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Lipid characterization of chestnut and willow honeybee-collected pollen: Impact of freeze-drying and microwave-assisted drying

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ABSTRACT

Honeybee-collected pollen is gaining attention as functional food, `due to its high content of bioactive compounds, such as essential amino acids, antioxidants, vitamins and lipids. Traditional conservation methods include drying in a hot air chamber or freezing. These techniques influence the pollen organoleptic properties and its content of nutraceutical compounds. We analysed the lipid component of two honeybee-collected pollens, *Castanea sativa* and *Salix alba*. Chestnut pollen was characterized by a higher level of omega-6 fatty acids, while willow pollen showed a higher concentration of omega-3 fatty acids and carotenoids. Furthermore, two novel conservation methods, freeze-drying and microwave-assisted drying, were proposed and their impact on the pollen lipid profile was assessed. Only the microwave treatment showed a damaging action on antioxidant compounds (i.e. reduction of tocopherols). Overall, this research shed light on the lipid profile of honeybee-collected pollen, highlighting the nutraceutical importance of pollen as a source of omega-6 and omega-3 polyunsaturated fatty acids.

1. Introduction

Honeybee-collected pollen is gaining attention as functional food for human consumption, due to its high content of bioactive compounds, such as essential amino acids, antioxidants, vitamins and lipids (Quan et al., 2008; Soares de Arruda et al., 2013; Krystyjan et al., 2015; Almeida et al., 2016). Pollen is the basis of bee food. Nurse bees are major consumers of pollen, from which they produce jelly in their food glands with which they feed the larvae. Adult bees also consume some pollen (Craane, 1990; Pernal and Currie, 2000; Brodschneider and Crailsheim, 2010; Di Pasquale et al., 2013). Besides the nitrogenous substances, present in the form of complex proteins or free amino acids, the bee-collected pollen is composed by water, sugars, vitamins of B group, provitamin A, folic acid, minerals and lipids (Leja et al., 2007; Campos et al., 2003, 2008; Nicolson, 2011; Soares de Arruda et al., 2013).

Currently, many countries, as Brazil, Poland, Bulgaria and Switzerland, have established guidelines about the physical, chemical and microbiological standards that the pollen for human consumption must fit (Canale et al., 2016). Besides this, each type of pollen has specific nutritional characteristics that reflect its botanical origin (Serra Bonvehì, 1988). However, while extensive research focused on the proteins and antioxidants available in honeybee-collected pollen, its lipid profile has been scarcely investigated. Frequently, analyses of the chemical composition have concentrated on pollen from plants of interest to apiculture. Relatively few plant species pollens have been studied for fatty acid composition. Most of the research work on pollen lipids has concentrated on the sterols, e.g. cholesterol, as they are also essential for honeybees (Manning, 2001). Some works studied the fatty acid (FA) content of different pollen types and found unsaturated and saturated fatty acids (Battaglini and Bosi, 1965, 1968; Mărgăoan et al., 2014). Differences between the pollen types were concerned much more with the proportions of FA than to their diversity.

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The commercial collection of pollen is carried out by beekeepers, using special traps placed at the entrance of the hives (Contessi, 2009). It is worthy to note that the nutraceutical quality of honeybee-collected pollen decline over time (Pernal and Currie, 2000). In addition, the conditioning carried out on the fresh pollen collected by bees before storage for human consumption changes its nutritional and functional value. To reduce the water content, the honeybee-collected fresh pollen is usually conditioned with little standardized methods (Serra Bonvehì and Lòpez Alegret, 1986; Canale et al., 2016).

From a technological point of view, the knowledge of the different factors contributing to the production of high quality dried pollen is scarce. Currently, the drying process is conducted at low temperatures, with short exposure times, in order to avoid the risk of formation of Maillard's compounds (Collin et al., 1995). Furthermore, a detrimental effect of the drying process on the content of antioxidants, such as polyphenols and flavonoids, has been highlighted. This leads to a lower functional value of bee-collected pollen for human consumption (Serra Bonvehì et al., 2001).

Knowledge reported above outlined that the optimization and standardization of the technologies employed to process the bee-collected fresh pollen is crucial (Canale et al., 2016). In this research, we analysed the lipid component of two honeybee-collected pollens, *Castanea sativa* and *Salix alba*, shedding light on the abundance of omega-6 and omega-3 polyunsaturated fatty acids, sterols, carotenoids and tocopherols. Furthermore, two novel conservation methods, freeze-drying and microwave-assisted drying, were proposed and their impact on the complete lipid profile of the two pollens was assessed.

2. Materials and methods

2.1. Pollen samples

Honeybee-collected chestnut pollen was harvested by a beekeeper in July 2015 in chestnut grows located in Castelnuovo Garfagnana (44°06′22.7"N 10°24′02.7"E, Lucca, Italy), using a pollen trap (A. Metalori, Italy). Honeybee-collected willow pollen was harvested by a beekeeper in April 2015 in *Salix* orchards located in Massa Macinaia (43°47′45.6"N 10°32′03.2"E, Capannori, Lucca, Italy), using the pollen trap mentioned above. Chestnut and willow pollen samples were immediately frozen and transferred to the laboratories for further conditioning. For both honeybee-collected pollens, their monofloral origin was identified by colour and light microscopy examination (400× magnification) (Erdtman, 1969). Chestnut and willow pollen types were identified by comparison available pollen atlas databases (Erdtman, 1969; Ricciardelli d'Albore, 1998; Marghitas et al., 2009). Post-conditioning, all analytical results were compared with the fresh untreated pollen sample (UP). Eight pollen samples were collected for the analyses, 4 samples per species (i.e. chestnut and willow) and each sample was analysed in triplicate to reduce the individual variability. Moreover, 8 samples were collected to evaluate the effect of post-harvest conditioning: (*i*) 2 untreated samples (control), (*ii*) 2 conventionally dried samples [i.e. bee-pollen dried at 32 °C for 24 h in a Northwest Technology (Italy) cool-air dryer, http://www.northwest-technology.com/], (*iii*) 2 freeze-drying samples and (*iv*) 2 microwave-dried samples.

2.2. Freeze-drying

Pollen freeze-drying was carried out using a lyophiliser Heto PowerDry® LL1500 with four manifold connecting to 100 mL flasks filled with 70 g of honeybee-collected fresh pollen (previously preserved in a freezer at −20 °C). During the whole process, the temperature inside the condensation chamber was −115 °C, with full vacuum. The exchange of heat in this type of equipment it has by convection with the temperature of the test room, which was continuously conditioned at 25 °C. The maximum temperature reached by the sample during the process is 25 °C. The liquid inside the condensation chamber was sampled. The samples, at the end of the treatment, were sealed and stored at a temperature of −20° C for subsequent analysis.

The detailed procedure for pollen freeze-drying was carried out as follows: (*i*) keep the flasks and the samples to be treated in a freezer at −20° C until the time of treatment; (*ii*) switch equipment until the temperature reaches −100° C inside the condensation chamber and the pump power for the vacuum; (*iii*) insert the sample into the sample and determine the initial weight; (*iv*) house the flasks on the structure of the freeze-dryer; (*v*) create full vacuum in the flasks; (*vi*) treat the pollen sample for 9 h of freeze-drying (the exposure period was determined by preliminary tests followed by thermogravimetric analysis, TGA) (*vii*) determine the final weight (*viii*) seal the samples and store them at −20° C until analysis.

After the freeze-drying treatment for 9 h, TGA was carried on, in order to evaluate the residual water content. The sample was then stored in freezer at −20 °C, waiting for the analyses. TGA conducted at 120 °C showed that the residual water content was 6.0% for chestnut pollen and 6.3% for willow pollen.

2.3. Microwave-assisted drying

Microwave (MW) drying was carried out following the method described by Canale et al. (2016). The tests were conducted at the absolute pressure of 50 mbar. The MW power was 150 W and the exposure time was 30 min for both pollens). At the end of the treatment, the pollen sample was immediately weighed and its temperature measured with a K-type thermocouple. The pollen was transferred into an airtight container and stored at −20 °C until the analyses. TGA conducted at 120 °C showed that the residual water content was 6.4% for chestnut pollen and 10.3% for willow pollen.

2.4. Fatty acids composition

Total lipids (TL) were extracted according to Rodriguez-Estrada et al. (1997), with some modifications. Briefly, 15 g of pollen was inserted in a bottle with 200 mL of chloroform:methanol (1:1) solution and homogenized by Ultraturrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Subsequently, samples were stored at 60 °C and then 100 mL of chloroform were added. After filtration, 100 mL of K_2CO_3 1 M solution were added and the samples were stored at 4 °C overnight. The apolar phase was separated and stored a −20 °C.

Total lipids extract was separated into free fatty acids (FFA), neutral (NL) and polar lipids (PL), using a solid-phase extraction procedure described by Kaluzny et al. (1985), with Supelclean LC—NH2 SPE cartridges (Supelco, Bellefonte, USA). Briefly, 10 mg of lipid sample was applied to solid-phase extraction cartridges previously conditioned with hexane. The NL fraction was eluted with 4 mL of chloroform: isopropanol (4:1) and collected. Then the cartridges were washed with 4 mL of 2% acetic acid in diethyl ether solution for the elution of FFA, which collected in a separated tube. Finally, the PL fraction was eluted with 4 mL of methanol and collected. The three fractions were dried under nitrogen flow and then dissolved in hexane for preparation of fatty acid methyl esters (FAMEs).

FAME of TL was prepared by acid trans-methylation according Christie (1993), while NL and PL fractions were methylated by a basic trans-methylation following the procedure described by