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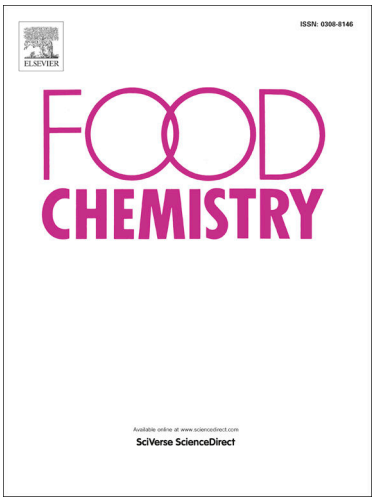

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Unveiling the Techno-Functional and Bioactive Properties of Bee Pollen as an Added-Value Food Ingredient

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Abstract

Bee pollen is an imperative product for human use. Seven bee pollens were harvested from Morocco, and their chemical, biological and techno-functional properties were studied. All samples showed acceptable physicochemical and nutritional quality with a mean energy value of 239 kcal/100g. FTIR spectra confirmed the presence of major constituents like carbohydrates, lipids, proteins and polyphenols. Moreover, pollens exhibited good techno-functional properties, like carbohydrate solubility (34.47-59.27 g/100g), protein solubility (7.28-23.31 g/100g), emulsifying stability (16.52-45.38 min), emulsifying activity (9.83-25.05 g/m^3) water absorption capacity (1.06-2.19 g/g), oil absorption capacity (1.15-3.50 g/g) and water-oil absorption index (0.62-1.25). Bee pollen extracts revealed potent antioxidant capacity and digestive enzyme inhibitory activity associated with the presence of fifteen phenolic compounds belonging to flavons, flavonols, flavanones, flavan-3-ols, hydroxybenzoic and hydroxycinnamic acids, and stilbenes families. Present data indicate the possible application of bee pollen as a useful nutritional, bioactive and anti-foaming ingredient, replacing synthetic products in food industries.

Keywords: Antihyperglycemic; Antioxidant; Bee products; Functional claim; Nutritional value; Physicochemical characterization.

1. Introduction

Nowadays, the research of new safer, and more active molecules from functional foods is a leading tendency in green chemistry. This trend is reinforced by scientific data demonstrating the importance of functional nutrients in the prevention and treatment of many illnesses.

In the last years, consumers have increasingly expected and demanded a wider variety of food options, especially those free of chemical additives and rich in bioactive constituents (Anjos et al., 2019). Bee pollen is a treasure trove of active natural metabolites, it is the bees' main source of nutrients such as minerals, protein, carbohydrates, vitamins (A, C, and E), and lipids including fatty acids, Omega-3 and Omega-6 (Estevinho et al., 2012). Besides its nutritional values, bee pollen presents an inexhaustible source of powerful antioxidant compounds like resveratrol, quercetin, kaempferol, cinnamic and caffeic acids (Laaroussi, Bakour, et al., 2020). It is a popular beehive product widely used in traditional medicine for the prevention and self-treatment of various pathologies and has attracted the interest of many researchers worldwide. Several human health-promoting effects have been referred to bee pollen extracts, including, cardioprotective, anti-inflammatory, anti-cancer, and hepatoprotective effects (Li et al., 2018). Furthermore, new research has shown that bee pollen offers promising benefits for Parkinson's disease, depression, and polycystic ovarian syndrome (Miyata et al., 2022). Owing to its well-known nutritional and medicinal benefits, bee pollen is commonly used as a natural dietary supplement. Recently, there is a considerable interest to utilize bee pollen in food systems as a functional ingredient to enrich the product quality characteristics (Kostić et al., 2015). In this context, bee pollen has been used in the enrichment of yogurt, cheese, bread, and fermented beverages (like kombucha, white wines, malt and milk beverages) as a (bio)techno-functional ingredient with strong

characteristics of the final products (Kostić, Milinčić, Barać, et al., 2020).

Some research has been carried out on different bee products, whose composition is strongly associated with specific geographical conditions and botanical origins. However, limited studies have been reported on the chemical characterization and biological activities of Moroccan bee pollen. Thus, the present work was designed to evaluate the physicochemical composition, the bioactive phenolic profile, and the antioxidant activity and digestive enzymes' inhibitory actions, as well as techno-functional properties of Moroccan mono-floral and poly-floral bee pollens with potential future industrial applications.

2. Material and methods

2.1. Bee pollen samples and extracts preparation

Seven bee pollen samples were harvested using specific bee pollen traps in May 2018 from healthy hives located in different geographical areas in Morocco (see Table S1(Supplementary material)). One gram of each sample was macerated in 30 mL of ethanol (70%, v/v) under mechanical stirring (170 rpm) for one week in the dark at room temperature. The supernatant of the extracts was previously filtered and collected for in vitro tests.

2.2.Palynological analysis of bee pollens

Qualitative and quantitative analysis of pollen grains was performed according to the International Commission for Bee Botany (ICBB) (Louveaux et al., 1978).

2.3. Physicochemical composition of bee pollens

The moisture content (%) was determined by drying 3 g of each pollen sample in an oven for 24 h at 105 °C. The moisture was calculated using the following equation (1).

$$\textbf{Moisture}\ (\%) = \frac{(\textbf{Wf} - \textbf{Wd})}{\textbf{Wf}} \times \textbf{100} \qquad (\textbf{1})$$

In which: Wf = weight of the sample and Wd = weight of the dry sample.

2.3.2. Water activity

The water activity (a_w) of the bee pollen samples was measured using a water activity meter (Model ms1, Novasina AG, Lachen, Switzerland).

2.3.3. Ash content

The ash content was measured at 500 °C for 14 h, then the residue was weighed 3 times. The ash (%) was determined as follows (equation 2):

$$\textbf{Ash}\ (\%) = \frac{\textbf{mA}}{\textbf{mBP}} \times \textbf{100} \qquad (\textbf{2})$$

Where mA = the net mass of the ashes, and mBP = the gross mass of bee pollen.

2.3.4. pH

The pH value of the pollen solution 30 % (w/v) was determined using a pH apparatus (ST2100-F, Ohaus Corporation, Parsippany, New Jersey, USA).

2.3.5. Protein content

The soluble protein content was analyzed using the Bradford methodology with some modifications (Bradford, 1976). Each pollen extract (20 µL) was mixed with 230 µL of Bradford dye reagent. The absorbance was measured at 595 nm after 5 min in a dark

Vermont, USA). Bovine albumin serum (BSA) was used to perform the standard curve (33–1000 mg/L, R^2 = 0.994) and the results were expressed as milligram of BSA equivalents per gram of dry bee pollen (g BSA/100g dw).

The total protein content of bee pollens was assessed by colorimetric titration and quantification of total nitrogen (Kjeltec Analyser, FOSS, Hillerød, Denmark) after sample HNO_3 digestion using a Kjeldahl digestor (Tecator, FOSS, Hillerød, Denmark), applying the nitrogen conversion factor (N × 6.25). Results were expressed as a gram of total proteins by 100g of dry weight of bee pollen (g/100g dw) (Laaroussi et al., 2021).

2.3.6. Total soluble carbohydrates

The total soluble carbohydrate content (TSC) was evaluated using the phenol-sulfuric acid method (Ferreira-Santos, Nunes, et al., 2020). For this, 50 µL of the extract was mixed with 150 µL of H_2SO_4 (96–98% (v/v)). Then, 30 µL of phenol reagent (5%) was added and the final solution was heated for 5min at 90 °C. The absorbance was measured at 490 nm using UV/Vis spectrophotometer, after cooling down at room temperature for 5 min. The calibration curve (R^2 = 0.992) was made using glucose as a standard (10–600 mg/L). The TSC content was expressed as gram of glucose equivalents (GlcE) per 100 g of dry pollen (g GlcE/100g dw).

2.3.7. Lipid content

The lipid content was determined through extraction with an automated Soxhlet system (SOXTEC 8000, FOSS analytical, Hillerød, Denmark) using petroleum ether at 80 °C for 12 h (Ferreira-Santos, Genisheva, et al., 2020).

The total energy of all pollen samples was calculated using the following equation (3) (Bakour et al., 2019):

$$\textbf{Energy (Kcal)} = \mathbf{4} \times (\textbf{g proteins} + \textbf{g carbohydrates}) + \mathbf{9} \times (\textbf{g lipids}) \quad (3)$$

2.3.9. Determination of minerals

Minerals identification and quantification were evaluated using the calcination method followed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Laaroussi et al., 2021). The elements quantification was achieved using different concentrations of standards (K, Ca, Mg, Fe, Na, Zn, Cu, Ni, Cd, and Pb). The results were expressed in mg of mineral per kilogram of dry bee pollen (mg/kg dw). All samples were analyzed in triplicate.

2.3.10. Fourier Transform Infrared Spectroscopy

Bonding arrangement and functional groups of constituents present in the raw bee pollens were determined by Fourier Transform Infrared Spectroscopy (FTIR) using an ALPHA II- Bruker spectrometer (Ettlingen, Germany) with a diamond-composite attenuated total reflectance (ATR) cell. The FTIR spectra were recorded in the range of 4000–400 cm^{-1}, with 60 scan cycles per sample at a resolution of 4 cm^{-1} (Ferreira-Santos, Genisheva, et al., 2020).

2.4. Chemical composition of bee pollen extracts

2.4.1. Total phenolic content

The total phenolic content (TPC) was quantified by the Folin-Ciocalteu methodology using UV/Vis spectrophotometer (Ferreira-Santos, Genisheva, et al., 2020). Gallic acid

the results were expressed in mg gallic acid equivalent (GAE) per gram of dry bee pollen (mg GAE/g dw).

2.4.2. Total flavonoids content

Total flavonoids content (TFC) was measured following the colorimetric procedure described by Ferreira-Santos et al (Ferreira-Santos, Genisheva, et al., 2020) using UV/Vis spectrophotometer. Quercetin (2.6–142 mg/L) was used to perform the standard curve (R^2 = 0.998) and the results were expressed in milligram of quercetin equivalent (QE) per gram of dry bee pollen (mg QE/g dw).

2.4.3. Identification and quantification of phenolic compounds

Hydroethanolic extracts of pollen samples were analyzed using a ShimatzuNexpera X2 ultra performance liquid chromatography (UPLC) chromatograph equipped with Diode Array Detector (DAD) (Shimadzu, SPD-M20A, Kyoto, Japan) following the method described by Ferreira-Santos et al (2020). Separation was executed at 40 °C with a flow rate of 0.4 mL/min on a C18 reversed-phase column by Waters (Acquity UPLC® BEHcolumn, 2.1 mm × 100 mm, 1.7 μm particle size) and a pre-column of the same material. HPLC grade solvents water/formic acid 0.1% (A) and acetonitrile (B) were used. The elution gradient for solvent B was as follows: from 0.0 to 5.5 min eluent B at 5%, from 5.5 to 17 min linearly increasing from 5 to 60%, from 17.0 to 18.5 min a linearly increasing from 60 to 100%; finally, the column was equilibrated at 5% for 11.5 min. The identification of the phenolic compounds was made by comparing their UV spectra and retention times with that of corresponding standards. Quantification was carried out using calibration curves for each phenolic compound using concentrations

(LOQ) were calculated (data not shown). In all compounds, the coefficient of linear correlation (R^2) was higher than 0.990. Different wavelengths (209–370 nm) were used for the quantification and identification of the target compounds. The values of individual phenolic compounds were expressed in milligrams per kilogram of dry pollen (mg/kg dw). All analyses were made in triplicate. All standards were of analytical grade and procured from Sigma Aldrich (St. Louis, USA).

2.5.In vitro antioxidant and antihyperglycemic activities

2.5.1. Antioxidant activity

Three methods to unveil the antioxidant activity of bee pollen samples were used: 2,2-Diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation-based ($ABTS^{\bullet+}$), and Ferric Reducing Antioxidant Power (FRAP) assays (Ferreira-Santos, Genisheva, et al., 2020). All assays were determined spectrophotometrically using UV/Vis spectrophotometer.

For the FRAP assay, a calibration curve was prepared using ferrous sulfate as a reference (800–100 µM, R^2 = 0.995). FRAP values are expressed as micromoles of ferrous equivalent per g of bee pollen (µmol Fe^{2+}/g).

Trolox was used as standard with a concentration range of 250–15 µmol/L(R^2 = 0.997) for DPPH assay, and 800–30 µmol/L (R^2 = 0.998) for ABTS assay, and a corresponding control (ethanol 70%) was used. Equation (4) demonstrates the calculation of the radical scavenging activity for DPPH and ABTS methods in % inhibition.

$$\% \textbf{ Inhibition} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \times \textbf{100} \qquad (\textbf{4})$$

expressed concentrations required to inhibit 50% (IC_{50}) of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals (μg/mL).

2.5.2. Digestive enzymes' inhibitory activity

The bee pollen extracts action in the inhibition of digestive α-amylase and α-glucosidase were determined colorimetrically using UV/Vis spectrophotometer, as described previously by Ferreira-Santos et al (2020).

For the α-amylase assay, 500 μL of α-amylase solution (0.5 mg/mL) was incubated with 500 μL of different concentrations of each pollen extract at 37 °C for 15 min. Ethanol 70% and acarbose were used as a negative and positive control, respectively. Afterward, 500 μL of starch solution (1%) was added and the mixture was incubated for 15 min at 37 °C. Immediately, 1 mL of dinitrosalicylic acid color reagent was added to the reaction and placed for 10 min in a boiling water bath. The resulting mixture was diluted 10 times and the absorbance was read at 540 nm.

Concerning α-glucosidase assay, a mixture of bee pollen extract and p-nitrophenyl-R-d-glucopyranoside (pNPG, 3 mM) was added to the α-glucosidase solution (10U/mL), then incubated for 15 min at 37 °C, and the reaction was interrupted by adding sodium carbonate ($Na_2CO_{3)}$ solution (1 M). The intensity of produced p-nitrophenol coloration was measured at 400 nm. α-amylase and α-glucosidase inhibitory activities (%) were calculated using equation (4). The pollen concentration required to inhibit 50 % (IC_{50}) of α-amylase and α-glucosidase activities were calculated from a dose-response curve, and the results were expressed in μg/mL.

2.6. Techno-functional properties

emulsifying properties, as well as water and oil absorption capacity) were determined as described previously by Kostić et al (2015).

Carbohydrate and protein solubilities are expressed as the content (g/100 g dw) of soluble fractions of these compounds compared to the total carbohydrate and total protein contents (g/100g). The emulsion stability index (ESI) is expressed in (min) while the emulsion activity index (EAI) is defined as (g/m^3). Water (WAC) and oil (OAC) absorption capacity are given as g/g of sample. The water-oil absorption index (WOAI) was calculated by the following formula:

$$\boldsymbol{WOAI = \frac{WAC}{OAC}} \qquad (\mathbf{5})$$

2.7. Statistical analysis

The extraction and analyses were performed in triplicate and the data are presented as mean ± standard deviation (SD) values. GraphPad Prism software (version 6.0; GraphPad Software, Inc., San Diego, CA, U.S.A.) was used for statistical analyses. The analysis of variance (ANOVA) and the least significant difference test were used to determine statistically different values at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Palynological origin of bee pollen samples

The palynological analysis of bee-collected pollen samples is summarized in Table S1 (Supplementary material). Based on the classification of Campos and co-workers (2008), only one bee pollen sample - BP4- has been considered as mono-floral (>80%) originated from *Reseda luteola* (81%), while, six samples BP1, BP2, BP3, BP5, BP6 and BP7 provide evidence for different botanical species and are grouped under poly-floral

occurred in BP5 as dominant pollen (>45%) is a native plant and considered one of the most authentic melliferous plants of the Ifrane area of Morocco.

3.2. Physicochemical analysis and energetic value of bee pollens

Over these last decades, the quality and safety of functional foods have been the main concerns of consumers and healthcare organizations around the world. As shown in Table 1, the chemical composition of bee pollen loads showed wide variation between studied samples, which is possibly related to the specific botanical sources and pedo-climatic conditions of growth plant species (soil nature, harvesting period, and climate type) (Estevinho et al., 2012).

Moisture and a_w are essential factors influencing the 'shelf lifetime' and organoleptic characteristics of bee pollen (Coronel et al., 2004). In fact, the high moisture content constitutes a favorable environment for fermentation and microbial contamination, especially by yeasts and molds. This imposes the need for bee pollen hygienic guidelines, especially fresh bee pollen.

Concerning moisture (water content), our samples showed values oscillating from 3.16 ± 0.01% in BP1 to 14.08 ± 0.09% in BP2 with a mean value of 5.09 ± 4.24%, which is within the maximum limit of 6%, reported by Campos et al (2008). Moreover, the obtained values are lower than those of fresh Romanian (17 to 31%) and other Moroccan (11 to 27%) bee pollen samples (Asmae et al., 2021; Spulber et al., 2018), that presented a higher percent of pollen humidity than that allowed by the French regulation (6%) (Bogdanov, 2004). This could be attributed to poor storage conditions and/or inadequate drying processes.

The recorded values for a_w were between 0.24 ± 0.03 in BP4 and 0.41 ± 0.01 in BP7 with a mean value of 0.32 ± 0.06, which are similar to the ones reported by Estevinho et al (2012) for Portuguese samples. This results indicated that our bee-collected samples are safe against

the minimum reported value for foodborne bacteria), and less than 0.61 is considered inadequate for osmophilic yeasts growth in bee pollen and other food stuffs rich in monosaccharides (Sagona et al., 2017).

Ash content in all studied samples (2.14 - 3.51 %) was less than the maximum limit set by the guideline of Campos et al (2008) at a maximum value of 6%.

The pH values varied between 4.13 ± 0.01 in BP1 collected from Sefrou and 5.12 ± 0.03 in BP3 harvested from Taza. Similar values were found for Argentinian and Moroccan bee pollens in which pH values oscillated from 3.8 to 5.4 and from 4.19 to 4.99, respectively (Asmae et al., 2021; Coronel et al., 2004). These results are within the pH range fixed by the Argentinian legislation (from 4 to 6) (Coronel et al., 2004).

From the nutritional and energetic point of view, carbohydrates represent the main source of honey bees' nutrition and are the principal components of bee pollen (13-55 g/100g) (Bogdanov, 2004). The average of carbohydrate content was 23.26 ± 2.26 g/100g dw with a range from 18.52 ± 0.19 g/100g dw (BP1) to 46.44 ± 0.55 g/100g dw (BP5). These results are within the range reported by Bertoncelj et al (2018) for twenty-eight Slovenian bee pollen samples. After carbohydrates, proteins are the most abundant compounds in bee pollen. Total and soluble proteins showed wide variations among samples, BP5 (*Thymus vulgaris*, 56%) harvested from Ifrane area had the lowest values, 19.18 ± 0.56 g/100g dw and 16.63 ± 3.66 gBSA/100g dw, respectively. However, the highest contents were found in Timahdite bee pollen (BP7 sample) (*Cytisus* sp., 48%). The current data follows those obtained for Serbian bee pollen samples (14.81 - 37.25 g/100g) (Kostić et al., 2015) and agree with the results of Bogdanov (2016) which reported the content of protein at a range of 10 - 40 g/100 g of bee pollen.

0.93 g/100g dw. These values are in agreement with other bee pollen from different countries (Kostić et al., 2015; Kostić, Milinčić, Trifunović, et al., 2020) and fit into the range proposed by Bogdanov (1-10 g/100g) (Bogdanov, 2004). The energy values of studied pollens varied between 196.3 ± 1.0 kcal/100g dw in BP3 and 247.1 ± 2.2 kcal/100g dw in BP7 (on average 239.7 ± 20.9 kcal/100g dw). These values were slightly lower than those reported by Kostić and co-workers (2015) for Serbian pollens (351 - 396 kcal/100g), possibly due to differences in the composition of bee pollens.

3.3. Mineral composition of bee pollens

Regarding mineral composition, ten elements were investigated, and the results are represented in Table 2. Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Sodium (Na) are grouped as macro-nutrients. These elements have been found at high amounts in several bee products - honey, and bee pollen (Asmae et al., 2021; Laaroussi, Bouddine, et al., 2020).

K and Ca were the abundant metals present in all examined bee pollens with concentrations reaching from 1049.9 to 2972.6 mg/kg dw for K, and 980.3 to 2343.6 mg/kg dw for Ca. These results were in agreement with our previous study, in which K and Ca were the main representative mineral in eight monofloral bee pollen samples harvested from different apiaries in Morocco (Asmae et al., 2021). These two minerals are important because contribute largely to the regulation of electrolyte exchange and maintaining the acid-base balance of the human body. Mg, Fe and Na are detected also in high amounts with an average value of 527.9, 99.3, and 15.58 mg/kg dw, respectively. Owing to their crucial role in the good physiologic and metabolic processes of animal and human organisms, the presence of diverse minerals in the human diet is indispensable. Clinical evidence has reported that the intake of dietary foods rich in Mg was inversely linked with the incidence of Type 2 diabetes, obesity, and cardio-metabolic

homeostasis by enhancing glucose uptake and improving insulin sensitivity (Khatun Kali et al., 2022). Zinc (Zn), Copper (Cu), and Nickel (Ni) presented as minor minerals showed values oscillating from 14.90 (BP1) to 33.56 mg/kg dw (BP3) for Zn, from 1.12 (BP3) to 8.15 mg/kg dw (BP6) for Cu, and from 0.05 (BP5) to 0.19 mg/kg dw (BP3) for Ni. Cu was not detected in BP2 and BP4 collected from Taounate and Tafajight areas, respectively. These concentrations fall generally within the range values described in literature 0.86-53.5 mg/kg for Zn, 3.16-18.5 mg/kg for Cu, and 0.35-3.70 mg/kg for Ni (Tutun et al., 2022). The mineral composition of bee pollen depends on several factors, such as soil nature, climatic conditions, plant species, and harvest period, which may explain the variation between the studied samples.

Recently, the importance of Zn in public health and human nutrition has attracted the attention of several health organizations and researchers worldwide (Bakour et al., 2022). Regarding heavy metals, Cd was not detected in all studied bee pollens, except in BP5 where its concentration (0.015 mg/kg) was within the safety limit suggested by the EU Legislation at (0.05 mg/kg) (Commission Regulation (EC), 2006). Lead-Pb concentration (0.076 mg/kg in BP1 and 0.103 mg/kg in BP4) was lower than the allowable maximum limit (0.5 mg/kg) fixed for the bee pollen described by Campos et al (2008). Heavy metals content has been the object of many studies, for instance, Altunatmaz et al (2017) studied 24 Turkish bee pollen samples and reported that Cd and Pb concentrations were-ranged from 0.025 to 0.181 mg/kg and from 0.065 to 0.479 mg/kg, respectively. These heavy metals might have been transported by bees during foraging from contaminated water, atmosphere, flowers, nectar, or/and honeydew (Laaroussi et al., 2021). Therefore, it is recommended to monitor the bee-collected pollen destined for human consumption.

It is most important to mention that bee-collected pollen grains are surrounded by two strong membranes namely, exine and intine, which protect their content against different

complex structure influences also its digestibility. In addition, the absence of specific enzymes for the digestion of the outer and inner layers of bee pollen in the human gastrointestinal system complicates and decreases the effectiveness process of its digestion and thus, reduces the bioavailability of its functional ingreedients, including phenolic compounds and mineral elements (Aylanc et al., 2021).

Pohl and coworkers (2020) studied the *in vitro* gastrointestinal digestion of bee pollen and evaluated the bioavailability of some minerals (Mg, Ca, Mn, Cu, Zn, and Fe). Accordingly, Mg and Ca are the most bioaccessible elements (70-85%), and Mn, Cu, Zn, and Fe showed bioaccessibilty ranged between 27 and 43%. Authors documented that bioaccecebility of all studied bee pollen minerals improved by 15% for Mg, 30% for Mn, and 20 % for Ca, Cu, Fe, and Zn, respectively, after a mixture with dionized water and incubated overnight. Outcome of this study, reaffirm the importance of its mixture with water as a good method of use for a better bioavailability of its nutrients and minerals.

Given these results, and taking into account the heterogeneous safety risks/hazards of bee pollen, the incorporation of safe organic bee pollen harvested from ecological zones as a supplementary ingredient in the daily diet may offer promising health benefits due to its high nutritional value.

3.4. Structural characterization of bee pollens

The FTIR spectra of bee-collected pollens are regrouped in Figure 1. The spectra are recorded between 4000 and 400 cm^{-1}, and show different typical absorption areas dominated by the largest water band (O-H stretching vibration) at 3275 cm^{-1}. This band overlapped with the N-H stretching vibration of proteins at 3500-3300 cm^{-1} (Ibrahim et al., 2018). Peaks at 2922 and 2852 cm^{-1} are attributed to the C-H stretching vibration bonds of sugars, glucans, and lipids

(Sefrou area) and BP3 (Taza area) which have the highest intense peaks (Figure 1B). Between 2800 and 1800 cm^{-1}, only BP3 and BP4 present smaller peaks at 2361 cm^{-1} which are assigned to O=C=O stretching mode of absorbed carbon dioxide (CO_2). The band identified at 1735 cm^{-1} is linked to the stretching mode of carbonyl moiety and asymmetric bending vibration C=O of flavonoids, lipids and amino acids. This peak is less intense in pollen harvested from Timahdite, BP7 (*Cytisus* sp., 48 %), and more intense in bee pollen from Sefrou area (BP1) and showed similarities between poly-floral samples BP6 (Fez) and BP3 (Taza) (Figure 1C), which explain the influence of botanical source on the chemical composition of bee collected pollens. A shoulder peak at 1711 cm^{-1} is related to the stretching mode of carboxylic moiety. The large band in the 1680-1600 cm^{-1} region, especially at 1735 cm^{-1} corresponds to the stretching mode of carbonyl moiety and asymmetric bending vibration C=O, which constitutes the chemical skeleton of hemicellulose and functional group of flavonoids, lipids and amino acids (Ibrahim et al., 2018). Absorptions at 1545 and 1516 cm^{-1} are usually due to the bending mode of CH_2 presents in the chemical structure of amides II and C=C stretching vibrations of phenolic acids (Castiglioni et al., 2019). Moreover, the peaks between 1440 and 1370 cm^{-1} represent C-H deformation vibration, OH stretching vibrations, and CH_3 bending vibration obtained from cellulose, lipids, ketone and aldehyde functional groups, glucose, and fructose. The 1200–500 cm^{-1} range is the so-called fingerprint region, in which an intense peak with shoulders at 1027 cm^{-1} is observed for all pollens, corresponding to the C–C, C–N, and C–O stretching vibrations of proteins and sugars. Absorbance bands between 921 and 700 cm^{-1} result from vibrational modes of C–OH present in the chemical structure of saccharides. The IR spectra interpretation clearly shows differences between the bee pollen samples, corroborating the results obtained in the other chemical determinations.

Bee pollen has been mentioned as a polyphenol-rich food with health-promoting benefits. As indicated in Table 3, bee pollens contain different amounts of phenolic compounds. The total phenolic content (TPC) varied from 12.40 ± 0.59 to 16.85 ± 0.07 mg GAE/g dw for Taounate (BP4) and Tafajight (BP2) samples, respectively. These results are within the range of values reported by Asmae et al (2021) for eight monofloral bee pollen samples harvested from different localities in Morocco (8.07 ± 1.03 to 32.38 ± 0.15 mg GAE/g), and lower than that found in Portuguese bee pollen (35.05 ± 0.5 mg GAE/g) (Asmae et al., 2021). The recorded flavonoid content ranged between 1.51 ± 0.09 in BP3 to 4.57 ± 0.14 mg QE/g in BP7, which was lower than that reported by Anjos and co-workers (2019) for a Portuguese collected bee pollen sample (6.99 ± 0.3 mg QE/g). The quantitative and qualitative composition of natural antioxidants from bee pollen are highly variable and depend on many factors including pedo-climatic characteristics, botanical origin, and conditioning storage. This might explain the recorded variability of TPC and TFC between the analysed samples.

A total of 15 phenolic compounds derived from hydroxycinnamic acid, hydroxybenzoic acid, flavonoid, and stilbenes were tentatively identified and quantified by UHPLC-DAD in each bee-collected pollen sample. The quantification of individual phenolic compounds was carried out to highlight the influence of specific plant origin on the chemical profile of bee pollen and thus the studied biological activities. As shown in Table 3, kaempferol was not detected in BP3 from the Taza area and was quantified in a lower amount in other samples. However, rutin was the predominant compound detected in BP1 (175.1 ± 11.1 mg/kg dw), BP3 (139.6 ± 8.5 mg/kg dw), BP4 (150.9 ± 4.2 mg/kg dw), and BP6 (343.6 ± 19.3 mg/kg dw). Quercetin was predominant in BP5 (275.3 ± 24.7 mg/kg dw), while hesperidin is the main phenolic in BP2 and BP7 with values of 275.3 ± 24.7 and 297.4 ± 12.5 mg/kg dw, respectively. Ferulic acid was found in similar concentrations in the seven samples of bee pollen. In another study, many

quantified as the most predominant components in bee pollens from different geographical origins (Bayram et al., 2021). The reported health-promoting effects of these phenolics are diverse and may include anti-cancer, anti-inflammatory, antioxidant, antimicrobial, and anti-aging properties. In addition to the impact of geographical origin on the phytochemical composition of bee-hive products, several studies have shown that the phenolic composition of bee pollens depends strongly on the floral origin and composition of pollen grain. In a recent study, Gercek et al (2021) stated that the type and the concentration of phenolic compounds in heterofloral bee pollen samples from Turkey varied depending on the botanical origin. In the same line, Asmae et al (2021) showed a significant difference of phenolic content between bee pollen samples collected from different apiaries from Morocco, which migh explained and reaffirme the influence floral species origin on the chemical composition of bee-collected pollen by the presence of secondary pollen.

The use of different solvents and the method of extract preparation, the analytical techniques for quantification of biocompounds, as well as the growing areas of plant origin and its specific pedo-climatic conditions, can influence and explain the wide differences in the phenolic profile between the studied samples. In fact, the phytochemical composition differences of bee pollens reflect the interaction between the specific plants' pollen and the growing environments. It is interesting to note that rosmarinic and ellagic acids were the most abundant phenolic acids present in all analyzed samples, except in the multifloral sample harvested from the Fez area (BP6) in which cinnamic acid was the predominant hydroxycinnamic acid (261.1 ± 8.2 mg/kg dw). However, sample BP6 had the highest concentrations of apigenin and naringin with values of 70.9 mg/kg dw and 117.0 mg/kg dw, respectively. Bee pollen samples, BP2 and BP4 accounted the highest concentrations of *o*-coumaric acid, 45.3 mg/kg dw and 50.4 mg/kg dw, respectively. Vanillic acid and gallic acid were identified in all samples. However, their

quantification limit). Chlorogenic acid was present only in BP1, BP2, BP6, and BP7 samples at concentrations between 14.7 and 16.8 mg/kg dw of pollen, which reaffirms the influence of pollen flowers' origin on the chemical composition of bee pollen. In turn, this extensive variation of secondary metabolites influences the antioxidant ability of bee pollen and gives it a wide range of pharmacological and biological functionalities. For instance, hesperidin, a flavanone mainly present in beehive products has emerged as a new powerful therapeutic molecule able to modulate several cardiovascular diseases (CVDs) risk factors. In fact, Xuguang et al (2019) have reported that hesperidin enhances the glucose uptake in lipopolysaccharide (LPS)-induced insulin-resistant HepG2 cells by modulating the insulin receptor substrate 1 (IRS1)- glucose transporter (GLUT)-2 pathway via toll-like receptor (TLR)-4. In the same context, several lines of evidence suggest that rutin, maintains glucose homeostasis, lipid profile and prevents diabetes-associated microvascular and macrovascular complications through the modulation of Akt-mediated insulin signaling pathway and/or adenosine monophosphate-activated protein kinase (AMPK) activation (Lim et al., 2021). It has been reported that quercetin, a distinctive bioactive flavonoid, plays a crucial role in the prevention of several human cancer,like breast, colon, liver, and lungs, via different mechanisms of action (Rauf et al., 2018). A previous study has shown that treatment of breast cancer cells with quercetin increases cell apoptosis and inhibits cell cycle progression by suppressing the phosphomitogen-activated protein kinases (p38MAPKs) and down-regulating the expression of cyclin-dependent kinase inhibitor 1 (CyclinD1), tumor-suppressor protein 21 (p21) (Nguyen et al., 2017). Moreover, quercetin has been documented to have several neuropharmacological effects and prevents/improves many neurological dysffunctions and neurodegenerative brain disorders including parkinson’s, alzheimer’s, cognitive impairment, and huntington’s diseases (Islam et al., 2021). Also, the hydroxycinnamic acids like cinnamic

range of health benefit effects such as anti-inflammatory and antiviral effects (Medrado et al., 2016).

3.6. In vitro antioxidant and antihyperglycemic activities of bee pollen extracts

3.6.1. Antioxidant activity

Nowadays, the antioxidant activity of natural products and their extracts is a subject of great interest in the food, pharmaceutical, and cosmetic industries. The essential step to measure the antioxidant capacity of the active ingredients is based on the selection of the right method. For that, to measure different mechanisms of action, the antioxidant activity of examined bee pollen loads was assessed by three recommended *in vitro* assays, like DPPH, ABTS, and FRAP.

The free radical scavenging activity (DPPH and ABTS methods) are colorimetric methods commonly used for the assessment of the antioxidant activity of various substances present in plants, foods, beverages, or natural extracts. These assays are based on the reduction of the chemical radicals in the presence of hydrogen-donating antioxidant agents. These tests are valid, simple, rapid, accurate, reliable, and economic methods to evaluate the free radical scavenging activity of natural antioxidants, since the radical is stable and commercially available (Ferreira-Santos, Genisheva, et al., 2020). As mechanism of action, in the presence of hydrogen-donating antioxidants, the DPPH assay measures the free radical scavenging activity of the examined extracts by reducing the $DPPH^{\bullet}$ (radical) to non-radical DPPH-H form. While, the ABTS test is principally based on the chemical interaction between specific antioxidant molecules and ABTS radical-cation ($ABTS^{\bullet+}$), leading to the quenching of hydrogen atom by the $ABTS^{\bullet+}$ nitrogen atom and thus, inducing the mixture decolorization (Laaroussi et al., 2021).

antioxidant molecules to reduce ferric ions (Fe^{3+} to Fe^{2+}) through electron-donating antioxidants (Ferreira-Santos, Genisheva, et al., 2020).

As shown in Table 4, all bee pollens presented potential antioxidant activities with significant differences between them. Regarding DPPH and ABTS tests, the highest antiradical capacities were attributed to BP5, a poly-floral bee pollen sample with a predominance of *Thymus vulgaris* pollen grains (56%) harvested from Ifrane, with the concentrations required to inhibit 50% of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals were 42.16 ± 0.19 µg/mL and 323.8 ± 16.7 µg/mL, respectively. However, the lowest $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ antiradical activities were expressed by BP4, a monofloral bee pollen (*Reseda luteola*, 81%) (IC_{50}= 73.87 ± 0.05 µg/mL), and BP1 (IC_{50}= 499.1 ± 11.3 µg/mL), respectively. These values are lower than those reported previously by our research team for different Moroccan bee pollen samples in which the IC_{50} values were between 0.245 to 0.283 mg/mL for DPPH and between 0.190 to 0.896 mg/mL for ABTS (Asmae et al., 2021), and higher than that observed for Trolox as standard reference (IC_{50} = 10.8 ± 0.1 µg/mL for DPPH, and IC_{50} = 23.2 ± 4.0 µg/mL for ABTS).

Regarding the FRAP assay, the antioxidant reducing power activity was generally different between samples, and between the seven samples, oscillating from 129.8 ± 0.9 in BP5 to 307.2 ± 1.9 µmol Fe^{2+}/g in BP6, which is higher than Trolox activity (136.1 ± 1.0 µmol Fe^{2+}/g) (Table 4).

Bee pollen load extracts showed powerful and wide differences of antioxidant capacities, which are most often linked to dominant and secondary flora pollen grain and their specific individual phytochemical components (Table 3).

In addition, all samples show good antioxidant capacity, which can lead to affirm its potential as a bioactive agent, although further tests in cell lines and animal models are necessary to confirm this activity *in vivo*. However, our research group has confirmed the antioxidant and

associated with oxidative stress (Laaroussi, Bakour, et al., 2020).

3.6.2. Antihyperglycemic activity

Nowadays, natural compounds are known for their broad spectrum of biological properties and advantageous health effects, where antioxidant and antidiabetic actions are important for body homeostasis. The uncontrolled elevated blood glucose concentrations may lead to the overproduction of reactive oxygen species (ROS) accompanied by oxidative stress. This harmful state is strongly involved in the development of serious pathophysiological complications such as hepatorenal dysfunction, CVDs, atherosclerosis, and other metabolic abnormalities (Laaroussi, Bakour, et al., 2020). α-Amylase and α-glucosidase are the pivotal enzymes involved in the process of dietary carbohydrate digestion and glucose release.

So, the inhibition of both enzymes contributes to the management of diabetes mellitus through the delaying of glucose absorption and thus, lowering the postprandial blood glucose level. Therefore, postprandial glucose (PPG) regulation is one of the target strategies for preventing and controlling diabetes, especially type 2 diabetes mellitus (T2DM). For that, extracts of seven bee pollen loads were evaluated for their α-amylase and α-glucosidase inhibitory activities. The obtained results showed that all samples had inhibitory actions on α-amylase and α-glucosidase activities (see Table 4). Sample harvested from Ifrane (BP5) that presented the greatest antioxidant activity, displayed the best α-amylase inhibitory activity with an IC_{50} value of 171.32 ±3.13 µg/mL, while, the pollen extract from Sefrou (BP1) exhibited the poorest inhibition (IC_{50} = 979.26 ± 7.34 µg/mL). These values were higher than that expressed by acarbose (IC_{50}=35.42 ± 1.0 µg/mL) and similar to those reported by Araújo and co-workers for nine Brasilian mono- and multi-floral bee pollen samples, where IC_{50} values were between 16.44 ± 0.79 and 1015.9 ± 12.16 µg/mL (Araújo et al., 2017).

structure, affecting their stability, bioaccessibility and possible beneficial effects. Nevertheless, some studies evidenced that phenolic compounds have the potential to inhibit digestive enzymes because of their ability to bind non-covalently into the active site residues of enzymes (Lim et al., 2022). In particular the flavonoids, due to its three-ring structure and wide variation in chemical structure, are potential natural drugs with α-amylase and α-glucosidase inhibition specificity. Lim et al (2019) reported some general structural requirements of three simple flavonoids as natural inhibitors of digestive enzymes. Their study showed that the double bond between C_2 and C_3 of the C-ring of quercetin and luteolin appeared particularly important to inhibit porcine pancreatic α-amylase, whereas the hydroxyl group (OH) of quercetin and eriodictyol at C_3 of the C-ring was related to inhibition of α-glucosidases from the rat intestine. Moreover, other studies show that several flavonoid glycosides such as tiliroside, isorhamnetin, among others, appear to be more stable under digestive process and better retained in the circulatory system than their aglycones (Antunes-Ricardo et al., 2017; Goto et al., 2012). In our work, only simple phenolic compounds were identified (by standards comparison using HPLC-DAD detector), without discarding the possibility of the presence of linked compounds that may influence their stability, accessibility, and action in the inhibition of α-amylase and α-glucosidase, preventing or treating some metabolic diseases.

The final step of dietary carbohydrate hydrolysis to absorbable monosaccharides is mediated by the α-glucosidase enzymes in the epithelium tissue of the small intestine. The current results showed that bee pollen extracts displayed different α-glucosidase inhibitory activities with IC_{50} values ranging from 107.99 ± 5.16 μg/mL to 661.05 ± 18.03 μg/mL. The lowest IC_{50} values (greater activity) were recorded for the BP5 and BP6 samples. These results may be related to a high number of secondary metabolites, such as phenolic acids and especially flavonoids, associated with high antioxidant activity; as well as the structure of chemical compounds.

which have the lowest TPC and TFC (see Table 3). It is well-known that α-glucosidase cleaves the glycosidic bonds of complex carbohydrates and releases absorbable monosaccharides by Asp481 and Asp647 as common catalytic residues in all α-glucosidase types (Okuyama et al., 2001). In addition, α-amylase aromatic residues, in particular, Phe256, Trp58, Trp59, and Tyr151 have been reported to have direct interactions with dietary carbohydrates polymers and play a pivotal role in substrate binding, enzyme activity, and catalysis (Evaristus et al., 2018). Thus, understanding the role of each domain provides an excellent basis for the research and selection of active natural enzyme inhibitors.

It has been recognised that some phenolic compounds exhibited potent α-amylase and α-glucosidase inhibitory actions. Gu et al (2016) have reported that flavonoids, inhibit α-amylase and α-glucosidase activities through a specific binding (hydrogen bonds) between the hydroxyl groups of their aromatic ring and the active site of α-amylase and α-glucosidase (functional amino acids) as well as by conjugated π-system between the A and C rings; and the indole Trp59 of the enzymes. These specific interactions hinder the reaction between these enzymes and dietary starch. Furthermore, Tadera et al (2006) reported that flavonoids component belonging to isoflavone (genistein, daidzein) and flavonol (quercetin, kaempferol, myricetin) groups were more potent inhibitors of both enzymes than those belonging to flavone (luteolin and apigenin), flavanone (naringenin and hesperidin), flavan-3-ol (catechin, epicatechin, and epigallocatechin) groups. This may explain the highest α-amylase and α-glucosidase inhibitory effect of BP5 rich in flavonols, especially quercetin (275.3 mg/kg dw), and the lowest enzymes inhibition of BP3 and BP4 that had the lowest content in flavanols (quercetin and kaempferol). As mentioned before, the number and the position of hydroxyl groups on the A and B rings and hydroxylation at the 3-position on the C ring of flavonoids principally affect their efficacy against both carbohydrate-digesting enzymes (Tadera et al., 2006) suggesting that α-amylase

components than total phenolic compounds content or antioxidant activity. Finally, the binary or multiple interactions between distinct bioactive ingredients of this bio-valuable food product (bee pollen) provide it the ability to control the first line of diabetes management by reducing the glucose absorption.

Current data were in accordance with the results of our previous study conducted *in vivo* (male Wistar rats), in which bee pollen extract (100 and 200 mg/kg body weight) decreased significantly the absorption of D-glucose and improved the hyperglycemia (Laaroussi, Bakour, et al., 2020). These findings indicate that bee pollen load extracts have a powerful inhibitory effect on these carbohydrate digestive enzymes and thus can reduce glucose absorption and prevent hyperglycemia and related metabolic syndromes.

3.7. Techno-functional properties

3.7.1. Protein solubility

Due to its impact on gelation, emulsification, and foaming, protein solubility was considered as the most important of techno-functional property (Kostić et al., 2015). The protein solubility of studied bee pollen samples varied between 7.28 ± 0.6 g/100g dw in BP6 and 23.31 ± 1.6 g/100g dw in BP7 (Table 5). This range was in agreement with the ones reported by Kostić et al (Kostić et al., 2015) for Serbian samples (from 2.79 to 25.9 g/100 g dw). In addition to amino acid composition, structure and conformation of proteins (e.g., hydrophobicity and surface) and processing conditions; the complex composition of bee pollen influences highly its solubility through the possible interactions between proteins and other constituents (e.g., carbohydrates, salts, phenolic compounds, and lipids) (Kostić, Milinčić, Trifunović, et al., 2020). In this sense, the BP7 and BP5 samples are promising for the food industry, since high protein solubility is desired for optimal functionality in food processing applications.

3.7.2. Carbohydrate solubility

Bee pollen is generally rich in sugars, which could influence the taste and sweetness of food products, thus, carbohydrate solubility should be taken into account when bee pollen is considered as an active ingredient or additive for food application. According to the obtained results (Table 5), carbohydrates solubility varied significantly among samples (except samples BP4 and BP7) from 34.47 g/100g dw in BP1 (polyfloral) to 59.27 g/100g dw in BP7. In addition to sugars contained in pollen flowers, high values of carbohydrate solubility are linked to the presence of nectar and honey in the bee pollen grains. Generally, the sugar composition of bee pollen loads is mainly represented by water-soluble mono and disaccharides such as fructose, sucrose, and glucose. Besides, bee pollen contains also soluble polysaccharides like pectin and starch which contribute largely to carbohydrate solubility. Additionally, insoluble polysaccharides (cellulose, callose, glucan, lignin, and sporopollenin) are present in bee pollen loads. Thus, the ratio of insoluble and soluble carbohydrates as well as the interaction of sugars with other bee pollen constituents (proteins, lipids, etc.), might influence the carbohydrate solubility. Kostić and co-workers (2015) have shown that lipid and total protein contents correlated positively with carbohydrate solubility ($r = 0.45$, $r = 0.39$, $p < 0.05$, respectively) which is not the case in the present work, as shown in the Table S2 (Supplementary Material).

3.7.3. Emulsifying properties

Emulsions play an essential role in the stability and formulation of several food products (like coffee creamers, mayonnaises, cream liqueurs, ice creams, infant formulae, etc.). These products are required to be prepared in specific conditions to avoid undesirable reactions such as aggregation and occlusion and thus, ensure emulsion stability over the lifetime. ESI and EAI are two main parameters used to assess the emulsifying functionalities of numerous food

variations were observed among samples in both parameters except between BP1 and BP3. ESI oscillated between 16.52 min in BP2 and 45.4 min in BP7 with a mean value of 25.8 min, whereas, EAI ranged from 9.8 in BP7 to 25.1 g/m^3 in BP6, with an average value of 16.14 ± 6.26 g/m^3. The obtained ESI and EAI results were in line with those reported by Kostić and co-workers (2015) for twenty-six Serbian pollens, in which ESI values varied between 19.6 and 49.3 min and EAI values ranged from 10.40 to 24.52 g/m^3. In addition, current results go in hand with those documented for several protein-containing products such as low-lipid soy flours, kidney bean, and pea protein isolates (Barac et al., 2010; Wani, Sogi, Wani, et al., 2013). This data indicates that bee pollen possesses good emulsifying properties that may be of industrial interest. A significant positive correlation was established between protein solubility and ESI ($r = 0.90$, $p < 0.01$), while, a negative correlation was observed between EAI and protein solubility ($p < 0.05$) (Table S2 (Supplementary Material)), indicating the influence of protein solubility on the emulsifying functionalities of bee pollen products. Also, Yan and Zhou (2021) have documented that soluble proteins enhance the emulsifying stability of the walnut protein by improving their absorption to oil/water interfaces.

Kostić et al (2015) have reported that protein fractions with molecular weights (MWs) of 50-25 kDa contribute largely to the emulsion stability of bee pollen samples, whereas, higher fractions (50-80 kDa) were correlated negatively with emulsion stability, pointing out lower MWs protein fractions might highly adsorbed to the oil/water interfaces and form interfacial films more stable than proteins with higher MWs. A positive correlation between emulsifying properties and protein solubility has been demonstrated (Phillips et al., 1994). Although proteins are valued as one of the major natural food emulsifiers, other active ingredients such as lipids, especially polar ones, act as good emulsifiers which are desirable in many food applications. In oil/water emulsion, all together, proteins and other amphiphilic emulsifiers such

interface material. Several surfactants including proteins reduce the interfacial tensions by altering the viscoelastic characteristics of the interface and inhibiting the coalescence (Dalgleish, 2006). Moreover, major metal ions such as Ca, Fe, and K as well as polysaccharides such as starch and pectin contained in bee pollen interact with the interfacial material and induce its stabilization (Han et al., 2020).

3.7.4. Water absorption capacity (WAC)

Owing to its effect on the yield and quality of food products, WAC is economically an important step to be validated by the food, cosmetic, and nutraceutical industries. The organoleptic and the texture of end-food products are strongly related to the WAC of its components. WAC is generally based on the interactions between proteins and water. Analysed bee pollen samples showed WAC values varied between 1.06 g/g in polyfloral pollen harvested from Taounate (BP2) and 2.19 g/g in bee pollen collected from Timahdite (BP7) (Table 5). These values are higher than those reported by Thakur and Nanda (2020) for thirty-five Indian bee pollen samples where WAC oscillated from 0.47 g/g (coriander bee pollen) to 0.72 g/g (coconut bee pollen), and similar to those registered for twenty-six samples collected from different areas of Serbia (0.92-2.25 g/g) (Kostić et al., 2015). Differences could be related to several factors such as charged/uncharged sugars, composition, and conformation of proteins, particularly insoluble proteins containing hydrophilic parts, polar lipids, and minerals that play a crucial role in the water uptake process (Kostić, Milinčić, Trifunović, et al., 2020). Furthermore, it was suggested that polysaccharide-protein interactions influence highly the shelf-life and texture of most food products. Thus, the wide WAC variability obtained between bee pollens confirms differences in their carbohydrate, lipid, and protein contents (Table 1). It has been documented that polar lipids with their charged regions could also contribute to better water uptake.

and lipids content (Table S2 (Supplementary Material)), which is probably linked to the water uptake effect of polar and charged regions of lipids (Kostić et al., 2015).

3.7.5. Oil absorption capacity (OAC) and water-oil absorption index (WOAI)

The OAC of several food products or their dietary molecules depends mostly on their ability to physically entrap oil through a complicated capillary-attraction process. This characteristic is highly dependent on the presence of hydrophobic portions of insoluble compounds. Similar to the OAC values found in many organic foods such as black gram (1.1-3.2 g/g) and kidney bean (2.2-3.2 g/g) (Wani, Sogi, & Gill, 2013; Wani, Sogi, Wani, et al., 2013), bee pollen samples showed different oil absorption capacities, where Taounate sample (BP2) had the lowest OAC values (1.15 g/g), while, the highest value (3.50 g/g) was expressed by the sample originates of Timahdite (BP7) (Table 5). In addition to the lipophilic amino acid residues, the high OAC of bee pollen has been attributed to the sporopollenin polymer complex, a major component of pollen exine (Thakur & Nanda, 2020). This component, in reality, traps oil in its matrix and thus improves the oil absorption ability.

Regarding the WOAI all samples showed values lower than 1, except multi-floral pollen collected from Taza (BP3) in which WOAI=1.25 ± 0.003, demonstrating the better hydrophilic than hydrophobic characteristics of bee harvested pollens.

4. Conclusions

The outcome of the present work provides insight into the nutritional composition, chemical and structural characterization, bioactive, and techno-functional properties of different monofloral and multifloral bee pollens from Morocco.

composition in the pollen raw material, as revealed by the palynological analysis, and by their nutritional and chemical composition. The current data shows that, namely, proteins, lipids, and carbohydrates as essential macro-nutrients are present in considerable amounts in studied bee pollens. Results exhibited desirable techno-functional characteristics important for food industry applications, as well as high antioxidant and antihyperglycemic activities associated with the presence of several phenolic compounds.

Overall, the combination of nutritive potential, bioactive characteristics, and specific techno-functional properties of bee pollens suggests its possible application as a multipurpose bio-functional ingredient either in food, nutraceutical, or pharmaceutical industries.

Author Contributions

Conceptualization, B.L, J.A.T, H.L, and P.F.-S.; Investigation, H.L, Z.G, P.F.-S, M.B and D.O.; Writing-original draft, H.L.; Supervision, B.L and J.A.T.; Visualization B.L, H.L, P.F.-S. D.O and M.B; Writing-review and editing D.O, B.L, J.A.T, A.E, Z.G, M.B, and P.F.-S. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Table 1. Physicochemical parameters and energetic value of bee pollen samples.

Bee Pollen	Moisture (%)	a_w	Ashes (%)	pH	S.proteins (g BSA/100g dw)	T.proteins (g/100g dw)	S. carbohydrates (g GlcE/100g dw)	Lipids (g/100g dw)	Energy (kcal/100g dw)
BP1	3.16 ± 0.0^{e}	0.25 ± 0.03^{c}	3.18 ± 0.03^{b}	4.13 ± 0.01^{e}	28.52 ± 3.86^{a}	32.00 ± 0.43^{b}	18.52 ± 0.19^{e}	3.74 ± 0.56^{ab}	235.74 ± 1.23^{c}
BP2	14.08 ± 0.09^{a}	0.33 ± 0.01^{ab}	2.22 ± 0.04^{e}	4.76 ± 0.05^{c}	28.85 ± 4.05^{a}	30.90 ± 0.15^{b}	22.86 ± 0.49^{b}	5.20 ± 0.16^{a}	261.84 ± 2.04^{a}
BP3	3.18 ± 0.00^{e}	0.32 ± 0.02^{ab}	3.51 ± 0.03^{a}	5.12 ± 0.03^{a}	19.40 ± 3.98^{ab}	20.56 ± 0.68^{d}	20.02 ± 0.85^{d}	3.78 ± 0.58^{ab}	196.34 ± 0.98^{f}
BP4	9.52 ± 0.58^{b}	0.39 ± 0.01^{a}	2.14 ± 0.01^{e}	4.38 ± 0.03^{d}	16.91 ± 3.22^{ab}	19.21 ± 0.28^{e}	23.24 ± 0.46^{b}	4.08 ± 0.45^{ab}	206.52 ± 2.15^{e}
BP5	3.45 ± 0.03^{e}	0.24 ± 0.03^{c}	2.83 ± 0.02^{c}	4.19 ± 0.02^{e}	16.63 ± 3.66^{ab}	19.18 ± 0.56^{e}	46.44 ± 0.55^{a}	4.99 ± 0.20^{a}	227.39 ± 1.86^{d}
BP6	6.39 ± 0.34^{c}	0.36 ± 0.01^{a}	2.14 ± 0.03^{e}	5.00 ± 0.02^{b}	27.77 ± 3.66^{a}	28.21 ± 0.28^{c}	23.27 ± 0.80^{b}	4.20 ± 0.29^{a}	243.72 ± 1.56^{b}
BP7	4.12 ± 0.02^{d}	0.41 ± 0.01^{a}	2.73 ± 0.04^{d}	4.41 ± 0.01^{d}	31.17 ± 3.81^{a}	33.50 ± 0.25^{a}	23.53 ± 0.10^{b}	2.11 ± 0.20^{c}	247.11 ± 2.18^{b}
Mean ± SD	5.09 ± 4.24	0.32 ± 0.06	2.78 ± 0.45	4.39 ± 0.34	27.83 ± 5.23	27.87 ± 5.23	23.26 ± 2.26	4.14 ± 0.93	239.73 ± 20.9
French Regulation	Maximum of 6	-	Between 2 and 6	-	Between 10 and 40	Between 10 and 40	Between 13 and 55	Between 1 and 10	-

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$). a_w: water activity; S.proteins: soluble proteins; T.proteins: total proteins; S. carbohydrates: soluble carbohydrates; dw: dry weight.

Table 2. Major and minor mineral elements of bee pollen samples.

Samples	Major minerals (mg/kg dw)				
	K	**Ca**	**Mg**	**Fe**	**Na**
BP1	1745.8 ± 23.5 ^{d}	1267.6 ± 12.6 ^{f}	409.3 ± 12.6 ^{c}	95.45 ± 4.2 ^{c}	14.9 ± 1.0^{ab}
BP2	2729.7 ± 35.8 ^{b}	2163.9 ± 8.3 ^{b}	130.3 ± 6.5 ^{e}	62.58 ± 4.5 ^{f}	18.3 ± 0.9 ^{a}
BP3	2972.6 ± 21.3 ^{a}	1679.9 ± 27.7 ^{d}	769.1 ± 28.9 ^{a}	157.58 ± 11.0 ^{a}	14.0 ± 2.4 ^{ab}
BP4	1780.2 ± 7.8 ^{d}	2343.6 ± 45.5 ^{a}	807.6 ± 5.9 ^{a}	77.40 ± 2.1 ^{e}	09.5 ± 1.4^{ab}
BP5	2141.7 ± 5.7 ^{c}	980.3 ± 7.8 ^{g}	582.9 ± 22.4 ^{b}	96.70 ± 2.8 ^{c}	15.1 ± 1.1^{ab}
BP6	1336.8 ± 15.4 ^{e}	1371.4 ± 6.3 ^{e}	625.3 ± 8.7 ^{b}	106.09 ± 5.3 ^{b}	25.2 ± 4.4 ^{a}
BP7	1049.9 ± 29.9 ^{f}	1766.1 ± 32.8 ^{c}	371.2 ± 22.9 ^{d}	85.39 ± 0.4 ^{d}	12.1 ± 4.1 ^{ab}
	Minor minerals (mg/kg dw)				
	Zn	**Cu**	**Ni**	**Cd**	**Pb**
BP1	14.9 ± 3.3 ^{b}	2.64 ± 0.70 ^{c}	1.06 ± 0.02 ^{a}	n.d.	0.08 ± 0.03
BP2	27.3 ± 2.4 ^{a}	n.d.	0.13 ± 0.02^{bc}	n.d.	n.d.
BP3	21.5 ± 2.8 ^{b}	1.12 ± 0.04 ^{cd}	0.19 ± 0.01 ^{b}	n.d.	n.d.
BP4	33.6 ± 2.9 ^{a}	n.d.	0.07 ± 0.02 ^{d}	n.d.	0.10 ± 0.03
BP5	18.4 ± 0.4 ^{b}	5.22 ± 0.94 ^{b}	0.05 ± 0.01 ^{d}	0.015 ± 0.007	n.d.
BP6	20.3 ± 2.3 ^{b}	8.15 ± 0.72 ^{a}	0.12 ± 0.02^{bc}	n.d.	n.d.
BP7	29.8 ± 3.9 ^{a}	3.18 ± 0.04 ^{c}	0.16 ± 0.01 ^{b}	n.d.	n.d.

Values of mineral elements are expressed as concentration (mg/kg dw) mean ± SD of 3 experiments. Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$). n.d.: not detected; dw: dry weight.

Table 3. Total phenolics (TPC) and flavonoids (TFC) content, and individual phenolic compounds identification and quantification of bee pollen.

	Bee pollen samples						
	BP1	**BP2**	**BP3**	**BP4**	**BP5**	**BP6**	**BP7**
TPC (mg GAE/g dw)	15.35 ± 0.87^{a}	16.85 ± 0.07^{a}	13.59 ± 0.46^{b}	12.40 ± 0.59^{b}	16.28 ± 0.63^{a}	15.66 ± 0.40^{a}	16.81 ± 0.45^{a}
TFC (mg QE/g dw)	2.87 ± 0.08^{c}	3.68 ± 0.19^{ab}	1.51 ± 0.28^{d}	1.68 ± 0.14^{d}	2.96 ± 0.19^{c}	4.15 ± 0.25^{a}	4.57 ± 0.14^{a}
Hydroxycinnamic acids (mg/kg dw)							
o-Coumaric acid	22.7 ± 0.0^{b}	45.3 ± 5.4^{a}	13.9 ± 0.1^{bc}	50.4 ± 1.6^{a}	19.1 ± 0.5^{b}	n.d.	16.9 ± 2.6^{b}
Ferulic acid	18.1 ± 0.1^{bc}	17.8 ± 0.1^{bc}	19.1 ± 0.2^{b}	19.1 ± 0.0^{b}	22.2 ± 1.2^{a}	18.3 ± 0.1^{b}	18.0 ± 0.0^{b}
Cinnamic acid	65.2 ± 3.1^{b}	4.8 ± 0.6^{d}	80.8 ± 6.0^{b}	20.2 ± 0.2^{d}	17.8 ± 0.8^{d}	261.1 ± 8.2^{a}	49.9 ± 11.5^{bc}
Rosmarinic acid	167.7 ± 13.6^{a}	87.0 ± 0.6^{c}	138.3 ± 14.4^{b}	101.6 ± 3.4^{c}	73.2 ± 2.2 ^{cd}	133.6 ± 0.7^{b}	68.2 ± 8.8^{cd}
Chlorogenic acid	15.8 ± 0.1^{b}	14.6 ± 0.2^{c}	n.d.	n.d.	n.d.	14.7 ± 0.0^{c}	16.8 ± 0.3^{a}
Hydroxybenzoic acids (mg/kg dw)							
Ellagic acid	128.1± 0.9^{b}	108.3 ± 0.5^{c}	22.1 ± 1.4^{f}	31.6 ± 0.7^{e}	98.5 ± 1.3^{d}	138.6 ± 0.9^{a}	12.9 ± 0.1^{g}
Vanillic acid	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Gallic acid	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Flavonoids (mg/kg dw)							
Naringin	96.00 ± 7.5^{b}	66.0 ± 2.3^{c}	29.8 ± 3.8^{de}	46.4 ± 4.6^{d}	36.9 ± 3.4^{d}	117.0 ± 2.3^{a}	44.9 ± 12.6^{d}
Hesperidin	16.3 ± 12.8 ^{e}	553.1 ± 11.8^{a}	122.5 ± 16.6^{c}	91.8 ± 0.5^{d}	28.8 ± 1^{e}	87.2 ± 3.2^{d}	297.4 ± 12.5^{b}
Apigenin	40.0±3.6^{b}	37.3 ± 0.6^{b}	30.2 ± 0.8^{bc}	32.9 ± 1.5^{b}	22.5 ± 1.2^{bc}	70.9 ± 6.0^{a}	27.3 ± 5.5^{bc}
Rutin	175.1 ±11.1^{b}	174.7 ± 3.8^{b}	139.6 ± 8.5^{bc}	150.9 ± 4.2^{b}	115.1 ± 3.1^{bc}	343.6 ± 19.3^{a}	159.4 ± 19.9^{b}
Quercetin	44.5 ± 6.6^{b}	31.2 ± 0.7^{b}	10.9 ± 0.0^{c}	10.3 ± 0.3^{c}	275.3 ± 24.7^{a}	48.7 ± 0.2^{b}	16.1 ± 2.3^{c}
Kaempferol	6.1 ± 0.1^{d}	3.9 ± 0.1^{e}	n.d.	8.8 ± 0.2^{a}	6.6 ± 0.2^{c}	7.8 ± 0.2^{b}	8.1 ± 0.1^{b}
Stilbene (mg/kg dw)							
Resveratrol	88.6 ± 9.1^{c}	174.3 ± 4.4^{b}	58.5 ± 13^{d}	37.3 ± 0.7^{d}	44.2 ± 1.9^{d}	257.9 ± 2.5^{a}	85.4 ± 13.2^{c}

Values of individual phenolic compounds are expressed as concentration (mg/kg dw) mean ± SD of 3 experiments. Values in the same line followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$). BP: bee pole, TPC: total phenolic content; TFC: total flavonoids content; n.d.: not detected; n.q: not quantified.

Table 4. Antioxidant and antihyperglycemic activities of bee pollen extracts.

Antioxidant activity								
Bee pollen samples	BP1	BP2	BP3	BP4	BP5	BP6	BP7	Trolox
DPPH IC_{50} (µg/mL)	63.84 ± 0.7^{b}	50.9 ± 5.4^{bc}	57.85 ± 1.7^{b}	73.87 ± 0.1^{a}	42.2 ± 0.2^{d}	44.3 ± 5.2^{d}	57.5 ± 3.7^{b}	10.81 ± 0.1^{e}
ABTS IC_{50} (µg/mL)	499.1 ± 11.3^{a}	369.7 ± 2.6^{c}	449.5 ± 20.1^{ab}	469.0 ± 17.1^{a}	323.8 ± 16.7^{d}	398.8 ± 15.5^{c}	405.6 ± 13.9^{c}	23.15 ± 4.0^{e}
FRAP (µmol Fe^{2+}/g)	237.4 ± 5.0^{b}	226.1 ± 5.7^{c}	202.0 ± 2.5^{d}	184.2 ± 3.0^{e}	129.8 ± 0.9^{g}	307.2 ± 1.9^{a}	171.6 ± 1.0^{f}	136.1 ± 1.0^{g}
Antihyperglycemic activity								
Bee pollen samples	BP1	BP2	BP3	BP4	BP5	BP6	BP7	Acarbose
α-Amylase IC_{50} (µg/mL)	979.3 ± 7.3^{a}	251.05 ± 12.2^{c}	521.4 ± 11.3^{b}	976.9 ± 15.5^{a}	171.3 ± 3.1^{d}	201.1 ± 11.0^{d}	547.8 ± 14.5^{b}	35.4 ± 1.0^{e}
α-Glucosidase IC_{50} (µg/mL)	412.3 ± 6.9^{d}	308.94 ± 6.5^{e}	661.1 ± 18.0^{b}	561.94 ± 16.3^{c}	129.0 ± 9.2^{f}	108.0 ± 5.2^{f}	339.8 ± 15.0^{e}	11000 ± 1.0 ^{a}

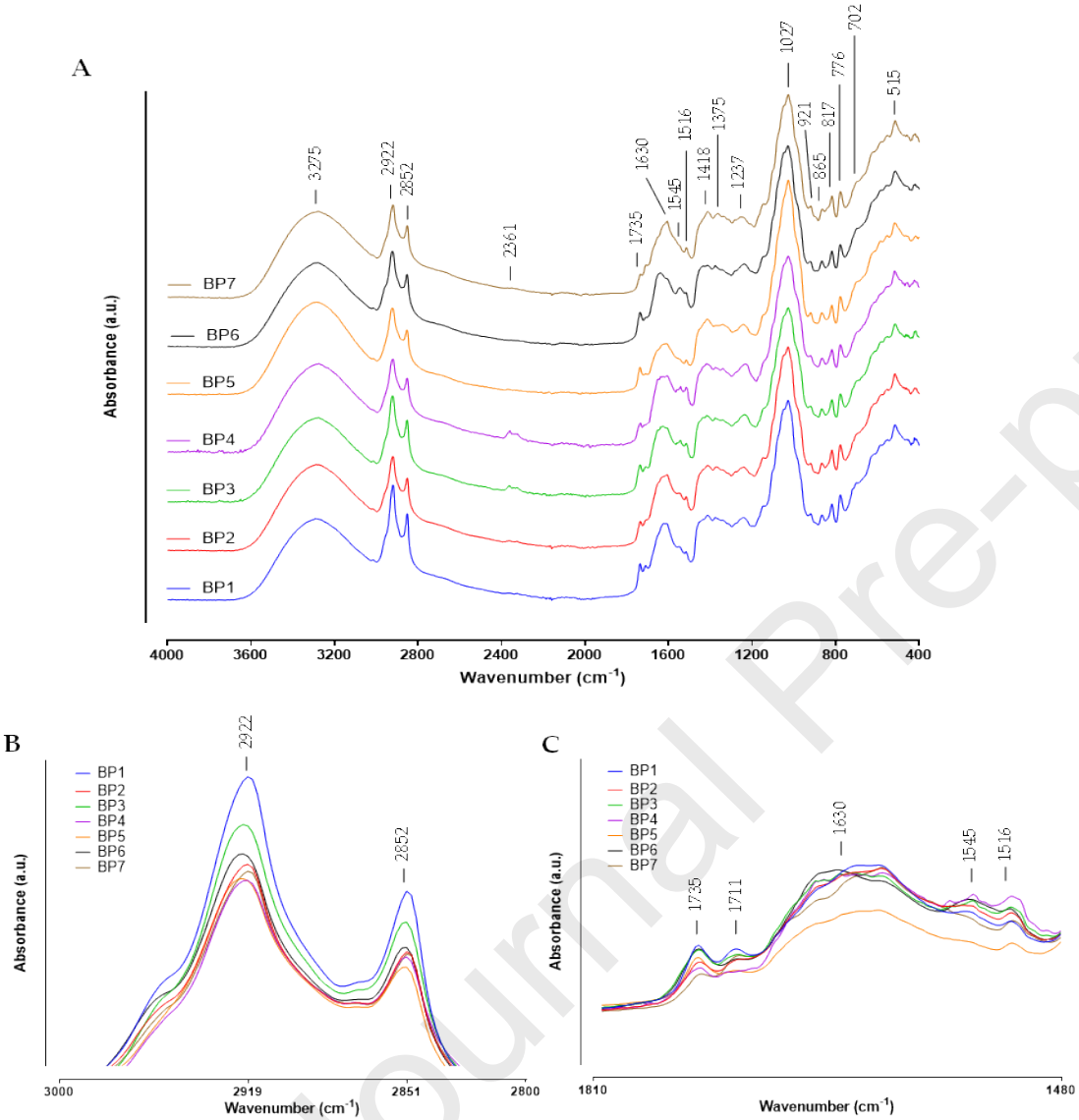
Values of phenolic compounds are expressed as concentration (mg/kg) mean ± SD of 3 experiments. Values in the same line followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$). n.q: not quantified; DPPH**: 2,2-Diphenyl-1-picrylhydrazyl assay; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay; FRAP:** Ferric Reducing Antioxidant Power assay.

Table 5. Techno-functional properties of bee pollen extracts.

Beepollen samples	Carbohydrate solubility (g/100 g dw)	Protein solubility (g/100 g dw)	ESI (min)	EAI (g/m^3)	WAC (g/g)	OAC (g/g)	WOAI
BP1	34.47 ± 0.75 ^{f}	13.11 ± 0.45 ^{d}	20.74 ± 0.55 ^{d}	10.81 ± 0.27 ^{e}	1.24 ± 0.08 ^{c}	1.74 ± 0.04 ^{d}	0.71±0.005^{c}
BP2	44.70 ± 1.70 ^{d}	8.19 ± 0.48 ^{e}	16.52 ± 0.32 ^{e}	23.66 ± 0.16 ^{b}	1.06 ± 0.04 ^{cd}	1.15 ± 0.02 ^{f}	0.92±0.002 ^{b}
BP3	37.76 ± 0.39 ^{e}	13.35 ± 0.26 ^{d}	21.96 ± 0.68 ^{d}	11.53 ± 0.40 ^{e}	1.70 ± 0.06 ^{b}	1.35 ± 0.01 ^{e}	1.25±0.003 ^{a}
BP4	57.39 ± 0.47 ^{b}	15.79 ± 0.33 ^{c}	26.53 ± 0.98 ^{c}	18.28 ± 0.28 ^{c}	1.36 ± 0.04 ^{c}	1.91 ± 0.04 ^{b}	0.71±0.001 ^{c}
BP5	75.33 ± 0.74 ^{a}	16.73 ± 0.89^{C}	32.69 ± 0.91 ^{b}	13.84 ± 0.32 ^{d}	1.06 ± 0.08 ^{cd}	1.56 ± 0.04 ^{c}	0.67±0.003 ^{d}
BP6	51.99 ± 1.18 ^{c}	7.28 ± 0.56 ^{e}	16.68 ± 0.74 ^{e}	25.05 ± 0.16 ^{a}	1.2 ± 0.06 ^{c}	1.31 ± 0.01 ^{e}	0.91±0.007^{b}
BP7	59.27 ± 0.41 ^{b}	23.31 ± 1.62 ^{a}	45.38 ± 0.65 ^{a}	9.83 ± 0.24 ^{f}	2.19 ±0.05 ^{a}	3.50 ± 0.06 ^{a}	0.62±0.002 ^{e}
Min.	34.47 ± 0.75	7.28 ± 0.56	16.52 ± 0.32	9.83 ± 0.24	1.06 ± 0.04	1.15 ± 0.02	0.62±0.002
Max.	59.27 ± 0.41	23.31 ± 1.62	45.38 ± 0.65	25.05 ± 0.16	2.19 ±0.05	3.50 ± 0.06	1.25±0.003
Mean ± SD	51.55 ± 14.07	14.57 ± 6.00	25.78 ± 10.33	16.14 ± 6.26	1.40 ± 0.41	1.78 ± 0.79	0.82 ± 0.21

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$). ESI: emulsifying stability; EAI: emulsifying activity; WAC: water absorption capacity; OAC: Oil absorption capacity; WOAI: water-oil absorption index; dw: dry weight.

Figure 1.



Highlights

- Bee pollens shows suitable physicochemical and nutritional values
- Bee pollen is a polyphenol-rich superfood with health-promoting benefits
- Pollen extracts have strong antioxidant and antihyperglycemic activity
- Bee pollen as a functional ingredient can improve the quality of food products