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Effects of drying techniques on chemical composition and volatile constituents of bee pollen

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Abstract: Bee pollen is used as a food supplement by humans as it is rich in carbohydrates, proteins, lipids, vitamins, minerals and trace elements. Bee pollen has many biological activities such as antibacterial, antifungal, antitumor and antioxidant. Fresh bee pollen is not suitable for long-term storage because of its moisture content. In order to protect the nutrient content and freshness, the bee pollen can be dried by using different drying techniques. In this study, the biochemical characterization of the bee pollen samples dried by different techniques and drying effects on the biochemical properties of bee pollen were determined. Moisture, total lipid and protein, pH and total phenolic content of pollen samples were determined. The results ranged 6.23–20.62%, 4.98–5.57%, 16.812–1.477%, 4.08–4.33 and 15.2–22.73 mg GAE g^{-1}, respectively. All samples are rich in squalene and methyl octadecanoate. It is clear that bee pollen bioactive components will be less damaged by using drying methods performed under more moderate conditions like lyophilization than when the traditional method is used.

Keywords: phenolics; lyophilization; oven-dried; palynological analysis; volatile compounds

Bee pollen is a bee product collected by honeybees (Mărgăoan et al. 2010). It is a protein source that is needed for honeybee lives and feeding of their offspring. In addition, it has biological activities such as antibacterial, antifungal, antitumor, antioxidant, immunomodulatory, antiaging, antiosteoporotic and antianaemic ones (Özkök & Sorkun 2006; Bogdanov 2011; Sorucu 2019). Bee pollen is used as a food supplement by humans as it is rich in carbohydrates, proteins, amino acids, lipids, vitamins, minerals and trace elements (Isidorov et al. 2009).

The colour, shape and chemical content of bee pollen vary depending on plant species, geographical features of the region where it is collected and production techniques (Corvucci et al. 2016). In general, bee pollen contains 35% of carbohydrates, 20% of protein, 20% of water, 5% of lipids, around 20% of other ingredients (Mayda et al. 2020).

Fresh bee pollen loses its nutritional value as a result of long-term storage in unsuitable conditions (Dietz 1984; Özkök & Sorkun 2007). Because of that drying methods are used in order to protect the nutrient content and freshness of bee pollen. Also drying methods reduce the moisture content of bee pollen and thus prevent the microbial growth, facilitate the preservation of the product and extend the shelf life.

Drying in the oven is a common drying method. Lyophilization (freeze drying), which is a newer drying technique, is the process of drying the substances by removing the water by sublimation, that is, directly converting the water from the solid state to gas.

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In this method, the sensory properties and nutritional value of the dried product are more suitable than in traditional methods (Kasper et al. 2013; Gaidhani et al. 2015). It is clear that bee pollen bioactive components will be less damaged by using drying methods performed under more moderate conditions than when traditional methods are used.

In this study, biochemical characterization of bee pollen samples dried by different techniques and drying effects on the biochemical properties of bee pollen were determined. Besides, the botanical origin of bee pollen samples was determined.

MATERIAL AND METHODS

Material. Fresh bee pollen samples were supplied by local bee keepers in the city of Amasya, Turkey, in the 2019 season. N-Methyl-N-(trimethylsiliyl)-trifloroacetamide (MSTFA) was obtained from Cova Chem, LLC (USA). $FeSO_4$ $7H_2O$, methanol, gallic acid, and ethanol were purchased from Sigma-Aldrich (USA). All other reagents were of analytical grade.

Palynological (botanical origin) analysis. Pollen samples were first examined macroscopically and the plant particles, insect residues etc. were removed. Secondly, pollen samples were prepared for microscopic analysis. Mixed pollen samples of 2 g were weighed and 13 mL of ethanol was added, after vortexing until homogenized they were centrifuged at 3 500 rpm for 20 min. After centrifugation, the supernatant was discarded and 13 mL of 50% glycerin-water mixture was added to the remaining pellet, vortexed for about a minute and centrifuged at 3 500 rpm for 20 min. After centrifugation, the supernatant was poured, the tubes were inverted on blotter paper and the pellet was allowed to dry. The pollen at the bottom of the tube was taken with the glycerin-gelatin matrix with the help of a dissecting needle and put on the slide, melted on the hot plate and covered with the coverslip, and then, minimally one hour it was waited for drying of the preparations. Three preparations were prepared for each sample (Mayda et al. 2019).

Drying process of bee pollen samples. Two different drying techniques were used. The one was a classical method, oven dried, and the other was lyophilization. Fresh bee pollen samples were dried in an oven (Nuve EN 400; Nuve, Turkey) at 50 °C to obtain oven-dried bee pollen samples during 8 h. Drying process of fresh bee pollen was carried out by using an Operon freeze dryer at –50 °C under 0.1 mbar pressure during 24 hours.

Preparation of extracts. Bee pollen was ground and about 3 g of ground bee pollen were weighed. An aliquot of 20 mL of ethanol (absolute) was added to the weighed bee pollen and mixed on a constant speed shaker for about 12 hours. Then the resulting mixture was filtered and the final volume was completed to 30 mL.

Moisture content. Moisture content analysis was performed according to European Commission Regulation EC 152.2009 (Mayda et al. 2020).

pH. After dissolving 10 g of bee pollen in 90 mL of pure water, pH was measured using a pre-calibrated pH meter (Yetim & Kesmen, 2008).

Total phenolic content. Total phenolic content of ethanol bee pollen extracts (BPE) was determined by using the Folin-Ciocalteu method (Singleton & Rossi 1965; Singleton et al. 1999). Gallic acid was used as standard. Results were expressed as mg GAE mL^{-1}.

Determination of total protein. Total protein content analysis was performed according to AOAC Official Method 990.03 (Mayda et al. 2020).

Determination of lipid content. The lipid content was determined by using the AOAC method 920.39 (Mayda et al. 2020).

Determination of chemical composition by GC-MS. Main chemical composition of bee pollen extracts (BPE) was determined by gas chromatography coupled with mass spectrometry (GC-MS). Samples were analyzed after derivatization with bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) to clarify the chemical composition. Shortly, bee pollen extracts were dried using a rotary evaporator and 5 mg of dried residue was mixed with 50 µL of dry pyridine and 75 µL of BSTFA. This reaction mixture was heated at 80 °C for 20 min. GC-MS analysis was applied with an Agilent 7890A GC system (Agilent, USA) equipped with HP5-MS capillary column (30 m × 0.25 mm × 0.5 mm). The oven temperature was programmed from 75 to 325 °C at a rate of 5 °C min^{-1}, and a 15-min hold at 325°C. Helium was used as a carrier gas at a flow rate of 0.8 mL min^{-1}. The split ratio was 1 : 50, the injector temperature 300 °C, and the ionization voltage 70 eV (Bankova et al. 2000). Identification of the compounds was performed using commercial libraries such as Wiley (Bankova et al. 2000).

RESULTS AND DISCUSSION

Pollen grains can vary in colour from yellow to green, red to black depending on the plant source (Corvucci et al. 2016). Pollen samples can be determined according to their botanical origins by microscopic analysis (Corvucci et al. 2016). In each of the pollen prepara-

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Table 1. Plant sources of bee pollen

Fresh bee pollen	Plant sources			
	dominant (≥ 45 %)	secondary (16–44%)	minor (3–15%)	trace (< 3%)
	Brassicaceae: 50.45%	Rosaceae: 18.34% Papaveraceae: 16.51%	Fabaceae: 9.17%	Apiaceae: 2.75% Lamiaceae: 1.83% Poaceae: 0.91%

tions, 500 plant taxa were counted and plant species were determined. Pollen families are classified as dominant (D) (≥ 45%), secondary (S) (16–44%), minor (M) (3–15%), and trace (T) (< 3%) according to their density (Mayda et al. 2020). In this study, the pollen count of the family Brassicaceae members was found to be dominant with 50.45%. This family was followed by Rosaceae 18.34% and Papaveraceae 16.51% as secondary, Fabaceae 9.17% as minor and Apiaceae 2.75%, Lamiaceae 1.83% and Poaceae 0.91% as trace families (Table 1).

In this study, moisture, total lipid and protein, pH and total phenolic content of pollen samples were determined. The results ranged from 6.23% to 20.62% (moisture), 4.98% to 5.57% (lipid), 16.812% to 1.477% (protein), 4.08 to 4.33 (pH) and 15.2 mg GAE g^{-1} to 22.73 mg GAE g^{-1} (total phenolic content) (Table 2).

In order to be bee pollens preserved for a long time, they must be dried using various techniques. In this study, biological activities and chemical compositions of lyophilized and oven-dried bee pollen samples were compared. According to the data obtained, it was seen that the lyophilized bee pollen preserved more than 90% of the protein content (Table 2). In addition, GC-MS analysis revealed that the pollen dried in the oven lost most of its volatile components, while the lyophilized bee pollen contained most of the components (Table 3).

Phenolic compounds are bioactive substances that are not synthesized by the human body (Sorucu 2019). They have specific metabolic or physiological actions and contribute to disease prevention if regularly present in the diet (Sorucu 2019). Those compounds are secondary metabolites of plants whose profiles are species-specific (De-Melo et al. 2016). Total phenolic content was found to be the lowest in oven-dried sample (Table 2). This result could be explained by the activity of specific enzymes like polyphenol oxidase and peroxidase. These enzymes may oxidize the phenolic compounds when pollen samples are dried in the oven (Silva et al. 2009; De-Melo et al. 2016). In dehydrated bee pollen, the total phenolic content can vary from 5.4 to 132.4 mg GAE g^{-1} (De-Melo et al. 2016); therefore, the results of this study were consistent with literature.

In this study, the chemical composition of pollen samples was determined using GC-MS. It was determined that fresh pollen samples contain components such as carvacrol, farnesol, methyl linoleate, methyl octadecanoate, ethyl linoleate, 3,4-dimethoxybenzaldehyde (Table 3). In the study of Neto et al. (2017), it was stated that stingless bee pollen contains components similar to our study. In the study carried out by Carpes et al. (2013), bee pollen samples collected from Brazil were found to contain benzoic acid. In our study, benzoic acid was also detected. As a result of this study, it was revealed that the pollen dried in the oven loses many components that it contains compared to the fresh pollen sample (Table 3).

Melo et al. (2011) stated that pollen samples lyophilized in their drying process using different drying techniques had lower moisture content. Feás et al. (2012) reported that the pH of bee pollen they studied ranged between 4.3 and 5.2. It is seen that our study is compatible with the literature data.

Table 2. Bioactivity of bee pollen samples

Bee pollen	Moisture (%)	pH	Protein (%)	Lipid (%)	Total phenolics (mg GAE g^{-1})
Fresh	20.62 ± 1.08	4.08 ± 0.02	17.477 ± 1.023	5.57 ± 0.03	15.27 ± 0.77
Oven-dried	6.23 ± 0.44	4.33 ± 0.01	16.812 ± 0.947	4.98 ± 0.01	20.88 ± 0.45
Lyophilized	11.01 ± 0.51	4.30 ± 0.02	17.416 ± 1.270	5.33 ± 0.03	22.73 ± 1.02

The results of the analysis were obtained in three replicates and the results are given as mean values with standard deviations (SD) on the basis of dry weight

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Table 3. Chemical composition of bee pollen samples

Compounds	Bee pollen		
	fresh	oven-dried	lyophilized
		(%)	
Hexadecan-1-ol	0.27	ND	0.01
Octadecan-1-ol	0.43	ND	0.22
Cinnamyl alcohol	0.01	ND	ND
Tridecan-2-one	0.12	ND	ND
3,4-Dimethoxybenzaldehyde	0.51	ND	0.02
Methyl pentadecanoate	1.71	0.28	0.79
Methyl hexadec-9-enoate	5.86	0.11	2.96
Methyl hexadecanoate	17.01	4.40	9.05
Ethyl hexadecanoate	0.63	ND	0.02
Methyl linoleate	1.42	0.04	0.37
Methyl octadec-9-enoate	18.48	2.16	10.33
Methyl octadecanoate	7.31	1.18	3.22
Ethyl linoleate	0.48	ND	0.08
2-Ethylhexyl 4-methoxycinnamate	0.70	0.14	0.26
Ethyl octadecanoate	0.33	0.08	0.09
Methyl eicosanoate	0.22	ND	0.01
Methyl tricosanoate	0.26	ND	0.01
n-Tetradecane	0.08	ND	ND
n-Docosane	0.93	0.05	0.27
n-Tetracosane	1.65	0.03	0.76
n-Pentacosane	1.02	0.22	0.48
Carvacrol	0.12	ND	0.06
Farnesol	1.45	ND	0.05
Squalene	22.15	6.15	14.64
Benzoic acid	0.05	0.01	0.02

The results are given as % peak areas of GC-MS; ND – not defined

Vasconcelos et al. (2017) stated that the lipid content of bee pollen obtained in different periods ranged from 4.07 to 5.62%. Melo et al. (2016) reported that the lipid content ranged from 1 to 13 g 100 g^{-1} and the protein content was below 10 g 100 g^{-1}. Işık et al. (2019a) found out that the amount of fresh pollen protein is 30.36 ± 0.63 g 100g^{-1} and the amount of lipid is 5.50 ± 0.04 g 100 g^{-1}. They concluded that the amount of protein decreased as a result of their infrared drying (Işık et al. 2019a). External factors such as temperature and radiation cause disruption in the structure of proteins. Therefore, it is seen that the amount of protein decreases to 17.44 ± 0.50 g 100 g^{-1} (Işık et al. 2019a). Işık et al. (2019b) investigated the effects of different drying temperatures on the biological activity of bee pollen in another study. According to this study, it was stated that the humidity and protein amount decreased with increasing temperature values.

In the study conducted by Özkök and Sorkun (2006) on bee pollen samples belonging to 14 different plant taxa, they found that the protein amount of wet pollen samples ranged from 13.60% to 23.77% and in the dryness of the same samples, these values ranged from 13.54% to 24.25%. In the same study, they found that the amount of lipid in 14 plant taxa ranged from 3.53% to 8.75% (Özkök and Sorkun 2007). In our study, protein amounts were found as 17.477% in fresh pollen, 16.812% in dried pollen in drying oven, and 17.416% in lyophilized pollen. On the other hand, lipid amounts were found as 5.57% in fresh pollen, 4.98% in dried pollen in drying oven, and 5.33% in lyophilized pollen. These results are compatible with all previous studies.

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CONCLUSION

Fresh bee pollen contains some moisture content. This moisture content makes the bee pollen a good source for microbial growth. In order to avoid the microbial growth bee pollen samples should be dried. Drying process could alter the chemical composition of bee pollen samples. In this study we compared the effect of drying process on the chemical composition of pollen samples. Although lower moisture content was achieved for oven-dried pollen samples, this drying process had negative effects on the nutritional value of pollen samples when compared to a freeze drying technique. Not only the volatile and free fatty acid content of oven-dried pollen samples decreased dramatically but also protein, lipid and total phenolic contents decreased as well. Freeze drying could be a better drying process for bee pollen samples by taking into account its nutritional value.

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