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Dried Vs Fresh-Frozen Bee Pollen: Botanical Sensory Profiling

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Abstract

This study obtains the sensory description of different botanical bee pollen (BP) profiles and shows the differences between dried and fresh-frozen BP organoleptic characterization. Fifty-four (n=54) samples of fresh-frozen (n=27) and dried (n=27) BP pellets were analyzed for botanical and descriptive sensory aspects. The palynological results identified unifloral (*Echium* sp.), bifloral (*Citrus* sp. and *Cistus* sp.), and polyfloral (Leguminoseae, Rosaceae, and Myrtaceae families) BP. The use of Hierarchical Cluster Analysis revealed that four different groups were separated, corresponding to all dried BP, fresh-frozen *Echium* sp., fresh-frozen polyfloral and fresh-frozen *Citrus* sp. and *Cistus* sp. Discriminant Analysis returned satisfactory results as 83.3% of all BP samples were correctly classified. No classification for different botanical origin in dried BP samples was possible based on their sensory properties. However, all fresh-frozen BP samples were differentiated according to their sensory profile coinciding with the results of the Principal Component Analysis (PCA). The first two discriminant functions explained 94% of the variance. The sensory profile for fresh-frozen BP was defined and the classification precision was also achieved. On the contrary, all samples that went under drying treatment presented the same sensory profile. These results suggested that sensory profile could be used as predictor to classify fresh-frozen BP based on its botanical origin.

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Introduction

Since immemorial time honey and other bee products which beehives provide us with, have been used due to their nutritional and medical functions.[1-4] Bee pollen (BP) results from the combination of the flower pollen collected by foraging honeybees (*Apis mellifera*) together with nectar and their mouth secretions. When honeybees visit flowers

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of entomophilous species they get covered with pollen dust. Then, during the grooming process, the honeybees collect the pollen grains all over their body and moisten them with regurgitated nectar and their own salivary glands secretions, the bees pack everything together as pollen loads or pollen pellets and finally, they pressed the loads into their pollen basket of their hind legs.[5,6] Thus, honeybees use pollen to feed their larvae in the early stages of development, becoming even more vital than nectar for the production of brood.[7] Apart from proteins, BP also contains all the essential amino-acids, lipids (omega-3 and omega-6), carotenoids, phenolic compounds, flavonoids, sterols, terpenes, vitamins, minerals, some carbohydrates, etc.[8,9,4-7] Not surprisingly, in view of its chemical composition it is considered as a 'perfectly complete food'.[10] Moreover, the composition of the BP is known to vary as it is closely linked to the botanical source which is harvested from. Hence, BP would have different characteristics depending on the floral species,[8] geographic areas, soil types, climatic conditions and even beekeeping routines.[4,11] Although the use of BP has survived in traditional folk medicine for centuries, and ancient civilizations have been aware of its therapeutic and healing properties, it was not until the Second World War that bee collected pollen was used for human nutrition on an industrial scale after pollen traps were developed.[12,13]

The different commercial types of BP depend on their industrial treatment. The oven-drying method is considered to be the most commonly used method to preserve BP. The drying process is carried out industrially in special ovens at a maximum temperature of 50ºC to reduce its moisture (5-8%) and prevent spoilage.[14] Fresh BP, frequently marketed as fresh-frozen BP, refers to samples which are frozen immediately after harvesting. By freezing, the BP keeps its freshness while ensuring its preservation. BP defrosts quickly without any significant deterioration just before consumption. Some research studies highlight undesirable changes in the chemical composition and organoleptic characteristics of BP depending on chosen conservation method.[15]

With the raising awareness of the positive aspects of healthy dietary patterns, mainly in developed countries, modern consumers perceive natural products to be less harmful than processed ones. In this context, BP is claimed to be the perfect natural food. It has been widely used as a dietary supplement for both human and animal nutrition, and nowadays it also has been called to be part of the food industry as an ingredient.[11]

Whereas there is now a large number of literature reports that have focused about the health promoting effects of BP. As such, anti-inflammatory, antioxidant, anti-androgenic, antimicrobial, anti-carcinogenic, hepatoprotective and immunomodulatory activities, scarce research appears to have been undertaken on describing its sensory characteristics. [4,12-14,16-21] Even though in the food industry, grading methods are widely used to separate products in different quality categories, such methods are often based on organoleptic attributes. Descriptive sensory analysis remains the most versatile, thorough, and practical sensory technique, offering in-depth insights into all of a product's sensory characteristics.[22] Descriptive analysis refers to a technique commonly used to identify, measure, and describe the sensory characteristics of food, relying on the evaluations provided by trained individuals.[23] Recognized authors set in their sensory study of honey that profiling approach involves identifying the most notable characteristics for each type of unifloral honey, along with their corresponding reference standards, and utilizing them to establish a comprehensive reference profile.[24] Non published literature was found on the sensory description of botanical bee pollen profiles nor about differences between dried and fresh frozen bee pollen organoleptic characterization. It is also important to evaluate how incorporating monofloral and multifloral bee pollen into food products influences their quality, to gain a deeper understanding of how the botanical origin affects product characteristics.[25] Taking the above points into account and considering sensory evaluation as a vital key to establish the quality of products, the aims of this study were (i) elaborating, for the first time, a vocabulary to establish a sensory characterization of different types of BP. The development of this lexicon is absolutely necessary before creating a profile sheet to perform a generic descriptive analysis of BP, and (ii) obtaining a complete characterization for BP by a sensory descriptive analysis, in order to identify their organoleptic attributes and set sensory profiles for different types of BP according to their botanical origin (unifloral, bifloral and polyfloral) and

depending on the preservation treatment (dried or fresh frozen).

Materials and Methods

Bee Pollen Samples

This study was conducted with fifty-four (n=54) samples of fresh-frozen (n=27) and dried (n=27) BP pellets. The fresh-frozen BP was collected by different beekeepers (March – June 2022). Standard pollen traps were fitted to the entrance of the beehive and emptied daily in sterile plastic bags. Then, the samples were delivered to the laboratory, and they were manually cleaned, separated into groups according to their colour and kept at freezing temperature (-18ºC) up to their analysis.

The dried BP samples were both from the market and beekeepers. The dried BP samples numbered 28-36 were collected by beekeepers and analyzed after a heat-treatment in drying-ovens (45ºC). The rest of the dried BP samples for this study (numbered 37-54) were purchased from local stores and herbalist´s and kept in the dark at room temperature (20ºC) until analysis.

Table 1:Description of the BP samples used in the study

Sample identification	Origin of the sample	Type of BP
1-7	Collected by beekeepers	Fresh BP frozen in the laboratory after collection
28-36	Collected by beekeepers	Fresh BP dried in the laboratory after collection
37-54	Commercial BP	Dried BP purchased from local stores and herbalist´s

Fresh BP samples collected from beekeepers were subdivided into two different subsamples, one subsample of fresh BP was frozen and then assessed (samples 1-27) and the other one was dried and then analyzed (samples 28-36). The detailed description of the samples is shown in Table 1.

Botanical Analysis of Pollen Loads

Pollen pellets present a huge variation of colours and morphological features. The colour of BP depends on the botanical source and chemical composition and is said to range from ‘cream’ to ‘dark purple’ or ‘all shades from black to white’.[26,27] BP is classified as unifloral when it comes from a particular botanical group. However, honeybees sometimes visit different plant species in one collecting-trip, blending the different pollen together in the same pollen load, this is known as pollen-assemblage.[14] According to some authors pollen pellets are classified as monofloral when they originate from a specific botanical taxon, contain a single dominant pollen type at a frequency exceeding 90%, or include no accessory pollen with a frequency surpassing 60%.[25] However, this study has followed the indications that consider predominant pollen at > 80% frequency as monofloral.[6,9]

In this study, botanical analysis of BP was performed following revised methodology.[9,14,28] A sample of 2g of BP (this is more or less 250-300 pollen loads) was considered to be representative. The loads were classified and separated according to their colour. Each sample was washed with 50% ethanol and left for 30 minutes. After centrifugation for 3 minutes at 2000 rpm, supernatant was removed. This process was repeated until obtaining a homogeneous suspension. Final sediment was suspended in 20 ml of distilled water. One drop of this suspension was mixed with glycerin jelly and basic fuchsine and placed on a slide. The pollen slides were analyzed using a light microscope (Nikon Labophot-2 microscope, Nikon, Tokyo, Japan) at magnifications of 400x and 1000x to identify the botanical sources of the pollen types. Counts were expressed as pollen frequency classes after counting a minimum of 500 pollen grains on three slides per sample.[29] For comparison and recognition purposes, a reference collection of the University of Córdoba and pollen morphology guides were used.

Descriptive Sensory Analysis

Assessors

A group of ten evaluators, consisting of six women and four men aged between 27 and 55 years,

was prepared to take part in this research. The participants were employees and researchers affiliated with the Food Science and Technology Department at the University of Córdoba in Spain. The panel had been previously chosen and trained in accordance with the guidelines of Standard ISO 8586:2014.[30] The selection process involved conducting tests for detection, recognition, and discrimination, as well as assessing the candidates' capacity to recall and articulate sensory perceptions. This panel has been conducting different beverages and food sensory testing for a number of years. The panel was tasked with creating a completely new lexicon for BP. It was explained that certain attributes might be common across all types of BP, while others could be unique to only a few. Panelists were encouraged to be as detailed as possible when identifying attributes. To minimize potential biases, no information about the BP samples was shared with the panelists. In fact, samples were randomly labelled, using three-digit alphanumeric-codes. The panel participated in two training sessions, each lasting two hours, to practice BP testing. These sessions took place in a sensory laboratory outfitted with a circular table for group training and individual booths, all designed in compliance with the ISO 8589:2010 standards.[31] The training sessions were held in the morning from 10:00 to 12:00. During the sessions, panelists individually assessed the samples, recording the descriptors they observed. After completing their evaluations, the panel leader guided a discussion to achieve agreement on the descriptors identified for each sample. After agreeing on the descriptors, the panel worked on refining the odour and flavour notes and proposing references for each descriptor. Whenever possible, they aimed to use representative reference products that clearly exhibited the specific attributes in question. For BP these specific attributes had the following references: Bencyl acetate, 1 g/100 g ethanol (floral), cis-3-hexenol, 1 drop in 50 ml water (green-leafy), acetyl-pyridine (burn-roasted), and 2-6-Dimethylcyclohexanol (earthy).

Creation of the Profile Sheet

There are no previous studies providing a profile sheet to evaluate BP. Only few works exist about the sensory profile of BP, although their results were limited because neither of them fully identified nor described all evaluable sensory attributes in BP.[8,15,32] Nevertheless, those previous studies were also considered for the creation of the profile sheet. They were grouped by odour families odour and aroma attributes following different classifications proposed by different authors for honeys, for various teas, and, for chemicals associated with green.[24,33-35]

As it has been suggested, the first step was to obtain a detailed list of possible assessable attributes and to provide the assessors with reference material to identify the descriptors.[36] For the sensory evaluation of BP did not exist a consolidated vocabulary or any sensory wheel. Therefore, seven sessions were conducted to perform a triangle test for identifying differences between the samples and then, to familiarize the panelists with the samples. The selected descriptors included in the profile sheet were obtained after a Principal Components Analysis. Finally, a total of 25 sensory attributes were included in the profile sheet (see supplementary material - S1). Each sensory attribute (except colour) was scored on a structured five-point scale (0 for total absence and 5 for strongest perception).[37] The sensory studied attributes were: two attributes for appearance (cleanliness degree and colour), one for tactile texture properties (sensory determination of finger texture of BP), one for texture properties in the mouth, one for the water content, one for the pollen-dust content, five for odour (global odour intensity, floral, burn-roasted, green-leafy and earthy), four for the basic tastes (sweetness, acidity, saltiness and bitterness), five for aroma (global aroma intensity, floral, burn-roasted, green-leafy and earthy), three for trigeminal sensations (freshness, astringency and hot), persistence and aftertaste.

Sensory Evaluation of the Samples (Panelists)

Each sample (20g) was put into a glass vial (6x2 cm) and covered with a watch glass for sensory analysis. The samples were prepared one hour before the analysis to allow headspace to equilibrate and were served at room temperature (20°C). Four samples (two for dried and two for fresh-frozen BP), labelled with 3-digited random numbers, were served, one at a time, over a session. Mineral water was used as cleanser between samples.

Tasting Procedure

The tasting procedure was performed as follows: Firstly, the assessor uncovered the vial and breathed over the top of it, then they shook the sample and

breathed again to score the odour (orthonasal) attributes. Secondly, a small amount of BP was put in the mouth using a disposable plastic teaspoon, BP was dissolved (if possible) before being slowly chewed and swallowed, perceiving the aroma (retronasal odour) and persistence attributes. The scores for the different studied attributes were obtained by consensus according to the ISO 13299:2016 guidelines.[38]

Statistical Analysis

Statistical analysis was performed to evaluate the association between the characterization of bee pollen sensory profiles and their defining organoleptic attributes.

Descriptive Statistics

Descriptive statistics (mean ± standard deviation) were calculated using Microsoft Excel 2010.

Exploratory Hierarchical Cluster Analysis (HCA): Identify Natural Grouping Based on Sensory Descriptors, Botanical Origin, and Pollen Type

Hierarchical Cluster Analysis (HCA) was performed to assess the similarities and dissimilarities among pollen groups by evaluating pollen sensory descriptors through the formation of natural major clusters. Preset groups of pollen depended on their botanical origin (*Echium* sp., polyfloral and *Citrus* sp./ *Cistus* sp.) and type (fresh/dried). The use of HCA was suggested in other affine pollen research.[39-41]

The Hierarchical Cluster routine of the Classify pack in the Analyze set in SPSS v.26 (SPSS Inc., Chicago, Illinois, U.S.A.) was used to perform HCA. Between-groups linkage was performed to graphically depict a dendrogram representing the relationships across pollen types (groups).

Between-groups linkage aims to identify clusters by measuring the average distance between all pairs of observations from different groups. This method, also known as Unweighted Pair Group Method with Arithmetic Mean (UPGMA), helps ensure that clusters are formed based on the overall similarity between groups, rather than individual elements.

Euclidean distances across pollen types using Ward's methods were calculated as this is often considered superior to other methods when within cluster variance is heterogenous, due to its ability to minimize the within-cluster variance, leading to more compact and homogenous clusters. Descriptive statistics were calculated with SPSS v.26 (SPSS Inc., Chicago, Illinois, U.S.A.).[40,42]

Ward's distances calculation method ensures that the clusters formed are more meaningful and representative of the underlying data structure, which is crucial for accurately assessing the subtle variations in pollen features.[41] Additionally, Ward's method tends to be more robust in handling different data distributions, making it a preferred choice for complex datasets like pollen analysis.[42] As pre-treatment of data was carried transform values of variables (average zero and standard deviation 1) called Z scores. The dendrogram similarity scales that are generated by the SPSS program range from zero (greater similarity) to 25 (lower similarity).

Principal Component Analysis (PCA): Examine Relationships Among Pollen Sensory Attributes

Principal Component Analysis (PCA) was performed to determine the existence of relationship across the sensory attributes presented in Table 2 as well as to extract major components which may agglomerate related attributes. The suitability of PCA to perform sensory evaluation of pollen has already been reported.[43]

The Cronbach's alpha test was conducted to determine the internal consistency and reliability of the data set for the performance of PCA as suggested by other studies of the same nature.[44-45] This coefficient represents the reliability of the data collected and it normally ranges from 0 to 1. Higher values indicate a higher level of internal consistency. A Cronbach's alpha value of 0.7 or greater is minimally sufficiently consistent to indicate and consider the items to be acceptable for further analysis.

Sample suitability for PCA was indicated by Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Barlett's test of sphericity. In general, KMO values between 0.8 and 1 indicate the sampling is adequate.[46] Pollen sensory attributes are considered to be significantly loaded components when they reach values of ≥|0.5|, as it has been suggested.[47]

PCA is preliminarily performed prior to Discriminant Analysis (DA), to reduce the number of variables to

those most influential in differentiating across pollen samples depending on their origin and type. DA then determines the percentage of pollen samples assigned to their initial classifications. Variables with a discriminant loading of ≥|0.40| are considered significant, while nonsignificant variables are excluded using the stepwise procedure. Variables with larger absolute coefficient values have greater discriminating ability. Data were standardized.

Table 2: BP sensory attributes included in the sensory analysis sheet

Category	Attribute
Appearance	
	Cleanliness Degree
	Colour
Tactile Texture Properties	Sensory Determination of Finger Texture of BP
Texture in Mouth	Texture in the Mouth
Water Content	Water Content
Pollen-Dust Content	Pollen-Dust Content
Odour	Global Odour Intensity
	Floral Odour
	Burn-Roasted Odour
	Green-Leafy Odour
	Earthy Odour
Basic Tastes	Sweetness
	Acidity
	Saltiness
	Bitterness
Aroma	Global Aroma Intensity
	Floral Aroma
	Burn-Roasted Aroma
	Green-Leafy Aroma
	Earthy Aroma
Trigeminal Sensations	Freshness
	Astringency
	Hot
Other Attributes	Persistence
	Aftertaste

Discriminant Analysis (DA): Predict BP Sample Pollen Group Membership and Classify Pollen Discriminant Attributes

Multicollinearity was analyzed to ensure predictor independence and detect strong linear relationships which may suggest a lack of orthogonality. Forward and backward stepwise selection methods produced identical variables, but the forward selection method was chosen for its shorter runtime. Canonical discriminant analysis was conducted using SPSS version 26.0 (SPSS Inc., Chicago, Illinois, U.S.A.). Variance inflation factor (VIF) was used to test for multicollinearity.[48]

Wilks' lambda test is used to evaluate the significant contributions of variables to the discriminant function. As Wilks' lambda approaches 0, the contribution of the variable to the discriminant function increases. The significance of Wilks' lambda is tested using a $\chi 2$ test. If the significance is below 0.05, the function effectively explains the group assignment.[48]

Equal covariance matrices assumption in discriminant function analysis was tested using Pillai's trace criterion.[48] A significance level of ≤0.05 indicates statistical significance.

Afterwards DA, the probability of BP samples belonging to one group of BP type or another was calculated using the hit ratio parameter. This parameter calculates the relative distance between the observation and the centroid of the nearest group, representing the percentage of correctly classified cases. Classification accuracy is achieved if the rate is at least 25% higher than the chance rate.

Leave-one-out cross-validation assesses the significance and validity of the discriminant functions. Press' Q statistic supports these results by comparing the discriminating power of the cross-validated function, calculated as: Press Q′=[n−(n′K)]2n(K−1), where n is the number of observations, n′ is the number of correctly classified observations, and K is the number of groups. Press' Q should be compared to the critical value of 6.63 for χ2 at a 0.01 significance level. A Press' Q value exceeding 6.63 indicates that the classification is significantly better than chance.

Results

Palynological Results

The results of the palynological analysis of BP revealed that not all samples analysed were characterized as pollen-assemblages. Samples were classified as multifloral when different pollen types were present. On the contrary, samples were considered unifloral when containing more than 80% of one pollen type.[6,9]

Table 3: Results of the BP botanical analysis

Sample identification	Colour of pollen pellets	Botanical origin	BP treatment	Pollen type*
1-9	purple	Unifloral (*Echium* sp.)	Fresh-frozen	FE
10-18	bright yellow	Bifloral (*Citrus* sp. + *Cistus* sp.)	Fresh-frozen	FC
19-27	mixed-colour	Polyfloral	Fresh-frozen	FP
28-36	purple	Unifloral (*Echium* sp.)	Dried	DE
37-45	bright yellow	Bifloral (*Citrus* sp. + *Cistus* sp.)	Dried	DC
46-54	mixed-colour	Polyfloral	Dried	DP

*FE: fresh-frozen Echium BP; FC: fresh-frozen Citrus and Cistus BP; FP: fresh-frozen polyfloral BP; DE: dried Echium BP; DC: dried Cistus and Citrus BP; DP: dried polyfloral BP.

Table 3 shows the results of the botanical origin. The most representative (>80%) botanical source identified in purple pollen pellets belonged to the Boraginaceae family. This type was considered as unifloral *Echium* type BP. Whereas bright yellow pollen pellets belonged to *Cistus* sp. and the Rutaceae family (*Citrus* sp. mainly) and was classified as bifloral BP. Finally, mixed-colour BP resulted to belong to different botanical families which most representative ones were Leguminoseae, Rosaceae and Myrtaceae. This mixed-colour BP was considered multifloral. In similar way, in a Portuguese study using BP as ingredient in black pudding it was raisen as predominant pollen *Cistus ladanifer* (42.6%) followed by *Echium* spp. (13.6%) and Apiaceae (13.2%). 8.6% of pollen of Cistaceae family was also found.[49] *Cistus ladanifer* pollen is very usual in Mediterranean regions. This type of BP has been consumed for centuries due to its remarkable nutritional value and medicinal and nutraceutical potential.

In contrast to what happens with some floral species, up to now, studies have not identified any kind of toxic compounds in *Cistus ladanifer* pollen. This BP is mainly composed of flavonoid glycosides of quercetin, myricetin and kaempferol, twenty-two free amino acids and it is considered a good source of mineral micronutrients (Cu, Fe, Mn, and Zn).[50]

Even though scarce references on BP sensory studies exist, several applications of BP as functional ingredients are found due to its bioactive properties. In this sense our botanical identification of *Cistus* pollen coincides with other authors including this unifloral type of BP as an antioxidant, being natural alternative to prevent the lipid oxidation in black pudding.[49] Furthermore, the use of BP as antioxidant

improves the product quality and consumer acceptance and does not affect their traditional flavor. In fact, the BP possessed the richest profile of chemical classes among other bee products (12 from 13 chemical groups). Its volatile profile included 13 alkanes, five aldehydes, two acids, two benzene derivatives, two ketones, two esters, and one compound from other groups (sulfoxides, alcohols, pyrroles, furans, lactones, and monoterpenes). The main chemical group in BP was represented by alkanes (65.5% of total volatiles).[51]

Table 4: Descriptive statistics for the BP (FE-Fresh Echium; FC-Fresh Citrus; FP-Fresh Polyfloral; DE-Dried Echium; DC-Dried Citrus; DP-Dried Polyfloral) sensory analysis (mean ± SD)

	FE	FC	FP	DE	DC	DP
Cleanliness	4.3 ± 0.5	3.3 ± 0.5	3.7 ± 0.7	4.3 ± 0.5	3.3 ± 0.5	4.9 ± 0.2
Global intensity odour	3.6 ± 0.5	3.7 ± 0.3	4.1 ± 0.4	0.9 ± 0.3	0.6 ± 0.2	0.7 ± 0.3
Floral odour	0.2 ± 0.3	3.9 ± 0.4	1.1 ± 0.2	0 ± 0	0.1 ± 0.2	0.1 ± 0.2
Burn-roasted odour	0 ± 0	0 ± 0	0 ± 0	3.2 ± 0.5	3.9 ± 0.5	3.9 ± 0.5
Green leafy odour	1.7 ± 0.5	0.9 ± 0.2	3.7 ± 0.4	0 ± 0	0 ± 0	0 ± 0
Earthy odour	4.2 ± 0.5	0.8 ± 0.3	0.7 ± 0.3	0 ± 0	0 ± 0	0 ± 0
Finger texture	4 ± 0	4 ± 0	4 ± 0	0.3 ± 0.5	0.2 ± 0.4	0.2 ± 0.3
Mouth texture	1 ± 0	1 ± 0	1 ± 0	2 ± 0	2 ± 0	2 ± 0
Water content	2 ± 0	1.9 ± 0.2	1.9 ± 0.2	0 ± 0	0 ± 0	0 ± 0
Pollen dust	3.3 ± 0.4	3.3 ± 0.4	2.4 ± 0.6	0 ± 0	0 ± 0	0 ± 0
Sweetness	0.1 ± 0.3	0.6 ± 0.5	0.6 ± 0.4	2.9 ± 0.6	3.1 ± 0.4	3.5 ± 0.5
Acidity	0.9 ± 0.8	1.3 ± 1.1	0.9 ± 0.9	0.3 ± 0.4	0.5 ± 0.4	0 ± 0
Salty	3.3 ± 0.5	2.4 ± 0.8	2.3 ± 0.4	0.4 ± 0.5	0.3 ± 0.4	0.6 ± 0.3
Bitter	3.3 ± 0.7	2.3 ± 0.4	2.3 ± 0.7	1.2 ± 0.4	0.4 ± 0.4	0.6 ± 0.6
Global intensity aroma	4.4 ± 0.3	3.4 ± 0.5	3.1 ± 0.6	2.2 ± 0.3	2 ± 0.5	1.8 ± 0.5
Floral aroma	0.1 ± 0.2	3.7 ± 0.4	1.7 ± 0.6	0.3 ± 0.4	0.3 ± 0.4	0.2 ± 0.4
Green leafy aroma	1.2 ± 0.3	0.8 ± 0.4	2.2 ± 0.8	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.3
Burn-roasted aroma	0 ± 0	0 ± 0	0.1 ± 0.2	3.1 ± 0.5	3.2 ± 0.5	3.6 ± 0.5
Earthy aroma	4.2 ± 0.5	0.1 ± 0.2	0.5 ± 0.6	0 ± 0	0 ± 0	0 ± 0
Hot	2.9 ± 1	2.8 ± 1.3	1.8 ± 0.6	0 ± 0	0 ± 0	0 ± 0
Freshness	1.2 ± 0.3	4 ± 1.3	1.7 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Astringency	0 ± 0	2.2 ± 2.6	1.1 ± 2.2	0 ± 0	0 ± 0	0 ± 0
Persistence	2.9 ± 0.7	2.4 ± 0.5	2.6 ± 0.4	0.6 ± 0.4	0.4 ± 0.5	0.2 ± 0.4
Aftertaste	1.6 ± 0.6	0 ± 0	0.4 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Acceptability	3.6 ± 0.3	3.8 ± 0.4	2.8 ± 0.4	1.5 ± 0.6	0.8 ± 0.5	1.8 ± 1

Sensory Analysis

The mean and standard deviation (SD) obtained from the sensory analysis are listed in Table 4. For the six different groups of samples spider diagrams of sensory profiles are shown in Fig 1.

Statistical Analysis

Exploratory Hierarchical Cluster Analysis (HCA): Identify Natural Grouping Based on Sensory Descriptors, Botanical Origin, and Pollen Type

Fig.2 reports the results from HCA. Four clusters were formed, as follows: Group 1 all dried BP samples with no difference on their botanical origin (samples 28-54), group 2 including all fresh-frozen *Echium* sp. (FE) samples, group 3 including all fresh-frozen polyfloral (FP) samples (19-27) and group 4 including all fresh-frozen *Citrus* sp. and *Cistus* sp. (FC) samples (10-18). The dendrogram in Fig. 2 shows the similarities between the analyzed samples.

These different sensory profiles were obtained based in 5 points scale following other studies on sensory evaluation of BP products.[52] As it can be observed,

the dried BP profiles are similar and undifferentiated for the three types of botanical origin. The attributes characterizing this profile are burn/roasted odor and aroma, and sweetness. On the contrary, the fresh frozen BP profile lacks these attributes and is characterized by higher freshness, persistence, and global intensity odor and aroma, with different notes depending on the botanical source: earthy, salty, and bitter for *Echium*; floral for *Citrus* and *Cistus*; green leafy for polyfloral. In contrast, some experience of adding dried bee pollen to white wines obtained a sensory profile with main attributes of floral and fruity odors.[53]

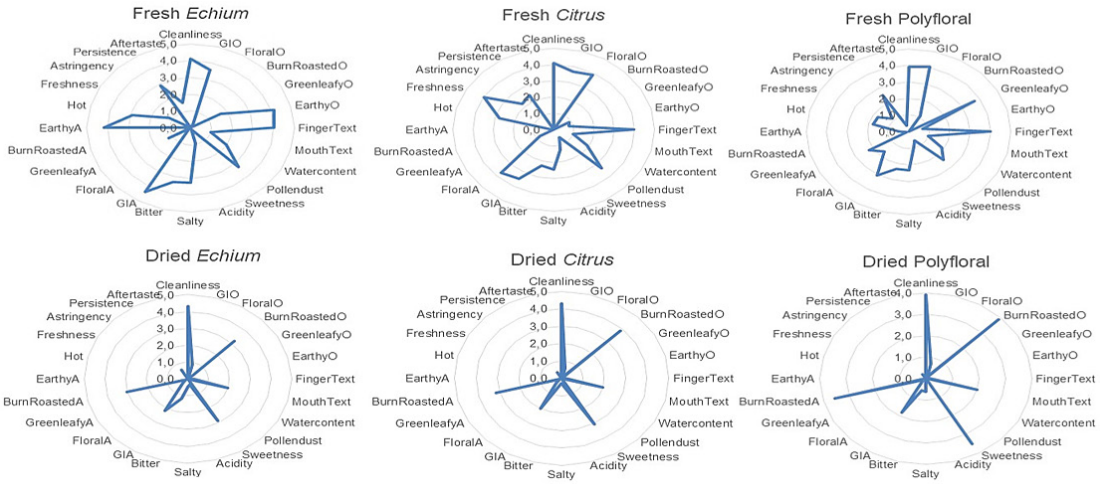

Fig. 1: Spider diagrams of mean scores for sensory attributes of BP samples

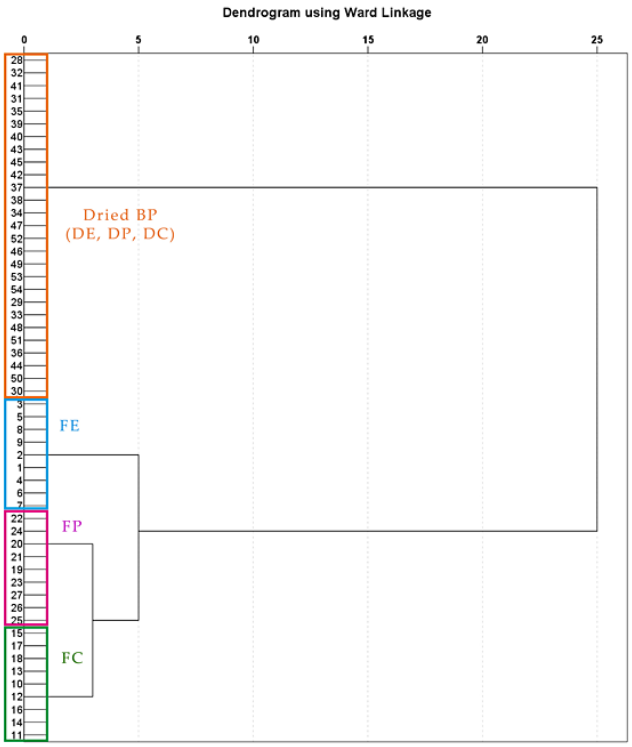

Fig.2. Dendrogram of Hierarchical Cluster Analysis for the BP samples

Principal Component Analysis (PCA): Examine Relationships among Pollen Sensory Attributes

Cronbach's alpha value obtained in this study was 0.771 which indicated the adequate reliability of the data set. The variables 'cleanliness', 'mouth texture' and 'sweetness' were deleted from the list for further analysis as they returned Cronbach's alpha values of 0.783, 0.836 and 0.830, respectively.

The suitability of the data for Principal Component Analysis (PCA) was evaluated using the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity. The KMO value was 0.896, exceeding the threshold of 0.6, indicating that the data were appropriate for PCA. Bartlett's test of sphericity was significant ($p < 0.005$), further supporting the applicability of PCA for dimensionality reduction and data interpretation.

The dataset was organized into six groups based on their botanical origin and type of bee pollen (BP): FE, FC, FP, DE, DC, and DP (see Table 3). PCA was performed, and components with eigenvalues greater than 1 were retained. This analysis identified three principal components (PCs), which collectively explained 85.544% of the total variance: PC1 (63.88%), PC2 (15.883%), and PC3 (5.781%).

Table 5 presents the factor loadings for the three PCs, with a threshold of 0.5 used as the cutoff for significant loadings. The highest factor loadings for each variable are highlighted in bold. PC1 demonstrated strong correlations (factor loadings close to 0.9) with variables such as water content, finger texture, burn-roasted aroma, burn-roasted odor, pollen dust, global intensity of odor (GIO), persistence, salty taste, hot taste, bitterness, and global intensity of aroma (GIA).

PC2 was primarily associated with floral odor and aroma, while PC3 correlated with green-leafy odor and aroma.

Table 5: Pollen sensory attributes component loadings from PCA

	PC1	PC2	PC3
Water content	0.986	0.027	-0.033
Finger Text	0.981	0.068	-0.042
Burn/Roasted A	-0.976	-0.064	0.032
Burn/Roasted O	-0.975	-0.070	0.044
Pollen dust	0.967	0.053	0.141
GIO	0.963	0.115	-0.157
Persistence	0.931	-0.035	-0.050
Salty	0.910	-0.137	0.049
Hot	0.864	0.07	0.201
Bitter	0.862	-0.237	0.055
GIA	0.850	-0.262	0.20
Green leafy A	0.604	-0.030	-0.620
Green leafy O	0.529	-0.043	-0.619
Freshness	0.534	0.547	0.266
Earthy O	0.696	-0.638	0.219
Acidity	0.526	0.224	-0.105
Floral A	0.525	0.779	0.196
Earthy A	0.581	-0.556	0.199
Floral O	0.558	0.562	0.292
Aftertaste	0.563	-0.515	0.112
Astringency	0.344	0.549	-0.147

Fig.3 illustrates the separation of the six BP groups based on PCA results. Fresh-frozen BP samples showed clear differentiation by botanical origin, correlating with distinct sets of variables. In contrast, dried BP samples clustered together, indicating a lack of botanical discrimination among these samples.

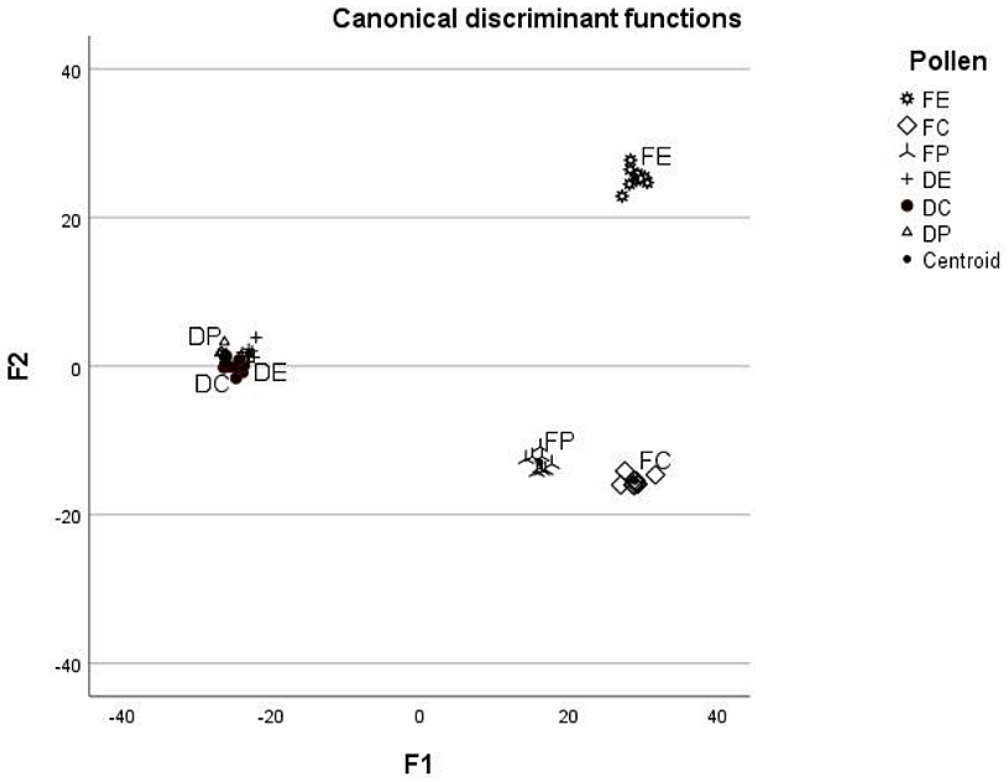

Fig. 3. Canonical discriminant functions biplot of the BP samples (FE-Fresh Echium; FC-Fresh Citrus; FP-Fresh Polyfloral; DE-Dried Echium; DC-Dried Citrus; DP-Dried Polyfloral)

Canonical Discriminant Analysis (CDA): Predict BP Sample Pollen Group Membership and Classify Pollen Discriminant Attributes

All variables reported VIF values≤5, hence were retained in the discriminant analysis. DA results showed that functions F1 and F2 were relevant enough to be used to create the canonical discriminant functions biplot depicted in Fig 3. The results for the analysis of DA efficiency parameters to determine the significance of each canonical discriminant function is presented in Table 6. The discriminant power of function F1 is high with eigenvalues of 397,765 and explanation of the variance of 84% (See Table 7). Functions F1 and F2 together explained 94% of the variance (Table 8).

Table 6: Canonical discriminant functions

Test of functions	Wilks's Lambda	Chi-square	df	Sig.
1 a 5	0.000	636.469	45	0.000
2 a 5	0.000	363.998	32	0.000
3 a 5	0.016	187.503	21	0.000
4 a 5	0.461	35.191	12	0.000
5	0.735	14.028	5	0.015

Table 7: Canonical discriminant analysis and variance explanation

Function	Eigenvalue	Variance %	Cumulative %	Canonical correlation
1	397.765	84.0	84.0	0.999
2	47.376	10.0	94.0	0.990
3	27.432	5.8	99.8	0.982
4	0.592	0.1	99.9	0.610
5	0.361	0.1	100.0	0.515

DA returned satisfactory results as 83.3% of the BP samples were correctly classified (Table 8). Yet, dried BP samples were not properly classified, as all of them presented the same sensory profile and hence the same correlation of variables (burn-roasted aroma and odour).

Table 8: Predicted classification for BP samples (FE-Fresh Echium; FC-Fresh Citrus; FP-Fresh Polyfloral; DE-Dried Echium; DC-Dried Citrus; DP-Dried Polyfloral)

Pollen			FE	FC	FP	DE	DC	DP	Total
Original	Counting	FE	9	0	0	0	0	0	9
		FC	0	9	0	0	0	0	9
		FP	0	0	9	0	0	0	9
		DE	0	0	0	6	2	1	9
		DC	0	0	0	1	5	3	9
		DP	0	0	0	0	2	7	9
	%	FE	100.0	0.0	0.0	0.0	0.0	0.0	100.0
		FC	0.0	100.0	0.0	0.0	0.0	0.0	100.0
		FP	0.0	0.0	100.0	0.0	0.0	0.0	100.0
		DE	0.0	0.0	0.0	66.7	22.2	11.1	100.0
		DC	0.0	0.0	0.0	11.1	55.6	33.3	100.0
		DP	0.0	0.0	0.0	0.0	22.2	77.8	100.0
Cross-Validation	Counting	FE	9	0	0	0	0	0	9
		FC	0	9	0	0	0	0	9
		FP	0	0	9	0	0	0	9
		DE	0	0	0	6	2	1	9
		DC	0	0	0	3	3	3	9
		DP	0	0	0	2	3	4	9
	%	FE	100.0	0.0	0.0	0.0	0.0	0.0	100.0
		FC	0.0	100.0	0.0	0.0	0.0	0.0	100.0
		FP	0.0	0.0	100.0	0.0	0.0	0.0	100.0
		DE	0.0	0.0	0.0	66.7	22.2	11.1	100.0
		DC	0.0	0.0	0.0	33.3	33.3	33.3	100.0
		DP	0.0	0.0	0.0	22.2	33.3	44.4	100.0

83.3% of the cases originally grouped were properly classified.

Discussion

Our findings coincide with other authors according to which the colour profiles were different for the five different studied bee pollen samples (sunflower, red clover, rapeseed and two polyfloral).[43] In addition, these authors carried out a quantitative descriptive

profile (QDP) method in their sensory evaluation of bee pollen, but different attributes of odour/flavour were used: sweet, sour, floral and hay. From this study it was obtained the complete characterization of five different pollen samples by appearance, odour, taste/flavour, and texture parameters.

Relating to the bee pollen treatment certain studies performed an instrumental sensory evaluation that allowed to establish a distinct difference in smelling profile between wet and dehydrated BP samples, which indicates the strong influence of dehydration process.[54] These authors set dried BP shows higher changes on smelling profile at a higher temperature (50°C), which indicates it is occurring a spoilage associated to lipids rancidity and BP samples with a lower water activity (dehydrated cabin) present a fast fat rancidity process. Reinforcing this outcome other authors comparing the organoleptic characteristics of BP obtained the chiller method at 4°C for 14 days retained quality attributes better than the BP oven-drying method at 40°C.[55]

Our results coincide with some research about the green character of the fresh frozen BP. These authors set that, generally, green can be characterized as unripe, peapod, grassy/leafy, viney, fruity or combinations of those.[35] Additional attributes that are important to the green character included musty/earthy, pungent, bitter, overall sweet, and floral.

Grassy/leafy is described as a green aromatic associated with newly cut grass and leafy plants; characterized by sweet and pungent character. In this way, the term earthy is described as humus-like aromatics that may or may not include damp soil, decaying vegetation, or cellar-like characteristics, and floral as sweet, light, slightly perfume impression associated with flowers.

Conclusion

Sensory evaluation is a useful tool to define the sensory profile of BP, and it can provide sufficient information about it related with its preservation treatment and botanical origin. As mentioned before, this is the first study characterizing the sensory profile of different samples of unifloral, bifloral and polyfloral both, dried and fresh-frozen BP. A questionnaire for the descriptive sensory analysis was created (see supplementary material). The results obtained from the analysis revealed that there is a relation between the treatment BP is given and its sensory profile.

Therefore, the fresh-frozen unifloral BP presents the same organoleptic properties as the original plant (*Echium* sp.) characterized by both earthy/musty odour and aroma, astringency and persistence, while the multifloral BP has variable properties as was composed of different pollen types. *Citrus-Cistus* fresh-frozen BP presented a sensory profile characterized by its floral odour and aroma and freshness. All fresh-frozen BP presented high values for pollen dust and water content attributes.

The sensory profile for fresh-frozen BP samples was defined and their classification accuracy was also achieved. On the contrary, all BP samples that went under drying treatment presented the same sensory profile making no differences between the three different botanical origins. Dried BP samples presented low values for water content due to the heat treatment and no pollen dust. Further research on sensory characterization of BP is needed for the purpose of including this natural product as routine cooking ingredient taking advantage not only of its nutritional aspects but also of its texture and emulsifying properties, odour, aroma and colorant attributes. And with the final objective of rescuing the BP from the dietary products line at the supermarkets, becoming a more polyvalent food.

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Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from other Sources

Not Applicable.

Author contributions

- **Salud Serrano:** Conceptualization, Writing – Original Draft, Supervision, Project Administration.
- **Francisco Javier Navas:** Data Collection, Analysis, Review & Editing.
- **Inmaculada Rodríguez:** Visualization, Methodology, Writing – Original Draft, Analysis, Review & Editing .

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