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Physicochemical parameters and content of B-complex vitamins: an exploratory study of bee pollen from southern Brazilian states

Parámetros físico-químicos y contenido de vitaminas del complejo B: un estudio exploratorio del polen apícola del sur de Brasil

Bianca Rodrigues de S[1], José Gasparotto S[1], Adriane Alexandre Machado DM[1], Daniel Granato[2], Elias da Silva A[1], Alex da Silva de F[3], Ortrud Monika B[4,5], Aroni Sattler[6], Ligia Bicudo de AM[1].

1. Department of Food and Experimental Nutrition, Pharmaceutical Sciences School, University of São Paulo, 05580-000, São Paulo, Brazil.
2. Department of Food Engineering, State University of Ponta Grossa, 84030-900, Ponta Grossa, Paraná, Brazil.
3. Laboratory of Marine Geology, Federal Fluminense University, 24210-340, Niterói, Rio de Janeiro, Brazil.
4. Laboratory of Palynology, Department of Geology, Institute of Geosciences, Federal University of Rio de Janeiro, 21949-900, Rio de Janeiro, Brazil.
5. Viral Morphology and Morphogenesis Laboratory, Oswaldo Cruz Institute, Fiocruz, 21040-360, Rio de Janeiro, Rio de Janeiro, Brazil.
6. Faculty of Agronomy, Federal University of Rio Grande do Sul, 91540-000, Porto Alegre, Rio Grande do Sul, Brazil.

Dirigir correspondencia a: Adriane A. M. de Melo.
Laboratório de Análise de Alimentos.
Av. Professor Lineu Prestes, 580, Bloco 13-A,
CEP: 05580-000, Cidade Universitária, São Paulo, SP, Brasil.
Tel.: +55 11 3091 3684.
E-mail: adriane.melo@uol.com.br

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ABSTRACT

This study aimed to determine the presence of B-complex vitamins and some physicochemical parameters in bee pollen samples from the southern Brazilian states of Paraná, Santa Catarina and Rio Grande do Sul and, then, to identify their correlations with the geographical and botanical origin of the samples using multivariate statistical techniques. B-complex vitamins were determined by HPLC and mean contents were 0.79 mg/100 g (vitamin B_1), 0.88 mg/100 g (vitamin B_2), 5.31 mg/100 g (sum of vitamin B_3 vitamers) and 4.42 mg/100 g (sum of B_6 vitamers). The physicochemical parameters of the samples were consistent with those reported in the literature. The results showed that bee pollen is an important source of B-complex vitamins and multivariate statistical exploratory techniques suggested its nutritional content should be evaluated locally.

Keywords: Bee pollen; Physicochemical parameters; B-complex vitamins; Botanical origin; Chemometrics.

RESUMEN

El objetivo de este estudio fue determinar la presencia de vitaminas del complejo B y algunos parámetros físico-químicos en muestras de polen apícola oriundas de los Estados del Sur de Brasil (Paraná, Santa Catarina y Rio Grande do Sul); y correlacionar estos resultados con el origen botánico y geográfico, usando un análisis estadístico multivariado. Las vitaminas del complejo B fueron determinadas por HPLC y su contenido fue de 0,79 mg/100 g (vitamina B_1); 0,88 mg/100 g (vitamina B_2); 5,31 mg/100 g (suma de los compuestos de vitamina B_3); y 4,42 mg/100 g (suma de los compuestos de vitamina B_6). Los parámetros físico-químicos de las muestras fueron consistentes con los reportados en la literatura. Los resultados mostraron que el polen apícola fue una fuente importante de vitaminas del complejo B, y las técnicas multivariadas sugirieron que su contenido nutricional debe ser evaluado localmente.

Palabras clave: Polen apícola; Parámetros físico-químicos; Vitaminas del complejo B; Origen botánico; Quimiométricos.

INTRODUCTION

Bee pollen (BP) has been shown to be a potentially functional food rich in bioactive compounds, including water- and fat-soluble vitamins and phenolic compounds[1,2]. Its protein content of up to 62 g/100 g, coupled with varying amounts of all essential amino acids and essential minerals, make it appealing for human consumption[2,3].

Water-soluble vitamins (B-complex) and their by-products have been widely investigated for their role in energy metabolism as enzyme cofactors[4]. The study of B-complex vitamins in bee pollen samples from Brazil is still in an early stage. The first research on this topic was conducted by Arruda et al.[5] and reported high levels of B-complex vitamins in bee pollen from the southeastern state of Sao Paulo, Brazil.

Levels of B vitamins and antioxidant vitamins (A, C and E) in bee pollen have been correlated with its botanical origin and the content of each pollen type in the pollen mix[1,5,6,7]. Identification and characterization of bioactive compounds in bee products along with reliable data on geographical and botanical origin inspire confidence among consumers and researchers and ensure optimal production and market recognition[1,8,9].

Brazil's flora is highly diverse. In particular, southern Brazilian states feature different biomes such as Cerrado, Atlantic Forest and Pampa. Therefore, it would be interesting to investigate bee pollen composition produced in these regions. Thus, the purpose of this study was to determine physicochemical parameters, including levels of B_1, B_2 and vitamers of B_3 and B_6, in BP from three southern Brazilian states and to identify their correlations with the geographical and botanical origin of the samples using multivariate statistical techniques.

MATERIALS AND METHODS

Bee pollen samples

Samples of unprocessed (*in natura*) BP were collected at apiaries located in southern Brazil (Paraná, Santa Catarina and Rio Grande do Sul) between August 2011 and December 2012. Table 1 shows details of the 28 BP used in this study, including state, city and sampling month/year. The samples were sent to the Food Analysis Laboratory of the Faculty of Pharmaceutical Sciences, University of São Paulo (São Paulo, Brazil), where they were coded and, finally, dehydrated in a Fabbe-Primar drying oven at 42 °C for 20 h, as recommended by Brazilian legislation[10].

Table 1. Details of the 28 bee pollen samples from the southern Brazilian states of Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS).

Samples	State	City	Sampling month/year
PR 01	Paraná	União da Vitória	November/2011
PR 02		União da Vitória	November/2011
PR 03		União da Vitória	November/2011
PR 04		União da Vitória	November/2011
PR 05		Lapa	December/2011
PR 06		Palmeira	December/2011
SC 01	Santa Catarina	São José	December/2011
SC 02		São José	December/2011
SC 03		São José	December/2011
SC 04		Balneário Gaivota	December/2011
SC 05		Içara	August/2012
RS 01		Cruz Alta	August/201
RS 02		São Gabriel	September/2011
RS 03		São Gabriel	September/2011
RS 04		São Gabriel	October/2011
RS 05		Erechim	October/2011
RS 06		Novo Hamburgo	October/2011
RS 07		Novo Hamburgo	November/ 2011
RS 08		Ijuí	December/ 2011
RS 09	Rio Grande	Cruz Alta	December/ 2011
RS 10	do Sul	Cruz Alta	March/2012
RS 11		Jacutinga	March/2012
RS 12		Cambará do Sul	October/2012
RS 13		Cambará do Sul	October /2012
RS 14		Cambará do Sul	October /2012
RS 15		Cambará do Sul	November /2012
RS 16		Cambará do Sul	December /2012
RS 17		Cambará do Sul	December /2012

Physicochemical characterization

All determinations below were carried out, in triplicate, as described by Almeida-Muradian et al.[11]. Moisture: samples were placed on an electronic precision balance (Micronal B160) equipped with an infrared dryer (Mettler Toledo LP16) and moisture content was gravimetrically determined. Total nitrogen: nitrogen was determined by the micro-Kjeldahl method. To convert the value to protein content, a factor of 6.25 was used. Lipid content: samples were defatted by the intermittent Soxhlet method using diethyl ether. Ash content: bee pollen samples were placed in an oven and heated to constant weight at 550 °C; ash content was gravimetrically determined. Total dietary fiber: an enzymatic-gravimetric method was employed to determine fiber content. Total sugar content: it was calculated by deducting all contents above from 100%. Free acidity: samples were titrated with NaOH (0.1 mol/L). pH - samples were diluted with 50 mL of distilled water before pH was read. Reducing sugars (glucose and fructose): analysis was performed using HPLC.

Quantification of B vitamins (B_1, B_2, B3 and B_6 vitamers)

The methods described by Arruda et al.[5] were adopted. Simultaneous extraction of thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinic acid and nicotinamide (vitamin B_3), and pyridoxamine, pyridoxal and piridoxol (vitamin B_6) was performed. Both vitamins B_1 and B_2 were eluted from the same column under the same mobile phase conditions and detected by fluorescence, but chromatographic analysis of thiamine was preceded by oxidation. Vitamins B3 and B6 were separated on two different columns under completely different mobile phase conditions. B6 vitamers were detected directly by fluorescence whereas determination of fluorescent B3 vitamin was carried out after a post-column reaction. Chromatographic conditions are described in Table 2.

Calibration curves were prepared using standard vitamin solutions: B1 (concentration range: 1.42 to 56.8 ng/20 µL, R^2= 0.999, p< 0.001); B_2 (range: 2.67 to 133.2 ng/20 µL, R^2= 1.000, p< 0.001); B_3 vitamers: nicotinic acid (range: 5.54 to 554 ng/20 µL, R^2= 0.999) and nicotinamide (range: 5.53 to 553.5 ng/20 µL, R^2= 0.999, p< 0.001); B_6 vitamers: pyridoxamine (range: 2.43 to 97.2 ng/20 µL, R^2= 0.999, p< 0.001), pyridoxal (range: 2.60 to 103.9 ng/20 µL, R^2= 0.999, p< 0.001) and piridoxol (range: 2.45 to 98 ng/20 µL, R^2= 0.999, p< 0.001).

The results were compared with reference values for Recommended Daily Intake (DRI) for vitamins B_1, B_2, B_3

Table 2. Chromatographic conditions used for the analysis of each B vitamin in bee pollen samples.

Conditions	B_1	B_2	B_3 (nicotinamide and nicotinic acid)	B_6 (pyridoxamine, pyridoxal, and piridoxol)
Mobile phase	Phosphate buffer (pH 7.2) and dimethylformamide (85:15 v/v)	Phosphate buffer (pH 7,2) and dimethylformamide (85:15 v/v)	Phosphate buffer, hydrogen peroxide and copper sulphate	Phosphate buffer (pH 2.5) and acetonitrile (96:4 v/v)
Flow rate (mL/min)	1.0	1.5	1.5	0.6
Injection volume (µL)	20	20	20	20
Stationary phase/Column	C_{18} (RP-18, 250 x 4.6 mm, 5 µm spherical) with Shim-pack VP-ODS pre-column (10 x 4.6 mm, 5 µm)	C_{18} (RP-18, 250 x 4.6 mm, 5 µm spherical) with Shim-pack VP-ODS pre-column (10 x 4,6 mm, 5 µm)	C_{18} (Luna C_{18} 250 x 4.6 mm, 5 µm spherical) with Phenomenex pre-column (10 x 10 mm, 5 µm)	C_{18} (Superspher 100 RP-18 endcapped, 250 x 4.0 mm, 5 µm) with Linchrospher 100 RP-18 pre-column (4 x 4 mm, 5 µm)
Wavelength (nm)	Excitation: 368 Emission: 440	Excitation: 450 Emission: 530	Excitation: 322 Emission: 380	Excitation: 296 Emission: 390
Column temperature (°C)	20	45	20	20
Conversion Reaction	Pre-column	*	Post-column	*

* No conversion reaction was carried out because these vitamins are naturally fluorescent.

and B_6 of 1.2, 1.3, 16 and 1.6 mg, respectively[12], and they were classified into two categories: high-content (30% DRI per serving) and source (15% DRI per serving)[13].

Determination of botanical origin

Samples were analyzed and classified as described by Barth et al.[14]. A 2-g-sample was weighed, washed with ethanol and resuspended in a water:glycerol solution (50:50 v/v). One drop of this homogenized suspension was placed on a microscope slide and 500 pollen grains per sample were counted. Pollen types were classified into predominant pollen (>45% pollen grains), accessory pollen (between 16% and 45%) or important isolated pollen (between 3% and 15%).

Data processing and multivariate statistical analysis

Results were expressed as means ± standard deviation of three independent samples. In order to compare physicochemical properties and B vitamin content of samples from the three southern states of Brazil, BP samples from each state were grouped and compared using one-way analysis of variances (ANOVA). When applicable, the post-hoc Duncan's multiple range at $p< 0.05$ was used to separate the means[15].

Multivariate statistical techniques, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were used to identify correlations of physicochemical properties and B vitamin content of BP samples with their geographical origins. Samples (n=28) were grouped in rows and physicochemical characteristics and chemical composition (n=19) were grouped in columns, totaling 532 data points. In order to give equal importance to all variables, data were scaled to unit variance prior to analysis[16]. Statistica 7.0 (Statsoft, USA) was used for all statistical analyses.

RESULTS

Contents of vitamins B_1, B_2, B_3 and B_6

Vitamins B_1, B_2, vitamers of B_3 and B_6 were detected in all BP samples. Table 3 shows the content of each vitamin and its vitamers in dehydrated BP samples as well as the percent of Recommended Daily Intake (% RDI) for each vitamin in a 25-g serving of dehydrated BP.

Thiamine content ranged between 0.46 and 1.83 mg/100 g of dehydrated BP. Based on the % DRI of B1 per 25-g serving in this study, 57% of the BP samples (n=28) can be classified as a "source" food and 7% can be classified as a "high-content" food. B1 levels showed were not statistically significant different ($p< 0.05$) among samples of the three states. Riboflavin occurred in similar quantities to thiamine and the majority of the BP samples, 68% and 7%, fell into the "source" and "high-content" categories, respectively. No significant difference ($p> 0.05$) in vitamin B2 levels was observed among BP samples of the three states.

Nicotinic acid and nicotinamide were detected in the range of 0.68-3.93 mg and 2.40-5.54 mg/100 g, respectively; total niacin was between 2.67-8.69 mg/100 g. In this study, the samples of the three Southern Brazilian states failed to meet Brazilian nutrition standards for vitamin B_3; levels were below the minimum requirements for niacin or even below the limit of detection (LoD) (0.24 mg/100 g for nicotinic acid and 2.22 mg/100 g for nicotinamide). Nicotinamide content of BP samples from Rio Grande do Sul was higher ($p< 0.05$) than that of samples from Paraná and Santa Catarina states.

Of the three vitamers of B6, only pyridoxal and pyridoxamine were detected in all samples. Some BP samples even exceeded 100% DRI for B6 per 25-g serving. Almost all samples (93%) fell into the "high-content" category for pyridoxine; the remainder fitted the "source" category. Rio Grande do Sul state provided samples with significantly higher amounts of total B6 than the state of Paraná. No significant difference ($p> 0.05$) in vitamin B6 levels was found between samples of Rio Grande do Sul and Santa Catarina.

Physicochemical composition

The results of the physicochemical analysis of the 28 BP samples, as well as the standard values set by Brazilian and Argentine legislation, members of Southern Common Market (MERCOSUL), are described in Table 4.

Moisture content ranged between 1.00 g and 6.73 g/100 g. All 28 samples complied with Argentine legislation (max 8 g/100 g)[17], but 46% did not comply with Brazilian legislation (max 4 g/100 g)[10]; most of them were from Rio Grande do Sul (n=17). There was no significant difference in the mean moisture content between states ($p> 0.05$).

Total sugars ranged from 43.59 g to 71.64 g/100 g; and glucose and fructose levels varied from 4.3 g to 8.9 g/100 g and 3.44 g to 7.0 g/100 g, respectively. Total average sugar content was lower in samples from Rio Grande do Sul than those of the other two states ($p< 0.05$). Protein levels in BP samples were above the minimum value recommended by Brazilian legislation[10]. Only four samples (PR06, SC02, SC05 and SC03) were outside the range recommended for protein content by Argentine legislation[17]. Lipids were within the range of 0.37 g to 14 g/100 g; and four samples (PR06, SC02, SC03 and RS09) were below the limit of 1.8 g set by Brazilian legislation[10]. Total ash content varied (1.05-2.83 g/100 g) and met the maximum limit of 4 g/100 g established by Brazilian and Argentine legislation[10,17].

In this study, total fiber content ranged from 12.79 to 20.30 g/100 g, which was above the minimum value established by Brazilian legislation[10]. No significant difference in average dietary fiber content was observed among samples of the three Brazilian states.

Free acidity was in the range from 220 to 665 mEq/kg; and most samples were above the limit of 300 mEq/kg set by Brazilian legislation[10]. pH values of all samples fell between the lower (pH 4.0) and upper (pH 6.0) limits established by legislation[10]. There was no significant difference among free acidity averages and pH values averages for each state.

Table 3. Contents of B1, B2, B3 and B6 vitamers in 28 dehydrated bee pollen samples from Southern Brazilian states and their percent of Daily Recommended Intake (DRI) in a 25-g serving.

Samples¥	B_1 (mg/100 g)	% DRI	B_2 (mg/100 g)	% DRI	Nicotinic acid (mg/100 g)	Nicotinamide (mg/100 g)	Total B_3 (mg/100 g)#	% DRI	Pyridoxamine (mg/100 g)	Pyridoxal mg/100 g)	Piridoxol (mg/100 g)	Total B_6 (mg/100 g)#	% DRI
PR 01	0.91±0.11	19.9**	0.79±0.02	15.2**	0.70±0.08	3.27±0.04	3.97±0.12	6.2	0.36±0.03	0.42±0.04	<LoD	0.78±0.07	15.0**
PR 02	0.58±0.03	12.1	0.78±0.01	14.9	0.68±0.11	2.73±0.12	3.41±0.24	5.3	0.26±0.02	1.48±0.16	<LoD	1.74±0.18	33.5***
PR 03	1.37±0.39	28.5**	1.86±0.01	35.7***	2.60±0.12	2.90±0.13	5.50±0.26	8.6	0.66±0.02	4.73±0.14	<LoD	5.40±0.16	103.9***
PR 04	0.91±0.06	18.9**	1.62±0.06	31.1***	0.95±0.21	4.20±0.08	5.15±0.28	8.1	0.50±0.06	5.14±0.06	<LoD	5.64±0.13	108.5***
PR 05	1.03±0.04	21.4**	0.55±0.22	10.5	1.80±0.12	3.97±0.18	5.77±0.30	9.0	0.44±0.01	2.63±0.03	<LoD	3.07±0.04	59.0***
PR 06	0.65±0.03	13.5	1.11±0.02	21.4**	3.93±0.25	<LoD	3.93±0.25	6.1	0.29±0.05	1.12±0.03	<LoD	1.41±0.08	27.2**
PR average	**0.91±0.28**	**-**	**0.95±0.52**	**-**	**1.38±1.29**	**3.09±1.41[ab]**	**4.56±0.97**	**-**	**0.40±0.15[b]**	**2.06±1.96**	**-**	**2.41±2.09[b]**	**-**
SC 01	0.65±0.40	13.6	1.08±0.01	20.7**	1.31±0.08	3.39±0.08	4.70±0.15	7.3	0.53±0.01	2.97±0.04	<LoD	3.50±0.05	67.3***
SC 02	0.76±0.45	15.9**	1.01±0.01	19.5**	2.18±0.03	2.72±0.05	4.91±0.09	7.7	0.75±0.12	2.28±0.05	<LoD	3.03±0.18	58.3***
SC 03	0.91±0.07	18.9**	1.62±0.03	31.3***	2.67±0.06	<LoD	2.67±0.06	4.2	0.83±0.02	1.82±0.04	0.07±0.00	2.72±0.06	52.3***
SC 04	0.80±0.08	16.7**	1.28±0.02	24.6**	2.71±0.12	<LoD	2.71±0.12	4.2	0.48±0.06	4.12±0.05	<LoD	4.59±0.11	88.3***
SC 05	1.10±0.15	22.9**	0.84±0.01	16.2**	3.17±0.14	4.57±0.03	7.74±0.17	12.1	0.70±0.01	4.88±0.10	<LoD	5.58±0.11	107.3***
SC average	**0.80±0.17**	**-**	**1.08±0.30**	**-**	**2.67±0.71**	**2.72±1.51[b]**	**4.70±2.08**	**-**	**0.70±0.15[a]**	**2.97±1.27**	**-**	**3.5±1.18[ab]**	**-**
RS 01	1.57±0.38	32.7***	1.06±0.01	20.4**	1.02±0.34	2.40±1.07	3.43±1.41	5.4	0.43±0.03	6.70±0.30	<LoD	7.13±0.33	137.1***
RS 02	0.49±0.13	10.3	0.97±0.09	18.6**	2.17±0.18	4.16±0.51	6.33±0.69	9.9	0.50±0.05	5.76±1.44	<LoD	6.26±1.49	120.4***
RS 03	0.51±0.08	10.7	0.87±0.01	16.7**	1.64±0.53	3.00±0.88	4.65±1.41	7.3	0.40±0.07	5.09±1.41	<LoD	5.49±1.47	105.5***
RS 04	1.23±0.17	25.5**	0.84±0.01	16.1**	1.07±0.10	3.25±0.15	4.32±0.25	6.8	0.46±0.02	5.59±0.43	<LoD	6.04±0.45	116.2***
RS 05	1.83±0.09	38.0***	0.76±0.03	14.6	2.50±0.09	2.96±0.11	5.46±0.20	8.5	0.48±0.02	3.24±0.20	<LoD	3.72±0.22	71.5***
RS 06	0.94±0.38	19.6**	0.55±0.14	10.6	0.91±0.14	5.34±0.29	6.25±0.43	9.8	0.65±0.10	2.91±0.21	<LoD	3.55±0.31	68.4***
RS 07	0.57±0.22	11.9	0.79±0.13	15.3**	0.96±0.09	3.79±0.08	4.75±0.17	7.4	0.52±0.03	5.53±0.10	<LoD	6.05±0.13	116.3***
RS 08	1.34±0.06	27.9**	0.92±0.04	17.7**	1.27±0.02	5.54±0.35	6.81±0.37	10.6	0.74±0.05	3.60±0.16	<LoD	4.34±0.21	83.4***
RS 09	0.73±0.47	15.3**	0.92±0.05	17.6**	2.08±0.04	3.76±0.21	5.84±0.25	9.1	0.63±0.07	2.29±0.02	<LoD	2.91±0.09	56.0***
RS 10	0.79±0.02	16.5**	0.80±0.08	15.4**	1.68±0.12	4.82±0.09	6.50±0.21	10.2	0.73±0.01	2.41±0.01	<LoD	3.14±0.03	60.4***
RS 11	0.78±0.19	16.2**	0.84±0.04	16.2**	3.38±0.17	5.31±0.18	8.69±0.35	13.6	0.95±0.06	3.82±0.14	<LoD	4.78±0.20	92.4***
RS 12	0.71±0.21	14.8	1.49±0.11	28.6**	1.24±0.12	4.87±0.26	6.12±0.38	9.6	0.51±0.02	4.54±0.63	0.11±0.00	5.16±0.65	99.3***
RS 13	0.71±0.02	14.8	1.53±0.04	29.5**	1.22±0.03	4.47±0.12	5.69±0.15	8.9	0.55±0.02	4.22±0.30	0.11±0.00	4.87±0.32	93.7***
RS 14	0.63±0.11	13.1	0.86±0.04	16.6**	1.32±0.31	4.76±0.34	6.08±0.65	9.5	0.49±0.00	4.60±0.12	0.09±0.00	5.18±0.12	99.6***
RS 15	0.46±0.25	9.6	0.89±0.04	17.2**	1.29±0.47	5.16±0.09	6.44±0.56	10.1	0.49±0.01	3.94±0.15	0.10±0.00	4.53±0.16	87.1***
RS 16	0.68±0.10	14.2	0.70±0.01	13.4	1.67±0.10	3.33±0.06	4.99±0.16	7.8	0.53±0.01	3.43±0.10	0.11±0.00	4.07±0.11	78.3***
RS 17	1.24±0.12	25.8**	0.40±0.03	7.7	1.15±0.10	3.19±0.11	4.34±0.20	6.8	0.50±0.02	3.31±0.21	0.13±0.01	3.94±0.24	75.7***
RS average	**0.73±0.40**	**-**	**0.86±0.28**	**-**	**2.67±0.71**	**4.16±0.99a**	**5.84±1.22**	**-**	**0.51±0.14ab**	**3.94±1.25**	**-**	**4.78±1.18a**	**-**
Total average (n=28)	0.79±0.34	-	0.88±0.35	-	1.48±0.86	3.58±1.33	5.31±1.41	-	0.51±0.16	3.71±1.53	-	4.42±1.54	-
p-value (ANOVA)	0.952	-	0.192	-	0.154	0.019	0.195	-	0.031	0.064	0.212	0.040	-

All results are expressed as mean ± standard deviation (n=3). ¥PR: Paraná state; SC: Santa Catarina state; RS: Rio Grande do Sul state. # Sum of contents of B3 and B6 vitamers. Limits of detection (LoD): B1 = 0.06 mg/100 g; B2 = 0.12 mg/100 g; nicotinic acid = 0.24 mg/100 g; nicotinamide = 2.22 mg/100 g. Means followed by different letters in the same column are statistically different ($p < 0.05$). ** - "source food" (> 15%DRI); *** - "high-content food" (> 30%DRI) as described in Brazil (1998).

Table 4. Physicochemical data of 28 dehydrated bee pollen samples and standards of Brazilian10 and Argentine legislation18.

Sample¥	Moisture (g/100 g)	Ash (g/100 g)	Lipids (g/100 g)	Proteins (g/100 g)	Total dietary fiber (g/100 g)	Total sugars* (g/100 g)	Fructose (g/100 g)	Glucose (g/100 g)	Calories (kcal)#	Free acidity (mEq/kg)	pH
PR 01	2.73±0.24+	1.79±0.05	4.47±0.61	17.13±1.04+	16.35±1.15	58.61±1.19	6.27±0.47	8.29±0.46	343±4	353±5	5.41±0.00
PR 02	2.66±0.11+	1.05±0.28	6.17±0.29	15.19±1.30+	13.98±0.28	61.67±1.44	6.59±0.50	8.48±0.65	363±3	280±6	4.62±0.02
PR 03	2.42±0.27+	1.96±0.04	2.86±0.19	15.76±0.10+	19.81±0.73	57.34±1.13	5.77±0.25	6.59±0.10	318±6	504±10	5.36±0.04
PR 04	3.72±0.10+	2.31±0.39	4.04±0.97	22.43±1.48+	18.48±0.93	50.04±1.16	5.46±0.25	8.12±0.34	326±7	337±1	5.73±0.01
PR 05	3.69±0.13+	1.60±0.17	3.80±0.47	19.51±1.69+	15.69±0.37	55.83±1.98	4.75±0.20	5.71±0.16	336±5	455±17	4.43±0.00
PR 06	4.73±0.08+	1.59±0.53	0.37±0.01	13.28±0.00+	12.79±0.29	67.66±0.43	3.94±0.59	4.96±0.49	327±2	529±6	4.11±0.01
PR average	3.21±0.88	1.70±0.42	3.92±1.93	16.45±3.29b	16.02±2.65	57.98±5.90a	5.62±0.98	7.36±1.49	332±16	404±101	4.99±0.64
SC 01	3.37±0.22+	1.93±0.41	3.54±0.20+	15.44±0.48+	19.08±0.29	56.98±0.75	5.24±0.49	5.34±0.40	322±3	428±7	4.40±0.01
SC 02	1.55±0.12+	1.57±0.12	1.11±0.38+	13.29±0.27+	15.15±1.72	67.46±1.37	5.64±0.25	7.95±0.32	333±6	665±18	4.97±0.01
SC 03	1.00±0.01+	1.31±0.12	0.91±0.05+	12.25±0.31+	14.40±1.51	71.64±0.78	5.11±0.26	6.75±0.34	344±3	340±5	4.52±0.02
SC 04	5.10±0.17+	2.15±0.12	1.89±0.40+	20.24±0.21+	15.98±0.20	54.60±0.27	6.12±0.27	7.52±0.32	316±3	220±5	5.10±0.02
SC 05	6.73±0.18	2.83±0.09	14.00±1.00	14.73±0.63	18.07±0.42	48.45±0.28	4.58±0.48	5.85±0.42	309±1	290±11	5.11±0.01
SC average	3.37±2.40	1.93±0.58	1.89±5.53	14.73±3.08b	15.98±1.98	56.98±9.52a	5.10±1.31	6.48±1.71	322±14	340±172	4.97±0.34
RS 01	2.84±0.24+	2.20±0.01	4.68±0.44	22.99±0.05+	19.09±0.98	48.12±0.80	6.62±0.02	7.97±0.09	327±4	521±5	5.30±0.02
RS 02	3.68±0.07+	2.71±0.06	6.20±0.44	24.46±0.18+	17.68±1.04	44.81±1.00	7.00±0.25	8.50±0.23	333±3	359±6	5.05±0.01
RS 03	3.25±0.22+	2.24±0.04	5.13±0.90	20.90±0.18+	15.48±1.01	53.60±1.82	5.60±0.53	6.76±0.86	344±6	273±6	5.00±0.01
RS 04	2.76±0.28+	2.66±0.10	2.53±0.06	23.23±1.36+	18.49±0.39	51.24±0.73	4.60±0.62	5.96±0.62	317±2	293±5	5.05±0.01
RS 05	2.74±0.12+	2.41±0.04	2.19±0.18	22.36±0.10+	17.97±1.99	51.34±0.33	4.88±0.23	5.64±0.23	315±1	290±6	4.25±0.01
RS 06	4.44±0.07+	2.54±0.02	2.52±0.58	26.76±0.11+	20.30±0.54	43.64±0.72	5.19±0.15	6.78±0.20	304±4	244±6	5.30±0.01
RS 07	3.79±0.16+	2.16±0.06	4.56±0.32	20.59±0.11+	18.67±0.20	50.36±0.43	5.89±0.41	7.83±0.34	325±1	320±6	5.18±0.03
RS 08	4.19±0.24+	1.56±0.10	1.97±0.06	21.57±0.21+	13.85±0.60	56.81±0.44	5.34±0.30	7.29±0.40	331±2	401±11	4.77±0.01
RS 09	2.54±0.11+	2.42±0.12	1.59±0.10	24.95±1.07+	19.34±0.26	48.60±0.73	4.98±0.06	6.38±0.13	309±1	379±6	5.42±0.05
RS 10	5.33±0.32+	1.89±0.07	4.56±0.28	16.76±0.76+	16.73±0.59	55.25±0.82	5.34±0.23	7.16±0.25	329±1	495±1	4.67±0.01
RS 11	4.28±0.01+	1.06±0.39	2.58±0.09	22.88±0.20+	15.55±0.37	45.83±0.44	4.43±0.18	6.18±0.29	368±1	383±0	4.92±0.02
RS 12	5.42±0.10	2.19±0.05	7.42±0.38	24.68±0.60	15.84±2.15	44.43±1.77	5.11±0.16	6.61±0.21	343±7	356±9	4.51±0.01
RS 13	5.73±0.07	2.14±0.03	7.33±0.13	22.05±0.14	17.75±0.52	44.01±0.46	5.10±0.27	6.48±0.79	330±3	468±6	4.61±0.01
RS 14	5.51±0.08	2.31±0.02	4.53±0.11	21.29±0.11	16.91±1.20	48.35±0.56	4.18±0.15	5.27±0.14	319±2	261±5	4.06±0.02
RS 15	5.32±0.07	2.61±0.35	7.24±0.42	21.18±0.22	19.40±1.91	43.59±2.80	3.44±0.14	4.32±0.16	324±8	224±6	4.69±0.01
RS 16	5.76±0.11	2.23±0.03	8.41±0.13	20.15±0.34	19.63±0.25	43.77±0.16	6.86±0.23	8.86±0.28	331±1	344±5	4.43±0.02
RS 17	5.61±0.08	2.17±0.01	5.47±0.18	22.46±0.67	17.67±2.17	45.33±1.48	5.52±0.15	6.71±0.10	320±4	330±5	4.55±0.01
RS average	4.28±1.19	2.23±0.41	4.56±2.16	22.36±2.26a	17.75±1.75	48.12±4.30b	5.24±0.70	6.76±0.91	322±14	344±86	4.77±0.40
Total average (n=28)	3.76±1.42	2.17±0.47	4.26±2.86	21.04±3.98	17.68±2.03	50.80±7.78	5.29±0.86	6.73±1.18	327±15	348±106	4.85±0.43
Brazilian legislation	Max. 4	Max. 4	Min. 1.8	Min. 8	Min. 2.0	14.5-55	-	-	-	-	-
Argentine legislation	Max. 8	Max. 4	-	15-28	-	45-55	-	-	-	-	-
p-value (ANOVA)	0.281	0.076	0.765	<0.001	0.240	<0.001	0.663	0.606	0.436	0.463	0.805

All results are expressed as mean ± standard deviation (n=3). ¥PR: Paraná state; SC: Santa Catarina state; RS: Rio Grande do Sul state. *Value calculated by difference. # Calories were calculated by multiplying grams of protein, carbohydrate and lipids by 4,4 and 9 kcal/g, respectively. Means followed by different letters in the same column are statistically different ($p < 0.05$). + Results presented by Sattler et al. (2015).

Pollen analysis

Table 5 shows the pollen types and their frequency of occurrence in the samples. In this study, 37 pollen types were identified in BP samples of southern Brazil. *Eucalyptus* pollen occurred in 64% of the samples and *Machaerium* pollen in 32% of BP samples. Some pollen types have been observed in only one sample, for example: Crotalaria (PR02), *Andira* (SC03) and *Dalbergia* (RS05).

Correlations of the pollen types with physicochemical parameters

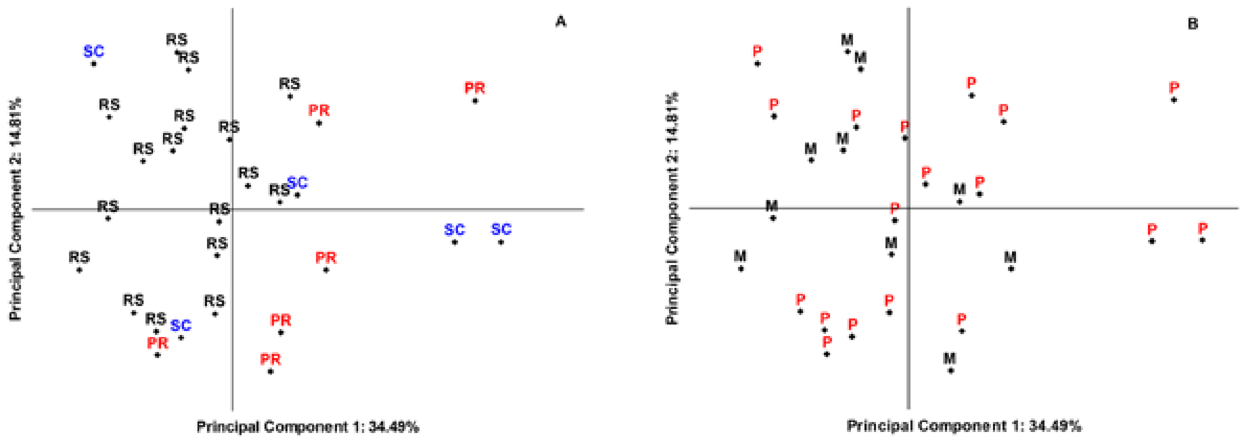
The correlations of physicochemical properties and B vitamin content of BP samples with their geographical origins were identified. PCA scores and loading plots of the two principal components (PC1 and PC 2) are shown in Figure 1 and the HCA dendrogram (Figure 2).

The sample distribution in Figure 1A shows that BP samples from Rio Grande do Sul were more clustered, revealing close similarity, compared to those from other states (Figure 1A). Despite the small number of samples from Paraná (n=6) and Santa Catarina (n=5), this plot illustrates a significant difference among their chemical composition and physicochemical properties. Figure 1B, on the other hand, displays a clear separation of samples revealing no significant influence of monofloral or polyfloral BP on the chemical composition and physicochemical properties of samples. However, 7 out of 10 monofloral samples presented the highest content of protein, ash, vitamin B6, and total dietary fiber (samples on the left-hand side). BP samples from Rio Grande do Sul also showed high content of the same variables. Levels of acidity and total carbohydrates (samples on the right-hand side) were greatest in samples of Paraná (Figures 1A, 1B and 1C). The HCA dendrogram corroborates the PCA observations: there was no clear distinction of BP samples of Southern Brazil in terms of physicochemical properties and chemical composition.

Table 5. Relative frequency (%) and classification of pollen types (botanical taxa) in dehydrated bee pollen samples.

Sample¥	Botanical taxa / Frequency (%) / Classification*
PR 1	*Eucalyptus 26.9 (AP) - Machaerium 49.9 (PP) - Phoradendron 5.8 (IIP) - Senecio 13.4 (IIP)*
PR 2	*Crotalaria 13.6 (IIP) - Eucalyptus 9 (IIP) - Piptocarpha 72 (PP)*
PR 3	*Eucalyptus 14.6 (IIP) - Ilex 7.4 (IIP) - Machaerium 62.1 (PP) - Rosaceae 5.6 (IIP)*
PR 4	*Eucalyptus 21.8 (AP) - Ilex 7.1 (IIP) - Machaerium 65.8 (PP)*
PR 5	*Brassica napus 6 (IIP) - Eucalyptus - 83.9 (PP) - Machaerium 5.1(IIP)*
PR 6	*Celtis 22.6 (AP) - Eucalyptus 13 (IIP) - Euterpe 38 (AP) - Ilex 23.9 (AP)*
SC 1	*Eucalyptus 15 (IIP) - Eupatorium 16.1 (AP) - Euterpe 32.6 (AP) - Ilex 4.5 (IIP) - Machaerium 10.2 (IIP) - Piper 14.7 (IIP) - Senecio 3.9 (IIP)*
SC 2	*Eucalyptus 22.2 (AP) - Lithraea 31.8 (AP) - Montanoa 21.2 (AP) - NI 17 (AP)*
SC 3	*Andira 98.5 (PP)*
SC 4	*Eupatorium 58.1(PP) - Eugenia 12.4 (IIP) - Ilex 7.4 (IIP) - Machaerium 9.8 (IIP) - Vernonia 6.3 (IIP)*
SC 5	*Asteraceae 15.8 (AP) - Brassica 77.3 (PP) - Eucalyptus 6.3 (IIP)*
RS 1	*Brassica napus 80.5 (PP) - Eucalyptus 19.4 (AP)*
RS 2	*Brassica napus 84.6 (PP) - Eucalyptus 9.2 (IIP) - Machaerium 5.2 (IIP)*
RS 3	*Brassica napus 48.5 (PP) - Caryca 13.3 (IIP) - Eucalyptus 16.4 (AP) - Machaerium 16.6 (AP) - Psychotria 4.7 (IIP)*
RS 4	*Caesalpiniaceae 56.1 (PP) - Eucalyptus 32.3 (AP)*
RS 5	*Dalbergia 16.4 (AP) - Eucalyptus 70.6 (PP) - Lithraea 10.5 (IIP)*
RS 6	*Mimosa scabrella 99.2 (PP)*
RS 7	*Eupatorium 6.4 (IIP) - Ilex 42 (AP) - Mimosa scabrella 43.7 (AP)*
RS 8	*Ambrosia 35.5 (AP) - Eupatorium 61.2 (PP)*
RS 9	*Eucalyptus 20.9 (AP) - Euterpe 10.1 (IIP) - Melastomataceae 25.6 (AP) - Piper 6.3 (IIP) - Poaceae 31.4 (AP)*
RS 10	*Asteraceae 4.8 (IIP) - Borreria 5.3 (IIP) - Eucalyptus 21.8 (AP) - Euterpe 16 (AP) - Machaerium 13.1 (IIP) - Phrygilanthus 3.5 (IIP) - Protium 4.4 (IIP) - Rubiaceae 12.3 (IIP) - Tapirira 3.8 (IIP)*
RS 11	*Melastomataceae 40.2 (AP) - Myrcia 32.7 (AP) - Piper 22.9 (AP)*
RS 12	*Mimosa scabrella 81.6 (PP) - Poaceae 5.8 (IIP) - Vernonia 5.8 (IIP) - NI 3.7 (IIP)*
RS 13	*Mimosa scabrella 94.8 (PP)*
RS 14	*Baccharis 4.7 (IIP) - Ilex 7.1 (IIP) - Mimosa scabrella 86 (PP)*
RS 15	*Asteraceae 20.5 (AP) - Ilex 71.8 (PP) - Poaceae 7.3 (IIP) - Podocarpus 3.8 (IIP)*
RS 16	*Asteraceae 3 (IIP) - Eucalyptus 75.7 (PP) - Ilex 4.5 (IIP) - Lamanonia 15 (IIP)*
RS 17	*Eucalyptus 69.8 (PP) - Eupatorium 29.2 (AP)*

¥PR: Paraná state; SC: Santa Catarina state; RS: Rio Grande do Sul state. *PP = predominant pollen type (> 45% of pollen grains); AP = accessory pollen type (between 16% and 45%); IIP = important isolated pollen type (between 3% and 15%); NI=not identified.



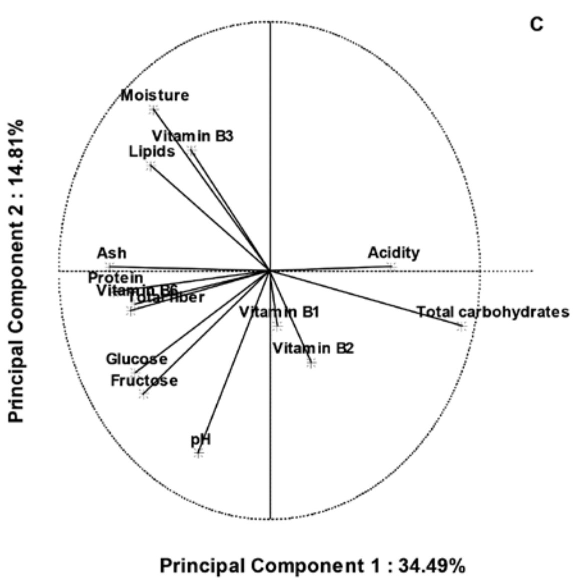


Figure 1. Principal Component Analysis (PCA) scores plots of PC1 versus PC2 for the 28 bee pollen samples of different geographical origins (1A) and different compositions (pollen type: M- monofloral and P - polyfloral) (1B); PCA factor loading plot of variables along PC1 and PC2 (1C).

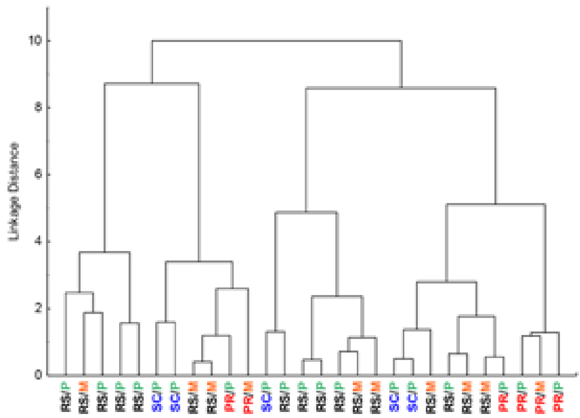


Figure 2. Hierarchical Cluster Analysis (HCA) dendrogram of the 28 bee pollen samples analyzed in this study. Note that: M - monofloral and P - polyfloral pollen types; SC - Santa Catarina, RS - Rio Grande do Sul, and PR - Paraná state.

DISCUSSION

Thiamine values in the current study were consistent with results in Arruda et al.[5,18] who reported values between 0.64-1.01 mg of vitamin B1/100 g of dehydrated BP from the southeastern Brazilian city of Pariquera-Açu and to dehydrated BP from São Paulo state, Brazil (0.5-1.3 mg/100 g) reported by in De-Melo et al[19]. However, concerning riboflavin content, De-Melo et al.[19] reported only 0.4-0.6 mg of vitamin B2/100 g; and Arruda et al.[5,18] found higher riboflavin levels (1.77- 2.56 mg/100g). BP from Southeastern Brazil have been reported to display higher levels of niacin - 7.27-14.43 mg/100 g whereas vitamin B_6 levels in this study were higher and showed far greater variation among samples than those of the same region (0.33 to 0.77 mg/100 g)[5,18]. The content of vitamins in BP can vary according to botanical origin[2]. De-Melo et al.[19] evaluated the influence of dehydration conditions on B-complex vitamin content, but the results were inconclusive.

Carpes et al.[20] also reported high moisture content for BP (n= 36) of Southern Brazil, with the highest values observed in the states of Paraná and Rio Grande do Sul.

Also, the moisture level of 154 BP samples from all over Brazil (including 49 samples from Paraná, Santa Catarina and Rio Grande do Sul) were analyzed by Karl Fischer and all state moisture content averages were over 4%[21]. However, a quantitative comparison among these moisture content data is difficult as methods used by researchers were different. Argentine legislation[17] sets a maximum moisture content of 8 g/100 g and establishes the analytical method for determination (oven under vacuum at 65°C). Brazilian legislation[10], however, does not provide any methods for quality control of BP.

The nutritional value of BP varies according to botanical and geographical origin[2]. Carpes et al.[20] and Modro et al.[22] have reported similar variations in the sugar content of BP samples (41.10 g to 54.43 g/100 g and 60.40 g to 77.22 g/100 g, respectively). Marchini et al.23 however, found lower sugar content (13.2-36 g/100 g) in BP samples (n=13) from the city of Piracicaba (SP, Brazil). Martins et al.[24] observed greater state averages for reducing sugars in BP samples of the same three Brazilian states. It is also noted that the values found were lower than those reported by De-Melo et al.[19] for dehydrated BP from São Paulo, Brazil (glucose: 13.3-18.2 g/100 g; fructose: 18.7-26.9 g/100 g).

In BP, protein levels can vary between 2.5 and 62 g/100 g of sample, according to botanical origin[2]. In this study, the results were similar to those reported by Carpes et al.[20], between 15 and 27.7 g/100 g. The highest content of protein in samples of Rio Grande do Sul could be explained by the predominance of *Brassica napus* and *Mimosa scabrela* pollen types. *B. napus* BP contains protein levels between 23 and 27.3 g/100 g; and *M. scabrella* BP contains values between 11.7 and 33.9 g/100 g[2].

Total lipid content average is consistent with the value reported by Carpes et al.[20] (4.90 g/100 g). Bastos et al.[25] have found lipid levels between 6.1 g and 14 g/100 g in commercial samples of dehydrated BP from São Paulo and Minas Gerais, Brazil. In BP, lipid content generally varies according to the composition of its plant of origin; however, the dehydration process can influence the results of the analysis[19].

The total ash content average in this study is consistent with those reported in Almeida-Muradian et al.[11], Arruda et al.[5], Carpes et al.[20], Marchini et al.[23], Martins et al.[24] and Melo et al.[26], between 2.4 and 3.1 g/100 g. These values are influenced by the botanical origin and mainly by the geographical origin of the BP2,[27]. Besides this, ash content can also indicate the accidental presence of inorganic contaminants such as sand, soot or metals in BP[11].

Dietary fiber is the portion of food that is not digested by enzymes in the human gastrointestinal tract. Fiber is beneficial as it has been associated with a decreased risk of obesity, diabetes and cardiovascular problems[28,29]. Few studies on the chemical composition of BP have measured dietary fiber content in samples. Carpes et al.[20], analyzed dehydrated BP samples from the same three Brazilian states but reported dietary fibers below 4.9 g/100 g BP.

Averages for free acidity of dehydrated BP samples from the same southern Brazilian states as reported by Martins et al.[24] were lower (Paraná, 292 mEq/kg; Santa Catarina, 264 mEq/kg and Rio Grande do Sul, 263 mEq/kg) and compliant with Brazilian requirements compared to our samples. In fact, great variation in free acidity measurements is expected as Brazilian legislation[10] does not establish any analytical methods for its determination. Besides this, it is possible that the results vary according to the botanical origin of the BP samples.

Few authors have determined pH values of BP, however, similar values have been reported in BP samples from southeastern Brazilian states in at least one other study[23].

Many studies have been conducted in different Brazilian regions in order to identify nutrient sources used by bees for their survival[30,31]. Ideal sources include plants that provide great amounts of nectar and pollen. However, potential flowers sources for beekeeping has been found to differ across regions in Brazil[32,33]. Barth el al.[14], also reported Eucalyptus and Brassica pollen types in BP samples of the Santa Catarina state; and *Eucalyptus, Eupatorium, Vernonia* and *Montanoa*, pollen types in BP samples from Paraná. In southern Brazil, *Eucalyptus pollen grains appears* to be an important source of pollen for the bees.

Kaškonienė et al.[34], Morgano et al.[35] and Sattler et al.[1] have used multivariate statistical techniques to identify correlations of physicochemical properties of BP samples with geographical origin, processing conditions and botanical sources. However, to the best of our knowledge, this is the first study to attempt to correlate the levels of B complex vitamins in BP samples with the same variables. Mărgăoan et al.[36], evaluated the carotenoid and fatty acid profiles of 16 BP samples from Romania, and conducted multivariate statistical analysis to identify similarities among pollen types and chemical parameters. PCA analysis revealed a clear separation of pollen samples (the first two principal components explaining 89% of variance of data); total carotenoid content, lutein content, β-cryptoxanthin content, myristic acid (14:0) concentration, and predominant pollen type *Filipendula ulmaria* (polifloral) contributed significantly to the clear distinctions observed. The composition of identified volatile and non-volatile compounds, radical scavenging activities, and total amounts of phenolic compounds and flavonoids of 14 BP samples of the Baltic region have been reported to vary according to their geographical origin, probably because of their different botanical compositions[32]. Although our results showed no clear distinction of BP samples, multivariate statistical analysis can be useful in identifying the composition of BP samples and should be used to provide more accurate interpretation of physicochemical data.

CONCLUSIONS

Quantitative analysis of the B vitamins and their vitamers in BP samples of Southern Brazil followed by multivariate statistical analysis showed that BP is a good source of these nutrients and that their content should be evaluated locally. No differences in the levels of B_3 and B_6 vitamins in dehydrated BP samples of Southeastern Brazil have been reported in the literature, and the samples of Southern Brazil analyzed in this study also showed no difference. The significant amounts of B complex vitamins found in BP suggest that it could be a natural dietary source of these compounds and an alternative to currently available supplements.

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