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Physicochemical and microbiological characterization of cassava flower honey samples produced by africanized honeybees

Caracterização físico-química e microbiológica de amostras de mel de flores de mandioca produzido por abelhas africanizadas

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Abstract

Cassava producers in the region of Marília-São Paulo are integrating their farming activity with beekeeping to diversify their income. The aim of this study was to evaluate the physicochemical and microbiological quality of honey samples produced by Africanized honeybees *Apis mellifera* from cassava flower in 2008. Analysis were carried out for pH, total soluble solids (TSS), acidity, moisture, reducing and total sugars, apparent sucrose, hydroxymethylfurfural, color, ash, proteins, water insoluble solids, diastasic activity, mineral content, microbiological evaluations, and mineral and hydrocyanic acid (HCN) content. The honey samples showed physicochemical and microbiological characteristics favorable to commercialization, with the exception of apparent sucrose and acidity, which show the need for a narrow focus of attention to the honey maturation degree at the harvest time and more careful monitoring during production and processing. The commercialization of Brazilian cassava honey, still little explored, can be widely spread in the market since the levels of hydrocyanic acid (HCN) showed no consumption risk; in addition the simultaneous production of honey and cassava provides an alternative to family income increase.
Keywords: *Manihot esculenta Crantz; hydrocyanic acid; beekeeping.*

Resumo

Produtores de mandioca da região de Marília, Estado de São Paulo, estão consorciando a atividade da apicultura em meio à cultura visando diversificar a renda obtida pela propriedade rural. Este trabalho teve como objetivo avaliar a qualidade físico-química e microbiológica do mel produzido por abelhas *Apis mellifera* africanizadas, elaborado a partir de flores de mandioca, no ano de 2008. Foram realizadas análises de pH, sólidos solúveis totais (SST), acidez, umidade, açúcares redutores e totais, sacarose aparente, hidroximetilfurfural, cor, cinzas, proteínas, sólidos insolúveis em água, atividade diastásica, teor de minerais e monitoramento dos teores de ácido cianídrico (HCN), além de avaliações microbiológicas. O mel apresentou características físico-químicas e microbiológicas favoráveis à comercialização, com exceção da sacarose aparente e da acidez, sendo necessária atenção na maturação do mel no momento da coleta e melhor monitoramento nas fases de produção e processamento. A comercialização brasileira do mel de mandioca é ainda pouco explorada, mas pode ser amplamente disseminada no mercado, visto que os valores de HCN não apresentaram risco ao consumidor e a produção do mel paralelamente ao plantio da mandioca proporciona uma alternativa de aumento na renda familiar.
Palavras-chave: *Manihot esculenta Crantz; ácido cianídrico; apicultura.*

1 Introduction

In Brazil, beekeeping has become attractive to many farmers and various institutions since it is an activity from which they can obtain good economic, ecological, and social results (EVANGELISTA-RODRIGUEZ et al., 2005; WELKE et al., 2008). Brazilian honey production, in 2007, was 34,747 t, and the state of São Paulo accounted for 2,332.19 t. In 2009, the production of São Paulo state reached 2,103 t, of which approximately 5,253 t were exported to other countries (INSTITUTO..., 2010; SILVA, 2011). Beekeeping is a production system that can be developed simultaneously with other agricultural activities; thus, some farmers in the cities of Marília and Ocauçu integrated beekeeping with cassava (*Manihot esculenta Crantz*) production focusing on diversifying their production. Cassava is a plant that has significant economic importance in Brazil. Brazilian crop in 2008 was 25.8 t (INSTITUTO..., 2010). There are few reports on the quality of honey produced from cassava flowers in the literature. The main pollinator of these flowers is *Apis mellifera*, which are attracted by their fragrance, pollen, and nectar featuring a perfect interaction between pollination agent and the cassava flower (SILVA et al., 2001).

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Brazilian law defines honey as a food product produced by honeybees from the nectar of blossoms or secretions of plant-sucking insects that are on living parts of plants that bees collect, transform, combine with their own specific substances, store, and let ripen in the honeycombs of the hive (BRASIL, 2000a).

The determination of honey physicochemical parameters is important for its characterization, taking into consideration the great botanical diversity, and to ensure the quality of this product in the market (WELKE et al., 2008; MENDES et al., 2009). Microbiological evaluation is necessary because most of the honey sold in the country does not go through a pasteurization process. Regional characterization of honeys is also of fundamental importance taking into account the great botanical diversity and climate variability in each region (WELKE et al., 2008).

In the case of honey produced by cassava flowers, the main element to be analyzed is hydrocyanic acid (HCN), found in high concentrations in the plant and present in the honey derived from its flowers. HCN is a volatile and highly toxic compound that inhibits respiratory chain enzymatic activity of living beings (CHISTÉ; COHEN, 2008). The maximum tolerated concentration of HCN is 4 mg.kg^{-1} of the multi-mixture (BRASIL, 2000b). According to the World Health Organization - WHO, the lethal dose (LD_{50}) of HCN is 10 mg. kg^{-1} body weight (CHISTÉ; COHEN, 2008). The purpose of this study was to evaluate the physicochemical and microbiological parameters of honey samples to establish a quality standard of cassava honey.

2 Materials and methods

Honey samples were collected from 12 hives located on the border of the cities of Marília and Ocauçu in the state of São Paulo, Brazil, a planted area with 400 ha of industrial varieties of cassava which flower in May. The extraction of honey samples produced by Africanized honeybees analyzed was carried out between June and July 2008. The following step, in August 2008, chemical and microbiological analysis were performed and the content of hydrocyanic acid (HCN) was determined.

The HCN content was analyzed every 15 days to observe its possible stabilization during 120 days of storage at room temperature (25 °C). All tests were performed in triplicate.

The samples were obtained from 12 hives of Africanized honeybees *Apis mellifera* located on a farm with 400 ha of industrial plantation of cassava varieties, on the border of the cities of Marilia and Ocauçu in the state of São Paulo.

The physicochemical and microbiological analyzes were performed within 30 days after collection. The levels of hydrocyanic acid in the honey samples were determined every 15 days for a period of 120 days, during which the honey was stored in 1 kg-nontoxic plastic bottles under ambient conditions (25 °C). All analyzes were performed in triplicate.

2.1 Physicochemical analysis

Moisture content of the honey samples was determined by refractometry using the AOAC 969,38b Method (ASSOCIATION..., 1997). The hydroxymethylfurfural (HMF) was determined by quantitative spectrometry with l = 284 and 336 nm using the AOAC 980,23 method (ASSOCIATION..., 1997), recommended by the Normative Instruction of the Ministry of Agriculture and Supply (Instrução Normativa do Ministério da Agricultura e do Abastecimento) (BRASIL, 2000a). The analysis of the free acidity was performed according to the AOAC 962,19 method (ASSOCIATION..., 1997). This procedure is recommended by the Technical Regulation of Identity and Quality of Honey (Regulamento Técnico de Identidade e Qualidade do Mel) (BRASIL, 2000b). The pH values were determined using a digital pH meter (Hanna Instruments, model pH 300). The ash content was obtained by calcination in a muffle furnace by the Almeida-Muradian and Bera (2008) method. The minerals content was determined by nitro-perchloric digestion using the Malavolta, Vitti and Oliveira et al. method (1989), and the determination was done by atomic absorption spectrometry (Varian - mod. Spectra A 10 Plus). Phosphorus (P) was determined using a UV/VIS spectrophotometer according to the method described by Pavan et al. (1992). The Lane-Eynon method (INSTITUTO..., 2005) was used for determination of total and reducing sugars and apparent sucrose. The determination of protein content was done using the semi-micro Kjeldahl method (0.2 g of sample). The conversion factor used was 6.25 (SILVA; QUEIROZ, 2002). The content of insoluble solids was determined by the Almeida-Muradian and Bera (2008) method, and the diastasic activity was determined according to Marchini, Sodré and Moreti (2004). Quantification of hydrogen cyanide (HCN) was performed according to the Teles method (1972) during 120 days of storage at room temperature (25 °C). The color of honey was determined according to Marchini, Sodré and Moreti (2004) using the Pfund scale.

2.2 Microbiological analysis

The honey samples were transported in an ice box to the Food Microbiology Laboratory of the Federal Technological University of Paraná - FTUPR *Campus* Campo Mourão for microbiological analyses. The presence/absence of *Salmonella* sp., coliform group, moulds, and yeasts were analyzed according to official methods (BRASIL, 2003).

3 Results and discussion

3.1 Physicochemical analysis

The results of physicochemical analysis of the honey samples produced by Africanized honeybees from cassava flower are shown in Table 1. National legislation and Mercosul have no parameters for pH values. The pH values were in accordance with the values reported by Almeida-Muradian and Bera (2008), between 3.3 and 4.6. Although pH analysis is not currently required for the quality control of Brazilian honey samples, it is useful auxiliary variable for testing quality because it is an important parameter during extraction and storage of honey. The pH values influence the texture, stability, and shelf life of honey; therefore, different pH values may indicate fermentation or honey adulteration (WELKE et al., 2008). All honey samples were acidic and the pH was influenced by botanical origin and concentration of different acids in its composition, besides being related to formation of HMF in honey (MARCHINI; SODRÉ; MORETI, 2004).

The acidity of the analyzed honey samples met the values established by Brazilian legislation (BRASIL, 2000a), but it was above the standard value established by the Mercosur's Technical Regulation, a maximum of 40.00 $mEq.kg^{-1}$. Evangelista-Rodrigues et al. (2005) evaluated two honey samples produced by *Apis mellifera* in the state of Paraiba and found values that ranged between 41.66 and 35.00 $mEq.kg^{-1}$. The honey acidity is due to the variation of organic acids caused by different sources of nectar, by the action of the glucose oxidase enzyme which generates gluconic acid, by the action of bacteria during honey ripening, and even by the amount of minerals found in honey.

The moisture content is lower than the maximum amount established by Brazilian legislation and Mercosur. The moisture content of honey is influenced by botanical origin, climatic conditions, harvest season, and the degree of honey maturation, and it is a very important parameter during storage. Moisture content below 21.0% prevents undesirable processes of honey fermentation (SODRÉ et al., 2007).

The TSS content found was 80° Brix. The TSS helps determine the moisture content. Reducing sugars' values were close to the minimum levels established. The total sugars averaged 76.10 $g.100\ g^{-1}$; nevertheless, there are no limits set by legislation. Bendini and Souza (2008) reported an average of 81.25 $g.100\ g^{-1}$ in the honeyof cashew flower. The sucrose content is above the standards set by legislation. Marchini, Sodré and Moreti (2004) reported that sucrose represents on average 2.0 - 3.0% of carbohydrates, and when it is above these values it indicates, in most cases, adulteration or premature harvest of honey, *i.e.,* it is a product in which sucrose was not completely transformed into glucose and fructose by the invertase enzyme.

HMF, known as non-enzymatic browning, is a major breakdown product during carbohydrate degradation (SPANO et al., 2008). Fresh honey has small amounts of HMF, but with prolonged storage at high room temperature, this level can rise changing the nutritional value of the product. Thus, the determination of HMF is an indicator of honey quality because when it is formed, it indicates loss of enzymatic activity enzymes of glucose oxidase, for example. The average value obtained was 5.14 $mg.kg^{-1}$ indicating that the sample is below the maximum value established by national and international legislations, and it is also below the values found by Bendini and Souza (2008), 14.21 $mg.kg^{-1}$, and Alves et al. (2005), 5.79 kg^{-1}.

The ash content can be an indicator of good manufacturing practices. Therefore, the high percentage of ash found in the

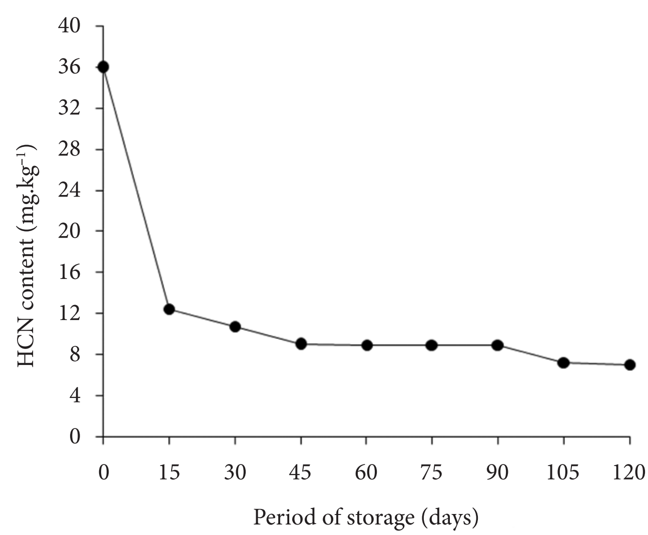

Figure 1. HCN contents of cassava flower honey during storage at room temperature.

Table 1. Physicochemical analysis of honey samples produced by Africanized honeybees from cassava flower in the cities of Marília and Ocauçu, state of São Paulo, Brazil, 2008.

Physicochemical parameters	Average values (±δ)	Mercosul, 1999	IN n.11, 2000
pH	4.14 ± 0.01	-	-
Acidity $mEq.kg^{-1}$	49.87 ± 0.20	Max. 40.00	Max. 50.00
Moisture ($g.100\ g^{-1}$)	18.13 ± 0.01	Max. 20.00	Max. 20.00
Total Soluble Solids (°Brix)	80.00 ± 0.01	-	-
Reducing Sugars ($g.100\ g^{-1}$)	65.27 ± 1.15	Min. 65.00	Min. 65.00
Apparent sucrose ($g.100\ g^{-1}$)	10.83 ± 1.33	Max. 5.00	Max. 6.00
Total sugars ($g.100\ g^{-1}$)	76.10 ± 1.78	-	-
HMF ($mg.kg^{-1}$)	5.14 ± 1.12	Max. 40.00	Max. 60.00
Ash ($g.100\ g^{-1}$)	0.45 ± 0.06	Max. 0.60	Max. 0.60
Protein ($g.100\ g^{-1}$)	3.29 ± 0.20	-	-
Insoluble solids in water (%)	0.04 ± 0.01	Max. 0.10	Max. 0.10
Diastasic activity (Goethe scale)	10.33 ± 1.55	Min. 8.00	Min. 8.00
Coloring	Dark Amber	-	-

±δ - standard deviation; HMF – Hydroximethylfufural; (-) Unrequired default.

honey samples evaluated may indicate improper handling or the presence of contaminants in the product (ALMEIDA-MURADIAN; BERA, 2008). The value found in this study, 0.45%, is higher than that found by Sodré et al. (2007), who obtained an average of 0.18%; Bendini and Souza (2008) found averages of 0.20% of total weight and ranged between 0.18 and 0.30% in cashew flower honey samples produced in the state of Ceará.

The value found for protein content was 3.29 g.100 g^{-1}. This determination is not established in legislation, but this evaluation is important to characterize the cassava-flower honey. Fresh honey produced in São Paulo state evaluated by Bera and Almeida-Muradian (2007) had lower values ranging from 0.34% to 0.73%. Sodré et al. (2007) analyzed honey samples produced in the state of Ceará and obtained values of 0.35%.

The diastasic activity of honey is in accordance with legislation (MERCOSUL, 1999; BRASIL, 2000a). Evaluating honey samples produced in Portugal, Mendes et al. (1998) obtained results that ranged from 2 to 22 in the Goethe scale. Diastase is one of the enzymes in honey, which has the function of digesting the starch molecule and is very sensitive to heat and, therefore, able to indicate the degrees of conservation and overheating of the product (MENDES et al., 2009). The absence of this enzyme indicates adulteration or use of procedures such as temperatures above 60 °C during processing, addition of inverted sugar, and improper storage conditions. Thus, the value obtained in this experiment indicate good beekeeping practices during the production of honey of cassava.

The content of insoluble solids in water met the legislation requirements (MERCOSUL, 1999; BRASIL, 2000a). The solids insoluble in water are parts of bees (legs, wings, etc.), wax residue, and other elements related to honey or its processing. Performing this analysis makes possible to detect impurities in the honey, a measure of hygienic control (SILVA et al., 2006). The honey sample were dark amber-colored due, perhaps, to the high content of minerals (Table 2). Mendes et al. (1998) observed colors ranging from light to dark amber in honey produced in Portugal. The color of honey varies according to its floral origin and age and storage temperature since overheating can darken the honey. Couto and Couto (2006) stated that several studies have shown that darker honeys could have four to six times more minerals than clear honey; the major minerals in honey are manganese, potassium, sodium, and iron. Thus, dark honey may be an indicator of quality.

The mineral content of cassava flowers are shown in Table 2. The content of calcium (Ca) present in one kilogram (kg) of honey produced from cassava flower accounted for 38.60% of the RDI. The content of magnesium (Mg) accounted for 80.77% of the RDI (BRASIL, 2005). Potassium (K) limit value has not been established the law. Phosphorus (P) represented only 2.00%. Copper (Cu) was present in concentrations five times higher than that recommended by RDI (BRASIL, 2005).

The contents of some minerals differed from those found by Santos et al. (2008), who analyzed the honey of the region of Bahia and obtained: Ca, 0.032 $g.kg^{-1}$; Mg, 0.015 $g.kg^{-1}$; K, 0.555 $g.kg^{-1}$; and Cu, 0.0004 $g.kg^{-1}$. As previously discussed and reported by Santos et al. (2008), K is the mineral of highest concentration in the honey samples evaluated. The other minerals, K, Ca, and Mg have higher concentrations than those reported in the literature. This is due to the different vegetation visited by honeybees and the type of soil. Cu was the mineral found in the smallest amounts in cassava flower honey. The levels of minerals influence on other characteristics of honey. The contents of K and Ca influence honey acidity (MARCHINI; SODRÉ; MORETI, 2004). This fact was observed in the honey investigated in this study, which has high concentration of K and Ca and, consequently, high acidity .

In Figure 1, it can be seen a decrease in the concentration of cyanide (HCN) in the honey samples during storage at room temperature showing a great reduction in the first days after harvesting, ranged from 36.00 to 7.03 $mg.kg^{-1}$. Cassava is the most important food crop among those that contain hydrocyanic acid. All cassava tissues, except for the seeds, contain large quantities of cyanogenic glycosides, linamarin, and lotaustralin with significant differences among varieties (CARDOSO JÚNIOR et al., 2005). The hydrolysis of cyanogenic glycosides is greatly favored in acidic medium (HELBIG; BUCHWEITZ; GIGANTE, 2008). Thus, when plants containing these glycosides are ingested and come in contact with the stomach pH, they find an ideal medium to release hydrocyanic acid.

It is known that the LD_{50} value of the HCN is 10 $mg.kg^{-1}$ of body weight (CHISTÉ; COHEN, 2008), and thus the HCN content found in the cassava honey studied, 36.00 $mg.kg^{-1}$ can be considered safe for consumption since if an 60 kg-individual consumes 15 g of cassava honey, he or she will be consuming 0.54 mg of HCN, which is lower than the LD_{50}.

3.2 Microbiological analysis

According to Alves et al. (2011), honey microbiology can be divided into two groups: those based on honey and those considered as secondary contamination related to processing. Among the former are molds and yeasts, which under normal conditions of moisture, do not interfere with the honey quality and are not pathogenic. In the second group, coliforms at 35 °C can be found, in addition to yeasts and molds, which can indicate poor hygiene, and coliforms at 45 °C indicate the sanitary conditions and can cause illnesses. The microbiological analysis of cassava flower honey produced by Africanized honeybees is presented in Table 3. Brazilian legislation and the Technical

Table 2. Mineral contents of honey samples produced by Africanized honeybees from cassava flowers in the cities of Marília and Ocauçu, state of São Paulo, Brazil, 2008.

Minerals	Average contents	RDI*
Ca	386 $mg.kg^{-1}$	1000 mg
Mg	210 $mg.kg^{-1}$	260 mg
K	2.110 $g.kg^{-1}$	-
P	14 $mg.kg^{-1}$	700 mg
Cu	5,000 mcg	900 mcg

*RDI – Recommended Daily Ingestion (BRASIL, 2005).

Table 3. Microbiological values of honey samples in cassava flower produced by Africanized honeybee in the state of Sao Paulo, Brazil, 2008.

Analysis	Values
Coliforms at 35 °C (NMP*.g^{-1})	<3.0
Coliforms at 45 °C (NMP.g^{-1})	<3.0
Moulds and yeasts (CFU**.g^{-1})	<10^1
Salmonella sp. (25 g)	Absent

*MPN Most probable number; **CFU colony forming unit.

Regulation of Honey Identity and Quality of Mercosul do not require microbiological analysis of honey; however, the use of adequate hygienic practices during the product handling is required (MERCOSUL, 1999; BRASIL, 2000a). On the other hand, the RDC n. 12 (BRASIL, 2001) addresses the need for microbiological standards for foods.

The evaluation of coliform groups at 35 °C and 45 °C indicates good sanitary quality of the product. The microorganism indicator of Coliforms at 45 °C is *Escherichia coli*, a gram-negative pathogenic bacteria, indicating fecal contamination or poor hygiene during production affecting the shelf life of the product. Microbiological evaluations performed in honey samples produced in the state of Minas Gerais had values similar to those obtained in this study (SILVA et al., 2008). The authors also report that values <3.0 NMP.g^{-1} may indicate good hygienic conditions during the processing of honey resulting in satisfactory sanitary quality.

The results of the yeast and mould analysis show growth lower than 10 CFU. Alves et al. (2009), evaluating the presence of yeasts and molds in honey samples produced in the region of the upper Paraná River, obtained 3.8×10^4 CFU, a value higher than that obtained in this study (<10^1 CFU). Yeast and mold are inherent microorganisms in honey, which under normal conditions of moisture do not interfere with honey quality and are not pathogenic (MENDES et al., 2009). According to Good Manufacturing Practice (GMC) of Resolution n.15 (MERCOSUL, 1994), published before resolution 56/99 (MERCOSUL, 1999), honey should not present values of molds and yeasts above 1.0×10^2 CFU, and the results of this study meet this requirement. Snowdon and Cliver (1996) believe that this value is considered acceptable since spores of microorganisms can be mixed with honey during the harvesting nectar and pollen by the bees entering and leaving the hive, and these may also come from the stomach of bees since they regurgitate during the production of honey.

Salmonella sp. was not detected; these bacteria can cause severe food poisoning and therefore should not be present in any type of food. This absence of microorganisms may have been favored by the pH range found in the samples, average of 4.14, leading to inhibition of microorganisms' multiplication (ALVES et al., 2009). These authors also reported that moisture content below 20% is sufficient to inhibit the development of moulds and yeasts in honey under environmental conditions.

Although honey presents antimicrobial compounds such as hydrogen peroxide (H_2O_2) and phytochemical (phenolic acids, terpenes, pinocembrim, benzyl alcohol, and lysozyme) (SNOWDON; CLIVER, 1996), it should not be considered microbiologically sterile since it is subject to contamination (SILVA et al., 2008). According Sereia et al. (2011), to obtain satisfactory values for the honey microbiota, there is a need to implement standard operational procedures (SOPs) for the production and processing of honey to ensure microbiological quality.

4 Conclusion

The honey obtained from the pollinated flower cassava showed physicochemical and microbiological characteristics favorable to commercialization, with the exception of apparent sucrose and acidity, which show the need for a narrow focus of attention to the honey maturation degree at the harvest time and more careful monitoring during production and processing.

Cassava Brazilian honey has great potential for commercialization since the values of HCN showed no consumption risk, and the production of honey and cassava provides an alternative to family income increase.

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