian clover (*Trifolium alexandrinum*), and maize (*Zea mays*) were evaluated, for the proximate chemical composition, energy value, amino acid, and antioxidants contents. The pollen samples' energy values ranged from 415.98 to 429.19 kcal. Egyptian clover has a greater total amino acid content (232.2 mg/g) than eucalyptus (180.5 mg/g) and maize (162.1 mg/g). Leucine, lysine, valine, threonine, and phenylalanine were the highest essential amino acid concentrations in the examined pollen samples. Compared to other non-essential amino acid types, proline, glutamic, and aspartic acids were found to have the highest concentrations in both pollen samples. The minimum necessary amount of essential amino acids for adult humans was entirely satisfied by the eucalyptus pollen.

Acknowledgements

The authors extend their appreciation to Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia for providing support for this study. All thanks go to the Dean of the Faculty of Agriculture in Cairo, Al-Azhar University.

Funding

This research was funded by the College of Science and HumanitiesHuraymila, Imam Mohammed Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia and the Faculty of Agriculture at Al-Azhar University, Cairo, Egypt.

REFERENCES

1. Almeida-Muradian, L.B., Pamplona, L.C., Coimbra, S., Barth, O.M. 2005. Chemical composition and botanical evaluation of dried bee pollen pellets. Journal of Food Composition and Analysis, 18(1), 105–111. https://doi.org/10.1016/J.JFCA.2003.10.008
2. Amarasinghe, N.K., Wickramasinghe, I., Wijesekara, I., Thilakarathna, G., Deyalage, S.T. 2021. Functional, physicochemical, and antioxidant properties of flour and cookies from two different banana varieties (Musa acuminata cv. Pisang awak and Musa acuminata cv. Red dacca). International Journal of Food Science, 2021. https://doi.org/10.1155/2021/6681687

3. Bakour, M., Fernandes, Â., Barros, L., Sokovic, M., Ferreira, I.C.F.R., Badiaa L. 2019. Bee bread as a functional product: Chemical composition and bioactive properties. LWT, 109, 276–282. https://doi.org/10.1016/J.LWT.2019.02.008
4. Barbieri, D., Gabriele, M., Summa, M., Colosimo, R., Leonardi, D., Domenici, V., Pucci, L. 2020. Antioxidant, nutraceutical properties, and fluorescence spectral profiles of bee pollen samples from different botanical origins. Antioxidants 9(10), 1001. https://doi.org/10.3390/ANTIOX9101001
5. Bishop, C.A., Machate, T., Henning, T., Henkel, J., Püschel, G., Weber, D., Weitkunat, K. 2022. Detrimental effects of branched-chain amino acids in glucose tolerance can be attributed to valine induced glucotoxicity in skeletal muscle. Nutrition & Diabetes, 12(1), 1–9.
6. Block, R.J., Durrum, E.L., Zweig, G. 2016. A manual of paper chromatography and paper electrophoresis. Elsevier.
7. Bujang, J.S., Zakaria, M.H., Ramaiya, S.D. 2021. Chemical constituents and phytochemical properties of floral maize pollen. PLOS ONE, 16(2), e0247327. https://doi.org/10.1371/JOURNAL.PONE.0247327
8. Campos, M.G., Frigerio, C., Bobiş, O., Urcan, A.C., Gomes, N.G.M. 2021. Infrared irradiation drying impact on bee pollen: Case study on the phenolic composition of eucalyptus globulus labill and salix atrocinerea brot. Pollens. Processes, 9(5), 890. https://doi.org/10.3390/PR9050890
9. Domenci, V., Gabriele, M., Parri, E., Felicioli, A., Sagona, S., Pozzo, L., Biondi, C., Pucci, L. 2017. Phytochemical composition and antioxidant activity of Tuscan bee pollen of different botanic origins. Italian Journal Of Food Science, 27(2), 120–131. https://doi.org/10.14674/1120-1770/IJFS.V191
10. Feas, X., Vazquez-Tato, M.P., Estevinho, L., Seijas, J.A., Iglesias, A. 2012. Organic bee pollen: botanical origin, nutritional value, bioactive compounds, antioxidant activity and microbiological quality. Molecules, 17(7), 8359–8377. https://doi.org/10.3390/MOLECULES17078359
11. Food and Agriculture Organization of the United Nations. 1991. Amino acid scoring pattern. In: Protein quality evaluation –Report of the Joint FAO/WHO Expert Consultation.
12. Food and Agriculture Organization of the United Nations. 2007. Protein and amino acid requirements in human nutrition: Report of an FAO/WHO/UNU Expert Consultation. WHO Technical Report Series No. 935. World Health Organization, Geneva.
13. Food and Agriculture Organization of the United Nations. 2011. Dietary protein quality evaluation in human nutrition.
14. Food and Agriculture Organization of the United Nations. 2013. Chapter 4-Food and Nutrition Paper 92. Rome. 29.
15. Food and Agriculture Organization of the United Nations. 2017. State of Food Insecurity and Nutrition in the World. Rome.
16. Gercek, Y.C., Celik, S., Bayram, A.S. 2021. Screening of plant pollen sources, polyphenolic compounds, fatty acids and antioxidant/antimicrobial activity from bee pollen. Molecules, 27(1), 117. https://doi.org/10.3390/MOLECULES27010117
17. Günalan, E., Turhan, S., Yıldırım, B., Kaya Cebioğlu, İ., Çonak, Ö. 2022. Quality and reliability analysis of youtube videos about branched-chain amino acids supplements: A pilot study. In U. Acaröz (Eds.).. International Congress on Biological and Health Sciences, ICBH, 342.
18. Kalaycıoğlu, Z., Kaygusuz, H., Döker, S., Kolaylı, S., Erim, F.B. 2017. Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. LWT, 84, 402–408. https://doi.org/10.1016/J.LWT.2017.06.003
19. Keskin, M., Özkök, A. 2020. Effects of drying techniques on chemical composition and volatile constituents of bee pollen. Czech Journal of Food Sciences, 38(4), 203–208. https://doi.org/10.17221/79/2020-CJFS
20. Khalifa, S.A.M., Elashal, M., Kieliszek, M., Ghazala, N.E., Farag, M.A., Saeed, A., Xiao, J., Zou, X., Khatib, A., Göransson, U., El-Seedi, H.R. 2020. Recent insights into chemical and pharmacological studies of bee bread. Trends in Food Science & Technology, 97, 300–316. https://doi.org/10.1016/J.TIFS.2019.08.021
21. Kieliszek, M., Piwowarek, K., Kot, A.M., Błażejak, S., Chlebowska-Śmigiel, A., Wolska, I. 2018. Pollen and bee bread as new health-oriented products: A review. Trends in Food Science & Technology, 71, 170–180. https://doi.org/10.1016/J.TIFS.2017.10.021
22. Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., Olczyk, K. 2015. Bee pollen: Chemical composition and therapeutic application. Evidence-Based Complementary and Alternative Medicine.
23. Kostić, A., Milinčić, D.D., Barać, M.B., Shariati, M.A., Tešić, Ž.L., Pešić, M.B. 2020. The Application of Pollen as a Functional Food and Feed Ingredient—The Present and Perspectives. Biomolecules, 10(1), 84. https://doi.org/10.3390/BIOM10010084
24. Li, Q.Q., Wang, K., Marcucci, M.C., Sawaya, A.C.H.F., Hu, L., Xue, X.F., Wu, L. M., Hu, F.L. 2018. Nutrient-rich bee pollen: A treasure trove of active natural metabolites. Journal of Functional Foods, 49, 472–484. https://doi.org/10.1016/J.JFF.2018.09.008

25. Lorini, A., da Rosa, C.C.B., Oliveira, L.T., Wobeto, C. 2020. Chemical composition and microbiological quality of bee pollen. Scientia Agraria Paranaensis, 229–235.
26. Mayda, N., Özkök, A., Ecem Bayram, N., Gerçek, Y.C., Sorkun, K. 2020. Bee bread and bee pollen of different plant sources: Determination of phenolic content, antioxidant activity, fatty acid and element profiles. Journal of Food Measurement and Characterization, 14(4), 1795–1809. https://doi.org/10.1007/S11694-020-00427-Y/TABLES/5
27. Mosić, M., Trifković, J., Vovk, I., Gašić, U., Tešić, Ž., Šikoparija, B., Milojković-Opsenica, D. 2019. Phenolic composition influences the health-promoting potential of bee-pollen. Biomolecules, 9(12), 783. https://doi.org/10.3390/BIOM9120783
28. National Institutes of Health Office of Dietary Supplements. 2017. Dietary supplements for exercise and athletic performance.
29. Official methods of analysis of the association. 2016. Official methods of analysis of the association of official analytical chemists international (20th Edition). Journal of the Association of Official Agricultural Chemists, Maryland.
30. Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., Estevinho, L.M. 2014. Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology, 63, 233–239. https://doi.org/10.1016/J.FCT.2013.11.010
31. Sadeq, O., Mechchate, H., Es-Safi, I., Bouhrim, M., Jawhari, F.Z., Ouassou, H., Kharchoufa, L., Alzain, M.N., Alzamel, N.M., Mohamed Al Kamaly, O., Bouyahya, A., Benoutman, A., Imtara, H. 2021. Phytochemical screening, antioxidant and antibacterial activities of pollen extracts from micromeria fruticosa, achillea fragrantissima, and phoenix dactylifera. Plants, 10(4), 676. https://doi.org/10.3390/PLANTS10040676
32. Sakooei-Vayghan, R., Peighambardoust, S.H., Hesari, J., Soltanzadeh, M., Peressini, D. 2020. Properties of dried apricots pretreated by ultrasound-assisted osmotic dehydration and application of active coatings. Food Technology and Biotechnology, 58(3), 249–259. https://doi.org/10.17113/FTB.58.03.20.6471
33. Soares de Arruda, V.A., Vieria dos Santos, A., Figueiredo Sampaio, D., da Silva Araújo, E., de Castro Peixoto, A. L., Estevinho, L. M., de Almeida-Muradian, L. B. 2020. Brazilian bee pollen: Phenolic content, antioxidant properties and antimicrobial activity, 60(5), 775–783. https://doi.org/10.1080/00218839.2020.1840854
34. Taha, E.K.A., Al-Kahtani, S., Taha, R. 2019. Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia. Saudi Journal of Biological Sciences, 26(2), 232–237. https://doi.org/10.1016/J.SJBS.2017.06.003
35. Thakur, M., Nanda, V. 2020a. Exploring the physical, functional, thermal, and textural properties of bee pollen from different botanical origins of India. Journal of Food Process Engineering, 43(1), e12935. https://doi.org/10.1111/JFPE.12935
36. Thakur, M., Nanda, V. 2020b. Composition and functionality of bee pollen: A review. Trends in Food Science & Technology, 98, 82–106. https://doi.org/10.1016/J.TIFS.2020.02.001
37. White, J.W., D.LW. 1980. Honey composition and properties. In: Beekeeping in the United States Agricultural Handbook (Revised). Washington, DC: US Department of Agriculture, Government Printing Office, 82–91.
38. Wu, W., Wang, K., Qiao, J., Dong, J., Li, Z., Zhang, H. 2019a. Improving nutrient release of wall-disrupted bee pollen with a combination of ultrasonication and high shear technique. Journal of the Science of Food and Agriculture, 99(2), 564–575. https://doi.org/10.1002/JSFA.9216
39. Žilić, S., Vančetović, J., Janković, M., Maksimović, V. 2014. Chemical composition, bioactive compounds, antioxidant capacity and stability of floral maize (Zea mays L.) pollen. Journal of Functional Foods, 10, 65–74. https://doi.org/10.1016/J.JFF.2014.05.007