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Bee-pollen retailed in Tuscany (Italy): labelling, palynological, microbiological, and mycotoxicological profile

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Highlights

- Bacterial contamination of bee pollen can be high, especially in fresh samples
- Presence of pathogenic microorganisms can be considered uncommon
- Bee pollen contamination by mycotoxins must be considered
- Mislabelled samples are present at retail level

Bee-pollen retailed in Tuscany (Italy): labelling, palynological, microbiological, and mycotoxicological profile

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Abstract

Bee pollen is the result of flower pollen collecting and processing by honey bees. Due to its nutritional profile, it is considered a functional food. The present work aimed at evaluating labelling, pH, Aw, microbiological and mycotoxicological profile of 29 bee pollen samples (dried and fresh) purchased at retail in Tuscany (Italy). Only one pollen lacked more than two mandatory indications. Aw ranged from 0.19 to 0.77, while pH from 4.50 to 6.10. Fresh samples presented higher bacterial loads than dried samples. Total bacterial counts, *Enterobacteriaceae*, yeasts and molds and lactic acid bacteria ranges were 2.2-6.0, <2-4.1, <1-4 and <2-6.0 log CFU/g, respectively. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and sulphite-reducing clostridia loads were lower than the detection limit; *Salmonella* spp. and *Listeria monocytogenes* were always absent. As for mycotoxins, total aflatoxins were detected in all samples (5.2-34.4 µg/kg). Higher deoxynivalenol concentrations were detected (maximum: 179.7 µg/kg). Since the occurrence of spoilage microorganisms seems to be frequent, leading to possible alterations of the organoleptic profile, the microbiological quality of fresh bee pollen should be monitored. The risk related to the presence of pathogenic microorganisms seems to be very low, while attention must be paid to the presence of toxic metabolites.

Keywords: bee pollen, labelling, microbiological quality, total aflatoxins, deoxynivalenol~~mycotoxins~~

1. Introduction

Bee pollen is the product resulting from flower pollens agglutination with nectar (and/or honey) and salivary substances (including antigerminative substances) by worker honey bees, and collected at the hive entrance (Campos, Frigerio, Lopes, & Bogdanov, 2010). Due to its high nutritional value, the intake of bee pollen as dietary supplement ~~is~~ was currently ~~often~~ proposed ~~recommended~~ (Thakur & Nanda, 2020). Indeed, bee pollen contains remarkable quantities of all the essential amino acids, vitamins A, D, E, K, C, bioflavonoids, and the vitamin B ~~-~~complex, pantothenic acid (B5) and niacin (B3) (Carpes, Mourão De Alencar, & Masson, 2009). Moreover, ~~it~~ bee pollen shows several biological activities, such as antioxidant, antibacterial, anti-carcinogenic and hepatoprotective (Li et al., 2018). ~~Currently~~Today, bee pollen is available ~~bee pollen can be found on the~~on the market in various forms ~~various forms~~, such as granules, capsules, tablets, pellets, and powder, although fresh frozen or dried grains remain the most common (Kostić et al., 2020).

Being very hygroscopic and therefore very moist, fresh pollen can easily support the growth of several microorganisms (DeGrandi-Hoffman, Chen, & Simonds, 2013); however, ~~currently,~~ no specific legislation for this product is currently available in the European Union.

Microbiological contamination is one of the most important criteria ~~for~~ to determine bee pollen ~~the~~ quality ~~of bee pollen~~ and several environmental factors may influence it ~~by the growth of microscopic fungi and bacteria~~ (Xue et al., 2014). If on the one hand many researches focused on the chemical composition of bee pollen ~~from~~ of different ~~plants~~ botanical and geographical origins (Almeida-Muradian et al., 2005; Costa, Morgano, Ferreira, & Milani, 2017; De-Melo & de Almeida-Muradian, 2017), less information is available on the parameters affecting microbial growth (e.g. ~~activity~~ water activity, pH) and ~~more generally on bee pollen~~ microbial contamination (Estevinho, Rodrigues, Pereira, & Feás, 2012; Mauriello, De Prisco, Di Prisco, La Storia, & Caprio, 2017; Sagona et al., 2017). Moreover, most of the available ~~a lot of~~

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~~information~~ data~~is~~ ~~are available~~ on bee pollen come from a limited number of~~some~~ countries such as Argentina, Portugal, and Brazil, while no studies were performed in other parts of the world (Costa et al., ~~, Morgano, Ferreira, & Milani,~~ 2017; Estevinho et al~~, Rodrigues, Pereira, & Feás~~, 2012; Fernández et al., 2020b).

While the occurrence of bacterial pathogens or potential pathogens, such as pathogenic *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus* seems to be rare, the contamination with molds ~~is~~ was often ~~observed~~ reported (Kačániová et al., 2011; Nardoni, D'Ascenzi, Rocchigiani, Moretti, & Mancianti, 2016). This could be a major issue, since the production of mycotoxins by these microorganisms in bee pollen has already been demonstrated~~reported~~ (González, Hinojo, Mateo, Medina, & Jiménez, 2005; Kačániová et al., 2011). Mycotoxins are toxic compounds naturally produced by molds, ~~(fungi)~~ which may contaminate several agricultural commodities both before and after harvest, wherever humidity and temperature create ~~the~~ suitable conditions (Murphy, Hendrich, Landgren, & Bryant, 2006). Thousands of mycotoxins exist, but only a few constitute a considerable food safety hazard~~s~~. The genera *Aspergillus*, *Fusarium*, and *Penicillium* produce the most prominent mycotoxins: aflatoxins, deoxynivalenol, zearalenone, ochratoxin, and fumonisin (Murphy et al.~~, Hendrich, Landgren, & Bryant~~, 2006).

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Lastly, bee pollen labelling represents an overlooked aspect. Indeed, at the best of our knowledge, only one study performed in Portugal looked at the labels of marketed bee pollen (Nogueira, Iglesias, Feás, & Estevinho, 2012), verifying their compliance with current legislation, while in Italy this evaluation was never carried out.

Thus, ~~T~~the present work aimed ~~to~~ at verifying the compliance to the labelling legislation of bee pollen samples retailed in Tuscany (Italy), ~~to~~ determining~~e~~ some of their physicochemical parameters (pH and Aw), their palynological and microbiological profiles, and the presence of mycotoxins (aflatoxins and deoxynivalenol).

2. Material and Methods

2.1 Bee pollen samples

Twenty-nine bee pollen samples were included in this study. They were purchased directly from beekeepers, supermarkets, ~~or~~ and farmers' markets ~~and included in this study~~ and ~~. They were~~ identified by progressive numbers from 1 to 29. Five samples were sold as bulk products, while 24~~5~~ were packaged. Eighteen out of the 29 bee pollen samples were driedd ~~(one of which frozen)~~, 10 were fresh frozen and one was fresh and packaged in a modified atmosphere at refrigeration temperature (Table S1).

2.2 Labelling analysis

~~Bee pollen can be sold as a food product or as an ingredient of food supplements. Italian laws regulate the production and the commercialization of food supplements by requiring an authorization for the production plants and the approval of the label by the Ministry of Health (D. Lgs. 169/2004). In most cases, these requirements do not fit with beekeeping realities and bee pollen is often sold as a food product. Therefore, t~~The label of the 24~~5~~ prepacked bee pollen samples was analyzed in accordance with the requirements of current European Union and Italian laws for food products.

The general laws on food identification (Directive 2011/91/EU) and on the provision of food information to consumers (Reg. (EU) 1169/2011) apply to both honey and bee products. In case of non-perishable single ingredient products, the list of mandatory indications, in addition to the geographical origin, is limited to: trade name; name and address of the food business operator responsible for the food information; date of minimum durability ('best before' date); special storage conditions (only for frozen bee pollen); lot identification; and net quantity

expressed in units of mass. A minimum font size has been set for all mandatory information on food labels (1.2 mm or 0.9 mm if the largest surface is less than 80 cm²). Other information not required by the law can be included on food labels (e.g. botanical origin of bee pollen). Labels of 245 bee pollen samples were analyzed for presence and accuracy of nutritional information and consumer advertising. This information shall be accurate, and shall not be ambiguous, confusing, or misleading for the consumer. Moreover, any nutrition or health claim shall be done in accordancein compliance with Reg. (EC) 1924/2006 on nutrition and health claims made onof foods.

2.3 Palynological identification

For each bee-pollen sample, 10 g of pollen loads were grouped by color. Then, each color group was weighted. Three single pollen loads of each color were prepared for the identification as follows: they wereby washing themed with distilled water and employing glycerin jelly for permanent preparations was employed. Pollen grains identification was performed by optical microscopy with total magnification (400X and 1000X). A reference pollen collection of Pisa University (Italy) and different pollen morphology guides were used for the recognition of the pollen types (Hodges, 1984; Palmieri et al., 2017; Ricciardelli-D'Albore, 1997, 1998; Ricciardelli-D'Albore & Persano, 1978). In case a pollen grain cannotould not be identified as far as the genus or species, it was associated in larger groups or (forms, or types), as suggested by Louveaux, Maurizio & Vorwohl Louveaux (197867). The botanical composition was calculated based on the weight of each color group.

2.4 pH and activity water activity (Aw) determinations

Each pollen sample was subjected to the determination of pH and Aw. The pH was determined in aqueous phase obtained after mixing 5 g of pollen in 20 ml of distilled water using a pH-

meter (XS-instruments, Bormarc srl, Carpi-Modena, Italy). ~~The pH was determined in aqueous phase obtained after mixing 5 g of pollen in 20 ml of distilled water. A pH glass electrode connected to a pH meter (XS instruments, Bormarc srl, Carpi Modena, Italy) was employed.~~ The Aw was determined using a water activity meter (HC2-AW-SONDA AW, Rotronic, Italy) according to the manufacturer's instructions.

2.5 Microbiological ~~determinations~~analysis

Ten grams of each pollen sample were diluted in 90 mL of sterile saline solution; then ten-fold dilutions were performed using the same diluent. Total bacterial count (TBC) was determined according to the ISO 4833 (ISO, 2003a) method. *Enterobacteriaceae* were enumerated by the ISO 21528-2 (ISO, 2004) method. *Escherichia coli* counts were determined according to the ISO 16649-2 (ISO, 2001) method. Lactic acid bacteria were determined according to the ISO 15214 (ISO, 1998) method. Yeasts and molds enumerations were carried based on the ISO 21527-2 (ISO, 2008) on Yeast Extract Glucose Chloramphenicol (YGC) agar. For sulphite-reducing clostridia and *Bacillus cereus* spores counting, aliquots of the dilutions were thermally treated at 80 °C for 10 min and counts were performed according to ISO 15213 (ISO, 2003b) and ISO 7932 (ISO, 2004b), respectively. Coagulase-positive and negative staphylococci were enumerated on Baird Parker agar with Egg Yolk Tellurite Emulsion by ISO 6888-1:1999 method (ISO, 1999). ~~Ten grams of each pollen sample were diluted in 90 mL of sterile saline solution; then ten-fold dilutions were performed using the same diluent. Total bacterial count was determined on Plate Count Agar (PCA) incubated at 30°C for 72 h. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose agar (VRBGA) after 24 hours at 37°C. *Escherichia coli* counts were determined on Tryptone Bile X-glucuronide agar after incubation at 37°C for 24 hours. Lactic acid bacteria were determined by anaerobic growth on de Man Rogosa Sharpe (MRS) agar incubated at 37°C for 48 hours. Yeasts and molds enumerations~~

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~~were carried out using Yeast Extract Glucose Chloramphenicol (YGC) agar after an incubation of 5 days at 25°C. For sulphite-reducing clostridia and *Bacillus cereus* spores counting, aliquots of the dilutions were thermally treated at 80 °C for 10 min and spreaded on Tryptose Sulfite Cycloserine agar with incubation at 37°C for 48 hours in anaerobiosis and on Mannitol Egg Yolk Polymyxin (MYP) agar, with incubation at 37°C for 24 hours, respectively. *Salmonella* spp. was evaluated following the UNI EN ISO 6579:2008 standard. Coagulase-positive and negative staphylococci were enumerated on Baird Parker agar with Egg Yolk Tellurite Emulsion incubated at 37°C for 48 hours.~~ Microbial counts were expressed as log colony-forming units ~~(log CFU/g)~~ per gram of bee pollen (log CFU/g). The presence of *Salmonella* spp. and *Listeria monocytogenes* were evaluated following the UNI EN ISO 6579:2008 standard and~~The presence of *Listeria monocytogenes* was evaluated following the~~ UNI EN ISO 11290-1:2005 standard, respectively. ~~Microbial counts were expressed as log colony-forming units (log CFU/g) per gram of bee pollen.~~ All media and supplements were purchased at Thermo Fisher Scientific (Milan, Italy).

2.6 Mycotoxins determination

Total aflatoxins (AFL) and deoxynivalenol (DON) contents of bee pollen were determined by ~~Total aflatoxins (AFL) and deoxynivalenol (DON) content of bee pollen was determined by~~ direct competitive enzyme-linked immunosorbent assays (ELISA). Samples were dried at 50°C overnight and milled to a fine powder. Extraction of samples was carried out in distilled water for DON and in methanol/water (70:30 v/v) for AFL. The Veratox quantitative test kits (Neogen, USA) were used and the ELISA procedure performed following the manufacturer's recommendations. Absorbance was determined using Sinergy HTX multi-scan reader (BioTek, Winooski, VT, USA) at 650 nm. A calibration curve for the standards for each toxin dilution

was plotted using the standards concentration against the percentage inhibition of the standards. Using a microwell reader, the tests provided sample results in µg/kg for all mycotoxins.

2.7 Statistical analysis

Normal distribution of data was assessed by Shapiro-Wilk test. Since data from analysis on Aw, DON, *Enterobacteriaceae*, ~~total bacterial count (~~TBC~~)~~ and lactic acid bacteria were not normally distributed, differences among pollen samples were assessed by the Mann-Whitney U-test pairwise comparisons. Data from pH determination, molds and mycotoxins contents resulted instead normally distributed and differences among pollen samples were assessed by the ANOVA test, followed by T-Student test. The correlation among investigated parameters was assessed by using Spearman test. All the statistics were performed using JMP software (SAS Institute, 2008) and Graph Pad Prism software (S. Diego, CA USA) with a 2-tailed α-error pre-set at 0.05.

3. Results

3.1 Labelling analysis

Table 1 shows the results of the labelling analysis. Overall, the analyzed labels ~~each label~~ did not lack more than two mandatory indications, except for one sample (sample 24, dried), the label of which showed only ~~lacking all the mandatory indications, except for~~ the ~~name of the~~ food business operator's name. All the 24~~5~~ prepacked products reported the name of the food business operator. Regarding trade name, address of the food business operator, date of minimum durability and net quantity, only one of the samples (4.2%) did not present this information. Two samples (8.3%) did not report the lot. Four samples (16.7~~16~~%) reported on the label "multifloral pollen" or "flower pollen" and five samples (20.8%) were sold as monofloral: two of them as "chestnut pollen", one as "coriander pollen"; one as "alexandrine

clover /ash tree" and one as “ivy pollen”. For most of them (62.54%), no specific indication was declared. In ~~Twenty two~~ 21 prepacked pollen samples (87.58%) the geographical origin was declared (Italy) were from Italy and three products (12.5%) had no indications on geographical origin. Regarding storage conditions, which must be indicated only for frozen products, 8~~9~~ out of 99~~10~~ fresh frozen prepacked samples presented this information on the label. The facultative nutritional labelling and consumer advertising (for people affected by allergies) were correctly reported ~~correctly~~ and presented in 3 samples (12.5%) and in ~~7~~6 samples (25.0~~8~~%), respectively.

3.2 Palynological analysis

Table 2 shows the pollen spectra of 29 bee pollens retailed in Tuscany (Italy). The pollen samples presented different pollen types composition ranging from 2 (sample 23) to 15 (sample 26) types. *Rubus* and *Castanea* were the most abundant pollen ~~species~~genera, being observed in 20 and 17 of the 29 investigated pollen, respectively. There was no botanical species present in all the analyzed pollen samples. This could be related to the variability of pollen species presence based on geographical origin, from the beekeeping flora into the area of origin.

By palynological analysis, the pollen blends 11 and 19 were confirmed ~~to be~~as chestnut pollen, as reported on their label, with 92.2% and 76.4% of *Castanea* pollen, respectively. Sample 29 was also confirmed to be ivy pollen (65.6 %), as reported ~~in~~ on the label. The pollen blend 21 indicated as “alexandrine clover /ash tree ~~*Trifolium alexandrinum* and ash~~ mix” presented, instead, a prevalence of *Castanea* (91.4%). Pollen 22, indicated as “coriander pollen”, reported a prevalence of *Trifolium pretense* (80.8%) and only 11% of *Coriandrum sativum*, however ~~it~~ such a percentage could be enough to give ~~particular~~peculiar organoleptic characteristic to ~~this~~ the blend.

3.3 pH and Aw determinations

Table 3 shows ~~A~~a summary of the results obtained for pH and Aw determinations for fresh and dried bee pollen ~~is shown in Table 3~~, while Table S1 reports detailed results for each sample. As for pH, ~~the~~ values ~~detected in the present work~~ ranged from 4.50 (sample 2, fresh pollen stored in modified atmosphere packaging) to 6.10 (sample 7, fresh frozen pollen), with an average pH value of 5.20. Considering the categories, ~~it was possible to observe~~ similar average pH values were observed, ~~which~~ in particular~~were~~ 5.10 ± 0.44 for fresh samples and ~~of~~ 5.20 ± 0.55 for dried samples with no statistical difference between the two sample ~~types~~ categories ($p>0.05$). Concerning Aw, values ranged from a minimum of 0.19 (sample 6, dried) to a maximum of 0.77 (sample 27, fresh frozen), with values for dried samples lower than those for fresh samples (0.33 ± 0.07 and 0.67 ± 0.07, respectively; $p<0.0001$).

3.4 Microbiological analysis

Table 3 shows a summary of results of microbiological determinations for fresh and dried bee pollen, while detailed results for each sample are reported in Table S1.

As for the total bacterial count, the highest concentration was detected in sample 25 (6 log CFU/g) (fresh frozen pollen). Six out of 29 (20.7%) analyzed pollen samples presented a concentration higher than 5 log CFU/g. Among these samples, 5 were fresh, while one was dried (sample 22). Sample 29 (dried pollen) presented instead the lowest concentration (2 log CFU/g). Total bacterial count mean value for fresh pollen samples was higher than that for dried samples (4.6 ± 1.0 and 3.1 ± 0.8 log CFU/g, respectively, $p=0.006$).

Enterobacteriaceae were detected in 7 out of 29 (24.1%) samples with concentrations higher than 2 log CFU/g. As for sample category (dried and fresh samples), *Enterobacteriaceae* were detected in 5.5% of dried pollen samples and 54.5% of fresh pollen samples. Except for one case, ~~A~~all ~~positive~~ samples with detectable *Enterobacteriaceae* ~~but one~~ were fresh. Among

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these, the highest value (4.1 log CFU/g) was detected in sample 27 (fresh frozen pollen), while the lowest (3 log CFU/g) in sample 22, which was the only ~~positive~~ dried pollen sample with detectable *Enterobacteriaceae*. No statistical difference was observed between the two samples categories ($p>0.05$). All samples presented an *E. coli* load <2 log CFU/g and resulted negative for the presence of *Salmonella* spp. For these determinations, no statistical differences were observed between fresh and dried samples ($p>0.05$).

As for Gram-positive microorganisms, *S. aureus* concentrations were always <2 log CFU/g and all samples were negative for the presence of *L. monocytogenes*. Similarly, sulfite-reducing clostridia and *B. cereus* were always <2 log CFU/g. For these determinations, no statistical differences were observed between fresh and dried samples ($p>0.05$).

Considering lactic acid bacteria, 51.7% of the samples showed detectable levels. Among dried and fresh samples, 27.8% and 90.9%, respectively, presented lactic acid bacteria, with a maximum concentration of 6 log CFU/g in sample 19 (fresh frozen). Fresh pollen samples presented a higher concentration of lactic acid bacteria than dried samples (5.0±1.3 and 2.2±0.9 log CFU/g, respectively; $p<0.0001$).

As for yeasts and molds, 89.6% of samples ~~was~~ resulted contaminated~~positive~~. Considering the sample category, 88.9% of dried pollen samples and 90.9% of fresh pollen samples ~~were positive~~showed detectable levels, but no statistical difference was observed between the two categories in terms of concentrations ($p>0.05$). All ~~positive~~ samples with detectable levels p~~p~~resented a concentration lower than 5 log CFU/g, with the highest value of 4 log CFU/g detected in sample 27 (fresh frozen pollen).

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3.5 Mycotoxins determination

Mycotoxins concentrations for fresh and dried bee pollen samples are reported in Table 3. AFL were detected in all bee pollen samples analyzed, whereas DON was detected in 25 out of 29

samples (86.2%). AFL were detected at concentrations ranging from 5.2 to 34.7 µg/kg (dried bee pollen samples) and 14.0 to 34.4 µg/kg (fresh bee pollen samples). DON was detected at concentrations ranging from < LOD to 172.9 (dried) and < LOD to 179.7 µg/kg (fresh frozen). No statistically significant differences ($p > 0.05$) for both AFL and DON content were found among dried and fresh bee pollen samples.

3.6 Statistical analysis

Table 4 shows the correlation matrix of the studied parameters. Those categories always showing values under the detection limit were not reported. The pH and Aw were negatively correlated ($p<0.05$). Aw values resulted positively correlated with ~~the~~ TBC ~~values of total bacterial count~~ ($p<0.05$), *Enterobacteriaceae* ($p<0.01$), and lactic acid bacteria ($p<0.01$). *Enterobacteriaceae* and lactic acid bacteria concentrations were also positively correlated ($p<0.0001$). Yeasts and molds and ~~total bacterial counts~~TBC values were positively correlated ($p<0.05$), while aflatoxins were negatively correlated with *Enterobacteriaceae* ($p<0.01$) and positively correlated with lactic acid bacteria ($p<0.01$).

4. Discussion

4.1 Labelling analysis

Bee pollen can be sold as a food product or as an ingredient ~~of~~for food supplements. Italian laws regulate the production and the commercialization of food supplements by requiring an authorization for the production plants and the approval of the label by the Ministry of Health (D. Lgs. 169/2004). In most cases, these requirements do not fit with beekeeping realities and bee pollen is often sold as a food product. Currently, general legislation for food labelling applies to bee products. This would allow to prevent traders and consumers to be misled by confusing and incomplete indications on the labels. However, no specific regulations for bee

pollen labelling is available, and this could be confusing for both beekeepers and consumers. ~~Today, bee pollen is considered as a functional food and it is mostly commercialized as a food product, rather than a food supplement. Currently, general legislation for food labelling applies to bee products. This would allow to prevent traders and consumers to be misled by confusing and incomplete indications on the labels. However, no specific regulations for bee pollen labelling is available, and this could be confusing for both beekeepers and consumers.~~ As for our results, among the 24~~5~~ packaged pollen samples examined, only one was seriously mislabelled, lacking ~~many~~ several mandatory indications. Nogueira ~~, Iglesias, Feás, & Estevinho~~et al. (2012) reported a slightly better situation, with all the products' labels correctly showing the sales name, date of minimum durability, lot and net quantity. As for the botanical origin, most of the samples did not present information on the label ~~or simply refer to "flower pollen" or "multifloral pollen"~~. While this aspect could be under looked, it should be taken into consideration by beekeepers. Indeed, when possible, the correct attribution of bee pollen to a specific botanical species, would allow the consumer to choose a product with peculiar and pleasant organoleptic characteristics.

4.2 Palynological analysis

Differently from honey, which is a product regulated at both national and international levels, there are no regulations specifying the definition of "monofloral pollen". Here, we chose to use the expression "prevalence of" when the pollen from a botanical species was present at least at 50% of the total.

Among the 29 pollen blends analyzed in this study, 16 (55.2%) reported a prevalence of a particular botanical species. The determination of the monoflorality could be very important since it could increase the economic value of the product. Indeed, while the floral origin does not seem to influence the nutritional value, it seems to be correlated to the abundance of

bioactive compounds, such as polyphenols and flavonoids (Domínguez-Valhondo, Bohoyo Gil, Hernández, & González-Gómez, 2011; Zhang, Wang, Wang, & Li, 2015). If one the one hand a monofloral pollen could be characterized by the presence of a peculiar molecule, a multifloral pollen could present a wider variety of bioactive compounds. For example, several For examplestudies were performed on *Cistus* bee pollen revealing its, it has been observed that the *Cistus* pollen has stimulatingnt effects on the bone system of in an animal modelthe rat, anti-inflammatory, hypoallergenic, and antioxidant effects, *in vitro* and *in vivo* (Hamamoto, Ishiyama, & Yamaguchi, 2006.; Ishikawa et al., 2008; Maruyama, Sakamoto, Araki, & Hara, 2010; Nagai, Inoue, Inoue, & Suzuki, 2002; Nagai, Inoue, Suzuki, Myoda, & Nagashima, 2005; Yamaguchi, Hamamoto, Uchiyama, Ishiyama, & Hashimoto, 2006; Yamaguchi, Hamamoto, Uchiyama, Ishiyama, & Hashimoto, 2007). Thus, the identification of the botanical origin seems to be fundamental especially when specific therapeutic properties are associated to a botanical species, bearing in mind that factors other than the sole floral species (geographical origin, chemical composition of the soil, and technological treatments applied) could determine wide variations in bee pollen chemical composition. (such as geographical origin, chemical composition of the soil, and treatment applied to obtain the final product).Furthermore, palynological analysis allows investigating the flora of a geographical area, the possible changes in the biodiversity of botanical species and the presence of new invasive, exotic species.

4.3 pH and Aw and pH determinations

pH and Aw are among the main factors influencing the microbial growth in the food matrix;, thus, they should be monitored in order toto understand the potential health risks for the consumer. In accordance with results by other authors (Feás, Vázquez-Tato, Estevinho, Seijas, & Iglesias, 2012; Nogueira et al., 2012; Estevinho et al., 2012; Coronel, Grasso, Pereira, &

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Fernández, 2004; Marchini, Reis, & Moreti, 2006), ~~detected for~~ pH ~~, detected~~ values showed a wide range of variation (from 4.5 to 6.1), with average values higher than 5 for both dried and fresh samples~~5~~4.5. Since this condition allows the growth of most microorganisms, reducing bee pollen moisture and Aw content seems to be of great importance. Indeed, ~~,H~~ high Aw values combined with neutral or slightly acidic pH, ~~could represent a~~support ~~risk to the health of consumers, as they promote microbial~~microorganisms proliferation, including ~~especially regarding yeasts and~~ toxigenic molds ~~(Gonzalez et al., 2005)~~.

Bacteria, especially Gram-negative, generally need Aw values higher than molds. Most of the spoilage bacteria do not develop at values lower than 0.91, while some spoilage molds are also able to proliferate at values of 0.80, with some xerophile molds ~~capable of~~ growing at values ~~equal to~~ of 0.65 (Jay, Loessner, & Golden, 2008). ~~Thus, data obtained in the present work suggest that bee pollen does not represent a substrate able to promote microbial development.~~

Focusing on bee pollen, different Aw values were reported, with variation due to the treatment undergone by the specific ~~Sagona et al. (2017) analyzing~~ samples. For example, considering fresh frozen bee pollen samples from ~~of~~ *Castanea* and *Eucalypstus*~~fresh frozen pollen, from *Castanea* and *Eucalypstus*~~ Sagona et al. (2017) ~~showed~~ reported Aw values of 0.52 and 0.59, respectively, while analyzing 22 dried bee pollen samples, ~~,~~ Feás et al. ~~, Vázquez-Tato, Estevinho, Seijas, & Iglesias~~ (2012) reported lower values (from 0.21 to 0.37) ~~analyzing 22 bee pollen samples, detected Aw values ranging from 0.21 to 0.37, due to the drying treatment undergone by the samples~~. Carpes et al. ~~, Mourão De Alencar, & Masson~~ (2009) and Serra Bonvehi & Escolà Jordà (1997) obtained similar values. Nogueira et al. ~~, Iglesias, Feás, & Estevinho~~ (2012), as well as Estevinho et al. ~~, Rodrigues, Pereira, & Feás~~ (2012), reported Aw values from 0.26 to 0.43, and from 0.32 to 0.55, respectively. Our results are generally consistent with those ~~previously~~ obtained by other authors. ~~and~~ The significant positive correlation between Aw and some of the targeted microorganisms (TBC, *Enterobacteriaceae*,

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and lactic acid bacteria) highlights the importance of the maintenance of adequate storage conditions, especially when considering fresh samples. High bacterial counts were also sporadically detected in dried samples, this would imply a massive contamination of the bee pollen before the drying treatment or ~~uncorrect~~incorrect production ~~-~~practices.

~~As for pH, observed values are in accordance with most of those reported in the studies by other authors: Feás, Vázquez-Tato, Estevinho, Seijas, & Iglesias (2012) found pH values ranging from 4.3 to 5.2; Nogueira, Iglesias, Feás, & Estevinho (2012) from 4.23 to 5.15, in compliance with the parameters established by Brazilian legislation (4-6.0) (Brazilian Legislation, 2001). Estevinho, Rodrigues, Pereira, & Feás (2012) detected values from 4.33 to 6.33, in accordance with Coronel, Grasso, Pereira, & Fernández (2004) and Marchini, Reis, & Moreti (2006).~~

4.4 Microbiological ~~determinations~~analysis

The evaluation of total bacterial count in bee pollen samples represents a good index of the microbiological quality of the product (De-Melo et al., 2016): the concentration of these microorganisms is strictly related to bee pollen harvesting, processing and storage hygienic conditions. A high concentration of total mesophilic bacteria could be suggestive of high contamination of raw material, improperly cleaned and sanitized working surfaces, inappropriate time/temperature conditions for production and storage or a combination of these factors (De-Melo et al., 2016; Estevinho et al., ~~, Rodrigues, Pereira, & Feás,~~ 2012; Feás et al., ~~, Vázquez-Tato, Estevinho, Seijas, & Iglesias,~~ 2012; Nogueira et al., ~~, Iglesias, Feás, & Estevinho,~~ 2012). An inappropriate management of all these phases would eventually affect bee pollen organoleptic characteristics and safety (presence of bacterial pathogens, production of biogenic amines etc.).

Different authors reported concentration of total mesophilic bacteria in dried bee pollen ranging from very low values (<10 CFU/g) to 10^3 CFU/g (Estevinho et al., ~~, Rodrigues, Pereira, & Feás,~~

2012; Feás et al., ~~Vázquez-Tato, Estevinho, Seijas, & Iglesias~~, 2012; Nogueira et al., ~~Iglesias, Feás, & Estevinho~~, 2012). More recently, de Arruda et al. (2017) detected the presence of mesophilic bacteria in 56% of 62 analyzed dried bee pollen samples, with values ranging from <10 to 1,260 CFU/g. The presence of bacteria in dried bee pollen samples could be due to the temperature employed in the dehydration process, which is not able to completely deactivate them; however, contaminations could also occur in the post-treatment phase. As concerns our results, none of the analyzed samples presented a concentration of mesophilic bacteria lower than the detection limit (<1 log CFU/g), with values sometimes higher than 5 log CFU/g. This would suggest a not always optimal management of bee pollen harvesting, processing, and storage phases.~~.~~

For several food categories, *Enterobacteriaceae* family is employed as an index of process hygiene. The detection of bacteria belonging to this family could be indicative of the occurrence of anomalies during the transformation phase or inappropriate handling/storage conditions after a thermal treatment, especially for fresh bee pollen samples. In our specific case, all the dried bee pollen samples, except one (sample 22), presented *Enterobacteriaceae* concentrations lower than the detection limit (<2 log CFU/g). This is in accordance with recent results by Fernández, Rodríguez, Sánchez, Pérez, & Gallez (2020a), who were not able to detect *Enterobacteriaceae* in 48 dried bee pollen samples from Argentina stored at room and cold temperatures for two years. Other authors who chose instead to evaluate the ~~amount~~number of coliforms (total or fecal) as fecal contamination index, reported heterogeneous results. For example, analyzing 45 samples, De- Melo, Estevinho, & Almeida- Muradian (2015) detected different concentrations of total coliforms, ranging from very low values (<10 CFU/g) to higher concentrations (2,800 CFU/g) (De- Melo et al., ~~Estevinho, & Almeida- Muradian~~, 2015); while Feás et al. , ~~Vázquez-Tato, Estevinho, Seijas, & Iglesias~~ (2012) targeting fecal coliform by MPN method highlighted low concentrations in all 22 analyzed samples. As for fresh bee

pollen samples, we detected a higher content of *Enterobacteriaceae*, which was an expected result. However, it is important to highlight that *E. coli* and *Salmonella* spp were absent in all the samples ~~resulted negative for the presence of *E. coli* and *Salmonella* spp~~. This would allow excluding a fecal contamination of bee pollen samples and the presence of other Gram-negative pathogenic bacteria. Thus, the high *Enterobacteriaceae* content in some of the analyzed fresh bee pollen samples seems to be due to the presence of environmental microorganisms rather than intestinal. As for pathogenic Gram-negative bacteria, most of the available studies confirmed that their occurrence in bee pollen is rare (De- Melo et al.,~~, Estevinho, & Almeida- Muradian,~~ 2015; Estevinho et al., ~~, Rodrigues, Pereira, & Feás,~~ 2012; Feás et al.,~~ Vázquez-Tato, Estevinho, Seijas, & Iglesias~~, 2012; Nogueira et al.,~~ Iglesias, Feás, & Estevinho~~, 2012). Indeed, currently only Belhadj, Harzallah, Dahamna, & Khennouf, (2014) reported the presence of *S. enterica* in some samples from Algeria and Egypt, while de Arruda et al. (2017) highlighted the presence of *E. coli* in 11.3% of the analysed samples even if in low amount (max 53 CFU/g). Considering Gram-positive bacteria, consistently with other authors, the concentration of ~~we did not detect~~ coagulase positive and negative staphylococci was always under the detection limit (De- Melo et al.,~~, Estevinho, & Almeida- Muradian,~~ 2015; Estevinho et al., ~~, Rodrigues, Pereira, & Feás,~~ 2012; Fernández et al., ~~, Rodríguez, Sánchez, Pérez, & Gallez,~~ 2020a).

As for *L. monocytogenes* and *B. cereus*, few studies considered their presence in bee pollen samples. Among these, the research by Mauriello et al. ~~, De Prisco, Di Prisco, La Storia, & Caprio~~ (2017) presented an extensive microbiological characterization of bee pollen samples harvested from Vesuvian area (Italy), including the research for pathogenic microorganisms. Consistently with our results, the authors were not able to detect *L. monocytogenes* among the investigated samples. On the contrary, Belhadj et al. ~~, Harzallah, Dahamna, & Khennouf~~ (2014) detected *L. monocytogenes* in 10 analyzed samples, while other authors (de Arruda et al., 2017; De- Melo et al.,~~, Estevinho, & Almeida- Muradian,~~ 2015; Estevinho et al.,~~ Rodrigues, Pereira,~~

& Feás, 2012; Feás et al., ; Vázquez-Tato, Estevinho, Seijas, & Iglesias, 2012; Nogueira et al., Iglesias, Feás, & Estevinho, 2012) did not include the research of *L. monocytogenes* in their analysis. Considering *B. cereus*, Mauriello et al. ; De Prisco, Di Prisco, La Storia, & Caprio (2017) reported its occurrence in fresh harvested bee pollen and observed how the storage for several months at -18°C was not always able to decrease this microbial content under the detection limit, while the drying treatment was effective in completely inhibiting *B. cereus* growth.

As for clostridial spores, despite the wide distribution of these microorganisms in the honey bee production environment, previously reported data would suggest a minor role of honey bees as reservoirs and spreaders (Wojtacka et al., 2020). In particular, as for the presence of sulphite-reducing clostridia spores of sulphite-reducing clostridia, our results confirms this trend, being are in accordance with most authors reporting their absence in all analyzed samples (Coronel et al., ; Grasso, Pereira, & Fernández, 2004; de Arruda et al., 2017; De-Melo et al., 2016; Estevinho et al.,; Rodrigues, Pereira, & Feás, 2012; Feás et al., ; Vázquez-Tato, Estevinho, Seijas, & Iglesias, 2012; Nogueira et al.,; Iglesias, Feás, & Estevinho, 2012).

Lactic acid bacteria were observed as the predominant contaminants of bee pollen grains by culture dependent and independent approaches, with *Lactobacillus kunkeii* as the main identified species (Anderson et al., 2014; Corby-Harris & Anderson, 2014; Endo & Salminen, 2013). However, none of the available research on the microbial content of bee pollen samples purchased on the retail market considered their presence. Our results are in accordance with those by Mauriello et al. ; De Prisco, Di Prisco, La Storia, & Caprio (2017) confirming that lactic acid bacteria represent most of the bacterial population of bee pollen. These microorganisms importantly contribute to bee bread production and storage in the hive (Vásquez & Olofsson, 2009) and could be considered a characterizing/functional bee pollen

microbial population, also preventing the proliferation of other microorganisms by producing antibacterial compounds.

Finally, as concerns yeasts and molds, most of the authors observed concentrations higher than 10^4 CFU/g (Belhadj et al., ~~, Harzallah, Dahamna, & Khennouf,~~ 2014; De- Melo et al.,~~, Estevinho, & Almeida- Muradian,~~ 2015; Estevinho et al.,~~ Rodrigues, Pereira, & Feás~~, 2012; Fernández et al., ~~, Rodríguez, Sánchez, Pérez, & Gallez,~~ 2020a). Not only yeasts and molds are responsible for changes in the organoleptic characteristics of the product, but molds could also represent a safety problem since they can produce allergenic substances and mycotoxins. From a biological point of view, high mold loads could be correlated with inadequate conditions of the working environments and, thus, represent an indication of efficient or inadequate apiaries management (Nogueira et al., ~~, Iglesias, Feás, & Estevinho,~~ 2012).

4.5 Mycotoxins determination

Results of the present study indicate that samples of bee pollen were contaminated with mycotoxins. Comparing the AFL and DON concentrations of the present study with data obtained for bee pollen in Serbia and Slovakia, the quantity of these mycotoxins is in the similar level (Kačániová et al., 2011). The drying and freezing do not protect bee pollen against mycotoxins contamination, and it could be necessary to set up mycotoxin limits for bee pollen employed as food or feed. No mandatory limits for mycotoxins in honey and honey bee products are provided by the EU legislation. Considering that limits must be set based on different factors (food matrix characteristics, probability of contamination by potential mycotoxins producing fungi and of their multiplication, doses presumably consumed), we could refer to the maximum accepted concentrations of total aflatoxins established by EU Regulation 1881/2006, which must not exceed 15 µg/kg in food or components for food production. Our results demonstrated that values more than twice as high could be present in pollen. As for DON, the same regulation

set the most rigorous maximum permissible concentration at 200 µg/kg (processed cereal-based foods and baby foods for infants and young children); thus, our values (maximum 179.7 µg/kg) seem to be reassuring. Anyway, concerns exist due to cross contamination of pollen samples as confirmed by the present study. ~~Previously, s~~ Several studies (Smith, Madec, Coton, & Hymery, 2016) confirmed that some combined mycotoxins have a more distinct harmful effect on human health. It is therefore of the highest importance to evaluate the toxicological impact of mycotoxins combinations on animal and human health risk. We did not detect any positive correlation between mycotoxins and molds concentrations. This could be explained by the fact that mycotoxins are stable compounds, able to persist in the food matrix after several technological treatments and for a long time (Bullerman & Bianchini, 2007). On the contrary, the mycotoxigenic molds could have been no longer viable in the analyzed samples. Moreover, we only performed an enumeration and not an identification of the molds, thus we cannot affirm they were actually those responsible for mycotoxins production.

5. Conclusions

This work represents a preliminary survey on some physicochemical parameters (Aw and pH), palynological, microbiological and mycotoxicological profile of commercial bee pollen purchased in Tuscany (Italy). At the same time, the compliance of labels to the current legislation was also verified. Our results suggest that attention must be paid at the microbiological quality of this product; especially when considering fresh bee pollen. Indeed, some environmental microorganisms, such as lactic acid bacteria, *Enterobacteriaceae* and yeast and molds could be present in relevant concentrations, possibly leading to the alteration or to accumulation of toxic metabolites, such as mycotoxins or biogenic amines. On the other hand, all samples were negative for the main food pathogens. ~~, which confirms the observation by other authors.~~

Conflict of interest statement

Authors have no competing interests to declare.

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Table 4 Correlation matrix of some studied parameters

Parameters		Aw	pH	Entero	TBC	YM	LAB	AFL	DON
Aw	R	1	**-0.39**	**0.51**	**0.46**	0.16	**0.55**	-0.26	-0.21
	p	-	**<0.05**	**<0.01**	**<0.05**	>0.05	**<0.01**	>0.05	>0.05
pH	R	**-0.39**	1	0.12	0.06	0.06	-0.03	-0.20	0.07
	p	**<0.05**	-	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Entero	R	**0.51**	0.12	1	**0.71**	0.29	**0.74**	**-0.46**	0.05
	p	**<0.01**	>0.05	-	**<0.001**	>0.05	**<0.001**	**<0.01**	>0.05
TBC	R	**0.46**	0.06	**0.71**	1	**0.41**	**0.77**	-0.35	0.33
	p	**<0.05**	>0.05	**<0.001**	-	**<0.05**	**<0.001**	>0.05	>0.05
YM	R	0.16	0.06	0.29	**0.41**	1	0.28	-0.06	0.02
	p	>0.05	>0.05	>0.05	**<0.05**	-	>0.05	>0.05	>0.05
LAB	R	**0.55**	-0.03	**0.74**	**0.77**	0.28	1	**0.55**	0.34
	p	**<0.01**	>0.05	**<0.001**	**<0.001**	>0.05	-	**<0.01**	>0.05
AFL	R	-0.26	-0.20	**-0.46**	-0.35	-0.06	**0.55**	1	-0.25
	p	>0.05	>0.05	**<0.01**	>0.05	>0.05	**<0.01**	-	>0.05
DON	R	-0.21	0.07	0.05	0.33	0.02	0.34	-0.25	1
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	-

Results showing a statistically significant positive or negative correlation are indicated in bold.
TBC: total bacterial count; Entero: *Enterobacteriaceae*; LAB: lactic acid bacteria; YM: yeast and molds, AFL: total aflatoxins, DON: deoxynivalenol.

Table 1 Results of labelling analysis on ~~29~~ 24 bee pollen samples

Indication	Presence on the label
Trade name	95.8~~6~~%
Name of the food business operator	100.0%
Address of the food business operator	95.8~~6~~%
Date of minimum durability	95.8~~6~~%
Lot	91.7~~2~~%
Net quantity (g or kg)	95.8~~6~~%
Geographical origin	87.5~~8~~%
Botanical origin	37.5~~36~~%
Storage conditions*	88.8~~90~~%
Nutritional information**	12.5%
Consumer advertising**	25.0~~8~~%

* required only for frozen bee pollen

** not required on the label

Table 2

Table 2 Botanical origin of ~~P~~pollen grains (%) ~~percentage of~~ in the 29 analysed bee pollens samples ~~retailed in Tuscany (Italy)~~. Percentage of a pollen grain lower than 0.1%, was indicated as "presence"~~For percentages lower than 0.1%, only the presence was reported~~.

Sample ~~number~~ID	Label indication on the botanical origin	~~P~~pollen grain percentages
1	"Flower pollen"	*Castanea* 83.9%; *Rubus* f. 7.9%; *Olea* f. 5.9%; Compositae form H 1.3%; *Acer* f. 1%; *Parthenocissus* 0.2%; *Echium* 0.1%; presence of Compositae form A; *Gleditsia*; *Salix*; *Laurus*; honeydew indicators.
2	~~Not reported~~~~None~~None	*Castanea* 30.8%; *Hedera* 27.5%; *Rubus* f. 23.6%; *Laurus* 14.9%; Compositae form H 2.3%; *Sedum* 0.5%; *Papaver* 0.4%; *Trifolium repens* gr. 0.2%; presence of *Convolvulus*; Compositae form S; honeydew indicators.
3	~~Not reported~~None	*Quercus ilex* gr. 75.1%; *Sedum* 4.9%; Compositae form T 4.6%; *Potentilla* f. 4.6%; *Echium* 4.1%; *Campanula* f. 3.2%; *Papaver* 2.2%; *Cistus* f.1.1%; *Convolvulus* 0.1%; presence of *Salix*; Cruciferae; *Erica* f.; Compositae form S; *Pinus* f.; honeydew indicators.
4	"Multifloral pollen"	*Castanea* 22.7%; *Trifolium pratense* gr. 20.7%; *Hedysarum* 18.3%; *Prunus* f. 16.2%; *Vicia* 7.4%; *Onobrychis* 7.4%; Compositae form H 6.9%; Umbelliferae 0.4%; presence of Compositae form S; *Rubus* f.; honeydew indicators.
5	~~Not reported~~None	*Cistus ladanifer* 44.2%; *Prunus* f. 20.8%; *Echium* 20.6%; Compositae form T 6%; *Melilotus* 4.5%; *Campanula* f. 3.5%; Compositae form S 0.5%; presence of Labiatae form M; *Olea* f.; Compositae form A; *Sinapis* f.; *Tilia*.
6	~~Not reported~~None	*Rubus* f. 34.7%; *Olea* f. 29.1%; *Quercus ilex* gr. 28.7%; *Castanea* 2.8%; *Trifolium repens* gr. 1.5%; *Sedum* 1.5%; *Prunus* f. 1.2%; *Cistus* 0.3%; Compositae form H 0.2%; presence of *Castanea*; *Olea* f.; *Hedera*; Compositae form T; *Quercus robur* gr.; *Rosa* f.; honeydew indicators.
7	~~Not reported~~None	*Rubus* f. 35.1%; *Trifolium repens* gr. 20.8%; *Sinapis* f. 11.3%; *Castanea* 8.9%; *Hedysarum* 8.1%; *Trifolium pretense* gr 8.1%; *Cistus* f. 5.9%; *Papaver* 1.8%; presence of *Rubus* f.
8	~~Not reported~~None	*Castanea* 65%; *Sedum* 10.2%; *Sinapis* f. 9.9%; Compositae form T 4.1%; Umbelliferae 3.8%; *Erica* f. 2.7%; *Trifolium pratense* gr. 2.3%; Compositae form H 1%; *Eucalyptus* f. 0.8%; presence of *Olea* f.; *Vicia* f.; *Rubus* f.; *Erica* f.; *Cistus ladanifer*;

		Magnoliacea; *Hedera*; *Prunus* f.; Compositae form S; Cruciferae.
9	~~Not reported~~None	*Prunus* f. 31.1%; *Cistus ladanifer* 30.4%; *Echium* 16.1%; Compositae form A 7%; *Trifolium pretense* gr. 6.6%; *Brassica* f. 3.7%; Compositae fom T 1.8%; *Campanula* f. 1.8%; Compositae form S 0.9%; *Potentilla* f. 0.6%; presence of Ranuncolaceae; *Olea* f.; Labiatae; Graminaceae; *Tilia*.
10	~~Not reported~~None	*Cistus ladanifer* 66%; *Rubus* f. 19.8%; Compositae form T 5.6%; *Campanula* f. 4.8%; *Echium* 1.4%; Compositae form S 1.2%; *Eucalyptus* f. 1.1%; *Prunus* f. 0.3%; presence of Compositae form H; *Acer* f.; Compositae form A; *Quercus ilex* gr.; *Vicia* f.; *Olea* f.; *Potentilla* f.; Compositae form H.
11	"Chestnut pollen"	*Castanea* 92.2%; *Rubus* f. 5.4%; *Citrus* 1.6%; *Trifolium repens* gr. 0.4%; *Trifolium pretense* gr. 0.4%; presence of Compositae form H; *Eucalyptus* f.; *Lonicera caprifolium*; *Parthenocissus*; *Prunus* f.; *Olea* f.; *Potentilla* f.; *Rosa* f.; Cruciferae; *Cistus* f.; *Sedum*; honeydew indicators.
12	"Multifloral pollen"	*Cistus ladanifer* 74.6%; *Calluna* 13%; *Echium* 4.7%; *Sedum* 3.6%; Compositae form S 1.4%; *Vitis* 1.3%; Liliaceae 0.9%; *Olea* f. 0.4%; *Trifolium repens* gr. 0.1%; presence of *Castanea*; *Plantago* f.; Graminaceae; Compositae form H; Compositae form T; *Hedera*; Umbelliferae; Palmae; honeydew indicators.
13	~~Not reported~~None	*Castanea* 90.7%; *Rubus* f. 8.1%; *Ephedra* f. 1.1%; presence of *Olea* f.; Umbelliferae; *Rosa* f.; *Cistus* f.; *Parthenocissus*; *Sedum*; honeydew indicators.
14	~~Not reported~~None	*Fraxinus/Olea* f. 65.2%; *Prunus* f. 25.4%; Labiatae form L 4.7%; *Plantago* f. 3.9%; *Papaver* 0.5%; *Laurus* 0.3%; presence of *Erica* f.; Compositae form T; *Allium*; *Parthenocissus*; *Brassica* f.; Campanulacea; *Vicia* f.; Rosacea; *Potentilla* f.
15	~~Not reported~~None	*Hedera* 85.1%; Compositae form H 8.4%; *Laurus* 6.5%; presence of *Papaver*.
16	~~Not reported~~None	*Hedera* 55.6%; *Castanea* 39.3%; *Rubus* f. 5.1%; presence of Compositae form H.
17	"Flower pollen"	*Trifolium repens* gr. 33.6%; *Trifolium pratense* gr. 23.1%; *Cistus ladanifer* 27.7%; Cruciferae 8.8%; *Echium* 5.8%; *Vitis* 1%; presence of Compositae form T; *Hedera*; Umbelliferae; Rosacea; *Castanea*; honeydew indicators.
18	~~Not reported~~None	*Castanea* 20.7%; *Rubus* f. 20.6%; *Tilia* 18.4%; *Fraxinus/Olea* f. 15.4%; *Prunus* f. 9.7%; *Loranthus* 8.7%; *Trifolium pratense* gr. 3.6%; *Calluna* 1.3%; Compositae form T 0.8%; Papaver 0.7%; presence of *Plantago* f.; Cruciferae; *Hedera*; *Salix*; *Gleditsia*; Corylaceae; *Quercus robur* gr.; *Pinus*; *Acer* f.; *Rosa*

		f.; *Onobrychis*; Compositae form H; *Potentilla* f.; *Tilia*; *Rubus* f.; *Castanea*; *Borago.*
19	"Chestnut pollen"	*Castanea* 76.4%; *Rubus* f. 20.8%; *Allium* 1.4%; *Potentilla* f. 0.7%; *Papaver* 0.7%; *Cistus* f. 0.1%; presence of *Trifolium repens* gr.
20	~~Not reported~~None	*Fraxinus/Olea* f. 46.5%; *Vicia* f. 12.5%; Compositae form T 11.3%; *Hedera* 8%; *Rubus* f. 5%; *Erica* f. 4.9%; *Melilotus* 4.5%; *Prunus* f. 4%; *Brassica* f. 1.4%; *Hedysarum* 1%; *Papaver* 0.8%; Caryophyllaceae 0.1%; presence of Compositae form H; *Castanea*; *Plantago* f.; Campanulaceae; *Borago*; *Allium*; *Pinus*; Umbelliferae; Compositae form S.; *Trifolium incarnatum* gr.
21	"~~*Trifolium alexandrinum*~~Alexandrine clover and ~~-~~ ash tree pollen mix"	*Castanea* 91.4%; *Rubus* f. 5.5%; *Myrtus/Syzygium* f. 2.1%; *Cistus* f. 0.5%; *Acer* f. 0.2%; Umbelliferae 0.2%.
22	"Coriander pollen"	*Trifolium pratense* gr. 80.8%; *Coriandrum sativum* 11%; *Sinapis* f. 4.6%; *Allium* 1%; *Hedysarum* 0.9%; *Sedum* 0.6%; *Rubus* f. 0.6% *Papaver* 0.4%; Compositae form H 0.2%; presence of *Eucalyptus* f.; *Hedera*; Rosacea; *Asphodelus*; *Pinus*.
23	~~Not reported~~None	*Castanea* 84.1%; *Rubus* f. 15.9%; presence of *Convolvulus*.
24	~~Not reported~~None	*Castanea* 26.8%; *Sinapis* f. 22.9%; *Allium* 15.2%; *Rubus* f. 14.2%; *Hedera* 6.2%; *Prunus* f. 5.4%; *Onobrychis* 2.8%; *Asparagus* f. 2.7%; Compositae form H 2.1%; *Cistus* f. 1.4%; Umbelliferae 0.3%; presence of Compositae form S; *Borago*; *Pinus*; *Medicago sativa*; *Fraxinus* f.; *Reseda* f.; *Citrus* f.; Compositae form S; *Knautia arvensis*; *Trifolium repens* gr.; *Brassica* f.; *Olea* f.; *Hedysarum*; Compositae form T; *Cornus sanguinea*; *Eucalyptus* f.; *Laurus*; *Trifolium pratense* gr.; *Erica* f.; *Lonicera* f.; *Quercus robur* gr.; *Acer* f.
25	~~Not reported~~None	*Prunus* f. 28.8%; *Castanea* 24.5%; *Olea* f. 24%; *Rubus* 20.2%; *Trifolium pratense* gr. 0.7%; Compositae form T 0.6%; Umbelliferae 0.5%; *Amorpha* f. 0.4%; *Erica* f. 0.3%; presence of Labiatae form M; *Sinapis* f.; Compositae form H; Corylaceae; *Sambucus* f.; *Fagopyrum*; *Acacia*; *Convolvolus*; *Rumex;* *Eucalyptus* f.; *Quercus robur* gr.; *Vitex*; *Allium*; *Vicia* f.; *Cistus* f.; Cruciferae; *Hedera*; *Onobrychis*; *Polygonum* f.; Graminacea.
26	~~Not reported~~None	*Quercus ilex* gr. 45%; *Olea* f. 12.9%; *Cistus ladanifer* 12.5%; *Echium* 6.6%; *Papaver* 3.8%; *Parthenocissus* 3.8%; *Trifolium repens* gr. 2.6%; Liliaceae 2.4%; *Prunus* F. 2.9%; *Quercus robur* gr. 1.9%; *Rumex* 1.9%; Campanulaceae 1.2%; *Rubus* f. 1.2%; *Potentilla* f. 1%; *Calluna* 0.3%; presence of Compositae form T; Compositae form S;

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		Umbelliferae; *Brassica* f.; *Calystegia* f.; *Borago*; *Eucalyptus* f.; *Helianthus* f.; Labiatae form L.; *Acer* f.
27	~~Not reported~~None	*Olea* f. 37.4%; *Lotus* f. 14.5%; *Trifolium incarnatum* gr. 12%; *Rubus* f. 10.6%; *Sedum* 10.6%; *Hedysarum* 8.5%; *Trifolium pratense* gr. 3.6%; *Papaver* 1.8%; *Sinapis* f. 1%; presence of *Trifolium repens* gr.; *Sambucus* f.; Liliaceae; *Prunus* f.
28	~~Not reported~~None	*Rubus* f. 86.2%; *Papaver* 11.4%; Compositae form H 2.1%; *Sambucus* f. 0.3%: presence of *Laurus*; *Tilia*; *sedum*.
29	"Ivy pollen"	*Hedera* 65.6%; Compositae form H 28.8%; *Mercurialis* 4%; *Potentilla* f. 0.5%; *Sinapis* f. 0.5%; *Brassica* f. 0.5%; *Laurus* 0.2%; presence of *Lonicera* f.

f.=form; gr.=group

Table

Table 3 Results obtained for pH and Aw determinations, microbial counts, and mycotoxins determinations. Samples were grouped by treatment (dried and fresh samples).

	Dried bee pollen			Fresh bee pollen			
	mean	**max**	**min**	**mean**	**max**	**min**	**P value**
pH	5.2±0.4	6.0	4.5	5.1±0.5	6.1	4.5	0.7976
~~a~~Aw	0.33±0.07	0.47	0.19	0.67±0.07	0.77	0.58	<0.0001
Total bacterial count log (CFU/g)	3.1±0.8	5.1	2	4.6±1.0	6.0	2.7	0.0064
***Enterobacteriaceae* log (CFU/g)**	1.8±0.3	3.0	<2.0	2.8±1.1	4.1	<2.0	0.4274
***Escherichia coli* log (CFU/g)**	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	1
***Salmonella* spp. (presence/absence in 10 g)**	absent	absent	absent	absent	absent	absent	1
Coagulase positive and negative staphylococci log (CFU/g)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	1
***Listeria monocytogenes* (presence/absence in 10 g)**	absent	absent	absent	absent	absent	absent	1
Sulphite-reducing clostridia log (CFU/g)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	1
***Bacillus cereus* log (CFU/g)**	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	1
Lactic acid bacteria log (CFU/g)	2.2±0.9	4.6	<2.0	5.0±1.3	6.0	<2.0	<0.0001
Yeasts and molds log (CFU/g)	2.4±0.8	3.7	<1.0	2.7±1.0	4.0	<1.0	0.1807
Aflatoxins (ppb)	25.6±7.0	34.7	5.2	21.2±5.5	34.4	14.0	0.084
DON (ppp)	28.6±39.3	172.9	<LOD	45.1±64.6	179.7	<LOD	0.399

LOD: limit of detection

Table

Table S1 Twenty-nine bee pollen samples analyzed in this study. For Total bacterial count, *Enterobacteriaceae, E. coli*, coagulase-positive staphylococci, sulphite-reducing clostridia, *Bacillus cereus*, lactic acid bacteria, and yeasts and molds results are expressed as log CFU/g; while for *L. monocytogenes* and *Salmonella* spp. the absence/presence in 10 g was determined. As for mycotoxins, total aflatoxins and deoxynivalenol results are expressed as µg/kg.

		pH	aw	TBC	Entero	*E. coli*	*Salmonella* spp.	CPS	*L. monocytogenes*	SRC	*B. cereus*	LAB	YM	AFL	DON
1	Dried	4.98	0.35	4.1	<2	<2	absent	<2	absent	<2	<2	<2	3.7	29.5	15.9
2	Fresh §	4.5	0.68	4.1	<2	<2	absent	<2	absent	<2	<2	3.9	3.7	34.4	<LOD
3	Dried	4.93	0.41	2.4	<2	<2	absent	<2	absent	<2	<2	<2	1.8	29.5	<LOD
4	Fresh *	4.86	0.66	5.3	3.3	<2	absent	<2	absent	<2	<2	5.9	2.7	20.3	51.0
5	Dried	4.6	0.47	3.5	<2	<2	absent	<2	absent	<2	<2	<2	1.5	33.0	4.5
6	Dried	5.98	0.19	3.7	<2	<2	absent	<2	absent	<2	<2	<2	2.0	33.0	29.5
7	Fresh *ff	6.1	0.62	5.0	3.9	<2	absent	<2	absent	<2	<2	5.7	3.1	14.0	<LOD
8	Dried	5.44	0.25	2.6	<2	<2	absent	<2	absent	<2	<2	2.0	3.0	5.2	40.6
9	Dried	5.04	0.36	3.2	<2	<2	absent	<2	absent	<2	<2	<2	2.6	28.5	11.7
10	Dried	5.4	0.25	3.1	<2	<2	absent	<2	absent	<2	<2	<2	2.2	23.3	23.0
11	Dried	5.57	0.28	3.2	<2	<2	absent	<2	absent	<2	<2	<2	3.6	26.2	18.8
12	Dried	5.29	0.31	2.6	<2	<2	absent	<2	absent	<2	<2	<2	2.5	29.9	4.2
13	Dried	5.6	0.35	3.3	<2	<2	absent	<2	absent	<2	<2	<2	2.0	23.9	<LOD
14	Dried	5.54	0.33	2.7	<2	<2	absent	<2	absent	<2	<2	<2	3.3	22.2	29.2
15	Fresh *ff	4.8	0.76	2.7	<2	<2	absent	<2	absent	<2	<2	<2	<1	18.4	10.0
16	Dried	5.9	0.32	3.2	<2	<2	absent	<2	absent	<2	<2	3.7	<1	23.7	39.6
17	Dried	4.8	0.36	2.5	<2	<2	absent	<2	absent	<2	<2	<2	<1	30.6	45.1
18	Dried	4.84	0.32	4.7	<2	<2	absent	<2	absent	<2	<2	4.6	3.3	26.2	172.9
19	Fresh *ff	4.67	0.73	5.0	3.8	<2	absent	<2	absent	<2	<2	6.0	3.0	16.1	5.5
20	Fresh *ff	4.74	0.64	4.9	<2	<2	absent	<2	absent	<2	<2	5.6	2.4	22.2	162.8
21	Fresh *ff	5.4	0.6	4.4	3.4	<2	absent	<2	absent	<2	<2	5.8	1.7	23.7	16.9
22	Dried *	4.51	0.36	5.1	3.0	<2	absent	<2	absent	<2	<2	4.1	2.0	15.5	47.1

23	Fresh *~~fr~~	5.75	0.58	4.8	<2	<2	absent	<2	absent	<2	<2	5.3	1.9	17.0	42.9
24	Dried	4.98	0.28	2.7	<2	<2	absent	<2	absent	<2	<2	2.7	1.0	22.0	22.4
25	Fresh *~~fr~~	5.16	0.61	6.0	3.8	<2	absent	<2	absent	<2	<2	5.8	3.4	25.9	179.7
26	Dried	5.14	0.33	2.2	<2	<2	absent	<2	absent	<2	<2	<2	1.9	24.8	10.4
27	Fresh *~~fr~~	5.77	0.77	5.4	4.1	<2	absent	<2	absent	<2	<2	5.6	4.0	20.2	11.0
28	Fresh *~~fr~~	4.65	0.75	3.1	<2	<2	absent	<2	absent	<2	<2	4.3	3.3	20.7	15.9
29	Dried	4.61	0.43	2.0	<2	<2	absent	<2	absent	<2	<2	<2	2.0	34.7	<LOD

§: in modified atmosphere packaging; ~~fresh fr: fresh frozen;~~ *: frozen; TBC: Total bacterial count; Entero: *Enterobacteriaceae*, CPS: coagulase-positive staphylococci; SRC: sulphite-reducing clostridia; LAB: lactic acid bacteria; YM: Yeast and moulds; AFL: total aflatoxins; DON: deoxynivalenol; LOD: limit of detection

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*Conflict of Interest Form

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: