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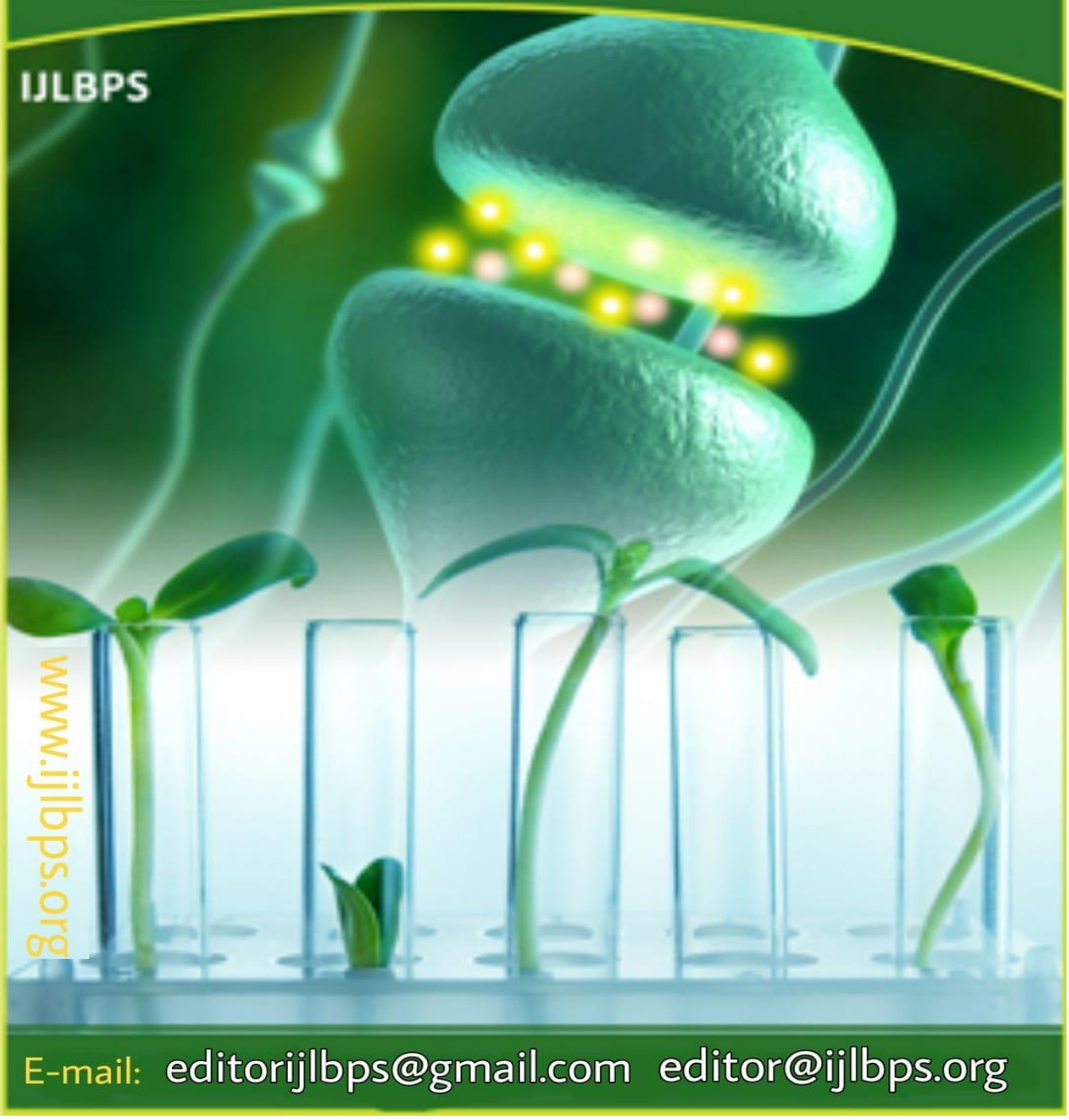

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Analysis of bee pollen and bee bread (Perga) under a microscope and comparison with their outer layers

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ABSTRACT

Background and Aims: Bee pollen and bee bread (perga) are utilized as dietary supplements because of their high nutritional content and therapeutic properties. When compared to bee pollen, research shows that bee bread is more bioavailable. Some research have proposed that the reason for this is because the exine layers of pollen in bee bread become fragmented. The exine layer may be compromised, although no conclusive microscopic evidence has been found. For the first time, this research compared pollen grains from bee pollen samples with those from bee bread to determine whether the exine layers of the pollen grains were broken during fermentation.

Approach: Bee bread and pollen were gathered from the same hives and processed into slides for light and scanning electron microscopy analysis. Under the microscope, we examined and contrasted the two pollen slides.

The results showed that following fermentation, the exine layers of the pollen grains in bee bread did not show any signs of deformation.

Conclusion: The increased bioavailability of bee bread has been attributed, in several studies, to the pollen grains' deformation at the exine structure. However, thorough microscopical evidence using light and scanning electron microscopes has not been found to support it. After fermentation, our research found that the exine structures of bee bread pollen did not deform.

Subjects covered: microscopy, exine layer, bee pollen, bee bread (perga), and bees.

INTRODUCTION

Because of its high nutritional content, plant pollen supplies honey bees with their essential protein requirements (Standifer, 1980). Honey bees gather pollen and keep it on their third set of legs as a pollen load (Alataş, Yalçın, & Öztürk, 1997; Almeida-Muradian, Pamplona, Coimbra, & Barth, 2005). These loads of pollen are referred to as "bee pollen" (also known as corbicular pollen or pollen gathered by bees) (Fuenmayor et al., 2014; Kňazovická et al., 2019). To make use of these pollen loads as food, honey bees must ferment them. As a result, the pollen is crushed and stored in the honeycomb before being mixed with the saliva secretions of the bees. Beeswax is then applied to the honeycomb (Nagai, Nagashima, Myoda, & Inoue, 2004). The presence of certain microorganisms in honey bee digestive secretions allows for fermentation to occur. These microbes include Lactic acid bacteria (LAB), Bifidobacterium spp., Saccharomyces spp., Pseudomonas spp., Streptococcus spp., and others (Gilliam, Wickerham, Morton, & Martin, 1974; Olofsson & Vásquez, 2008). The pollen fermented in honeycombs is known as "bee bread" or "perga" (Herbert & Shimanuki, 1978; Nagai et al., 2004; Silici, 2014). The process takes around two weeks to finish. It follows that bee bread contains and benefits from probiotic microbes (Kieliszek et al., 2018).According to

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many studies (Campos et al., 2008; Morais, Moreira, Feás, & Estevinho, 2011; Markiewiczśukowska et al., 2013; Özkök, 2018), bee pollen has a high nutritional and polyphenolic content in addition to numerous therapeutic benefits, including antioxidant, antiallergenic, immunomodulator, anticarcinogen, and hepatoprotective properties. Bee pollen has been a dietary supplement for humans for ages because of these qualities (Özkök, 2018; Howell & Champie, 1981). Nevertheless, new research has shown that human digestive enzymes can only break down about half of pollen grains due to their thick and lengthy cell walls.

Campos, Frigerio, Lopes, and Bogdanov (2010) found that bee pollen was poorly digestible and had low bioavailability compared to fermented bee bread. Many theories have been advanced by scholars to account for this phenomenon. Pollen protoplasts are released and their bioavailability is enhanced, according to some researchers (Mutsaers, Blitterswijk, Leven, Kerkvliet, & Waerdt, 2005; Zuluaga, Serratob, & Quicazana, 2015). This process is facilitated by the presence of acidic substances that are formed during bee bread fermentation and by a low pH value. However, this scenario is quite unlikely given the exine layer's structure.

According to research by D'Albore (1997) and Wiermann and Gutz (1992), the pollen structure reveals that there are two primary layers—the exine and the intine—that surround each pollen grain. Sporobollin has a high molecular weight and an exine layer structure that is composed of polymerized mono- and carboxylic fatty acids (Heslop-Harrison, 1968). The pollen grains are protected by the exine layer, which is tough to clean and digest and especially resistant to strong acids (HNO3, HF, HCL) and high heat (about 400-500°C).

According to research conducted by Özkök (2018), Martin and Byers (1965), Morgan, Flynn, Sena, and Bull (2014), and Southworth (1990), pollen may maintain its integrity for an extended period of time due to its stable exine structure. The protoplast of the pollen is encased in the intine layer, which is located underneath the exine. Wiermann and Gubatz (1992), Kapp (1969), and Halbritter et al. (2018) all agree that intine is less stable than exine and is rich in polysaccharides. It also includes protein components in its structure. During pollen germination, the intine layer gives rise to the germination tube. The pollen surface's exine weakening or removal forms apertures, which are gaps on the exine layer. These apertures enable the germination tube to grow outward (Kapp, 1969). Because exine is quite durable, it stands to reason that the exine layer will be difficult to breakdown during fermentation. A review of the relevant literature revealed that there was a lack of thorough microscopic analysis of the pollen's structure after fermentation. Light and scanning electron microscopy were used to analyze pollen grains in bee bread and pollen samples collected from the same hive. This research sought to provide light on the condition in the exine layer by analyzing the pollen structure after fermentation.

MATERIAL AND METHODS

Gathering specimens

The same hive in Bursa, Turkey, was used to gather pollen and bee bread samples. For a full year's worth of data, we combined pollen samples taken from beehives every 15 days using pollen traps. From the same hive, using honeycomb samples gathered after harvest to represent a full year, bee bread samples were extracted using a specialized bee bread technique. Prior to microscopic examination, samples of bee pollen and bread were stored at a temperature of +4 °C.

Using a light microscope to analyze the samples

The samples of bee pollen and bee bread were homogenized by mixing them separately. The procedure for preparing pollen slides was described by Mayda, Özkök, Bayram, Gerçek, and Sorkun (2020). The pollen grains were held to the surface of the lamels by fixing the slides with glycerin gelatin and inverting them. After twelve hours, the pollen slides were prepared for microscopic analysis, which was carried out using a Nicon Eclipse E400 light microscope.

Analyzing the specimens with a scanning electron microscope

Bee pollen and bee bread were produced for scanning electron microscopy (SEM) analysis in accordance with the procedure described by Doğan and Erdem (2018), with a few adjustments. Every homogenized sample was weighed at two grams. After being macerated in 10 ml of distilled water, the mixture was vortexed until it was completely homogenized.

The liquid above the solid was collected after 20 minutes of centrifugation at 3500 rpm. The samples were left at room temperature for one night after being mixed with 3 ml of glutaraldehyde and 0.1 M 7.2 pH phosphate buffer for each fixation. The tubes were centrifuged for 20 minutes after each of three washes with phosphate buffer after fixing. To eliminate any

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remaining particles, the mixture was filtered through gauze before the final centrifugation. The samples were immersed in several concentrations of ethanol (25, 50, 75, 90%, and 100%) for 5 minutes each, followed by 20 minutes of centrifugation of the water contained therein. The liquid portion that remained after centrifugation was finished was collected. After 10 minutes of inversion on blotting paper, the tubes were filtered. Using a glass Pasteur pipette, we extracted the remaining samples from the tube and distributed them over the stabs that had been coated with carbon tape earlier. The stabs were dried in the oven at 25°C for the whole night.

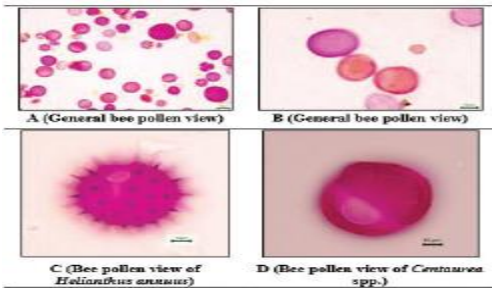
RESULTS AND DISCUSSION

Light microscopy analysis of bee pollen and bread samples revealed that the exine layers of the pollen grains were intact. Similar to bee pollen samples, the structural integrity of the pollen grains in bee bread was retained (Figure 1).

There was no evidence of fragmentation in the exine layers of the pollen grains when the bee bread and pollen samples were examined using the scanning electron microscope. The structural integrity of the bee pollen grains was retained in the bee bread as well (Figure 2).

Bee pollen and bee bread have comparable nutritional qualities.

Mayda et al. (2020) found that compared to bee pollen, bee bread may have a little lower protein, lipid, and antioxidant capacity. However, bee bread supposedly has less sugar, higher vitamin K, and



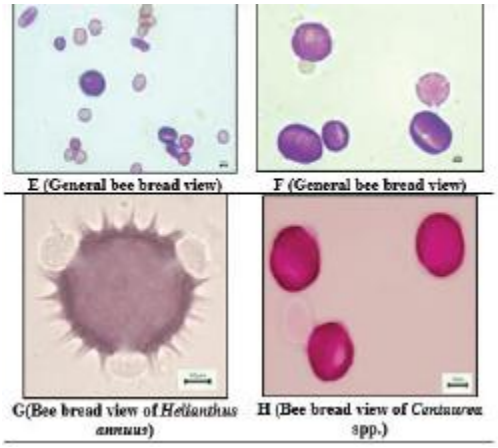


Figure 1. Pollen photos of bee pollen (A-D) and bee bread (E-H from light microscope.

plant material (Ivanišová et al., 2015). Research has shown that bee bread has a higher bioavailability compared to bee pollen, even if both have identical nutritional contents (Campos et al., 2010; Zuluaga et al., 2015).

According to research by Campos et al. (2010), between 48 and 59 percent of bee pollen may be broken down by living organisms. Bell et al. (1983) found that both types of commercial bee pollen had very poor bioavailability when tested on mice. The digestion rate of bee pollen was measured at 63 grams of digested protein per 100 grams of total protein in a research carried out by Zuluaga et al. (2015).Conversely, 79 grams of digested protein per 100 grams of total protein was found to be the rate of digestion for bee bread.

Several scholars have proposed numerous explanations for the increased bioavailability of bee bread. Hypothesized to induce distortion and disintegration in the pollen wall are the acidic byproducts of fermentation in bee bread. The increased bioavailability of bee bread is a result of this exine layer deformation that enables pollen protoplasts to emerge (Mutsaers et al., 2005; Zuluaga et al., 2015).

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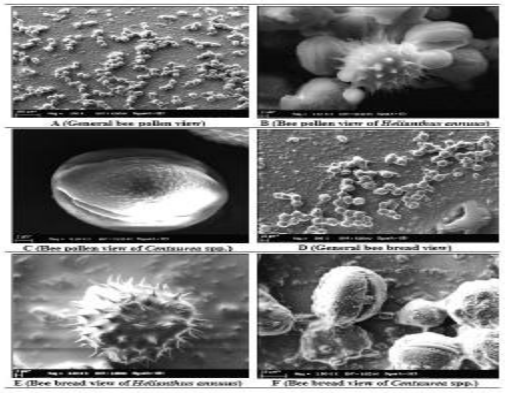

Figure 2. Pollen photos of bee pollen (A-C) and bee bread (D-F) from SEM.

Likewise, Gönül (2016) clarified the exine structural fragmentation and investigated the high bioavailability of fermented pollen. Unfortunately, the light microscope used for the experiment lacked the resolution to adequately investigate the exine layer's surface.

This research used light microscopy and a scanning electron microscope (SEM) to analyze pollen grains in bee bread and pollen samples obtained from the same hives.

Gathering samples from the same hive ensures that you get ones with comparable botanical origins, which is crucial since the wall structure's durability varies depending on the botanical origin. According to Campbell (1991) and Twiddle and Bunting (2010), entomophilic plant pollen has a higher chemical content and longer-lasting sporoderm than amenemophilic plant pollen. According to Hall (1981), pollen from entomophilic plants, such as Taraxacum spp., is more resistant than pollen from anemophilic plants, such Corylus spp. and Quercus spp.

Morphological analyses carried out under a light microscope revealed that the pollen grains in bee bread did not exhibit any surface deformation. The scanning electron microscope backed up the light microscope's findings as well. So, contrary to what was previously thought, the exine layers of the pollen grains in bee bread did not exhibit any deformation (Dustmann, 2007; Bobiset al., 2017). The structural integrity of the pollen grains was maintained, just as in bee pollen.

Although it encases the pollen protoplast, the intine, which lies under the exine, is structurally less robust and stable than the exine (Kapp, 1969). The integrity of the intine may be impacted by a variety of chemical and mechanical processes. Intine is removed in Erdtman's acetolysis process, whereas the

Pollen grains treated with acidic chemicals retain their exine structure (Erdtman, 1957). The study's findings revealed

that the exine structure is not deformed by the acidic chemicals that occur during fermentation. But, if acidic chemicals penetrate the exine's surface holes and reach the intine surface, the intine surface can become deformed. One possible explanation is that Protoplast may break the intine and get out of the holes in this fashion. Both Bobis et al. (2017) and Dustmann (2007) made similar points.

The increased bioavailability of bee bread, due to the presence of water and sugar from honey, was elucidated by Dustman (2007). Pollen grains experience an osmotic shock due to the concentration difference between sugar and water. Bioavailability rises as a result of the pollen's release from the apertures caused by this osmotic shock (Komosinska-Vassev, Olczyk, Kaźmierczak, Mencner, & Olczyk, 2015). Just as honey's sugars and water enhance bee bread's internal composition, osmotic pressure allows them to be absorbed via the aperture, as Bobis et al. (2017) noted.

CONCLUSION

Microscopical evidence is lacking to support the widespread belief that the pollen grains' distortion in the exine structure accounts for bee bread's increased bioavailability.

Given the exine's sturdy construction, this seems implausible. Our investigation found that the exine structures of pollen in bee bread did not deform during fermentation. The pollen protoplast, however, might emerge from the intine structure once acidic molecules created by acidic activity reach the exine's surface apertures and distort them. In bee bread, the protoplast content may be released due to the osmotic pressure of the sugar and water derived from honey. Bee bread's prebiotic quality also likely contributes to its ease of digestion. After reviewing the research, we can conclude that the intine layer deformation and interaction with acidic chemicals are responsible for bee bread's high bioavailability.

This allows the pollen to be released via the openings.

Bee bread is said to have enhanced intestinal absorption and bioavailability due to its probiotic

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properties. Further research is required to address the topic of how to explain the bioavailability of bee bread.

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