Revista ARACÊ
ISSN: 2358-2472

CHARACTERISTICS OF POLLEN QUALITY AND USE: A LITERATURE REVIEW

https://doi.org/10.56238/arev6n2-208

Submitted on: 30/09/2024 **Publication date:** 30/10/2024

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ABSTRACT

Pollen is a natural product collected by bees and is rich in nutrients such as protein, vitamins, minerals, and antioxidants. It has been increasingly sought after by the natural food market, for its benefits to human health. The objective of this study was to carry out a literature review on pollen, addressing: nutritional components, production and processing, quality analysis and applications in the food industry. The research methodology included

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the selection of sources, exploratory and selective reading, and recording of information. A systematic literature review was carried out in databases such as Scielo, Capes Journals, SB/UFCG, BDTD/USP, RI/UFS, BD/IPB, TEDE/UFMA published in the last 10 years (2012 to 2022). 46 national articles and 1 international article available online in full text were used. Information was obtained ranging from the collection of pollen to its applications in the food industry, which include the production of functional foods, such as cereal bars, energy drinks and food supplements. The work also highlights the importance of pollen quality, including collection in pesticide-free areas and proper storage. As well as, it highlights the positive impacts that are generated by beekeeping and meliponiculture, reaching the three axes of sustainability: ecological, economic and social.

Keywords: Pollen. Melipona. Application. Quality.

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INTRODUCTION

Beekeeping in Brazil began in 1839 with Father Antônio Carneiro Aureliano, who imported from Portugal some swarms of *Apis mellifera* bees, known as "bees of the kingdom" or "black bees", as well as other subspecies brought by immigrants. Initially, four subspecies were introduced: *Apis mellifera mellifera*, *Apis mellifera ligustica*, *Apis mellifera carnica,* and *Apis mellifera caucasica*, which did not reach the productivity desired by beekeepers (Fepe, 2020).

Seeking to improve the scenario, in 1956 Prof. Dr. Warwick Estevam Kerr, a specialist in bee genetics, who was part of a national program to improve honey production, traveled to South Africa and Tanganyika, on an official mission of the Brazilian government, to bring fertilized queens, of the subspecies Apis mellifera scutellata, known as "African bees" (Fepe, 2020; Salomé, 2020).

Normative Instruction No. 3, of January 19, 2001, defines bee pollen as the result of the agglutination of flower pollen, carried out by worker bees, through nectar and its salivary substances, which is collected at the entrance to the hive. The quality of pollen is influenced by its chemical composition, which varies according to several factors, such as the type of flower, agroecological conditions and collection methods (Melo, 2015).

The components of bee pollen ensure that it is an excellent complementary food for humans, with high nutritional value, being rich in proteins, carbohydrates, lipids, minerals, and vitamins (Milfont, 2020). These substances are present in pollen grains because they are nutritional reserves that would be used when pollination is completed and plant germination occurs (Souza, 2014).

The nutrient content in pollen varies according to the plant species it comes from, the environmental conditions and the nutritional status of the plant. The shape, size, appearance, and color of pollen grains can also vary due to these conditions (Melo *et al*, 2009). It can vary according to the season, air temperature, pH and soil fertility, in addition to the characteristics of the flora itself (Bendini & Souza, 2020).

Melipona pollen has rich characteristics and has been increasingly sought after by the natural food market. The pollen of stingless bees, also known as melipona or samburá pollen, is deposited in the colony in cerumen pots, which makes it relatively easy to extract when compared to the system applied to Apis (Villas-Boas, 2012). They generally have a high moisture content that gives them an unpleasant appearance for commercialization and with a greater probability of deterioration.

According to Bogdanov (2015), the consumption of bee pollen or its derivatives, as a dietary supplement for humans, has grown rapidly. In addition, interest in pollen has been increasing, due to its nutritional content and biological activity.

Thus, the present study aimed to carry out a literature review on Apis *mellifera* and *Melipona* pollen, through the survey of bibliographic data on the quality and application characteristics of bee and melipona pollen, nutritional properties and applications of pollen in the food industry and to highlight the importance of pollen quality for a better use of the raw material.

METHODOLOGY

The research was carried out through access available via the internet. A systematic literature review was carried out in databases such as Scielo, Capes Journals, SB/UFCG, BDTD/USP, RI/UFS, BD/IPB, TEDE/UFMA published in the last 12 years (2012 to 2024). 46 national articles and 2 international articles available online in full text were used. The following descriptors were applied: beekeeping, physicochemical characteristics of bee pollen, composition of bee pollen, bee pollen, history of beekeeping, meliponiculture, physicochemical characteristics of samburá, pollen collection and processing.

RESULTS

HISTORY OF BEEKEEPING AND MELIPONICULTURE IN BRAZIL

Africanized bees arose from an accidental hybridization between African and European bees. Although they are very productive and resistant to diseases, they caused a great impact at the beginning of their dispersion, due to their aggressive behavior (Terças *et al*., 2017). After the development of appropriate management techniques for Africanized bees, in the 70s, beekeeping began to be developed in all Brazilian states (Tomazini, 2019).

In Brazil, more than 300 species of stingless bees are known to show heterogeneity in various aspects such as color, size, shape, nesting habits and number of individuals in each nest (Afonso, 2012).

Initially developed by the Indians, Brazilian meliponiculture was over time practiced in a traditional way by small and medium-sized producers, especially by those who used family labor in agricultural activities, being considered a complementary economic activity (Silva & Paz, 2012).

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In the northeast of Brazil, the breeding of stingless bees has always been developed in a rustic way. In some quilombola communities in Paraíba, for example, the breeding of meliponines is still very rustic, with the use of tenements (trunks). However, younger breeders are already starting to adopt rustic boxes for breeding and are less attached to traditional customs (Carvalho *et al*., 2014).

Brazil contains the greatest diversity of meliponines on the planet, The use of the various products of the hive of these bees is millennial among the peoples of the Americas (Venturieri, 2008). Meliponiculture is one of the few activities in the world that fits into the four major axes of sustainability. It generates a positive environmental impact, is economically viable, is socially accepted and culturally important for the educational proposal it plays in the interaction with society (França, 2011).

IMPORTANCE OF BEES

Being part of the order *Hymenoptera*, bees, as well as wasps and ants, belong to the superfamily known as *Apoidae* (Silva, 2021). Bees, despite being most often known for the production of honey, are also capable of providing wax, propolis, pollen, royal jelly, among others. Since its economic importance is not only based on the supply of these products, it is estimated that at least a third of human food has a direct or indirect dependence on pollination (Silva, 2021).

The main characteristic of this species is its total dependence on floral products, such as nectar and pollen, as a source of food and income for its young. Bees occupy a very important key position in the maintenance of plant diversity, as they are the main pollinating agents (Souza, 2021).

When visiting the flowers to collect pollen and nectar, a protein and energy source, respectively, bees involuntarily promote this key ecological service for food production, maintenance, and conservation of ecosystems, ensuring the perpetuation of thousands of plant species (Pinto *et al*., 2018).

POLLEN PRODUCTION IN BRAZIL

Beekeeping is a practice of great potential in Brazil due to favorable factors such as climate and flora diversity (Paula *et al*, 2006; Francisco *et al*, 2019). It is one of the few agricultural activities that meets the three requirements of sustainability: economic, social and ecological. Thus, it provides income for the beekeeper, employs family or contracted

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labor, and contributes to the preservation of native flora, as it is from it that nectar and pollen are extracted, essential components for the life of the hives (Paula *et al*, 2006; Francisco *et al*, 2019).

The production of bee pollen in Brazil began, in a modest way, at the end of the 80's. In recent years, the favorable market for the consumption of natural products, complementary to the diet or with therapeutic effects, has stimulated and promoted this modality of the beekeeping production chain (Rocha *et al*, 2002).

Currently, the demand for bee products has been increasing in Brazil and in the world due to the favorable market for the consumption of these natural products with functional and/or nutritional properties complementary to the diet or with therapeutic effects (Jacob, 2014; Ferreira, 2012; Martins, 2010).

In the Brazilian northeast, pollen obtained from native flora still predominates (Freire *et al*, 2012; Francisco *et al*, 2019) It is one of the few regions in the world with the potential to produce organic bee products in large quantities, due to the diversity of plant species and microclimates, along with vast areas of unexplored land, free from conventional agricultural activity (Morgano *et al*, 2012; Francisco *et al*, 2019).

Meliponiculture is an important activity for various types of people, especially for traditional communities and family farmers who depend on it as their main source of income or relevant additional contribution (Ribeiro *et al*. 2019). The term used to refer to meliponiculture was first used by researcher Paulo Nogueira Neto in 1953, which refers to the breeding of bees that have their stinger atrophied, making it impossible for them to use it as a defensive, for this reason, they came to be popularly known as stingless bees (ASF) (Farias, 2019).

Species of social bees native to the Americas, producers of honey and propolis, have been subject to management practices since the times of pre-European colonization civilizations. This relationship is so intimate that bees of the Meliponini tribe are commonly called stingless bees or indigenous bees (Muñoz, 2016).

In the semi-arid region of Brazil, a species of stingless bee is gaining prominence due to the quality of its melipona pollen and other products. The jandaíra bee (Melipona subnitida) is often bred and used in meliponiculture in the region (Dantas *et al*., 2020). The high content of essential amino acids, minerals, flavonoids and D-mannitol sugar add functional characteristics to this food (Silva *et al*., 2006; Silva *et al*., 2013).

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Information on the pollen collected by stingless bees is still not widely disseminated, due to the diversity of bee species and the vegetation, climate and soil of ecosystems distributed in Brazil and in the world. Thus, its commercialization is still informal (Mendonça Neto *et al*., 2021).

As most beekeepers do not have records and do not issue purchase and sale notes, there are no real estimates of the economic movement of meliponiculture in Brazil. The municipality of Santa Rosa de Lima, in the state of Santa Catarina, is collecting this data. It is considered a center of meliponiculture: about 70 families maintain more than 10,000 mother colonies, which can be sold between 150 and 400 reais (Koser *et al.*, 2020).

POLLEN CHARACTERISTICS

Definitions and functions

The pollen (from the Greek "pales" = flour or powder) of flowers is the set of male reproductive elements of the flowers, in which are found the gametes that will fertilize the ovules to later generate seeds. It is composed of a large proportion of proteins (16 to 40%) of high biological value (contains all the essential amino acids), as well as numerous vitamins, mainly C and B3 (Costa *et al.*, 2011; Braga 2019).

Bee pollen is the result of the agglutination of flower pollen, carried out by worker bees, involving nectar and its salivary substances, which is collected at the entrance of the hive (Brasil, 2001; Ramalho, 2018). It is one of the richest and purest natural food supplements packaged by bees in granules and later harvested from hives by humans (Bogdanov, 2017; Francisco *et al.*, 2019). It is abundant in compounds that can benefit humans, such as vitamins, antioxidant substances, enzymes, and phenolic compounds, and can prevent various diseases existing in today's society, in addition to being a source of nutrients for a balanced diet (Arruda, 2013; Freitas *et al.*, 2020).

The pollen grains have the essential biological function of transporting the male gamete to the stigma, the female reproductive organ of the flowers, completing the pollination process. This transport can occur through the wind, through animals, or by water (Freitas *et al*., 2020).

The pollen of stingless bees, because it is different from pollen in natura, receives special names: saburá or samburá, depending on the region of Brazil and has been increasingly sought after by the natural food market (Villas-Bôas, 2012). They are stored in cerumen jars built for food storage, known as "pot pollen", it is a fermented product, rich in nutrients, from meliponiculture, a traditional and millennial activity that consists of the rational breeding of stingless bees, an economic and sustainable activity (Francisco *et al*., 2016; Neto *et al*., 2021).

Melipona pollen, a product made by stingless bees from flower pollen collected from various botanical sources, contains bioactive compounds, minerals, fatty acids, proteins, essential amino acids, and fibers (Nogueira *et al*., 2012; Perusso, 2022). Its quality is directly related to its microbiological, physicochemical and biological characteristics, which, in turn, vary according to climatic conditions, the soil of the region where it is produced, the botanical origin, the processing of the product and the practices employed during collection (Campos *et al*., 2008; Perusso, 2022).

Sensory characteristics

The aroma, as well as the color of the pollen, should be characteristic, according to the floral origin. The aspects are heterogeneous grains, of varying shape and sizes, tending to spherical with a characteristic flavor (Brasil, 2001).

Apis pollen can be used as a natural sweetener due to the presence of natural sugars such as fructose and glucose. These sugars provide a sweet taste to the compost pollen, making it a potential natural sweetener for food products. Additionally, compost pollen contains other bioactive compounds, such as polyphenols, which have antioxidant properties, which may provide additional health benefits when used as sweeteners (Khalifa *et al., 2021).*

Melipona pollen has a high acidity content due to the reactions that occur during the processing of this product by bees during storage in pollen jars (Alves & Santos 2018) thus, it is a naturally acidic food, due to the presence of gluconic and lactic acids found in the final product (Kalaycioğlu *et al*., 2017).

The composition of melipona pollen has a higher quantity and quality of nutrients than that of flower pollen before harvest. This is due to the fact that bees add salivary secretions with their enzymes, add nectar and enrich the final product with nutrients that allow it to be a functional food with biological activities and a higher nutritional proportion compared to flower pollen (Mendonça Neto *et al*., 2021).

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Physicochemical characteristics of pollen

The chemical composition of pollen can vary depending on the plant and geographic origin and the set of other factors such as climatic conditions, soil type and the activities of the beekeeper (Feás *et al.*, 2012; Francisco *et al.*, 2020). Each type of pollen has its own specific characteristics related to the genetics of the flower species visited by bees and that can influence biological properties (Komosinsca *et al.*, 2015; Alves *et al.*, 2020).

Bee pollen is rich in phenolic compounds, which are known for their antioxidant and antimicrobial properties. Studies show that different ethanolic concentrations of pollen extracts exhibit different antioxidant and antibacterial activities, which is related to the phenolic content extracted. Some studies also suggest that the antimicrobial activity of bee pollen is related to the total number of phenolic compounds present (Caldas *et al.*, 2019). Bee pollen is a rich source of flavonoids, which have antioxidant, anti-inflammatory, and antimicrobial properties (Perusso, 2022).

In its composition, bee pollen has 10% to 40% protein, 13% to 55% carbohydrates, 1% to 13% lipids, 0.3% to 20% crude fiber and 2% to 6% ash. It has all the essential amino acids, as well as enzymes, linoleic and linolenic fatty acids and several important mineral elements, such as potassium, calcium, magnesium, iron, manganese, zinc, phosphorus, among others, and vitamins such as β-carotene, vitamins C, D, E, B vitamins and folic acid (Campos *et al.*, 2008; Bogdanov, 2016; Negrão; Orsi, 2018; Embrapa, 2020).

Stingless bee pollen has been used for a long time, especially among natural food enthusiasts, as a supplement to the human diet, probably due to its richness in proteins, lipids, vitamins and mineral salts. It has β-carotene in its composition as provitamin A, vitamins C, E, D and B complex, in addition to being a source of carbohydrates and having all the essential amino acids (Estevinho *et al.*, 2012).

Study conducted by Alves *et al*. (2018) with melipona pollen samples from the interior of Bahia, the following mean values were observed: 44.71% moisture, 1.84% ash, 4.25% lipids, 23.88% proteins, 0.87% fibers, 24.48% carbohydrates, pH 3.75, a free acidity of 150.57 meq/100 kg, a water activity of 0.92 and a total energy value of 231.33 kcal/100 g. In addition, microbiological analyses revealed that the product was satisfactory and free of pathogens.

Nunes (2017), when performing a physicochemical analysis on 11 samples of Melipona fasciculata pollen from the Maranhão Amazon, found the following average values: 35.88% moisture, 2.02% ash, 3.14% lipids, 23.47% proteins, 35.49% carbohydrates, 3.63 pH and 132.30 mEq/kg free acidity.

Mesquita (2021), when analyzing the physicochemical composition of 16 melipona pollen samples collected from Melipona fasciculata (Tiúba) hives, from four different locations in the northeast region of Maranhão, found the following mean values: 25.4% for moisture, 3.1% for ash, 10.6% for lipids, 23.2% for proteins, 31.7% for carbohydrates, 3.9 for pH, and 125.7 mEq/kg for free acidity.

In order to evaluate the attributes of bee pollen that make it a healthy food suitable for human consumption, the characteristics of five samples of Christmas bee pollen were examined, namely ash, moisture, proteins, carbohydrates, lipids and organic matter. The mean values for each category were calculated as follows: ash (2.84%), proteins (12.95%), carbohydrates (81.45%), moisture (22.54%) and lipids (2.74%) (Melo *et al.,* 2015).

Despite being a product with great market potential in Brazil, the production and commercialization of melipona pollen still suffers several obstacles, such as the physical-chemical and microbiological characteristics of the product still remain unknown, as few studies have been carried out to characterize it. The lack of knowledge in the area is an obstacle to the development of standardization techniques that drive the regulation, production, and commercialization of the product on a large scale (Alves & Carvalho, 2018; Alves, Sodré & Carvalho, 2018).

Microbiological characteristics of pollen

Taking into account the amount of nutrients present in bee pollen, a wide variety of microorganisms could develop in this product. When assessing the quality of a food, it is important to determine its safety from a microbiological point of view. The evaluation of the microbiological quality of a product provides information on the conditions of processing, storage and distribution for consumption, shelf life and consequently the risk to public health (Arruda, 2013).

Food safety with an emphasis on qualitative aspects can be understood as the acquisition by the consumer of good quality food, free of contaminants of a physical (glass, stone), chemical (pesticides) and biological (pathogenic organisms) nature (Arruda, 2013).

The high moisture content, when in natura, and the nutritive value of pollen favor the proliferation of a range of microorganisms, therefore, the creation of one or more obstacles, such as the use of freezing and dehydration, are important to avoid this proliferation (De-

Melo, 2015). Although efficient to control microbial development because it reduces aw in the final product, the dehydration of this product is carried out at a maximum temperature of 42ºC and a time ranging from 10 to 30 hours, so that the process is not drastic enough to eliminate the population of microorganisms, especially spores of mesophilic bacteria and fungi (De-Melo, 2015). Bee pollen has antimicrobial properties (against Gram-positive and Gram-negative bacteria), caused by the presence of flavonoids and phenolic acids (Rzepecka-Stojko *et al.*, 2015).

In the pollen of meliponines there seems to be an association with some bacteria, at least for some species of Melipona. It is believed that, in Apis Mellifera, the association of stored pollen with microorganisms may be responsible for the fermentation or pre-digestion of stored food. This association can also contribute by adding certain organoleptic properties to the pollen, which are specific to each bee species. Among other factors, such microorganisms can produce chemical substances such as fatty acids and antibiotics that inhibit competing organisms and contribute to improving the preservation of this product (Ferreira, 2013).

The success of the technique in preserving the pollen of Apis and meliponines bees is largely due to the microbial flora added by these bees to the still fresh pollen when it arrives at the hive. Yeasts and lactic acid bacteria are recognized as the microorganisms responsible for exerting the fermentation of stored pollen, which increases its acidity and inactivates microorganisms (Mohammad *et al.*, 2021).

POLLEN COLLECTION AND PROCESSING

Collection and processing of bee pollen

The bee pollen collector is the equipment used to remove the bee pollen acorns from the hind legs of bees when they return to the hive. The equipment must retain 70% of the pollen, since approximately 30% of the bee pollen must pass through the collector to be used to supply the food needs of the hive (Epagri, 2017).

All the pollen, when collected by the campeiras, receives salivary secretions that the bees use to assist in the aggregation of the grains to the body of the bees and later in the corbiculae, which are located in the third pair of legs. However, the pollen collected by bees can give rise to two different products, which are "bee bread" and bee pollen (Milfont, 2011; Milfont, 2020).

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Considering the high hygroscopic capacity of pollen, the choice of collector must consider some factors, such as the climatic conditions of the region in which the apiary is located, as well as the management to be adopted. The external collectors (figure 1) are fixed at the entrance of the hive, and are necessary for the daily collection of pollen, since it is more exposed to humidity. In the internal collectors (figure 2), pollen is less exposed to humidity, being recommended in regions that have higher relative humidity and rainfall (Fepe, 2020; Epagri, 2017; Alves, 2013).

Figure 1. Pollen collector (external)

Source: ciadaabelha.commercesuite.com.br

Figure 2. Pollen collector (internal)

Source: acervodigital.ufpr.br

During collection, the smoke should be used moderately and the smoker should not be directed to the collectors, in order to prevent the pollen from absorbing the smell of the smoke.

The drawer with pollen should be replaced by a sanitized one and the collected pollen should be transferred to properly clean, dry containers (trays or buckets) with lids, for transport to the handling room. The drawers of the collectors removed must be sanitized at each collection (Epagri, 2017; Lopes *et al*., 2022).

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The collected bee pollen must be taken to a suitable environment, where all the necessary steps for processing/processing will be carried out to originate the product "dehydrated bee pollen", in order to maintain its properties and provide longer "shelf life" (Barreto *et al*., 2006; Lopes *et al*., 2022). The environment and the actions for processing must follow the standards established in the Technical Regulation on Hygienic-Sanitary Conditions and Good Preparation Practices for Food Elaborating/Industrializing Establishments, which are contained in Ordinance No. 368, of 09/04/1997, of the Ministry of Agriculture, Livestock and Supply (Brasil, 2001).

In the environment for handling and processing the product, the newly collected pollen must initially be subjected to pre-cleaning. To do this, the pollen must be distributed in trays (non-toxic plastic or stainless steel) and, with the help of stainless steel tweezers, impurities such as dead bees, leaves or other impurities must be removed. Then, the product must be packed in containers with lids, suitable for food, and taken to the freezer for freezing for at least 48 hours. Freezing will serve to kill insect eggs and larvae, mites and to prevent the proliferation of fungi and bacteria (Milfont *et al*., 2011; Bogdanov, 2016; Lopes *et al*., 2022).

To carry out the next processing steps, the bee pollen must be thawed, preferably slowly, so that the frozen water inside the pollen can gradually reintegrate. For this, the product must be kept in a refrigerator for a period of 4 to 8 hours (Milfont *et al*., 2011; Lopes *et al*., 2022).

After thawing, the product must go through the dehydration process. This step can be carried out by means of greenhouses (figure 3) with air circulation. The pollen must be distributed in thin layers, in trays and screens placed in the form of shelves in the greenhouse, so that the process occurs homogeneously. The temperature of the oven must be maintained between 40 °C and 42 °C for a period of 8 to 12 hours, on average, after which the product must have a humidity of no more than 4% (Brasil, 2001).

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Figure 3. Pollen drying kiln

Source: www.apisfilanis.com

The next step after dehydration is aeration or ventilation, which consists of subjecting the product to a ventilation system in order to remove smaller and lighter impurities, such as bee fragments, plant residues, dust, propolis, and other foreign elements that still remain in the pollen (Lopes *et al*., 2022).

For commercialization, dehydrated bee pollen can be packaged in various packages such as glass or non-toxic plastic containers (jars, bags or buckets) suitable for packaging food. Filling should be carried out quickly and in a dry environment, preferably with dehumidifiers to avoid the absorption of moisture by the product (Lopes *et al*., 2022). The storage of processed and bottled bee pollen should be done in a dry, ventilated place and away from light, in order to preserve the characteristics of the product and maintain its nutritional properties (Milfont *et al*., 2011; Lee *et al*., 2022).

Collection and processing of Melipona pollen

The pollen of stingless bees is deposited in the colony in cerumen pots, which makes it relatively easy to extract when compared to the system applied to Apis. As much as the pollen of stingless bees is stored in the colony in exclusive jars, it is common for the set of jars of this product to be adhered in some portion to a set of honey jars. To prevent the pollen from being "contaminated" with the honey, it is recommended that the honey from the adjacent jars be extracted before removing the pollen jars. The processing stages mentioned below were described by Villas-Bôas (2018).

After all the honey has been sucked or drained, the pollen pots should be removed from the colony or honeycomb with a serrated knife or other utensils that aid their removal. Jars should be deposited in an appropriate storage container, preferably a non-toxic plastic box with a lid. At this time, all care is valid so that the bees are not deposited in the container with the pollen pots.

Once the storage container is full, it should be transferred to the processing site, such as the warehouse, and cooled. In addition to preserving the product, cooling helps the execution of the first stage of processing, which is the opening of the cerumen pots to access the pollen in natura.

The first stage of pollen processing is unwrapping. In an appropriate place, on a clean surface – stainless steel table or masonry countertop – pollen agglomerates must be manually removed from the cerumen pots and stored in the dehydration trays. Concomitantly, screening is carried out, that is, the visual classification of pollen clusters, separating those that are suitable for processing and eliminating unwanted ones.

A pollen cluster can be classified as unsuitable for processing if it has the following characteristics: presence of honey that may have run off during extraction; presence of any type of insect larva; pasty pollen (without the presence of the characteristic granules). The pollen clusters selected and stored in the trays then go on to a sequence of three stages of dehydration:

- First dehydration: In this step, the pollen clumps are partially dehydrated for easier handling and fragmentation. For this, 4 hours in an oven (from 40°C to 42°C) or 24 hours in a refrigerator (with a minimum spacing of 10 cm on all sides of the tray for air circulation) are required. Next, the partially dehydrated agglomerates should be fragmented into smaller granules (increasing the contact surface for the second dehydration) and deposited in another tray. Concomitantly, another sorting should be carried out, eliminating residues of cerumen and/or unsuitable agglomerates that have passed the first sorting.
- Second dehydration: In this step, the pollen fragments are partially dehydrated to make sifting viable. For this, another 4 hours in the oven or another 24 hours in the refrigerator are needed. The fragments are then sifted into another tray, acquiring the grainy texture of the final product. The size of the granules can vary according to the sieve used by the producer. 1 mm to 3 mm is suggested.
- Third dehydration: Finally, the pollen, now granulated, must go through the third stage of dehydration. For this, approximately 4 more hours in the oven or another 24 hours in the refrigerator are needed. The time should be enough for the final product to have a maximum of 4% moisture.

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Granulated and dehydrated pollen should be immediately bottled – to avoid moisture absorption – and sent for storage and/or commercialization. Ideally, the packaged product should not be exposed directly to the action of light to reduce the degradation of nutritional properties.

POLLEN QUALITY CONTROL

The quality of the final product is related to carrying out the collection and processing stages properly, so that the original characteristics of the product are preserved as much as possible (Lopes *et al*., 2022). The quality of pollen is influenced by its chemical composition, which varies according to several factors, such as the type of flower, agroecological conditions and collection methods. (Melo, 2015; Lema, 2020).

To ensure the quality of bee pollen, MAPA instituted, through Normative Instruction No. 3/2001, the Technical Regulation of Identity and Quality (RTIQ) of bee pollen, which determines criteria for its classification, as well as sensory, compositional and physicochemical requirements (Brasil, 2001; Ferreira, 2020).

Table 1. Physicochemical requirements for bee pollen and dehydrated bee pollen

Parameter	Reference values	
	Bee pollen	Dehydrated bee pollen
Humidity (%)	Max. 30	Max. 4
Ash(%)	Max. 4	Max. 4
Lipids (%)	Min. 1,8	Min. 1,8
Proteins (%)	Min. 8	Min. 8
Total sugars (%)	14.5 to 55.0	14.55 to 55.0
Crude fibre (%)	Min. 2	Min. 2
Free acidity (mEq/kg)	Max. 300	Max. 300
Ph	4 to 6	4 to 6

Source: Brazil, 2001; Ferreira, 2020.

Humidity

The moisture content can be considered one of the main indicators of the quality that affects the characteristics of bee pollen and combined with high values of water activity (aw), favor an environment conducive to the development of microorganisms. The transfer of moisture from the environment to the bee pollen can promote microbial growth and product spoilage. In this way, high moisture levels promote the growth of microorganisms, and low levels inhibit their growth (Orvalho, 2023).

Ashes

The ash content assessment is carried out to verify whether the pollen has undergone changes due to environmental pollution or inadequate practices for collecting and processing the product (Nogueira *et al*., 2012). High levels of ash indicate the presence of inorganic contaminants, such as silica, soil, sand, fumigator soot and metals (Almeida-Muradian *et al*., 2012), which can signal irregularities in the production process and/or processing.

Lipids

The lipid content in bee pollen can be differentiated depending on the floral species visited and the environmental and climatic conditions (Oliveira *et al*., 2020). Lipid levels lower than those established by current legislation may indicate the degradation of lipids present in bee pollen.

Proteins

Research carried out on the protein content of bee pollen collected in various regions of Brazil has shown that it can vary from 15% to 28%, depending on the location and botanical origin (Nascimento *et al*., 2018). The great variability of protein levels found in bee pollen can be explained by the diversity of floral origin and biological, ecological and geographical factors related to its harvest and also by factors inherent to its handling and storage (Estevinho *et al*., 2012).

Total sugars

Carbohydrates are the largest constituent of bee pollen, accounting for almost two-thirds of the total dry weight of pollen (Li *et al*., 2018). The total sugar content outside the parameter established by Brazilian legislation, i.e., with levels above 55%, may suggest the addition of sugar to the product, and with levels below 14.5%, it may be due to the oxidation of sugars in the pollen from a normal aging process or due to excessive exposure to high temperatures (Almeida-Muradian *et al*., 2012).

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Crude Fiber

The legislation for bee pollen also determines the use of crude fiber analysis, which basically consists of determining cellulose with small amounts of lignin and hemicellulose (Embrapa, 2022). Crude fiber represents the residue of substances from cell walls. According to current legislation, the crude fiber content allowed in pollen is at least 2% w/w, on a dry basis (Brasil, 2001).

pH and free acidity

Measurements involving acidity (pH and free acidity) are important parameters in food analysis because they are associated with the conservation status of pollen, since decomposition processes (such as oxidation and fermentation) usually alter these parameters. In addition to these criteria, Brazilian legislation does not authorize the use of additives in bee pollen and determines that organic and inorganic contaminants cannot be present in quantities higher than the limits established in the specific regulation (Ferreira, 2020; Brazil, 2001).

There is still no legislation and standardization that promotes the formal commercialization of melipona pollen. In an attempt to contribute to the elaboration of appropriate legislation for pollen collected by stingless bees, several researchers sought to characterize melipona pollen from different species of stingless bees (Alves & Santos, 2018; Bárbara *et al*., 2015).

According to Neto *et al*. (2021), after collecting the pollen, the jars are closed for natural fermentation. The colors vary from yellow to brown, with a pH around 2.6 with a low number of microorganisms. Melipona pollen, i.e., stingless bees (ASF), as well as their honeys, generally have a high moisture content that gives it an unpleasant appearance for commercialization and with a greater probability of deterioration (Lima et al, 2018).

USE OF POLLEN IN INDUSTRY

Use of pollen in medicinal products

Pollen is known as a food-medicine, with beneficial effects on human health such as the proper functioning of the intestinal flora, the prevention of prostate problems, desensitization of allergies, degenerative diseases, atherosclerosis and neoplasms. Its production does not generate contaminants to the environment and does not pose risks to humans (Lima, 2018).

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In addition to its usefulness as a food supplement, pollen is used in other sectors, such as: in pharmacology, cosmetics, food, in beekeeping as food for bees in periods of drought and in the monitoring of environmental pollution (Jacb, 2014; Ramalho, 2018). Bee pollen is used in the production of dietary supplements in the form of tablets, capsules, and granules, and alcohol and aqueous extracts are also made from it (Rzepecka-Stojko et al., 2015).

Use of pollen in food

As it is a natural unprocessed substance, bee pollen is one of the richest raw materials in terms of quality and quantity of nutrients. There is a synergy between these nutrients that favors their use by the human body and for this reason the enrichment of food products with bee pollen has been a possibility increasingly tested by researchers (Serafini, 2013).

In view of the beneficial characteristics described, the insertion of pollen in everyday foods, such as bakery products, is an option to insert it into the diet in a pleasant way, as it is currently consumed mainly as supplements in encapsulated form (Krystyjan et al., 2015).

The concern in the search for healthier foods has extended to the food industry, which often resorts to the use of synthetic food additives, particularly substances with antioxidant activity (Lema, 2020).

Stingless bee pollen has also been gaining ground in haute cuisine, being used mainly as a seasoning, due to its strong flavors (Villas-Bôas, 2018). Bee pollen is used in the production of dietary supplements in the form of tablets, capsules, and granules, and alcohol and aqueous extracts are also made from it (Rzepecka-Stojko et al., 2015).

In a study carried out by Machado et al (2018), we sought to evaluate the effects of the use of pollen, in different concentrations, in mead. Where the results showed that at low concentrations, the added pollen can be an appropriate activator to overcome nutrient limitations.

Another research carried out by Freitas et al (2020), it was decided to partially replace the use of wheat with pollen in the preparation of cake, with a view to the nutritional enrichment of the final product. According to the authors, the results showed that enrichment interferes with the browning of the cake and makes it softer. Factors that were observed in the proportion of 10% pollen, on the other hand, it was found that these characteristics do not make the product unpleasant.

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Lima (2018), aimed in his work to prepare a cereal bar supplemented with melipona pollen with formulations of 5% and 10% pollen. At the end of the study, the formulation with 5% pollen was the one that obtained the best scores for the ideal scale and the highest purchase intention (60%) on the part of the tasters.

In a study conducted by Braga *et al,. (2019),* where the objective was to elaborate and characterize the mousse of siriguela added to bee pollen aiming at the creation of a functional product. An experimental work was carried out on the elaboration and formulation of siriguela mousse with different concentrations of bee pollen. The results of phenolic compounds and vitamin C indicated that the addition of bee pollen promoted an increase in bioactive compounds. Therefore, it is concluded that the addition of bee pollen to mousse generates a product with potential as a functional food due to the presence of phenolic compounds and vitamin C, but sensory acceptance tests are necessary.

A study developed by Brochard *et al,.* (2021). aimed to make fortified doughs incorporating chestnut flour (25–55%) and pollen powder (5–20%), alone or in combination. It was noted that the incorporation of powdered pollen (up to 20%) in pasta prepared with wheat flour and water or fresh egg reduced the cooking time and cooking yield, both in fresh and dry pasta.

CONCLUSION

Pollen is a functional food with several beneficial health properties promoting a better quality of life for those who consume it. Proper processing of pollen is crucial to maintain its properties and prevent the occurrence of microorganisms due to its high moisture content and nutritional value. There is a need to create legislation for Melipona pollen, to establish quality parameters for stingless bee pollen, which will help ensure its safety and efficacy in the food industry.

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