Evaluation of the influence of seasonality and landscape on the physicochemical characteristics of propolis

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Abstract

This study aimed at verifying the influence of the seasonality and landscape of the place of apiaries in the production and physicochemical quality of propolis produced in Cáceres-MT. Within August/2006 and July/2007 twenty-three propolis samples were collected monthly. The physicochemical quality of propolis was determined by the characteristic: loss by drying, wax, mechanical mass, oxidation activity, dry extract, flavonoids and total phenolics. The production of propolis was analyzed through correlation with seasonality (dry and wet). The same statistic model was used to correlate the production with the landscape (highland and flood plain). For each physicochemical characteristic were used statistic model of correlation described for the production of propolis. The seasonality did not influence on propolis production and on physicochemical quality. The landscape did not influence on propolis production and physicochemical quality loss by drying and mechanical mass, however, it determined the physicochemical quality for wax (x=22.44%), oxidation activity (x=9.73"), dry extract (x=22.31%), flavonoids (x=1.94%) and total phenolics (x=0.02%) in the highland of the Pantanal of Cáceres. This way it concludes that the production and physicochemical quality of propolis were not influenced by seasonality. The landscape influenced positively on physicochemical quality of propolis in the highland of the Pantanal of Cáceres.

Keywords: *Apis mellifera*; landscape; flavonoids; antioxidant; food control quality.

1 Introduction

Propolis is a resin collected by bees from several parts of plants such as sprouts, floral buttons and resinous exudate, which when mixed with their salivary secretions originate a material of various colors and consistency (GHISALBERTI, 1979).

The quality control of propolis for human consumption in Brazil is assured by the rules for Regulation Technical Identity and Quality of Propolis (BRASIL, 2001a, b).

Propolis has been researched by several authors aiming at analyzing the factors that can cause variations in its quality and biological activity such as the geographical region of origin and vegetation (SFORCIN et al., 2000; BASTOS, 2001; FUNARI; FERRO, 2006; SILVA et al., 2006; SOUSA et al., 2007; COSTA et al., 2008; BARBOSA et al., 2009). According to some authors, seasonality causes changes in the physicochemical quality (BANKOVA et al., 1998; INOUE et al., 2007; SILVA et al., 2006) and productivity (PUKER et al., 2010; SOUZA et al., 2010) of propolis.

Currently, the investigation of the chemical characteristics of propolis includes the use diverse methods and technologies, which have been applied mainly for determination of its chemical components, such as flavonoids, phenolics and antioxidant activity (CABRAL et al., 2009; ALENCAR et al.; 2007; LUO et al., 2011; MARÓSTICA JUNIOR et al., 2008; ALVES; KUBOTA, 2013; FISCHER et al., 2010; OLDONI et al., 2011; SILVA et al., 2012; NUNES et al., 2009; COTTICA et al., 2011).

The green propolis of the Cerrado of Minas Gerais is an example of a product that meets international quality standards (LIMA, 2006). Studies conducted in Mato Grosso have shown that black propolis produced in Cáceres and brown propolis produced in Colíder meet standard quality, and local beekeepers use the extracts of propolis as natural antibiotcs (LOUREIRO; GALBIATI; DA SILVA, 2008; SOARES; GALBIATI, 2011).

According to those studies, vegetation explains the quality of the propolis; nevertheless, it does not specify the effect of the different vegetation landscapes and their interaction with seasonality on the physicochemical quality of the propolis. The present study is a comparative study of the physicochemical quality of two different types of propolis collected at different times, from different places and landscapes in Pantanal.

Accordingly, the current research aimed at verifying the influence of seasonality and landscape of the apiaries on the production and physicochemical quality of propolis in two apiaries located in Cáceres-MT. The hypotheses tested were: i) propolis production does not vary between the dry and wet seasons at which it was collected and the apiary landscape (highland and flood plain); ii) the physicochemical quality of the propolis is determined by seasonality and the apiary landscape.

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2 Materials and methods

2.1 Place of study

The present study was carried out in the city of Cáceres in the state of Mato Grosso, Northern Pantanal (SOUZA; LANI; SOUSA, 2009). The apiaries were located on the Nossa Senhora Aparecida farm, (FNSAp apiary), in the coordinates 16° 00' 02" S and 57° 39' 55" W, and on Girau farm (FG apiary), in the coordinates 16° 04' 55" S and 57° 37' 25" W (Figure 1).

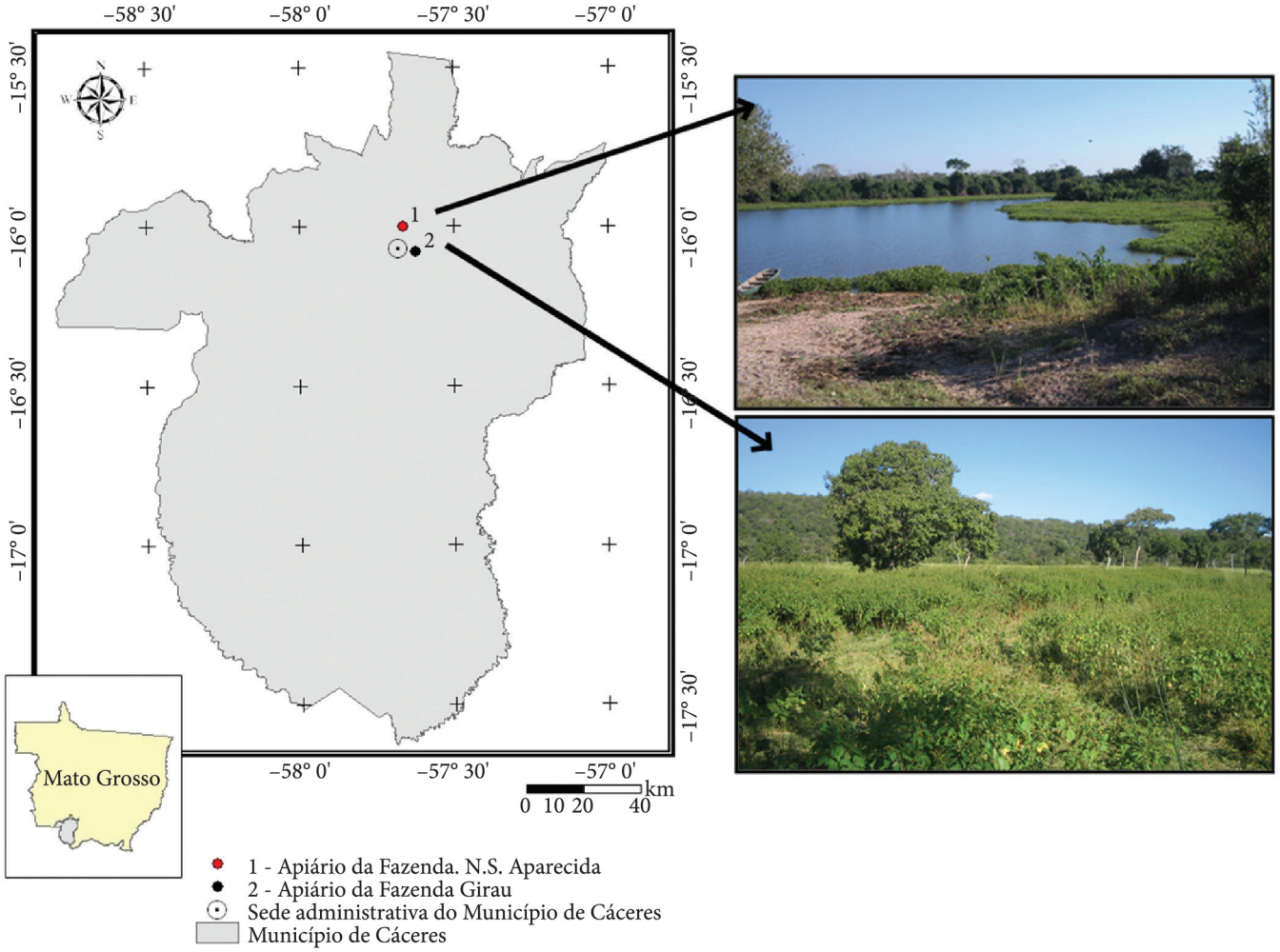
The landscape of Nossa Senhora Aparecida apiary is characterized by a flood plain and Seasonal Alluvial Semidecidual Forest combined with the Savanna Forest (1066.79 ha), pasture (924.85 ha), Savanna Park with Swampy areas (692.16 ha), and floodable areas (134.3 ha) (AMARAL et al., 2012). The size of stains was quantified by Amaral et al. (2012), who used geoprocessing tools.

The Girau farm's apiary landscape is characterized by highland pasture (2081,6 ha), Forested Savanna (603.64 ha), and flooded areas (1.97 ha). The *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf of that farm has several adult trees; most of them are Sucupira (*Pterodon emarginatus* Vogel) (AMARAL et al., 2012), unlike the cultivated pastures in the other apiary studied.

The propolis was collected monthly between August/2006 and July/2007. In each apiary, africanized honeybees, *Apis mellifera,* housed in three beehives of Langstroth type, chosen at random, were used. A beehive with a CPI collector (intelligent collector of propolis), was placed on the nest in each beehive; a total of six beehives were investigated, three in each apiary.

Seasonality was characterized by dry season, from May to October, and wet season from November to April. The State of Mato Grosso has two well-defined seasons: dry (May to September) and wet (November to March) (DUARTE, 1988). April is considered a rainy season due to monthly precipitation of 100 mm, and October is considered dry season, with precipitations of 76 mm (CENTRO..., 2009).

The experimental design for propolis collection was used in 2 apiaries and 6 beehives: one apiary and 3 beehives in each type of landscape (highland and flood plain), three beehives



Fonte: Malha Municipal digital do Instituto Brasileiro de Geografia e Estatística.
Elaboração: Eliezer Rangel de Campos Soares

Figure 1. Apiary Nossa Senhora Aparecida: flood plain typical of flooding pulse concept (1) and apiary Girau: highland, without the presence of flooding pulse (2) in the Pantanal of Cáceres, Mato Grosso.

per apiary with monthly sample collection, which resulted in a sample composed of 23 samples (apiary/month).

In the month of September there was no propolis production in the FG apiary. The collection in the three beehives composed one sample per month since the amount of propolis of the individual samples were insufficient to analyze all physicochemical characteristics.

2.2 Physicochemical analysis

The physicochemical analyses of the propolis we performed at the "Fundação Ezequiel Dias (FUNED)", at the Laboratory of Plant Resources and organo-therapeutic products, in Belo Horizonte - MG, for loss due to desiccation, mechanic mass, wax, oxidation activity, dry extract, flavonoids, and total phenolics.

The loss due to desiccation of the gross propolis was determined at 5.0 g of gross propolis, based on the loss of water due to desiccation in a muffle at 105 °C until constant weight was reached (COTTICA et al., 2011).

The analysis of wax, mechanical mass, oxidation activity, and flavonoids of the propolis evaluated was determined at 1.0 g of gross propolis (PREGNOLATTO; PREGNOLATTO, 1985).

2.3 Preparation and analysis of alcoholic extract of propolis (AEP)

The alcoholic extracts of propolis (AEP) were prepared with 50 g of gross propolis and 100 ml of 70% ethyl alcohol for seven days at room temperature. Later, the extract was filtered using a paper filter, and the supernatant was stored in amber flasks at room temperature, as adapted by Bastos (2001).

The propolis dry extract was determined based on the extract weight loss after the water removal (desiccation at 105 °C) (PREGNOLATTO; PREGNOLATTO, 1985).

A total of 100 µL of alcoholic extracts of propolis were used for the quantification of flavonoids in the propolis extract adding, separately, a solution of nitrate of aluminum and a solution of potassium acetate; a solution of ethanol 80% was added until the volume reached of 25 ml, and it was homogenized. The flavonoid concentration was obtained by the construction of the calibration curve using different concentrations of a quercetin solution. Absorbance was measured with a spectrophotometer at 415 nm (PARK; KOO, 1996) wavelength using the UVPROB 2.21 software program.

To determine the total phenolics in the propolis extract, an aliquot of 100 µL of propolis extract was added in a volumetric balloon containing 35 ml of distilled water; next, 4 ml of the reagent of Folin-Ciocalteau were added under agitation. Six mL of a solution of sodium carbonate (20%) were added between 1 and 8 minutes. The volume was completed (50 ml) with distilled water and was homogenized. After two hours, absorbance was measured with a spectrophotometer at 760 nm (WOISKY; SALATINO, 1998), and the UVPROB-2.21 software program was used. A gallic acid standard curve was built to quantify the total phenolics.

2.4 Statistical analysis

Production (y) and physicochemical quality of the propolis (y) were correlated with seasonality (x) and landscape (x) using Pearson correlation coefficient when the variable y was normally distributed and the Spearman correlation for non-Normal distribution.

Propolis production was analyzed by correlation with seasonality (dry and wet). The same statistical model was used to correlate production with landscape (highland and flood plain).

Each physicochemical characteristic of the correlation statistical models described for propolis production, was associated to the variable y.

The Shapiro-Wilk test was used for testing the distribution of errors. The free software of R statistical analysis, version 2.12.0, was used for the statistical analysis.

3 Results and discussion

3.1 Propolis production

Propolis production in the apiaries studied was not correlated with seasonality (GL=1,21; r^2=–0.13; P=0.55); there was no significant difference in the production between the seasons (dry=104.14±44.01 g and wet=96.77±55.39 g).

The difference in the propolis production was not correlated with the landscape of the apiaries (GL=1,21; r^2=0.35; P=0.10); there was no significant difference between the average production in the landscapes investigated (highland= 117.645±59.82 g and flood plain= 84.38±32.03 g).

The propolis production throughout the year found in the present study was similar to that found in Cerrado-Pantanal ecotone region, which is composed of pasture diversity and remaining Savana areas (PUKER et al., 2010).

However, in the municipality of Botucatu, SP, seasonality influenced the production of propolis, what is not in agreement with the results found in this present study. Therefore, the influence of seasonality can be related with geographical places with significantly different climates, such as those in Botucatu. On the other hand, in places where seasonal variation is not very wide, there were no differences in the production of propolis like that observed in the city of Cáceres (BANKOVA et al., 1998; INOUE et al., 2007).

3.2 Propolis quality

The quality of propolis was not significantly correlated with seasonality for any of the physicochemical characteristics analyzed (Table 1).

The physicochemical characteristics that were significantly correlated with landscape were wax, oxidation activity, dry extract, flavonoids, and total phenolics (Table 1). The characteristics loss due to desiccation and mechanic mass were not correlated with landscape (P>0.05).

The values loss due to desiccation of propolis from Pantanal, obtained either from the highland or flood plain, met the requirements of the Brazilian legislation (Table 2). These results corroborate with those of some other studies (SOUSA et al., 2007) and with the study carried out in the Microrregião Brejo, which showed that there was not influence of the pluviometric precipitation on the loss due to desiccation (SILVA et al., 2006).

The wax content was positively correlated with the FG apiary highland landscape, with a lower content of wax (x=22.44%) than that of the flood plain landscape (x=31.37%) of the FNSAp apiary in the flood plain, and only four samples showed physicochemical quality higher than 25%, the maximum value established by the Brazilian legislation (BRASIL, 2001a), which corresponds to 36% of the samples.

The samples of the FG apiary with values higher that those established by the legislation were collected during the dry season in the highland (x=28.41 %) (Table 2). The samples of the flood plain apiary show poor quality for the characteristic wax since all samples had higher values than those established by the legislation (dry season=30.32 % and wet season=32.42 %).

Seasonality can cause variation in the wax content associated to the area of propolis collection; in the Savana of Minas Gerais state, there was no variation in the dry and wet season (BASTOS, 2001), and in Paraíba, the high wax content was found in the dry season (SILVA et al., 2006). However, in the present study, a variation in the wax content was observed within the same area caused by the landscape.

The propolis mechanic mass value was higher than the maximum limit established by the legislation (BRASIL, 2001a) (Table 2) in the highland and flood plain during both seasons.

The oxidation activity of the propolis was positively correlated with the landscape of highland with shorter period of time (x=9.73 seconds) than that of the flood plain (x=417.83 seconds) (Table 1). The FG apiary exhibited quality for that characteristic, ranging from 3 to 29 seconds. The samples of the apiary of the flood plain did not exhibit quality for this characteristic, with values higher than 22 seconds, which are higher than those established by the Brazilian legislation (BRASIL, 2001a).

The oxidation activity varied as a function of the Brazilian areas' vegetation in the Savana of Minas Gerais state that showed oxidation activity varying between 3.3 to 40.6 seconds with average of 12.23 seconds (BASTOS, 2001) and in the ecotone Cerrado-Southern Pantanal, Mato Grosso do Sul, between 8.1 to 12.7 seconds with an average 10.6 seconds (PUKER et al., 2010). Similar values were observed in the Cerrado of Mato Grosso (OLIVEIRA et al., 2012). These results indicate influence of the landscape, resulting in a propolis with similar quality as that obtained in highland of the Pantanal of Cáceres.

However, some researches consider this propolis as having physicochemical quality with values higher than those established by Brazilian legislation, with an oxidation index between 3.0 a 54 according to Sousa et al. (2007) and Tagliacollo and Orsi (2011).

Table 1. Correlation between the physicochemical characteristics of propolis from Pantanal of Cáceres, MT, with seasonality and the landscape by the Pearson[1] and Spearman[2] (r^2) tests.

	Seasonality			Landscape		
	Dry $\chi\pm\sigma$	Wet $\chi\pm\sigma$	r^2	Upland	Flood plain	r^2
LD[1]	6.35±3.31	7.24±1.95	0.17^{ns}	7.74±2.29	5.96±2.79	0.34^{ns}
Wax[2]	29.45±5.05	24.94±10.99	–0.14^{ns}	22.44±10.46	31.37±3.67	–0.50**
MM[1]	66.01±12.16	57.31±11.2	–0.36^{ns}	66.41±14.20	56.96±8.42	0.39^{ns}
OA[2]	226.73±217.74	218.92±224.13	0.08^{ns}	9.73±7.56	417.83±80.13	–0.87**
Dry extract[2]	13.79±11.27	12.19±8.08	–0.07^{ns}	22.31±3.75	4.38±1.25	0.87**
Flavonoides[2]	0.99±1.01	1.00±0.91	–0.01^{ns}	1.94±0.23	0.13±0.06	0.87**
Fenolic[2]	0.01±0.01	0.02±0.01	0.05^{ns}	0.02±0.00	0.01±0.00	1.00**

LD=Loss due to desiccation; MM= mechanic mass; OA=oxidation activity; Fenolic= total phenolics. **significant to 1%. ns not significant.

Table 2. Physicochemical characteristics of propolis from Pantanal of Cáceres, MT, according to seasonality (dry and wet seasons) and landscape (highland and flood plain) in comparison with the quality patterns of the Brazilian Normative Instruction (NI)*.

Parameter		NI*	Highland		Flood plain	
			Dry $\chi\pm\sigma$	Wet $\chi\pm\sigma$	Dry $\chi\pm\sigma$	Wet $\chi\pm\sigma$
LD	%	8[1]	7.14±3.06	8.24±1.52	5.69±3.65	6.23±1.89
Wax	%	25[1]	28.41±7.05	17.46±10.67	30.32±3.05	32.42±4.19
MM	%	40[1]	72.87±14.10	61.02±12.94	60.3±7.09	53.61±8.88
AO	s	22[1]	9.20±11.28	10.17±3.49	408±89.30	427.67±79.93
Dry extract	%	11[2]	25.46±2.12	19.69±2.53	4.07±0.96	4.69±1.50
Flavonoids[3]	%	0.25[2]	2.03±0.21	1.85±0.24	0.12±0.05	0.14±0.07
Fenolic[4]	%	0.5[2]	0.02±0.00	0.02±0.00	0.01±0.00	0.01±0.00

*NI 03/2001 (BRASIL, 2001b); [1]maximum and [2]minimum value established for the quality of the Brazilian propolis (BRASIL, 2001b); [3]equivalent to quercetin; [4]equivalent to gallic acid.

The FG apiary in the highland showed positive correlation with the largest contents of dry extract (x=22.31%) in comparison with those of the apiary in the flood plain (x=4.38%) (Table 1); all samples of the highland were higher than the minimum limit established by the Brazilian legislation (11%) (Table 2).

The dry extract values of the Brazilian propolis samples evaluated were higher than that established by the Brazilian legislation (FUNARI; FERRO, 2006; SOUZA et al., 2010; TAGLIACOLLO; ORSI, 2011), in terms of quality. However, for the flood plain propolis, the dry extract content was smaller at 11% (Table 2), which can be attributed to the landscape in the Pantanal of Cáceres, MT.

The flavonoid contents were positively correlated with the highland landscape (x=1.94%) (Table 1). The FG apiary, in the highland, showed good quality for flavonoid content, which varied from 1.56 to 2.31%; value was considered as medium and high flavonoids content (BRASIL, 2001b), respectively, similar to what was observed in another study (WOISKY; SALATINO, 1998).

In the FNSAp apiary, in the flood plain, propolis did not exhibit quality for flavonoid content (x=0.12 to 0.14 %, Table 2), which were lower than the minimum value of 0.25% established by the Brazilian legislation.

The content of flavonoids of the Brazilian propolis exhibited quality in other areas of the States of Rio Grande do Sul (ALVES; KUBOTA, 2013) and São Paulo (COSTA et al., 2008; FUNARI; FERRO, 2006; TAGLIACOLLO; ORSI, 2011).

In the samples of propolis collected in Botucatu-SP, no significant differences in the flavonoid content due to the seasonality were observed, confirming that there was not effect of seasonality (SOUZA et al., 2010), similarly to what was found in the current research.

The total phenolics of the propolis from the Pantanal, despite the significant correlation with landscape (Table 1), did not meet the quality requirement established by the Brazilian legislation (BRASIL, 2001b) (minimum of 0.50 % (Table 2)).

The variation in the chemical quality of the propolis obtained from different places can be explained by the biodiversity of each area where the apiaries are located (SOUSA et al., 2007; MARCUCCI, 1996). The highland landscape contributed to quality propolis, confirming the importance of biodiversity and variation in the vegetation of the apiary.

The results found in this study showed that the highland propolis showed quality suitable for human consumption, as welll as Brazilian green and red propolis (BASTOS, 2001; ALENCAR et al., 2007) and the propolis extracts sold at the informal market of São Paulo according to the legislation (TAGLIACOLLO; ORSI, 2011).

This diversity of the propolis in Brazil is reflected on its several therapeutic properties such as antimicrobial, anti-inflammatory, immunomodulatory, and anticancer activities (FISCHER et al., 2010; SILVA et al., 2012; MARCUCCI, 1996; SFORCIN; BANKOVA, 2011). According to the results obtained, a standardization and a further detailed study on the chemical composition of propolis (BANKOVA, 2005; SFORCIN; BANKOVA, 2011) are necessary.

4 Conclusions

Seasonality was not significantly correlated with production and physicochemical quality of the propolis, indicating that the propolis can be collected throughout the whole year.

The landscape of the apiary located in the highland contributed to the physicochemical quality of propolis from Pantanal of Cáceres.

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