QUALIDADE DE PÓLEN APÍCOLA DESIDRATADO

DEHYDRATED APICULTURE POLLEN QUALITY

SILVA, Elen Vanessa Costa da ^{1*}; REIS, Igor Fernando de Araújo¹; ALMEIDA, Leandro das Neves Tolosa de¹; PRAZERES, Alan Reis dos¹; SILVA, Josyane Brasil da¹; OLIVEIRA, Andrey Carlos do Sacramento de²

¹ Universidade do Estado do Pará, Departamento de Tecnologia de Alimentos, Tv. Eneas Pinheiro 2626, cep 66.095-100, Belém – PA, Brasil. (fone: +55 91 981140062)

² Universidade Federal do Pará, Br Km 62, Castanhal, PA, Brasil.

* Autor correspondente
e-mail: elen.vanessa@uepa.br

Received 11 September 2018; received in revised form 18 October 2018; accepted 22 October 2018

RESUMO

O pólen das flores é recolhido pelas abelhas, posteriormente aglutinado e transportado para a colmeia, onde é colocado nos alvéolos dos favos de mel. Devido o aumento no consumo de produtos naturais, observou-se uma crescente utilização e comercialização do pólen apícola, justificando a necessidade de um maior controle de qualidade quanto às características físico-químicas e microbiológicas desse produto. Desta forma, a pesquisa objetivou determinar as características físico-químicas, microbiológicas e sensoriais de quatro marcas de pólen desidratado comercializadas em casas de produtos naturais em diferentes pontos da cidade de Belém-Pará. Foram realizadas as análises físico-químicas (umidade, cinzas, lipídios, proteínas, pH, acidez livre, açúcares total), microbiológicas (Bactérias aeróbias mesófilas, Fungos e leveduras, Coliformes a 45°, *Salmonella sp* e *Staphylococcus aureus*) e sensorial. Os resultados obtidos encontraram-se dentro dos parâmetros de qualidade permitidos pela legislação brasileira para pólen desidratado. Quanto a análise microbiológica, todas as marcas apresentaram ausência de *Staphylococcus aureus* e *Salmonella spp*. Para a análise sensorial, a marca C apresentou melhor resultado tanto para sabor quanto para aroma, confirmando a qualidade do pólen desidratado comercializado em Belém-Pa.

Palavras-Chaves: apicultura; proteína; legislação; microbiológica; sensorial.

ABSTRACT

Due to the increase in the consumption of natural products, there has been a growing use and commercialization of bee pollen, thus, the need for a greater quality control regarding the physical-chemical and microbiological characteristics of this product is justified. In this way, the research aimed to determine the physical-chemical, microbiological and sensorial characteristics of four brands of dehydrated pollen, marketed in natural products houses in different parts of the city of Belém-Pará. Microbiological (aerobic mesophilic bacteria, fungi and yeasts, Coliformes at 45°, *Salmonella sp* and *Staphylococcus aureus*) and sensorial analyzes were carried out in the physicochemical analyzes (moisture, ashes, lipids, proteins, pH, free acidity, total sugars). The results obtained were within the parameters of quality allowed by the Brazilian legislation for dehydrated pollen. Regarding the microbiological analysis, all brands showed absence of *Staphylococcus aureus* and *Salmonella spp*. For the sensorial analysis, the C mark presented better results for both flavor and aroma, confirming the quality of the dehydrated pollen commercialized in Belém-Pa.

Keywords: apiculture; protein; legislation; microbiological; sensorial.

INTRODUCTION

Bee pollen is the result of the pollen agglutination of flowers by worker bees, involving nectar and its salivary substances, which is collected at the entrance of the hive (BRASIL, 2001).

The commercial production of pollen has gained the attention of beekeepers in several countries with bee potential. China, USA, and Japan are the main importers and consumers. Spain, China, Australia, Argentina, the world's largest producers (ESTEVINHO *et al.*, 2012).

With increasing consumption of natural products, pollen has been used and marketed more frequently, increasing the need for quality control of physico-chemical and microbiological characteristics. In Brazil, the pollen legislation is incipient, since it indicates the limits, but does not stipulate the methods of analysis, so the quality control of bee pollen requires standardization of methods (MURADIAN, 2009).

The chemical and nutritional composition is variable and depends on the floral origin (MELO *et al.*, 2009). Pollen must meet the quality and certification criteria before marketing. Apolar pollen has been prominent both because it contains nutritional substances such as carbohydrates, proteins, amino acids, lipids, vitamins, minerals and micronutrient traits, as well as its therapeutic properties, such as antioxidant activity (SOMERVILLE, 2005; ALMARAZ-ABARCA *et al.*, 2007) and is one of the few foods that has all the essential amino acids (ESTEVINHO *et al.*, 2012), such as lysine, tryptophan, histidine, leucine, isoleucine, methionine, phenylalanine, glutamic acid, arginine, cystine, trionine, valine RIBEIRO *et al.*, 2007), in addition to carotenoids, flavonoids and phytosterols being the reason for their use as alternative food and / or food supplement.

The aim of this study was to evaluate the physicochemical composition, the microbiological and sensorial quality of four samples of dehydrated pollen commercialized in the region of Belém, Pará.

MATERIAL AND METHODS

The samples of bee pollen used in the study came from four commercial establishments of natural products located in different points of Belém-PA. The pollen samples were named as

samples A1, A2, A3, A4 for each brand and taken to the Food Laboratory of the State University of Pará (UEPA).

Chemical and physical methods for analysis of dehydrated pollen (moisture, protein, lipids, ash (fixed mineral residue), pH, determination of reducing sugars (air) and total sugars, titratable acidity, water activity (aw) followed the Instituto Adolfo Lutz (2005), and the methods described in Normative Instruction N°. 3 of January 19, 2001 approving the Technical Regulations on the Identity and Quality of Apitoxin, Bee Wax, Royal Jelly, Lyophilized Royal Jelly, Bee Pollen, Pollen Desiccated Beekeeping, Propolis and Propolis Extract, (BRASIL, 2001).

Humidity: based on the weight loss of the sample by gravimetric method, subjecting the sample in an oven with circulation and renewal of air at a temperature of 105 ° C, until obtaining constant weight.

Ashes: the sample was charred until smoke cessation ceased and then calcined in muffle at 550 ° C until constant weight was obtained.

Lipids: was obtained by the direct extraction method, using the Soxhlet type extractor, using n-hexane as the solvent.

Proteins: based on the determination of total nitrogen and was conducted according to the micro-Kjeldahl digestion method, where the existing nitrogen is transformed into ammonia, using the empirical factor 6,25.

Aw: was performed on AQUALAB model 4TEV water activity analyzer.

Acidity: It was carried out by titration using as indicator phenolphthalein solution and titration with 0.1 M sodium hydroxide solution until pink.

Reducing and Total Sugars: They were made through the titration method with solutions of Fehling A and B, according to AOAC (1997), method n ° 920.183b.

Due to the lack of specific microbiological parameters for bee pollen in the Technical Regulation on Food Microbiological Standards of ANVISA (BRASIL, 2001), the microorganisms related to similar food groups (group 10, flours, pasta, bakery, processed and packaged, and the like, including granola and unpactised cereal mix, with or without additives and the like).

The four brands of dehydrated bee pollen were submitted to analyzes of mesophilic aerobic bacteria, fungi and yeasts, Coliforms at 45 °, *Salmonella* sp and *Staphylococcus aureus* (BRASIL, 2003).

The hedonic scale acceptance test was performed for the aroma and flavor attributes for the four brands of dehydrated pollen according to the Adolfo Lutz Institute (2005).

The results of the physical-chemical analyzes were evaluated through the mean analysis, standard deviation, variance (ANOVA), at a significance level of 5% and Tukey test for comparison between means.

RESULTS AND DISCUSSION:

The results of the physico-chemical characteristics of the four brands of pollen marketed in Belém (Tab. 2) are in accordance with the parameters required by the Brazilian legislation (2001). From the statistical analysis, it was observed that the samples presented significant difference for the protein, total sugars and free acidity analyzes at the level of 5%.

The analysis of moisture in samples A, B, C and D was within the parameters required by the legislation that is of a maximum of 4% and did not present differences among themselves at the level of 5%, this fact is probably due to the correct process drying (time and temperature) during the processing and storage (closed packages) of the bee pollen during the commercialization stages. These results were similar to the values found by Oliveira (2006) when analyzing samples collected in São Paulo where they found values from 2.5 to 3.5% for dehydrated pollen samples. In the work of Carpes, (2008) that evaluated the pollen of the southern region of Brazil, the moisture content ranged from 1.69% to 7.84%, and 53% of the samples were submitted under brazilian legislation.

The Brazilian legislation establishes for ashes values of maximum 4% m / m dry basis for pollen, being the value found in this work for samples A, B, C and D within the standards established by the legislation of which the same did not differ at a level of 5%. Indicating that there were no manipulation failures and the pollen did not present content of vegetal residues, dust, propolis and fragments of bees (legs and wings). Melo *et al.* (2009) found an average ash value of

3.08% in samples of dehydrated bee pollen. Azevedo *et al.* (2009) analyzing samples of pollen from Ceara found value between 3.0 and 3.6% for ash. Martins (2010) found 1.33 to 4.13 g / 100g for ash in samples of bee pollen from all Brazilian states.

The values found in this work for protein analysis are within the standards required by Brazilian law, with values varying between 12.38% and 23.57%, differing among them at the 5% level. Pollen is one of the few foods that contains all the essential amino acids that the organism alone can not synthesize (LEGLER, 2002).

This work found values similar to those of Martins (2010), which found 12.28 to 27.07 g / 100g for proteins in samples of bee pollen among all Brazilian states. The work of Carpes (2008) that evaluated the pollen of the southern region of Brazil, the protein content ranged from 15.04 to 27.69%.

The values found in this study average between 15.49% and 32.40%, being within the standards of the legislation (Brazil, 2001), differentiating each other at the level of 5%. A, B, C and D obtained different results according to tukey test. This difference can be related to the fact that the bee mixes nectar to the pollen at the time of collection, raising its sugar content.

This work found approximate values for the work of Marchini *et al.* (2006) analyzing samples of pollen from Piracicaba, found a mean value of total sugar of 28.4% and lower than the results found by Arruda (2009), who found a mean value of 64, 80 ± 0,18 for dehydrated bee pollen collected in the Atlantic Forest Zone, Ribeira Valley - state of São Paulo, Brazil.

The values found in this work average between 170.47 and 228.68 mEq / kg, according to the Brazilian legislation (Brazil, 2001) the results are within the standards that are maximum of 300 mEq / kg, and differ between 5% level. The acid content of the pollen is directly related to the floral origin from which it was collected. Marchini *et al.* (2006) analyzing samples of pollen from Piracicaba found a mean free acid value of 207 meq / kg. Campos *et al.* (2009) found an average value of 213 mEq / kg.

The Brazilian legislation establishes parameters for pH between 4 and 6 in pollen samples, the value found in this work being 4.5 ± 0 for sample A, from 4.5 ± 0 for sample B, 4.0 ± 0 for sample C and 4.2 ± 0 for sample D. The

samples present statistical difference at 5% level. The pH value can be influenced by the pH of the nectar, soil or plant species. Mandibular bee substances added to pollen when transporting to the hive may also alter the pH of the pollen. Azevedo *et al.* (2009) analyzing samples of Ceara pollen found value between 4.31 and 4.59 for pH.

The lipid value for sample B did not present significant difference in relation to sample A and C, also in relation to sample D at 5% level in the tukey test. According to Brazilian legislation (BRASIL, 2001), it can be verified that all values for lipids are above the minimum allowable value (1.8%) and differ from each other at the level of 5%.

Similar values were found in the work of Oliveira, (2010) that analyzed samples of bee pollen collected in establishments in the city of Pindamonhangaba presented values ranging from 4.8% to 7.99%, with an average of 6.88%. Melo *et al.* (2009) found an average lipid value of 4.97% in samples of dehydrated bee pollen.

The Brazilian legislation does not establish parameters for water activity in pollen samples, and the value found in this work for the sample C and D is statistically different from each other at 5% level. Barreto *et al.* (2005) states that for pollen to be considered dehydrated, the water activity should be at most 0.57 (Wa), so all samples are in compliance.

We found lower values than Oliveira (2010) found values of water activity ranging from 0.31 to 0.52, with an average of 0.39 in samples of bee pollen collected in establishments in the municipality of Pindamonhangaba.

According to Tab. 2, the analyzed pollen sample, brand A presented low value for 45 ° Coliforms, Mesophilic Aerobes and Fungi and Yeasts. According to Tab. 2, the B and C marks showed high values in Mesophilic Aerobes and Fungi and Yeasts, and low values for Coliforms at 45 °, in the D mark obtained high values for the Aerobic Mesophilic and Fungos and Yeasts analyzes and a high value of Coliforms at 45 °, this can be justified by the higher degree of aw compared to the other samples.

The mesophilic aerobic count or standard plate count in a food product reflects the sanitary quality of the food or raw material, as well as processing, handling and storage conditions. The presence of mesophilic bacteria in large numbers

may indicate contaminated raw material; cleaning and disinfection of unsuitable surfaces; sufficient hygiene in the production or preservation of food; inadequate time / temperature conditions during food production or preservation, or a combination of these circumstances (FRANCO and LANDGRAF, 2005).

All samples were absent for analysis of *Staphylococcus aureus* and *Salmonella* sp in 25 g of product.

It is important to point out that it is possible to significantly reduce the presence of pathogenic microorganisms through the dehydration process, but they can still develop due to the beekeeper's negligence in hygiene and handling standards, promoting health risk (VASCONCELOS, 2009).

For the evaluators the brand C presented a greater acceptability for the evaluation of flavor and aroma presenting frequency of 96.67% and 93.33%, respectively.

CONCLUSION

The results of the physico-chemical characteristics of the four brands of dehydrated pollen sold in Belém-Pa were in accordance with the parameters required by the legislation. The B and C marks presented high values in Mesophilic Aerobes and Fungi and Yeasts, in the D mark, the values are out of the standard for the analyzes of Coliforms at 45 ° C, Mesophilic Aerobes and Fungi and Yeasts. All samples were absent for analysis of *Staphylococcus aureus* and *Salmonella* sp in 25g of product. According to the sensorial analysis the brand C presented a greater acceptability for the evaluation of flavor and aroma.

REFERENCES:

1. Almaraz-Abarca, N.; Campos, M. G.; Ávila-Reyes, J. A.; Jiménez, N. N.; Corral, J. H.; González-Valdez, L. S. *Journal of Food Composition and Analysis*. **2007**, 20, 320.
2. Arruda, V. A. S. *PhD thesis*, University of São Paulo, Brazil, 2009.
3. Azevedo, et, al. *Anais do Congresso Brasileiro de Química*. Porto Alegre,

- Brasil, 2009.
4. Barreto, L. M. R. C.; Funari, S.R.C.; Orsi, R.O. *Boletim de Indústria Animal*, **2005**, 62: 250.
 5. BRASIL. Ministério de Agricultura e do Abastecimento. Instrução Normativa nº 3, de 19 de janeiro de 2001. Regulamento Técnico de Identidade e Qualidade do Pólen Apícola. Brasília (DF).
 6. BRASIL. Instrução Normativa n. 3, de 19 de janeiro de 2001. Regulamentos Técnicos de Identidade e Qualidade de Apitoxina, Cera de Abelha, Geleia Real, Geleia Real Liofilizada, Pólen Apícola, Própolis e Extrato de Própolis. Brasília (DF).
 7. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 62, de 26 de agosto de 2003. Oficializa os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Brasília (DF).
 8. Campos, L. N. de O. et al. Trabalho de Conclusão de Curso, Universidade de Taubaté. Brasil, 2009.
 9. Carpes, S.T. et al. *Química Nova*, **2008**, 3, 1660-1664.
 10. Estevinho et al. *Food and Chemical Toxicology*, **2012**, 50, 340.
 11. Franco, B. D. G. M.; Landgraf, M. *Microbiologia dos alimentos*. Atheneu. 182 p, 2005
 12. Lengler, C. B. Anais do I Encontro de Apicultores do Mercosul. São Paulo, Brasil, 2002
 13. Marchini, L. C.; Reis, V. D. A. dos; Moreti, A. C. de C. C. *Ciência Rural*, **2006**, 36: 1050.
 14. Martins, M. C. T. *PhD thesis*, Universidade Estadual de Campinas, Brasil, 2010.
 15. Melo, I. L. P. D. et al. *Revista do Instituto Adolfo Lutz*, **2009**, 68, 500.
 16. Muradian, L.B. A. *PhD thesis*. Universidade de São Paulo, Brasil, 2009.
 17. Oliveira, K. C. L. S. *PhD thesis*. Universidade de São Paulo, Brasil, 2006.
 18. Oliveira, I.V. Monografia em Biologia. Universidade de Taubaté, 2010.
 19. Ribeiro, J.G.; Silva, R.A. *Tecnologia & Desenvolvimento Sustentável*, **2007**, 1, 50.
 20. Somerville, D. C. *Journal of Experimental Agriculture*, **2005**, 45: 1800.
 21. Vasconcelos, M.R.S. Dissertação de Mestrado, Universidade Federal de Alagoas, Brasil, 2009.

Table 1. Results of physical-chemical analyzes

Analyzes	Sample A	Sample B	Sample C	Sample D	BRASIL,2001
Humidity	3.54 ^a ± 0.03	3.34 ^a ± 0.05	2.75 ^a ± 0.01	2.53 ^a ± 0.01	Max 4%
Ashes	3.17 ^a ± 0.01	3.39 ^a ± 0.01	3.69 ^a ± 0.01	3.14 ^a ± 0.01	Max 4% m/m base seca
Proteins	23.57 ^c ± 0.01	16.59 ^d ± 0.02	20.46 ^b ± 0.02	12.38 ^a ± 0.03	Min 8% m/m base seca
Total sugar	15.49 ^a ± 0.11	29.41 ^c ± 0.01	23.87 ^b ± 0.03	32.40 ^d ± 0.07	14,5 a 55 % m/m Base seca
Free acidity	138.58 ^a ± 0.18	170.47 ^b ± 0.11	228.68 ^d ± 0.06	193.29 ^c ± 0.01	Max 300 mEq/Kg
pH	4.5 ± 0 ^a	4.5 ± 0 ^a	4.0 ± 0 ^b	4.2 ± 0 ^c	4 a 6
Lipids	1.80 ^a ± 0.03	3.07 ^{ab} ± 0.01	2.46 ^a ± 0.01	4.11 ^b ± 0.01	Min 1,8% m/m base seca
Aw	0.2331 ^{ab} ± 0.11	0.2976 ^{ab} ± 0.06	0.2149 ^a ± 0.01	0.3544 ^b ± 0.01	-----

Table 2. Results of dehydrated bee pollen marketed in Belém, Pará state, Brazil.

Analyzes	Sample A	Sample B	Sample C	Sample D
Coliforms at 45 °(UFC/g)	<1,0 x 10 ¹	<1,0 x 10 ¹	<1,0 x 10 ¹	1,5 x 10 ²
Mesophilic aerobes (UFC/g)	9,0x10 ¹	1,9x10 ²	2,1x10 ²	1,9x10 ⁴
Fungi and Yeast (UFC/g)	9,0x10 ⁻¹	2,9x10 ³	5,5x10 ²	1,0x10 ⁴
<i>Staphylococcus aureus</i>	Absence	Absence	Absence	Absence
<i>Salmonella</i> sp	Absence	Absence	Absence	Absence