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Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil

Composição química e atividade de sequestro do radical livre de pólen apícola de Apis mellifera da Região Sul do Brasil

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Summary

Bee pollen is an agglomerate of pollen grains from various botanical sources, which are collected by the bees and mixed with nectar and secretion from the hypopharyngeal glands such as β-glycosidase enzymes. Bee pollen has a complex chemical composition constituted of carbohydrates, proteins, aminoacids, vitamins and minerals, and is considered a good nutritional source, beneficial to health, particularly because of the presence of phenolic compounds with antioxidant activity. Therefore, the aim of this study was to assess the nutritional composition and antioxidant activity of the bee pollen produced in the Southern region of Brazil. The content of humidity, water activity, protein, total sugars, reducing sugars, crude fiber, lipids and minerals, as well as the content of total phenolic compounds, flavonoids, and antioxidant activity by the DPPH free radical scavenging method (2,2 diphenyl-1-picryl-hydrazyl) were determined. The mean contents of humidity, protein and reducing sugars were 4.19, 20.47 and 48%, respectively, and the predominant minerals were phosphorus, potassium, calcium and magnesium. The contents of phenolic compounds and total flavonoids were 30.46 ± 8.22 mg of GAE.g^{-1} pollen and 8.92 ± 5.5 mg of quercetin.g^{-1} pollen, respectively. High scavenging activities were found for the free radical DPPH, with EC_{50} (minimum concentration required for the antioxidant to reduce the initial concentration of the DPPH by 50%) values that ranged from 810 to 4690 μg.mL^{-1}. The bee pollen of Santa Catarina showed high antioxidant activity probably due to the high content of phenolic compounds present in pollen.

Key words: *Bee pollen; Phenolic compounds; Flavonoids; Antioxidant activity; DPPH, EC_{50}.*

Resumo

O pólen apícola possui uma composição química complexa constituída por carboidratos, proteínas, aminoácidos, vitaminas e minerais, sendo considerada uma boa fonte nutricional com benefícios para a saúde, principalmente pela presença de compostos fenólicos com atividade antioxidante. Portanto, o objetivo deste estudo foi avaliar a composição nutricional e a atividade antioxidante do pólen apícola produzido na região sul do Brasil. Foram determinados o teor de umidade, atividade de água, proteína, açúcares totais, açúcares redutores, fibra bruta, lipídeos e minerais, além do teor de compostos fenólicos totais, flavonoides e a atividade antioxidante pelo método de sequestro do radical livre DPPH (2,2 difenil-1-picril hidrazil). Os teores médios de umidade, proteína e açúcares redutores foram de 4,19, 20,47 e 48%, respectivamente, e os minerais predominantes foram o fósforo, potássio, cálcio e magnésio. Os teores de compostos fenólicos e flavonoides totais foram de 30,46 ± 8,22 mg de EAG.g^{-1} pólen (EAG: equivalentes de ácido gálico) e 8,92 ± 5,5 mg de quercetina.g–1 pólen, respectivamente. Foram encontradas altas atividades de sequestro para o radical livre DPPH, com valores de EC50 que variaram de 810a 4690 μg.mL^{-1}. O pólen apícola de Santa Catarina apresentou alta atividade antioxidante provavelmente em função do alto teor de compostos fenólicos presentes nesse pólen.

Palavras-chave: *Pólen apícola; Compostos fenólicos; Flavonóides; Atividade antioxidante; DPPH; EC_{50}.*

1 Introduction

Bee pollen has been used for many years in both traditional medicine and supplementary nutrition, as well as in alternative diets, mainly due to its nutritional properties and health benefits (SERRA BONVEHÍ and ESCOLÁ JORDÁ, 1997; ISLA et al., 2001; KROYER and HEGEDUS, 2001; ALMEIDA-MURADIAN et al., 2005). Its nutritional composition consists of proteins, lipids, sugars, fibers, mineral salts (calcium, chlorine, copper, iron, magnesium, iodine, molybdenum, selenium, strontium, stannum, boron, fluoride, vanadium, chromium, phosphorus, potassium, sulfur, aluminum, iron, manganese, and zinc), aminoacids and vitamins (A, B, C, D, E) (WESH and MARSTON, 1983; MARCHINI et al., 2006).

In addition to this, pollen also has high contents of polyphenolic substances, chiefly flavonoids with antioxidant (KROYER and HEGEDUS, 2001; CAMPOS et al., 2003) and antimicrobial activity (GARCÍA et al., 2001; BASIM et al., 2006). During the last decade, interest in the study of phenolic compounds has increased greatly, mainly due to the antioxidant capacity of these substances in scavenging free radicals that are harmful to human health (DORMAN et al., 2003). The antioxidant activity of phenolic compounds depends on the chemical structure and can be determined by the action of the molecule as reducing agents (speed of free radical inactivation, reactivity with other antioxidants and metal chelating potential). *In vitro* trials have demonstrated that some flavonoids have greater antioxidant activity than vitamins E and C (RICE-EVANS et al., 1996; ALMARAZ-ABARCA et al., 2007). Epidemiologic studies have also demonstrated positive correlation between the increase in phenolic compound consumption with antioxidant action (JAVANMARDI et al., 2003) and reduction of the risk of cardiovascular diseases and certain types of cancer (RICE-EVANS and MILLER, 1994; COOK and SAMMAN, 1996).

The biological properties of phenolic compounds are related to the activity that each phenol exercises in a certain medium, and the chemical structure of flavonoids favors antioxidant action. The high capacity of the phenolic constituents to neutralize the species reactive to oxygen are strongly associated with their structure, such as conjugated double bonds and number of hydroxyls in the aromatic ring of flavonoids and cinnamic acid derivatives (CAMPOS et al., 1997). The radical DPPH is widely used to test the free radical scavenger capacity in apicultural products such as, pollen (CAMPOS et al., 2003; SILVA et al., 2006; LEJA et al., 2007), propolis (LU et al., 2003; KUMAZAWA et al., 2004) and honey (MEDA et al., 2005). The antioxidant activity using the stable free radical DPPH is based on the transfer of electrons from an antioxidant compound to a free radical, DPPH which, when it is reduced, loses its purple coloring. Thus, only the reducing power of the antioxidant is assessed, which becomes oxidized when it donates an electron, and for this reason does not detect pro-oxidant substances (BRAND-WILLIAMS et al., 1995).

Bee pollen production is a recent activity in Brazil, having begun in the late 1980s. However, the country has the potential of being a large world producer of high quality pollen, particularly because of the great diversity of tropical flora and the resistance of the *Apis mellifera* in to diseases of bees. Therefore, the aim of this study was to assess the nutritional composition and DPPH scavenging activity of the bee pollen produced in the Southern region of Brazil, by bees of the Africanized *A. mellifera* species.

2 Material and methods

2.1 Bee pollen samples

Thirty-six dehydrated bee pollen samples were collected by beekeepers from different locations in the Southern region of Brazil, sixteen being from the State of Paraná, ten from the State of Santa Catarina and ten from the State of Rio Grande do Sul, during the period from 2005 to 2006. After collection, the bee pollens were sent to the laboratory and each sample was separately crushed in commercial blender, homogenized and stored between –12 to –15 °C in freezer for later analysis.

2.2 Ethanolic pollen extract preparation (EPE)

The ethanolic pollen extracts used for determining the contents of total phenolic compounds, total flavonoids and antioxidant activity were prepared by extracting two grams of crushed pollen in 15 mL of ethanol (70%) in a water bath at 70 °C, for 30 min. Next the samples were filtered and stored in tubes with a screw-on cap, at –5 °C (PARK, 1998).

2.3 Water activity (A_w)

Water activity was determined at 22 °C, using a PawKit water activity meter (Decagon), in accordance with the methodology described by AOAC (2000) (Water Activity method 32.004-32.009).

2.4 Moisture, protein content, crude fibers, lipids, ash and sugar content

The humidity content was determined in an oven at 60 °C, until a constant weight of previously crushed 2 g pollen dry basis sample was obtained method 935.29 of AOAC (1997). Total nitrogen was determined by the Kjeldahl method from a 0.7 g crushed pollen sample, using a factor of 6.25 for conversion into protein beside method 991.20 of AOAC (1997). Crude fibers were quantified by

gravimetry from two grams of crushed and defatted pollen, in accordance with the methodology described by AOAC (1997) (fibers method 991.43). Total lipids were determined by gravimetry from the extract of 2 g of crushed pollen with petroleum ether, in a Soxhlet apparatus, for approximately 4 h (FOLCH et al., 1957). Ash was determined by gravimetry, after incineration to a constant weight in a furnace at 550 °C (ash method 942.05) (AOAC, 1997). Total sugar and reducing sugar contents were determined spectrophotmetrically at 510 nm, in accordance with the *Somogyi-Nelson* method (SOMOGYI, 1945). All analyses were performed in triplicate and dry basis.

2.5 Mineral content

The minerals Fe, Ca, Zn, K, Na, Cu, Mg and Mn were determined after incineration of 1.5 g of pollen at 550 °C, until a constant weight was obtained. Next, the ash was solubilized with 25 mL of HNO_3 50%, heated in a water bath for 30 min, filtered and the minerals determined by atomic absorption spectrophotometry (Varian Model Spectra AA 100 & 200). The phosphorus content was determined by an spectrophotometer (Shimadzu model UV-Vis 1601 PC) (AOAC, 2000) (method 986.11).

2.6 Total phenolic and flavonoid contents

Total content of polyphenols in the EPE was determined by the colorimetric method of Folin-Ciocalteau (SINGLETON et al., 1999) by mixing an aliquot of 0.5 mL of the EPE (1:25) with 2.5 mL of Folin-Ciocalteau reagent diluted to 1:10 and 2.0 mL of 4% Na_2CO_3. The absorbance was measured at 740 nm after two hours of incubation in the dark at ambient temperature. The results of the total phenolic contents were expressed as gallic acid equivalents (mg GAE.g^{-1} bee pollen dry basis).

The content of total flavonoids in the EPE was determined in accordance with the method described by Park et al. (1995), with some modifications. An aliquot of 0.5 mL of the EPE (1:10) was mixed with 4.3 mL of 80% ethanol, 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M potassium acetate. After 40 min at ambient temperature, the absorbance was measured at 415 nm in a spectrophotometer (UV-Vis Mini 1240, Shimadzu Co.). The total quantity of flavonoids was calculated as quercetin equivalents (mg quercetin/g bee pollen dry basis).

2.7 Free radical-scavenger activity

The antioxidant activity of the compounds present in the EPE was determined by means of DPPH (2,2 diphenyl-1-picryl-hydrazyl) free radical scavenger capacity. An aliquot of 0.5 mL of the EPE solution was placed in a test tube containing 3 mL of absolute ethanol and 0.3 mL of the 0.5 mM DPPH ethanolic solution. The standard solutions of BHT (butylated hydroxyl-toluene), BHA (butylated hydroxyanisole) and α-tocoferol were assessed at the final concentration of 90 mg.mL^{-1}. The decrease in absorbance at 517 was determined in a spectrophotometer (UV-Vis Mini 1240, Shimadzu Co.), after 100 min of reaction. A tube containing 3.5 mL of ethanol and 0.3 mL of 0.3 mM DPPH was used as negative control. The blank sample was made by adding 3.3 mL of ethanol and 0.5 mL of the EPE. The antioxidant activity was expressed in terms of EC_{50} (minimum concentration required for the antioxidant to reduce the initial concentration of the DPPH by 50%). The lower the EC_{50} value, the greater the antioxidant capacity of the compounds present in the EPE. The EC_{50} values were calculated by means of a linear regression between the concentration in μg.mL^{-1} (axis of the abscissas) and the mean percentage of antioxidant activity, (ordinal axis), according to the formula described by Mensor et al. (2001) (Equation 1).

$$\%AA = 100 - \{ [(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\} \quad (1)$$

2.8 Statistical analysis

The student's-*t* test was used to compare the antioxidant activity with the positive controls and the Tukey test to assess the differences between the means at a level of $p < 0.05$, by means of SAS V9 (2004) software. All the values represented the mean of three repetitions with the standard deviation (n = 3) and the coefficient of variation (CV).

3 Results and discussion

The bee pollen samples from the Southern region of Brazil demonstrated a homogeneous nutritional composition, with the exception of the humidity content, which presented a coefficient of variation of over 40% (Table 1).

The humidity content ranged from 1.69 to 7.84%, and 47% of the samples were outside of the Brazilian legislation that allows the sale of pollen with a maximum humidity content of 4% (BRASIL, 2001). Among the three states analyzed, the pollen from Paraná (PR samples) was one with the highest number of specimens, 50% of which had contents above the limit set by the legislation (Table 1).

The mean water activity of the samples was 0.38 ± 0.04, which was characteristic of dehydrated foods and recommended for good storage stability (Table 1). The water activity could favor microbiological contamination, particularly by fungi and yeasts. Pollen is highly hygroscopic, and is therefore affected by environmental conditions.

The total protein content ranged from 15.04 to 27.69%, and the protein contents of the samples from the State of Santa Catarina (SC) differed statistically from those of the States of Rio Grande do Sul (RS) and Paraná (PR)

Table 1. Nutrient composition of bee pollen samples.

Samples*	aW**	Humidity (%)	Protein % (N x 6.25)	Crude fibers (%)	Lipids (%)	Ash (%)	TS*** (g.100 g^{-1})	RS**** (g.100 g^{-1})
PR 01	0.3 ± 0.0^{a}	6.6 ± 0.1^{a}	18.9 ± 0.2^{b}	3.5 ± 0.0^{a}	4.2 ± 0.1^{a}	2.1 ± 0.1^{a}	54.6 ± 2.1^{a}	48.7 ± 2.2a
PR 02	0.3 ± 0.0^{a}	6.6 ± 0.1^{a}	18.6 ± 0.4^{b}	3.1 ± 0.0^{a}	4.1 ± 0.1^{a}	1.9 ± 0.0^{a}	58.8 ± 0.2^{a}	56.6 ± 0.4^{a}
PR 03	0.4 ± 0.0^{a}	5.3 ± 0.1^{a}	18.9 ± 0.7^{b}	3.6 ± 0.1^{a}	5.1 ± 0.1^{a}	3.2 ± 0.0^{a}	55.3 ± 0.2^{a}	52.8 ± 0.6^{a}
PR 04	0.4 ± 0.0^{a}	7.8 ± 0.1^{a}	15.0 ± 0.2^{b}	2.7 ± 0.1^{a}	4.9 ± 0.1^{a}	3.1 ± 0.0^{a}	58.6 ± 0.2^{a}	56.3 ± 0.5^{a}
PR 05	0.5 ± 0.0^{a}	6.3 ± 0.1^{a}	15.4 ± 0.7^{b}	2.5 ± 0.1^{a}	4.7 ± 0.0^{a}	3.2 ± 0.0^{a}	60.3 ± 0.5^{a}	55.9 ± 0.8^{a}
PR 06	0.4 ± 0.0^{a}	3.3 ± 0.4^{b}	21.7 ± 0.4^{b}	4.8 ± 0.1^{a}	5.2 ± 0.0^{a}	2.5 ± 0.0^{a}	55.2 ± 0.5^{a}	52.6 ± 0.3^{a}
PR 07	0.3 ± 0.0^{a}	1.9 ± 0.0^{c}	22.4 ± 0.8^{b}	3.2 ± 0.0^{a}	5.7 ± 0.0^{a}	2.9 ± 0.1^{a}	51.3 ± 0.4^{a}	45.1 ± 0.7^{a}
PR 08	0.4 ± 0.0^{a}	3.3 ± 0.1^{b}	22.3 ± 0.4^{b}	2.8 ± 0.1^{a}	6.5 ± 0.1^{a}	2.9 ± 0.0^{a}	52.6 ± 0.4^{a}	50.9 ± 0.1^{a}
PR 09	0.4 ± 0.0^{a}	3.1 ± 0.1^{b}	21.5 ± 0.3^{b}	3.0 ± 0.1^{a}	5.4 ± 0.0^{a}	3.9 ± 0.1^{a}	50.8 ± 0.2^{a}	49.5 ± 0.7^{a}
PR 10	0.4 ± 0.0^{a}	3.1 ± 0.1^{b}	21.8 ± 0.3^{b}	3.9 ± 0.0^{a}	3.7 ± 0.0^{a}	3.7 ± 0.0^{a}	50.3 ± 0.3^{a}	47.0 ± 0.6^{a}
PR 11	0.4 ± 0.0^{a}	3.0 ± 0.0^{b}	21.9 ± 0.4^{b}	3.9 ± 0.0^{a}	4.2 ± 0.0^{a}	3.8 ± 0.1^{a}	50.1 ± 0.5^{a}	47.6 ± 0.4^{a}
PR 12	0.4 ± 0.0^{a}	4.4 ± 0.2^{a}	22.5 ± 0.1^{b}	3.9 ± 0.2^{a}	4.9 ± 0.1^{a}	3.8 ± 0.0^{a}	48.7 ± 0.2^{a}	45.1 ± 0.5^{a}
PR 13	0.4 ± 0.0^{a}	4.6 ± 0.0^{a}	21.8 ± 0.6^{b}	2.8 ± 0.1^{a}	5.7 ± 0.1^{a}	2.9 ± 0.0^{a}	52.2 ± 0.2^{a}	48.7 ± 0.4^{a}
PR 14	0.4 ± 0.0^{a}	1.7 ± 0.0^{c}	20.1 ± 0.2^{b}	3.0 ± 0.1^{a}	6.0 ± 0.0^{a}	2.9 ± 0.0^{a}	50.3 ± 0.5^{a}	48.0 ± 0.2^{a}
PR 15	0.4 ± 0.0^{a}	3.4 ± 0.2^{a}	20.9 ± 0.3^{b}	3.5 ± 0.0^{a}	5.2 ± 0.0^{a}	3.0 ± 0.1^{a}	50.0 ± 0.6^{a}	48.0 ± 0.6^{a}
PR 16	0.4 ± 0.0^{a}	5.8 ± 0.7^{a}	21.5 ± 0.1^{b}	3.4 ± 0.1^{a}	5.5 ± 0.0^{a}	2.2 ± 0.0^{a}	50.5 ± 0.0^{a}	47.0 ± 0.0^{a}
SC 01	0.4 ± 0.0^{a}	2.1 ± 0.2^{a}	22.9 ± 0.4^{a}	3.7 ± 0.0^{a}	4.2 ± 0.0^{a}	3.4 ± 0.0^{a}	48.4 ± 0.5^{a}	44.2 ± 3.7^{a}
SC 02	0.4 ± 0.0^{a}	2.9 ± 0.1^{a}	27.7 ± 0.3^{a}	2.2 ± 0.0^{a}	4.1 ± 0.1^{a}	3.3 ± 0.0^{a}	42.8 ± 0.3^{a}	40.4 ± 1.0^{a}
SC 03	0.4 ± 0.0^{a}	3.8 ± 0.1^{a}	22.6 ± 1.5^{a}	4.0 ± 0.0^{a}	5.4 ± 0.1^{a}	3.1 ± 0.0^{a}	49.7 ± 0.2^{a}	45.9 ± 1.0^{a}
SC 04	0.3 ± 0.0^{a}	3.4 ± 0.2^{b}	20.9 ± 0.7^{a}	3.5 ± 0.1^{a}	5.2 ± 0.0^{a}	3.0 ± 0.1^{a}	55.0 ± 0.4^{a}	50.4 ± 0.2^{a}
SC 05	0.5 ± 0.0^{a}	4.8 ± 0.1^{a}	22.9 ± 0.8^{a}	3.4 ± 0.1^{a}	5.3 ± 0.0^{a}	2.9 ± 0.0^{a}	53.3 ± 0.47^{a}	50.4 ± 0.6^{a}
SC 06	0.4 ± 0.0^{a}	4.2 ± 0.6^{a}	24.9 ± 0.2^{a}	2.9 ± 0.0^{a}	4.9 ± 0.1^{a}	2.4 ± 0.1^{a}	52.8 ± 0.2^{a}	50.4 ± 0.7^{a}
SC 07	0.4 ± 0.0^{a}	2.3 ± 0.1^{b}	19.0 ± 0.6^{a}	3.6 ± 0.1^{a}	3.9 ± 0.0^{a}	2.6 ± 0.0^{a}	51.2 ± 0.6^{a}	49.8 ± 0.2^{a}
SC 08	0.4 ± 0.0^{a}	2.9 ± 0.0^{b}	21.5 ± 0.2^{a}	2.8 ± 0.1^{a}	4.5 ± 0.0^{a}	2.5 ± 0.0^{a}	49.4 ± 0.2^{a}	47.6 ± 0.5^{a}
SC 09	0.4 ± 0.0^{a}	2.3 ± 0.2^{b}	24.4 ± 0.3^{a}	4.3 ± 0.1^{a}	4.4 ± 0.0^{a}	3.2 ± 0.0^{a}	50.5 ± 0.4^{a}	46.2 ± 0.2^{a}
SC 10	0.4 ± 0.0^{a}	5.6 ± 0.6^{a}	19.1 ± 1.5^{b}	4.6 ± 0.0^{a}	3.9 ± 0.0^{a}	3.1 ± 0.0^{a}	51.3 ± 0.2^{a}	49.0 ± 0.5^{a}
RS 01	0.4 ± 0.0^{a}	3.7 ± 0.6^{b}	20.0 ± 0.1^{b}	3.5 ± 0.1^{a}	4.0 ± 0.0^{a}	2.6 ± 0.1^{a}	60.4 ± 0.6^{a}	55.0 ± 0.7^{a}
RS 02	0.4 ± 0.0^{a}	1.9 ± 0.0^{c}	18.9 ± 0.5^{b}	3.5 ± 0.0^{a}	4.6 ± 0.0^{a}	1.9 ± 0.0^{a}	49.4 ± 0.3^{a}	45.4 ± 0.2^{a}
RS 03	0.3 ± 0.0^{a}	6.9 ± 0.0^{a}	18.2 ± 0.2^{b}	3.4 ± 0.1^{a}	4.9 ± 0.0^{a}	2.9 ± 0.0^{a}	54.2 ± 0.8^{a}	49.0 ± 0.9^{a}
RS 04	0.4 ± 0.0^{a}	7.3 ± 0.0^{a}	16.4 ± 0.9^{b}	3.2 ± 0.1^{a}	4.2 ± 0.0^{a}	3.0 ± 0.0^{a}	57.7 ± 0.5^{a}	53.0 ± 0.2^{a}
RS 05	0.5 ± 0.0^{a}	5.1 ± 0.1^{a}	18.5 ± 0.2^{b}	3.9 ± 0.0^{a}	5.6 ± 0.0^{a}	3.0 ± 0.0^{a}	54.7 ± 0.5^{a}	52.2 ± 0.3^{a}
RS 06	0.4 ± 0.0^{a}	6.7 ± 0.1^{a}	17.1 ± 0.1^{b}	3.1 ± 0.0^{a}	5.0 ± 0.0^{a}	2.9 ± 0.0^{a}	55.0 ± 0.9^{a}	48.4 ± 0.4^{a}
RS 07	0.4 ± 0.0^{a}	3.9 ± 0.1^{b}	19.3 ± 0.1^{b}	3.3 ± 0.1^{a}	4.5 ± 0.1^{a}	3.3 ± 0.0^{a}	41.1 ± 0.4^{a}	38.2 ± 0.7^{a}
RS 08	0.4 ± 0.0^{a}	5.9 ± 0.0^{a}	19.4 ± 0.0^{b}	3.2 ± 0.0^{a}	4.9 ± 0.0^{a}	2.8 ± 0.1^{a}	46.5 ± 0.6^{a}	41.5 ± 0.8^{a}
RS 09	0.4 ± 0.0^{a}	2.4 ± 0.1^{b}	19.9 ± 0.1^{b}	4.3 ± 0.0^{a}	5.4 ± 0.1^{a}	2.6 ± 0.1^{a}	51.1 ± 0.4^{a}	50.2 ± 0.8^{a}
RS 10	0.4 ± 0.0^{a}	4.8 ± 0.0^{a}	18.0 ± 0.1^{b}	3.5 ± 0.1^{a}	5.0 ± 0.1^{a}	2.9 ± 0.0^{a}	51.4 ± 0.2^{a}	48.7 ± 0.3^{a}
$V_{máx.}$	0.5	7.8	27.7	4.9	6.5	3.91	60.4	56.6
$V_{mín.}$	0.3	1.7	15.0	2.2	3.7	1.90	41.1	38.2
Mean ± SD	0.4 ± 0.0	4.2 ± 1.7	20.5 ± 2.6	3.4 ± 0.6	4.9 ± 0.7	2.9 ± 0.5	52.1 ± 4.22	48.8 ± 4.2
C.V(%)	10.6	40.6	12. 9	16.5	13.4	16.33	8.09	8.53

* PR: Parana samples, SC: Santa Catarina samples, RS: Rio Grande do Sul samples; ** Water Activity (22 °C); *** Total Sugar (g.100 g^{-1} drybasis); **** Reducing Sugars (g.100 g^{-1} drybasis); Means followedby the same letter do not differ statistically by the Tukey test ($p < 0.05$).

(Table 1). Higher protein contents of 25.9% have also been found in bee pollen produced in the Southeast of Australia (SOMERVILLE and NICOL, 2006). The greatest part of the nitrogen present in pollen is in the protein fraction, this being the second most abundant group of nutrients, after the carbohydrates. All the samples analyzed presented a protein content of over 8% thus being in accordance with the Brazilian technical regulation (BRASIL, 2001). The high protein content (20.47 ± 2.64%) and reducing sugars (48.79 ± 4.16%) and low lipid content found (4.86 ± 0.65%) make pollen and excellent food supplement (Table 1). This result corroborates those of Almeida-Muradian et al. (2005), who assessed Brazilian pollen samples and found a content of humidity, proteins, lipids and ash of 7.4, 20, 6 and 2.2%, respectively. The high content of reducing sugars in pollen can be justified by the presence of

honey or nectar in the fluid that cements the pollen grains (STANLEY and LINSKENS, 1974). According Qian et al. (2008) the calcium ligand exchange chromatography with PAD detection was capable of resolving a range of sugars although as has been observed previously it is difficult to get a method which will completely separate all possible sugar structures. The current method separated three common hexoses and sucrose with good resolution. As well as confirming the identity of fructose, glucose and sucrose in the bee pollen it was possible to tentatively identify melezitose as a minor component in the bee pollen from Spain, Israel, China and from Romania.

The lipid content of the samples (4.86 ± 0.65 g.100 g^{-1} dry basis) (Table 1) was similar to that described by Somerville (2005), who found a variation from 0% of lipids for *Eucalyptus macrorhyncha* pollen and 11.2% for *Hypochoeris radicata* pollen in the Southeast of Australia.

The predominant minerals in the samples were phosphorus (6923.63 ± 872.53 mg.kg^{-1} of pollen), followed by potassium (5116.76 ± 528.89 mg.kg^{-1} of pollen), calcium (1031.98 ± 377.51 mg.kg^{-1} of pollen) and magnesium (754.64 ± 184.17 mg.kg^{-1} of pollen) (Table 2). Statistical analysis by the Tukey test demonstrated no significant

Table 2. Mineral content of bee pollen samples.*

Samples**	Ca	Cu	Fe	P	Mg	Mn	K	Na	Zn
PR 01	1035.3 ± 8.2^{a}	15.5 ± 0.8^{a}	139.5 ± 0.6^{a}	7345.5 ± 75.1^{a}	763.4 ± 20.4^{a}	73.3 ± 3.6^{a}	5727.4 ± 268.6^{a}	206.5 ± 7.7^{a}	53.1 ± 1.2^{a,b}
PR 02	1036.3 ± 20.0^{a}	15.6 ± 0.8^{a}	145.1 ± 3.4^{a}	7364.3 ± 259.9^{a}	785.5 ± 41.4^{a}	75.4 ± 4.7^{a}	5419.7 ± 439.2^{a}	212.9 ± 18.9^{a}	54.5 ± 0.7^{a,b}
PR 03	1515.7 ± 64.1^{a}	11.4 ± 2.3^{a}	76.8 ± 2.7^{a}	7277.3 ± 361.9^{a}	946.9 ± 99.5^{a}	43.6 ± 4.2^{a}	4820.4 ± 295.3^{a}	459.4 ± 22.1^{a}	46.2 ± 5.9^{b}
PR 04	1195.8 ± 6.2^{a}	14.2 ± 1.1^{a}	120.5 ± 3.0^{a}	7328.0 ± 269.9^{a}	831.9 ± 42.5^{a}	64.1 ± 2.6^{a}	5322.5 ± 48.6^{a}	292.9 ± 9.5^{a}	51.3 ± 1.2^{a,b}
PR 05	1249.3 ± 22.6^{a}	13.7 ± 1.5^{a}	114.1 ± 1.3^{a}	7323.5 ± 21.7^{a}	854.8 ± 62.8^{a}	61.0 ± 0.6^{a}	5187.5 ± 43.9^{a}	321.7 ± 0.8^{a}	50.6 ± 0.7^{a,b}
PR 06	1320.3 ± 24.4^{a}	13.1 ± 0.6^{a}	103.8 ± 8.5^{a}	7309.9 ± 227.4^{a}	877.9 ± 41.5^{a}	56.2 ± 0.6^{a}	5110.1 ± 80.4^{a}	358.0 ± 17.8^{a}	49.4 ± 8.8^{b}
PR 07	1195.8 ± 55.7^{a}	14.2 ± 0.3^{a}	120.5 ± 3.1^{a}	7329.0 ± 253.6^{a}	831.9 ± 36.5^{a}	64.1 ± 1.4^{a}	5322.5 ± 80.9^{a}	292.9 ± 7.8^{a}	51.3 ± 2.0^{a,b}
PR 08	1123.6 ± 127.0^{a}	10.4 ± 0.9^{a}	57.5 ± 1.5^{a}	6132.7 ± 217.8^{a}	714.4 ± 3.5^{a}	97.5 ± 3.1^{a}	5645.7 ± 198.4^{a}	73.5 ± 1.8^{b}	61.2 ± 0.9^{a}
PR 09	1213.2 ± 52.2^{a}	12.5 ± 0.5^{a}	93.9 ± 1.1^{a}	6923.9 ± 86.7^{a}	808.1 ± 0.8^{a}	72.6 ± 0.5^{a}	5359.4 ± 80.9^{a}	241.5 ± 0.8^{a}	53.9 ± 0.3^{a,b}
PR 10	1177.5 ± 6.8^{a}	12.4 ± 0.1^{a}	90.6 ± 3.7^{a}	6795.2 ± 37.7^{a}	784.8 ± 15.4^{a}	78.1 ± 1.9^{a}	5442.5 ± 10.9^{a}	202.6 ± 1.1^{a}	55.5 ± 0.8^{a,b}
PR 11	975.1 ± 50.4^{a}	9.8 ± 0.3^{a}	68.6 ± 6.7^{a}	6404.6 ± 217.4^{a}	738.3 ± 23.1^{a}	83.3 ± 7.3^{a}	5318.0 ± 231.6^{a}	19.7 ± 4.4^{c}	48.7 ± 2.2^{b}
PR 12	860.3 ± 88.7^{a}	9.2 ± 0.9^{a}	38.6 ± 3.8^{a}	5724.1 ± 447.2^{a}	463.3 ± 7.8^{a}	51.5 ± 6.5^{a}	3722.5 ± 237.3^{a}	161.8 ± 8.8^{a}	43.0 ± 2.2^{b}
PR 13	1764.6 ± 89.8^{a}	11.5 ± 1.6^{a}	58.2 ± 6.5^{a}	7155.5 ± 78.2^{a}	1095.9 ± 32.5^{a}	63.7 ± 4.2^{a}	6363.7 ± 24.7^{a}	231.1 ± 63.0^{a}	55.2 ± 4.7^{a,b}
PR 14	1383.4 ± 11.0^{a}	10.3 ± 0.6^{a}	66.9 ± 2.1^{a}	8865.3 ± 229.9^{a}	924.3 ± 10.2^{a}	129.4 ± 5.0^{a}	5586.8 ± 33.8^{a}	106.8 ± 9.1^{a}	53.7 ± 2.2^{a,b}
PR 15	628.1 ± 35.7^{a}	8.2 ± 0.1^{a}	34.6 ± 0.8^{a}	6609.5 ± 240.3^{a}	713.4 ± 12.2^{a}	98.9 ± 1.8^{a}	5516.8 ± 159.6^{a}	42.7 ± 4.6^{b}	42.7 ± 4.2^{a,b}
PR 16	1190.6 ± 47.7^{a}	10.8 ± 0.6^{a}	57.6 ± 0.7^{a}	7748.2 ± 47.3^{a}	953.6 ± 36.5^{a}	63.5 ± 0.6^{a}	6274.0 ± 78.7^{a}	221.66 ± 0.6^{a}	53.9 ± 1.7^{a,b}
SC 01	938.3 ± 62.4^{a}	13.3 ± 1.4^{a}	103.3 ± 2.3^{a}	5872.4 ± 235.7^{a}	536.0 ± 32.0^{a}	50.2 ± 3.0^{a}	4556.3 ± 297.3^{a,b}	202.5 ± 22.4^{a}	55.6 ± 4.6^{b}
SC 02	1388.8 ± 69.1^{a}	9.3 ± 0.5^{a}	71.2 ± 2.5^{a}	8769.4 ± 149.0^{a}	1039.5 ± 111.0^{a}	25.3 ± 1.5^{a}	5136.0 ± 428.8^{a,b}	636.4 ± 87.5^{a}	42.4 ± 1.7^{b}

* mg.kg^{-1} of bee pollen dry basis; ** PR: Parana samples; SC: Santa Catarina samples; RS: Rio Grande do Sul samples; Means followed by the same letter do not differ statistically by the Tukey test ($p < 0.05$).

Table 2. Continued...

Samples**	Ca	Cu	Fe	P	Mg	Mn	K	Na	Zn
SC 03	866.7 ± 75.2^{a}	16.4 ± 0.1^{a}	115.6 ± 8.1^{a}	6958.8 ± 69.7^{a}	558.2 ± 8.2^{a}	59.4 ± 1.4^{a}	5270.1 ± 135.2^{a,b}	227.7 ± 40.0^{a}	60.6 ± 0.7^{a}
SC 04	808.8 ± 19.9^{a}	10.7 ± 0.3^{a}	35.4 ± 0.5^{a}	7691.8 ± 8.2^{a}	799.0 ± 1.9^{a}	110.7 ± 0.6^{a}	5348.8 ± 72.7^{a,b}	229.7 ± 5.0^{a}	45.7 ± 0.4^{b}
SC 05	792.9 ± 7.35^{a}	9.9 ± 0.4^{a}	48.8 ± 0.3^{a}	6079.8 ± 125.9^{a}	544.6 ± 4.7^{a}	83.0 ± 5.0^{a}	4976.1 ± 78.7^{a,b}	55.4 ± 0.7^{b}	36.1 ± 0.7^{b}
SC 06	816.0 ± 19.1^{a}	11.3 ± 0.2^{a}	35.0 ± 1.1^{a}	7433.8 ± 48.7^{a}	818.6 ± 29.9^{a}	110.9 ± 3.2^{a}	5347.0 ± 284.6^{a,b}	239.6 ± 23.6^{a}	46.7 ± 0.1^{b}
SC 07	750.8 ± 22.0^{a}	9.1 ± 0.2^{a}	49.7 ± 4.8^{a}	5913.5 ± 86.9^{a}	534.7 ± 10.9^{a}	59.3 ± 2.1^{a}	4762.8 ± 110.4^{a,b}	47.0 ± 2.7^{b}	38.0 ± 0.3^{b}
SC 08	1518.6 ± 36.2^{a}	10.3 ± 0.4^{a}	48.5 ± 0.5^{a}	5921.3 ± 67.2^{a}	530.3 ± 6.3^{a}	58.9 ± 0.9^{a}	4792.0 ± 61.1^{a,b}	54.9 ± 0.7^{b}	38.9 ± 0.8^{b}
SC 09	868.1 ± 50.0^{a}	13.7 ± 0.3^{a}	51.3 ± 1.3^{a}	8224.5 ± 72.6^{a}	881.3 ± 6.0^{a}	69.4 ± 0.8^{a}	5073.3 ± 76.0^{a,b}	168.3 ± 20.1^{a}	49.6 ± 4.8^{b}
SC 10	870.3 ± 5.5^{a}	8.8 ± 0.3^{a}	35.9 ± 0.3^{a}	5868.6 ± 32.1^{a}	547.9 ± 26.4^{a}	59.8 ± 0.6^{a}	4715.3 ± 71.0^{a,b}	48.7 ± 0.5^{b}	37.1 ± 0.1^{b}
RS 01	843.2 ± 85.0^{a}	12.0 ± 3.7^{a}	54.4 ± 2.3^{a}	6134.7 ± 20. 7^{a}	575.9 ± 46.9^{a}	43.5 ± 3.6^{a}	5074.9 ± 85.6^{b}	202.2 ± 24.6^{a}	48.3 ± 7.1^{b}
RS 02	1086.7 ± 96.2^{a}	14.9 ± 0.1^{a}	107.6 ± 3.4^{a}	8456.6 ± 34.61^{a}	908.9 ± 36.9^{a}	48.9 ± 1.5^{a}	5452.5 ± 95.5^{b}	379.9 ± 27.6^{a}	66.9 ± 2.0^{a}
RS 03	291.6 ± 11.4^{a}	4.5 ± 0.2^{a}	69.0 ± 1.5^{a}	6073.2 ± 22.7^{a}	612.8 ± 2.4^{a}	26.50 ± 1.7^{b}	4435.6 ± 82.5^{b}	12.9 ± 0.6^{c}	51.5 ± 0.3^{b}
RS 04	301.8 ± 23.6^{a}	4.8 ± 0.3^{a}	71.6 ± 7.4^{a}	6128.7 ± 64.7^{a}	623.0 ± 6.2^{a}	28.9 ± 3.6^{b}	4462.8 ± 62.9^{b}	13.2 ± 1.2^{c}	51.6 ± 0.7^{b}
RS 05	2149.2 ± 88.8^{a}	11.9 ± 0.1^{a}	27.9 ± 5.0^{a}	7455.4 ± 60.9^{a}	1235.1 ± 21.4^{a}	83.4 ± 2.8^{a}	5133.1 ± 272.4^{b}	323.5 ± 66.9^{a}	55.8 ± 1.3^{b}
RS 06	540.4 ± 44.7^{a}	10.0 ± 1.1^{a}	203.3 ± 15.9^{a}	6119.9 ± 75.9^{a}	809.3 ± 56.6^{a}	24.9 ± 2.5^{b}	4389.8 ± 63.8^{b}	262.0 ± 32.8^{a}	82.1 ± 3.7^{a}
RS 07	964.2 ± 25.7^{a}	17.8 ± 2.2^{a}	101.3 ± 2.5^{a}	7006.3 ± 91.1^{a}	695.5 ± 17.4^{a}	57.5 ± 1.1^{a}	4898.8 ± 221.6^{b}	272.1 ± 11.9^{a}	60.8 ± 7.1^{a}
RS 08	732.0 ± 95.7^{a}	7.0 ± 0.1^{a}	136.9 ± 17.9^{a}	6932.8 ± 80.4^{a}	532.5 ± 8.5^{a}	51.2 ± 5.6^{a}	4782.7 ± 255.2^{b}	193.6 ± 32.5^{a}	40.8 ± 1.4^{b}
RS 09	726.0 ± 82.0^{a}	10.8 ± 0.6^{a}	36.5 ± 1.2^{a}	5647.9 ± 90.0^{a}	540.0 ± 47.5^{a}	18.7 ± 1.3^{b}	4329.6 ± 110.2^{b}	72.1 ± 7.1^{b}	39.1 ± 2.0b
$V_{máx.}$	2149.2	17.8	203.3	8865.3	1235.1	129.4	6363.7	636.4	82.1
$V_{mín.}$	291.6	4.5	27.9	5647.9	463.3	18.7	3722.5	12.9	36.1
Mean ± SD	1031.9 ± 377.5	11.4 ± 2.9	79.7 ± 40.3	6923.6 ± 872.6	754.6 ± 184.2	64.2 ± 25.5	5116.8 ± 528.9	202.5 ± 136.9	50.6 ± 9.2
CV (%)	36.58	26.0	50.6	12.6	24.4	39.7	10.3	67.6	18.3

* mg.kg^{-1} of bee pollen dry basis; ** PR: Parana samples; SC: Santa Catarina samples; RS: Rio Grande do Sul samples; Means followed by the same letter do not differ statistically by the Tukey test ($p < 0.05$).

difference between the contents of calcium, copper, iron, phosphorus, magnesium, manganese and sodium in the pollen samples of the South of Brazil. However, for potassium, the samples from the State of Paraná (PR) presented the highest contents and differed statistically from the samples of the State of Rio Grande do Sul (RS) (Table 2). The mean zinc content was 50.63 mg.kg^{-1} of pollen and the samples from the State of Rio Grande do Sul (RS) presented the highest contents probably because of differences on the floral origin of pollen and on the plant growth conditions, such as soil and geographic origin. According to Stanley and Linskens (1974) there are differences in the mineral content of pollen collected by bees and pollen collected directly from the flower.

Serra Bonvehí and Escolá Jordá (1997) established parameters for the quality of Spanish bee pollen. In this study both Fe (39,2 mg.kg^{-1} of bee pollen) and Zn (33,9 mg.kg^{-1} of bee pollen) present suprass the 15% established for Recommended Dietary Allowance (RDA). According to Wesh and Marston (1983) the presence of

zinc, copper, iron and a high rate of potassium/sodium make bee pollen an interesting food for diets with a defined electrolytic balance.

The pollen samples from the South of Brazil present a heterogeneous phenolic content. The total phenolic compound contents range from 19.28 to 48.90 mg (mg GAE.g^{-1} of pollen), with a mean value of 30.77 ± 8.22 mg (Figure 1). Among the samples SC 03, PR 03 and RS 07 had the highest levels of total phenolic compounds (48.90, 46.09 and 43.24 mg GAE.g^{-1} de pollen, respectively). These results were higher than those presented in bee pollen in the region of Vienna in Austria which was found values of 8.2 mg.g^{-1} of phenolic compounds in bee pollen *in natura*, and was increased to 24.6 mg.g^{-1} when the analyses were made from the ethanolic extract (KROYER and HEGEDUS, 2001).

The total flavonoid contents expressed in quercetin equivalents ranged from 2.10 mg to 28.33 mg of mg.g^{-1} of pollen (Figure 1). The bee pollen of SC 03 sample that showed higher levels of phenolic compounds also showed the highest level of flavonoids (28.33 mg of quercetin.g^{-1} bee pollen). Leja et al. (2007) studied the phenolic constituents (total phenolics, phenylpropanoids, flavonoids and antocyanins) and the antioxidant capacity of bee pollen from 12 different species from the region of Krakow in Poland. In this study, a large variety of phenolic compounds were found in the analyzed species, and in the majority of the samples, the antioxidant activity was related to the phenylpropanoid content. It was also found that the contribution of the flavonoids to the total phenolic compound contents differed considerably as a function of the floral origin, from 4.78% (*L. purpureum*) to 37.3% (*C. angustifolium*). Serra Bonvehí et al. (2001), determined total phenolic compounds and total flavonoids in 11 bee pollen samples from Spain. Fifteen compounds were separated by HPLC and 13 were identified and quantified. The predominant compounds were the flavonoids quercetin and miricetin and *trans* cinnamic acid.

The scavenger activity of the free radical DPPH was expressed in terms of EC_{50}. Lower values indicates better antioxidant capacity of the bee pollen extracts. The EC_{50} values for the extracts ranged from 810 ± 60 µg.mL^{-1} to 4690 ± 290 µg.mL^{-1}, with a mean value of 1920 ± 130 µg.mL^{-1} (Figure 2). The pollen from Santa Catarina (RS) presented the lowest EC_{50} values, and consequently the greatest antioxidant activity in terms of scavenging the free radical DPPH (Figure 2). Each pollen type has its own specific characteristics related to the genetics of the floral species and plantations visited by the bees and may influence in the biological properties. In according Carpes (2008) twenty-two pollen types were identified in the 36 bee pollen samples from the Southern region of Brazil. With exception of one sample, which was classified as monofloral (Asteraceae), all the other samples of bee pollen presented at least two pollen types and are characterized by the presence of Asteraceae *Brassicaceae, Myrtaceae, Arecaceae,*

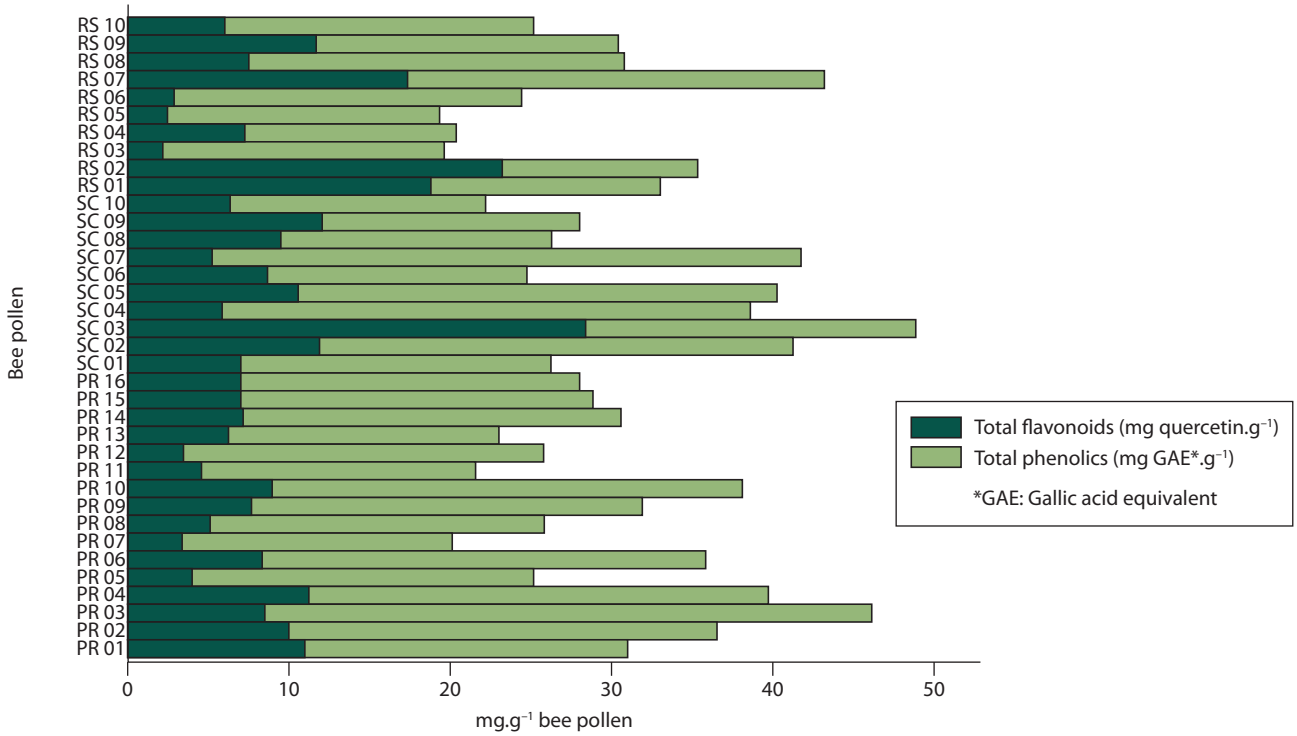

Figure 1. Total phenolic compounds (mg GAE.g^{-1} bee pollen dry basis), total flavonoids content (mg quercetin/g bee pollen dry basis). Data are means (n = 3).

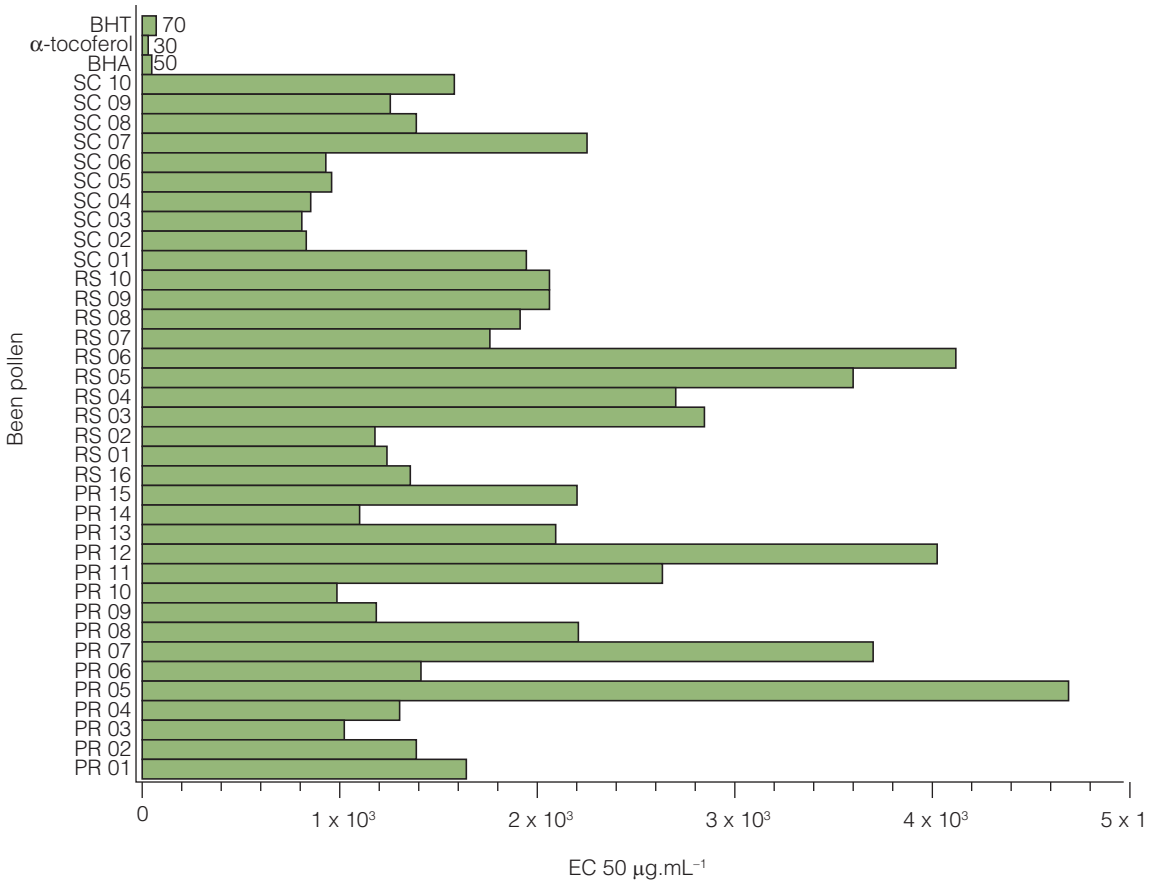

Figure 2. Free radical-scavenger activity of the bee pollen. Data are means (n = 3).

Euphorbiaceae, Anacardiaceae, Mimosaceae, Rosaceae, Leguminosaceae Sapindaceae e *Loranthaceae*.

According to Campos et al. (2003), the antioxidant activity of pollen is largely as a result of the phenolic compounds and flavonoids that have free radical scavenger activity, although other constituents such as, proteins and vitamins may also contribute to this property. These authors found EC_{50} values that ranged from 40 to 500 $\mu g.mL^{-1}$ in pollen samples collected in Portugal and New Zealand. The results of antioxidant activities of samples from the South of Brazil were superior to those found by Meda et al. (2005), who analyzed 27 samples with different geographic origins (eastern, western, south-western and central parts) of Burkina Faso (an African country) and found a mean EC_{50} value of 10.60 ± 7.30 ($mg.mL^{-1}$). As found by other authors (ALMARAZ-ABARCA et al., 2007) the mesquite pollen extracts showed antioxidant activity related to the flavonol concentration in both the in vitro-biological system and the in vivo system, even though this activity is lower than that showed by vitamin E.

The EC_{50} values found for the positive controls BHT, BHA and α-tocoferol were 70, 50 and 30 $\mu g.mL^{-1}$, respectively (Figure 2). However, these substances were used pure and are recognized as antioxidants with high free radical scavenger capacity. Therefore, in addition to being a good nutritional reserve of proteins, carbohydrates and minerals, the bee pollen samples from Southern Brazil are also a good source of phenolic compounds with antioxidant activity.

4 Conclusion

Considering the high protein content, reducing sugars and low lipid content found in bee pollen, make pollen an excellent food supplement. Each bee pollen generally contained different polyphenolic, and each of these compounds possesses differing amounts of antioxidant activity. Bee pollen from Southern Brazil may supply substantial antioxidants, which, in turn, may provide health-promoting effects to consumers. However, due to the diversity and complexity of the bee pollen, further investigations should be performed to evaluate the profile of polyphenolic composition.

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