

# Microscopic examination and comparison of exine layer of bee pollen and bee bread (Perga)

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## ABSTRACT

**Background and Aims:** Thanks to their high nutritional content and therapeutic effects, bee pollen and bee bread (perga) are used as a food supplement. Studies have shown that bee bread has more bioavailability than bee pollen. This situation has been explained by the fragmentation of the exine layers of pollen in bee bread in some studies. However, there is no clear microscopic study showing that the exine layer is broken. This study investigated for the first time whether the pollen grains in bee bread were fragmented in the exine layers after fermentation, in comparison with the pollen grains in bee pollen samples.

**Methods:** Bee pollen and bee bread samples were collected from the same hives and pollen slides were prepared for examination with light and SEM microscopes. Both of the pollen slides were compared and microscopic photographs were taken.

**Results:** No deformation was observed in the exine layers of the pollen grains in bee bread after fermentation.

**Conclusion:** In many studies, the higher bioavailability of bee bread has been explained by the deformation at exine structure of the pollen grains. But it has not been supported microscopically in detail with both light and SEM microscopes. Our study's conclusion was that no deformation was observed in the exine structures of the pollen in bee bread after fermentation.

**Keywords:** Bee bread (perga), Bee pollen, Exine layer, Microscopic analysis

## INTRODUCTION

Plant pollen provides the basic protein needs of honey bees due to its high nutritional content (Standifer, 1980). Pollen is collected by honey bees and stored as pollen loads on the 3<sup>rd</sup> pair of legs (Alataş, Yalçın, & Öztürk, 1997; Almeida-Muradian, Pamplona, Coimbra, & Barth, 2005). These pollen loads are defined as "bee pollen" (corbicular pollen, bee-collected pollen) (Fuenmayor et al., 2014; Kňazovická et al., 2019). These pollen loads must be fermented in order to be used as a nutrient by honey bees. Therefore, the pollen is stored in the honeycomb, it is compressed, and saliva secretions of honeybees are added. Then, the honeycomb is covered with bee wax (Nagai, Nagashima, Myoda, & Inoue, 2004). Fermentation takes place thanks to microorganisms (Lactic acid bacteria (LAB), *Bifidobacterium* spp., *Saccharomyces* spp., *Pseudomonas* spp., *Streptococcus* spp., etc.) naturally found in the digestive secretions of the honey bees (Gilliam, Wickerham, Morton, & Martin, 1974; Olofsson & Vásquez, 2008). Fermentation is completed in about two weeks and the pollen stored in the honeycombs is called "bee bread" or "perga" (Herbert & Shimanuki, 1978; Nagai et al., 2004; Silici, 2014). So bee bread is probiotic due to the presence and activities of probiotic microorganisms in it (Kieliszek et al., 2018).

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Bee pollen has high nutritional and polyphenolic content and many therapeutic effects, such as antioxidant, antiallergenic, immunomodulator, anticarcinogen, hepatoprotective (Campos et al., 2008; Morais, Moreira, Feás, & Estevinho, 2011; Markiewicz-Żukowska et al., 2013; Özkök, 2018). Due to these properties, bee pollen has been used as a food supplement in human nutrition for centuries (Özkök, 2018; Howell & Champie, 1981). However, in recent years studies have shown that the dense and durable wall structure of pollen grains can't be fully digested (only 48% to 59% in the human body) by the digestive enzymes in the living body. In contrast, fermented bee bread was observed to have higher digestibility and bioavailability than bee pollen (Campos, Frigerio, Lopes, & Bogdanov, 2010). This situation has been explained by researchers in different ways. Some researchers suggest that the presence of acidic substances formed after fermentation of bee bread and low pH value break down the exine layer of pollen and in this way the pollen protoplast comes out and its bioavailability increases (Mutsaers, Blitterswijk, Leven, Kerkvliet, & Waerd, 2005; Zuluaga, Serratob, & Quicazana, 2015). But considering the structure of the exine layer, the possibility of this situation is doubtful.

When the structure of the pollen is examined, each of the pollen grains is surrounded by a sporoderm layer, which consists of two main layers, exine and intine (D'Albore, 1997; Wiermann & Gubatz, 1992). The exine layer structure of the sporopollenin consists of the polymerization of monocarboxylic and carboxylic fatty acids and has a high molecular weight (Heslop-Harrison, 1968). The exine layer, which is very resistant and difficult to purify and digest, provides protection of the pollen, and pollen grains are highly resistant even against strong acids (HNO<sub>3</sub>, HF, HCL) and at high temperatures (about 400-500°C). Studies have revealed that the pollen can remain unharmed for many years thanks to its stable exine structure (Özkök, 2018; Martin & Byers, 1965; Morgan, Flynn, Sena, & Bull, 2014; Southworth, 1990). The intine layer beneath the exine surrounds the protoplast of the pollen. In addition to being rich in polysaccharides, the intine layer also contains protein compounds in its structure and is not as stable as exine (Wiermann & Gubatz, 1992; Kapp, 1969; Halbritter et al., 2018). The germination tube formed during germination of the pollen originates from the intine layer. The apertures (gaps on the exine layer), formed by the weakening or disappearance of the exine on the pollen surface, allow the outward development of the germination tube (Kapp, 1969). Considering the stable nature of exine, it is suggested that the exine layer cannot be easily degraded after fermentation. When the studies on the subject were examined, it was seen that microscopic examinations regarding the structure of the pollen after fermentation were insufficient. In this study, pollen grains in bee pollen and bee bread samples (obtained from the same hive) were examined through light microscopy and a scanning electron microscope (SEM). By examining the pollen structure after fermentation, this study aimed to clarify the situation in the exine layer.

## MATERIAL AND METHODS

### Collecting samples

Bee pollen and bee bread were samples collected from the same hive from Bursa city in Turkey. Bee pollen samples were

collected with pollen traps attached to the hive every 15 days and mixed to represent an entire year. Bee bread samples were collected with a special bee bread tool from the same hive, from honeycomb that was collected after the honey harvest to represent an entire year. Bee pollen and bee bread samples were kept at +4 °C until microscopic analysis.

### Examining the samples with light microscope

Bee pollen samples and bee bread samples were separately homogenized through mixing. Pollen slides were prepared according to the method of Mayda, Özkök, Bayram, Gerçek & Sorkun (2020). Slides were fixed with glycerin gelatin and inverted to hold the pollen grains to the lamella's surface. Pollen slides were ready for microscopic examination after 12 hours, and they were examined and photographed under a Nikon Eclipse E400 light microscope.

### Examining the samples with scanning electron microscope (SEM)

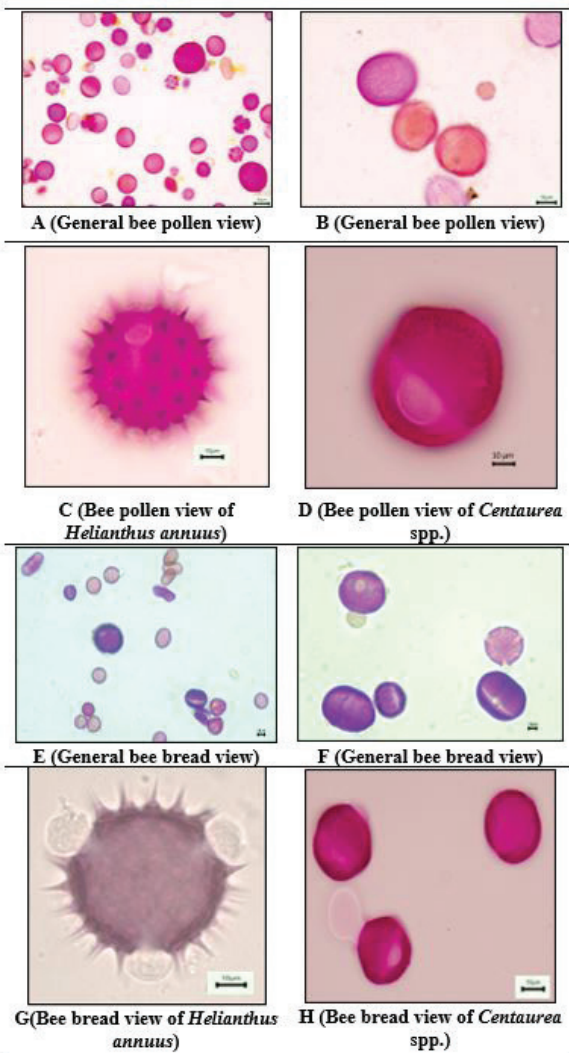
Pollen slides of bee pollen and bee bread were prepared for examination with SEM according to the method of Doğan & Erdem (2018), with some modifications. Two grams of each homogenized sample were weighed. 10 ml of distilled water were added and they were macerated, then vortexed until homogenized. After centrifuging at 3500 rpm for 20 minutes, the supernatant was removed. 3 ml of glutaraldehyde and 0.1 M 7.2 pH phosphate buffer were added to each sample for each fixation and the samples were kept at room temperature for one night. After fixation, the tubes were washed 3 times with phosphate buffer and centrifuged 20 minutes each time. Before the last centrifuge, the mixture was filtered through gauze and unwanted particles were removed. Samples were kept in ethanol series of 25%, 50%, 75%, 90% and 100% for 5 minutes each time, and the water in it was centrifuged for 20 minutes each time. After the centrifugation process was completed, the supernatant part was removed. The tubes were inverted on a blotter paper and allowed to filter for 10 minutes. The samples remaining at the bottom of the tube were removed with the help of a glass Pasteur pipette and spread over the stabs previously covered with carbon tape. Stabs were left to dry overnight in the oven at 25°C.

## RESULTS AND DISCUSSION

As a result of the examination of bee pollen and bee bread samples under light microscope, no fragmentation was observed in the exine layers of the pollen grains. Pollen grains in bee bread preserved their structural integrity as in bee pollen samples (Figure 1).

In an examination of the bee pollen and bee bread samples with the SEM, no fragmentation was observed in the exine layers of the pollen grains. Pollen grains in the bee bread preserved their structural integrity as in the bee pollen samples (Figure 2).

The nutritional properties of bee pollen and bee bread are similar. It has been observed that protein, lipid content and antioxidant capacity of bee bread may decrease slightly compared to bee pollen (Mayda et al., 2020). But it has also been reported that bee bread contains more vitamin K, reduced sugar and

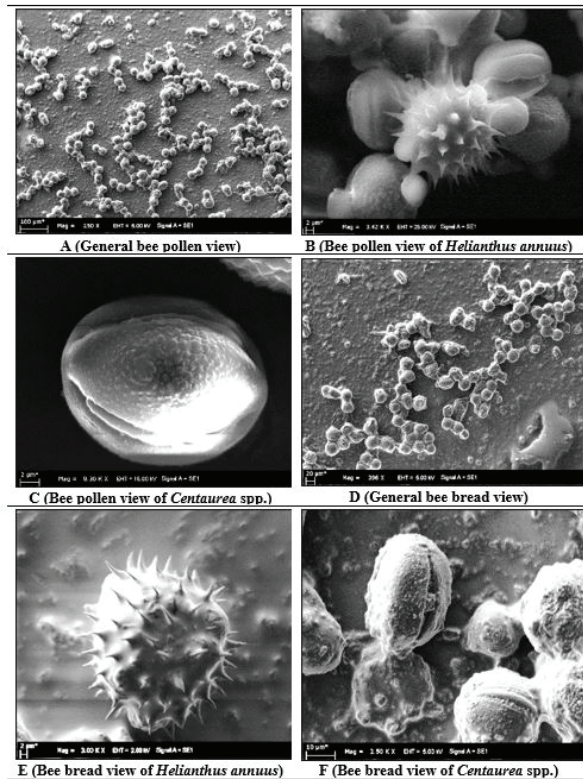


**Figure 1.** Pollen photos of bee pollen (A-D) and bee bread (E-H from light microscope.

fiber (Ivanišová et al., 2015). Although their nutritional contents are similar, studies show that bee bread has more bioavailability in the human body than bee pollen (Campos et al., 2010; Zuluaga et al., 2015).

Campos et al., (2010) reported that bee pollen can be digested 48%-59% in the living body. Bell et al., (1983) examined the bio-availability of two different commercial bee pollen in mice and observed that both were quite low. In a study conducted by Zuluaga et al. (2015), bee pollen was digested at the rate of 63 g (digested protein) / 100 g (total protein). On the other hand, it was observed that bee bread was digested at the rate of 79 g (digested protein) / 100 g (total protein).

The high bioavailability of bee bread has been explained by various researchers in different ways. It is suggested that the acidic products formed as a result of fermentation in bee bread cause deformation and disintegration in the pollen wall. This deformation occurring in the exine layer allows the emergence of pollen protoplast and thus higher bioavailability of bee bread (Mutsaers et al., 2005; Zuluaga et al., 2015).



**Figure 2.** Pollen photos of bee pollen (A-C) and bee bread (D-F) from SEM.

Similarly, Gönül (2016) studied the high bioavailability of the fermented pollen and explained the fragmentation occurring in the exine structure. However, the investigation was carried out under a light microscope did not have sufficient detail to examine the surface of the exine layer.

In this study, pollen grains in bee pollen and bee bread samples collected from the same hives were examined with both light microscopy and Scanning Electron Microscope (SEM). The collection of samples from the same hive is important in terms of obtaining samples with similar botanical origins, because the stability of the wall structure changes according to the botanical origin. Sporoderm is richer and more durable in entomophilic plant pollen in terms of chemical content than anemophilous plant pollen (Campbell, 1991; Twiddle & Bunting, 2010). Hall (1981) reported that pollen of *Taraxacum* spp., which is an entomophilic plant, is more resistant than pollen of *Corylus* spp. and *Quercus* spp., which are anemophilic plants.

As a result of morphological examinations performed under the light microscope, no deformation was observed on the surface of the pollen grains in bee bread. Examinations made with the light microscope were also supported by the SEM. Thus, it was revealed that there was no deformation in the exine layers of the pollen grains in bee bread, as explained in some studies (Dustmann, 2007; Bobiset al., 2017). Pollen grains' structural integrity was preserved as in bee pollen.

The intine, located below the exine and enveloping the protoplast of the pollen, does not have as durable and stable a structure as the exine (Kapp, 1969). Various mechanical and chemi-

cal effects can affect the integrity of the intine. In the acetolysis method developed by Erdtman, intine is eliminated, while the exine structure is preserved in pollen grains treated with acidic substances (Erdtman, 1957). As a result of this study, it was found that the acidic substances occurring as a result of fermentation do not cause deformation of the exine structure. However, acidic substances reaching the intine surface through the openings on the surface of the exine can cause deformations on the intine surface. In this way, it has been suggested that Protoplast can escape from the apertures by disrupting the intine. Similar comments were made by Dustmann (2007) and Bobis et al. (2017). Dustman (2007) explained the high bioavailability of bee bread with the presence of water and sugar from honey. The difference in concentration between sugar and water causes an osmotic shock in the pollen grains. Due to this osmotic shock, the content of pollen is released from the openings (aperture) and bioavailability increases (Komosinska-Vassev, Olczyk, Kaźmierczak, Mencner, & Olczyk, 2015). Similarly, Bobis et al., (2017) explained that sugars and water from honey are absorbed through the aperture of as a result of osmotic pressure and enrich the internal composition of bee bread.

## CONCLUSION

In many studies, the high bioavailability of bee bread has been explained by the deformation of the pollen grains in the exine structure, but this has not been supported in detail microscopically. Considering the stable and durable structure of the exine, this seems unlikely. The conclusion of our study is that no deformation was observed in the exine structures of pollen in bee bread after fermentation. However, it is thought that acidic substances formed as a result of acidic activity may enter the apertures on the surface of the exine, causing deformations in the intine structure and the pollen protoplast may come out of these openings. It is also possible that the protoplast content is released as a result of the osmotic pressure of the sugar and water sourced from honey in the bee bread. In addition, the prebiotic property of bee bread may be an effective factor in its easy digestibility. Examining the literature data, we can say that the high bioavailability of bee bread is due to contact with acidic substances and deformation of the intine layer.

As a result of this, pollen content can go out from the apertures. Moreover, it is believed that the fact that bee bread is a probiotic substance increases its absorption and bioavailability in the intestine. Explanations on the bioavailability of bee bread are still insufficient; this question needs to be investigated in further studies.

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