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Classification of Wild Honeys of Different Mesoregions from Paraná State, Brazil, by Principal Component Analysis

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Classificação de Méis Silvestres de Diferentes Mesorregiões do Estado do Paraná, Brasil, pela Análise de Componentes Principais

Resumo: No Brasil a caracterização da origem botânica e/ou geográfica de méis através da análise físico-química empregando ferramentas quimiométricas é relativamente recente e realizada para os méis mais comercializados ou típicos de uma determinada região. No entanto, até o momento não foram encontrados estudos de caracterização da origem geográfica de méis silvestres do Estado do Paraná, Brasil. Desta forma, neste trabalho foi realizada a classificação de 31 amostras de méis silvestres provenientes de diferentes mesorregiões do Estado do Paraná, Brasil, pela Análise de Componentes Principais (PCA). Os resultados indicaram que os parâmetros pH, condutividade elétrica e HMF são determinantes para a identificação da origem geográfica dos méis silvestres das mesorregiões Centro Sul, Sudeste e Oeste, sugerindo que as regiões produtoras possuem diferentes condições climáticas e topológicas, grande diversidade floral e diferentes tipos de processamento dos méis.

Palavras-chave: Mel silvestre; origem geográfica; mesorregião; análise de componentes principais.

Abstract

In Brazil, the botanical and/or geographical origin characterization from honeys by physicochemical analysis using chemometric tools is relatively recent and held to the most marketed or typical honeys from a particular region. However, until the moment, not were found studies of geographical origin characterization of wild honeys from Paraná State, Brazil. Thus, in this work the geographic classification of 31 wild honey samples of different mesoregions from Paraná State, Brazil, was carried out by Principal Components Analysis (PCA). The results indicated that pH, electrical conductivity and HMF are key parameters to identify the geographical origin of wild honeys from South Centre, Southeast and West mesoregions suggesting that the producing regions have different climatic and topological conditions, great floral diversity and different honey processing types.

Keywords: Wild honeys; geographic origin; mesoregion; principal components analysis.

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Classification of Wild Honeys of Different Mesoregions from Paraná State, Brazil, by Principal Component Analysis

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1. Introduction

Most of the honey produced in the world is sold under the name "honey". Generally this means that it was produced with contributions from nectar and honeydew of several species of plants and therefore is a mixture of different types of honeys.[1,2]

This intrinsic variability is not always desirable from a commercial point of view when seeking a consistent product with the most current regulations. On the other hand, it allows provide to the consumer a typical number of honeys with special features resulting from the particular source. This demonstrates the importance of the correct and reliable origin characterization from honeys as a way to protect consumers and preserve the reputation of the floral and geographical designation.[1,2]

The physicochemical analysis adopted in quality control of honeys produces very reproducible results, even when data from several laboratories are used to analyze samples from different countries. The analytical methods utilized for the origin classification of honey are basically the same as those adopted for the quality control of this product.[3] Recently, several studies have been performed in order to identify which physicochemical characteristics could be used to characterize the floral and geographical origin and establish ranges for these parameters in various types of honeys.[1,3,6-8] This characterization type has provided subsidies for developing new standards and legislations, including the distinction between floral types of honey in different countries, especially in Europe, besides the determination of the geographical origin. This was observed in the last revision of the Codex Alimentarius, published in 2001 and by the European Directive 110/CE published in 2002, in which specific limits depending on the floral source with respect to the parameters of moisture, sugar composition and electrical conductivity were defined.[1,9,10]

Another way to identify the origin of honeys using physicochemical characteristics is based on the application of sophisticated statistical methods such as principal component analysis (PCA), linear discriminant analysis (LDA) and cluster analysis (Cluster) (CA) to parameters such as the composition of sugars and their ratios, electrical conductivity, color, specific rotation, moisture, ash, pH, proline, mineral composition and acidity. This characterization form has achieved good results in the attribution of botanical and geographical origins of honeys.[1,6-8,11]

In Brazil, the characterization of the botanical and/or geographical origin of honeys by physicochemical analysis using chemometric tools is relatively recent and it is carried out for the most marketed or typical honeys from a particular region.[5,7,8,12-15] Most studies are still focused on the determination of the physicochemical quality of the honeys in relation to the requirements of Brazilian legislation.[16-20] These facts demonstrate the importance of conduct scientific researches to establish specific limits for the different types of honeys produced in Brazil. This could facilitate the trade relations in the national and international levels through aggregation of commercial value by assigning the origin of product. Moreover, it is also clear that, it is not always easy to determine the botanical and geographical origin of honeys, due to the variability in their composition, especially for wild honeys. Therefore, characterization studies should seek the best strategies for the designation of origin, which points to the application of different chemometric tools to physicochemical parameters (individuals or in group).

The southern region of Brazil is responsible for most of the honey production in the country. The Paraná State is the second largest producer with 16.4 % of Brazilian honey production in 2012.[21] It is divided into 10 administrative mesoregions, with different climatic, topological conditions and a great floral diversity, and between them stands out in honey production, the Southeast, Eastern Center, Metropolitan, Southwest and West mesoregions.[22] However, despite the economic importance of the Paraná State for Brazilian honey production, studies related to characterization of physicochemical quality of honeys produced in this State are still scarce[23-25] and by consequence, researches dedicated to designation of origin (botanical or geographical) of the honeys also still were not realized. Thus, in this work, at the first time, the geographical origin classification of 31 wild honey samples from different mesoregions of Paraná State, Brazil, using physicochemical parameters (moisture, color, pH, electrical conductivity, HMF and free acidity) was carried out by Principal Components Analysis (PCA).

2. Materials and Methods

2.1. Honey Sampling

Thirty-one wild honey samples were collected from apiaries and beekeeping associations located in different mesoregions from the Paraná State between the years 2010 and 2012 (Table 1, Figure 1). The Paraná State is divided in ten mesoregions that presented different topological and climatic conditions and great floral diversity. North region is characterized by hot summers with low incidence of frost, plane ground with gently rolling, hills with flat tops and good soil fertility. South region is characterized by the absence of dry season with harsh winter, severe and frequent frosts, rainy and mild summers, rugged topography and soils with low fertility. West region is characterized by harsh winter with average rainfall and frosts, rainy summers and high temperature, flat relief, small ripples and high fertility soils. Collection sites of wild honey samples studied in this work were distributed in these regions.

The samples were classified by beekeepers as wild honeys and it were stored in polyethylene bottles at room temperature in the dark until the realization of physicochemical analyzes.

2.2. Physicochemical Analyzes

Determination of parameters moisture (method 173/IV), hydroxymethylfurfural (HMF) (method 175/IV), pH and free acidity (method 174/IV) was carried out according to Physicochemical Methods for Food Analysis(2008). [26]Electrical conductivity was determined according to methodology described by Honey European Community [3]and color classification was obtained according to method recommended by BRASIL (1981).[27] All physicochemical analyzes were realized in duplicate and the data were expressed as means and standard deviations.

2.3. Statistical Analysis

Due the existence of differences in climate, soil and vegetation in the mesoregions producing honey in Paraná State, Brazil, the Principal Components Analysis (PCA) was applied to physicochemical parameters (moisture, pH, color, free acidity and HMF) in order to identify the chemical variables capable of characterizing the geographic origin from wild honeys produced in each area. PCA was used to achieve a reduction of dimension while retaining the maximum amount of variability present in data and observing a primary evaluation of the between-class similarity. [9] All statistical analysis was realized using MATLAB software (Matlab & Simulink-R2006a, OPENCADD ADVANCED TECHNOLOGY, São Paulo, Brazil).

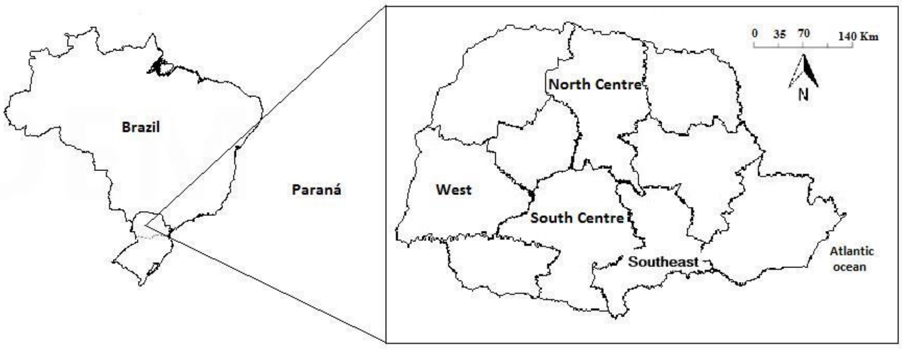


Figure 1. Collection sites from wild honey samples at the different mesoregions from Paraná State, Brazil

Table1. Distribution of wild honey samples from Paraná State by cities and mesoregions

Mesoregions	Number of Samples by Mesoregion	Cities[a]
West (WE)	10	Toledo (1) Realeza (1) Cascavel (1) Mercedes (1) Entre Rios do Oeste (1) Foz do Iguaçu (1) Guaraniaçú (1) Nova Santa Rosa (1) Santa Helena (1) Terra Roxa (1)
South Centre (SC)	08	Santa Maria do Oeste (1) Guarapuava (2) Turvo (1) Nova Laranjeiras (1) Marquinho (1) Laranjeiras do Sul (2)
North Centre (NC)	06	Manoel Ribas (2) Mandaguaçu (1) Rolândia (1) Londrina (1) Maringá (1)
Southeast (SE)	07	Cruz Machado (2) Barra Mansa (1) Prudentópolis (4)

[a] number of wild honey samples collected in each city described in parenthesis.

3. Results and Discussion

3.1. Physicochemical Quality Evaluation

The results of physicochemical analyzes of wild honey samples from each mesoregion studied are presented in Table 2. Data are expressed as means, standard deviations, minimum values, maximum values and limits in the national and/or international legislations.

The parameter color is important of a commercial point of view since lighter color honeys are preferred by consumers. Honey color is determined by its botanical source and by the local climate and soil conditions. Storage, light, heat and potential enzymatic reactions may also affect color.[28] Recently, several researches have demonstrated that the honey colors also can be influenced by concentration of phenolic compounds.[29,30] Darker honeys tend to have higher antioxidant activity and an increased concentration of phenolic compounds.[30]

The wild honey samples studied in this work presented almost every colors established by Pfund classification, which demonstrates the floral diversity of collection regions of honeys. It was verified a predominance of dark amber and amber colors in all mesoregions investigated (Figure 2) and lighter honeys were found only in the Southeast and West mesoregions. Colors diversity between wild honey samples was observed also by Lacerda *et al.* (2010)[8] for honeys produced in Southeast Bahia, Brazil.

Table 2. Results of physicochemical analyzes for wild honey samples of different mesoregions from Paraná State, Brazil

Physicochemical Parameters		Mesoregions			
		SC	SE	NC	WE
Moisture (%)	Mean	17.8	18.6	18.3	17.8
	SD	1.4	1.1	1.2	1.3
	Min.	15.5	16.6	16.0	15.7
	Max.	19.7	20.0	20.0	19.9
	Limit	20.0[a]			
HMF ($mg\ kg^{-1}$)	Mean	6.4	10.7	36.6	40.0
	SD	6.1	4.0	29.2	38.3
	Min.	2.6	4.6	7.6	8.7
	Max.	21.3	15.9	78.4	133.3
	Limit	60.0[a]/80.0[b]			
pH	Mean	4.31	3.82	3.90	3.64
	SD	0.59	0.43	0.44	0.21
	Min.	3.50	3.30	3.40	3.40
	Max.	5.50	4.50	4.60	3.90
	Limit	3.0 – 4.5[c]			
Electrical Conductivity ($mS\ cm^{-1}$)	Mean	0.599	0.468	0.457	0.246
	SD	0.121	0.167	0.237	0.111
	Min.	0.407	0.283	0.296	0.104
	Max.	0.726	0.742	0.883	0.421
	Limit	0.80[b]			
Free Acidity ($meq\ kg^{-1}$)	Mean	20.3	24.5	30.5	21.8
	SD	5.3	5.4	13.4	7.4
	Min.	13.6	16.3	16.5	8.5
	Max.	35.1	35.5	62.3	35.0
	Limit	40.0[a]/50.0[b]			

[a]national legislation; [b] international legislation; [c] Crane (1990); SD = standard deviation, Min.= minimum value, Max.= maximum value; SC = South Centre, WE = West, SE = Southeast, NC = North Centre

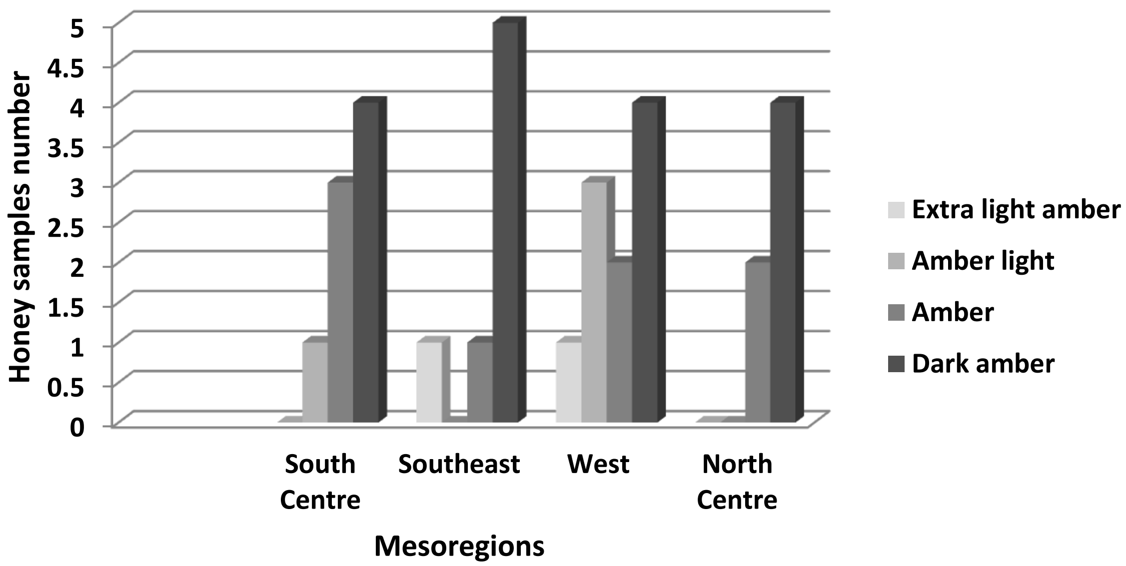


Figure 2. Color classification of wild honey samples by mesoregions from Paraná State

The most of wild honey samples showed compliance with national and/or international legislations for moisture (100%), HMF (87%), free acidity (97%) and electrical conductivity (97%).[9,31] These results demonstrate that the wild honeys produced in Paraná State, Brazil have high quality. However, in relation to each physicochemical parameter some observations should be realized.

In literature, several researchers have pointed out that the moisture contents in honey are influenced by climate conditions of producing region, floral origin, time of honey harvesting and degree of maturation. Beside this, moisture values can be indicative of the tendency to fermentation and crystallization of honeys.[6,13-14,32]In all mesoregions studied were identified honey samples with elevated moisture contents. This fact may be related to period of honeys production and processing. These samples were produced and/or processed in November to March months, period in that there is a great incidence of rainfall in mesoregions investigated. According Marchini, Moreti and Otsuk[13] higher moisture contents in honey samples may be caused by not operculated combs or period and/or inadequate storage conditions which allow the absorption of water from the environment.

The results for HMF analysis demonstrate that 13% of wild honeys presented values for this contaminant upper allowed by national legislation (60 mg kg^{-1}).[31] The HMF is a contaminant that is formed by the decomposition of fructose in the presence of acids and its content may increase with rising temperatures, improper storage, invert sugar addition, acidity, pH, moisture and minerals contained in honey. Therefore, this parameter is considered an indicator of the quality of the honey and high levels may indicate a decline in nutritional value by destruction of some enzymes and heat-labile vitamins.[32] The more elevated levels of HMF in wild honeys were found in samples produced and processed in North Centre and West mesoregions. These mesoregions are characterized by a warmer climate with higher temperatures during periods of production, processing and storage of honeys in relation to mild climate conditions of mesoregions South Centre and Southeast. According to White Junior (1992),[33] the honey produced in subtropical regions can contain higher HMF concentrations without overheating or adulteration due to the normally high local temperatures.

The honey acidity is caused by organic acids from different nectar sources, by glucose-oxidase enzyme action, by minerals, by ions as phosphate and by equilibrium of lactones with its organic acids corresponding. This parameter is very important because it confer chemical and organoleptic characteristics to honey, beside contribute for its stability against the microorganism development.[32,35] So, it is used to identify fermentation process and to assess storage conditions, contributing for an evaluation of honey quality.[14] In this work, only one wild honey sample (3.0 %), produced and processed in North Centre mesoregion, showed a value upper allowed by national (50 meq kg^{-1}) and international legislations (40 meq kg^{-1}).It was observed also for this sample that the values for other physicochemical parameters (moisture and HMF) were not altered. According to Rodríguez et al. (2004)[36], honey harvest time and botanical origin can impact acidity. Other cause for this behavior is the use of inadequate beekeeping practices such as reuse of combs, which favors the increase of the amount of acids in the honey not directly influencing other factors such as HMF.[16,37]

The pH values of wild honey samples ranged from 3.30 to 5.50 and they were similar to those found by Alves *et al.* (2011)[24] for honeys produced in Parana Islands, in Paraná State, Brazil. Between the mesoregions investigated, it were found lower pH values for West mesoregion samples and higher pH values for honeys from South Centre mesoregion. According to Crane (1983)[38] the pH value can be directly related to the floristic composition in the areas of collection and influenced by the pH of nectar, differences in soil composition or association of plant species during the preparation of honey. Due to these factors, the pH has been extensively used in studies for botanical and geographic origins classification of honeys.

The electrical conductivity values for wild honeys ranged from 0.104 a 0.883 mS cm^{-1}. The lower values were observed in samples from West mesoregion while the higher values were determined in South Centre and North Centre mesoregions. This parameter, in honey, depends on the nectar composition of the predominant plants in the production area for their formation. It is also closely related to the concentration of mineral salts, organic acids and proteins and has been proved useful for discriminating honey of different botanical and geographical origins.[7]

3.2. Geographic Origin Classification by Principal Component Analysis (PCA)

In honey quality control and origin characterization (botanical and/or geographical) several physicochemical parameters are commonly used such as color, moisture, sugars, insoluble solids, diastasis, protein, ash, pH, free acidity, HMF, minerals, phenolic compounds and electrical conductivity. [11-20] In this work, a reduced number of variables (pH, free acidity, electrical conductivity, color, moisture and HMF) which have been recognized in the literature because it contains important information for the geographical classification of honeys was selected. According to Lopez *et al.* (1996),[38] the selection of a small number of key variables increases the reliability of mathematical classification, eliminates features with minor information and allows a visual examination of the data set by two-dimensional plot of the key features. Thus, the principal component analysis (PCA) was applied to the autoscaled data from physicochemical parameters HMF, pH, electrical conductivity, color, free acidity and moisture for verify which of these were important for geographic classification of wild honeys from Parana State, grouped in producing mesoregions.

Analysis of PCA results showed that 73.51% of the total variance in the data is explained by the first two components (Figure 3). The first principal component (PC1 with 43.29% of total variance) was strongly influenced by pH, electrical conductivity and HMF (Figure 3a) while for the second principal component (PC2 with 30.22% of the total variance) the physicochemical parameters free acidity and moisture were more important (Figure 3b). The color variable was removed from the PCA because it was located close to the axis in the two principal components (PC1 and PC2) (Figures 3a and 3b) and thus it do not contribute to the geographical origin classification of wild honeys samples investigated in this study. This result can be related to fact that wild honeys samples analyzed presented a predominance of dark amber and amber colors in all mesoregions investigated as illustrated in Figure 2.

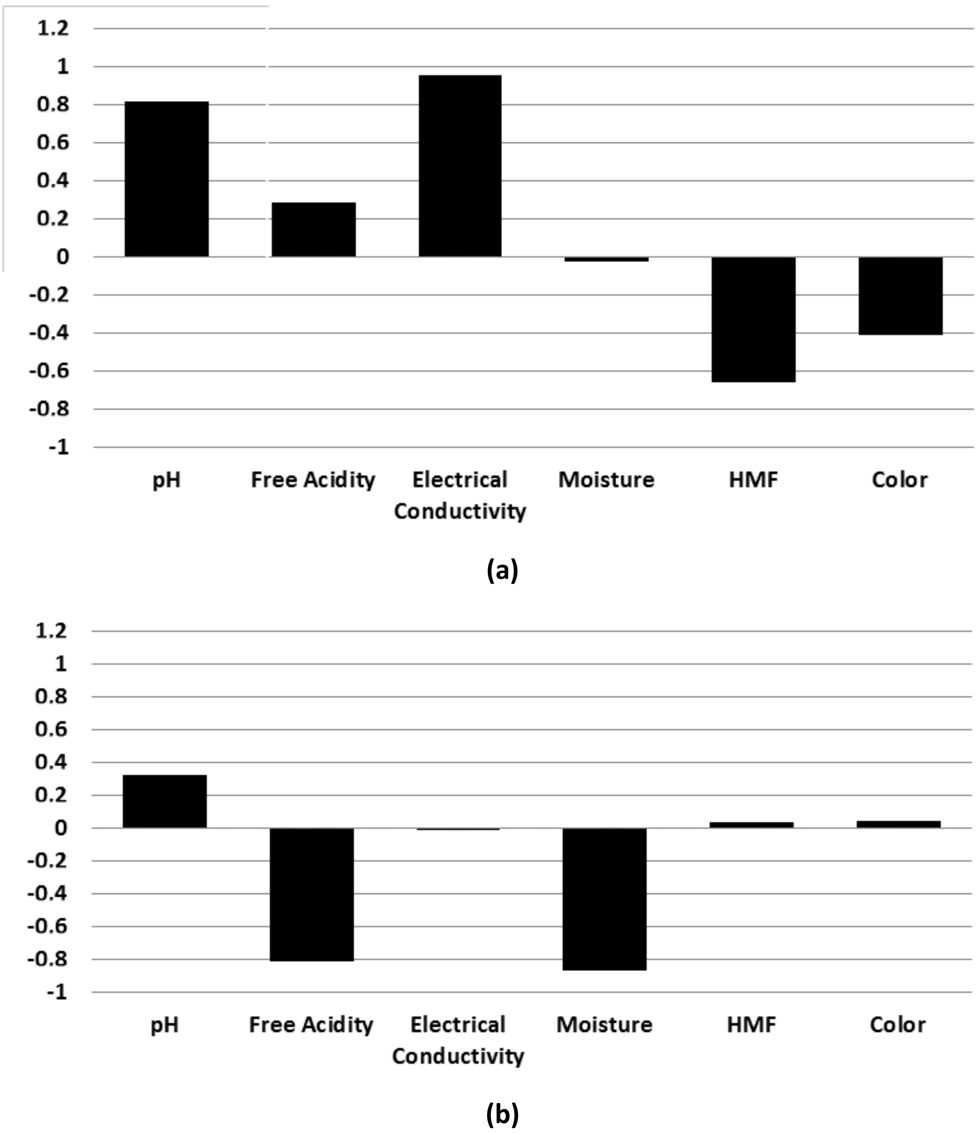
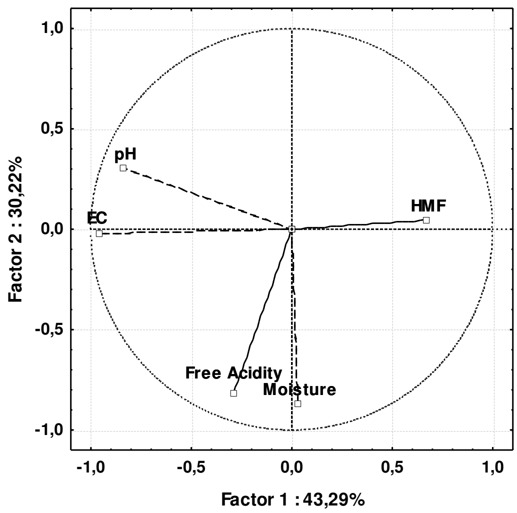


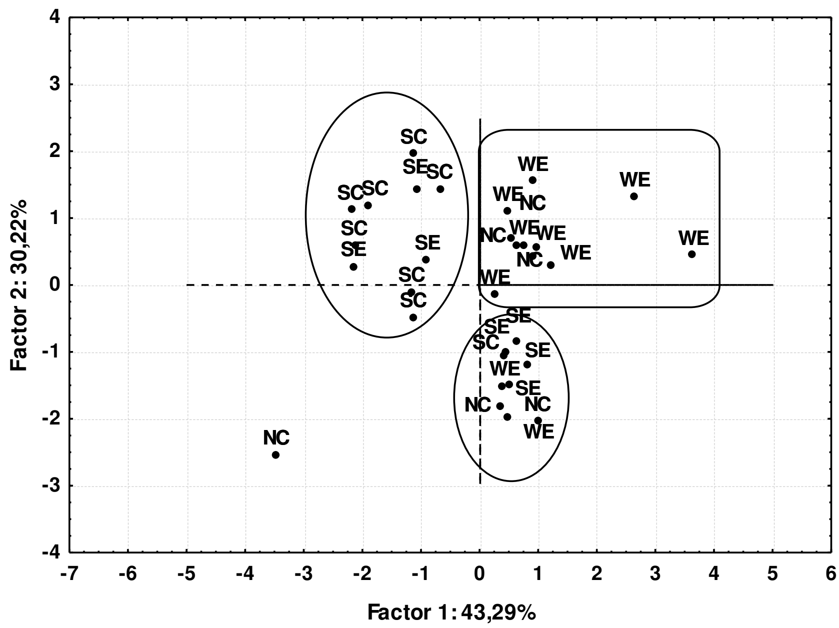
Figure 3. Variable loadings in the two principal components: (a) PC1 and (b) PC2 for wild honey samples

The first principal component (PC1) showed a contrast between the pH, electrical conductivity and HMF it which were inversely correlated (Table 2, Figures 3a and 4a). The parameters pH and electrical conductivity showed negative scores suggesting a contribution of geographical and botanical origins from wild honey samples, while positive scores were related to HMF parameter that is most influenced by the beekeeping practices, processing type and storage conditions. It was also observed that the variables pH and electrical conductivity are important for the classification of honeys produced in the Center South (CS) and Southeast (SU) mesoregions, while the HMF was decisive for the honeys produced in the West (OE) and North Centre (NC) mesoregions (Figures 4a and 4b).

The second principal component (PC2) presented a strong negative correlation between moisture and free acidity parameters (Table 2, Figures 3-b 4-a). These variables showed negative scores suggesting that the quality of honeys can be affected by beekeeping practices, adopted processing type and storage conditions of this product.



(a)



(b)

Figure 4. (a) Plot of the loadings from two principal components (PC1 and PC2) and (b) plot of scores from principal components for the separation of honey samples by geographic origin

The moisture parameter did not allow a clear separation of honeys in relation to geographic origin. It was important for some honey samples of all investigated mesoregions (South Centre (SC), West (OE), North Centre (NC) and Southeast (SU)) (Figure 4b).This fact clearly demonstrates that this is a parameter very connected to climatic conditions at the time of production and processing in the mesoregions investigated and therefore it is difficult to control, but that affects the quality of honeys, and difficult the geographic origin classification. The same behavior was observed for the free acidity parameter that showed negative scores, also not allowing a separation between wild honeys samples produced in the different mesoregions studied (Table 2, Figures 3-b and 4-b). It was verified that a honey sample from North Centre (NC) mesoregion showed the highest value of free acidity. This can be explained by the use of inappropriate beekeeping practices as reuse of combs that favors raising the amount of acids in honey not directly influencing other factors such as HMF.[16]It is also important to note that the wild honeys samples from North Centre mesoregion were located in different quadrants in the PCA being characterized by high moisture, free acidity and HMF contents. So, these honey samples presented a low physicochemical quality that can be attributed to inadequate beekeeping practices and processing.

4. Conclusions

The most of wild honey samples coming from the four mesoregions (South Centre, Southeast, North Centre and West) investigated showed accordance with national and international regulations in regarding the parameters HMF, moisture, pH, free acidity and electrical conductivity. Besides this, it was observed a predominance of colors dark amber and amber in all mesoregions studied and lighter colored honeys (extralight amber and amber light) were observed in West and Southeast mesoregions. Honey samples from North Centre mesoregion differed from the other ones for presenting high moisture, HMF and free acidity contents suggesting a lack of standardization of beekeeping practices and adopted processing.

The wild honeys because of the contribution of various nectar sources in their preparation are known for not having a defined chemical composition and thus it becomes difficult to classify its geographical origin by univariate methods. In this study we observed that the application of multivariate methods such as PCA, allowed, of an unprecedented way, the separation of wild honeys of different mesoregions the Paraná State by their physicochemical characteristics. Honeys samples from South Centre and Southeast mesoregions characterized by high pH and electrical conductivity values and low free acidity and HMF contents were located in PCA on an opposite quadrant to the honeys from West mesoregion, it which were characterized by low electrical conductivity and pH values and high HMF contents. This behavior was attributed to differences in soil composition, vegetation and climate conditions and processing type of each one of these mesoregions. The honey samples from North Centre mesoregion were distributed in different quadrants of the PCA, indicating that the implementation of good beekeeping practices and adequate processing are necessary to ensure the correct denomination of geographic origin of wild honeys from this mesoregion.

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