

Analytical, Nutritional and Clinical Methods Section

Determination of oligosaccharides in Brazilian honeys of different botanical origin

J.M. Da Costa Leite^a, L.C. Trugo^b, L.S.M. Costa^a, L.M.C. Quinteiro^c,
O.M. Barth^d, V.M.L. Dutra^d, C.A.B. De Maria^{a,*}

^aInstituto Biomédico, UNI-RIO, Caju, Rua General Sampaio 71 apt. 911 CEP 20931 350 Rio de Janeiro, Brazil

^bInstituto de Química, UFRJ, Brazil

^cInstituto de Ciências Exatas, UFRRJ, Brazil

^dInstituto Oswaldo Cruz, FIOCRUZ, Brazil

Received 6 August 1999; received in revised form 12 November 1999; accepted 12 November 1999

Abstract

The levels of 10 oligosaccharides in 70 genuine Brazilian honeys of different floral types are reported. The contents of sucrose and isomaltose were broad, ranging from mean values of 0.07–0.77 and 0.18–0.71%, respectively. The mean values for maltose were in the range 1.58 to 3.77%. The level of turanose (0.78–2.03%) was similar to that of nigerose (1.11–2.81%). Low amounts of melibiose (0.05–0.15%) and panose (0.03–0.08%) were found in Brazilian honeys. Maltotriose, melezitose and raffinose, were present with mean values of 0.24–1.03, 0.21–0.37 and 0.10–0.25%, respectively. Between Brazilian states, honeys from São Paulo had mean values for melibiose significantly ($P < 0.05$) lower than those from the Minas Gerais and Rio de Janeiro. The mean values for maltose and nigerose found in the Mato Grosso do Sul and Goiás states were significantly higher than from the Mato Grosso state. Honey samples from the Paraná state showed a mean value for maltotriose significantly higher than that from the Rio Grande do Sul state. The contents of maltose, nigerose, turanose and maltotriose proved to be useful for the differentiation of honey samples from different geographical regions and also may be valuable for testing the authenticity of Brazilian honeys. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Honey is produced by bees mainly from nectar of flowers or honeydew. Surveys of floral honey composition have established that fructose and glucose are the major carbohydrates, ranging from 65–80% of the total soluble solids (Costa et al., 1999; Donner, 1977; Siddiqui, 1970). Besides these sugars, other minor carbohydrates, chiefly di- and trisaccharides containing glucose and fructose residues, have been identified (Low & Sporns, 1988; Siddiqui & Furgala, 1967, 1968; Swallow & Low, 1990). Siddiqui and Furgala (1967, 1968), after separation and identification of sugars by paper chromatography, estimated the levels of nine disaccharides (e.g. maltose and turanose) and eight trisaccharides (e.g. maltotriose and panose). The content of minor carbohydrates in floral honey has been more accurately determined by

high performance liquid chromatography (HPLC) (Földházi, 1994; Swallow & Low, 1990) or gas chromatography (GC) (Low & Sporns, 1988; Mateo & Bosch-Reig, 1997, 1998). Swallow and Low, using a HPLC method with a pulsed amperometric detector, measured 20 sugars including rare oligosaccharides (e.g. isopanose and laminaritriose). In addition to the floral honey oligosaccharides only composed of glucose and fructose, some workers detected small amounts of raffinose which is formed of galactose, glucose and fructose moieties (Mateo & Bosch-Reig, 1997; White, Meloy, Probst & Huser, 1986; Zürcher, Maurizio & Hadorn, 1975).

Some studies have been carried out in order to investigate the origin of the honey oligosaccharides. Maurizio (1975a) and Percival (1961), studying the carbohydrates of nectar of the majority of plant families visited by honeybees, noted that variable amounts of sucrose, glucose and fructose were present therein. Other sugars, such as raffinose and melezitose, were mainly found in honeydew, a sweet liquid secreted by

* Corresponding author. Tel.: +55-21509-7251.

E-mail address: cabm@acd.ufirj.br (C.A.B. De Maria)

some species of plant-sucking insects, which is gathered by bees during periods of low nectar availability (Lombard, Buffa, Manino & Patetta, 1984; Maurizio, 1975b). However, the large variety of honey oligosaccharides has been attributed to the ability of the honeybee α -D-glucosidase to catalyse the transference of α -D-glucopyranosyl groups from sucrose to an acceptor carbohydrate (Donner, 1977; Siddiqui, 1970; White & Maher, 1953). Only a little trans- α -D-fructosylating activity was detected in a honey enzymatic preparation (White & Maher, 1953), but there is at present no direct evidence for the presence of an α -D-fructosidase in honey. Doner (1977) and Siddiqui (1970) reviewing the origin of honey oligosaccharides, demonstrated the occurrence of both α -D-glucopyranosyl- and α -D-fructofuranosyl-transferring enzymes in osmophilic yeasts. These micro-organisms become active with an increase in the moisture content of the product and thus could influence the carbohydrate profile of honey (Tilbury, 1980).

Low and Sporns (1988) and Mateo and Bosch-Reig (1997, 1998) have emphasized that the complex mixture of oligosaccharides in honey may be useful in determining the floral type. Mateo and Bosch-Reig (1997) attempted to characterize seven Spanish unifloral honey types by means of GC profiles of carbohydrates. They concluded that the ratio glucose/water and contents of fructose, glucose, sucrose and maltose, selected by discriminant analysis, provided an overall percentage of correct classifications of 71.6%.

There is a vast number of floral species characteristic of Brazil visited by the honeybees. As a result, the potential for the production of Brazilian honeys with peculiar sensorial properties is substantial. However, reports of the chemical composition of Brazilian honeys are scarce (Bastos, Dayrell, Sampaio & Jolk, 1996; Cortopassi-Laurino & Gelli, 1991). In a recent study, we investigated the levels of water, total acidity, free proline, diastase activity, hydroxymethylfurfural, fructose and glucose of authentic honeys from 14 Brazilian states (Costa et al., 1999). In the present work, we analyse the content of major oligosaccharides of these same Brazilian honey samples.

2. Materials and methods

2.1. Honeys

Apis mellifera honey samples of different botanical origins were obtained from apiarists and beekeepers' associations. The samples were produced in 14 Brazilian states from south, southeast, northeast and midwest regions and they were harvested between 1996 and 1997. Honeys were stored in plastic bottles at -18°C under N_2 atmosphere. Before analysis, crystallized honeys were defrosted at 30°C and homogenized by gently stirring thoroughly for 3 min. The honey samples used were as

follows: 3 *Citrus* sp., 18 *Eucalyptus* sp., 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 19 multifloral honeys. Extra-floral honeys are prepared by the bees from other raw material sources (e.g. plant exudate, fruits, etc.) except honeydew. All samples were analysed in three triplicates.

2.2. Reagents and standards

Methanol and acetonitrile were of HPLC grade (Fluka, USA). The oligosaccharide standards were purchased from Sigma (USA) and itemized as follows.

2.2.1. Disaccharides

Sucrose (α -D-glucopyranosyl- β -D-fructofuranoside), turanose [*O*- α -D-glucopyranosyl-(1 \rightarrow 3)-D-fructose], maltose [*O*- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose], nigerose [*O*- α -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose], melibiose [*O*- α -D-galactopyranosyl-(1 \rightarrow 6)-D-glucopyranose], isomaltose [*O*- α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose].

2.2.2. Trisaccharides

Maltotriose [*O*- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose], melezitose [*O*- α -glucopyranosyl-(1 \rightarrow 3)- β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranoside], panose [*O*- α -D-glucopyranosyl (1 \rightarrow 6)-*O*- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose], raffinose [*O*- α -D-galactopyranosyl-(1 \rightarrow 6)-*O*- α -D-glucopyranosyl- β -D-fructofuranoside].

2.3. Procedure

The botanical origin was ascertained by pollen analysis in accordance with to the International Commission for Bee Botany (Louveaux, Maurizio & Varwohl, 1978).

The determination of oligosaccharides was based on the method described previously (Trugo, Farah & Cabral, 1995). A 5% (v/v) honey solution was prepared and diluted with acetonitrile in the proportion 1:1 (v/v), and the mixture centrifuged for 1 min in an Eppendorff-type centrifuge (Incibrás, Brazil). Each sugar standard was dissolved in acetonitrile:water (1:1, v/v) at a concentration of 2 mg/ml. Analyses were carried out in an HPLC system with a pump (Knauer, Germany), an injection valve of 20 μl loop (Reodhyne, USA) and a refractive index monitor (Waters, USA). Separation of oligosaccharides was on a Lichrosphere 5-NH₂ (250 \times 4 mm, i.d., Merck, Brazil) column using acetonitrile:water (85:15, v/v) as mobile phase and of flow rate of 1 ml/min.

Each standard was injected separately, and both the standard mixture and a honey sample were spiked with

each individual sugar to confirm the identity of each oligosaccharide. Quantification was obtained by peak height comparison with standard of oligosaccharides. Results were expressed as g% for each sugar. Undetectable levels have been considered as zero for calculation purposes.

Statistical analysis of data was performed by one way analysis of variance (at the 0.05 significance level) using a statistical graphics system (STSC, 1986).

3. Results and discussion

3.1. Botanical origin

By employing the pollen analysis, it was realized that 48 and 27% samples were of the unifloral and multifloral type, respectively (Table 1). The presence of 17% extra-floral honeys reflected climatic conditions in which flower nectar was rare. Extensive studies on botanical origin of Brazilian honeys have been previously described by Barth (1989, 1990).

3.2. Disaccharides

As can be seen from Table 1, we were able to identify and quantify six major disaccharides in 70 genuine honeys. Maltose (mean = 3.05%) was the major oligosaccharide found in Brazilian honeys, which was in agreement with samples from some countries, e.g. Spanish honeys (mean = 3.96%) (Mateo & Bosch-Reig, 1997) and Hungarian honeys (mean = 3.36%) (Földházi, 1994), but not with others, e.g. USA honeys (mean = 7.31%) (Donner, 1977) and Canadian honeys (mean = 1.07%) (Siddiqui & Furgala, 1967). For turanose and nigerose, we found mean values of 0.78–2.03 and 1.11–2.81%, respectively, in contrast to lower values obtained by Siddiqui and Furgala (1967) of 0.17% for turanose and 0.06% for nigerose in Canadian honeys. The mean values for melibiose lay in the range of 0.05–0.15% which were lower than those of other disaccharides analysed. The detection of melibiose in honey samples is in accordance with reports from other authors (Pourtaillier, 1968; Serra, Gómez & Gonell, 1987). The distribution of sucrose and isomaltose was broad, reaching mean values of 0.07–0.77 and 0.18–0.71%, respectively. The reason for the variable levels of sucrose could be that a transglucosylation reaction is initiated by transference of the α -D-glucopyranosyl unit from sucrose to a acceptor molecule. The sucrose content for all the samples fell within the limits of the European Codex Honey Standards (≤ 5 g/100 g for honeys in general; up to 10 g/100 g for *Citrus* honey) and Brazilian legal regulations (≤ 10 g/100 g).

Between Brazilian regions, the mean value for maltose found in the southeast (1.58%) was significantly

($P < 0.05$) lower than those from northeast (3.16%), midwest (3.69%) and south (3.77%). Furthermore, *Eucalyptus* honeys from the southeast region showed a mean value for maltose (0.69%) significantly lower than those from the south region (4.03%). In relation to the nigerose, the highest mean value was encountered in the midwest region (2.81%). Honey samples from the northeast region had a mean value for turanose (0.78%) significantly lower than those from other Brazilian regions (1.73–2.03%). Consequently, between the different disaccharides analysed, maltose, nigerose and turanose proved to be useful for the differentiation of Brazilian honeys from different geographical regions.

With respect to the Brazilian states, honey samples from São Paulo (0.06%) showed a mean value for melibiose significantly ($P < 0.05$) lower than those from Minas gerais (0.21%) and Rio de Janeiro (0.23%). Thus, we suggest that the melibiose content may be a useful parameter to differentiate honeys from the São Paulo state from those from other states of the southeast region. The mean values for maltose and nigerose found in the Mato Grosso do Sul and Goiás states were significantly higher than those from the Mato Grosso state. These findings indicate that honey samples from the state of Mato Grosso could be distinguished those from other states of the midwest region on the basis of the maltose and nigerose levels.

3.3. Trisaccharides

The contents of maltotriose, panose, melezitose and raffinose of the analysed honey samples are shown in Table 1. The mean values for maltotriose ranged from 0.24 to 1.03% which were higher than the 0.02–0.23% maltotriose found in honeys from other geographical regions (Földházi, 1994; Swallow & Low, 1990). The low amount of panose (0.03–0.08%) in Brazilian honeys was similar to the results obtained for Canadian honeys (trace–0.09%) (Low & Sporns, 1988; Swallow & Low, 1990). The mean values for melezitose were in the range of 0.21–0.37% and higher than those reported in other countries (0.03–0.06%) (Földházi, 1994; Mateo & Bosch-Reig, 1997; Swallow & Low, 1990; Zürcher et al., 1975). In general, the presence of melezitose in floral honeys is considered to be a result of contamination with honeydew. For raffinose, we found mean values of 0.10–0.25% that were close to honeys from Spanish origin (0.16–0.34%) (Mateo & Bosch-Reig, 1997), but different from samples from Hungary (0–0.06%) (Földházi, 1994). The origin of raffinose in floral honey is not clear, but White et al. (1986) have reported that raffinose could be a nectar constituent or could arise from honeydew contamination.

The mean value for maltotriose found in the northeast region (0.24%) was significantly ($P < 0.05$) lower than those from south (0.69%), midwest (0.79%) and southeast (1.03%) regions. Between Brazilian states,

Table 1

Maltose, turanose, nigerose, melibiose, sucrose, isomaltose, maltotriose, panose, melezitose and raffinose contents of honeys of different botanical origin (g%)^{a,b}

Region/state ^c /Year ^d	Sample	mal	tur	nig	meb	suc	iso	mtr	pan	mel	raf
<i>Southeast</i>											
RJ/1996	<i>Eucalyptus</i> sp.	1.00	2.43	1.55	0.20	3.00	1.34	3.73	0.03	1.14	0.16
RJ/1996	<i>Palmae</i> + <i>Vernonia</i> sp.	0.67	0	0.90	0.20	0.54	1.31	2.65	0.03	0.70	0.20
RJ/1996	Multifloral	0.56	1.94	0.70	0.23	0.27	1.34	0.59	0.03	0.28	0.16
RJ/1997	Extra-floral	3.40	1.53	0.95	0.20	0.19	0	0.86	0.03	0.28	0.13
RJ/1997	Extra-floral	3.20	1.53	1.11	0.26	0.19	1.38	0.49	0.03	0.28	0.10
RJ/1996	Extra-floral	0.86	2.43	1.90	0.30	0.27	1.76	1.57	0.07	0.56	0.20
SP/1997	<i>Eucalyptus</i> sp.	0.82	2.27	1.55	0.03	0.67	1.22	1.62	0.02	0.42	0.10
SP/1997	<i>Eucalyptus</i> sp.	0.77	1.78	1.12	0.07	0.27	0	0.24	0	0.14	0
SP/1997	<i>Eucalyptus</i> sp.	0.52	1.62	1.12	0.03	0.54	0.64	0.13	0	0.07	0
SP/1997	<i>Eucalyptus</i> sp.	2.10	0.94	0.96	0.07	0.10	1.28	0.16	0.06	0.14	0.05
SP/1996	<i>Eucalyptus</i> sp.	3.80	4.65	2.40	0.21	0	0	0.22	0	0.14	0
SP/1997	<i>Eucalyptus</i> sp.	1.80	0.62	0.80	0	0	0	0	0	0	0
SP/1997	<i>Eucalyptus</i> sp.	2.40	1.00	1.04	0.05	0	0	0.32	0.05	0.17	0.10
SP/1997	<i>Citrus</i> sp.	0.90	3.24	1.00	0.07	0.81	1.02	1.43	0.07	0.35	0.23
SP/1996	<i>Citrus</i> sp.	0.21	3.56	0.86	0	8.37	0	1.90	0.03	0.56	0.13
SP/1997	<i>Citrus</i> sp.	0.97	2.43	1.46	0.03	0.67	0.90	1.13	0.03	0.28	0.13
SP/1997	<i>Piptadenia moniliformis</i>	2.00	1.30	0.88	0.05	0.48	0	0.59	0	0.14	0
MG/1997	<i>Eucalyptus</i> sp.	2.60	0.76	0.32	0.21	0.09	0	0.49	0.03	0.14	0.10
MG/1997	<i>Eucalyptus</i> sp.	2.60	1.65	1.27	0.20	0	1.50	0.43	0.07	0.28	0.13
MG/1996	<i>Eucalyptus</i> sp.	0.86	2.75	1.00	0.16	0.40	1.22	0.78	0.03	0.38	0.16
MG/1996	<i>Eucalyptus</i> sp.	1.05	2.43	2.06	0.13	1.35	1.47	0.70	0	0.56	0.20
MG/1996	Multifloral	0.90	2.60	1.30	0.11	1.08	1.49	3.78	0.07	1.00	0.20
MG/1997	Extra-floral	4.10	1.77	2.30	0.36	0	0	0.92	0.03	0.49	0.26
MG/1996	Extra-floral	0.73	2.38	1.55	0.20	0	0	0.09	0.02	0.14	0.20
MG/1996	Extra-floral	0.77	2.43	2.84	0.28	0	0	0.92	0.05	0.28	0.23
	Mean value	1.58	2.00	1.33	0.15	0.77	0.71	1.03	0.03	0.36	0.13
	Standard deviation	1.11	0.98	0.58	0.10	1.67	0.66	1.02	0.02	0.27	0.08
<i>South</i>											
RS/1997	<i>Eucalyptus</i> sp.	3.34	0.60	1.26	0.37	0	0	0.17	0.04	0	0.13
RS/1997	<i>Eucalyptus</i> sp.	3.70	0.83	1.60	0.04	0	0	0.04	0	0.05	0
RS/1997	<i>Eucalyptus</i> sp.	4.82	2.05	2.80	0	0	0	0.30	0.05	0.21	0.07
RS/1997	<i>Eucalyptus</i> sp.	4.27	2.14	2.24	0	0	0	0.10	0	0.14	0.02
RS/1997	<i>Schinus</i> sp.	5.64	2.60	3.36	0	0	0	0.13	0	0.10	0.10
RS/1997	<i>Anadenanthera</i> sp.	3.34	1.49	1.68	0	0	0	0.09	0.01	0.12	0.06
RS/1997	Multifloral	3.34	2.60	2.80	0	0	0	0.91	0.07	0.26	0.22
PR/1996	Multifloral	3.20	3.09	1.00	0.18	0.10	1.44	0.20	0	0.23	0.03
PR/1996	Multifloral	3.80	1.77	2.08	0	0.19	0	2.21	0.17	1.61	0.33
PR/1996	Honeydew	3.60	2.12	1.60	0	0.10	0	1.03	0.03	6.56	0.10
PR/1996	Multifloral	3.20	1.53	1.60	0.07	0.10	0	0.49	0.54	0.31	0.16
PR/1996	Extra-floral	3.80	1.77	1.20	0.03	0.10	0	1.18	0.07	0.35	0.13
SC/1997	Multifloral	3.40	1.42	1.52	0.10	0.10	0	1.65	0.07	0.70	0.13
SC/1997	Multifloral	3.33	0.21	1.12	0	0.27	1.57	1.24	0.03	0.49	0.10
	Mean value	3.77	1.73	1.85	0.05	0.07	0.21	0.69	0.08	0.37	0.11
	Standard deviation	0.68	0.77	0.69	0.10	0.08	0.53	0.66	0.13	0.39	0.08
<i>Northeast</i>											
PE/1997	Extra-floral	3.52	0	0.98	0.07	0.13	0	0.08	0.08	0.05	0.08
PE/1997	Extra-floral	3.71	0	0.84	0	0.13	0	0.22	0.02	0.14	0.06
PB/1997	<i>Mimosa caesalpiniaefolia</i>	3.34	0	0.28	0	0.13	0	0.15	0.02	0.23	0.05
PB/1997	<i>Mimosa caesalpiniaefolia</i>	3.71	0	1.12	0	0	0	0.05	0.03	0.09	0.10
PB/1997	<i>Piptadenia moniliformis</i>	3.15	0.93	1.00	0.15	0	0	0.09	0	0.09	0.06
PB/1997	Multifloral	3.34	0.93	1.12	0	0	0	0.04	0	0.05	0.08
CE/1997	Multifloral	2.97	0.74	0.56	0.48	0.27	0	0.07	0.02	0.05	0.06
CE/1997	Multifloral	3.71	0.93	0.98	0.04	0	0	0.22	0.02	0	0.08
CE/1997	<i>Hovenia dulcis</i>	4.08	0	0.84	0	1.62	0	0.64	0.04	1.37	0.19
PI/1997	<i>Vernonia</i> sp. + <i>Borreria verticillata</i>	2.79	1.60	2.96	0.04	0.13	0	0.09	0.10	0.05	0.13
PI/1997	<i>Mimosa verrucosa</i>	1.67	0.70	0.59	0.03	1.35	0.35	0.09	0.02	0.19	0.10
PI/1997	<i>Dombeya</i> sp.	3.00	0.83	0.72	0	0.19	0	0.33	0.03	0.28	0.10
PI/1997	Multifloral	3.16	1.50	1.48	0.16	0.67	0.80	0.65	0.07	0.07	0.20

(continued on next page)

Table 1 (continued)

Region/state ^c /Year ^d	Sample	mal	tur	nig	meb	suc	iso	mtr	pan	mel	raf
PI/1997	<i>Piptadenia moniliformis</i>	3.71	1.62	1.26	0.11	0.27	0	0.43	0	0.21	0
PI/1997	Multifloral	2.79	1.30	1.48	0.06	0.40	0.80	0.49	0.03	0.21	0.20
PI/1997	Multifloral	2.73	1.10	1.18	0.09	0.94	0.56	0.18	0.04	0.16	0.08
PI/1997	Extra-floral	2.42	1.10	1.48	0.08	0.54	0.61	0.32	0.05	0.28	0.13
	Mean value	3.16	0.78	1.11	0.08	0.40	0.18	0.24	0.03	0.21	0.10
	Standard deviation	0.57	0.57	0.57	0.11	0.27	0.30	0.19	0.02	0.30	0.05
<i>Midwest</i>											
GO/1997	<i>Schinus</i> sp.	6.86	2.14	2.80	0	0.27	0	1.39	0.06	0.47	0.26
GO/1997	<i>Anadenanthera</i> sp. + <i>Citrus</i> sp.	4.36	2.19	3.08	0	0.86	0	0.55	0.03	0.66	0.06
GO/1997	<i>Anadenanthera</i> sp.	5.56	2.32	3.22	0	0.27	0	0.70	0.04	0.24	0.16
MT/1997	Multifloral	0.63	1.78	1.38	0.07	0	0.70	0.11	0.06	0.14	0.10
MT/1997	Multifloral	0.57	1.62	1.29	0.23	0.27	1.54	0.43	0.10	0.28	0.11
MT/1997	Multifloral	0.55	1.62	1.38	0.23	0.27	1.31	0.32	0.07	0.24	0.10
MT/1997	Extra-floral	0.63	1.94	1.29	0.16	0.67	1.34	1.08	0.02	0.35	0.13
MT/1997	Extra-floral	0.76	1.78	1.29	0.08	0.67	1.20	1.30	0.07	0.40	1.65
MS/1996	Multifloral	5.39	2.60	5.62	0.23	0.07	0	0.84	0.19	0.35	0.10
MS/1996	<i>Anadenanthera</i> sp. + <i>Citrus</i> sp.	5.95	2.90	3.85	0.13	0.81	1.31	1.24	0.03	0.42	0.23
MS/1997	<i>Eucalyptus</i> sp.	4.56	1.70	2.66	0.08	0.54	0.82	0.09	0.03	0.05	0.06
MS/1996	Multifloral	5.58	1.70	2.96	0.16	0.54	1.28	1.57	0.05	0.42	0.16
MS/1997	<i>Eucalyptus</i> sp.	4.28	1.70	3.85	0.04	0.13	0	0.09	0.08	0.14	0.10
MS/1996	<i>Schinus</i> sp. + <i>Hovenia dulcis</i>	6.04	2.50	4.74	0.16	0.15	0	1.40	0.03	0.49	0.30
	Mean value	3.69	2.03	2.81	0.11	0.40	0.68	0.79	0.06	0.33	0.25
	Standard deviation	2.38	0.40	1.34	0.08	0.27	0.62	0.52	0.04	0.16	0.39

^a The content of each oligosaccharide is expressed as g%.

^b Year, the year that sample was collected.

^c States: RJ — Rio de Janeiro, SP — São Paulo, MG — Minas Gerais, 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.

^d Mal, maltose; tur, turanose; nig, nigerose; meb, melibiose; suc, sucrose; iso, isomaltose; mtr, maltotriose; pan, panose; mel, melezitose; raf, raffinose.

honey samples from Piauí had mean values for maltotriose and raffinose significantly higher than those from Paraíba. Furthermore, the mean value for maltotriose found in the Paraná state (1.02%) was significantly higher than that from the Rio Grande do Sul state (0.25%). From the results shown herein, maltotriose was the trisaccharide that served best to differentiate Brazilian honeys from different geographical areas.

Our preliminary results indicate that the profile of oligosaccharides could be useful for the identification of the Brazilian region in which honey was produced and may also be useful for testing Brazilian honey authenticity. This kind of characterization could be useful for consumers who intend to purchase honeys from states with regulations that are rigorous in relation to agrotoxic utilization as well as the cultivation of transgenic plants.

Acknowledgements

We acknowledge financial support from Fundação de Amparo e Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We thank Dr. Ortrud M. Barth and the biologist Vania M.L. Dutra for the

floral type analysis. We thank the researcher Cristina Lourenzon (“Universidade Federal Rural do Rio de Janeiro, state of the Rio de Janeiro”), the apiarist Antonio L.D. Filho (“Cooperativa Apícola da Micro-região de Picos — CAMPIL, state of the Piauí”), the biochemist Liane Salomé (state of the “Santa Catarina”), Constantino Z. Filho (President of the “Associação Paulista de Apicultores, state of the São Paulo”), the chemist Mary Ribeiro (“Cooperativa de Apicultores do Rio de Janeiro (COAPI-RIO), state of the Rio de Janeiro”) and Waldyr R. Ozório (President of the “COAPI-RIO”) and Sebastião R. Gonzaga (“Associação Paranaense de Apicultores, state of the Paraná”) who provided honey samples. We thank the apiarists: Alessandra Brossmann (Industry Brossmann Ltda., state of the “Goiás”), Thales M. Prado and Lacy D.B. Tietzmann (apiarists, state of the “Rio Grande do Sul”), Juarez Parreira (apiarist, state of the “Mato Grosso do Sul”), José R. Erthal (apiarist, state of the “Mato Grosso”) who also provided honey samples.

References

- Barth, O. M. (1989). *O polén no mel Brasileiro* (pp. 1–180). Gráfica Luxor.

- Barth, O. M. (1990). Pollen in monofloral honeys from Brazil. *Journal of Apicultural Research*, 29, 89–94.
- Bastos, E. M., Dayrell, I. O., Sampaio, I. B., & Jolk, L. (1996). Correlation between pollen spectrum and physico-chemical properties of honeys produced in the Minas Gerais state (Brazil). *Ciência e Tecnologia de Alimentos*, 16, 146–152.
- Cortopassi-Laurino, M., & Gelli, D. S. (1991). Analyse pollinique, propriétés physico-chimiques et action antibactérienne des miels d'abeilles africanisées *Apis mellifera* et de Meliponinés du Brésil. *Apidologie*, 22, 61–73.
- Costa, L. S. M., Albuquerque, M. L. S., Trugo, L. C., Quinteiro, L. M. C., Barth, O. M., Ribeiro, M., & De Maria, C. A. B. (1999). Determination of non-volatile compounds of different botanical origin Brazilian honeys. *Food Chemistry*, 64, 1–6.
- Doner, L. M. (1977). The sugars of honey — a review. *Journal Science and Food Agriculture*, 28, 443–456.
- Földházi, G. (1994). Analysis and quantitation of sugars in honey of different botanical origin using high performance liquid chromatography. *Acta Alimentaria*, 23, 299–311.
- Lombard, A., Buffa, A., Manino, A., & Patetta, A. (1984). Identification of raffinose in honeydew. *Experientia*, 40, 178–180.
- Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of Melissopalynology. *Bee World*, 59, 139–157.
- Low, N. H., & Sporns, P. (1988). Analysis and quantitation of minor di- and trisaccharides in honey, using capillary gas chromatography. *Journal of Food Science*, 53, 558–561.
- Mateo, R., & Bosch-Reig, F. (1997). Sugar profiles of Spanish unifloral honeys. *Food Chemistry*, 60, 33–41.
- Mateo, R., & Bosch-Reig, F. (1998). Classification of spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugar, and pH. *Journal of Agricultural and Food Chemistry*, 46, 393–400.
- Maurizio, A. (1975a). How bees make honey. In E. Crane, *Honey, a comprehensive survey (1st ed)* (pp. 77–105). London, UK: Heinemann (in cooperation with the International Bee Research Association).
- Maurizio, A. (1975b). Microscopy of honey. In E. Crane, *Honey, a comprehensive survey (1st ed)* (pp. 240–257). London, UK: Heinemann (in cooperation with the International Bee Research Association).
- Percival, M. (1961). Types of nectars in angiosperms. *New Phytology*, 60, 235–281.
- Pourtallier, J. (1968). Über die benutzung der gaschromatographie für die bestimmung der zucker in honig. *Zeitschrift Bienenforschung*, 9, 217–221.
- Serra, J., Gómez, A., & Gonell, J. (1987). Composición, propiedades físico-químicas y espectro polínico de algunas mieles monoflorales españolas. *Alimentaria*, 24, 61–84.
- Siddiqui, I. R. (1970). The sugars of honey. *Advances in Carbohydrate Chemistry and Biochemistry*, 25, 285–309.
- Siddiqui, I. R., & Furgala, B. (1967). Isolation and characterization of oligosaccharides from honey. Part I. Disaccharides. *Journal of Apicultural Research*, 6, 139–145.
- Siddiqui, I. R., & Furgala, B. (1968). Isolation and characterization of oligosaccharides from honey. Part II. Trisaccharides. *Journal of Apicultural Research*, 7, 51–59.
- STSC (1986). *Statistical graphics system, user's guide*. Statistical Graphic Corporation; Rockville, MD, USA.
- Swallow, K. W., & Low, N. H. (1990). Analysis and quantitation of the carbohydrates in honey using high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 38, 1828–1832.
- Tilbury, R. H. (1980). Xerotolerant (osmophilic) yeasts. In F. A. Skinner, S. M. Passmore, & R. R. Davenport, *Biology and activities of yeasts (1st ed.)* (pp. 153–179). London, UK: Academic Press.
- Trugo, L. C., Farah, A., & Cabral, L. (1995). Oligosaccharide distribution in Brazilian soya bean cultivars. *Food Chemistry*, 52, 385–387.
- White Jr., J. W., & Maher, J. (1953). Transglucosidation by honey invertase. *Archives of Biochemistry and Biophysics*, 42, 360–367.
- White, J. W., Meloy, R., Probst, J., & Huser, W. (1986). Sugars containing galactose occur in honey. *Journal of Apicultural Research*, 25, 182–185.
- Zürcher, K., Maurizio, A., & Hadorn, H. (1975). Untersuchungen an handelshonigen mit spezieller berücksichtigung des zuckerspektrums. *Apidologie*, 6, 59–90.