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Determination of non-volatile compounds of different botanical origin Brazilian honeys

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Abstract

The levels of water, HMF, free proline, total acidity, diastase activity, fructose and glucose in 74 different floral type honeys from four Brazilian regions are reported. The majority of the samples showed adequate water and HMF contents indicating the use of good practices by beekeepers in Brazil. Large variation in the contents of proline (389–520 mg/kg) was found in the honeys from the four regions studied. These findings are presumably due to the more intensive labor of the bees on the collected nectar by adding gland secretions. The high amount of diastase (40–120°G) found in some honeys from the northeast region could be due to the visit of the bees to *Manhiot* sp. (cassava) flour factories to collect starch-rich food, consequently increasing the diastase activity in the honey. Xerotolerant yeasts may be responsible for higher total acidity and ratios below 1 for fructose/glucose found in some samples. The majority of the analysed honeys (82%) were within the limits of the European Codex Honey Standards and Brazilian legal regulations.

1. Introduction

Honey, a viscous and aromatic product appreciated since ancient Grecian times, is prepared by bees mainly from nectar of flowers or honeydew (Dustmann, 1993). The characteristics of texture, appearance, flavor and sweetness of honey, as well as its medicinal properties, have attracted thousands of consumers (Dustmann, 1993; Zumlai & Lulat, 1989). Furthermore, a great number of consumers are aware that refined sugar is associated with empty energy and thus they are looking for other more nutritious foods (Passmore & Eastwood, 1986). So, it is anticipated that the world trade of honey will grow consistently in the future.

The annual production of honey in Brazil is about 20,000 tons, which is substantially low considering the great diversity of the flora and climate of the country. As a consequence, honeys are widespread in different Brazilian regions. Despite the variety of honey types found in Brazil, published scientific literature on chemical composition is virtually scarce (Bastos, Dayrell, Sampaio, & Jolk, 1996; Cortopassi-Laurino & Gelli, 1991). Therefore, the purpose of this work was to investigate the principal constituents of the non-volatile fraction of genuine honey samples derived from different plant species encountered in different Brazilian states. The levels of water, total acidity, free proline, diastase activity, HMF, fructose and glucose are reported.

2. Materials and methods

2.1. Samples

Typical *Apis mellifera* honey samples of different floral types from 14 Brazilian states were obtained from apiarists and beekeeper's associations. These Brazilian states are representative of four regions: (1) South (Rio Grande do Sul, Paraná, Santa Catarina), (2) Southeast (Rio de Janeiro, São Paulo, Minas Gerais, Espírito Santo), (3) Northeast (Piauí, Ceará, Pernambuco, Paraíba) and (4) Midwest (Goiás, Mato Grosso, Mato

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Grosso do Sul). All the honeys were collected in 1996 or 1997 and stored in plastic bottles at −18°C under N_2 atmosphere. Before analysis, crystallised honeys, free from extraneous matter, were heated at 30°C and homogenized by gently stirring for 3 min.

Altogether 74 samples were examined: 3 *Citrus* sp., 18 *Eucalyptus* sp., 1 *Croton* sp., 1 *Antigonon leptopus*, 1 *Hovenia dulcis*, 1 *Dombeya* sp., 2 *Anadenanthera* sp., 3 *Piptadenia moniliformis*, 2 *Mimosa caesalpiniaefolia*, 2 *Schinus* sp., 1 *Mimosa verrucosa*, 1 *Schinus* sp. + *Hovenia dulcis*, 1 Palmae + Vernonia sp., 2 *Anadenanthera* sp. + *Citrus* sp., 1 *Vernonia* sp. + *Borreria verticillata*, 1 honeydew, 12 extra-floral and 21 multifloral honeys. The term extra-floral relates to honeys produced by the bees from other raw material sources (e.g. plant exudate, fruits etc) excluding excretions of plant-suckling insects. All samples were analysed in duplicate.

3. Methods

Diastase activity was measured photometrically in a Titertek Multiskan plus instrument (Eflab, Finland) according to the AOAC method 31.126 (AOAC). Results were calculated (as Gothe's degrees, °G) as ml of 1% starch hydrolysed by an enzyme in 1 g honey in 1 h. Photometrical analysis of free proline was followed the AOAC method 31.126 (AOAC). Results were expressed as mg proline/kg. Water in honeys was determined by refractometry following the AOAC method 31.119 (AOAC) using an Abbé Refractometer (Carl Zeiss, Germany) and obtaining correspondent percent water from the Chataway table. Total acidity was analysed by the titrimetric method using 0.1 N NaOH in accordance with the AOAC method 16.023 (AOAC). Results were expressed as milliequiv. $_{NaOH}$/kg. The determination of HMF was based on the method described previously (Lee, Rouseff, & Nagy, 1986). A 20% honey solution was clarified adding 0.5 ml of each Carrez I and II solutions and the mixture was centrifuged at 2000×***g*** for 5 min. One ml of clear supernatant was passed through the conditioned Sep-PAk C-18 cartridge (cartridge from Waters, USA, was conditioned with 2 ml of methanol followed by 5 ml of distilled water before use). After washing the cartridge with 0.5 ml of hexane, the HMF was eluted twice with 3 ml of ethyl acetate and dried with anhydrous sodium sulphate. Sugars were analysed by HPLC based on the previous work (Trugo, Farah, & Cabral, 1995). A 0.5% honey solution was prepared and diluted with pure acetonitrile in the proportion 1:1 (v/v), and the mixture centrifuged for 1 min in a Eppendorff centrifuge (Incibrás, Brazil). A HPLC system with a pump and a UV monitor (Knauer, Germany), a injection valve of 20 µl loop (Rheodyne, USA) and a refractive index (RI) detector (Waters, USA) were used for chromatography. Analyses were carried out by injecting 20 µl of sample or standards of HMF or fructose or glucose (Sigma, USA). For HMF analysis, the column was a Spherisorb ODS II C-18 (250 × 4 mm, i.d., Knauer, Germany) with acetonitrile:water (15:85, v/v at 1.3 ml/min) as mobile phase and detection of the effluent at 280 nm. For sugar analysis, a Lichrosphere-5-NH_2 (250 × 4 mm, i.d., Merck, Brazil) column was used with acetonitrile:water (85:15, v/v) at 1.5 ml/min and the effluent monitored with a RI detector. Quantification was achieved by peak height comparison with standards of HMF (keep in the dark) or fructose and glucose purchased from Sigma (USA). Results were expressed as mg HMF/kg or g% for each sugar.

Determination of floral type was based on pollen analyses described previously (Louveaux, Maurizio, & Varwohl, 1978). Statistical analysis of data was performed by one way analysis of variance (Tukey's test) using a statistical graphics system (STSC, 1986).

4. Results

Table 1 shows, for each honey sample, the floral type, diastase activity, the ratio fructose/glucose and contents of free proline, HMF, fructose, glucose, water and total acidity.

Analysis of the botanical origin showed that 16.2% samples were of the extra-floral type. Besides the most common unifloral honeys (e.g. *Eucalyptus* sp.), thirteen unifloral and five bifloral honeys, characteristic of Brazil, were identified (e.g. *Piptadenia moniliformis, Vernonia* sp. + *Borreria verticillata*). Descriptions of pollen grain morphology in Brazilian honeys have been previously published (Barth, 1989, 1990).

The mean values of 17.38–19.15% for water found in honeys of different Brazilian regions were lower than the average of 19.20% previously reported (Cortopassi-Laurino & Gelli, 1991). Nevertheless, all samples from the state of Paraíba had a water content in the range of 21–23%.

The free proline content of the analysed samples showed a wide variation of 63–1245 mg/kg. Brazilian *Eucalyptus* honeys had a mean value of 344 mg/kg for proline which was substantially higher in comparison with that (225 mg/kg) from other countries (Bouseta, Scheirman, & Collin, 1996; Davies, 1975). Between Brazilian regions, the highest mean value for proline (519 mg/kg) was found in the northeast.

The mean values for total acidity found in different Brazilian regions lay in the range of 8.20–50.0 meq/kg which agreed with those from other geographical origins (Kaushik, Joshi, & Gupta, 1993; Perez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1994). Between Brazilian regions, the highest total acidity mean values were found in the northeast (29.4%) and midwest (29.1%). Only three samples had a total acidity content above 40 meq/kg.

Table 1
Water, proline, total acidity, diastase activity (Goethe scale), HMF, fructose and glucose contents and ratio fructose/glucose of honeys of different botanical origin

Region/state[a]/year[b]	Sample	Water (%)	Proline (mg/kg)	Total acidity (millieq./kg)	Diastase (°G)	HMF[c] (mg/kg)	Fructose (g%)	Glucose (g%)	Fru/Glu[d]
Southeast									
RJ/1996	*Eucalyptus* sp.	18.20	227	26.0	10.0	9.80	36.7	32.4	1.14
RJ/1996	*Palmae* + *Vernonia* sp.	19.70	239	34.2	7.50	9.81	39.8	32.4	1.23
RJ/1996	Multifloral	19.80	325	33.7	13.2	30.00	39.6	34.0	1.17
RJ/1997	Extra-floral	17.90	410	17.4	20.0	5.76	44.4	31.7	1.40
RJ/1997	Extra-floral	19.40	341	14.2	19.5	4.44	43.5	31.1	1.40
RJ/1996	Extra-floral	17.80	325	24.3	7.70	14.40	37.8	34.0	1.11
SP/1997	*Eucalyptus* sp.	17.30	301	21.3	19.0	3.29	42.5	24.0	1.77
SP/1997	*Eucalyptus* sp.	17.70	328	28.3	15.0	6.58	45.2	31.8	1.42
SP/1997	*Eucalyptus* sp.	14.70	310	20.1	10.4	2.10	47.2	32.6	1.45
SP/1997	*Eucalyptus* sp.	18.30	154	16.8	18.2	nd	44.0	36.3	1.21
SP/1996	*Eucalyptus* sp.	18.10	134	16.7	9.80	16.40	40.3	33.1	1.22
SP/1997	*Eucalyptus* sp.	19.10	190	22.6	12.2	1.82	45.7	37.0	1.24
SP/1997	*Eucalyptus* sp.	19.50	340	37.9	13.3	9.09	46.0	34.9	1.32
SP/1997	*Citrus* sp.	16.70	133	9.0	7.80	4.80	46.1	29.4	1.57
SP/1996	*Citrus* sp.	17.79	804	13.7	8.45	1.98	43.0	34.7	1.24
SP/1997	*Citrus* sp.	17.50	233	15.0	8.75	3.60	43.7	31.4	1.39
SP/1997	*Piptadenia moniliformis*	16.90	140	19.8	10.2	2.72	43.0	33.7	1.28
MG/1997	*Eucalyptus* sp.	18.50	347	19.1	21.4	3.60	44.9	30.1	1.49
MG/1997	*Eucalyptus* sp.	18.90	380	19.8	16.2	3.10	43.3	33.3	1.30
MG/1996	*Eucalyptus* sp.	19.40	1245	17.1	9.65	9.26	40.0	32.0	1.25
MG/1996	*Eucalyptus* sp.	16.40	534	24.9	7.80	1.73	39.0	33.3	1.17
MG/1996	*Antigonon. leptopus*	18.00	773	21.8	10.0	1.75	42.0	38.7	1.09
MG/1996	Multifloral	17.00	840	18.9	8.20	5.67	40.0	34.1	1.17
MG/1997	Extra-floral	18.30	283	11.6	10.9	25.0	44.7	30.9	1.44
MG/1996	Extra-floral	19.60	386	44.5	8.30	8.10	39.4	35.5	1.11
MG/1996	Extra-floral	18.20	282	19.2	7.50	4.60	38.4	32.4	1.18
ES/1996	*Croton* sp.	18.80	509	50.0	16.7	6.52	41.0	33.0	1.24
Mean value		18.10	389	22.9	12.1	7.25	42.3	32.9	1.30
Standard deviation		1.15	2.52	9.56	4.38	6.91	2.81	2.67	0.16
South									
RS/1997	*Eucalyptus* sp.	19.30	162	25.8	10.5	3.97	40.8	29.9	1.40
RS/1997	*Eucalyptus* sp.	18.30	271	20.9	9.20	5.73	40.4	33.6	1.20
RS/1997	*Eucalyptus* sp.	18.50	408	24.3	12.5	6.21	39.7	33.2	1.19
RS/1997	*Eucalyptus* sp.	18.10	194	13.0	8.10	nd	40.1	37.9	1.06
RS/1997	*Schinus* sp.	17.50	840	25.3	20.0	4.29	43.7	30.7	1.43
RS/1997	*Anadenanthera* sp.	17.90	314	18.3	13.7	13.71	42.9	35.6	1.21
RS/1997	Multifloral	17.70	664	17.8	18.8	nd	43.3	32.9	1.33
PR/1996	Multifloral	19.80	423	35.4	22.2	5.18	43.2	35.5	1.22
PR/1996	Multifloral	19.20	178	33.7	13.4	4.25	40.8	32.4	1.26
PR/1996	*Honeydew*	19.40	172	36.4	18.5	21.27	40.1	30.2	1.33
PR/1996	Multifloral	20.19	699	27.2	21.5	9.27	39.6	33.4	1.18
PR/1996	Extra-floral	18.80	602	28.7	15.7	5.73	40.1	31.0	1.29
SC/1997	Multifloral	15.80	414	16.3	13.3	4.20	45.9	33.3	1.38
SC/1997	Multifloral	16.80	468	17.9	5.90	17.81	44.8	32.5	1.38
Mean value		18.38	415	24.3	14.5	7.26	41.8	33.0	1.28
Standard deviation		1.16	2.11	7.14	4.93	6.02	2.02	2.15	0.10
Northeast									
PE/1997	Extra-floral	17.80	971	29.6	100	1.82	40.8	32.7	1.25
PE/1997	Extra-floral	17.10	530	27.2	40.0	1.82	41.5	33.5	1.24
PB/1997	*Mimosa caesalpiniaefolia*	21.30	346	29.2	14.7	5.68	40.6	34.7	1.17
PB/1997	*Mimosa caesalpiniaefolia*	21.30	368	38.5	23.8	6.77	42.0	34.3	1.23
PB/1997	*Piptadenia moniliformis*	21.50	260	26.1	13.3	9.00	42.5	36.3	1.17
PB/1997	Multifloral	21.00	110	22.7	16.7	23.10	42.3	30.4	1.39
PB/1996	Multifloral	23.00	63	36.6	13.7	26.50	29.6	34.7	0.85
PB/1996	Multifloral	21.00	150	37.0	7.80	18.50	27.8	35.8	0.78
CE/1997	Multifloral	17.80	1099	48.9	66.7	3.60	41.1	33.5	1.23

Continued

Table 1 (*continued*)

Region/state[a]/year[b]	Sample	Water (%)	Proline (mg/kg)	Total acidity (millieq./kg)	Diastase (°G)	HMF[c] (mg/kg)	Fructose (g%)	Glucose (g%)	Fru/Glu[d]
CE/1997	Multifloral	17.00	476	26.6	46.9	2.72	42.7	33.9	1.26
CE/1997	*Hovenia dulcis*	17.10	380	14.6	33.3	3.30	45.3	33.1	1.37
PI/1997	*Vernonia* sp. + *B. orreria verticillata*	19.40	705	34.3	120.0	13.3	44.7	33.0	1.35
PI/1997	*Mimosa verrucosa*	16.60	967	34.5	17.1	6.98	44.2	35.8	1.24
PI/1997	*Dombeya* sp.	18.10	237	17.7	12.5	4.85	41.3	37.1	1.11
PI/1997	Multifloral	17.20	744	24.3	22.2	4.53	44.9	36.7	1.22
PI/1997	*Piptadenia moniliformis*	19.40	609	28.3	21.5	4.65	38.3	33.6	1.14
PI/1997	Multifloral	19.30	682	28.3	18.8	4.52	43.0	31.7	1.36
PI/1997	Multifloral	19.10	560	27.4	16.7	3.20	45.6	35.6	1.28
PI/1997	Extra-floral	19.00	610	27.8	13.8	6.15	43.7	32.6	1.34
Mean value		19.37	519	29.4	32.6	7.94	41.2	34.1	1.21
Standard deviation		1.94	2.91	7.54	30.2	7.01	4.64	1.71	0.16
Midwest									
GO/1997	*Schinus* sp.	16.10	155	8.22	6.65	8.75	41.6	33.7	1.24
GO/1997	*Anadenanthera* sp. + *Citrus* sp.	17.90	179	16.2	7.0	38.00	42.2	31.8	1.33
GO/1997	*Anadenanthera* sp.	17.30	809	30.6	10.0	27.60	39.6	31.9	1.24
MT/1997	Multifloral	18.40	519	41.9	13.0	14.50	42.3	33.4	1.27
MT/1997	Multifloral	19.10	476	38.3	13.0	8.30	41.3	32.9	1.26
MT/1997	Multifloral	18.70	473	38.4	11.6	8.30	39.8	31.8	1.25
MT/1997	Extra-floral	18.80	681	32.0	13.0	10.0	41.5	32.7	1.27
MT/1997	Extra-floral	18.90	419	30.9	18.8	4.60	42.5	32.2	1.32
MS/1996	Multifloral	15.70	595	36.4	17.2	26.50	39.3	29.9	1.31
MS/1996	*Anadenanthera* sp. + *Citrus* sp.	15.60	191	17.3	7.10	16.2	43.2	32.2	1.34
MS/1997	*Eucalyptus* sp.	16.60	255	28.7	6.9	7.85	41.3	35.2	1.17
MS/1996	Multifloral	16.80	344	33.1	8.2	1.80	40.3	33.6	1.20
MS/1997	*Eucalyptus* sp.	17.20	417	28.4	17.4	3.80	40.3	30.2	1.33
MS/1996	*Schinus* sp. + *Hovenia dulcis*	16.20	377	27.4	7.2	21.7	40.0	30.2	1.32
Mean value		17.38	421	29.1	11.2	14.1	41.1	32.3	1.27
Standard deviation		1.20	1.86	9.10	4.15	10.2	1.19	1.45	0.05

[a] States: RJ, Rio de Janeiro; SP, São Paulo; MG, Minas Gerais; ES, Espírito Santo; RS, Rio Grande do Sul; PR, Paraná; SC, Santa Catarina; PE, Pernambuco; PB, Paraíba; CE, Ceará; PI, Piauí; GO, Goiás; MT, Mato Grosso; MS, Mato Grosso do Sul.
[b] Year: the year that sample was collected.
[c] HMF: hydroxymethylfurfural.
[d] Fru/glu: ratio fructose/glucose.

The mean values for diastase activity found in different Brazilian regions lay in the range of 11–30°G which were close to those from other continents (Huidobro et al., 1995; Schade, Marsh, & Eckert, 1958). Conversely, 26% of samples from the Brazilian northeast region showed very high values of diastase activity (40–120°G). HMF in honeys fell within the range of 1.70–38.0 mg/kg, but only 17.58% samples had a HMF value above 15 mg/kg.

The fructose and glucose contents of the analysed samples were in the range of 22.8–47.2 and 24.0–38.7%, respectively which were similar to those from other geographical origins (Donner, 1977; Perez-Arquillué et al., 1994). Between Brazilian regions, the largest fructose content was found in *Eucalyptus* honeys from the southeast. Conversely, it was reported that *Eucalyptus* honeys from the Zona da Mata in the state of Minas Gerais (southeast region) had a low fructose level (Bastos et al., 1996). Only two samples from the state of Paraíba had the ratio fructose/glucose below 1 which was due to the low fructose value (< 30%).

5. Discussion

The great number of *Eucalyptus* honey samples found in the southeast and south regions appears to reflect the substitution of *Eucalyptus* culture for primary forest. Depending on the climatic conditions, when flower nectar is scarce, bees may use other food sources producing extra-floral honeys.

Our lower mean value for water in comparison with that previously reported (Cortopassi-Laurino & Gelli, 1991) was in part due to the current use of more adequated practices for honey management by beekeepers in Brazil. The water content is an important criterion for evaluating the grade of ripeness of the honey and its shelf-life (Dustmann, 1993). Between Brazilian regions, the northeast showed a mean value for water significantly ($p < 0.05$) higher than those from southeast and midwest. However, this result was due to the highest water content (> 21%) found in the samples from the state of Paraíba. The higher water value found was attributed to the earlier extraction of honey from hives. In general, a high amount of water causes the honey to ferment, to spoil and to lose flavor, with ensuing honey-quality loss.

Besides the water, the content of proline is also used as an appropriate parameter for identifying whether honeys are ripe or not. In accordance with previous work (Von Der Ohe, Dustmann, & Von Der Ohe, 1991), there should be present at least 200 mg proline/kg of honey. The majority of Brazilian honeys (80%) had an amount of proline above 200 mg/kg. In addition, a large average content of 389–520 mg/kg for proline was found in the four Brazilian regions studied. These findings were presumably due to the more intensive labor of the Brazilian bees on the collected nectar by adding gland secretions.

The mean values for total acidity found in the Brazilian northeast and midwest regions were significantly ($p < 0.05$) higher than that from southeast region. The occurrence of hot climatic conditions in the northeast and midwest regions favors the rapid growth of xerotolerant yeasts (eg *Saccharomyces rouxii*) that produce organic acids as secondary metabolites. These micro-organisms, capable of growth at low water activity values (< 0.85) or high solute concentrations, are normally found in honeys (Tilbury, 1980). Thus, we suggest that xerotolerant yeasts were responsible for raising the average total acidity found in the Brazilian northeast and midwest regions.

The mean value for diastase activity found in the Brazilian northeast region was significantly ($p < 0.05$) higher than those from the southeast, south and midwest regions. Furthermore, the high diastase activity ($> 40°G$) found in some samples from the northeast region was surprising. The apiaries were situated near to cassava flour factories. This tropical plant is rich in starchy roots. Thus, the foraging honey bees would visit these factories to collect the wet cassava mass as one of the raw material sources which would increase the diastase activity of the end-product, honey.

The mean value for HMF was higher in the Brazilian midwest region when compared with those from southeast, south and northeast regions. Nevertheless, all samples were classified as fresh pure honeys when HMF value alone was used as a criterion to evaluate the quality of the honeys. As previously reported (White, 1994), the content of HMF (maximum 40 mg/kg) alone provides all the information needed to estimate the total heat exposure of any honey. In contrast to this, combination of HMF content with diastase activity is widely used in evaluating the freshness of honey (Dustmann, Praagh, & Van Bote, 1985; Schade et al., 1958). The application of the last standard of judgement showed that 8% of samples had HMF contents above 15 mg/kg and diastase values of less than 8°G which were out of the limit recommended by European and Brazilian legislations.

In relation to the mean value for fructose, glucose and ratio fructose/glucose, there were no significant differences ($p < 0.05$) between Brazilian regions. However, *Eucalyptus* honeys from the state of São Paulo had a mean value for fructose (44.2%) considerably higher than those from other Brazilian states (40.2–41.3%) and than those produced in Spain (37.0–39.2%) (Mateo & Bosch-Reig, 1997). Consequently, we suggest that *Eucalyptus* honeys from the state of São Paulo may be typified on the basis of the fructose content. Interestingly, only two samples from the state of Paraíba that were collected in 1996 had a ratio fructose/glucose below 1. As stated by Auerbach and Bodlander (1976), honey samples with a ratio fructose/glucose of less than 1 should be classified as adulterated honeys. On the other hand, a peculiar metabolic activity of xerotolerant yeasts is that, when they are grown on foods containing some simple sugars as a carbon source, the fructose is preferentially assimilated; this is termed fructophily (Tilbury, 1980). Since xerotolerant yeasts grow commonly in honeys from the Brazilian northeast region, we suggest that micro-organisms were responsible for decreasing the ratio fructose/glucose in two honeys from the state of Paraíba. Therefore, a ratio fructose/glucose below 1 originates either by adulteration with burnt commercial sugar or by growth of xerotolerant yeasts. The majority of Brazilian honeys (82%) were within the limits of the European Codex Honey Standards and Brazilian legal regulations that set current maximum levels of 20–21% water, 40 meq/kg total acidity, 40 mg/kg HMF and a minimum of 8°G for diastase activity. Low diastase activity (minimum of 3°G) is permissible as long as HMF content does not exceed 15 mg/kg. Furthermore, the sum of fructose plus glucose showed that 97% analysed honeys were within the limit (> 60 g/100 g) proposal for future Europe Union and Codex Alimentarius Standards (Bogdanov, Martin, & Lullmann, 1997).

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