

PHYSICOCHEMICAL COMPOSITION OF *Apis mellifera* HONEY SAMPLES FROM SÃO PAULO STATE, BRAZIL

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This research, developed with *Apis mellifera* honey samples from producers of São Paulo State, Brazil, has the objective of verifying how eucalyptus, wild flower, and orange honey samples would be clustered, based on physicochemical characteristics. All the orange honey samples and some wild flower ones formed distinct groups, thus confirming that the floral source interferes with honey characteristics. Eucalyptus and some of the wild flower honey samples were clustered together because of the great floral source variation in the latter ones. The characteristics that influence sample clustering are acidity and electric conductivity on the X axis, and total sugars and pH on the Y axis.

Keywords: cluster analysis; *Apis mellifera*; honey composition.

INTRODUCTION

Honey in Brazil can be produced all year round owing to a diverse flora, as a consequence of the country's vast territory and climatic variability. Since honey is the result of dehydration and transformation of nectar collected by bees, the amount of honey produced from a given plant varies with factors that influence nectar yield and concentration, in addition to the concentration and proportion of its carbohydrates, number of flowers in the area, and number of days during which the flowers secrete nectar¹.

Honey composition basically depends on the nectar composition of each producing plant species, conferring specific characteristics to it, while climatic conditions and management on the part of the beekeeper have less influence². Knowledge about the floral source of honey is very important to characterize the product; therefore, the pollen analysis of honey is an important tool in recognizing bee forage plants as nectar and pollen supplies³.

The botanic origin of honey samples decisively affects their physicochemical characteristics. This allowed the formation of distinct groups or sub-groups when sample composition was studied by cluster analysis in samples from the State of Tocantins⁴, in Brazil. Several papers conducted using eucalyptus and citrus honey samples from different Brazilian regions make reference to the physicochemical composition of the samples, by analyzing moisture, electric conductivity, protein, ash content, pH, acidity, formaldehyde index, diastatic activity, HMF, total sugars, reducing sugars, apparent sucrose, and viscosity⁵⁻⁹.

This research, developed with *Apis mellifera* honey samples from various municipalities in the State of São Paulo, Brazil, aimed to verify how eucalyptus, wild, and orange honey samples would be clustered, based on their physicochemical characteristics.

EXPERIMENTAL

The honey samples (a 205 total) were obtained by means of direct contact with beekeepers from different localities in the State of São Paulo, Brazil, being collected during different seasons of the year depending on floral source (eucalyptus, wild, or orange), packaged in glass jars properly labeled with information about the production site, and then taken to the laboratory.

The physicochemical and pollen analyses of honeys produced by *Apis mellifera* were performed at the Beekeeping Laboratory of Departamento de Entomologia, Fitopatologia e Zoologia Agrícola of Escola Superior de Agricultura “Luiz de Queiroz”, Piracicaba campus, Universidade de São Paulo.

All samples were submitted to the acetolysis method and then submitted to a qualitative and a quantitative analysis¹⁰. The physicochemical analyses were carried out only after the honey samples were subjected to the pollen analysis.

Physicochemical analyses

The moisture of the various honey samples was determined by means of a manual Atago refractometer (natural light, room temperature), specific for use with honey¹¹. This device was adapted from the Abbé refractometer and has a high contrast field of view¹².

Ash determination by calcination in a muffle at 550 °C until constant weight, diastase index and Hydroxymethylfurfural was performed according to the methodology cited in the Brazilian legislation¹².

Acidity, pH and formaldehyde index were determined according to the methodology of Moraes Teixeira, in Marchini *et al.*¹³. Total sugars, reducing sugars, and apparent sucrose determinations were performed by the method established by Copersucar, with adaptations¹⁴.

Electric conductivity was obtained in a 20% honey dry matter solution at 20 °C¹⁴, using a Hanna, model HI 8820 conductivity meter to obtain the data.

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Honey proteins were determined following the micro-Kjeldahl method cited in the analytical regulations of Instituto Adolfo Lutz¹⁵; a factor of 6.25 was used in the protein value calculations.

Viscosity was determined by means of a model 100S Brookfield digital viscometer (25 ± 1 °C); the technique consists in measuring the torque generated by the resistance offered by the fluid to rotary movement¹⁶.

Cluster analysis

The data were analyzed by multivariate analysis, using the principal components analysis to evaluate the importance of each physicochemical trait studied over the total variation available¹⁷. This technique is based on the standardization and rotation of the orthogonal axes (physicochemical character), generating a new set of coordinates (principal components) not correlated among themselves¹⁸.

In the cluster analysis, the mean Euclidean distances for the properly standardized data were adopted as dissimilarity means, according to Bussab *et al.*¹⁹. Clusters were formed by the UPGA (unweighted pair-group average) method.

RESULTS AND DISCUSSION

Physicochemical analyses

Although the Brazilian legislation establishes that honey moisture should not exceed 20%¹², in the present work 17.82% of the 50 orange honey samples, 18.81% of the 128 wild honey samples, and 19.30% of the 27 eucalyptus ones (Table 1), totaling 48 samples (23.4% of the total) showed a moisture content higher than the allowed value. Therefore, a considerable percentage of these honey samples does not comply with the quality regulations for Brazilian honey¹², a fact also observed by Cano *et al.*⁶ in honey samples consumed in São Paulo, SP, Brazil, and by Cano *et al.*⁸ in Brazilian honey samples from eucalyptus and orange flowers, with average moisture contents near those obtained by Cortopassi-Laurino⁵, Costa *et al.*⁷, and those in the present work. The explanation for the high water content found in this experiment, especially in eucalyptus honey samples, could be that the honey was harvested from uncapped frames, in addition to the storage period; thus, the honey may have absorbed moisture from the environment.

Great variation in electric conductivity values was observed among the samples analyzed (Table 1). The mean ($1021.48 \mu\text{S cm}^{-1}$) obtained for eucalyptus honey samples from different localities in the State of São Paulo can be considered high when compared with the mean value of $448.60 \mu\text{S cm}^{-1}$, found for commercial eucalyptus honey samples from Spain²⁰, with an average of $628.83 \text{ mS cm}^{-1}$ observed for wild honey and $346.93 \mu\text{S cm}^{-1}$ verified for orange honey, while in the northern coast of the State of Bahia, Brazil, electric conductivity in honey samples ranged from 271.67 to $1634.00 \mu\text{S cm}^{-1}$, with an average of $780.70 \mu\text{S cm}^{-1}$ ²¹.

The mean protein percentages found were 0.31% for eucalyptus honey, 0.20% for wild honey, and 0.16% for orange honey (Table 1). These values were not very different from those obtained for different Italian honey samples^{22,23}.

The ash percentage found in honey expresses its richness in mineral material, and constitutes a frequently used parameter in determinations intended to verify honey quality. Brazilian law has established that the maximum ash content present in honey should be 0.60%¹². Therefore, based on the law, it can be seen that the eucalyptus, wild, and orange honey samples in our experiment showed mean ash percentages below the allowed maximum, and thus conform to the regulations for good quality honey (Table 1). The mean ash percentage in the eucalyptus honey samples analyzed

was 0.16%; this value is a little lower than the 0.20% found in commercial eucalyptus honey samples from Spain²⁰; for wild honey, the mean obtained was 0.26%, while for orange honey the mean was 0.24%, a little higher than the 0.20% value observed in the Spain samples²⁰.

The pH values of 3.62; 3.39; and 3.19 found in eucalyptus, wild, and orange honey, respectively (Table 1), are near those obtained for Brazilian^{21,24,25}, Argentine²⁶, and Portuguese honey²⁷.

The mean acidity values obtained were $33.85 \text{ mEq kg}^{-1}$ for eucalyptus honey, $27.79 \text{ mEq kg}^{-1}$ for wild honey, and $21.72 \text{ mEq kg}^{-1}$ for orange honey; these values are near those observed in various Brazilian honey samples^{21,24} and higher than the mean obtained by Costa *et al.*⁷. Twenty-one from 173 honey samples sold in the city of São Paulo, Brazil (36.2%) showed acidity values above the allowed limit⁶, while in the present work these values were 14.81% for eucalyptus honey, 2.34% for wild honey, and 2% for orange honey; these percentages are much lower than the 36.2% submitted by those authors.

The formaldehyde index values found for eucalyptus honey ranged from 5.00 to 12.50 mL kg^{-1} , with an average of 6.91 mL kg^{-1} ; from 4.00 to 20.50 mL kg^{-1} with an average of 9.91 mL kg^{-1} for wild honey; and from 4.70 to 9.50 mL kg^{-1} with an average of 6.91 mL kg^{-1} for orange honey. These values are near those obtained by Temiz²⁸ and Sodr e *et al.*²¹.

The diastatic activity for the 205 honey samples analyzed in the present work ranged from 1.10 to 38.50 (Gothe scale), with an average value of 15.77 for eucalyptus honey; 17.32 for wild honey; and 8.14 for orange honey (Table 1), while in honey samples of the State of São Paulo from different floral sources⁷ values ranged from 7.80 to 19.00 (Gothe scale); in honey from Portugal, values ranged from 13.00 to 51.10 (Gothe scale)²⁷, and in honey from the northern coastal region of the State of Bahia values ranged from 16.66 to 62.81 (Gothe scale), with a mean value of 34.11²¹. Because the limit established by Brazilian law¹² for diastatic activity is at least 8, we have that 1 eucalyptus honey sample (3.70%), 4 wild honey samples (2.25%), and 16 orange honey samples (32.00%) did not meet the specifications.

The average amounts of hydroxymethylfurfural (HMF) found in the honey samples analyzed were 17.46 mg kg^{-1} for eucalyptus honey, 15.14 mg kg^{-1} for wild honey, and 54.06 mg kg^{-1} for orange honey (Table 1) and the limit established by Brazilian law¹² is 60 mg kg^{-1} ; thus, one eucalyptus honey sample (3.70%), one wild honey sample (0.78%), and 14 orange honey samples (28%) did not meet these specifications. In honey samples from the northern coastal region of Bahia²¹ the values ranged from 1.50 to $136.00 \text{ mg kg}^{-1}$, with an average of 24.33 mg kg^{-1} . Costa *et al.*⁷ and Dayrell and Vital²⁹ verified that the majority of Brazilian samples showed adequate HMF contents, indicating the use of good practices by beekeepers, with values ranging from 1.10 to $248.20 \text{ mg kg}^{-1}$, while for Portuguese honey samples, values from 1.70 to 94.90 mg kg^{-1} were observed³⁰. White Jr.³¹ mentioned that honey samples from subtropical countries may have naturally high HMF values regardless of the fact that the honey was not overheated or adulterated, due to high temperatures; however, it is essential to quantify this component in order to check on product quality, as it is an indicator of heating, storage, and adulteration with inverted sugar.

The reducing sugars, total sugars, and apparent sucrose mean percentage values (Table 1) obtained for eucalyptus, wild, and orange honey samples are near those obtained by other authors in honey samples from Brazil³², and those obtained using honey samples from other countries^{26,27}. The mean values observed for reducing sugars and apparent sucrose are within the limits established by Brazilian law¹², although one wild honey sample

Table 1. Means, standard deviations, and variation intervals for moisture, electric conductivity, protein, ash, pH, acidity, formaldehyde index, diastase index, HMF, sugars, and viscosity in honey samples from different municipalities in the State of São Paulo

Parameter*	Brazilian legislation ¹²	Eucalyptus honey (27 samples)		Wild honey (128 samples)		Orange honey (50 samples)	
		mean ± sd	Variation interval	mean ± sd	Variation interval	mean ± sd	Variation interval
Moisture (%)	Maximum 20	20.84 ± 0.91	19.30 to 22.40	18.81 ± 1.42	16.00 to 23.40	17.82 ± 1.12	15.10 to 21.50
Electric conductivity (μS cm ⁻¹)	-	1,021.48 ± 471.55	331.00 to 2865.00	628.83 ± 377.67	160.71 to 2,200.00	346.93 ± 127.56	212.00 to 1089.70
Proteins (%)	-	0.31 ± 0.07	0.23 to 0.49	0.20 ± 0.11	0.05 to 0.58	0.16 ± 0.05	0.06 to 0.30
Ashes (%)	Maximum 0,60	0.16 ± 0.08	0.07 to 0.32	0.26 ± 0.18	0.02 a 0.92	0.24 ± 0.17	0.10 to 1.22
pH	3,30 a 4,60	3.62 ± 0.43	2.90 to 5.10	3.39 ± 0.48	2.30 to 5.00	3.19 ± 0.37	2.70 to 4.60
Acidity (mEq kg ⁻¹)	Maximum 50	33.85 ± 11.56	12.50 to 55.00	27.79 ± 12.53	14.00 to 75.50	21.72 ± 7.33	14.00 to 57.00
Formaldehyde index (mL kg ⁻¹)	-	6.91 ± 1.51	5.00 to 12.50	9.91 ± 3.58	4.00 to 20.50	6.23 ± 0.93	4.70 to 9.50
Diastase index	Minimum number 8 (Gothe) or 3 if the HMF < 15	15.77 ± 5.60	5.00 to 23.80	17.32 ± 9.01	5.00 to 38.50	8.14 ± 3.77	1.10 to 17.90
HMF (mg kg ⁻¹)	Maximum 60	17.46 ± 38.81	0.30 to 207.20	15.14 ± 5.72	1.00 to 122.00	54.06 ± 64.98	7.90 to 254.70
Total sugar (%)	74.87 ± 3.57	67.80 to 88.30	75.92 ± 3.09	68.20 to 85.00	76.53 ± 2.40	67.80 to 81.60	
Reducing sugars (%)	Minimum 65	72.34 ± 2.46	67.70 to 77.10	72.80 ± 3.59	53.20 to 80.00	74.14 ± 2.51	66.50 to 78.20
Apparent sucrose(%)	Maximum 6	2.40 ± 2.94	0.10 to 15.20	3.01 ± 3.42	0.10 to 27.40	2.26 ± 1.44	0.30 to 6.80
Viscosity (mPa.s)	-	-	-	-	-	1,461.16 ± 874.29	98.00 to 5,090.00

* All parameter means resulted from three replications; sd = standard deviation

showed a reducing sugars value of 53.20%, therefore below the minimum required by law, which is 65%. With regard to sucrose, one eucalyptus honey sample (3.27%), 14 wild honey samples (10.94%), and one orange sample (2.00%) had higher values than the 6% allowed by law; this fact was also observed by Cano *et al.*⁶, that is, 10.3% of honey samples sold in the city of São Paulo, São Paulo, Brazil, contained apparent sucrose values above those allowed by current Brazilian law¹².

It can be seen that there was considerably great variation in the observed values for viscosity in orange honey samples, with an average of 1,461.16 (mPa.s) (25 °C); such average is much smaller than the averages observed in honey samples of different botanical sources from Libya and Egypt (ranging from 49.51 to 472 Pa.s at 20 °C)³³, in *Calluna vulgaris* honey produced in Spain (viscosity variation from 5,198 to 17,325 cP (20.5 °C))³⁴, and honey samples from the states of Minas Gerais and Santa Catarina, in Brazil³². The variation interval from 0.098 to 5.09Pa.s observed in the present paper (25 °C) (Table 1) is near those obtained in Chinese honey of different floral sources at the same temperature³⁵ and those verified in samples from the states of Ceará, Piauí, and Pernambuco, in Brazil (mean viscosity of 1,607 mPa.s.)²¹.

Cluster analysis

The principal components and cluster analysis of the 205 honey samples was conducted for all 13 physicochemical characters shown in Table 1, and the variance estimate results (eigenvalues) obtained are presented in Table 2.

From Table 2 it can be observed that the first five components concentrated 75.39%. Mardia *et al.*¹⁷ stated that, when in a principal components analysis the first two or three components accumulate a relatively high percentage of total variation, in general above 70%, these would explain the variability manifested among evaluated varieties. In the present case, it can be seen that five principal components were required to explain 70% of total variance; thus, considerable variance dispersion can be verified in the material under study; this is why we chose to use Cluster analysis (Figure 1).

Table 2. Variance estimates (eigenvalues) and cumulative percentage of total variance (%), obtained by principal components analysis considering 205 honey samples and 13 physicochemical characters

Principal components	Honey samples	
	Eigenvalues	Cumulative percentage
Y ₁	3.466	26.67
Y ₂	2.285	44.23
Y ₃	1.577	56.36
Y ₄	1.347	66.73
Y ₅	1.126	75.39

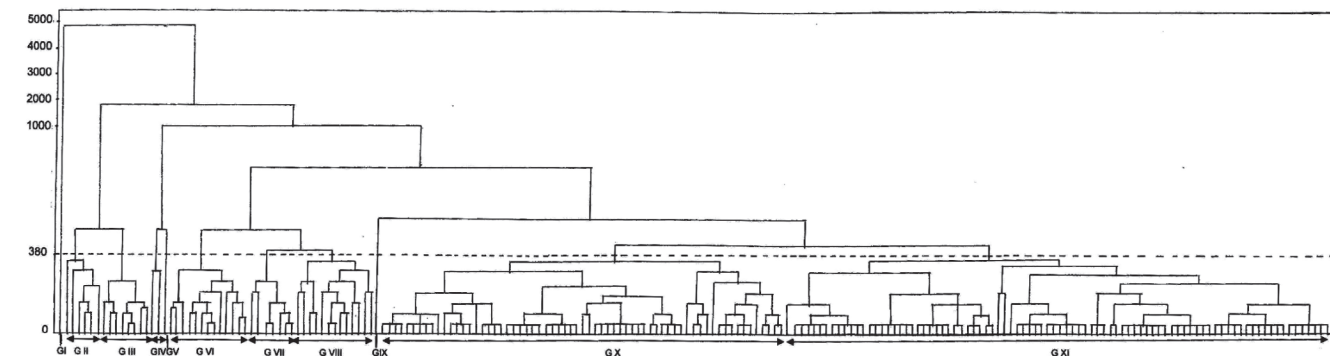
From Figure 1 it can be seen that five groups were formed, which can be identified as follows: 154 samples were clustered in group I; three samples in group III; 14 samples in group IV; and one sample in group V.

In the present work, the optimal cutoff point selected was 380, represented by the dashed line in Figure 1; this point was based on the identification of a plateau in the vertical direction, which means that many groups were formed at the same link distance; this distance is the optimal cutoff point for the phenogram, establishing the number of groups formed.

It can be observed that orange honey samples were all clustered in the first subgroup of group I, except for sample number 125, which, although also belonging in group I, did not fall within the first subgroup (Figure 1). The other subgroups that comprise group I are formed by eucalyptus and wild honey samples, which can be explained by the fact that samples declared as wild come from different floral sources, many of them polyfloral, without dominance of one or another plant species.

In Figure 1, it can also be seen that the other groups formed (II, III, IV, and V) are all composed of wild honey samples (totaling 51 samples, i.e., 40% of all wild honey samples).

From the principal components analysis, it can be verified that the characters that influenced honey sample clustering the most were acidity and electric conductivity on the X axis, and total sugars (AT) and pH on the Y axis.



LEGEND

Groups	Sample number	Floral source	Groups	Sample number	Floral source	Groups	Sample number	Floral source	Groups	Sample number	Floral source	Groups	Sample number	Floral source
GI	166	Orange	GVIII	132	Orange	GX	95	Eucalyptus	GXI	29	Wild	GXI	35	Wild
GII	169	Orange	GVIII	143	Orange	GX	10	Wild	GXI	193	Wild	GXI	13	Wild
GII	167	Orange	GVIII	152	Orange	GX	63	Wild	GXI	180	Wild	GXI	189	Wild
GII	170	Orange	GVIII	151	Orange	GX	62	Wild	GXI	44	Wild	GXI	192	Wild
GII	171	Orange	GVIII	142	Orange	GX	114	Eucalyptus	GXI	48	Wild	GXI	179	Wild
GII	165	Orange	GVIII	134	Orange	GX	78	Wild	GXI	28	Wild	GXI	94	Wild
GII	129	Orange	GVIII	139	Orange	GX	57	Wild	GXI	5	Wild	GXI	18	Wild
GIII	161	Orange	GVIII	131	Orange	GX	197	Wild	GXI	69	Wild	GXI	194	Wild
GIII	128	Orange	GVIII	163	Orange	GX	76	Wild	GXI	92	Wild	GXI	183	Wild
GIII	124	Orange	GVIII	127	Orange	GX	97	Eucalyptus	GXI	68	Wild	GXI	45	Wild
GIII	160	Orange	GIX	126	Orange	GX	115	Eucalyptus	GXI	61	Wild	GXI	91	Wild
GIII	159	Orange	GX	203	Wild	GX	100	Eucalyptus	GXI	79	Wild	GXI	56	Wild
GIII	158	Orange	GX	110	Eucalyptus	GX	31	Wild	GXI	42	Wild	GXI	85	Wild
GIII	123	Orange	GX	101	Eucalyptus	GX	113	Eucalyptus	GXI	15	Wild	GXI	27	Wild
GIII	122	Orange	GX	66	Wild	GX	89	Wild	GXI	11	Wild	GXI	190	Wild
GIV	199	Wild	GX	80	Wild	GX	105	Eucalyptus	GXI	74	Wild	GXI	191	Wild
GIV	174	Wild	GX	196	Wild	GX	70	Wild	GXI	60	Wild	GXI	181	Wild
GV	118	Orange	GX	112	Eucalyptus	GX	14	Wild	GXI	107	Eucalyptus	GXI	65	Wild
GVI	156	Orange	GX	99	Eucalyptus	GX	4	Wild	GXI	64	Wild	GXI	24	Wild
GVI	145	Orange	GX	49	Wild	GX	200	Wild	GXI	84	Wild	GXI	17	Wild
GVI	135	Orange	GX	20	Wild	GX	176	Wild	GXI	53	Wild	GXI	16	Wild
GVI	153	Orange	GX	201	Wild	GX	119	Eucalyptus	GXI	43	Wild	GXI	9	Wild
GVI	148	Orange	GX	173	Wild	GX	98	Eucalyptus	GXI	104	Eucalyptus	GXI	187	Wild
GVI	149	Orange	GX	121	Eucalyptus	GX	175	Wild	GXI	19	Wild	GXI	184	Wild
GVI	154	Orange	GX	108	Eucalyptus	GX	177	Wild	GXI	93	Wild	GXI	182	Wild
GVI	141	Orange	GX	103	Eucalyptus	GX	198	Wild	GXI	12	Wild	GXI	47	Wild
GVI	140	Orange	GX	86	Wild	GX	117	Eucalyptus	GXI	83	Wild	GXI	186	Wild
GVI	138	Orange	GX	205	Wild	GX	172	Wild	GXI	3	Wild	GXI	185	Wild
GVI	146	Orange	GX	96	Eucalyptus	GX	204	Wild	GXI	111	Eucalyptus	GXI	178	Wild
GVI	147	Orange	GX	71	Wild	GX	102	Eucalyptus	GXI	90	Wild	GXI	30	Wild
GVI	130	Orange	GX	6	Wild	GX	33	Wild	GXI	82	Wild	GXI	26	Wild
GVII	162	Orange	GX	88	Wild	GX	120	Eucalyptus	GXI	41	Wild	GXI	34	Wild
GVII	157	Orange	GX	81	Wild	GX	51	Wild	GXI	59	Wild	GXI	40	Wild
GVII	168	Orange	GX	38	Wild	GX	32	Wild	GXI	36	Wild	GXI	7	Wild
GVII	164	Orange	GX	50	Wild	GX	2	Wild	GXI	23	Wild	GXI	54	Wild
GVII	155	Orange	GX	109	Eucalyptus	GXI	125	Orange	GXI	77	Wild	GXI	39	Wild
GVII	150	Orange	GX	37	Wild	GXI	195	Wild	GXI	25	Wild	GXI	55	Wild
GVII	133	Orange	GX	106	Eucalyptus	GXI	202	Wild	GXI	58	Wild	GXI	75	Wild
GVIII	144	Orange	GX	87	Wild	GXI	69	Wild	GXI	22	Wild	GXI	21	Wild
GVIII	137	Orange	GX	72	Wild	GXI	46	Wild	GXI	188	Wild	GXI	8	Wild
GVIII	136	Orange	GX	116	Eucalyptus	GXI	73	Wild	GXI	52	Wild	GXI	1	Wild

Figure 1. UPGMA phenogram and mean Euclidean distances for 205 Apis mellifera honey samples from the State of São Paulo

According to Bogdanov³⁶, electric conductivity is considered a good criterion to indicate honey floral source, because it is influenced by acids and by ash content. Honey samples from different floral sources have different electric conductivity values.

CONCLUSIONS

Because the wild honey samples came from different floral sources, they did not all have the same behavior with regard to physicochemical characters; some were clustered together, while others were near eucalyptus honey.

Floral source crucially interferes with honey characteristics.

The mean values for the parameters analyzed, mentioned in current laws, are within the established limits, except for moisture in eucalyptus honey samples, which showed a mean a little above the maximum prescribed in the regulations.

Among the 205 honey samples from the State of São Paulo that were analyzed, orange honey samples form a distinct subgroup with regard to physicochemical characters.

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