Engineering in Agriculture, Environment and Food 11 (2018) 57–64

Contents lists available at ScienceDirect

Engineering in Agriculture, Environment and Food

journal homepage: www.elsevier.com/locate/eaef

Influence of drying-related operations on microbiological, structural and physicochemical aspects for processing of bee-pollen

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ARTICLE INFO

Keywords:
Bioactive compounds
Calorimetry
Exine
Quality
Thermogravimetry

ABSTRACT

Bee-pollen is an important source of nutritional and bioactive compounds. Given its high moisture content, bee-pollen is dried before commercialization; however, there are some drawbacks in the microbiological quality, which have a remarkable effect on the physicochemical stability. The aim of this study was to evaluate the influence of drying on bee-pollen at temperatures between 40 °C and 60 °C based on microbiological, structural and physical-chemical characteristics. The obtained results showed that bee-pollen dried at 40 and 50 °C had a significant increase in acidity after treatment, possibly due to the remain microbiological load. In contrast, a significant increase in flavonoids, phenolics and antioxidant activity was found for all thermal treatments; nonetheless, a loss of carotenoids by the effect of temperature was also obtained. Microscopy images showed a slight degradation in the bee-pollen structure, which may have caused the release of bioactive compounds, increasing the antioxidant capacity. A total ranking technique suggested the most adequate temperature for drying bee-pollen at 60 °C.

1. Introduction

Bee-pollen is a product collected from the hive and intended for human consumption because of its highly valued nutritional and bioactive composition. Besides carbohydrates, protein and lipids, it is a rich source of phenolic compounds and carotenoids (Zuluaga et al., 2016). Phenolic compounds, one of the main sources of secondary metabolites in bee-pollen, are responsible for the color of the grain (yellow, brown, red, purple, etc.), and its characteristic bitter taste (Bogdanov, 2011). Moreover, carotenoids are also important for color and other biological functions, such as antioxidant activity, pro-vitamin A activity, and the enhancement of the immune system (Fernández-García et al., 2012). This product must be considered as a complex food, not only for its microbiological quality, but also from the points of view of grain structure, as well as nutritional and bioactive composition.

Once collected, most beekeepers usually dry bee-pollen in order to increase its shelf –life and to avoid product spoilage. Once harvested, the bee-pollen grain has a moisture content which usually ranges from 20% to 30% (Campos et al., 2010); in consequence, it must be immediately subjected to moist reduction processes, until free water content is lowered to between 5 and 8% (Almeida-Muradian et al., 2005). Few countries such as Brazil, Argentina, Switzerland, Spain or Mexico have established official quality standards, setting temperature limits for bee-pollen drying lower than 42 °C (Almeida-Muradian et al., 2005; Bogdanov, 2011; Campos et al., 2010). However, moisture content is not the only aspect to take into account during bee-pollen processing.

From a structural point of view, it has been observed that physical processes in bee-pollen are necessary operations, since they are required to modify the external structure of the grain, known as exine, which is extremely rigid and limits the absorption capacity of nutrients into the human digestive tract (Choi et al., 2016). In addition, different studies assert that bee-pollen drying must be done at moderate temperatures in order to avoid the degradation of thermolabile compounds, such as vitamins or carotenoids (Campos et al., 2010; Domínguez-Valhondo et al., 2011; Oliveira, 2006).

Prior experience has showed us that a drying temperature close to 42 °C may not efficiently favor the removal of moisture and does not guarantee the elimination of microorganisms; on the contrary, it might even stimulate the growth of the microbial population. This aspect is particularly marked in tropical bee-pollens, where the microbiological loads from the surrounding environment to the apiaries are often high,

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https://doi.org/10.1016/j.eaef.2018.01.003
Received 23 June 2017; Received in revised form 12 December 2017; Accepted 12 January 2018
Available online 16 January 2018

even if Good Manufacturing Practices (GMP) are applied. While it is true that new technologies have been proposed for reducing moisture in bee-pollen with a minimum loss in thermolabile compounds, such as lyophilization, it still does not appear to be a feasible option either economical or technologically for beekeepers, in particular small and medium producers.

Based on this, a balance between a minimal loss of nutritional and bioactive compounds and a successful reduction in the microbiological load must be found, when thermal treatments are employed in bee-pollen, as it has happened in other kind of foodstuff (e.g. milk). The use of low temperature for bee-pollen drying has led to commercialize bee-pollen that in some cases have had an inadequate management and may even be unsuitable for human consumption, as it has already been reported (Deveza et al., 2015; Nardoni et al., 2016).

Likewise, it would be desirable a thermal treatment that could modify the microstructure of the exine, thus nutritional compounds would be more available. Different reports of plant origin foods, especially cereals or legumes, show that physical processes are a useful strategy for: i) modifying food structure, in order to increase the availability of nutritional and bioactive substances or eliminate anti-nutritional factors, and ii) reducing the microbial load, which affects the product safety (Hernández-Carrión et al., 2014).

In this context, this paper evaluates the influence of different drying temperatures on the microstructural, physical and chemical characteristics of bee-pollen, in order to ensure quality criteria related not only to safety, but also to nutritional and bioactive conditions. The aim was to study three different temperatures (40 °C, 50 °C and 60 °C) in a conventional hot air drying device and evaluate the structural, microbiological and bioactive composition of treated bee-pollen. Data were analyzed by means of ANOVA, meanwhile a Principal Component Analysis (PCA) was employed for a global comprehension of information. Finally, a Total Ranking Method was used as a multicriteria decision making technique to classify in an objective order the studied options, through desirability and utility functions for each attribute, giving as a result the selection of the best treatment.

2. Materials and methods

2.1. Samples and treatments

2.1.1. Bee-pollen

Bee-pollen samples for this study were taken from the most important region for this apicultural activity in Colombia, covering most commercially available varieties of this product in the country. This region is the Cundiboyacense high plateau, located at an altitude higher than 2500 m.a.s.l., on the central part of the Colombian eastern Andes. The palynological characterization revealed that *Hypochaeris radicata* and *Brassica* sp. were the most abundant plant families. Bee-pollen was stored in bags and kept in refrigeration (2 °C ± 1 °C) until thermal treatments assays.

2.1.2. Drying assays

Drying of bee-pollen grains was performed in a conventional hot air oven (Heraeus Vötsch VMT 07/35, Germany) at three different temperatures: 40, 50 and 60 °C, keeping a fixed air flow of 3 m/s. All drying assays were performed in triplicate. For each operation, 1.5 kg ± 0.1 kg of bee-pollen was used. Preliminary assays showed that at these temperatures, a drying time of six hours is enough to achieve a final moisture content lower than 8%.

2.2. Methods

2.2.1. Thermogravimetrical analysis

The measurement was carried out on a 2050 thermogravimetric analyzer (TA Instruments, USA), with Thermal Solutions Software. All analyses were performed with approximately 10 mg of sample. Measurements were made under a nitrogen atmosphere (100 cm^3/min) and a heating ramp of 10 °C/min.

2.2.2. Differential scanning calorimetry

The procedure was carried out as reported by Buitink et al. (1996). Approximately 10 mg of sample were placed in a sample container for this type of test, which was sealed and placed in the device under a hermetic and adiabatic environment. The thermograms were recorded between 25 and 200 °C in a modulated system at a rate of 10 °C/min, using nitrogen as a purge. A Modular DSC 2910 (TA Instruments, USA) was used with Thermal Solutions Software.

2.2.3. Scanning electron microscopy (SEM)

Samples were mounted on SEM stubs and sputter coated with gold. Specimens were examined and pictures obtained with FEI Quanta 200 Scanning Electron Microscope (Hillsboro, USA).

2.2.4. Microbiological analyses

Microbiological quality of bee-pollen was assessed according to procedures established by the American Public Health Association (Doores et al., 2013). A sample dilution was performed by weighing 10 g of bee-pollen and dissolving it in 90 mL of sterile 0.1% peptone water (Scharlau Chemie S. A, Spain). As many successive dilutions as necessary were done, by taking 1 mL of the prepared solution and dissolving it in sterile peptone water 9 mL.

2.2.4.1. Total aerobic bacteria. One mL from the first three dilutions were taken and placed in sterile Petri dishes, adding SPC agar (Standard Plate Count, Scharlau Chemie S. A, Spain) growth medium until its solidification. Dishes were incubated at 37 °C for 48 h. Counting was performed and reported as CFU/g pollen.

2.2.4.2. Molds and yeasts. One mL of inoculum was placed in sterile Petri dishes after adding 20 mL of OGY agar Oxytetracyclin Glucose Yeast, (Scharlau Chemie S. A, Spain). Dishes were incubated at 25 °C for seven days. Counting was reported as CFU/g pollen.

2.2.4.3. Salmonella. Initially, a pre-enrichment by weighing 25 g of bee-pollen and mixing it with 225 mL of lactose broth was performed (composition per liter: beef extract 3 g, peptone 5 g and lactose 5 g, Scharlau, Spain). This mixture was incubated for 24 h at 37 °C. From this selective enriched culture, 1 mL in 10 mL of a selenite and tetrathionate broth (Oxoid, UK) was added and incubated at 43 °C for 24 h. Finally, a depletion seeding in XLD (Xylose Lysine, Deoxycholate, Scharlau, Spain) and Hektoen agar (Scharlau, Spain) was done. Dishes were incubated at 35 °C for 48 h. A visible growing in any of the agars indicated the presence of *Salmonella.*

2.2.5. Physicochemical analyses

2.2.5.1. Moisture. Three g of sample was weighed and heated at 65 °C for 24 h. Moisture content was obtained by difference (Fuenmayor et al., 2014).

2.2.5.2. pH and acidity. A bee-pollen sample of 2.5 g was mixed with 35 mL of CO_2-free water and titration was started with NaOH (Chemi, Italy) 0.05 N until pH 8.5. pH was measured using a potentiometer previously to titration (Fuenmayor et al., 2014).

2.2.5.3. In vitro digestibility. A sample of 1.5 g of dried-defatted bee-pollen was mixed with 150 mL of a 0.002% pepsin (Sigma Aldrich, USA) in HCl (Chemi, Italy) 0.075 N solution. This mixture was subjected to enzymatic digestion by 16 h at 45 °C and kept in agitation. Then, the content was filtered and the protein of both, the non-digestible and digestible portions, was measured by the Kjehldahl method. Digestibility was expressed as the ratio among the protein content of the digestible part and the original protein content of the