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Evaluation of hygienic-sanitary quality of honey from *Apis mellifera* L. obtained in semi-arid region of Piauí, Brazil

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This study aimed to evaluate the quality of honey from *Apis mellifera* L. obtained in Piauí, Brazil. The completely randomized design (CRD) was used in the experiments. Two treatments of honey were prepared: one from beekeepers that use Extraction Units for Bee Products (EUBP) with Best practices for beekeeping (T1), and another one from those which use EUBP without the best practices (T2). Parameters analyzed were: moisture, water activity (aw), pH, acidity, color, detection of *Salmonella* spp., MPN.g^{-1} of coliforms at 35°C and at 45°C, counting of coagulase-positive *Staphylococcus*, standard counting of mesophilic heterotrophic bacteria and detection of yeast and filamentous fungi. The counting of mesophilic heterotrophic bacteria and yeast and filamentous fungi showed abnormalities ($p<0.05$) in the counting performed in $\log_{10}.g^{-1}$ with samples of T1 and T2, respectively. There were presence of fungi of various genus and species, especially *Aspergillus* spp. and *Penicillium* spp. The quality of honey from *Apis mellifera* bees from Piauí, Brazil, was satisfactory regarding parameters of moisture, aw, pH and HMF. Neither *Salmonella* spp., nor coliforms, nor coagulase-positive *Staphylococcus* were found. The presence of filamentous fungi in the samples reinforces the need for quality control of honey from Piauí, Brazil.

Key words: Physical chemistry, Hygiene and sanitary quality, Fungi, Best practices for beekeeping.

INTRODUCTION

Honey is one of the oldest food linked to the human history and had always attracted the attention of the man, especially because of their sweetening characteristics (Silva et al., 2004; Bera and Almeida-Muradian, 2007). This product is consumed worldwide because it is considered a natural and energetic sweetener, with predominance of sugars, glucose, fructose, saccharose (70% of carbohydrates) and the water in which the sugars are dissolved (Crane, 1983; Barros and Batista, 2008; Aroucha et al., 2008).

In Brazil, the commercial production of honey is related to the beekeeping, whose history had its beginning with the insertion of the European *Apis mellifera* bees in the State of Rio de Janeiro in 1839. After the development of adequate handling techniques in the 70's, the beekeeping turned out to be intensely practiced in all States of Brazil (Souza, 2004).

Furthermore, due to the high international demand for the product and the favorable exportation prices, the apiculture in Brazil changed from a craft activity focused in the domestic market to an entrepreneurial activity with more elaborate and productive techniques focused in the external market. Data from FAO unveil that Brazil has reached the seventh place in exports of honey, with a quantity of 22 thousand tons and a value of US$70,879, benefiting all regions of the country (FAO, 2011).

Regionally, the Northeastern production is in ascension. Between 1999 and 2005 it has reached 10.9 thousand tons and achieved the second place, behind of the South region of Brazil, which traditionally occupies the first place and achieved a production of 15.8 thousand tons of honey (IBGE, 2006). Such a fact reflected in 2009, when the Northeastern region was responsible for the production of 14.9 thousand tons of the whole Brazilian production, keeping its second place and approaching the South region, which produced 16.5 thousand tons of honey (IBGE, 2009).

Thus, like the other States of Northeast, Piauí has a high potential for honey production due to its environmental conditions and its melittophilous vegetation, which make of the beekeeping an outstanding activity in the State as well as in the country. It is of note that Piauí was able to insert honey as an important product among the worldwide exportation commodities. In 2005 and 2006, Piauí was the third biggest producer of honey of Brazil and in 2009 it became the fourth biggest producer in the country (Moura et al., 2013; IBGE, 2006, 2009).

This productive scenario must conform to numerous quality criteria and certifications, before its commercialization and exportation, once they are subject to frauds, adulteration and contamination due to inadequate manipulation (Silva et al., 2008). The microorganisms commonly found in this product are bacteria in its sporulated form, like *Bacillus*, yeasts and fungi, as the ones of the genus *Penicillium*, *Mucor*, *Aspergillus* and *Saccharomyces* (Snowdon and Cliver, 1996; Sodré et al., 2007).

Due to this, the concern with the quality of the honey produced in Piauí became relevant, as well as the knowledge of the microorganisms that are most used as quality indicators in order to conform to the market demands, especially the foreign market.

It is still a reality in the State of Piauí the existence of beekeepers that are in a craft category and ones that use methods for the control of the quality of the extraction established in some Extraction Units for Bee Products (EUBP). The use of the EUBP favors the security of the product when essential cares are taken in order to obtain a good quality honey. To do this, the EUBP and the implantation of Best Practices for Beekeeping (BPB) result in a quality improvement of the honey produced in the State and this necessity appeared with the arousing of exportations and the demands from the foreign market (Vilela, 2000; Moura et al., 2014).

Thus, to diagnose the quality of the honey of Piauí is important as a way to direct the support activities that will help to develop small and large producers. The aim of this study was to evaluate the quality of the honey of *Apis mellifera* L. bees obtained from beekeepers of Piauí that use the EUBP with the Best Practices for Beekeeping (BPA) and of that ones who use the EUBP without the Best Practices for Beekeeping.

MATERIALS AND METHODS

Initially a survey was conducted to assess the main municipalities regarding production and exportation of honey in Piauí; it was found that the cooperatives that suited the objectives of the study were concentrated in the central South region of the State, where semi arid climate predominate (Brasil, 2009). Inside that region, were randomly selected the cooperatives of the cities of Picos (07° 04' 37" S; 41° 28' 01" W), Simplício Mendes (07º51'14" S; 41º54'37" W) and São Raimundo Nonato (09°00'54" S; 42°41'56" W), to the acquisition of honey samples directly from the beekeepers.

The experimental design was the completely randomized design (CRD), with two treatments (T1 and T2) to the honey acquired from beekeepers, summing 54 samples of honey, with 27 collected for treatment. It was considered as treatments, in the scope of this study, the samples of honey from two groups of beekeepers (manipulators) were: the ones that use EUBP with Best Practices for Beekeeping (T1); and the ones that use EUBP without Best Practices for Beekeeping (T2).

Fifty four (54) samples of honey (27 per treatment) were collected,

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Table 1. Averages and standard deviation of physicochemical analyses of honey of *Apis mellifera* L bees.

Treatment	Physicochemical parameter					
	Moisture	aw	pH	Acidity (meq.kg^{-1})	Color (mm)	HMF (mg.kg^{-1})
T1	17.8^{b}±0.55	0.68^{b}±0.08	3.72^{a}±0.43	39.3^{a}±21.34	63.85^{a}±14.19	18.3^{a}±15.17
T2	18.2^{a}±0.96	0.76^{a}±0.03	3.52^{a}±0.37	59.1^{b}±24.24	61.14^{a}±13.59	16.1^{a}±3.77
P	0.041	0.0001	0.0719	0.0024	0.4779	0.4735
Maximum Reference Value (Brasil, 2000)	20.0	-	-	50.0	-	60.0

T1, Honey from beekeepers that use the EBUP with Best Practices for Beekeeping. T2, Honey from beekeepers that use the EBUP without Best Practices for Beekeeping. aw, Water activity; meq, milliequivalent. [a]Averages followed by the same letter in the lines and in columns do not differ between themselves by the Tukey's test ($p<0.05$).

from March to April of 2010, which were aseptically held in sterilized bottles of 350 ml of capacity, and wrapped in a plastic-bag for first-use food. The samples were sent to Laboratory of Microbiological Control of Food of the Food Processing, Research and Studies Center (NUEPPA), of the Centre for Agricultural Sciences, and the Interdisciplinary Laboratory for Advanced Materials (LIMAV), of the Natural Sciences Center, both centers belonging to the Federal University Piauí (UFPI), to perform the analyzes.

The physicochemical analyzes constituted the maturity indicators of the honey: moisture and water activity (aw); indicators of deterioration of the honey: pH, acidity and hydroxymethylfurfural (HMF); and the sensorial feature of color. All the analyses were performed in triplicate, following methods predetermined by the Brazilian laws (Brasil, 2000). The procedures used were in conformation with the analytical norms of the Association of Official Analytical Chemists (AOAC, 1998). The analyses of water activity (aw) were performed using the digital model of aw determiner Decagon Pawkit.

In all samples of honey sent to the laboratory, 25 gof honey were removed, weighted aseptically and then added to 225.0 mL of peptone saline 0.1%, in order to obtain an initial dilution of 10^{-1} and, from this dilution, decimal dilutions were prepared until 10^{-3} dilution was reached. The microbiological analyses of the study were focused on the occurrence of *Salmonella* spp., most probable number of coliforms at 35°C and at 45°C, coagulase-positive *Staphylococcus* count and standard counting of mesophilic aerobic microorganisms, which were based on methodologies described in 62nd Normative Instruction (Brasil, 2003).

The serial dilution in its decimal form as described by Pitt and Hocking (2009) was obeyed for standard counting in dishes of filamentous fungi and yeasts and for the identification of the fungi species. Inocula were aliquots of 0.1 mL per Petri dish, on the surface of the medium of Dichloran Rose Bengal Chloramphenicol (DRBC), in duplicate, in each of dilutions used to the general counting. The dishes with DRBC were incubated at 25ºC for seven days, in absence of light. All dishes were analyzed, having selected those which presented CFU.g^{-1} around 10 and 100. After the counting, the fungi colonies that were selected to identification were isolated and kept until the transplant to their correspondent medium, that was proper to each genus/specie, that is Spezieller Nalvistoffarmer Agar (SNA) for the genus *Fusarium* and Agar Estrato de Malte (MEA) for the genus *Aspergillus* and *Penicillium*.

The fungi colonies belonging to the genera *Aspergillus* and *Penicillium* were identified using identification keys described by Klich and Pitt (2002), based on sowing in four basic mediums: Czapek yeast extract agar (CYA); malt extract agar (MEA); Czapek yeast extract agar 20% sucrose (CY20S) and Agar 25% Glicerol Nitrate (G25N).

A conidial suspension was prepared from each strain in 0.5 mL of a medium constituted of 0.2% of Agar-agar and 0.05% of Tween 80TM, distributed in hemolysis tubes and previously sterilized at 121°C for 5 min (Pitt and Hocking, 2009). Then, a needle made of platinum was inserted into the conidial suspension and then transferred to three equidistant points in dishes containing CYA, MEA, CY20S and G25N. These dishes were incubated for seven days at 25ºC. Each strain was identified accordingly to the methods described by Pitt (1988) and Klich and Pitt (2002).

The HMF data underwent the *Kolmogorov-Smirnov* K-S proof for normality, and then underwent analyses of variance. For the variables related to the microbiological parameters, ANOVA test was performed with normalized data transformed to $\log_{10}^{(x+1)}$ and the F test was used to confront the existence of relevant differences between the average of the variables between the treatments. The Tukey's test at a significance level 5% was used to compare the averages, in accordance to the procedures established by the Statistical Analyses System (SAS, 1986). Frequencies of identification of the fungi genus and specie were calculated with the use of SPSS software, version 13.0.

RESULTS AND DISCUSSION

Results of physicochemical analyses were expressed by means of average calculation and standard deviation and compared between the treatments (T1 and T2), and to the values suggested by the 11th Normative Instruction of the Agriculture and Supply Ministry of Brazil, according to the Table 1 (Brasil, 2000). Except from acidity in T2, the samples of T1 and T2 were within the limits established by the Brazilian legislation.

The variables of moisture, water activity and acidity showed differences between the treatments (T1 and T2) in the samples of honey of *A. mellifera* bees of the semi arid region of Piauí. The average values for moisture were below 20% (Table 1) in accordance to the Brazilian legislation (Brasil, 2000), and were lesser than those found in the same region by Silva et al. (2004), who reported average values of 19.4% in the honeys associated to different studied crops. The results of moisture obtained in Northeast region varied between 17 and 20%, with average of 19.2% in honeys from the city of Crato, State of Ceará (de Araújo et al., 2006) and 18.7% in the other cities of that State (Sodré et al., 2007), and in the State of Paraíba (18.8%) (Rodrigues et al., 2008).

There were difference in moisture values between the

results of the two treatments of this study ($p<0.05$) and the samples of T2 with the largest percentiles (Table 1). The Africanized bees cap the honey when moisture is between 17 and 18% (Evangelista-Rodrigues et al., 2005). This indicates maturity (Brasil, 2000). This parameter of quality also can influence directly the stability of the honey and the microbial changes by the contamination attributed to the bees, the nectar, the environment and the inadequate handling during the whole processing of honey. The quantity of micro-organisms associated to moisture can favors the fermentation when the temperature is high and the storage is made in improper conditions. On the other hand, when analyzing this parameter, the samples of the honeys recently collected by the beekeeper offer probable stability from the microbiological point of view.

The result for the water activity (aw) showed an average value of 0.68 and 0.76, for T1 and T2 ($p<0.05$). Despite the fact that aw is not an obligatory parameter for quality evaluation according to the Brazilian legislation (Brasil, 2000), this parameter, together with the levels of moisture, assigns a better protection against the growing of microorganisms. Values over 0.60 may favor the growing of xerophilic fungi and osmophilic yeast, in addition to halophilic bacteria (Jay, 2005; Franco and Landgraf, 2008). The hygroscopic character of the honey favors the absorption of water in environments where the relative humidity of the air (RH) is superior to that of the honey. Even though the RH of the semi-arid region of Piauí (Brasil, 2005) does not favor the increasing of aw in the honey - since usually the honey handling is made in a hot and dry atmosphere, which is typical of the region – the average values of aw found in the samples are superior. This may be related to improper storage conditions of the honey after its extraction.

Moreover, these values have be shown to be superior when compared to the average values of aw of 0.58 to 0.60 found by Moura et al. (2014) that evaluated honeys in the semi arid region of Piauí; to the values of 0.49 to 0.66 in honeys from São Paulo (Denardi et al., 2005); to the Schalabitz et al. (2010) that described values between 0.54 to 0.62 in the region of Taquari Valley, in the State of Rio Grande do Sul; aw of 0.55 Moroccan honeys (Malika et al., 2005), and of Kacaniová et al. (2007), that found values between 0.46 to 0.66 in honeys from Slovakia.

There are no Standards defined for pH in current Brazilian legislation (Brasil, 2000). Nevertheless, this parameter is important to help in acidity evaluation and, in some degree, to foresee honey quality. There was no difference in pH values between both treatments whose average values found were 3.72 and 3.52 for T1 and T2, respectively (Table 1). They have shown numerically similar results from those obtained in studies with honeys of the Northeast region of Brazil (Silva et al., 2004; Evangelista-Rodrigues et al., 2005; Rodrigues et al., 2008), of the South region of Brazil (Mendonça et al., 2008; Welke et al., 2008) and over the world (Iurlina and Fritz, 2005; Malika et al., 2005; Tchoumboue et al., 2007). Such values may be attributed to different flowering composition, as well as nectaries, as Crane (1983) pointed out. Comparing with previous Brazilian legislation (Brasil, 1985) it can be noticed that the results of this study are in accordance with the recommended.

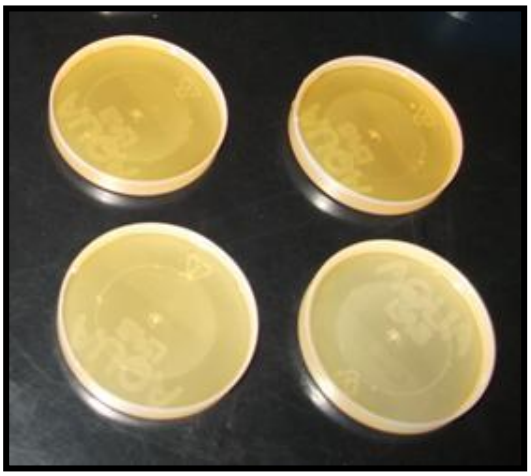

Figure 1. Average light-amber color found in studied honeys.

Regarding acidity, there were differences between the treatments ($p<0.05$), with T2 showing higher averages (Table 1) with 55.5% of the samples being above the limit established by the current Brazilian legislation (Brasil, 2000). The Brazilian norm established a maximum value of 50.0 $meq.kg^{-1}$, and the higher values indicate a high possibility of unwanted fermentation occurrence in honey. The results found in T2 were superior to that found by Sodré et al. (2007) that evaluated honeys from Piauí; Rodrigues et al. (2008) that observed values of acidity in honeys from the State of Paraíba; Finola et al. (2007), observing honeys from Argentina.

The color of honeys studied was similar in both treatments (Table 1) with predominance of a light amber color (Figure 1). These values were in accordance with the 11^{th} Normative Instruction (Brasil, 2000) which established that the color of the honey can vary from water-white to a dark amber color. So, the honeys from treatments 1 and 2 had a color that was attractive to the market, in a way that it characterizes the product as well valued by the foreign market (Brasil, 2009). It is of note the preference of the honey colorization by the Brazilian consumers, once the Brazilian honey is always dealt in a way that it can satisfy the European consumer preferences. This predominance of light amber color was also observed by Mendonça et al. (2008) and Moura et al. (2014).

Regarding hydroxymethylfurfural (HMF) there were no difference between the treatments T1 and T2 (Table 1) with the values being within limits established by the

Table 2. Microbiological parameters of honeys from *Apis mellifera* L. bees.

Treatments	*Salmonella* spp.	Coliforms 35 and 45°C (MPN. g^{-1})	*Coagulase-positive Staphylococcus* (CFU. g-1)	Mesophilic Heterotrophic Bacteria (CFU. g-1)	Yeast and filamentous fungi (CFU. g-1)
T1	Abs in 25 g	< 3.0	Abs in 0.1 g	1.22^{b}	2.03^{b}
T2	Abs in 25 g	< 3.0	Abs in 0.1 g	2.42^{a}	2.70^{a}

T1, Honey from beekeepers that use the EBUP with Best Practices for Beekeeping. T2, Honey from beekeepers that use the EBUP without Best Practices for Beekeeping Abs = absence; MPN.g^{-1} = most probable number per gram, expressed in logarithms of base 10; CFU.g^{-1} = colony-forming unit per gram, expressed in logarithms of base 10; ^{a} = Averages followed by the same letter in the lines and in the columns do not differ from each other in the Tukey's test ($p<0.05$).

Brazilian legislation (Brasil, 2000). The HMF value can identify deterioration of the honey related to the period of storage, sugar addition, pH, moisture and temperature conditions to which it underwent, apart from adulteration (White Junior, 1989, 1992). Therefore, HMF values of T1 and T2 samples of honeys suggest that they were not subjected to high temperatures and its analyses were performed quickly. The results obtained were close to those found by Sodré et al. (2007) in honeys from the State of Ceará; Bera and Almeida-Muradian (2007), in honeys from the State of São Paulo; and inferiors to those found by Arruda et al. (2005), in honeys from the State of Ceará; de Araújo et al. (2006); and Santos and Ribeiro (2009).

The results regarding the observed microbiological parameters are shown in Table 2. *Salmonella* spp. was absent from all samples of honey, and similar to those of Matuella and Torres (2000); Iurlina and Fritz (2005); Boff et al. (2008); Schlabitz et al. (2010); and Moura et al. (2014). Current Brazilian legislation (Brasil, 2000) does not determine microbiological parameters to honey, nonetheless the analysis of *Salmonella* spp. was the only microbiological parameter established by the previous legislation (Brasil, 1978). Bacteria like *Salmonella* spp. are capable of surviving in honey; however, they do not grow because of low values of water activity and pH (Snowdon and Cliver, 1996).

According to Table 2, in all samples analyzed the counting of coliforms at 35ºC and coliforms at 45ºC in both treatments results were lower than 3.0 MPN.g^{-1} which evidenced security about the presence of coliforms and enteric pathogens. These results can be justified by the physicochemical composition of the honey, that determines which microorganism will be capable of growing or not. Similar results were found by Iurlina and Fritz (2005); Boff et al. (2008); Barros and Batista (2008) and Moura et al. (2014). Silva et al. (2008) associated the absence of these microorganisms to proper hygiene conditions during the processing of honey, guaranteeing the hygienic-sanitary quality of this product.

The counting of coagulase-positive *Staphylococcus* showed absence in 0.1 g of the sample (Table 2). These results are similar to those found by Matuella and Torres (2000) and Schlabitz et al. (2010). The results of T1 and T2 showed that honey was obtained adequately, and that this product has inherent antimicrobial properties that reduce microbial growth and survival, and the properties of the high osmotic pressure and low water activity greatly contribute to this feature.

Microbiological analyzes in food are of fundamental importance for the prevention of diseases transmitted by them, and for the honey would not be different, since it is a widely consumed food in the world. The absence of *Salmonella* spp., coliforms at 35°C and 45°C and coagulase-positive *Staphylococcus* indicate that beekeepers, even when using Extraction Units for Bee Products without the Best Practices for Beekeeping (T2), kept the honey free of enteric bacteria, what represents a higher security to the consumer.

Factors such as moisture, aw and pH are limiting for the growth and development of microorganisms (Franco and Landgraf, 2008). Thus, the absence of the studied microorganisms may have been favored, both in T1 and in T2 (Table 1), by the moisture range, the water activity level and the pH level that were found in the analyzed samples, which are in conformity with the unfavorable conditions for such growth, especially regarding enteric bacteria, which in most cases can only tolerate an aw level of 0.86 and a pH level of 4.0 as a minimum.

There were differences between the treatments 1 and 2 regarding the count of mesophilic heterotrophic bacteria (Table 2). In the samples T1 and T2, the values in CFU.g^{-1} were 1.0×10^{4} and 5.0×10^{4}, respectively, and according to Franco and Landgraf (2008) in most foods the values above which sensorial changes can be detected are superior to 10^{6} CFU.g^{-1}. However, the results of the counting of these bacteria were numerically lower than those found by Iurlina and Fritz (2005); Barros and Batista (2008); Schlabitz et al. (2014). This counting is required to indicate the sanitary quality of foods; even if there have been no deterioration changes to them.

The results for fungi and yeast counting showed difference ($p<0.05$) between T1 and T2, with higher values for T2 (Table 2). However, when compared to other studies, they showed to be lower to those of Sodré et al. (2007); Kacaniová et al. (2007); Silva et al. (2008);

Table 3. Genera of fungi isolated from samples of *Apis mellifera* L. bees.

Fungi genus*	T1		T2		Total	
	N	%	N	%	N	%
Penicillium spp.	10	23.8	6	14.3	16	38.1
Aspergillus spp. and telemorfos	9	21.4	4	9.5	13	31
Cladosporium spp.	4	9.5	6	14.3	10	23.8
Fusarium spp.	1	2.4	0	0	1	2.4
Byssochlamys spp.	1	2.4	0	0	1	2.4
Curvularia spp.	1	2.4	0	0	1	2.4
Total	26	61.9	16	38.1	42	100

*Frequencies calculated with SPSS software of version 13.0. EUBP, Extraction Units for Bee Products; T1, honey from beekeepers that use the EBUP with Best Practices for Beekeeping.; T2, Honey from beekeepers that use the EBUP without Best Practices for Beekeeping. N, Numbers; %, values in percentile.

Table 4. Identification of fungi species in honeys from *A. mellifera* L. bees.

Fungi species*	T1		T2	
	N	%	N	%
Aspergillus flavus	5	33.3	1	8.3
Aspergillus niger and agregattes	2	13.3	3	25.0
Penicillium citreonigrum	3	20.0	0	0
Penicillium decubens	1	6.7	0	0
Penicillium waskmani	2	13.3	0	0
Penicillium restrictum	1	6.7	2	16.7
Penicillium implicatum	0	0	5	41.7
Penicillium islandicum	1	6.7	0	0
Penicillium felutanum	0	0	1	8.3
Total	15	100.0	12	100.0

*Frequencies calculated with SPSS software of version 13.0. EUBP, Extraction Units for Bee Products; T1, honey from beekeepers that use the EBUP with Best Practices for Beekeeping.; T2, Honey from beekeepers that use the EBUP without Best Practices for Beekeeping. N, Numbers; %, values in percentile.

Boff et al. (2008) and Lieven et al. (2009). These microorganisms can resist low levels of water activity and pH and for this reason they are usually found in honey.

Even though no sensorial changes associated to fermentation occurred in T2, the average acidity of 59 meq.kg^{-1} in the samples (Table 2) may correspond to a parameter indicating fermentation by yeasts. This found may be associated to contamination by primary sources or to the observance of the Best Practices for Beekeeping during the handling of the hives, which emphasizes the importance of continuous monitoring of the processing of honey in order to guarantee that a safe food is commercialized.

However, despite the fact that the counting of filamentous fungi and yeast presented significant difference between T1 and T2 (Table 2), the frequency of genera and species of isolated fungi (Tables 3 and 4) showed contradiction between both T1 and T2. The numbers of fungi genera and species were higher in T1 than in T2, even though T1 is considered to have used the Best Practices for Beekeeping during the handling of the hives. This fact can be conjectured when confronted to the parameter of aw > 0.60 (Table 1), which according to Denardi et al. (2005) would be a limiting factor for the development of filamentous fungi.

From the 54 samples of honey analyzed, filamentous fungi were found in 42 samples (78.0%). The fungi prevalent in the samples of honey (Table 1) were *Penicillium spp.* (38.1%), *Aspergillus* spp. and their teleomorphs (31.0%), *Cladosporium* spp. (23.8%) and *Fusarium* spp. (2.4%), and T1 showed a higher quantity of fungi genera (61.9%). Similar results were found by Kacaniová et al. (2007), when in 30 samples of honey the prevalent genera were *Aspergillus*, *Penicillium* and *Cladosporium*, with the found values higher than those found in T1 and T2 in the semi arid region of Piauí, Brazil. Tchoumboue et al. (2007) reported that in 49 samples of honey from West Cameroon, 18.4% were *Aspergillus*. The presence of fungi in foods, specially of the genera *Aspergillus*, *Penicillium* and *Fusarium* are undesirable because some species are capable of producing enzymes spoilage; as well as production mycotoxins, that

are toxic products of the secondary metabolism and nowadays they represent contamination risk for the environment, which implies serious harm to human health (Corrêa et al., 1997; Bando et al., 2007).

Among the fungi species identified in this study, those that were found in most of the cases were *Aspergillus flavus* (33.3%), followed by *Penicillium citreonigrum* (20.0%) in T1; and *Penicillium implicatum* (41.7%), followed by *Aspergillus niger* and its aggregates (25.0%) in T2 (Table 4). Among these, the incidence of *A. flavus* in the honeys of T1 (Table 4) must be highlighted and evaluated with caution, since this species is capable of producing aflatoxin (Klich and Pitt, 2002).

However, the presence of filamentous fungi which produce mycotoxins does not necessarily indicate their presence in the studied food, since, in order to produce mycotoxin, microorganisms require enabling environments such as those with inadequate values and/or high levels of moisture, water activity and pH (Franco and Landgraf, 2008).

Nevertheless, according to the physicochemical characteristics analyzed (Table 1), it is possible to notice that the honeys of T1 did not showed enabling conditions to the development and the multiplication of fungi, as well as to the production of mycotoxins.

Martins et al. (2003) identified three fungi genera (*Aspergillus*, *Penicillium* and *Mucor*) and two genera of yeast (*Sacharomyces* and *Candida*) in honeys from Portugal, including specially *A. flavus* (57.5%), followed by *A. niger* (51.3%); and *Penicillium* spp. and *Mucor* sp. were isolated in 38.8 and 31.3% of samples, respectively. Aflatoxins, however, were not present in the samples due to the fact that honeys do not offer an enabling medium to the development of secondary metabolites of the multiplication of those microorganisms.

Snowdon and Cliver (1996) affirm that bacterial spores, filamentous fungi and yeast can be acquired from primary sources related to the bees, or can be incorporated during the processing of the honey. The quality of honey can be affected by management during harvest. The quality of honey can be affected by handling during harvest. Thus, the beekeeper must perform appropriate procedures from the time of the harvesting of honey from beehives to its transport to the extraction unit, in order to interfere as little as possible in hygienic and sanitary quality.

Thus, the smaller the adoption of Best practices for beekeeping the higher is the contamination by microorganisms, especially by filamentous fungi and yeast, coupled with high levels of moisture and water activity which represent lower stability of the medium and high sanitary risk.

Conclusions

The quality of honey from *A. mellifera* L. bees produced in Piauí, Brazil was in conformity to moisture, water activity, pH and HMF parameters, especially in samples from beekeepers that use the EUBP. The honey did not show contamination by *Salmonella* spp., coliforms and coagulase-positive *Staphylococcus*. Given the results of mycotoxin-producing fungi in the honeys, it is suggested that producers have greater concern for the quality control and use of BPB in all honey production steps.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Aroucha EMM, Oliveira AJF, Nunes GHS, Maracajá PB, Santos MCA (2008). Qualidade do mel de abelha produzido pelos incubados da IAGRAM e comercializado no município de Mossoró/RN. Rev. Caatinga 21(1): 211-217.

Arruda CMF, Marchini IC, Moreti, ACCC, Otsuk IP, Sodré GS (2005). Características físico-químicas de méis da Chapada do Araripe/ Santana do Cariri. Acta. Sci. Anim. Sci. 27(1): 171-176.

Association of Analytical Chemists (AOAC) (1998). Official Methods of analysis. 15th. ed. Supl 2.

Bando E, Gonçales LN, Tamura NK, Machinski Junior M (2007). Biomarcadores para avaliação da exposição humana às micotoxinas. J. Bras. Patol. Med. Lab. 43(3): 175-180.

Barros HD, Batista E (2008). Avaliação físico-química e microbiológica de diferentes marcas de mel. Hig. Aliment. 22(166/167): 76-79.

Bera A, Almeida-Muradian IB (2007). Propriedades físico-químicas de amostras comerciais de mel com própolis do estado de São Paulo. Ciênc. Tecnol. Aliment. 27(1): 49-52.

Boff T, Rosa CS, Santos RCV (2008). Qualidade físico-química e microbiológica de méis comercializados nos principais supermercados de Santa Maria. RS. Hig. Aliment. 22(162): 57-62.

Brasil (1978). Ministério da Saúde. Comissão Nacional de Normas e Padrões para Alimentos. Resolução CNNPA n° 12 de 30 de março de 1978. Aprova as seguintes Normas Técnicas Especiais, do Estado de São Paulo, revistas pela CNNPA, relativas a alimentos e bebidas, para efeito em todo território brasileiro. Diário Oficial da União. 1978 jul. 24; Seção 1. p.11499. Disponível em:<http://portal.anvisa.gov.br/wps/wcm/connect/e57b7380474588a39266d63fbc4c6735/RESOLUCAO_12_1978.pdf?MOD=AJPERES>. Acesso em: 05 jul. 2015.

Brasil (1985). Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Inspeção de Produto Animal. Portaria n°6, de 25 de julho de 1985. Aprova as Normas Higiênico-Sanitárias e Tecnológicas para o Mel, cera de Abelhas e Derivados. Diário Oficial da União. 1985 jul. 02; Seção 1. p.11100. Disponível em:<http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=7916>. Acesso em: 05 jul. 2015.

Brasil (2000). Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 11, de 20 de outubro de 2000. Aprova o Regulamento Técnico de Identidade e Qualidade do Mel. Diário Oficial da União. 2000 out. 23; Seção 1. p. 16-17. Disponível em:<http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=160&data=23/10/2000>. Acesso em: 05 jul. 2010.

Brasil (2003). Ministério da Agricultura e do Abastecimento. Instrução Normativa nº 62, de 26 de agosto de 2003. Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Diário Oficial da União. 2003 set. 18; Seção 1. p. 14-51. Disponível em:<http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=18/09/2003&jornal=1&pagina=51&totalArquivos=256>. Acesso em: 05 jul. 2010.

Brasil (2005). Ministério da Integração Nacional. Secretaria de Políticas de Desenvolvimento Regional. Cartilha: Nova Delimitação do Semiárido

Brasileiro. 2005. Disponível em: <http://www.integracao.gov.br/desenvolvimentoregional/publicacoes/delimitacao.asp>. Acesso em: 2 mar. 2010.
Brasil (2009) Ministério do Desenvolvimento, Indústria e Comércio Exterior. Secretaria de Comércio Exterior. Estatísticas de comércio exterior (DEPLA*)*. Balança comercial brasileira por município. Brasília, DF: SECEX, 2009. Disponível em: <http:www.midic.gov.br//sitio/sistema/balança>. Acesso em: 11 dez. 2010.
Corrêa B, Galhardo M, Costa EO, Sabino M (1997). Distribution of molds and aflatoxins in dairy cattle feeds and raw milk. Rev. Microbiol. 28(4):279-83.
Crane E (1983). O livro do mel. São Paulo: Nobel, 226p.
de Araújo DR, Silva RHD, Sousa JS (2006). Avaliação da qualidade físico-química do mel comercializado na cidade do Crato, CE. Rev. Biol. Ciênc. Terra. 6(1): 51-55.
Denardi CAS, Nishimoto EJ, Balian SC, Telles EO (2005). Avaliação da atividade de água e da contaminação por bolores e leveduras em mel comercializado na cidade de São Paulo – SP, Brasil. Rev Inst. Adolfo Lutz. 64(2):219-222.
Evangelista-Ridrigues A, Silva EMS, Beserra EMF, Rodigues ML (2005). Análise físico-química dos méis das abelhas *Apis mellifera* e *Melipona scutellaris* produzidos em duas regiões no Estado da Paraíba. Ciênc. Rural 35(5):1166-1171.
Finola MS, Lasagno MC, Marioli JM (2007). Microbiological and chemical characterizations of honey from central Argentina. Food Chem. 100(4): 1649-1653.
Food and Agriculture Organization of the United Nations (FAO) (2011). Faostat: Trade exports country by commodity. FAO, 2011. Disponível em:< http://faostat.fao.org/site/342/default.aspx>. Acesso em: 02 jun. 2011.
Franco BDGM, Landgraf M (2008). Microbiologia dos alimentos. São Paulo: Atheneu, 182p.
Instituto Brasileiro de Geografia e Estatística (IBGE) (2006). Ministério do Planejamento, Orçamento e Gestão. Produção da Pecuária Municipal 2006. Rio de Janeiro, 34: 1-62. 2006. Disponível em: <http://www.ibge.gov.br/home/estatistica/economia/ppm/2006/ppm2006.pdf>. Acesso em: 6 dez. 2010.
Instituto Brasileiro de Geografia e Estatística (IBGE) (2009). Ministério do Planejamento, Orçamento e Gestão. Produção da Pecuária Municipal 2009. Rio de Janeiro, 37: 1-55. 2009. Disponível em: < http://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2009_v37_br.pdf>. Acesso em: 9 jun. 2015.
Iurlina MO, Fritz R (2005). Characterization of microorganisms in Argentina honeys from different sources. Int. J. Food Microbiol. 105(3):297-304.
Jay JM (2005). Microbiologia de alimentos. 6. ed. Porto Alegre: Artmed. 712p.
Kacaniová M, Sudzina M, Sudzinová J, Fikselová M, Cubon, J, Hascik P (2007). Microbiologigical and physico-chemical quality of honey collected from different slovak habitats. Slovak. J. Anim. Sci. 40(1):38-43.
Klich MA, Pitt JIA (2002). A Laboratory quide to commo *Aspergillus* species and their teleomorphs. CDIRO, Division of food research Sydney. Acdemic Press: Austrália, 89p.
Lieven M, Correia KR, Flor TL, Fortuna JL (2009). Avaliação da qualidade microbiológica do mel comercializado no extremo sul da Bahia. RBSP 33(4):544-552.
Malika N, Mohamed F, Chakib EA (2005). Microbiologia and physico-chemical properties of Moroccan honey. Int. J. Agric. Biol. 7(5):773-776.
Martins HM, Martins ML, Bernardo FMA (2003). *Bacillaceae* spores, fungi and aflatoxins determination in honey. RPCV 98(546): 85-88.
Matuella M, Torres VS (2000). Teste da qualidade microbiológica do mel produzido nos arredores do lixão do município de Chapecó – SC. Hig. Aliment. 14(70):73-77.
Mendonça K, Marchini LC, Souza BA, Almeida-Anacleto D, Moreti ACCC (2008). Caracterização físico-química de amostras de méis produzidas por *Apis mellifera* L. em fragmento de cerrado no município de Itirapina, São Paulo. Ciênc. Rural 38(6): 1748-1753.
Moura SG, Muratori MCS, Monte AM, Carneiro RM, Souza DC, Alencar LC (2013). Perfil sanitário dos apicultores Piauienses quanto às boas práticas apícolas. Scientia Plena 9(5): 1-6.
Moura SG, Muratori MCS, Monte AM, Carneiro RM, Souza DC, Moura JZ (2014). Qualidade do mel de *Apis mellifera* L. relacionadas às boas práticas apícolas. Rev. Bras. Saúde Prod. Anim. 15(3): 731-739.
Pitt JI (1988). Laboratory guide to commom *Penicillium* species. 2.ed. North Ride: CSIRO Division of Food Processing, 186p.
Pitt JI, Hocking AD (2009). Fungi and Food Spoilage. 3 ed. Springer Dordrecht. Heidelberg, London, New York, 2009, 519 p.
Rodrigues AE, Silva RA, Aquino IS, Gomes JP, Souza DL, Pereira WE (2008). Avaliação físico-química de méis de Apis melífera L., 1758 (*Hymenoptera* Apidae) produzidos na Paraíba, Brasil. Hig. Aliment. 22(163): 26-31.
Santos CS, Ribeiro AS (2009). Apicultura uma alternativa na busca do desenvolvimento sustentável. Rev. verde agroecologia desenvolv. Sustent. 4(3):1-6.
Schlabitz C, Silva SAF, Souza CFV (2010). Avaliação de Parâmetros físico-químicos e microbiológicos em mel. Rev. Bras. Prod. Agroind. 4(1):80-90.
Silva CL, Queiróz AJM, Figueirêdo RMF (2004). Caracterização físico-química de méis produzidos no Estado do Piauí para diferentes floradas. Rev. Bras. Eng. Agríc. Ambient. 8(2/3): 260-265.
Silva MBL, Chaves JBP, Message G, Gomes JC, Oliveira GL (2008). Qualidade microbiológica de méis produzidos por pequenos apicultores e de méis de entrepostos registrados no Serviço de Inspeção Federal no Estado de Minas Gerais. Alim. Nutr. 19(4): 417-420.
Snowdon, JA, Cliver, DO (1996). Microrganisms in honey. Int. J. Food Microbiol. 31: 1-26.
Sodré GS, Marchini IC, Zucchi OLAD, Nascimento Filho VF, Otsuk IP, Moreti ACCC (2007). Determination of chemical elements in africanized *Apis mellifera* (Hymenoptera: Apidae) honey samples from the State of Piauí. Brazil. Quim. Nova 30(4):920-924.
Souza DC (org.) (2004). Apicultura: manual do agente de desenvolvimento rural. Brasília: SEBRAE, 100p.
Statistical Analysis System (SAS) (1986). System for Linear Models. Cary: SAS Institute, 211p. Statistical Package for Social Sciences (S.P.S.S.), versão 13.0.
Tchoumboue J, Awah-Ndukum J, Fonteh FA, Dongock, ND, Pinta J, Mvondo ZA (2007). Physico-chemical and microbiological characteristics of honey the sudano-guinean zone of West Cameroon. Afr. J. Biotechnol. 6(7): 908-913.
Vilela SLO (2000). A importância das novas atividades agrícolas ante a globalização: a apicultura no Estado do Piauí. Teresina: Embrapa Meio-Norte, 228 p.
Welke JE, Reginato S, Ferreira D, Vincenzi R, Soares JM (2008). Caracterização físico-química de méis *Apis mellifera* L. da região noroeste do Estado do Rio Grande do Sul. Ciênc. Rural 38(6): 1737-1741.
White Junior JW (1989). La miel. In: DADANT, H. La colmena y la abeja mellifera. Montevideo: Hemisfério Sul, 1989. cap.1, p. 21-35.
White Junior JW (1992). Quality evaluation of honey: role of HMF and diastase assays. Part II. Am. Bee J. 132(12):792-794.