Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 1236–1241
Food Chemistry
www.elsevier.com/locate/foodchem

Chemical changes in the non-volatile fraction of Brazilian honeys during storage under tropical conditions

Ricardo F.A. Moreira [a,*], Carlos A.B. De Maria [a], Márcia Pietroluongo [b], Luiz C. Trugo [b,✠]

[a] *Lab. de Avaliação da Composição e Aroma de Produtos Alimentícios (LACAPA)/Lab. de Química de Alimentos (LQA), Disciplina de Bioquímica, Departamento de Ciências Fisiológicas, Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rua Frei Caneca, 94, 4º andar, Cidade Nova, Rio de Janeiro 20211-040, RJ, Brazil*

[b] *Dep. de Bioquímica, Inst. de Química, Universidade Federal do Rio de Janeiro (UFRJ), C.T. bloco A, Lab. 528, Ilha do Fundão, Rio de Janeiro 21949-900, RJ, Brazil*

Received 18 October 2005; received in revised form 23 January 2007; accepted 30 January 2007

Abstract

The chemical transformations of the non-volatile fraction of Brazilian honeys during storage in tropical condition were monitored. Five systems, namely: 1 – fresh samples; 2 and 3 – samples heated for 3 and 6 months at 35–40 °C; 4 and 5 – samples left under similar conditions to systems 2 and 3, but containing sodium metabisulphite (120 ppm), were tested. The major transformations during storage occurred in the free and lactone acidity, diastase activity and 5-hydroxymethylfurfural (5-HMF) content. Storage modified diastase activity and the content of 5-HMF to an extent that the samples could not remain classified as fresh honey. The action of sodium metabisulphite in the control of the 5-HMF formation seemed to be dependent on other honey characteristics, such as its botanical origin. Sodium metabisulphite also influenced the development of the internal esterification reaction that converts gluconic acid into its corresponding lactone.

Keywords: Non-volatile fraction; Brazilian honeys; Chemical transformations; Tropical conditions

1. Introduction

Due to commercial aspects, honey samples often have to be stored for one year before consumption. During storage period, many changes that positively or negatively influence the nutritional and sensory characteristics of these products can occur (Aparna & Rajalakshmi, 1999). Based on the commercial importance of honey, some studies have been carried out in order to monitor the chemical changes of samples during processing and storage (Bath & Singh, 2000; Visser, Allen, & Shaw, 1988). However, in these studies honey samples were submitted to temperatures higher than those normally experienced in nature (>50 °C), even considering the hottest regions of the planet. Sometimes, the problem is the too short exposure period. Fortunately, in the last years some researchers have also paid attention to the changes that occur in honey samples during long storage periods at temperatures close to room temperature (Cherchi, Porcu, Spanedda, & Tuberoso, 1997; Gonzales, Burin, & del Pilar Buera, 1999). However, due to the small number of physicochemical parameters monitored in these studies, the data interpretation has been too complex to reach any meaningful conclusions. The increase of knowledge in this area would be extremely important for developing tropical countries like Brazil. In these countries, the changes that occur in honey samples during storage must be more important due to the high average temperatures. The understanding of these modifications could be relevant to the development of the quality and, consequently, of the competitiveness of Brazilian honey in the national and foreign markets. For some Brazilian regions,

* Corresponding author. Tel./fax: +55 21 25319678.
E-mail addresses: ricfelipe@terra.com.br, ricfelipe1@yahoo.com.br (R.F.A. Moreira).
✠ In memoriam.

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.055

like its northeast, it could be vital, since honey culture is the only income source of some Brazilian wasteland communities.

The aim of the present work was to monitor the changes that occur in the non-volatile fraction of two northeast Brazilian honey, namely cashew (*Anacardium occidentale*) and marmeleiro (Croton species), stored for three and six months at 35–40 °C (simulating tropical conditions).

2. Materials and methods

2.1. Samples

Two samples of cashew honey (Anacardiaceae) and two of marmeleiro honey (Rosaceae) were obtained directly from reliable beekeepers from Ceará and Piauí States, respectively. These samples were harvested between 1997 and 1998 and immediately stored under nitrogen at −18 °C in small glass bottles often used by beekeepers to store this kind of product.

2.2. Storage experiments simulating tropical conditions

Forty grams of each honey sample were heated in glass bottles using an oven. The bottles were kept closed with appropriate metallic covers to guarantee a complete isolation of the samples from the external environment. The samples were kept at temperatures ranging from 35 to 40 °C over three and six months. Sodium metabisulphite was added in some of the bottles in an initial amount of 120 ppm. The study was carried out by employing five different systems: system 1 – fresh samples (cashew honey = C_1 and marmeleiro honey = M_1); systems 2 and 3 – *in natura* samples heated for 3 (C_2 and M_2) and 6 months (C_3 and M_3); systems 4 and 5 – sodium metabisulphite added samples heated for 3 (C_4 and M_4) and 6 months (C_5 and M_5).

2.3. Materials

Methanol and acetonitrile were of HPLC grade (Merck, Rio de Janeiro, Brazil). Standards of fructose (purity grade = 99.95%), glucose (99.5%), sucrose (99.5%), turanose (98%) and maltose (99%) were from Sigma (St. Louis, MO, USA). Proline (>99%) was obtained from Merck (Darmstadt, Germany). The 5-hydroxymethylfurfural (5-HMF) standard (99%) was purchased from Aldrich (Milwaukee, WI, USA). All other reagents were of analytical grade.

2.4. Non-volatile fraction analysis

The floral origin of the honeys was monitored by pollen analysis (Louveaux, Maurizio, & Vorwohl, 1978). Free acidity, lactone acidity, total acidity and pH were measured according to the AOAC (1984) method 31.160. The free acidity is the acidity titratable with sodium hydroxide up to the equivalence point; the lactone acidity corresponds to the combined acidity that is not directly titratable. The acidity of the lactones is obtained by adding an excess of sodium hydroxide to the honey solution and plotting the neutralization curve of the excess sodium hydroxide by a back titration with an acid; the total acidity is the sum of the free acidity and the lactone acidity (Bogdanov, Martin, & Lullmann, 1997). The moisture content of honey samples was determined by refractometry following the AOAC (1984) method 31.119 using an Abbé Refractometer (Carl Zeiss, Pirmasens, Germany) and the Chataway table. Photometrical analysis of free proline was carried out by the AOAC (1984) method 31.126. Results were expressed as mg of proline/kg of honey. Diastase number was measured photometrically in a Titertek Multiskan plus instrument (Eflab, Helsinki, Finland) according to the AOAC (1984) method 31.166. Results were calculated and expressed in Gothe units (°G). The determination of 5-HMF was based on a HPLC method described by Lee, Rouseff, and Nagy (1986). The mono- and oligosaccharides were analyzed by another HPLC method (Da Costa Leite et al., 2000). In both methods, quantification was achieved by peak height or area comparison with standards. In the first case, results were expressed as mg of 5-HMF/kg of honey and, in the second case, as g/100 g of honey for each sugar.

2.5. Statistical analysis of data

The variance analysis (ANOVA) was carried out to check statistical differences between the systems ($p < 0.05$). Triplicate analyses of each one of the samples (two samples of cashew honey and two samples of marmeleiro honey) were always carried out.

3. Results, discussion and conclusions

The results of the analyses carried out on the non-volatile fractions of honey samples are presented in Tables 1 and 2. As already mentioned, the following parameters were monitored in this study: moisture content, pH, free acidity, lactone acidity, total acidity, 5-HMF, proline, fructose, glucose, sucrose, turanose and maltose contents and diastase activity. No significant statistical differences ($p > 0.05$) were observed between the moisture contents of the fresh honey samples and their heated systems during storage for 3 or 6 months, with or without sodium metabisulphite (see Tables 1 and 2). These results showed that the glass bottles normally used in the Brazilian commerce (with metallic thread covers) were efficient for storing this kind of product (honey samples). These bottles adequately isolated the samples from the atmospheric air during the storage period, not allowing that this hygroscopic product gained water, becoming more susceptible to the osmophilic yeasts action. Another parameter that did not show any significant statistical variation ($p > 0.05$), staying in the range of 3.6–4.6 established by the Brazilian legal regulations (SIPA, 1997), was the pH of these systems in both honey samples. A previous work developed with *Helianthus annus*

Table 1
Moisture content, pH, free acidity, lactone acidity, total acidity, proline, 5-HMF and sugars contents and diastase activity of the systems related to the cashew honey in study

Parameters	C1	C2	C3	C4	C5
Moisture content (g/100 g of honey)	17.10 ± 0.40	18.64 ± 0.61	18.84 ± 0.56	17.32 ± 0.29	18.48 ± 0.35
pH	3.80 ± 0.10	3.75 ± 0.11	3.80 ± 0.07	3.88 ± 0.07	3.72 ± 0.10
Free acidity (meq/kg of honey)	$45.50 \pm 1.50^{A,B}$	37.80 ± 2.00^{A}	$34.00 \pm 2.00^{B,C}$	43.58 ± 0.90^{C}	44.32 ± 2.93
Lactone acidity (meq/kg of honey)	6.90 ± 0.10^{A}	$14.38 \pm 0.10^{A,B,C}$	$18.56 \pm 0.13^{A,D,E}$	$7.62 \pm 0.50^{B,D}$	$7.40 \pm 0.35^{C,E}$
Total acidity (meq/kg of honey)	52.40 ± 1.60	52.18 ± 2.10	52.56 ± 2.13	51.20 ± 1.40	51.72 ± 3.28
Proline content (mg/kg of honey)	1402.65 ± 68.20	1395.93 ± 161.98	1393.19 ± 206.71	1498.40 ± 121.48	1407.29 ± 217.66
5-HMF content (mg/kg of honey)	7.4 ± 0.3^{A}	18.87 ± 0.88^{A}	41.50 ± 0.89^{A}	12.70 ± 0.39^{A}	27.12 ± 1.36^{A}
Diastase number (°G)	$12.40 \pm 0.88^{A,B,C}$	$5.76 \pm 0.21^{A,C}$	$3.61 \pm 0.11^{A,B}$	$5.00 \pm 0.21^{B,D}$	$3.30 \pm 0.33^{C,D}$
Fructose content (g/100 g of honey)	33.30 ± 0.50	33.97 ± 0.40	34.50 ± 0.50	34.14 ± 0.13	33.83 ± 0.80
Glucose content (g/100 g of honey)	21.0 ± 0.40	21.50 ± 0.25	21.58 ± 0.47	21.63 ± 0.30	21.34 ± 1.05
Sucrose content (g/100 g of honey)	0.50 ± 0.02	0.44 ± 0.03	0.38 ± 0.04	0.41 ± 0.04	0.51 ± 0.02
Turanose content (g/100 g of honey)	0.84 ± 0.06	0.99 ± 0.13	1.11 ± 0.22	0.72 ± 0.04	0.72 ± 0.08
Maltose content (g/100 g of honey)	1.30 ± 0.20	1.55 ± 0.22	1.84 ± 0.17	1.72 ± 0.09	1.61 ± 0.09

According to the regulations of the Brazilian Legislation (SIPA, 1997) and to the European Codex Honey Standards (Bogdanov et al., 1997), a well processed and ready to be consumed honey must contain the following characteristics: maximum moisture content of 20–21 g/100 g of honey, reducing sugars content $\geqslant$65 g/100 g, sucrose content $\leqslant$5 g/100 g, free acidity $\leqslant$40 milliequivalents/kg, diastase number $\geqslant$8°G, 5-HMF content $\leqslant$40 mg/kg of honey. In a specific line of this table, the values marked with the same letters are statistically different ($p < 0.05$). C_1(cashew honey) = fresh samples; C_2 = *in nature* samples heated during 3 months; C_3 = *in nature* samples heated during 6 months; C_4 = sodium metabisulphite added samples heated during 3 months; C_5 = sodium metabisulphite added samples heated during 6 months. The average values that were shown in this table were obtained from triplicate analysis of each system.

Table 2
Moisture content, pH, free acidity, lactone acidity, total acidity, proline, 5-HMF and sugars contents and diastase activity of the systems related to the marmeleiro honey in study

Parameters	M1	M2	M3	M4	M5
Moisture content (g/100 g of honey)	20.50 ± 0.28	19.80 ± 0.33	20.15 ± 0.18	19.28 ± 0.37	19.60 ± 0.19
pH	3.70 ± 0.09	3.67 ± 0.05	3.74 ± 0.06	3.70 ± 0.06	3.59 ± 0.12
Free acidity (meq/kg of honey)	$23.60 \pm 0.40^{A,B}$	$19.20 \pm 0.30^{A,C,D}$	$17.66 \pm 0.50^{B,E,F}$	$24.43 \pm 1.05^{C,E}$	$25.06 \pm 1.26^{D,F}$
Lactone acidity (meq/kg of honey)	6.05 ± 0.95^{A}	$14.56 \pm 0.50^{A,B}$	$17.11 \pm 0.40^{A,C,D}$	$6.69 \pm 1.08^{B,C}$	6.70 ± 3.24^{D}
Total acidity (meq/kg of honey)	29.65 ± 1.35^{A}	33.76 ± 0.80	34.77 ± 0.90^{A}	31.12 ± 2.13	31.76 ± 4.50
Proline content (mg/kg of honey)	443.80 ± 6.00	418.06 ± 12.61	471.41 ± 45.37	473.93 ± 88.23	441.16 ± 121.00
5-HMF content (mg/kg of honey)	$2.80 \pm 0.20^{A,B,C}$	$9.21 \pm 0.18^{A,C}$	$17.31 \pm 0.14^{A,B}$	$10.62 \pm 1.38^{B,D}$	$18.97 \pm 1.05^{C,D}$
Diastase number (°G)	$10.55 \pm 0.95^{A,B,C}$	$4.43 \pm 0.12^{A,C}$	$3.50 \pm 0.23^{A,B}$	$4.30 \pm 0.12^{B,D}$	$3.28 \pm 0.25^{C,D}$
Fructose content (g/100 g of honey)	38.60 ± 0.40	38.24 ± 0.35	39.81 ± 0.25	37.18 ± 0.36	37.19 ± 0.70
Glucose content (g/100 g of honey)	26.35 ± 0.55	26.10 ± 0.37	27.62 ± 0.16	25.04 ± 0.27	24.55 ± 1.05
Sucrose content (g/100 g of honey)	0.12 ± 0.06	0.11 ± 0.03	0.18 ± 0.04	0.18 ± 0.05	0.13 ± 0.03
Turanose content (g/100 g of honey)	1.14 ± 0.04	1.26 ± 0.05	1.28 ± 0.03	1.26 ± 0.08	1.18 ± 0.05
Maltose content (g/100 g of honey)	2.10 ± 0.30	2.17 ± 0.07	2.50 ± 0.10	1.67 ± 0.18	1.80 ± 0.23

According to the regulations of the Brazilian Legislation (SIPA, 1997) and to the European Codex Honey Standards (Bogdanov et al., 1997), a well processed and ready to be consumed honey must contain the following characteristics: maximum moisture content of 20–21 g/100 g of honey, reducing sugars content $\geqslant$65 g/100 g, sucrose content $\leqslant$5 g/100 g, free acidity $\leqslant$40 milliequivalents/kg, diastase number $\geqslant$8°G, 5-HMF content $\leqslant$40 mg/kg of honey. In a specific line of this table, the values marked with the same letters are statistically different ($p < 0.05$). M_1 (marmeleiro honey) = fresh samples; M_2 = *in nature* samples heated during 3 months; M_3 = *in nature* samples heated during 6 months; M_4 = sodium metabisulphite added samples heated during 3 months; M_5 = sodium metabisulphite added samples heated during 6 months. The average values that were shown in this table were obtained from triplicate analysis of each system.

and *Eucalyptus lanceolatus* honey samples showed similar results (Bath & Singh, 2000).

In the present work, a significant reduction ($p < 0.05$) in the free acidity values of systems 2 and 3 (heated without sodium metabisulphite stored for 3 and 6 months, respectively) was observed when these values were compared to those obtained in the fresh honey. This reduction observed in systems 2 and 3 for both honey samples was followed by an increase in the lactone acidity of these specific systems. The comparison between the free acidity and the lactone acidity values of the fresh cashew honey and its systems (2 and 3) generated a correlation coefficient of −0.99951, while a correlation coefficient of −0.99954 between these parameters was found for the fresh marmeleiro honey and its systems 2 and 3. The honey acidity is a result of the presence of organic acids, particularly of the D-gluconic acid, in balance with its lactones (internal esters) and some inorganic ions like phosphate, chloride and sulphate, whose corresponding acids are honey components (Bath & Singh, 2000). The gluconic acid represents from 70% to 90% of the honey organic acids content (Moreira & De Maria, 2001; Stinson, Subers, Petty, & White, 1960) and it is produced from D-glucose in a reaction catalyzed by an enzyme known as glucose oxidase. In fact, glucose oxi-

dase first converts glucose into δ-gluconolactone (Mato et al., 1997) which in turn is hydrolyzed to gluconic acid (Fig. 1). Thus, there is a balance between the concentrations of the latter two compounds. Besides gluconolactone, glucose oxidase also produces hydrogen peroxide (called inhibine) that has bactericidal action (White, Subers, & Schepartz, 1963). The total acidity of systems 2 and 3 of the cashew honey could not be considered statistically different from the average value found in this fresh honey. Therefore, it appears that the hydrolysis reaction of the lactone was only displaced in the direction of its own production (Fig. 1). The reason for the displacement in this direction was not clear, but maybe the storage temperature (35–40 °C) and the pH of the systems influenced the reaction balance. It is known, for example, that the closing of the rings of the lactones that give bitter taste to citric juices is thermally encouraged (Kays & Wang, 2000). In addition to the earlier discussions, the activity of glucose oxidase was much reduced, otherwise a higher amount of glucose would be converted to gluconolactone and, thus, the balance would be shifted toward production of more gluconic acid. Thus, an increase in the total acidity of this product, due to an increase in the lactone acidity and/or free acidity, may be noticed. The low activity of this enzyme in the cashew honey could be related to the smaller moisture content showed by this honey, compared to marmeleiro honey. It is known that denser honey samples show a reduced activity of this enzyme (White, 1983). In the marmeleiro honey, where the moisture content is higher, the increase in the lactone acidity of systems 2 and 3 was not proportional to the free acidity reduction. Therefore, part of the lactone generated in these systems would be produced from the glucose oxidation reaction catalyzed by a glucose oxidase that is more active in this high moisture medium. The shift of the lactone hydrolysis reaction in the direction of its own production (Fig. 1) was still occurring, but probably it was being slowed by the production of the lactone from glucose and by the higher amount of water in this matrix. Thus, a significant statistical increase ($p < 0.05$) in the total acidity of the marmeleiro honey system 3 was observed in relation to the mean total acidity value in this fresh honey sample. In spite of the fact that there was no significant statistical difference between system 2 of this honey and its fresh form when analyzing this parameter, such tendency could be observed (see Table 2).

Sodium metabisulphite is a salt that efficiently controls Maillard reaction, enzymatic browning and microorganism development (Araújo, 1995; Kaushik, Joshi, & Gupta, 1993). This salt was added to a part of the honey samples submitted to heating in order to study the contribution of Maillard reaction to the changes observed in these natural products during storage. Based on the data obtained, this salt also seemed to influence the development of the internal esterification reaction that converts part of the honey acids into their lactones. Analyzing Table 1, someone skilled in the art can observe that the free, lactone and total acidity mean values of both sodium metabisulphite added honey samples heated during 3 (system 4) and 6 months (system 5) did not change statistically significantly compared with the values found for these parameters in the respective fresh samples. The internal esterification reaction was possibly blocked by sulphite. The internal esterification mechanism is complex and not yet completely understood, but the fact that this process is catalyzed by acids is well established (Bobbio & Bobbio, 1989). The role of acids is to protonate the carboxyl oxygen, making the carbon atom attached to it more susceptible to attack by a nucleophile, carried out in this case by a hydroxyl group in the same molecule in which the carboxyl group is attached. In an aqueous medium, the bisulphite ion hydrolysis produces the sulphite ion ($HSO_3^- + H_2O \leftrightarrows SO_3^{2-} + H_3O^+$). Despite the fact that this balance is favourable to higher concentrations of the bisulphite ion, the sulphite is a much more efficient nucleophile than the former, not needing acid catalysis to block the carbonyl group (C=O) by the irreversible formation of sulphonates. It is not possible to produce the intermediary carbanion specie if the carbonyl group is blocked, so the esterification reac-

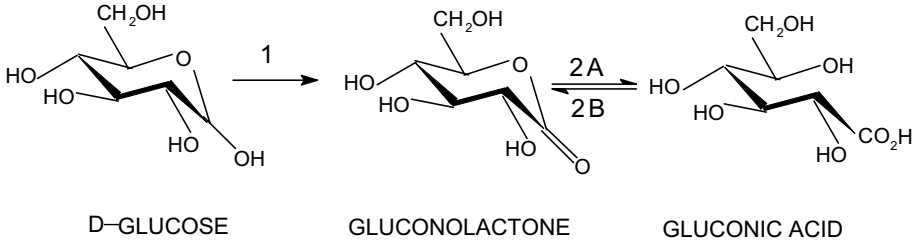

Fig. 1. Aldose oxidation (based on the book of Tedder et al., 1972).

Reaction 1: glucose oxidation catalyzed by glucose oxidase;

Reaction 2: gluconolactone hydrolysis → Reaction 2A: gluconolactone hydrolysis by itself.

Reaction 2: gluconolactone hydrolysis → Reaction 2B: internal esterification of gluconic acid

tion is affected. Besides blocking the shifting of the balance of the internal esterification reaction of the gluconic acid in the direction of lactone formation, the sulphite also seemed to block the gluconolactone production, catalyzed by glucose oxidase using glucose as its substrate.

As expected, no significant statistical differences ($p > 0.05$) existed between the proline content of the fresh honeys and their systems heated for 3 or 6 months, with or without sodium metabisulphite (see Tables 1 and 2). Thus, proline has good stability in the temperature range of 35–40 °C, as no pyrrol was detected in the volatile fraction of the cashew and marmeleiro honey samples that were heated for 3 and 6 months (data not shown). The fact that pyrrols are produced by the pyrolysis of proline through decarboxylation and dehydrogenation is well known in the scientific literature (Higman, Schmeltz, & Schlotzhauer, 1970). Proline is the major amino acid found in honey, representing from 80% to 90% of the total amount of free amino acids in this kind of food matrix. Proline content practically does not change when honey is heated and there is a high positive correlation (+0.96) between this parameter and the invertase (enzyme contained in honey) activity. So, Von der Ohe, Dustmann, and Von der Ohe (1991) have suggested that the proline content could be used as a marker of the original invertase activity in this matrix. In other words, it means that a low invertase activity can only be associated to an excessive heating of honey, if the proline content is high. If the proline content is small, the low invertase activity can be considered an intrinsic characteristic of that kind of honey and not a result of extreme heating of the product.

The 5-HMF mean content of cashew honey system 2 was statistically higher ($p < 0.05$) than that found in its fresh form, however this value was still below the limit (40 mg of 5-HMF/kg of honey) established by the Brazilian legal regulations (SIPA, 1997). The 5-HMF is produced from decomposition of hexoses catalyzed by heating (Bicchi, Belliardo, & Frattini, 1983) and, since it is a toxic compound, it makes honey not appropriate for consumption when found in a concentration greater than 40 mg/kg sample. After six months at 35–40 °C (system 3), the 5-HMF content exceeded this limit (see Table 1). In the cashew honey samples containing sodium metabisulphite (systems 4 and 5), the 5-HMF concentrations also increased ($p < 0.05$) compared to the values found in the fresh samples, but this increase occurred to a lower extent than that observed in systems 2 and 3 of this honey. The reduction of the 5-HMF production observed in the cashew honey heated for 6 months with sodium metabisulphite (system 5) was high enough to keep this parameter [(27.12 ± 1.36) mg of 5-HMF/kg of honey] under the limit established by the Brazilian regulations. This behaviour could be explained by the action of sulphite in the Maillard reaction. The sulphite is added to the dehydroreductone (Maillard reaction intermediary) from which the 5-HMF is formed by cyclization and dehydration. The sulphite combination with this intermediary generates stable sulphonic acids and, thus, the sequence of reactions that leads to the formation of melanoidins (pigments) and volatile compounds is interrupted (Bobbio & Bobbio, 1992). The 5-HMF content in the marmeleiro honey heated for 3 and 6 months without sodium metabisulphite (systems 2 and 3) also increased significantly ($p < 0.05$) in comparison with the value found for the corresponding fresh honey. However, curiously, systems 4 and 5 of this honey did not show values for this parameter significantly different from those observed in systems 2 and 3, respectively (see Table 2). Thus, sulphite was probably incapable of interrupting the formation of 5-HMF. Therefore, during storage period at this temperature range some processes might have occurred in variable ways depending on the intrinsic characteristics of each honey sample (e.g.: botanical origin, volatile and non-volatile fractions composition, etc.).

A significant reduction of the diastase (α-amylase) activity ($p < 0.05$) was observed in both honey samples when their fresh samples were compared with the corresponding systems 2 and 3. This information could be explained by the enzyme being exposed to heat that promoted its denaturation during the storage. It must be mentioned that after 3 months at this temperature range, a diastase activity lower than that established by the Brazilian regulations (8°G) for fresh honey (not heated) had already been reached. No significant statistical differences in this parameter (diastase activity) were observed between systems 2 and 4 and systems 3 and 5 of both honey samples, indicating that metabisulphite did not influence the loss of activity of this enzyme during honey storage at the temperature range used in the study.

The average concentrations of the major sugars (fructose, glucose, sucrose, turanose and maltose) that were found in both honeys (system 1) did not change in any significant statistical way after storage for 3 or 6 months at 35–40 °C (systems 2 and 3, respectively). Changes in the concentration of these sugars could be explained by the activity of the enzyme called invertase, also known as sucrase, that is responsible for hydrolysis of sucrose, releasing its monosaccharides fructose and glucose. This would result in an increase of the amount of these hexoses and a proportional reduction in the amount of sucrose, but these changes were not observed. Invertase has also a secondary transglucosidation activity responsible by the formation of oligosaccharides in honey (Siddiqui, 1970). In the transglucosidation reactions catalyzed by invertase, α-D-glucosyl groups are transferred to a receiving compound like glucose, fructose and sucrose. This process could generate an increase in the turanose and maltose contents during storage, but these changes were not observed. The invertase transglucosidation action can explain sucrose detection (even in very small amounts) in any honey. The low amounts of this disaccharide in honey samples probably are associated with the balance between its hydrolysis reaction (major reaction) and its production reaction (secondary reaction), both of which are catalyzed by this enzyme. The fact that we did not detect the expected variations in these sugars contents during the stor-

age period could be explained by the low activity of this enzyme in the systems, probably related to its denaturation by heating. It is known, for instance, that invertase is a more labile enzyme than diastase when exposed to heating (Dustmann, 1993). A reduction in the fructose and glucose contents during storage by the formation of furanic compounds (e.g. 5-HMF) is also not reasonable. Analyzing Tables 1 and 2, someone skilled in the art would understand that the amount of 5-HMF produced in the systems was negligible when compared to the initial amounts of these hexoses, so, the 5-HMF formation under these conditions would not change the content of hexoses in any significant statistical way. The glucose content can also be reduced during storage by its conversion to gluconic acid, catalyzed by glucose oxidase. However, as previously discussed, the activity of this enzyme appears to be extremely reduced, especially in the cashew samples. Nevertheless, as in the case of 5-HMF, the amounts of glucose consumed in this process would be negligible compared to their initial amounts, not allowing the observation of a significant statistical change during the storage. No significant statistical differences were observed in the contents of these five sugars between systems 2 and 4 and systems 3 and 5 for both honey samples, indicating that the addition of metabisulphite did not modify the sugar profiles in these systems.

In the studied systems, the major transformations due to storage were observed in the following parameters: free acidity, lactone acidity, diastase activity and 5-HMF content. The latter two parameters deserve more attention. The obtained data revealed that the storage of honey samples for 3 and 6 months at 35–40 °C could modify these parameters in such way that the samples could not be classified as fresh and not excessively heated honey according to the Brazilian regulations (SIPA, 1997).

Acknowledgements

We acknowledge the financial support of FAPERJ, CAPES and CNPq. This work is dedicated to the memory of Professor Luiz Carlos Trugo.

References

AOAC (1984). In S. Williams (Ed.), *Official methods of analysis*. Arlington, VA: Association of Official Analytical Chemists Inc.

Aparna, A. R., & Rajalakshmi, D. (1999). Honey – its characteristics, sensory aspects, and applications. *Food Reviews International, 15*, 455–471.

Araújo, J. M. A. (1995). *Química de Alimentos (Teoria e Prática)*. Viçosa (MG): Imprensa Universitária da Universidade Federal de Viçosa.

Bath, P. K., & Singh, N. (2000). Chemical changes in *Helianthus annus* and *Eucalyptus lanceolatus* honey during storage. *Journal of Food Quality, 23*, 443–451.

Bicchi, C., Belliardo, F., & Frattini, C. (1983). Identification of the volatile components of some piemontese honeys. *Journal of Agricultural Research, 22*(2), 130–136.

Bobbio, F. O., & Bobbio, P. A. (1989). *Introdução à Química de Alimentos*. São Paulo: Livraria Varela Ltda.

Bobbio, P. A., & Bobbio, F. O. (1992). *Química do Processamento de Alimentos*. São Paulo: Livraria Varela Ltda.

Bogdanov, S., Martin, P., & Lullmann, C. (1997). Harmonized methods of the European Honey Commission. *Apidologie*(extra issue), 1–10.

Cherchi, A., Porcu, M., Spanedda, L., & Tuberoso, C. I. G. (1997). Influence of ageing on the quality of honey. *Industria Conserve, 72*, 266–271.

Da Costa Leite, J. M., Trugo, L. C., Costa, L. S. M., Quinteiro, L. M. C., Barth, O. M., Dutra, V. M. L., et al. (2000). Determination of oligosaccharides in Brazilian honeys of different botanical origin. *Food Chemistry, 70*, 93–98.

Dustmann, J. H. (1993). Honey quality and its control. *American Bee Journal, 133*, 648–651.

Gonzales, A. P., Burin, L., & del Pilar Buera, M. (1999). Color changes during storage of honeys in relation to their composition and initial color. *Food Research International, 32*, 185–191.

Higman, E. B., Schmeltz, I., & Schlotzhauer, W. S. (1970). Products from the thermal degradation of some naturally occurring materials. *Journal of Agricultural and Food Chemistry, 18*, 636–639.

Kaushik, R., Joshi, V. K., & Gupta, J. K. (1993). Total soluble solids, acidity, pH and standard plate counts in the Indian honey as affected by different treatments and storage conditions. *Journal of Food Science and Technology, 30*, 442–443.

Kays, S. J., & Wang, Y. (2000). Thermally induced flavor compounds. *HortScience, 35*, 1002–1012.

Lee, H. S., Rouseff, R. L., & Nagy, S. (1986). HPLC determination of furfural and 5-hydroxymethylfurfural in citrus juices. *Journal of Food Science, 51*, 1075–1076.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World, 59*, 139–157.

Mato, I., Huidobro, J. F., Sánchez, M. P., Muniategui, S., Fernández-Muiño, M. A., & Sancho, M. T. (1997). Enzymatic determination of total D-gluconic acid in honey. *Journal of Agricultural and Food Chemistry, 45*, 3550–3553.

Moreira, R. F. A., & De Maria, C. A. B. (2001). Glicídios no mel. *Química Nova, 24*, 516–525.

Secretaria de Inspeção de Produto Animal (SIPA). (1997). Normas Higiênico-Sanitárias e Tecnológicas para Mel, Cera de abelhas e Derivados. Ministério da Agricultura – Portaria No. 367.

Siddiqui, I. R. (1970). The sugars of honey. In R. S. Tipson (Ed.). *Advances in carbohydrate chemistry and biochemistry* (Vol. 25, pp. 285). New York: Academic Press.

Stinson, E. E., Subers, M. H., Petty, J., & White, J. W. Jr., (1960). The composition of honey V. Separation and identification of the organic acids. *Arch. Biochem. Biophys., 89*, 6–12.

Tedder, J. W., Nechvatal, A., Murray, A. W., & Carnduff, J. (1972). *Basic organic chemistry. Part 4. Natural products* (1st ed.). New York: Wiley, pp. 44–46.

Visser, F. R., Allen, J. M., & Shaw, G. J. (1988). The effect of heat on the volatile flavor fraction from a unifloral honey. *Journal of Apicultural Research, 27*, 175–181.

Von der Ohe, W., Dustmann, J. H., & Von der Ohe, K. (1991). Prolin als kriterium der reif des honigs. *Deutsche Lebensmittel-Rundschau, 87*, 383–386.

White, J. W., Subers, M. H., & Schepartz, A. I. (1963). The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochemica et Biophysica Acta, 73*, 57–70.

White, J. W. Jr., (1983). Honey. *Advances in Food Research, 24*, 287–374.