

Effect of Solar Drying Process on Bioactive Compounds and Antioxidant Activity *In Vitro* of High Andean Region Bee Pollen

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Bee pollen is a hive product of common consumption with a known and remarkable content of bioactive and functional compounds, namely carotenoids and phenolic compounds. The bee pollen from the Colombian High Andean region has a prominent content of these compounds due to environmental and botanical conditions. In this work, the process of bee pollen drying by solar dehydration was evaluated to determine its influence on functional compounds and antioxidant activity. This process was carried out in a greenhouse type solar system located in the central region of Colombia into the High Andean region. Drying tests were conducted for two consecutive days during the dry season. The tests were developed for 8h, collecting samples at the beginning ($t = 0h$) and at the end ($t = 8h$) of the process. The contents of total carotenoids, total phenol compounds and antioxidant activity *in vitro* of samples were measured by spectrophotometric standard methods: a spectrophotometric measure of acetone extract for carotenoids, Folin-Ciocalteu for phenol compounds and TEAC/FRAP for antioxidant activity. The content of carotenoids in dry bee pollen was in average 0.9mg β -carotene/g. Besides, the content of phenolic compounds of bee pollen processed in the solar system was 16mg gallic acid/g. The content of carotenoids and phenol compounds in dry bee pollen did not change significantly from initial raw bee pollen. Furthermore, the antioxidant activity of raw bee pollen (0.85mmol Trolox/g) was maintained even after the drying process in the solar dehydration system. The results show that there was no impact of the solar drying process on bioactive compounds content of bee pollen. Therefore, this type of solar drying system is an actual alternative to traditional equipment for bee pollen drying at this region, favoring a lower energy cost and with a no evident influence on the product quality.

1. Introduction

Bee pollen is a hive product produced by bees from floral pollen grains by agglomeration and the addition of nectar and saliva (Margaoan, Marghitas et al. 2010). This food is considered a good source of nutrients as essential amino acids, minerals (Cu, Fe, Zn), reducing sugars and vitamins: β -carotene, tocopherol, niacin, thiamine and folic acid (Campos, Bogdanov et al. 2008). In addition, the bee pollen is a food potentially beneficial to human health, due to the presence of anti-inflammatory and antioxidant compounds such as phenols and carotenoids generated in plant metabolism with functional properties (Ares, Valverde et al. 2018). These properties help to prevention of coronary and neuro-degenerative diseases (Feás, Vazquez-Tato et al. 2012). The Colombian “Altiplano” Cundiboyacense, located in the mid-eastern Andean mountain chain between Boyacá and Cundinamarca with coordinates 5°31'56" N and 73°21'24" W, has a high potential to produce bee pollen with an annual average production of 40kg per hive (Zuluaga, Serrato et al. 2015). The bee pollen from this region has average levels of 24% protein, 6% fat and 3% minerals, and a content of

bioactive compounds due to metabolites of the botanical sources of the area (Zuluaga, Serrato et al. 2015). The main compounds of this type found in pollen are phenylpropanoids, flavonols (phenols) and β -carotene (Morais, Moreira et al. 2011).

The bee pollen is a product highly susceptible to chemical and microbiological degradation (Puig-Peña, Risco-Ríos et al. 2012). In Colombian apiaries, the harvest and storage conditions with long periods of exposure to adverse atmospheric conditions such as high humidity and large temperature fluctuations, favored this degradation. The drying allows the preservation of the product and ensures its quality and safety (González, Hinojo et al. 2005). Since some of the compounds with functional characteristics in pollen are unstable when exposed to temperature changes, drying conditions affect the final content of these components and process parameters such as drying speed (Isik, Ozdemir et al. 2018). In general, pollen is dried in hot-air drying cabinets. The temperature used by beekeepers for drying pollen varies between 40 and 50°C, due to the nutritional and functional degradation that it is believed to occur when dehydrated to higher temperatures, although previous studies have demonstrated that such degradation may not significantly influence the content of bioactive compounds, except for carotenoids (Pulido, Salazar et al. 2012).

On the other hand, solar drying equipment is widely used for drying of vegetable products, but its use for bee pollen has been wrongly discarded by the belief of negative impact on the nutritional and bioactive properties of this food for sun radiation (Fudholi, Sopian et al. 2010). The use of this system significantly generates energy savings and has a positive environmental impact, reducing the carbon footprint of the process (Sing, Lim et al. 2018). Colombia has a great potential of solar radiation as energy source, given its location in the equatorial strip (Fudholi, Sopian et al. 2015). Therefore, the objective of this work was aimed at evaluating the impact of the solar drying process on the bioactive characteristics of bee pollen, as an alternative to traditional hot-air cabinet drying, under the specific environmental conditions of Colombian High Andean region.

2. Materials and methods

2.1 Bee pollen

The bee pollen was extracted directly of hives from the southwest region of Boyacá, located principally at high Andean forest and Paramus zones, and transported to the beekeeping processing facilities near to apiaries, in the same region.

2.2 Drying process

Fresh raw bee pollen was arranged in trays of plastic mesh with wooden frame with an approximate 60cm wide by 90cm long (see Figure 1a). The pollen was distributed on the trays to a thin layer of uniform thickness of 1cm. Subsequently, the trays with the pollen were placed inside the drying systems.

First the bee pollen was dried for 8h in a traditional cabin system. The equipment consists of a wooden chamber of external dimensions 66.5cm x 95.0cm x 158.5cm, with wooden walls of 0.5cm thick, with insulating coating of polyurethane foam of 1.5cm thick and an air heating unit based on an electrical resistance of 1500W of power; the system works with forced air circulation using an axial flow fan with AC supply and a humid air vent tube in the upper side. The average temperature during process was 50°C and was used an air-flow speed of 2m/s. These tests were done by triplicate, to have a comparison baseline. A diagram outline of the tray dryer used in the trials is presented in Figure 1b below.

2.3 Solar drying process

The harvested raw bee pollen was dried in a solar dehydration system with greenhouse configuration (see Figure 2). The bee pollen was disposed on the previously described trays, trying to reach a thin layer of product, with around 700g in each tray and a total of 8 trays into the system. The drying solar process was carried out at two consecutive days for 8 h between the 7:00 a.m. and 15:00 p.m. hours, taking advantage of the higher solar brightness.

Bee pollen samples were taken from the system during each hour from 0 to 8 hour, during the drying processes. The samples were stored in hermetically sealed glass jars for later analysis in the laboratories of the Instituto de Ciencia y Tecnología de Alimentos (ICTA) of the Universidad Nacional de Colombia.

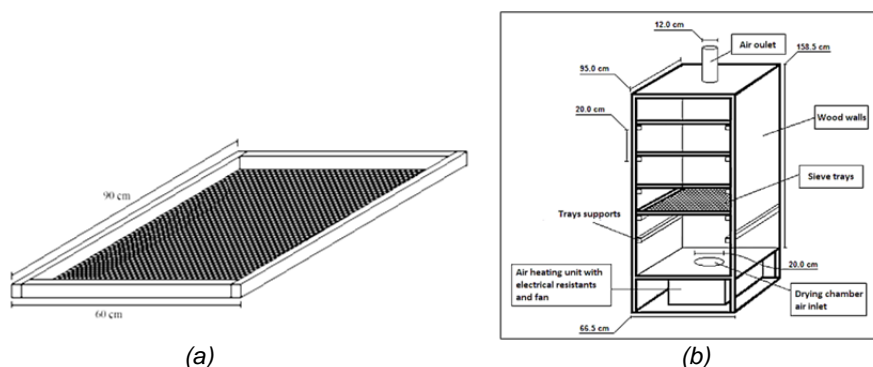


Figure 1. (a) Schematics of single tray with plastic mesh and wood frame and (b) perforated tray dryer used for drying bee pollen.

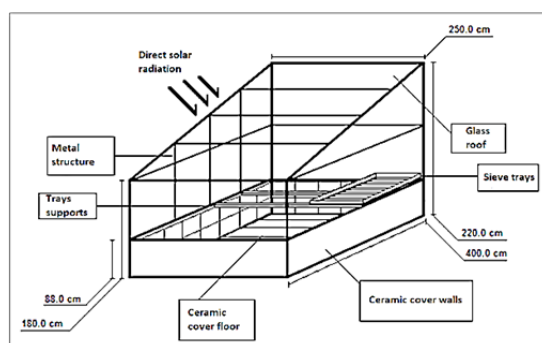


Figure 2. Diagram of the solar dryer used in bee pollen dehydration tests.

2.4 Bee pollen characterization

Total carotenoids content

The carotenoid content in the pollen of the initial and final samples of the drying process was measured. The measurement was made on the extracts of the samples, by means of the spectrophotometric method at an absorbance of 450nm. The extracts were obtained by diluting 50mg of the sample in 2mL of acetone for subsequent centrifugation and filtration; these extractions were carried out successively until the solution was discolored. Finally, the filtered solution was completed up to a volume of 25mL. The extracts thus prepared were arranged in quartz cells for measurement in an UV-Vis spectrophotometer (ThermoScientific, Genesys 10S). The results obtained were expressed as mg- β -carotene/g dry matter (Schulte, Mäder et al. 2009).

Total phenols content

The phenol content of the initial and final samples of dried pollen was measured by the Folin-Ciocalteu method. The extraction process was carried out by dissolution of 1g of the sample in ethanol (96%) as solvent (30mL) for 24h under dark conditions. Subsequently, the solution was filtered and transferred quantitatively to a volume of 100mL. Finally, the absorbance on aliquots of the sample extracts was measured by spectrophotometric method, at a wavelength of 765nm in an UV-Vis spectrophotometer (ThermoScientific, Genesys 10S). The absorbance results obtained for each sample were expressed as gallic acid/g dry matter (Stratil, Klejdus et al. 2006).

Antioxidant activity

The antioxidant activities of the initial and final samples of the drying process were obtained by TEAC and FRAP methods. The extraction process was carried out by dissolving 1g of the sample in ethanol (96%) as a solvent (30mL) for 24h under dark conditions. The solution was filtered and transferred quantitatively to a volume of 100mL. Finally, the absorbance on aliquots of sample extracts was measured by spectrophotometric method, at a wavelength of 734nm (TEAC) and 593nm (FRAP) (Brand-Williams, Cuvelier et al. 1995).

In both cases, an UV-Vis spectrophotometer (ThermoScientific, Genesys 10S) was used for the measurement. The absorbance results obtained for each sample, in both methods, were expressed as mmolTrolox/g dry matter (Marghitas, Stanciu et al. 2009).

2.5 Statistical Methodology

Drying tests on the product and analysis of bioactive compounds and antioxidant activity were carried out in triplicate. The results obtained were analyzed using a Completely Randomized (CR) model, independently evaluating the factors of application of the drying process to the product and the type of system used in the process. To determine the best conditions of pollen drying, an unplanned comparison test was made between the different levels of the factors using an Analysis of Variance (ANOVA) and a Tukey's HSD test, with a confidence level of 95%. The analysis was performed using the basic MS Excel®, and the statistical Statistix® and SAS® packages.

3. Results and discussion

3.1 Bioactive compound content and antioxidant activity

The results of the contents of bioactive compounds and the antioxidant activity of dry pollen were obtained in each one of the systems evaluated comparatively with fresh pollen (see Figures 3 to 6).

Although a slight decrease in the carotenoid content of the bee pollen subjected to the drying process (8h) can be observed in either of the two systems, with respect to fresh pollen (0h), consequent to that established in previous investigations, there are no significant differences on the carotenoid content due to the drying process in the solar system, either in the cabinet system (Barajas-Ortiz, Martínez et al. 2010).

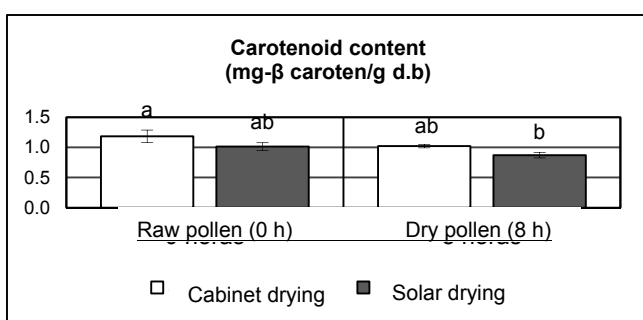


Figure 3. Carotenoid content of fresh pollen samples (0h) and dry pollen samples (8h) in both types of drying systems.

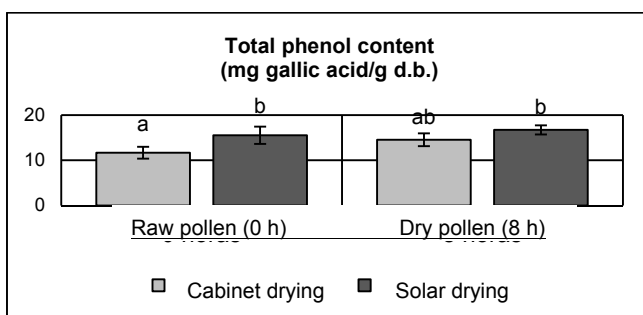


Figure 4. Total phenol content (Folin-Ciocalteu method) of fresh pollen samples (0h) and subjected to the drying process (8h) in both types of drying systems.

High levels of phenolic compounds were found in the evaluated pollen, according to those found by other researchers in bee pollen from other productive regions (Morais, Moreira et al. 2011). Additionally, it can be observed that there is an increase, although not significant, in total phenol content in the pollen subjected to the drying process, with respect to fresh pollen, for both drying systems. The differences in the values of antioxidant activity found by both techniques (TEAC/FRAP) due to the solar drying process are not significant in time, i.e. between fresh and dry pollen, however there is an increase in antioxidant activity throughout the process. Solar radiation therefore does not have a negative effect on this property under these conditions. A difference in the change in antioxidant activity of the dehydrated product is not evident when comparing both drying techniques employed, but if significant differences are observed between the pollen initially processed in each system, the cause of which is discussed below.

The temperatures used in the drying process in both systems do not generate a degradation of the antioxidant compounds evaluated in the pollen, and complementarily, the total antioxidant capacity of the product is not affected. Another condition to be considered in the solar drying system is the incident radiation on the product,

which despite being high for the region in which the tests were carried out, with a range between 5.0 and 6.5 kWh/m², did not adversely influence the content of bioactive compounds and the antioxidant capacity of the pollen and can be a beneficial factor for the reduction of microbial load of bee pollen due to the effect of UV radiation. The UV radiation also can have a potential effect in biosynthesis of secondary metabolites into the bee pollen structure even after its collection and harvest, due to its interaction with chemical structure of protection (sporopollenin) and the stimulation of the vegetal nucleus from pollen grains. The significant differences between the initial properties of bee pollen drying in cabinet system (collected in May, finishing the rainy season) and bee pollen drying in solar system (collected between June and July, starting the dry season) can be attributed to the time of harvesting of the pollen in the hive, having a direct effect on the content of compounds with bioactive properties (see in Figures 3 to 6).

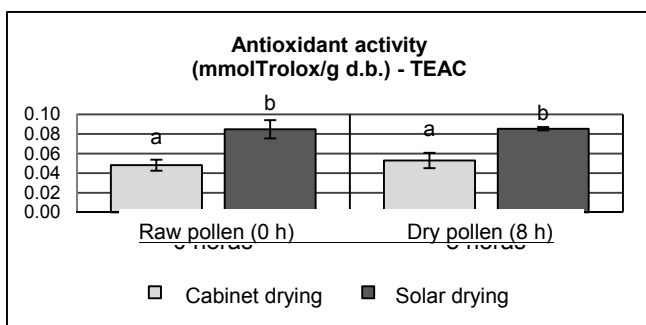


Figure 5. Antioxidant activity (TEAC method) of fresh pollen samples (0h) and subjected to the drying process (8h) in both types of drying systems.

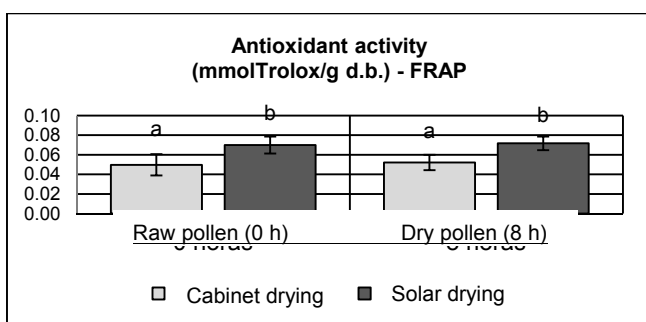


Figure 6. Antioxidant activity (FRAP method) of fresh pollen samples (0h) and those subjected to the drying process (8h) in both types of drying systems.

In the solar drying system, the climatic conditions of low radiation and high relative humidity, such as those that occurred on the days analyzed, lead to problems in the drying process, producing rehydration of the pollen, especially in the afternoon hours when there are lower temperature levels. According to these results, the optimal hours were determined for the process of drying in this system, as the ones between the 8:00 a.m. and the 14:00 p.m. hours. It can then be said that solar drying is an alternative to traditional cabin drying, as it reaches a moisture content favorable for subsequent storage and conservation of bee pollen. However, it is necessary to consider in this case the fluctuations in the temperature inside the system, due to the environmental factors that can affect the incident energy (solar radiation) into the system.

4. Conclusions

The temperature control and forced airflow inside the cabin drying system allow this to be more effective, having a higher drying rate and lower final product moisture content values, and at the same time more stable, having less variation in temperature and relative humidity inside the equipment during the process. On the other hand, the solar drying system has low effectiveness and unstable conditions inside due to environmental parameters but has lower energy requirements (electricity or combustibles) and subsequently costs for the producer, without an evident effect on the bioactive properties of bee pollen. The solar drying is a possible option to replace the traditional drying for bee pollen, since the physicochemical and bioactive properties of this food are not altered by the treatment carried out in this system, giving an added value to the product and decreasing the operation costs.

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