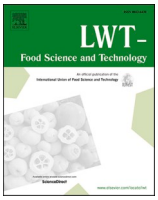
LWT - Food Science and Technology 170 (2022) 114065

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Characterization of carotenoid profile and α-tocopherol content in Andean bee pollen influenced by harvest time and particle size

Claudia Y. Salazar-González [a,b], Carla M. Stinco [c], Francisco J. Rodríguez-Pulido [c], Consuelo Díaz-Moreno [a], Carlos Fuenmayor [a], Francisco J. Heredia [c,*], M. Lourdes González-Miret [c]

[a] Instituto de Ciencia y Tecnología de Alimentos – ICTA, Universidad Nacional de Colombia, Carrera 30 # 45-03, Bogotá D.C, Colombia
[b] Departmento de Ingeniería Química y Ambiental, Universidad Nacional de Colombia, Carrera 30 # 45-03, Bogotá, Colombia
[c] Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012, Sevilla, Spain

ARTICLE INFO

Keywords:
Botanical origin
High Andean forest
Pigment profile
RRLC
Xanthophylls

ABSTRACT

Bee pollen (BP) is a natural product with remarkable nutritional and bioactive composition. Some of the compounds of interest are carotenoids with provitamin A activity, possessing an excellent antioxidant capacity and positive health effects. For that, the objective of this work was to characterize the botanical origin and carotenoid profile in two particle sizes of Colombian BP by Rapid Resolution Liquid Chromatography (RRLC). The carotenoid profile and α-tocopherol content were obtained, and ANOVA analysis was employed to explore data. The main carotenoids found were 9Z-zeaxanthin (65.15 ± 7.41–1104.98 ± 113.41 μg/g pollen), zeaxanthin (35.29 ± 2.08–354.30 ± 29.91 μg/g pollen), two zeaxanthin isomers (18.23 ± 3.73–227.69 ± 19.00 μg/g pollen) and β-cryptoxanthin (8.67 ± 1.27–34.25 ± 2.25 μg/g pollen). The statistical analysis showed significant differences between harvest time and particle size, proving the influence of climatic and botanic factors. The high content of macular and provitamin carotenoids was highlighted, which proposes BP as a significant source of important bioactive compounds. The carotenoids found allows characterizing BP in a complete seasonal cycle (January–December), research conducted for the first time in this product. Also, the information reported can be used to select harvest time of BP in order to include it as an ingredient, supplement, or raw material in the food industry, according to the carotenoid composition needed.

1. Introduction

Carotenoids are bioactive compounds produced by plants with many other functions than nutrition (Machado De-Melo et al., 2016; Saini et al., 2015; Song et al., 2016). They are currently gaining attention since several studies have demonstrated that carotenoids have a beneficial impact on the reduction of some degenerative syndromes such as cancer, cardiovascular disease, diabetes, cataracts, among others (Esquivel et al., 2019; Kim et al., 2016; Song et al., 2016). These secondary metabolites are widely present in Andean bee pollen (BP), generated especially for protection against environmental changes, mainly photo-oxidation (Cândido et al., 2015; Sarungallo et al., 2015). This product is collected from floral pollen agglutination by worker bees, and it has recently been highlighted due to its nutritional and bioactive composition, which depends mainly on its botanical source (Salazar-González & Díaz-Moreno, 2016).

Due to the importance in human health and the chemical variability that depends on the botanical source, it is very important to determine the carotenoid content both quantitatively and qualitatively. These determination have usually carried out by using conventional methodologies, such as thin layer and column chromatography; and more specific methods such as High-Efficiency Liquid Chromatography (HPLC), coupled with diode detectors mass spectrometry (MS) or nuclear magnetic resonance (NMR) (Saini et al., 2015). HPLC is the most used technique to control external factors such as light and temperature; since it is faster and needs less sample compared with other methodologies such as spectrophotometry. The latest advances have improved this technique, generating ultra-HPLC and Rapid Resolution Liquid Chromatography – RRLC (Stinco et al., 2014, 2019), which reduce the time of analysis and use of solvents.

Those methodologies have been used to identify carotenoids in

* Corresponding author.
E-mail address: heredia@us.es (F.J. Heredia).

https://doi.org/10.1016/j.lwt.2022.114065
Received 22 July 2022; Received in revised form 30 September 2022; Accepted 4 October 2022
Available online 5 October 2022

Abbreviations

ATOC	α-tocopherol
ZEA	all E-zeaxanthin
BCR	β-cryptoxanthin
PHY	phytoene
LUT	lutein
BP	bee pollen

several fruits and vegetables, including bee products, especially BP. Several studies have shown the relation between nutritional and bioactive compounds with botanical origin. For instance, in Brazilian BP higher proteins and flavonoids were found in the winter season, as well as predominance of the Myrtaceae family, while a high concentration of lipids and low ash content was related to spring season (Negrão & Orsi, 2018). Oliveira et al. (2009) found that floral species such *Raphanus* sp, *Eucalyptus* sp, *Macroptilium* sp, and *Mimosa caesalpineafolia* are related to high contents of vitamin E; *Anadenanthera* sp, Arecaceae type, and *Philodendron* sp. To vitamin C, and *Raphanus* sp., *Macroptilium* sp. And *Mimosa caesalpineafolia* to β-carotene.

With respect to carotenoids, the main compounds identified in BP up to date are β-carotene, β-cryptoxanthin, zeaxanthin, neoxanthin, antheraxanthin, α-carotene, lutein, and some traces of phytoene, measured by HPLC, HPLC-DAD, and thin-layer high-performance chromatography with Raman spectroscopy in samples from several botanical origins (Abd-Alla & Salem, 2020; Conte et al., 2017; Gardana et al., 2018; Kalaycıoğlu et al., 2017; Schulte et al., 2009; Şahin & Karkar, 2019; Žilić et al., 2014). In the case of Colombian BP, authors have found traces of lutein, zeaxanthin, β-carotene, and phytoene. After comparing the chromatographic, spectrophotometric, and mass spectrum characteristics, the zeaxanthin esterified with two lauric acid molecules was found to be the major compound. The second compound was lutein di-lauryl ester (Gardana et al., 2018).

Previous reports show that several factors influence pollen composition. Botanical origin is important because flowering varies across the year and each type of plant has a specific metabolism that generates different bioactive compounds, such as carotenoids (Machado De-Melo et al., 2016; Saini et al., 2015; Song et al., 2016). In South American Andean countries, such as Colombian forest areas, there is a great variety of floral resources that generate carotenoids in high quantity and quality when combined with high solar radiation, soil quality and diversity of insects (Chamorro et al., 2017). Also, bees can visit flowers in a distance of up to 9 km from their hive and collect approximately 48.6% of yellow pollen, which is related to carotenoid content (Machado De-Melo et al., 2016; Xu et al., 2013). Considering these factors, it is essential to identify the seasons where more quantity of carotenoids is generated in Colombian BP.

This work aimed to characterize the carotenoid profile of Colombian high Andean Forest BP in a year, considering particle size and botanical origin. As far as we know, this is the first time that a complete carotenoid profile of Colombian BP is made. Therefore, it is important not only to thoroughly describe the obtained results, but also to compare these results with those obtained by other authors in different regions in the world. It is expected a relationship between botanical origin, color and carotenoid content among the harvest year. Also, these findings could be used to select the harvest time of BP to include it as an ingredient, supplement, or raw material in the food industry, according to the carotenoid composition needed.

2. Material and methods

2.1. Samples

Bee pollen was collected monthly throughout 2016. The experimental unit was an apiary in the geographic region of the Colombian high Andean Forest (between 2.800 and 3.200 m above sea level). The coordinates are northwest: latitude (5°56′) and longitude (−72°59′); northeast: latitude (5°46′) and longitude (−73°42′); southwest: latitude (4°28′) and longitude (−74°05′), and southeast: latitude (4°52′) and longitude (−74°24’). BP was subjected to drying (60 °C for 4 h) and cleaning processes. The drying process was performed to avoid microbiological spoilage. Samples were stored at room temperature until analysis.

2.2. Chemicals

Extraction solvents were analytical-grade acetone and hexane from (VWR Seattle, WA, USA). Analytic solvents were HPLC-grade methanol and methyl-tert-butyl-ether (MTBE) from Merck (Darmstadt, Germany). Purified water was obtained from Barnstead Nanopure (Thermo Scientific, USA). α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin were purchased from Sigma-Aldrich (Steinheim, Germany), while phytoene was isolated from appropriate sources following standard procedures (Mapelli-Brahm et al., 2017). α-Tocopherol was purchased from Calbiochem Merck (Darmstadt, Germany).

2.3. Granulometric analysis

It was made using a test sieve with mesh numbers 7, 10, 12, 16, 18, and 30, according to a previous study made in Colombian BP (Salazar-González et al., 2018). The fractions selected for the tests were those from sieves 12 (1.68 mm) and 16 (1.19 mm), since they presented the most yellow and orange hues, and are expected to have a bigger quantity of carotenoids. The other sieves were considered impurities and discarded. A total of 24 samples were used.

2.4. Colorimetric analysis

The colorimetric analysis was performed in homogenized BP, using a Konica Minolta CM-5 colorimeter (Konica Minolta, New Jersey, USA). Color was determined using the tristimulus coordinates of the CIELAB space, which is represented in rectangular coordinates: lightness (L*), a* (green-red hues) and b* (blue-yellow hues). Cartesian coordinates a* and b* were also expressed as polar coordinates: chroma (C^*_{ab}) and hue (h_{ab}) (Salazar-González et al., 2018).

2.5. Palynological analysis

For the qualitative analysis, samples were acetolysed according to Erdtman (1969), as reported by Salazar-González et al. (2020). Pollen pellets were washed with acetic acid, followed by the mixture for acetolysis and centrifuging. The supernatant was decanted, and the sediment was washed with acetic acid and distilled water, followed by centrifuging. One slide of each sample was prepared by adding glycerine and using glycerin jelly and paraffin for permanent preparations. At least 400 pollen grains were counted. The identification of the pollen types was based on the Melisopalynology Laboratory from Universidad Nacional de Colombia, using a reference collection and pollen catalogs (Colinvaux et al., 1999; Roubik & Moreno, 1991; Velásquez, 1999).

2.6. Carotenoid analysis

The complete procedure is reported in a previous work from the author (Salazar-González et al., 2020). The extraction and saponification of carotenoids were carried out according to the method described

by Stinco et al. (2014) with some modifications. The chromatographic analysis was made according to Stinco et al. (2019). The validation procedure is also reported in this work. BP samples were analyzed by Rapid Resolution Liquid Chromatography (RRLC) in an Agilent 1260 system. A YMC C30 (150 × 4.6 mm, 3 μm) column and a C30 YMC pre-column (10mm × 4 mm, 3 μm) (Agilent, Dinslaken, Germany) were used as stationary and mobile phases: methanol, methyl-tert-butyl ether, and deionized water. The selected wavelengths were 285 nm for α-tocopherol and phytoene, and 450 nm for the rest of carotenoids. The identification and quantitative analysis of carotenoids was made by comparing their chromatographic and UV–Vis spectroscopic characteristics with their corresponding standards. The tentative identification of *Z*-isomers (*cis*-isomers) was achieved by comparing their chromatographic and spectroscopic features with those of a standard mixture of zeaxanthin, β-cryptoxanthin and lutein, obtained by iodine-catalyzed isomerization; and with data reported by other authors (Aman et al., 2005; Song et al., 2016; Zhang et al., 2016). External calibration was used for quantification and results were reported in μg carotenoid/g BP. The total carotenoid content (TCC) was assessed as the sum of the content of individual pigments in RRLC.

2.7. Statistical analysis

The results were reported as mean ± standard deviation (Excel®). Analysis of variance (ANOVA) and Tukey tests (95% confidence level) were carried out using the software Matlab® in order to evaluate differences among treatments (The Mathworks Inc., MA).

3. Results and discussion

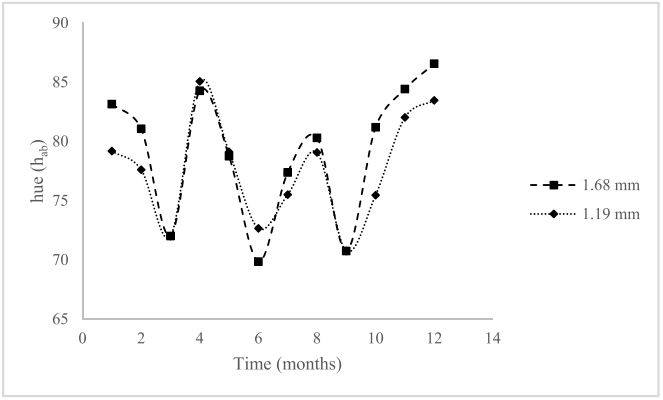
3.1. Colorimetric analysis

Fig. 1 shows the results of the colorimetric coordinates of bee pollen samples through a year. The smallest size fractions (1.19 mm) have the lowest hue values (h_{ab}) (Fig. 1a), which leads to the most orange tones; except in April, May, and June, when the pellets with size 1.68 mm have the highest orange colorations. In general, a seasonality is observed and it allows concluding that March, June, and September are characterized by orange tones, while January, April, August, and December have yellow tones. Similarly, chroma (Fig. 1b) presents higher values as the particle size decreases, which indicates that the tonality will be more vivid or with a more intense color.

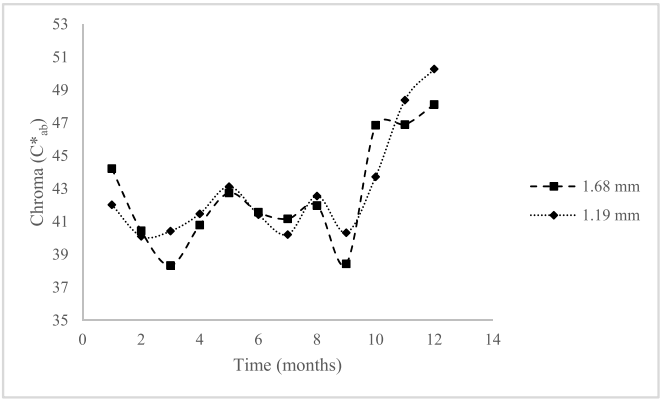
In various Slovakian BP samples, values for L* were found between 51.40 and 75.23; chroma (C^*_{ab}) between 10.42 and 39.76, and hue (h_{ab}) between −1.47 and 1.33 (Bleha et al., 2019). Compared with these results, the Colombian BP is more intense due to its higher chroma value, in addition to its totally different hues. Tunisian BP has yellow hue and is lighter and less intense than Colombian BP, as in the former L* values (84.37 ± 0.09) are higher, C^*_{ab} values (18.55 ± 0.15) are lower and the h_{ab} value (86.57 ± 0.08) places it in the yellow range (Sebii et al., 2019). In Indian pollen, values for L* were reported between 48.37 and 55.56; C^*_{ab} between 11.10 and 33.37, and h_{ab} between 68.58 and 73.91 (Thakur y Nanda, 2018), with hues from yellow to orange, lower lightness and intensity than Colombian BP. In a previous study of color characterization of Colombian high Andean BP, this product was catalogued with yellow-greenish shades, which differs from the findings of the present work.

3.2. Botanical origin

Different floral species were found in the Colombian high Andean Forest. For this reason, BP is usually multi-floral. Table 1 shows the most frequent pollen types through the year, being present in at least 15 samples. Additionally, 21 more palynomorphs were identified. However, they were found in few samples (one – six samples) or had a low frequency: *Myrcia mollis*, *Tecoma stans*, Euphorbiaceae type, *Pentacalia*



(a)



(b)

Fig. 1. Colorimetric coordinates: (a) hue and (b) chroma of each particle size.

type, *Hypericum* sp., *Acacia decurrens*, *Baccharis* type, *Bidens* type, *Piper* sp., *Ilex* sp., *Senecio* type, *Verbesina* type, *Impatiens balsamina, Brugmansia, Eirmocephala* sp., *Arcythophyllum, Spermacoce,* Passifloraceae, *Sida acuta, Psydium guajava* and *Hedyosmum.*

Palynomorphs in bold (dandelion, clover, eucalyptus, and turnip) could be considered as predominant pollen, according to Louveaux et al. (1978), who suggested that a content superior to 45% in one morphotype can be considered as monofloral pollen. Values between 16 and 45% are considered secondary pollen, such as *Hypochaeris radicata* in January and February in BP with 1.68 mm particle size. Values between 3 and 15% are considered important minor pollen, such as *Eucalyptus globulus* in February, April, August, and November in BP with 1.68 mm particle size. Values less than 3% are considered minor pollen.

Samples with predominant pollen could be the product of a flowering period of a single specific species; a large amount of pollen per flower unit, either with numerous stamens per flower or many small flowers per inflorescence; or an unusually long period of flowering (Chamorro et al., 2017). It is worth noting that *Hypochaeris radicata*, *Brassica* type, and *Trifolium pratense* are floral species found in all samples, which indicates a continuous flowering period throughout the year. *Hypochaeris radicata* was the most abundant specie and it was present in greater quantities in BP of size 1.19 mm than 1.68 mm in most of the months. Likewise, *Trifolium repens* was present mostly in 1.68 mm particles. The reason could be that those pollen types were more prone to agglomerate and form pellets from that specific size. The presence of this type of floral species is related to their proliferation in grasslands, farmland, and extensive forests, all of them characteristic of the high Andean forest (Chamorro et al., 2017).

The results obtained for the samples are similar to those previously reported for BP from the same region: Brassicaceae (*Brassica* vs.

Table 1
Most frequent pollen types present in BP samples.

	Frequency (%)																							
	Particle size 1.68 mm												Particle size 1.19 mm											
Palynomorph/month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Brassica type	7.3	**56**	2.6	7.7	9.9	0.0	0.0	**46**	9.8	**67**	39	**85**	23	14	6.8	3.3	10	11	38	**55**	24	24	7.7	**87**
Hypochaeris radicata	37	19	**56**	11	**58**	6.5	3.6	1.9	**46**	8.6	12	5.6	7.1	**59**	**72**	20	38	**74**	4.4	2.8	20	**46**	16	1.7
Eucalyptus globulus	0.0	7.2	0.9	6.1	0.0	0.0	**64**	12	0.0	0.0	9.3	0.0	0.0	1.1	2.6	**77**	12	0.0	9.1	0.0	0.0	2.2	0.0	0.0
Trifolium pratense	2.0	0.0	0.6	0.0	3.9	0.0	18	18	9.8	0.5	9.5	5.7	33	1.7	0.0	0.2	0.0	0.7	44	23	1.5	0.2	16	4.8
Trifolium repens	20	9.2	8.3	0.2	0.2	10	0.0	0.2	0.6	7.6	13	0.0	2.7	9.5	3.4	0.0	0.9	1.1	0.0	0.0	8.0	1.0	**46**	3.4
Acalypha diversifolia	0.0	0.0	0.0	**57**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gaiadendron punctatum	0.0	0.0	32	9.7	5.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.4	11	0.0	31	3.2	0.0	0.0	0.0	0.0	0.0	0.0
Cecropia peltata	0.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0	14	0.5	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	32	10	0.0	0.0
Viburnum	19	0.1	0.3	2.5	0.0	0.0	0.0	0.8	6.6	6.2	0.2	1.9	0.0	5.0	2.6	0.0	0.6	0.4	0.0	3.4	4.4	0.2	0.0	0.6
Weinmannia	0.0	3.2	0.0	0.0	6.4	5.5	6.0	0.6	0.0	8.3	0.4	0.0	0.0	3.9	0.2	0.0	5.8	0.7	0.3	0.0	4.7	2.7	0.0	0.4
Muehlenbeckia tamnifolia	0.0	0.0	0.0	0.0	0.0	**75**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.1	0.0	0.0	0.0	0.0	0.0	0.0

Palynomorphs in bold could be considered as predominant pollen.

Raphanus), *Hypochaeris radicata*, *Eucalyptus globulus*, *Trifolium repens*, *Trifolium pratense*, *Acalypha diversifolia Gaiadendron punctatum*, *Cecropia peltata*, *Rubus* sp., *Muehlenbeckia tamnifolia*, *Viburnum* sp., and *Weinmannia* sp. (Chamorro et al., 2017; Chamorro-García et al., 2013), except Rubus sp. These differences occur due to the geographic position and the great variety of floral species in Colombian Andes region, therefore, there are different floral resources in each location.

Each flower has different components and metabolisms to generate secondary metabolites, i.e. bioactives, hence the botanical origin is an important factor in the nutritional composition of BP (Negrão & Orsi, 2018; Oliveira et al., 2009). For this reason and given the biodiversity found in the Colombian high Andean Forest, bee pollen can be classified as multi-floral, which serves as an indicator to conclude that carotenoid composition, related to pigment profiles, could be variable.

3.3. Total carotenoid content (TCC)

TCC is shown in Fig. 2 considering the particle size and the harvest month. Particle size of 1.19 mm presented higher concentrations of total carotenoids respect to particles of 1.68 mm, except in April and June. This indicates that separation allowed obtaining more concentrated fractions in carotenoids.

TCC increase with the month progress between January–March and April–June for particles of 1.68 mm and between January–March and April–May for those of 1.19 mm. Statistically, significant differences were observed between different months for each particle size. However, in particles of 1.19 mm, there was higher homogeneity. Differences were probably caused by the synthesis of carotenoids, which depends on climatic conditions, radiation, and the amount of light which the plant is exposed (Books et al., 2018; Machado De-Melo et al., 2016; Song et al., 2016). Regarding climatic conditions, they are related to the harvest month. The months with the lowest TCC (January, April, August) are in

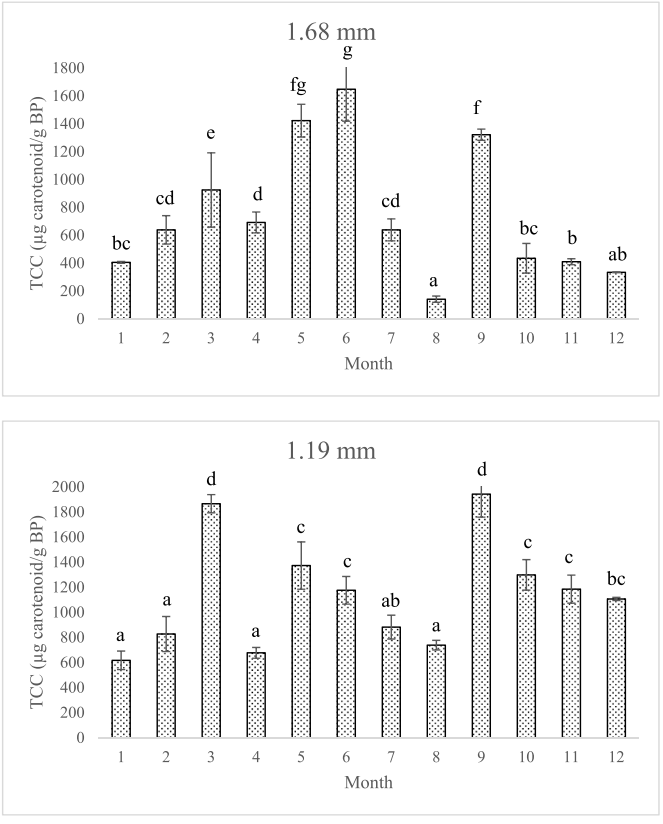


Fig. 2. Total carotenoid content in Andean BP samples according to particle size.

the rainy season of the country, which impedes the appearance of bioactive compounds, due to the decrease in temperature and radiation. The months with the highest TCC (March, June, September) are considered transitional since they combine dry and rainy periods. This condition generates the best conditions for the production of BP and secondary metabolites; availability of water favors the growth of the plant and radiation of the dry periods allows the production of carotenoids for photo-protection (Cândido et al., 2015; Sarungallo et al., 2015).

Additionally, bees use large and small flowers with diverse colors to collect pollen. This causes diversity in compounds and quantities (Chamorro-García et al., 2013). Other factors that affect the carotenoid content are prolonged storage and exposure to light or heat treatments (mainly drying) since carotenoids degrade with time and temperature (Fernández-Lara et al., 2015).

Compared with other countries, carotenoid content from Colombian BP (503.6–2149.0 mg β-carotene/kg BP) exceeded that in bee pollen from Spain (12.41 mg/kg) (Domínguez-Valhondo et al., 2011), Germany (8.78 mg/kg) (Schulte et al., 2009), Brazil (5.3–1233 mg/kg) (Gasparotto et al., 2015), Western Slovakia (223.10–261.33 mg/kg) (Fatrcová-Šramková et al., 2016), Chile (2.8–50.2 mg/kg) (Velásquez et al., 2017) and China (271.6 mg/kg) (Xu et al., 2011). Those differences are mainly generated by the greater synthesis that occurs in the high Andean area thanks to climatic conditions and differences in the carotenoids content in flower species (there exist differences between gender and family) (Velásquez et al., 2017). Regarding botanic origin, low carotenoid contents have been reported in *Brassica* sp pollen. This floral specie was found mainly in yellow pollen, therefore, a relationship can be established between the botanical origin and the carotenoid content, since samples with yellow coloration presented low carotenoid contents (Velásquez et al., 2017). The multi-floral character of BP samples will influence the composition of the carotenoid profile, generating a variability inherent to botanical diversity.

3.4. Carotenoid profile

The carotenoid profile (after saponification) of BP is shown in Fig. 3, where samples evidence a high number of carotenoids. Nine carotenoids were identified in addition to α-tocopherol: 9*Z*-zeaxanthin, zeaxanthin, 9,13-di-*Z*-zeaxanthin, 9,15-di-*Z*-zeaxanthin, β-cryptoxanthin, phytoene, 9 or 9′*Z*-β- cryptoxanthin, 9′*Z*-lutein and lutein. From the above carotenoids, zeaxanthin, β-cryptoxanthin, phytoene and lutein were also reported in other studies (Abd-Alla & Salem, 2020; Gasparotto et al., 2015; Karkar et al., 2020; Machado De-Melo et al., 2016; Schulte et al., 2009; Şahin & Karkar, 2019). These results are consistent with a previous study in Colombian BP, where traces of lutein, zeaxanthin, β-carotene, and phytoene were identified (Gardana et al., 2018).

The carotenoid profile was similar in all samples; however, quantities were variable. Table 2 and Table 3 summarize the carotenoids contents, where α-tocopherol is listed followed by the carotenoids in descending order of content. In most of the samples, those with smaller particle sizes (1.19 mm) had a significant quantity of carotenoids with respect to the bigger ones (1.68 mm). Significant differences were observed between the individual carotenoids throughout the year. These differences can be determined by the variations among botanical species (all samples were multifloral), since it is possible that each plant developed adaptations of the metabolic routes to generate carotenoids, which causes variation in the presence and quantity of them (Machado De-Melo et al., 2016; Song et al., 2016). Carotenoids are regulated by light, epigenetic mechanisms and redox state of signaling molecules (Books et al., 2018).

BP has mainly xanthophylls (zeaxanthin, β-cryptoxanthin, lutein and several isomers), which can come from exine (external layer of pollen). It has been reported that some bee pollen carotenoids are found in this layer, esterified with lipids (Domínguez-Valhondo et al., 2011; Salazar-González & Díaz-Moreno, 2016). The exine is composed of various organic and inorganic substances, among which sporopollenin is a very complex polymer that has been extensively studied (Kovacik et al., 2009). Its structure includes different substances, such as lipids, some types of carotenoids, tocopherols, provitamin A and vitamin D (Domínguez-Valhondo et al., 2011; Salazar-González & Díaz-Moreno, 2016). The radicals present in the structure of xanthophylls allow fatty acids to be linked with sporopollenin, which is why their major presence in bee pollen is observed. Phytoene, a colorless carotenoid that is a precursor of carotenoids, was also found.

After analyzing the content of zeaxanthin, as well as its isomers (9*Z*-zeaxanthin, 9,13-di-*Z*-zeaxanthin, 9,15-di-*Z*-zeaxanthin), it is observed that this xanthophyll is the major component in BP, since it is found throughout the year. The total of zeaxanthin contents (*cis* and *trans* isomers) was between 81 and 94% respect to the total carotenoids identified, while its isomers are only between 63 and 77%. Among them, 9*Z*-zeaxanthin was the one that presented the highest contents, with percentages between 42 and 56% of the total. This xanthophyll is found mainly in the months of March and September in the particle size of 1.19 mm and in June in 1.68 mm particles. The highest levels of 9,13-di-*Z*-zeaxanthin and 9,15-di-*Z*-zeaxanthin were found in September and

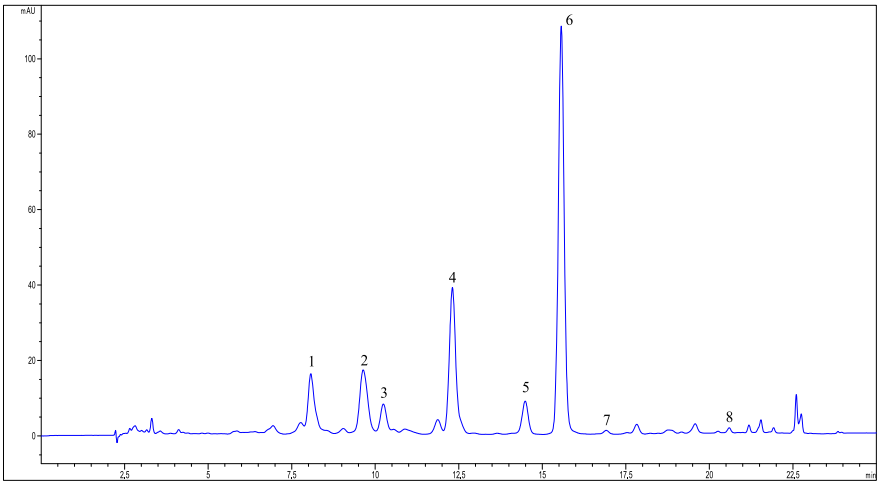


Fig. 3. Carotenoid profile of Colombian BP at 450 nm. Peaks: 1 = 9,15-di-*Z*-zeaxanthin; 2 = 9,13-di-*Z*-zeaxanthin; 3 = lutein; 4 = *all* E-zeaxanthin; 5 = 9′*Z*-lutein; 6 = 9*Z*-zeaxanthin; 7 = β-cryptoxanthin; 8 = 9 o 9′*Z*- β-cryptoxanthin.

Table 2
Carotenoid content and α-tocopherol in Andean Forest BP (μg/g BP): particle size 1.68 mm.

Month	ATOC	9Z-ZEA	ZEA	9,13Z-ZEA	9,15Z-ZEA	BCR	PHY	9Z-BCR	9Z-LUT	LUT
1	29.29 ± 3.20^{b}	201.55 ± 17.24^{bc}	101.22 ± 7.06^{bcd}	52.38 ± 3.77^{bc}	34.21 ± 0.04^{ab}	9.26 ± 0.34^{a}	ND	ND	13.75 ± 0.58^{b}	11.98 ± 0.26^{a}
2	40.98 ± 3.95^{cd}	321.92 ± 29.06^{cde}	121.22 ± 13.63^{d}	74.66 ± 8.78^{cd}	58.89 ± 5.35^{cd}	13.75 ± 0.43^{bc}	ND	43.16 ± 8.91^{c}	21.70 ± 4.82^{c}	14.54 ± 3.41^{a}
3	47.32 ± 7.07^{d}	608.65 ± 98.75^{f}	201.69 ± 22.04^{e}	132.07 ± 20.13^{e}	109.79 ± 17.55^{e}	8.67 ± 1.27^{a}	1.92 ± 0.73^{a}	ND	ND	ND
4	33.95 ± 0.47^{bc}	393.69 ± 39.33^{e}	123.93 ± 17.21^{d}	81.02 ± 8.28^{d}	71.18 ± 5.74^{d}	10.03 ± 0.31^{a}	6.20 ± 0.75^{c}	ND	9.28 ± 0.61^{ab}	ND
5	69.20 ± 4.22^{f}	815.23 ± 77.05^{g}	255.56 ± 12.94^{f}	178.54 ± 13.82^{fg}	148.82 ± 12.76^{fg}	15.33 ± 0.75^{cd}	9.25 ± 0.37^{d}	ND	ND	ND
6	65.17 ± 1.44^{ef}	945.09 ± 114.67^{h}	243.41 ± 27.48^{f}	181.10 ± 24.51^{g}	164.64 ± 20.36^{g}	19.29 ± 2.25^{e}	ND	25.99 ± 2.87^{b}	40.77 ± 6.05^{d}	50.03 ± 11.08^{b}
7	30.45 ± 0.00^{b}	345.01 ± 45.89^{de}	117.18 ± 11.96^{cd}	73.01 ± 8.59^{cd}	58.57 ± 7.14^{cd}	17.18 ± 2.19^{de}	ND	12.95 ± 1.54^{a}	15.19 ± 0.92^{b}	12.63 ± 1.22^{a}
8	ND	65.15 ± 7.41^{a}	35.29 ± 2.08^{a}	18.88 ± 1.83^{a}	18.23 ± 3.73^{a}	10.62 ± 0.93^{a}	4.28 ± 0.46^{b}	ND	ND	ND
9	59.81 ± 0.26^{e}	748.34 ± 29.92^{g}	238.70 ± 1.71^{f}	154.56 ± 8.13^{ef}	132.49 ± 5.89^{f}	22.49 ± 1.30^{f}	3.41 ± 0.91^{ab}	21.70 ± 0.91^{b}	ND	ND
10	30.73 ± 4.63^{b}	229.31 ± 56.72^{bcd}	85.77 ± 16.75^{bc}	51.81 ± 12.34^{bc}	43.66 ± 9.98^{bc}	14.18 ± 0.35^{c}	2.30 ± 0.16^{a}	11.30 ± 1.92^{a}	3.77 ± 1.00^{a}	ND
11	ND	205.78 ± 12.30^{bc}	87.15 ± 10.42^{bc}	43.11 ± 5.98^{ab}	32.54 ± 4.48^{ab}	10.24 ± 1.26^{a}	8.73 ± 1.38^{d}	10.06 ± 0.58^{a}	ND	ND
12	15.50 ± 0.04^{a}	156.63 ± 2.04^{ab}	77.68 ± 4.33^{b}	37.33 ± 0.17^{ab}	27.16 ± 0.47^{ab}	11.12 ± 0.50^{ab}	14.32 ± 0.19^{e}	9.77 ± 0.47^{a}	ND	ND

Month: January 1 until December 12. ND: Not detected. ATOC: α-tocopherol; 9Z-ZEA: 9Z-zeaxanthin; ZEA: *all* E-zeaxanthin; 9,13Z-ZEA: 9,13-di-Z-zeaxanthin; 9,15Z-ZEA: 9,15-di-Z-zeaxanthin; BCR: β-cryptoxanthin; PHY: phytoene; 9Z-BCR: 9 o 9′Z-β-cryptoxanthin; 9Z-LUT: 9′Z-lutein; LUT: lutein. Different letters in the same column indicate statistically significant differences.

Table 3
Carotenoid content and α-tocopherol in Andean Forest BP (μg/g BP): particle size 1.19 mm.

Month	ATOC	9Z-ZEA	ZEA	9,13Z-ZEA	9,15Z-ZEA	BCR	PHY	9Z-BCR	9Z-LUT	LUT
1	42.35 ± 2.35^{bc}	347.91 ± 40.78^{a}	109.33 ± 18.04^{a}	63.47 ± 2.93^{a}	58.18 ± 3.78^{a}	13.46 ± 0.51^{a}	14.30 ± 2.57^{bc}	18.35 ± 0.36^{ab}	ND	ND
2	52.21 ± 4.21^{cd}	438.74 ± 76.60^{a}	127.71 ± 21.77^{ab}	88.37 ± 13.69^{abc}	81.89 ± 6.62^{abc}	13.33 ± 4.61^{a}	ND	51.12 ± 17.18^{d}	25.89 ± 0.80^{b}	ND
3	82.02 ± 4.33^{f}	1084.64 ± 53.71^{e}	308.89 ± 40.92^{e}	223.70 ± 13.63^{g}	189.17 ± 15.31^{g}	17.84 ± 2.38^{abc}	17.06 ± 1.26^{c}	ND	ND	ND
4	25.49 ± 3.47^{a}	355.94 ± 51.70^{a}	115.43 ± 12.39^{a}	78.11 ± 0.42^{ab}	69.23 ± 0.16^{ab}	14.97 ± 0.71^{ab}	6.76 ± 0.29^{a}	17.78 ± 1.36^{ab}	ND	ND
5	58.68 ± 17.67^{d}	803.38 ± 109.00^{d}	233.64 ± 28.11^{d}	166.50 ± 26.46^{f}	143.03 ± 22.86^{f}	15.17 ± 1.60^{ab}	11.42 ± 0.48^{b}	ND	ND	ND
6	63.89 ± 6.02^{de}	682.05 ± 47.77^{cd}	183.13 ± 10.43^{bcd}	129.06 ± 8.54^{de}	115.20 ± 12.18^{e}	16.11 ± 1.91^{ab}	ND	18.93 ± 1.38^{ab}	24.56 ± 1.01^{b}	28.25 ± 2.59
7	39.97 ± 1.65^{abc}	495.81 ± 50.81^{ab}	159.73 ± 20.14^{abc}	103.86 ± 9.14^{bcd}	87.77 ± 7.45^{bcd}	18.57 ± 1.94^{bc}	ND	15.93 ± 1.68^{a}	9.95 ± 0.16^{a}	ND
8	32.52 ± 10.72^{ef}	403.80 ± 11.92^{a}	137.06 ± 12.78^{ab}	85.07 ± 2.75^{abc}	73.67 ± 0.87^{ab}	15.50 ± 0.73^{ab}	16.05 ± 0.85^{c}	15.20 ± 1.41^{a}	ND	ND
9	77.72 ± 7.63^{de}	1104.98 ± 113.41^{e}	354.30 ± 29.91^{e}	227.69 ± 19.00^{g}	195.88 ± 17.19^{g}	24.84 ± 1.48^{d}	5.90 ± 1.33^{a}	29.38 ± 3.03^{bc}	ND	ND
10	63.97 ± 0.25^{g}	724.78 ± 67.98^{cd}	213.33 ± 24.07^{cd}	142.44 ± 12.88^{ef}	127.02 ± 11.13^{ef}	34.25 ± 2.25^{e}	16.77 ± 2.03^{c}	39.98 ± 3.06^{cd}	ND	ND
11	112.35 ± 3.31^{f}	682.51 ± 75.86^{cd}	201.58 ± 21.64^{cd}	122.64 ± 14.35^{de}	110.49 ± 12.94^{de}	21.68 ± 2.81^{cd}	22.27 ± 1.67^{d}	33.17 ± 4.48^{c}	ND	ND
12	88.71 ± 2.01^{f}	631.20 ± 4.83^{bc}	181.02 ± 2.63^{bcd}	109.33 ± 0.90^{cd}	101.90 ± 0.50^{cde}	24.26 ± 1.87^{d}	29.03 ± 0.84^{e}	29.72 ± 2.80^{bc}	ND	ND

Month: January 1 until December 12. ND: Not detected. ATOC: α-tocopherol; 9Z-ZEA: 9Z-zeaxanthin; ZEA: *all* E-zeaxanthin; 9,13Z-ZEA: 9,13-di-Z-zeaxanthin; 9,15Z-ZEA: 9,15-di-Z-zeaxanthin; BCR: β-cryptoxanthin; PHY: phytoene; 9Z-BCR: 9 o 9′Z-β-cryptoxanthin; 9Z-LUT: 9′Z-lutein; LUT: lutein. Different letters in the same column indicate statistically significant differences.

March, in both particle sizes.

In nature, *all*-E isomers are the most common with respect to *cis* isomers, except in green leafy vegetables and many algae (9′Z-neoxanthin) or 15Z-phytoene (precursor of all carotenoids) (Fraser & Bramley, 2004). Surprisingly, the results showed that BP contained high quantity of zeaxanthin Z-isomers. This could be caused, because in BP it is necessary a thermal process such as lyophilization or convection drying for conservation. It is well known, that those processes can generate significant changes in carotenoid isomers (Saini et al., 2015).

In all samples analyzed, Colombian BP is approximately 10 times higher in zeaxanthin and its isomers (except in August for 1.68 mm particles) compared with values reported in the literature (0.20–36.38 μg/g BP) (Conte et al., 2017; Domínguez-Valhondo et al., 2011; Gardana et al., 2018; Şahin and Karkar, 2019; Karkar et al., 2020; Mărgăoan et al., 2014; Şahin y; Schulte et al., 2009).

Zeaxanthin is a macular carotenoid, important for ocular health, and has only few food sources in nature, therefore, BP is a good source giving its high contents found. The sum of zeaxanthin and its isomers from March, June and September in BP overcome the quantities of orange paprika. With respect to Goji berries (194–1860 μg/g), a widely

recognized matrix as zeaxanthin source (Hempel et al., 2017; Kulaitienė et al., 2020; Niro et al., 2017), BP samples from March, May, June, September, October and November with different particles sizes overcome the minimum values. Only BP from September with particles of 1.19 mm has more quantity of zeaxanthin (with isomers) than Goji berries. Compared with orange paprika (850–1514 μg/g) (Kim et al., 2016), BP had lower quantities but overcame corn (0.83–10.86 μg/g), yellow paprika (4.5–45.6 μg/g) and papaya (14.1 μg/g fresh weight) (Saini et al., 2015; Song et al., 2016).

Regarding the last macular xanthophyll in BP, lutein and its isomer 9′*Z*-lutein, the values found were between 0.8 and 5% respect to the total carotenoids, in 5 and 9 samples, respectively. In Colombia, BP has lower contents (12.63–50.03 μg/g BP) compared with other countries (0.81–476.30 μg/g BP) (Conte et al., 2017; Gardana et al., 2018; Schulte et al., 2009; Şahin & Karkar, 2019) and matrixes such as carrot (383.51 μg/g), pumpkin (74.48 μg/g), paprika (87.5–283.9 μg/g) (Kim et al., 2016; Saini et al., 2015; Stinco et al., 2014) and goji berries (46.0–58.0 μg/g) (Kulaitienė et al., 2020; Niro et al., 2017).

β-cryptoxanthin is an important carotenoid from the nutritional point of view (provitamin A) (Murillo et al., 2013; Saini et al., 2015). Its presence in all samples (including the 9 or 9′*Z*-BCR isomer) was between 1 and 8% respect the total carotenoids, reporting the highest contents between September and December for 1.19 mm particles, and in September for 1.68 mm particles. The values obtained are in the range of other studies on BP (1.31–44.67 μg/g BP) (Conte et al., 2017; Gardana et al., 2018; Karkar et al., 2020; Schulte et al., 2009; Şahin & Karkar, 2019). Compared to yellow and orange paprika (0.3–11.2 μg/g) (Kim et al., 2016) and corn (1.7 μg/g) (Saini et al., 2015; Song et al., 2016), BP had higher contents, however, it has similar or lower contents compared with goji berries (61.0 ± 1.4 μg/g) (Niro et al., 2017). It is remarkable the presence of a BCR isomer in BP because sources of this compound are scarce.

The existing variation in carotenoids and their amount is affected by two main variables: nutritional conditions of the plant and environmental conditions. In a study made in two genotypes of corn samples, mature and immature, Song et al. (2016) evaluated the carotenoid profile according to nutrients and humidity. Results show differences in composition, being lutein and zeaxanthin, the main compounds found. Regarding environmental conditions, Cândido et al. (2015) studied the effect of environmental temperature on the carotenoid content in buriti fruits from two locations in Brazil (Cerrado and Amazon). They found that samples from the Amazon had higher total carotenoids (52.86 mg/100g) than those from Cerrado (31.13 mg/100g), which allows suggesting that plants exposed to high temperature and direct sunlight incidence may increase carotenoids content to protect themselves from photo-oxidation (Sarungallo et al., 2015). In BP from the Andean forest, solar radiation is elevated in comparison with the rest of the country (IDEAM, 2005, 2017), which generates a great variety of colored plants, where bees collect pollen.

The study of the palynological analysis allows obtaining information on the botanical origin, which, is intrinsically related to the nutritional and bioactive composition (Negrão & Orsi, 2018; Oliveira et al., 2009). This work found that samples from March, June and September have the specie *Hypochaeris radicata* as predominant pollen, and according to Tables 2 and 3, 9*Z*-zeaxanthin is the majority carotenoid in those months, which shows a possible relationship between botanical origin and composition. This is in accordance with the study by Oliveira et al. (2009), who found a relationship between the content of β-carotene and the species *Raphanus* sp., *Macroptilium* sp. And *Mimosa caesalpineafolia* in BP from the Brazilian southeast. Regard to relation between color and carotenoids, the results showed that smaller particle size have more carotenoid content.

It is important to remark that the main carotenoid in BP is an isomer (9*Z*-zeaxanthin), unusual in fruits or vegetables, which makes BP an exceptional matrix and source of this macular carotenoid. Also, BP has the two macular carotenoids (zeaxanthin and lutein), present in orange, spinach, and maize, as well as β-cryptoxanthin, especially in samples with a particle size of 1.19 mm. This allows cataloging this matrix as a good source of these compounds and add value to this natural product. However, a study is still necessary to evaluate the bioavailability of zeaxanthin isomers, as it has not been reported before.

Respect to α-tocopherol content of Colombian BP samples, this is superior compared to BP from Italy (1.10–4.73 μg/g) (Conte et al., 2017) and have similar values to those of BP from Brazil (4.6–113.9 μg/g) (Gasparotto et al., 2015; Machado De-Melo et al., 2016). Compared with other foods, BP has higher quantities than eggs, cereals, grains, dairy and seafood products (18.11, 4.78, 7.32, 6.26 y 13.65 μg de a-tocopherol/g, respectively) (Pulido et al., 2012).

This is the first study that characterizes the carotenoid profile and α-tocopherol content of Colombian BP, performing sampling every month for a year. This allows knowing the botanical, geographical, and particle size influence of the high Andean Forest on the bioactive components of the fat-soluble fraction. For a better description of the carotenoid composition, it is advisable to perform a study with mass spectrometry to identify all the isomers found in the present study. It is also important to know if the drying process affect the formation of isomers and control its influence.

4. Conclusions

The development of a specific analytical methodology for BP allowed identifying and quantifying the content of carotenoids, which were xanthophylls, mainly 9*Z*-zeaxanthin, zeaxanthin, two isomers of zeaxanthin and β-cryptoxanthin. This identification is the first study carried out on Colombian BP in a harvest year considering the particle size and botanical origin. It allows obtaining deep knowledge of this matrix and suggests that this natural product can be considered as an excellent source of bioactive compounds beneficial to health as all the carotenoids identified are important for nutrition. The zeaxanthin content and its isomers make BP a good source of this compound due to the lack of natural products with this carotenoid, in contrast to lutein or β-carotene that are present is several foods.

CRediT authorship contribution statement

Claudia Y. Salazar-González: Investigation, Methodology, Formal analysis, Writing – original draft. **Carla M. Stinco:** Methodology, Supervision, Writing – original draft. **Francisco J. Rodríguez-Pulido:** Methodology, Writing – review & editing. **Consuelo Díaz-Moreno:** Conceptualization, Methodology, Visualization. **Carlos Fuenmayor:** Visualization. **Francisco J. Heredia:** Supervision, Writing – review & editing. **M. Lourdes González-Miret:** Writing – review & editing, Review and editing the second version.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgments

We thank Facultad de Ingeniería of Universidad Nacional de Colombia for its support to international mobility; Asociación Universitaria Iberoamericana de Posgrado -AUIP- with the program “Becas de Movilidad entre Universidades Andaluzas e Iberoamericanas Convocatoria 2017” and MINCIENCIAS - Fondo de Ciencia, tecnología e Innovación del Sistema General de Regalías, administrados por el Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación “Francisco José de Caldas”, Programa Colombia BIO y

Gobernación de Cundinamarca. Finally, we thank the staff of the Biology Service (SGI, Universidad de Sevilla) for the technical assistance.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114065.

References

Abd-Alla, A. E., & Salem, R. A. (2020). Impact of storage period on different types of bee pollen pigments. *Journal of Plant Protection and Pathology, 11*(1), 9–13. https://doi.org/10.21608/jppp.2020.68178

Aman, R., Biehl, J., Carle, R., Conrad, J., Beifuss, U., & Schieber, A. (2005). Application of HPLC coupled with DAD, APcI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. *Food Chemistry, 92*(4), 753–763. https://doi.org/10.1016/j.foodchem.2004.10.031

Bleha, R., Shevtsova, T., Kružík, V., Brindza, J., & Sinica, A. (2019). Morphology, physicochemical properties and antioxidant capacity of bee pollens. *Czech Journal of Food Sciences, 37*(1), 1–8. https://doi.org/10.17221/139/2018-CJFS

Books, B., Petropoulos, S., Ferreira, I. C. F. R., & Barros, L. (2018). *Phytochemicals in vegetables: A valuable source of bioactive compounds*. Bentham Science Publishers. https://doi.org/10.2174/9781681087399180101

Cândido, T. L. N., Silva, M. R., & Agostini-Costa, T. S. (2015). Bioactive compounds and antioxidant capacity of buriti (*Mauritia flexuosa L.f.*) from the Cerrado and Amazon biomes. *Food Chemistry, 177*(Supplement C), 313–319. https://doi.org/10.1016/j.foodchem.2015.01.041

Chamorro-García, F. J., León-Bonilla, D., & Nates-Parra, G. (2013). El Polen Apícola como Producto Forestal no Maderable en la Cordillera Oriental de Colombia. *Colombia Forestal, 16*, 53–66.

Chamorro, F. J., León, D., Montoya-Pfeiffer, P. M., Solarte, V. M., & Nates-Parra, G. (2017). Botanical origin and geographic differentiation of bee-pollen produced in high mountains from the Colombian eastern Andes. *Grana, 56*(5), 386–397. https://doi.org/10.1080/00173134.2017.1283440

Colinvaux, P., De Oliveira, P., & Moreno, J. (1999). *Amazon pollen manual. Part I.* Overseas Publisher Association.

Conte, G., Benelli, G., Serra, A., Signorini, F., Bientinesi, M., Nicolella, C., Mele, M., & Canale, A. (2017). Lipid characterization of chestnut and willow honeybee-collected pollen: Impact of freeze-drying and microwave-assisted drying. *Journal of Food Composition and Analysis, 55*, 12–19. https://doi.org/10.1016/j.jfca.2016.11.001

Domínguez-Valhondo, D., Bohoyo Diego, G., Hernández, M. T., & González-Gómez, D. (2011). Influence of the commercial processing and floral origin on bioactive and nutritional properties of honeybee-collected pollen. *International Journal of Food Science and Technology, 46*(10), 2204–2211. https://doi.org/10.1111/j.1365-2621.2011.02738.x

Erdtman, G. (1969). *Handbook of palynology. Morphology - taxonomy - Ecology. An introduction to the study of pollen grain and spores*. Hafner Publishing Company. https://doi.org/10.1002/fedr.19710810815

Esquivel, P., Schweiggert, R. M., Chacón-Ordóñez, T., Steingass, C. B., Carle, R., & Jiménez, V. M. (2019). Carotenoid assembly in fruits and vegetables. *Carotenoid Esters in Foods: Physical, Chemical and Biological Properties*, (13), 51–67.

Fatrcová-Šramková, K., Nôžková, J., Máriássyová, M., & Kačániová, M. (2016). Biologically active antimicrobial and antioxidant substances in the *Helianthus annuus L.* bee pollen. *Journal of Environmental Science and Health, Part B, 51*(3), 176–181. https://doi.org/10.1080/03601234.2015.1108811

Fernández-Lara, R., Gordillo, B., Rodríguez-Pulido, F. J., Lourdes González-Miret, M., del Villar-Martínez, A. A., Dávila-Ortiz, G., & Heredia, F. J. (2015). Assessment of the differences in the phenolic composition and color characteristics of new strawberry (*Fragaria x ananassa Duch.*) cultivars by HPLC–MS and Imaging Tristimulus Colorimetry. *Food Research International, 76*(3), 645–653. https://doi.org/10.1016/j.foodres.2015.07.038

Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research, 43*(3), 228–265. https://doi.org/10.1016/j.plipres.2003.10.002

Gardana, C., Del Bo', C., Quicazán, M. C., Corrrea, A. R., & Simonetti, P. (2018). Nutrients, phytochemicals and botanical origin of commercial bee pollen from different geographical areas. *Journal of Food Composition and Analysis, 73*, 29–38. https://doi.org/10.1016/j.jfca.2018.07.009

Gasparotto, J. A., Pereira de Melo, I. L., Granato, D., Araújo, E., da Silva de Freitas, A., Barth, O. M., Sattler, A., & de Almeida-Muradian, L. B. (2015). Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: A screening study. *Food Research International, 77*(2), 82–91. https://doi.org/10.1016/j.foodres.2015.09.013

Hempel, J., Schädle, C. N., Sprenger, J., Heller, A., Carle, R., & Schweiggert, R. M. (2017). Ultrastructural deposition forms and bioaccessibility of carotenoids and carotenoid esters from goji berries (*Lycium barbarum L.*). *Food Chemistry, 218*, 525–533. https://doi.org/10.1016/j.foodchem.2016.09.065

IDEAM. (2005). *Estudio de la caraterización climática de Bogotá y Cuenca Alta del Río Tunjuelo, i* p. 122).

IDEAM. (2017). *Características climatológicas de Ciudades principales y municipios turísticos*.

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 - Food Science and Technology, 84*, 402–408. https://doi.org/10.1016/j.lwt.2017.06.003

Karkar, B., Şahin, S., & Güneş, M. E. (2020). Evaluation of antioxidant properties and determination of phenolic and carotenoid profiles of chestnut bee pollen collected from Turkey. *Journal of Apicultural Research*, 1–10. https://doi.org/10.1080/00218839.2020.1844462

Kim, J.-S., An, C. G., Park, J.-S., Lim, Y. P., & Kim, S. (2016). Carotenoid profiling from 27 types of paprika (*Capsicum annuum L.*) with different colors, shapes, and cultivation methods. *Food Chemistry, 201*(Supplement C), 64–71. https://doi.org/10.1016/j.foodchem.2016.01.041

Kovacik, L., Plitzko, J. M., Grote, M., & Reichelt, R. (2009). Electron tomography of structures in the wall of hazel pollen grains. *Journal of Structural Biology, 166*(3), 263–271.

Kulaitienė, J., Vaitkevičienė, N., Jarienė, E., Černiauskienė, J., Jeznach, M., & Paulauskienė, A. (2020). Concentrations of minerals, soluble solids, vitamin C, carotenoids and toxigenic elements in organic goji berries (*Lycium barbarum L.*) cultivated in Lithuania. *Biological Agriculture & Horticulture, 36*(2), 130–140. https://doi.org/10.1080/01448765.2020.1748714

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World, 59*(4), 139–157. https://doi.org/10.1080/0005772X.1978.11097714

Machado De-Melo, A. A., Fernandes Estevinho, M. L. M., Gasparotto Sattler, J. A., Rodrigues Souza, B., Freitas, A. da S., Barth, O. M., & Almeida-Muradian, L. B. (2016). Effect of processing conditions on characteristics of dehydrated bee-pollen and correlation between quality parameters. *LWT - Food Science and Technology, 65*, 808–815. https://doi.org/10.1016/j.lwt.2015.09.014

Mapelli-Brahm, P., Corte-Real, J., Meléndez-Martínez, A. J., & Bohn, T. (2017). Bioaccessibility of phytoene and phytofluene is superior to other carotenoids from selected fruit and vegetable juices. *Food Chemistry, 229*, 304–311.

Mărgăoan, R., Mărghitaş, L. A., Dezmirean, D. S., Dulf, F. V., Bunea, A., Socaci, S. A., & Bobiş, O. (2014). Predominant and secondary pollen botanical origins influence the carotenoid and fatty acid profile in fresh honeybee-collected pollen. *Journal of Agricultural and Food Chemistry, 62*(27), 6306–6316. https://doi.org/10.1021/jf5020318

Murillo, E., Giuffrida, D., Menchaca, D., Dugo, P., Torre, G., Meléndez-Martinez, A. J., & Mondello, L. (2013). Native carotenoids composition of some tropical fruits. *Food Chemistry, 140*(4), 825–836. https://doi.org/10.1016/j.foodchem.2012.11.014

Negrão, A., & Orsi, R. (2018). Harvesting season and botanical origin interferes in production and nutritional composition of bee pollen. *Anais da Academia Brasileira de Ciências, 90*, 325–332.

Niro, S., Fratianni, A., Panfili, G., Falasca, L., Cinquanta, L., & Alam, M. (2017). Nutritional evaluation of fresh and dried goji berries cultivated in Italy. *Italian Journal of Food Science, 29*(July), 398–408.

Oliveira, K. C. L. S., Moriya, M., Azedo, R. A. B., Almeida-Muradian, L. B. de, Teixeira, E. W., Alves, M. L. T. M. F., & Moreti, A. C. de C. C. (2009). Relationship between botanical origin and antioxidants vitamins of bee-collected pollen. *Química Nova, 32*, 1099–1102. https://doi.org/10.1590/S0100-40422009000500003.

Pulido, N., Salazar, C., & Díaz, C. (2012). Cuantificación de vitamina E en muestras comerciales de polen apícola. *Vitae - Revista de La Facultad de Química Farmaceútica, 19*(1), 357–359.

Roubik, D., & Moreno, J. (1991). *Pollen and spores of barro Colorado island*.

Şahin, S., & Karkar, B. (2019). The antioxidant properties of the chestnut bee pollen extract and its preventive action against oxidatively induced damage in DNA bases. *Journal of Food Biochemistry*. https://doi.org/10.1111/jfbc.12888. Article e12888.

Saini, R. K., Nile, S. H., & Park, S. W. (2015). Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. *Food Research International, 76*(3), 735–750. https://doi.org/10.1016/j.foodres.2015.07.047

Salazar-González, C., & Díaz-Moreno, C. (2016). The nutritional and bioactive aptitude of bee pollen for a solid-state fermentation process. *Journal of Apicultural Research, 55*(2), 161–175. https://doi.org/10.1080/00218839.2016.1205824

Salazar-González, C. Y., Rodríguez-Pulido, F. J., Stinco, C. M., Terrab, A., Díaz-Moreno, C., Fuenmayor, C., & Heredia, F. J. (2020). Carotenoid profile determination of bee pollen by advanced digital image analysis. *Computers and Electronics in Agriculture, 175*(June), 1–8. https://doi.org/10.1016/j.compag.2020.105601

Salazar-González, C. Y., Rodríguez-Pulido, F. J., Terrab, A., Díaz-Moreno, C., Fuenmayor, C. A., & Heredia, F. J. (2018). Analysis of multifloral bee pollen pellets by advanced digital imaging applied to functional food ingredients. *Plant Foods for Human Nutrition, 73*(4), 328–335. https://doi.org/10.1007/s11130-018-0695-9

Sarungallo, Z. L., Hariyadi, P., Andarwulan, N., Purnomo, E. H., & Mitsuhiro, W. (2015). Analysis of α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene of *Pandanus conoideus* oil by high-performance liquid chromatography (HPLC). *Procedia Food Science, 3*, 231–243. https://doi.org/10.1016/j.profoo.2015.01.026

Schulte, F., Mäder, J., Kroh, L. W., Panne, U., & Kneipp, J. (2009). Characterization of pollen carotenoids with in situ and high-performance thin-layer chromatography supported resonant Raman spectroscopy. *Analytical Chemistry, 81*(20), 8426–8433.

Sebii, H., Karra, S., Bchir, B., Ghribi, A. M., Danthine, S. M., Blecker, C., Attia, H., & Besbes, S. (2019). Physico-chemical, surface and thermal properties of date palm pollen as a novel nutritive ingredient. *Advances in Food Technology and Nutritional Sciences, 5*(3), 84–91. https://doi.org/10.17140/AFTNSOJ-5-160

Song, J., Li, D., He, M., Chen, J., & Liu, C. (2016). Comparison of carotenoid composition in immature and mature grains of corn (*Zea mays L.*) varieties. *International Journal*

of Food Properties, 351–358. https://doi.org/10.1080/10942912.2015.1031245, 19 (March 2015).

Stinco, C. M., Benítez-González, A. M., Hernanz, D., Vicario, I. M., & Meléndez-Martínez, A. J. (2014). Development and validation of a rapid resolution liquid chromatography method for the screening of dietary plant isoprenoids: Carotenoids, tocopherols and chlorophylls. *Journal of Chromatography A, 1370*, 162–170. https://doi.org/10.1016/j.chroma.2014.10.044

Stinco, C. M., Benítez-González, A. M., Meléndez-Martínez, A. J., Hernanz, D., & Vicario, I. M. (2019). Simultaneous determination of dietary isoprenoids (carotenoids, chlorophylls and tocopherols) in human faeces by Rapid Resolution Liquid Chromatography. *Journal of Chromatography A, 1583*, 63–72. https://doi.org/10.1016/j.chroma.2018.11.010

Thakur, M., & Nanda, V. (2018). Exploring the physical, functional, thermal, and textural properties of bee pollen from different botanical origins of India. *Journal of Food Process Engineering*, 1–14. https://doi.org/10.1111/jfpe.12935. October.

Velásquez, C. (1999). *Atlas palinológico de la flora vascular paramuna de Colombia: Angiospermae*. Universidad Nacional de Colombia, Sede Medellín, Facultad de Ciencias. https://books.google.com.co/books?id=8jdiHQAACAAJ.

Velásquez, P., Rodríguez, K., Retamal, M., Giordano, A., Valenzuela, L. M., & Montenegro, G. (2017). Relation between composition, antioxidant and antibacterial activities and botanical origin of multi-floral bee pollen. *Journal of Applied Botany and Food Quality, 314*, 306–314. https://doi.org/10.5073/JABFQ.2017.090.038

Xu, Dong, J., Li, T., He, W., & Sun, L. (2013). Influence of storage temperature on lipid oxidation, stale flavor formation and discoloration in lotus (*Nelumbo nucifera*) bee pollen. *Journal of Food Processing and Preservation, 37*(2), 171–178. https://doi.org/10.1111/j.1745-4549.2011.00642.x

Xu, Dong, J., Mu, X., & Sun, L. (2011). Supercritical CO_2 extraction of oil, carotenoids, squalene and sterols from lotus (*Nelumbo nucifera Gaertn*) bee pollen. *Food and Bioproducts Processing, 89*, 47–52.

Zhang, Z., Xiao, Y., Li, D., & Liu, C. (2016). Identification and quantification of all-trans-zeaxanthin and its cis-isomers during illumination in a model system. *International Journal of Food Properties, 19*(6), 1282–1291. https://doi.org/10.1080/10942912.2015.1072209

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