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Biological Elements and Residues in Brazilian Honeys

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ABSTRACT

Honey is a product appreciated worldwide and is subject to easy tampering throughout its production cycle. A survey of 187 honey samples from different botanical origins and regions was conducted to identify their biological and other elements, using the Melissopalynology methodology to assess the quality control. In the sampling, biological elements are mainly represented by parts of plants, mites and microbes (65%), the amount of dirtiness is high (32%), calling attention to the presence of insect body fragments, starch grains and soot, such as the most frequent (24%). The level of elements due to the addition of the frequencies of microorganisms and dirtiness is 58% in the samples, which could be an indicator of contamination in studies of Melissopalynology. These results bring new data to assess some risks linked with honey consumption and also emphasize that there should be greater rigor in the basic quality control of this product. Monitoring program of honeys should be continuous throughout its production cycle, starting from the field activities, harvesting, processing, and storage until reaching the consumer's table.

Resumen

La miel es uno de los alimentos más apreciados en el mundo y por esta razón facilmente se detecta su adulteración a lo largo de su cadena de producción. En una encuesta por 187 muestras de miel de diferentes orígenes botánicos y regiones ha sido realizada para identificar sus elementos figurativos de la miel mediante el análisis melisopalinológico de modo a evaluar la calidad de este producto. En la muestra, los elementos biologicos son representados principalmente por residuos vegetales, ácaros y microbios (65%), La frecuencia de partículas de suciedad se ha notado alta (32%), a lo que se constato restos de insectos, gránulos de almidón y hollín, que son los de mayor frecuencia (24%). El nivel de elementos es 58% cuando se añade las frecuencias de microorganismos y partículas de suciedad presentes en las mieles, lo que puede ser un indicador de contaminación en estúdios de melisopalinologia. Esos resultados traen nuevos datos para evaluar algunos riesgos que hay que tener con el consumo de la miel además sugieren a que lleven un mayor rigor en el control básico de calidad en mieles. El programa de monitoreo debe ser continuo durante todo la cadena de producción, desde las primeras actividades en campo de las colmenas, durante la cosecha, en el procesamiento del producto, en su transporte, en el almacenamiento en la tienda y hasta en la mesa del consumidor.

INTRODUCTION

Honey is a popular natural food produced by honey bees (*Apis mellifera*), that is known to be an energetic and health beneficial nutritional option (Vanhanen et al., 2011).

Its complex composition, due mainly to the presence of organic and inorganic compounds, is strongly influenced by both natural and anthropogenic factors, which vary based on its geographical origins. Alves (2008), Pereira (2008) and Mendes et al. (2009) classified two types of sources that may promote the contamination of honey: primary sources from flower resources, water, air, soil, etc., when bees absorb unwanted elements; and secondary sources, such as procedures for bee management, harvesting, processing and storage of honey, practiced by beekeepers unintentionally. These conditions expose honey to physical, chemical and biological agents (Sebrae, 2009; Lorenzon et al., 2017). The actions of these related agents contaminate honeys silently. When the level of contamination is high, it is a significant indicator of a deficiency in bee health and of the state of the environment (Snowdon and Cliver, 1996).

In order to yield medicinal effects, and to be used as a natural sweetener, honey should be free of any contaminants, using also analyses of the types of elements for evaluating honey quality. Nowadays, there is an increasing interest in the study of biological systems, however this is not a trivial task, because the behavior of the biological system, biological objects and their reaction to human intervention is unpredictable (Mckee, 2003; Zacepins and Stalidzans, 2012). Currently, searches for trace element content of honey is more related to non-natural sources (eg Vincevica-Gaile et al., 2011; Alqarni et al., 2012; Solayman et al., 2016; Siliciet al., 2016), other studies pointed out some minerals associated with the soil where melliferous flora grows (eg Fernández-Torres et al., 2005, Ariyama et al., 2006; Baroni et al., 2009, Batista et al., 2012; Yücel and Sultanoğlu, 2013). However, the presence of biological elements should also be evaluated searching for signals of synergism they may show with the environment.

Due to the quick development of beekeeping in many countries, while preventing the honey quality, this study is aimedat presenting types of elements and residues in honey and also at evaluating its quality focused on microbiological and morphological methods.

METHODOLOGY

A set of 187 varieties of natural honeys were obtained by donation and purchase in stores and apiaries. The samples were gathered from 51 counties in the state of Rio de Janeiro, which is an important commercial center and the second largest economy in Brazil. Some beekeepers and entrepreneurs reported that they usually sell local and imported honeys in the domestic market.

Samples were prepared following Louveaux et al. (1978) methodology, using 10 grames of honey per sample. Light microscopy of 40x and 100x magnification was used to check the presence of trace elements, and to count and identify the pollen types. Acetolysis was excluded to prevent destruction of morphological components and to preserve honey quality. During the count of pollen grains, the presence of yeast, bacteria, crystal, fungi, starch grains, soot, raphides, vegetal tissue, honeydew and other components were registered and photographed by a digital camera. These elements were classified and their frequencies (%) were calculated.

RESULTS AND DISCUSSION

Two criteria for classification of elements were proposed: (a) biological: they help to evaluate the environment; and (b) dirtiness: they are strange and can be easy to find out. These criteria were adopted considering the variety of elements that occurred in the honey (Table 1) and based on the following reviewers. Barbieri et al. (2001) suggested qualifying the elements in honeys in organic or inorganic, live or inert, harmful or harmless. Ziobro (2000) considered dirtiness as heavy, light and easily sifted elements in honeys. Additionally, according to the Brazilian legislation (Brasil, 2000; 2010), elements of any nature that compromise the quality and identity of honey should be named as strange substances, such as insect body parts, larvae, sand and others.

Biological elements originated by plants occur in honeys when honey bees forage on flowers or other organs of the plants searching for trophic resources. These are elements found in honey analysis: (1) pollen grains, mainly associated with body hairs of bees; (2) fragments of vegetal tissue, such as raphides, parts of cell walls and sclerenchymatic cells; (3) grainy mass originated from leaf surfaces that can be found in a diffused form or as a compact and agglomerated form; (4) amyloplasts originated from broken pollen grains or from the air (atmospheric) sediments. In air sediments, starch grains are always larger than pollen amyloplasts. In honeys, starch grains are rare and can be identified as elements in the shape of a cross-malt (Barth personal observation; (5) algae from water; (6) oil, commonly from visited flowers of Malpighiaceae and Solanaceae; (7) fruit sediments (Fig. 1).

The microorganisms that occur in several places visited by bees are discharged as biological elements. They are also part of the industrial processes of honey, whose level can affect the honey's safety. These biological elements found in honeys included: (1) yeast, fungi, fungi spores and hyphae of fungi, (2) mites and (3) bacteria (Fig. 2).

Dirtiness or undesirable materials are non-components of honey. These residues appear under inappropriate sanitary conditions that can result in different levels of contamination. The presence of these elements in honeys make them a vehicle to detect pollutants, as follows: (1) synthetic fibers; (2) starch grains; (3) sand grains and crystals; (4) soot (caused by fumigation of beehives or industrial residues); (5) parts of equipment and materials; (6) fragments of insects (Fig.3).

The frequency of dirtiness in our honey samples is high (32%), highlighting the presence of fragments of insect bodies, starch grains and soot, this last residue being the most frequent one (24%). Mallman (2010) noticed the presence of dirtiness and other matters ranging between 29% and 71%, whose average level was high. Honey samples that came from the Brazilian northeast, which were registered by Sousa and Carneiro (2008), showed a level of 65% in dirtiness, putting these samples out of the Brazilian standards, thus disqualifying such honey. Lima Silva et al. (2008), Lieven et al. (2009) and Sereia et al. (2011) reported the level of contaminants and dirtiness higher than 50% affects the purity of honeys. Cordeiro et al. (2012) reported no impurities or other elements in honeys from the Brazilian northeast under microscopy analysis. Previous studies showed the most frequent dirtiness in honey samples: Mallman (2010) fragments of insect bodies and pieces of wood; Sousa and Carneiro (2008) mites, fragments of insect bodies and larvae; Martins et al. (2014) high level of fragments of insect bodies (68%).

In some honeydew samples, grainy mass, soot particles, spores, hyphae of fungi and frequent pollen grains from anemophilous plants, according to Barth (1989), were also found. However, the level of these elements in honeydew is relatively low (13%) and follows the standards of honey from other Brazilian regions (Barth, 1970; Freitas, 1991; Aires and Freitas, 2001).

When analyzing all honey samples presenting microorganisms and dirtiness, the level of elements rises to 58% (n=109), which requires careful assessment. As stated in the Brazilian legislation (Brasil, 2000; 2001), the honey must be free from contamination of chemical products, as well as from solid particles transported by the air, objects from manipulators and processing, other strange substances and strict occurrence of microorganisms.

The presence of yeast, fungi, spores, hyphae, bacteria and mites in honey samples in the present study is alarming as well, and reached the level of 26%. Some bee diseases caused by fungi have not yet been notified by law in Brazil, and the proposed analysis may offer an important signal. Researches conducted by Pacheco et al. (2009) and Keller et al. (2014) have shown the occurrence of the disease known as Brazilian sac brood (BSB), which is due to some pathogenic fungi and their mycotoxins. These authors recorded unusual frequency of fungal spores and hyphae in honeys, although honeys did not have structure that favors the growth and maintenance of fungi. Thus, the presence of this element in honeys could be an important indicator of high incidence of fungi in honey bee hives.

Previous researchers are warning about the increase of the biota in honeys. Lima Silva et al. (2008) warned that honey samples from Brazilian southeast were above the recommended limits of microorganisms; Sereia et al. (2011) in the south of Brazil, Lieven et al. (2009) and Sodré et al. (2007) in the Brazilian northeast reported similar results as well. Ananias (2010) reported the level of 33% (n=66) of colonies of fungi and yeasts from Brazilian central region. Santos et al. (2010) and Gois et al. (2015) also noticed similar levels in honeys from Brazilian northeast as well. Otherwise, the organic honey samples from some regions of southern Brazil were within the limits of safe food, according to Alves et al. (2009). As noted above, most of authors found microbes as such level that make them primarily responsible for the contamination of Brazilian honeys, indicating a deficiency in the application of good practices in beekeeping. Rosa et al. (2006) warned the presence of fungal filaments in foods, which can lead to deterioration by the action of certain enzymes and toxic metabolites.

The results of our study point out that the Melissopalinology analysis (as microscope analysis of foods) is an important instrument for evaluating certain kinds of adulteration of honeys, as also emphasized by Russman (1998) and Barbieri et al. (2001).

The complexity structure of honeys, providing nutritional and therapeutic values, the processes in the production cycle, from the region of honey production until its storage, including the consumers home, deserve more cautiousness. Likewise, the quality control of honeys should be strengthened following the current legislation in order to guarantee credibility and the consumer’s appreciation of the product.

Table 1.Classification and frequency (%) of structured elements in Brazilian honey samples.

Elements	Frequency	Elements	Frequency
Biological origin	*Total-65%*		
01.Grainy mass	14%	08.Yeast	10%
02.Vegetal tissue	10%	09.Fungi spores (9%), hyphae (1%)	10%
03.Amyloplasts	07%	10.Fungi	03%
04.Lumps and clots	03%	11.Bacteria	02%
05.Oil	02%	12.Mites	01%
06.Algae	01%	13.Sediments (from fruits)	01%
07.Pollen grains	01%		
Dirtiness	*Total– 32%*		
1.Insect fragments	13%	5.Sand/dirtiness grains	03%
2.Starch grains	06%	6.Residues of materials and equipments	01%
3.Soot	05%	7.Sintetic fibers	01%
4.Organic material	03%		
Others	*Total–3%*		
1.Crystals	01%	3.Wax	01%
2.Trichomes	01%		

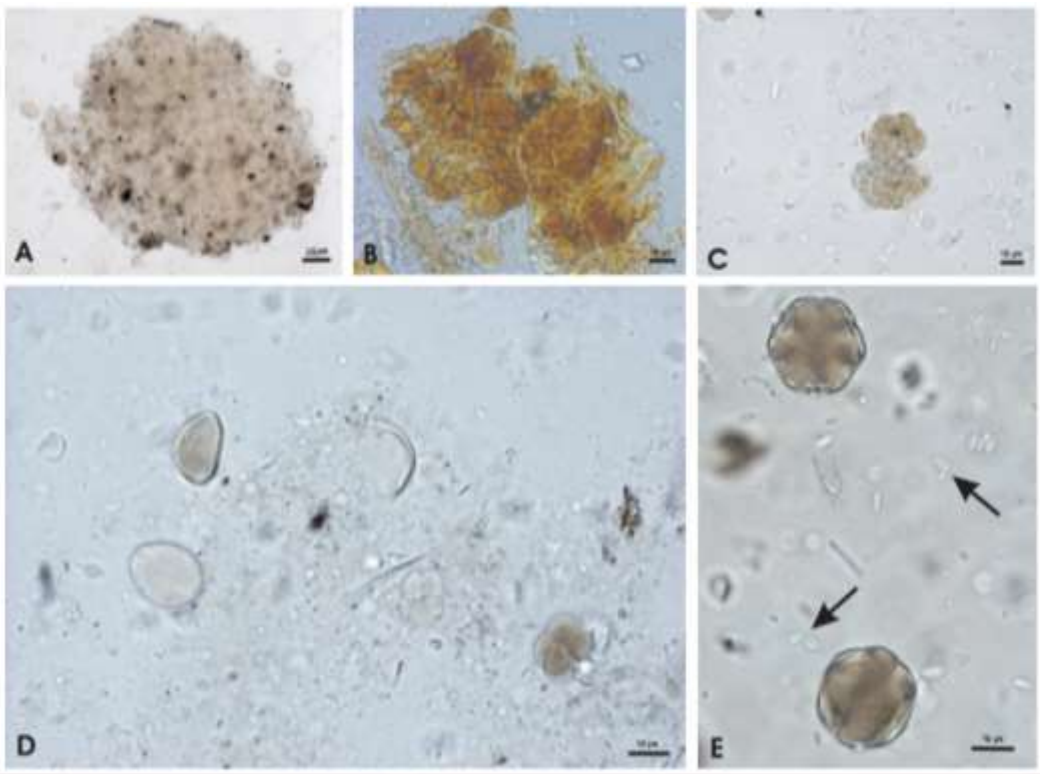

Figure 1. A – organic amorphous mass. B – vegetal tissue. C – algae colony. D – vegetal grainy mass and pollen grains. E – two pollen grains of Melastomataceae and yeast spores (arrows).

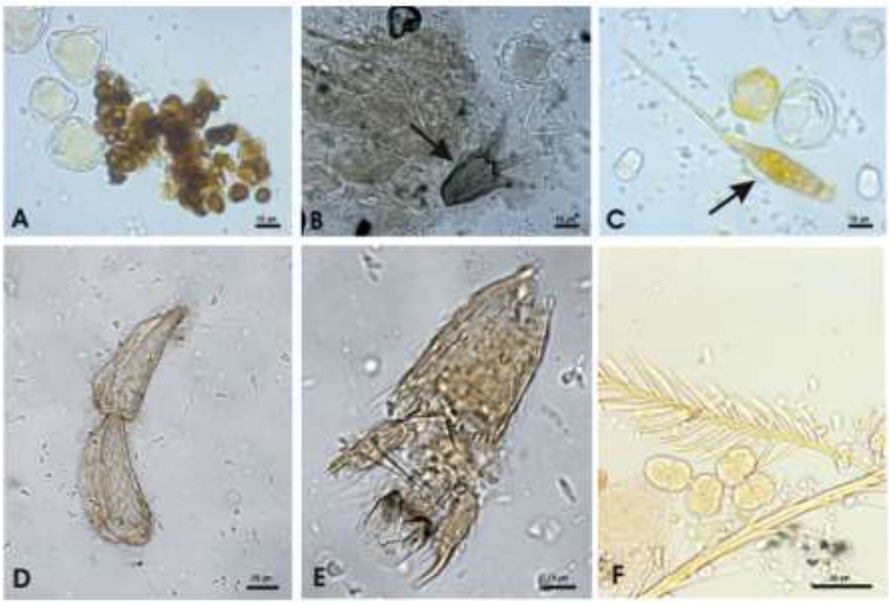

Figure 2. A–pollen grains and a group of dark spores of fungi.B–tetraspore of fungus (arrow). C–spore of *Alternaria* fungus (arrow). D–fragment of an insect leg. E–fragment of an insect carapace. F–insect bristles and *Mimosa* pollen grains.

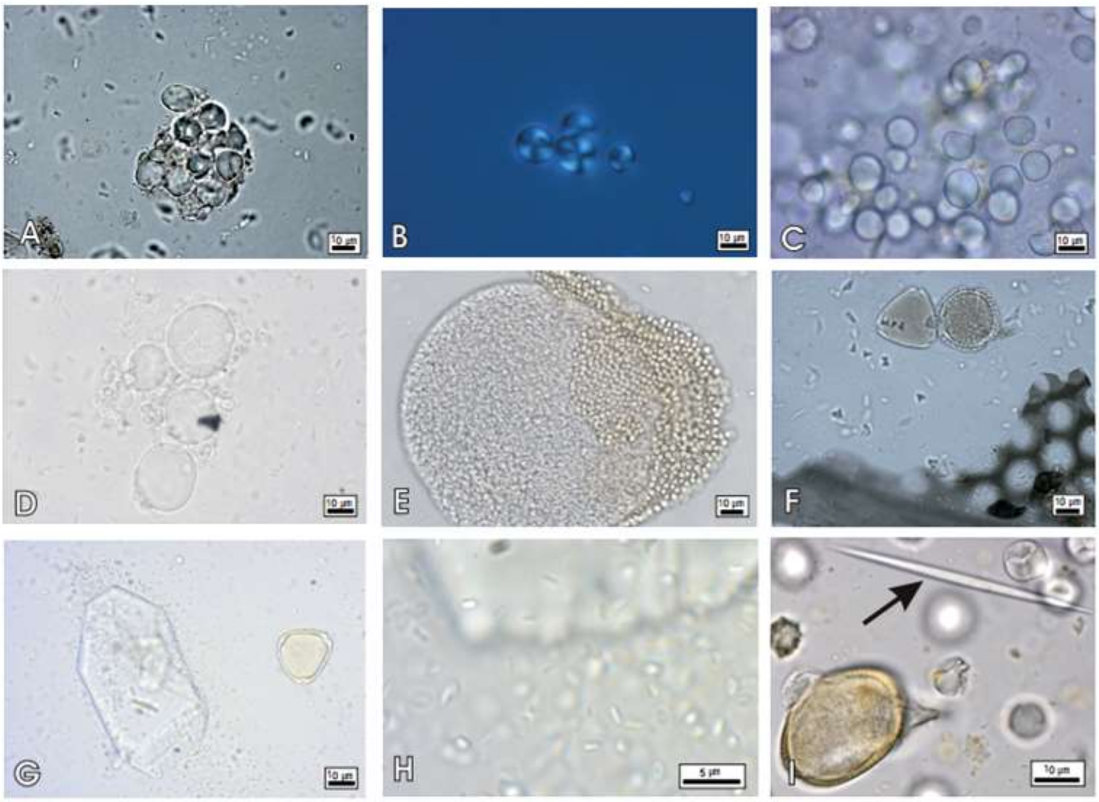

Figure 3. A–starch grains in transmitted light microscopy. B–starch grains in polarized light. C– bloated starch grains. D– extremely bloated starch grain. E–broken pollen grain of *Croton*, amyloplats filled the cytoplasm; exine is located on the right corner of the pollen grain. F–carbonized vegetal tissue and two pollen grains.G–silica crystal and one pollen grain of *Myrcia*.H–previous enlarged image. G- rod-shaped bacteria. I–raphide (arrow), pollen grain and crystals.

Competing interests

We showed the need and the relevance to adapt the bee products to the market reaching the best quality required by the legislation and to enhance the technical services. Thus, our team will be assisting producers who must achieve more competitive prices in the market of bee products.

Authors' contributions

M Lorenzon, S Linhares – They carefully organized the database and tables, reviewed and wrote the manuscript.
O Barth – She performed in the pollen and residues analysis of the experiment, made the scientific figures and reviewed the manuscript.

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