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Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: A screening study

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Ascorbic acid (PubChem CID: 54670067)
Beta-carotene (PubChem CID: 573)
Alpha-carotene (PubChem CID: 6419725)
Alpha-tocopherol (PubChem CID: 14985)
Beta-tocopherol (PubChem CID: 6857447)
Gamma-tocopherol (PubChem CID: 92729)
Delta-tocopherol (PubChem CID: 92094)
Glucose (PubChem CID: 53782692)
Fructose (PubChem CID: 53782691)

A B S T R A C T

Bee pollen (BP) has been increasingly studied because it contains a wide variety of bioactive compounds, including vitamins. Brazilian botanical diversity, together with the potential of local beekeeping production, makes BP's capacity as a food source of bioactive compounds major focus for research. In this scenario, the objective of this study was to screen and evaluate the chemical composition, including antioxidant vitamins, of BP samples from southern Brazil, and to correlate them with their botanical origin. Analyses of nutritional composition were performed to compare them with the quality parameters established by Brazilian and international regulations. Additionally, individual sugars and vitamins (C, E and pro-vitamin A) were quantified and microscopic analysis for taxon classification was performed to correlate with vitamins and nutritional composition. The results of the chemical analysis showed that the samples were in accordance with the relevant regulations. The composition of vitamins and pollen types varied among the samples. Some BP could be classified as a source of a particular vitamin in a standard dose (25 g). Lipid and protein content from Rio Grande do Sul presented higher mean values ($p < 0.05$) compared with the other two states. Some correlations between chemical composition and botanical taxon were observed. Principal component analysis showed that the samples from the states of Rio Grande do Sul and Paraná presented similarities in terms of composition for each location. HCA and PLSDA were not able to classify the samples based on the chemical markers used. The analysis of vitamins confirmed that BP from this region can be a good source of antioxidant vitamins and that it can provide important nutritional information to food researchers and bioactive compounds for consumers.

1. Introduction

The bee pollen (BP) is one of the hive products (as well as honey, royal jelly, and propolis) that is gaining prominence due to the presence of bioactive compounds that are associated with beneficial properties to health (Komosinska-Vassev, Olczyk, Kaźmierczak, Mencner, & Olczyk, 2015; Campos, Frigerio, Lopes, & Bogdanov, 2010). The BP composition varies due to plant species and is also influenced by age, nutritional condition of the plant and environmental conditions during pollen development (Arruda, Pereira, de Freitas, Barth, & Almeida-Muradian, 2013; Melo, Freitas, Barth, & Almeida-Muradian, 2009).

BP composition includes proteins, carbohydrates, lipids and minerals. It also contains some antioxidant vitamins, namely C, E, β-carotene, as well as vitamins from the B-complex (Arruda et al., 2013; Campos et al., 2008). Few studies have identified antioxidant vitamins in BP. Melo et al. (2009) and Oliveira, Moriya, Azedo, and Almeida-Muradian (2009) performed the quantification of α-tocopherol, vitamin C and β-carotene in BP samples from southeast region of Brazil. Vitamins were present in all samples in varying amounts, and some BP samples were considered dietary sources of antioxidant vitamins in a

Abbreviations: BP, bee pollen; GI, geographical indication; HPLC, high-performance liquid chromatography; GPS, global positioning system; PCA, principal component analysis; HCA, hierarchical cluster analysis; DRI, dietary reference intake.

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daily serving (25 g). However, Almeida-Muradian, Pamplona, Coimbra, and Barth (2005) reported the absence of β-carotene and vitamin C in the some collected samples from southern Brazil. According to these authors, the thermal process applied on BP samples can explain the lack of these vitamins, as such compounds are unstable at high temperatures.

In addition to vitamins, BP has phenolic compounds as confirmed by studies of Cheng, Ren, Lei, Zheng, and Cao (2013), Freire et al. (2012) and Carpes (2009). Besides its high nutritional value, some studies show that this food can exert antioxidant, anti-inflammatory, antimicrobial, anticancer, antimutagenic and immunomodulatory activities, as evidenced by cellular, animal and human studies (Pascoal, Rodrigues, Teixeira, Feás, & Estevinho, 2014; Xua, Gaoa, & Sunb, 2012; Graikou et al., 2011; Trautwein & Demonty, 2007). Another important factor is the microbiological quality of Brazilian bee pollen. A study conducted with 45 BP samples collected in nine Brazilian states showed that all the samples did not present sulfite-reducing clostridia spores, *Salmonella*, coagulase-positive *Staphylococcus* and *Escherichia coli*, which are microorganisms of public health concern (De-Melo, Estevino, & Almeida-Muradian, 2015).

Currently, the use of the geographical indication (GI) for bee products, especially honey, has been proposed because of a higher demand from consumers for differentiated products with regional identity (Karabagiasa, Badekaa, Kontakosb, Karabourniotic, & Kontominasa, 2014; Castro-Vázquez, Díaz-Maroto, de Torres, & Pérez-Coello, 2010). According to Luykx and Ruth (2008), the use of GI allows producers to obtain confidence from consumers, leading to market recognition and also a premium price.

The evaluation of the nutritional composition and quality parameters of bee pollen from southern Brazil, which has great botanical diversity and subtropical to tropical climate as compared to the rest of the country, brings useful information to both producers and consumers. Another important aspect is the growing number of studies that have been recognizing BP as a functional food (Komosinska-Vassev et al., 2015). These features are a great opportunity for the development of Brazilian beekeeping and consequently the production of hive products, including the BP. In this scenario, the main objective of this work was to screen and evaluate the chemical composition (proximate, antioxidant vitamins C, E, pro-vitamin A and sugars) of dehydrated bee pollen produced in georeferenced apiaries from southern Brazil. Chemical data were correlated with botanical origin and compared with the quality parameters established by Brazilian and international regulations.

2. Materials and methods

2.1. Sampling and processing (dehydration) of the samples

Samples of BP in natura were obtained from August 2011 to March 2012 at apiaries located in southern Brazil (States: Paraná, Santa Catarina and Rio Grande do Sul). Table 1 shows the information obtained from 21 samples of BP in natura including, apiary collection, sampling month, state/province, and geographical position of the apiaries (global positioning system — GPS). Although the number of BP was not large, it represents the number of BP produced in the region at that time.

To dehydrate the BP samples, a food brand Fabbe-Primar dryer oven was used at a temperature of 42 °C for 20 h (time/temperature were based on preliminary studies), as recommended by the Brazilian legislation (Brasil, 2001).

2.2. Determination of proximate composition, sugars (glucose and fructose) and vitamin C (ascorbic acid)

The following determinations were carried out in accordance with the methodologies described by Almeida-Muradian, Arruda, and Barreto (2012): moisture was gravimetrically determined using an electronic precision balance (Micronal B160), fitted with an infrared dryer (Mettler Toledo LP16); total nitrogen — proteins were measured by the micro-Kjeldahl method; lipids were measured using an intermittent Soxhlet extractor and ethyl ether as solvent; fixed mineral residue — ash was measured gravimetrically in an oven at 550 °C to constant weight. The extraction and determination of the sugars glucose and fructose were performed by HPLC (Almeida-Muradian et al., 2012). For the determination of vitamin C, the titrimetric method (AOAC, 1995) was used, which relies on the reduction of 2-6-dichlorophenol-indophenol (DCFI) by ascorbic acid. All analyses were performed in triplicate.

2.3. Analysis of vitamins by high-performance liquid chromatography (HPLC)

2.3.1. Sample preparation

The reagents used during the extraction procedures were of analytical grade. The HPLC analytical methods were prepared with HPLC grade solvents and all the standards were purchased from Sigma Aldrich (St. Louis, MO, USA). First, the samples were subjected to exhaustive extraction with cold acetone using a mechanical mixer (Turrax). Then, the material was submitted to vacuum filtration using a sintered glass filter and the filtrate was transferred to a separatory funnel containing petroleum ether. The solution was washed five times with distilled water to completely remove the acetone. The petroleum ether extract was collected, dried with anhydrous sodium sulfate, filtered and concentrated in a vacuum rotary evaporator (~35 °C). The obtained residue, containing tocopherols and carotenes, was fully evaporated in an atmosphere of nitrogen, resuspended in 10 mL of mobile phase, filtered through a 0.22 μm membrane and injected into the chromatograph system equipped with two Shimadzu (Japan) (LC-20AT) pumps, Shimadzu (SIL-20A Autosampler) autosampler; Shimadzu (RF-10AXL) Fluorescence detector; Software LC-Solution and Shimadzu CBM-20A (SCL-

Table 1
Information obtained from 21 samples of BP.

State	Samples	Apiaries	Sampling month/year	City	Location of apiaries — GPS
RS	1	A	Aug/11	Cruz Alta	S28°37′35.94″/O53°37′57.66″
RS	2–3–4	B	Aug–Sept.–Sept./11	São Gabriel	S30°15′27.72″/O54°28′59.41″
RS	5	C	Oct/11	São Gabriel	S30°27′13.09″/O54°22′08.51″
RS	6	D	Oct/11	Erechim	S27°37′17.05″/O52°15′28.91″
RS	7–8	E	Oct–Nov./11	Cambará do Sul	S28°54′45.06″/O50°04′58.45″
PR	9–10	F	Nov–Nov./11	União da Vitória	S26°10′50.78″/O51°07′16.25″
PR	11	G	Nov/11	União da Vitória	S26°10′38.80″/O51°05′46.30″
PR	12	H	Nov/11	União da Vitória	S26°10′36.45″/O51°05′45.17″
SC	13–14–15	I	Dec–Dec.–Dec./11	São José	S27°34′00.35″/O48°43′18.27″
SC	16	J	Dec/11	Urubici	S27°57′10.00″/O49°54′54.50″
RS	17	K	Dec/11	Ijuí	S28°21′41.14″/O53°50′34.84″
PR	18	L	Dec/11	Lapa	S25°48′50.27″/O49°46′48.27″
PR	19	M	Dec/11	Palmeira	S25°41′12.58″/O50°09′32.56″
RS	20–21	A	Dec/11–Mar./12	Cruz Alta	S28°37′35.94″/O53°37′57.66″