

# Chemical composition and physical properties of coffee (*Coffea robusta*) bee pollen in Daklak province, Vietnam

L.P.T. Quoc\* 

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 700000, Ho Chi Minh City, Vietnam

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## ABSTRACT

The main purpose of this study was to determine the chemical composition of monofloral bee-collected pollen from the coffee (*Coffea robusta*) plant and its pellet morphology. According to the results coffee bee pollen (CBP) has a high nutritional value. It contains a large percentage of protein, reducing sugar, and lipid. Also, vitamins (B<sub>2</sub> and E) and minerals (K, Ca, and P) were found in CBP. In addition, the results revealed that the total polyphenols content (TPC) was 10.62 mg GAE/g dry weight (DW) and antioxidant activity (AA) of CBP was 5.52  $\mu$ mol TE/g DW. Some physical properties of CBP pellet were recorded such as moisture, relative diameter, sphericity, bulk density, and colour parameters. The pollen pellet exhibits shapes of colour from light to dark yellow. These results pointed out that CBP can be used as a remarkable source of compounds with antioxidant activity and health-protective capacity for humans.

## KEYWORDS

bee pollen, *Coffea robusta*, composition, phenolic compounds, structure

\* Corresponding author. Tel.: +084 906 413 493. E-mail: lephamtanquoc@iu.edu.vn

## 1. INTRODUCTION

Bee pollen is collected from flowers by honeybees. It is a mixture of pollen, salivary substance, nectar, wax, and honey. It is made by worker honeybees and used as the main food source for the hive. Raw bee pollen was collected by pollen traps at the entrance of the hives (Li et al., 2018). Nowadays, bee pollen is considered to be a great resource in food processing; it contains a large amount of carbohydrate, protein, lipid, fibre, and phenolic compounds (Komosinska-Vashev et al., 2015). In addition, many essential amino acids are also found in bee pollen from floral sources; for instance, valine, leucine, isoleucine, phenylalanine, lysine, arginine, cysteine, tryptophan, and tyrosine (Taha et al., 2019). However, the chemical composition may significantly vary according to the plant source, climatic conditions, and geographic origin (Nogueira et al., 2012). Essentially, bee pollen has many benefits for human health. According to Li et al. (2018), bee pollen possesses antioxidant, antibacterial, anti-inflammatory, anticarcinogenic, and antiallergic properties.

In Vietnam, a large amount of bee pollen is collected from the coffee (*Coffea robusta*) plant. However, farmers in Vietnam store this material and primarily use it as food for the hive in severe weather conditions. Until now, none of the references have recorded studies about this material in Vietnam. Therefore, the objective of this study is to determine the main chemical composition, antioxidant activity, physical properties, and morphological structure of coffee bee pollen (CBP) in Daklak province (Vietnam) in order to investigate its nutritional value and physical properties.

## 2. MATERIALS AND METHODS

### 2.1. Pollen samples and chemicals

CBP was produced and collected by pollen traps in the coffee farm (Daklak province, Vietnam). Pollen pellets were solar-dried until the moisture was lower than 10%. Then, they were packed in a polyethylene (PE) bag and stored at room temperature (28–30 °C) for one month (Fig. 1). All chemicals used in this study were of analytical reagent grade.

### 2.2. Determination of moisture and ash content of CBP

To determine the moisture content, sample (1 g) was dried in a moisture analyser at 105 °C to constant weight. Before and after the drying process, sample weight was recorded. The difference in these weights represents the moisture lost and the percentage of moisture content was calculated.

To determine ash content, the powdered CBP (1 g) was placed in a porcelain crucible and then incinerated in an oven at 550 °C until white ash was obtained. Then, it was cooled in a desiccator and the obtained ash was weighed.

### 2.3. Chemical analysis

Essential nutrients of CBP were determined by various analytical methods. Protein, lipid, reducing sugar, and vitamin B<sub>2</sub> were analysed according to ISO/TS 16634-2:2009, FAO/FNP (1986), TCVN 4594:1988, and QTTN/KT3 077:2012, respectively; while vitamin E, potassium





Fig. 1. Coffee bee pollen (CBP) pellets

(K), calcium (Ca), and phosphorus (P) were determined following [AOAC \(2016\)](#) methods (including AOAC 992.03, 969.23, and 985.01, respectively).

#### 2.4. Preparation of extract of CBP

The dried CBP was ground and then passed through a sieve (diameter of 0.6 mm). The powdered CBP (2 g) was extracted with distilled water as the solvent (solid to solvent ratio was 1/20, w/v) using a microwave at the power of 314 W for 5 min. Next, the extract obtained was filtered by Whatman filter paper (No.4) and then TPC and AA were analysed.

#### 2.5. Determination of TPC and AA of CBP

The TPC was measured according to the method of [Siddiqua et al. \(2010\)](#), and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of CBP (mg GAE/g DW). The AA was determined by the procedure of [Quoc and Muoi \(2015\)](#), and the results were expressed as  $\mu\text{mol}$  of Trolox equivalent (TE) per gram of dry weight of CBP ( $\mu\text{mol TE/g DW}$ ).

#### 2.6. Determination of equivalent diameter (ED), angle of repose (AOR), bulk density (BD), and 1,000 pellets weight of CBP

According to [Thakur and Nanda \(2018\)](#), the ED was calculated following the formula  $ED = (L \times W \times T)^{1/3}$ , where  $L$ ,  $W$ , and  $T$  are the length, width, and thickness of the CBP pellet



(mm), respectively; whereas sphericity ( $S$ ) was estimated using the following expression:  $S = ED/L \times 100$  (%).

The AOR was determined according to the procedure of Liu (2011) with some minor changes. The CBP pellets were filled to the total height of the inox cylinder (internal diameter of 9 cm and height of 17 cm). The surfaces on which spreading took place were a smooth surface (polished granite surface). The cylinder was lifted at a slow velocity (approximately  $5 \text{ cm s}^{-1}$ ). The inverse tangent of the ratio of height and radius of the base of the cone was the AOR.

The CBP pellets were gently poured into an empty cylinder (1 L). The heap above the cylinder was then levelled with a blunt ruler. Next, the pellets inside the cylinder were weighed and the BD of CBP pellets was expressed as  $\text{kg m}^{-3}$ .

1,000 pollen pellets from a random sample were counted manually and then weighed in grams using a balance with an accuracy of 0.01 g.

## 2.7. Colour parameters

Colour parameters of samples were measured using a CS-10 colorimeter (China) with  $L^*$  (from darkness to lightness),  $a^*$  (from greenness to redness), and  $b^*$  (from blueness to yellowness) values.

## 2.8. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of CBP were recorded on a Bruker Tensor 27 (Germany) spectrophotometer in the  $4,000\text{--}400 \text{ cm}^{-1}$  region at the resolution of  $1 \text{ cm}^{-1}$  using potassium bromide (KBr) pellets mixed with sample before experiments.

## 2.9. Scanning electron micrograph

Micromorphology of samples was evaluated by a scanning electron microscope (SEM) (Jeol JSM-6400, Japan). SEM was used at 5 kV and the vacuum pressure was 0.04 Pa with various magnifications.

## 2.10. Data analysis

All analyses were performed in triplicate except for the physical properties analysis, which was done ten times and the obtained results were expressed as mean  $\pm$  standard deviation. All statistical analyses were performed using the software Microsoft Excel (version 14.0.4760.1000, USA).

# 3. RESULTS AND DISCUSSIONS

## 3.1. Chemical composition

The nutritional composition of CBP pellets is presented in Table 1. The moisture content of CBP pellets was less than 8%, which is similar to other scientific studies, for instance, oak bee pollen (4.4%) (Ghosh and Jung, 2017) or fresh pollen of sunflowers (6.78%) (Nicolson and Human, 2013). The low moisture of the CBP pellets in this study could be of help in long-term storage. Arruda et al. (2013) also pointed out that the moisture of pollen should be kept at a low level (4–8%).



Table 1. Chemical composition of CBP pellets

Nutrients	Content
Moisture (%)	6.76 ± 0.68
Ash (g/100 g)	3.23 ± 0.21
Protein (g/100 g)	27.80 ± 0.80
Lipid (g/100 g)	5.72 ± 0.29
Reducing sugar (g/100 g)	28.40 ± 0.85
Vitamin B <sub>2</sub> (mg/100 g)	0.06 ± 0.01
Vitamin E (mg/100 g)	3.11 ± 0.62
K (mg/100 g)	673 ± 135
Ca (mg/100 g)	166 ± 33
P (mg/100 g)	695 ± 139
TPC (mg GAE/g DW)	10.62 ± 0.18
AA (μmol TE/g DW)	5.52 ± 0.12

This preserves the nutrients in the pollen and dried pollen ensures safety for humans. However, if bee pollen is dried until the moisture level is less than 3%, discoloration and chemical reactions (Maillard reaction and lipid oxidation) will result in the rancidity of bee pollen (Nogueira et al., 2012).

Ash content is the inorganic component of pollen and it is approximately 3.23 g/100 g. This is lower than the results recorded by Ghosh and Jung (2017) for oak pollen (5.3%) and hardy kiwi pollen (5.2%) samples, but higher than that reported by Negrão and Orsi (2018) in bee pollen samples in Botucatu (Brazil) (2.22–2.58%).

Lipid, protein, and reducing sugar contents in CBP pellets were 5.72, 27.80, and 28.40 g/100 g, respectively. Although lipid content only accounts for a small portion (5.72 g/100 g) of this sample, it plays an important role in attracting bees (Singh et al., 1999). However, high lipid concentration is subject to the oxidation process, which leads to the rancidity of the product. In general, the lipid content in this study is similar to that of oak and hardy kiwi pollen (Ghosh and Jung, 2017). A high protein level was also found in CBP, and it is considered to be a rich nutrient source for bees and for humans. It was higher than bee pollen from various other sources such as alfalfa, date palm, summer squash, sunflower, and rape bee pollen (15.19–20.23 g/100 g DW) in the study reported by Taha et al. (2019). This proved that CBP pellets could be a valuable special food for humans in the future. Reducing sugars constituted the main portion with values going up to 28.40 g/100 g. It is also the major component in nectar sugar that sticks coffee pollen grains together. This result was higher than that reported by Chantarudee et al. (2012) for corn bee pollen (14.11 g/100 g) and lower than that studied by Taha (2015) for date palm bee pollen (Fructose and glucose contents were 21.3% and 17.06%, respectively). This shows that the chemical composition in bee pollen strongly depends on the initial materials and species of bees.

Many literature studies have argued that vitamins were detected in bee pollen, especially group-B vitamins, vitamin E, and vitamin C (Arruda et al., 2013; Chantarudee et al., 2012; Negrão and Orsi, 2018). In this study, we also found the presence of vitamin B<sub>2</sub> (riboflavin) at the low concentration of 0.06 mg/100 g. This is lower than that of bee pollen (1.73–2.56 mg/100 g) from Botucatu, Brazil (Arruda et al., 2013). Besides, CBP pellets also contain vitamin E (3.11 mg/100 g). Compared to other samples from elsewhere, this CBP sample contained a lower level of vitamin E



than that found in corn bee pollen from Nan province, Thailand (6.21 mg/100 g) (Chantarudee et al., 2012). These compounds are quite important and necessary to ensure the normal growth and health maintenance of animals as well as humans.

According to Komosinska-Vassev et al. (2015), macro- and micronutrients in bee pollen could exist in the amount of 1.6%. In the range of this study, the macronutrient content determined included only phosphorus (P), potassium (K), and calcium (Ca) as 695, 673, and 166 mg/100 g, respectively. In general, the predominant minerals were P and K, followed by Ca. The trend of the results is in agreement with the report of Carpes et al. (2009), who studied the composition of bee pollen from Southern Brazil. These minerals are essential components for cell protection and maintaining homeostasis, assist in the development and maintenance of bone tissue and in regulating the blood (Thakur and Nanda, 2020).

The TPC and AA of CBP were also determined in this study. They were 10.62 mg GAE/g DW and 5.52  $\mu$ mol TE/g DW, respectively. These bioactive compounds play an extremely important role in plant physiology and are involved in plant growth and reproduction. In addition, they have a high antioxidant capacity and prevent pathogens from infecting plants. Compared to other studies, TPC in CBP were lower than the values in organic bee pollen harvested in the Douro International Natural Park, Portugal (12.9–19.8 mg GAE/g of extract) (Feás et al., 2012) or bee pollen procured from various regions of India (15.50–25.63 mg GAE/g DW) (Thakur and Nanda, 2021); while they were higher than that in sunflower bee pollen in Vojvodina province, Serbia (2.9–3.8 mg GAE/g DW) (Kostić et al., 2019). The aqueous extract showed that the antioxidative activity of CBP was lower than that of bee pollen extract from Poland (0.30–0.69 mmol TE/g of extract) (Rzepecka-Stojko et al., 2016); these authors used pepsin and ethanol to extract phenolic compounds from bee pollen. These results prove that the TPC and AA are significantly affected by the source of the sample and the extraction method.

3.2. Physical properties

The physical properties of bee pollen are important parameters for processing and preservation of the products. As seen in Table 2, the ED obtained was only 2.51 mm, which is higher to that of coconut bee pollen (2.18 mm) and lower than that of coriander bee pollen (2.72 mm) (Thakur and Nanda, 2018). This could be explained by the fact that ED depends on the moisture of the

Table 2. Physical properties of CBP pellets

Physical properties	Value
Length of pollen pellet (mm)	3.28 ± 0.09
Width of pollen pellet (mm)	2.87 ± 0.16
Thickness of pollen pellet (mm)	1.69 ± 0.17
Equivalent diameter (ED, mm)	2.51 ± 0.06
Sphericity (S, %)	76.55 ± 2.80
1,000 pollen pellets weight (g)	4.06 ± 0.04
Bulk density (BD, kg m <sup>-3</sup> )	636.50 ± 1.98
Angle of repose (AOR,°)	15.99 ± 0.56
<i>L</i> <sup>*</sup>	62.37 ± 1.56
<i>a</i> <sup>*</sup>	9.54 ± 0.14
<i>b</i> <sup>*</sup>	35.04 ± 0.76





sample and the structure of CBP. Changes in the dimensions (length, width, thickness) of samples significantly affect sphericity. In this study, the CBP pellets had egg, oval, or sphere shape. Therefore, the sphericity of the sample was approximately 76.55%. It was higher than that of multi-floral bee pollen from India (70.69%) (Thakur and Nanda, 2018).

The 1,000 pellets weight and BD were 4.06 g and  $636.50 \text{ kg m}^{-3}$ , respectively. These parameters will vary with CBP sizes. Compared to other materials, the 1,000 pellets weight in this study was heavier than that of the seeds of *Arabidopsis thaliana* ecotypes Bur and Cvi (29.7–37.3 mg) (Huang et al., 2014) and lower than that of cereal seeds such as paddy (19.18 g), wheat (46.18 g), etc (Deivasigamani and Swaminathan, 2018). In addition, because the ED of the sample was quite small, the porosity of the pollen pellet could be low and the BD obtained was at the high level, approximately that of a grain of barley ( $566\text{--}615 \text{ kg m}^{-3}$ ) or canola ( $671 \text{ kg m}^{-3}$ ) (Nelson, 2002).

The AOR value is important for the design of the agricultural machine hopper and storage, and conveying equipment of grains. In this study, the low moisture content and the smooth and round pollen pellet could be both responsible for the low AOR (15.99°). Besides, although the amount of sugar in the sample was quite high, it did not cause clumping of the pellets. This is a remarkable advantage of CBP pellets, because the transporting process will require less energy (Teferri, 2019).

The colour is also a necessary standard in evaluating the quality of bee pollen. The  $L^*$ ,  $a^*$ , and  $b^*$  values of the samples were 62.37, 9.54, and 35.04, respectively. These results implied that the colour of CBP pellet was brighter, redder, and yellower than that of coconut, coriander, or rapeseed bee pollen (Thakur and Nanda, 2018). The different colours of samples could be affected by the difference in a gene or their various chemical compositions, especially pigments (Meléndez-Martínez et al., 2007).

### 3.3. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of CBP is shown in Fig. 2. The presence of –OH groups in CBP samples was determined by the wide band of approximately  $3,383 \text{ cm}^{-1}$ . In addition, intense absorption of approximately  $3,000\text{--}2,800 \text{ cm}^{-1}$  was recorded because of the distension of –CH, –CH<sub>2</sub>, –CH<sub>3</sub> (Coates, 2000). These bonds are quite popular in many organic compounds in food products related to carbohydrates. The spectral region from  $1,700$  to  $1,500 \text{ cm}^{-1}$  pointed out that aromatic ring (aryl) group is present. Besides, the absorption band at  $1,418 \text{ cm}^{-1}$  identified the methyl (–CH<sub>3</sub>) group in CBP. These results are similar to the findings of Thakur and Nanda (2018) for coconut bee pollen. Further, the mild peaks from  $1,270$  to  $1,230 \text{ cm}^{-1}$  were attributed to the aromatic ethers or aryl –O stretch. Moreover, the wavenumbers from  $1,090$  to  $1,020 \text{ cm}^{-1}$  also revealed the presence of primary amine group (C–N) in CBP (Coates, 2000). Wavenumbers ranging from  $900$  to  $700 \text{ cm}^{-1}$  can be assigned to saccharides (Castiglioni et al., 2019). In general, FTIR analysis showed that the components of CBP are multiform, however, they also were similar to those of other bee pollens.

### 3.4. Micromorphology

Figure 3 shows that the CBP pellet was formed by many tiny pollen grains (diameter of  $20 \mu\text{m}$ , ovoid to spheroidal). The adhesion of the pollen grains was considered to be related to the combination of flower pollen, nectar, enzymes, honey, wax, and bee secretions, especially aided by electrical charge (Vaknin et al., 2000). The surface of the pollen grain is not smooth; it is a surface with perforate ornamentation. These results are similar to those from *Silene mushaensis*



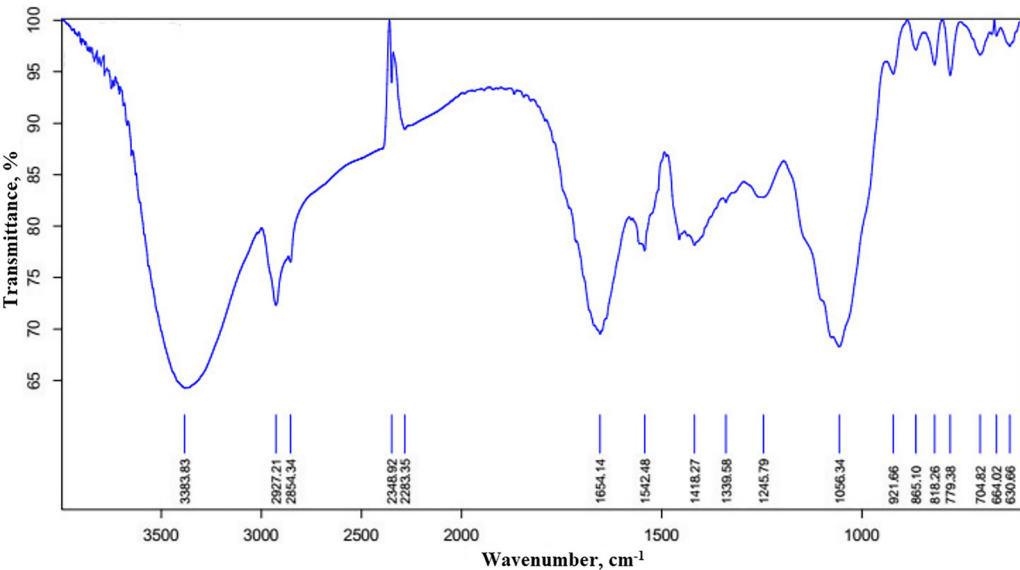


Fig. 2. FTIR spectra of CBP pellets

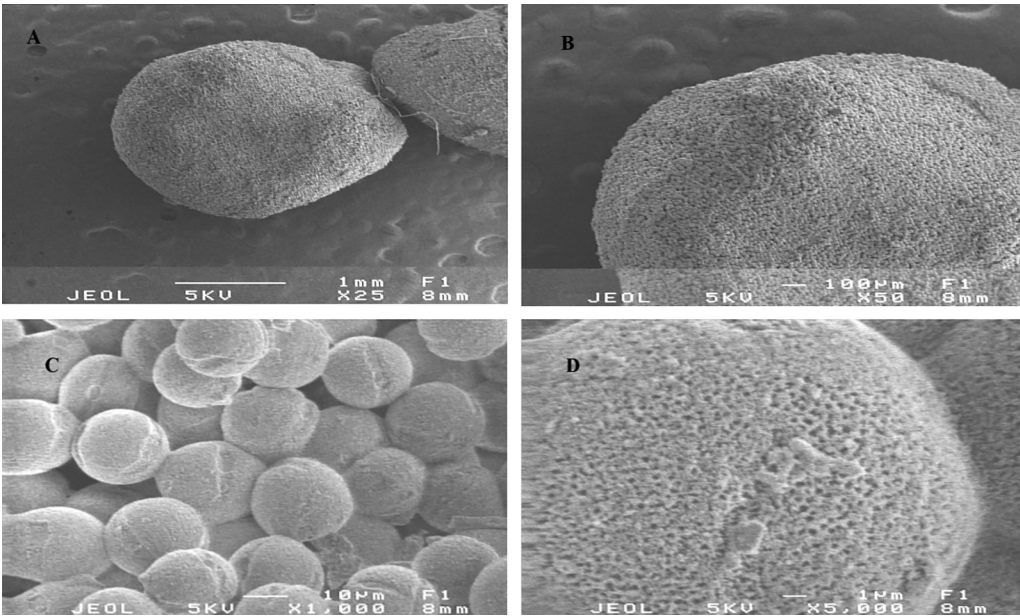


Fig. 3. SEM of bee pollen from *Coffea robusta*. (A) CBP pellet at a magnification of  $\times 25$ . (B) Surface of CBP pellet at a magnification of  $\times 50$ . (C) CBP grains at a magnification of  $\times 1,000$ . (D) Surface of CBP grain at a magnification of  $\times 5,000$



pollen recorded by Wan et al. (2018) and completely different from those from *Alcea ficifolia* pollen, which had the echinate (spiny) surface (Konzmann et al., 2019). This indicates that bees could easily collect the coffee pollen grains compared to other materials, because there are no long spines on the pollen grain surface, which can hinder pollen collection by bees.

## 4. CONCLUSIONS

In summary, the present study determined some major chemical components and evaluated the TPC and AA of CBP. In addition, the physical properties and micromorphology of CBP were also investigated. This material is a rich source of protein, sugar, vitamins, minerals, and phenolic compounds for bees and humans. These results also help to enhance the knowledge about CBP, which will become a supplemental food supply in the future.

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