



**USP**

Universidade de São Paulo  
Brasil



**Apimondia**  
Ukraine 2013

# **PYRIDOXOL, PYRIDOXAL AND PYRIDOXAMINE (VITAMIN B<sub>6</sub> VITAMERS) CONTENT IN BRAZILIAN DEHYDRATED BEE POLLEN**

Ligia Bicudo de Almeida Muradian,  
Bianca Rodrigues de Souza,  
José Augusto Gasparotto Sattler

UNIVERSITY OF SÃO PAULO - BRAZIL

# PHARMACEUTICAL SCIENCE SCHOOL UNIVERSITY OF SÃO PAULO, BRAZIL





# International Honey Commission

- World Network of Honey and Bee Product Science -

[Home](#) [Reports](#) [Publications](#) [Contact](#) [Members](#)



Welcome on the Homepage of the International Honey Commission (IHC).



An International Honey Commission (IHC) was formed in 1990 in order to create a new world honey standard. All modern routine honey analysis methods were collaboratively tested and compiled as "Harmonised methods of the European Honey Commission", published in *Apidologie*, extra issue, 1-59, 1997. Based on these method the Codex Alimentarius Standard and the EU Honey Directive were revised.

Next, the major European unifloral honeys were characterised in a special issue of *Apidologie* (2004), allowing the international trade with these honeys.



## Contact



Gudrun Beckh  
Head of IHC  
Quality Services International GmbH, Bremen, Germany

[gudrun.beckh@qsi-q3.de](mailto:gudrun.beckh@qsi-q3.de)



Maria Teresa Sancho  
Co-Chair of IHC  
Leader Working Group: Honey Analysis Methods

Departamento de Biotecnología y Ciencia de los Alimentos  
Universidad de Burgos, Spain

[mtsanco@ubu.es](mailto:mtsanco@ubu.es)



Ligia Bicudo de Almeida Muradian  
Co-Chair of IHC  
Leader Working Group: Non Apis Mellifera

Faculdade de Ciências da univ. De Sao Paulo, Brazil

[Ligia.muradian@gmail.com](mailto:Ligia.muradian@gmail.com)

# INTRODUCTION



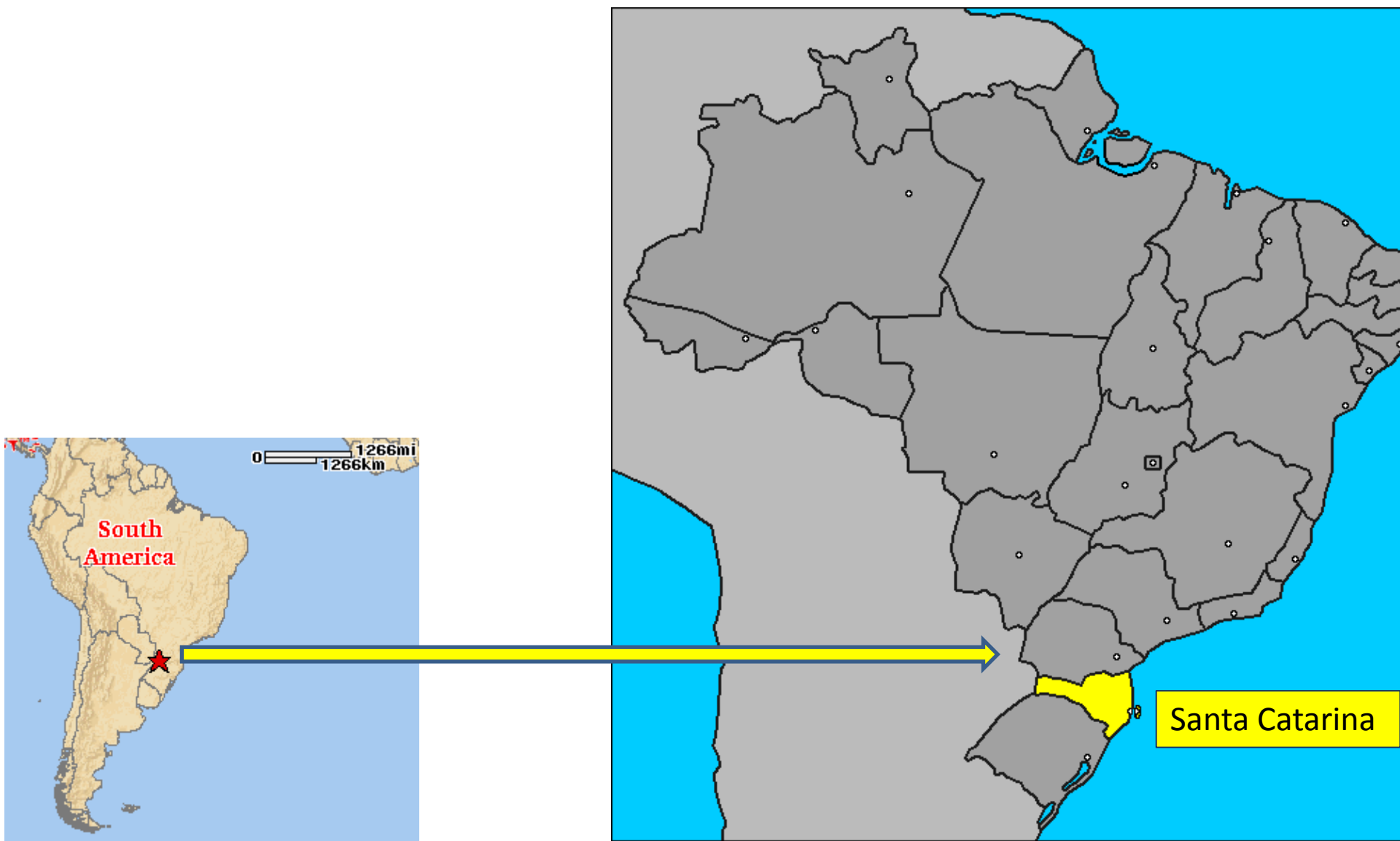
- Bee pollen consists of a "mix" of floral pollen, nectar and salivary secretions agglutinated in acorns that are collected in the entry of worker bees into the hive.
- Composition: proteins, carbohydrates, lipids, minerals, antioxidant vitamins, B complex vitamins, phenolic compounds (mainly flavonoids)
- The knowledge of the nutritional composition of food consumed in Brazil is of critical importance to evaluate nutrient supply and its consumption by populations.

# INTRODUCTION



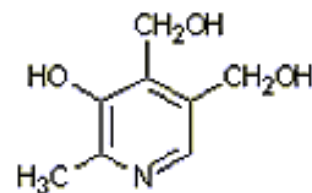
- Arruda *et al.* (2013) have determined the levels of pyridoxol, pyridoxal and pyridoxamine in bee pollen from Pariquera-Açu city, State of São Paulo, southeastern Brazil, however at concentrations that do not reach the Dietary Reference Intakes (DRI) per serving portion.

In southern Brazil, specifically in Santa Catarina there is a high bee pollen production which supplies the national market and exports to Colombia and Uruguay (South America)

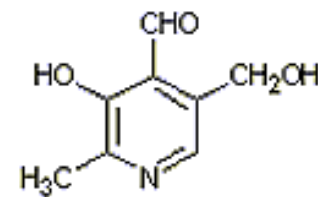


# OBJECTIVE

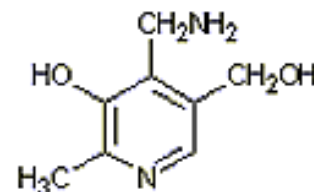
## Vitamin B6



Pyridoxine  
(Pyridoxol)



Pyridoxal



Pyridoxamine

- Due to the economic importance of this regional product, this study aimed to evaluate the contents of the three vitamin B<sub>6</sub> vitamers (**PYRIDOXOL, PYRIDOXAL AND PYRIDOXAMINE**) in lots of dehydrated bee pollen from *Apis mellifera* and harvested on different apiaries installed in the States of Paraná, Santa Catarina and Rio Grande do Sul, Brazil.



# MATERIAL AND METHODS



- 22 batches of dehydrated bee pollen were collected from hives of *Apis mellifera* during the period from August 2011 till August 2012.
- Samples were obtained in different places of Paraná, Santa Catarina and Rio Grande do Sul states, Brazil and were send to Food Analysis Laboratory/FCF-USP/Brazil.

# Paraná



- 18
- 17
- 10
- 11
- 8
- 9
- Palmeira
- Lapa
- União da Vitória

# Santa Catarina



- 14
- 13
- 12
- 21
- 5
- Jacutinga
- Erichim
- São José



- 16
- 19
- 20
- Ijuí
- Cruz Alta



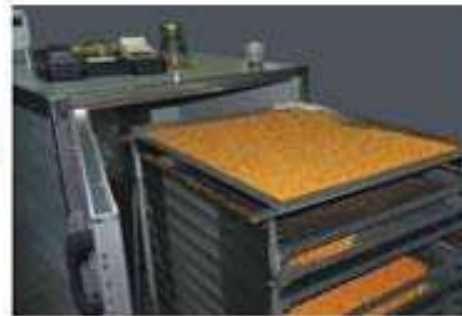
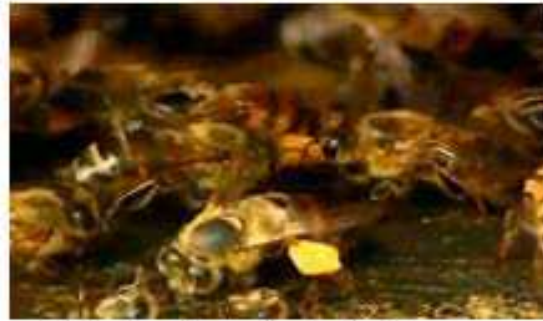
- 22
- 15
- Içará
- Balneário Gaivota

# Rio Grande do Sul



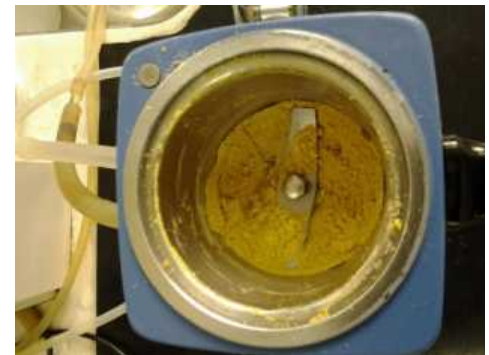
- 3
- 4
- 1
- 2
- São Gabriel
- 6
- 7
- Novo Hamburgo





# MATERIAL AND METHODS

- All batches were ground in analytical mill (IKA<sup>®</sup> Wercke - M20) during standardized time in three series of 30 seconds to carry out the analysis for the determination of the three vitamin B<sub>6</sub> vitamers.



# METHOD

Determination according to Arruda *et al.*  
(2013a)

Food and Chemical Toxicology 51 (2013) 143–148



Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: [www.elsevier.com/locate/foodchemtox](http://www.elsevier.com/locate/foodchemtox)



Presence and stability of B complex vitamins in bee pollen using different storage conditions

Vanilda Aparecida Soares de Arruda<sup>a</sup>, Aline Aparecida Santos Pereira<sup>a</sup>, Leticia M. Estevinho<sup>b,\*</sup>, Ligia Bicudo de Almeida-Muradian<sup>a</sup>



# METHOD

Determination according to Arruda *et al.*  
(2013b)

Journal of Food Composition and Analysis 29 (2013) 100–105



Contents lists available at SciVerse ScienceDirect

Journal of Food Composition and Analysis

journal homepage: [www.elsevier.com/locate/jfca](http://www.elsevier.com/locate/jfca)



Original Research Article

Dried bee pollen: B complex vitamins, physicochemical and botanical composition

Vanilda Aparecida Soares de Arruda <sup>a</sup>, Aline Aparecida Santos Pereira <sup>a</sup>, Alex Silva de Freitas <sup>b</sup>,  
Ortrud Monika Barth <sup>b,c</sup>, Ligia Bicudo de Almeida-Muradian <sup>a,\*</sup>

# EXTRACTION METHOD



Weight 5 g



Add 50 mL of 0,1 M HCl



98 °C during 30 min



pH adjustment to 4.6 with 2.5 M sodium acetate



42 °C for 2 h



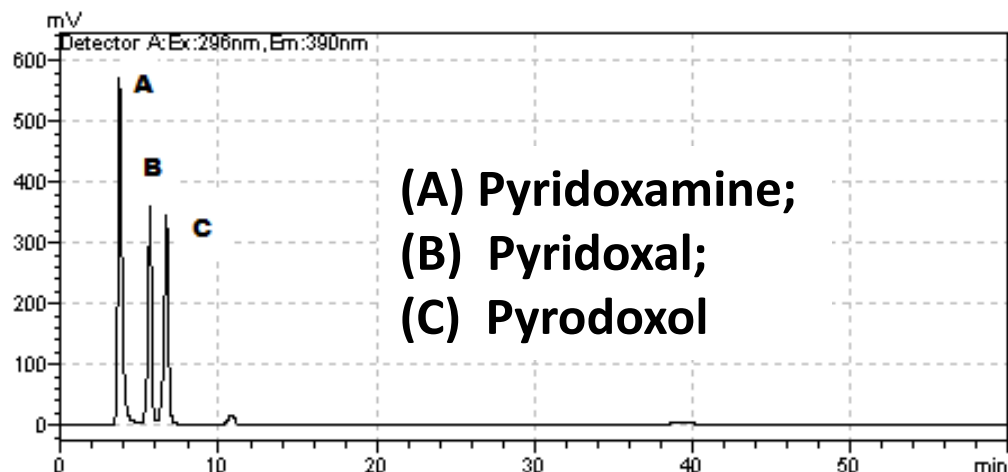
Add 5 g of Taka diastase

Transfer to a 100 mL volumetric flask

Filtration of the mixture through filter paper

Filtration of the extract through 0.45 µm cellulose ester membrane

# CHROMATOGRAPHIC CONDITIONS

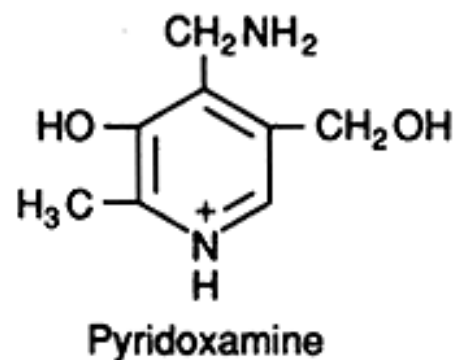
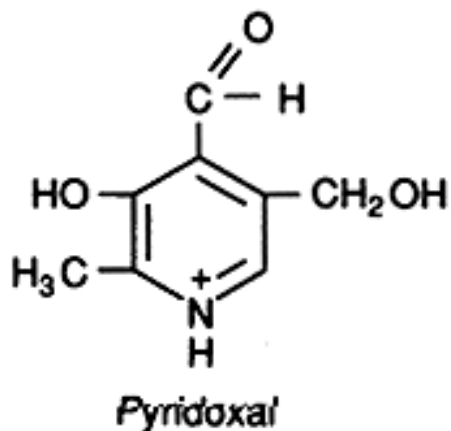


- 20  $\mu\text{L}$  were injected in HPLC
- column  $\text{C}_{18}$  reversed-phase Superspher 100 RP-18 endcapped 5  $\mu\text{m}$ /250 x 4.0 mm
- pre-column 5  $\mu\text{m}$ /4 x 4 mm Lichrospher 100 RP-18
- Mobile phase: phosphate buffer pH 2.5 (0.68%  $\text{KH}_2\text{PO}_4$ ) with ion pair (0.014% PIC 7) and acetonitrile (96:4); flow 0.6 mL/min;
- Detection by fluorescence: Ex 296 nm; Em 390 nm.

- The experiment were performed in triplicate ( $n = 3$ ) and the results were expressed as mean  $\pm$  standard deviation.

# RESULTS AND DISCUSSION

- Regarding the concentrations of vitamin B<sub>6</sub> **only pyridoxal and pyridoxamine** could be quantified in **all analyzed samples**.



Samples	Pyridoxamine (mg / 100 g)*	Pyridoxal (mg / 100 g)*	Pyridoxol (mg / 100 g)*	Vitamin B <sub>6</sub> (mg / 100 g)
1	0.43 ± 0.03	6.70 ± 0.30	0.02 ± 0.00	7.15 ± 0.33
2	0.50 ± 0.05	5.76 ± 1.44	0.01 ± 0.00	6.27 ± 1.49
3	0.40 ± 0.07	5.09 ± 1.41	BOQ	5.49 ± 1.47
4	0.46 ± 0.02	5.59 ± 0.43	BOQ	6.04 ± 0.45
5	0.48 ± 0.02	3.24 ± 0.20	0.02 ± 0.00	3.74 ± 0.22
6	0.65 ± 0.10	2.91 ± 0.21	BOQ	3.55 ± 0.31
7	0.52 ± 0.03	5.53 ± 0.10	BOQ	6.05 ± 0.13
8	0.36 ± 0.03	0.42 ± 0.04	BOQ	0.78 ± 0.07
9	0.26 ± 0.02	1.48 ± 0.16	BOQ	1.74 ± 0.18
10	0.66 ± 0.02	4.73 ± 0.14	0.02 ± 0.01	5.42 ± 0.16
11	0.50 ± 0.06	5.14 ± 0.06	BOQ	5.64 ± 0.13
12	0.53 ± 0.01	2.97 ± 0.04	BOQ	3.50 ± 0.05
13	0.75 ± 0.12	2.28 ± 0.05	0.04 ± 0.00	3.07 ± 0.18
14	0.83 ± 0.02	1.82 ± 0.04	0.07 ± 0.00	2.72 ± 0.06
15	0.48 ± 0.06	4.12 ± 0.05	BOQ	4.59 ± 0.11
16	0.74 ± 0.05	3.60 ± 0.16	BOQ	4.34 ± 0.21
17	0.44 ± 0.01	2.63 ± 0.03	0.01 ± 0.01	3.08 ± 0.05
18	0.29 ± 0.05	1.12 ± 0.03	BOQ	1.41 ± 0.08
19	0.63 ± 0.07	2.29 ± 0.02	BOQ	2.91 ± 0.09
20	0.73 ± 0.01	2.41 ± 0.01	BOQ	3.14 ± 0.03
21	0.95 ± 0.06	3.82 ± 0.14	0.03 ± 0.01	4.81 ± 0.21
22	0.70 ± 0.01	4.88 ± 0.10	0.05 ± 0.01	5.63 ± 0.12

\*A ± SD (Average ± Standard Deviation), n=3, BOQ (Below limit of quantification).



Samples	Vitamin B <sub>6</sub> (mg / 100 g)	Vitamin B <sub>6</sub> (mg / 25 g)
1	7,15	1,79
2	6,27	1,57
3	5,49	1,37
4	6,04	1,51
5	3,74	0,94
6	3,55	0,89
7	6,05	1,51
8	0,78	0,20
9	1,74	0,44
10	5,42	1,36
11	5,64	1,41
12	3,5	0,88
13	3,07	0,77
14	2,72	0,68
15	4,59	1,15
16	4,34	1,09
17	3,08	0,77
18	1,41	0,35
19	2,91	0,73
20	3,14	0,79
21	4,81	1,20
22	5,63	1,41

DRI ( 1.3 mg vitamin B<sub>6</sub>/ day)

Portion suggested: 25g

Source (portion of 25 g): min 15% DRI = 0.19 mg

**Rich (portion of 25 g): min 30% DRI = 0.39 mg**

<b>Food</b>	<b>Vitamin B6 (USDA)</b> <b>(mg / 100 g)</b>
Dehydrated bee pollen	0.78 – 7.15
	<b>(USDA)</b>
Wheat germ	1.300
Chicken Liver (cooked)	0.840
Banana	0.367
Chicken	0.310
Lentils	0.164
Oat (raw)	0.119
Beans (cooked)	0.093
White rice (cooked)	0.026

# CONCLUSION

- The consume of dehydrated bee pollen from the Southern region of Brazil could be an excellent complement in regarding of the necessary dairy consumption of vitamin B<sub>6</sub> by the population.

THANK YOU !!!



Conselho Nacional de Desenvolvimento  
Científico e Tecnológico



[ligiabi@usp.br](mailto:ligiabi@usp.br)

