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5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment

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ABSTRACT

This study aimed to assess 5-hydroximethylfurfural and carbohydrates (fructose, glucose, and sucrose) in 13 stingless bee honey samples before and after thermal treatment using a capillary electrophoresis method. The methods were validated for the parameters of linearity, matrix effects, precision, and accuracy. A factorial design was implemented to determine optimal thermal treatment conditions and then verify the postprocedural 5-HMF formation, but once 5-HMF were <LOQ was not possible to build a response surface. The methods applied to samples obtained from Brazil, expressed good linearity, precision, and accuracy. None of the thirteen *in natura* samples presented 5-HMF, and carbohydrate levels ranged from 48.59% to 69.36%. In the same conditions of thermal treatment, *Apis mellifera* honey presented higher 5-HMF content than stingless bee honey. Results suggest that a high temperature related to briefer thermal treatment could be an efficient way to extend shelf life without affecting 5-HMF content in stingless bee honey.

1. Introduction

For centuries bees have been kept for the extraction of honey, pollen, propolis, and wax, this activity has been considered profitable and sustainable in many countries (Nogueira-Neto, 1997). There are two fields of bee management: apiculture and meliponiculture. Apiculture refers to the widely popular rational management of *Apis mellifera*, while meliponiculture refers the rational management of stingless bees, which has recently experienced increased interest due to the bees' stinglessness (Oliveira, Celi, Müller, Fernandes, & Nahum, 2012).

In Brazil several native bee species produce honey and are known as indigenous bees, stingless bees, or *Meliponinae*. For some time, honey has not only been consumed by the local population as a source of energy and sustenance but also cultivated for its medicinal properties (Guerrini et al., 2009; Silva et al., 2013). Most honey production in Brazil occurs in the northeast and northern regions, though production has recently increased in the southeast (i.e., São Paulo) and the southern (i.e., Paraná and Santa Catarina) region's tropical and subtropical climates.

Honey is a natural food produced by honey bees from the nectar of a variety of plants and consists mainly of glucose and fructose. Its other minor compounds include minerals, phenolic compounds, organic acids, protein, amino acids, vitamins, enzymes, and other phytochemicals (Brasil & Pecuária e Abastecimento, 2000; Da Silva et al., 2013; Silva et al., 2013). Different honeys' characteristics can derive from species, floral origin, and geographic region due to parameters of quality and identification stipulated by regulatory agencies. The Brazilian Ministry of Agriculture and Livestock (Portuguese acronym MAPA) is the agency responsible for the fiscalization of physicochemical properties, purity, and deterioration of only *Apis mellifera* honey (Brasil, 2000). The lack of legislation for stingless bee honey stems from its lower production rate and lower shelf life and causes limited worldwide distribution of the product compared to *Apis mellifera* honey (Guerrini et al., 2009).

Stingless bee honey is a product valued by the local Brazilian population due to its medicinal properties. For Da Silva et al. (2013), the growing interest in the honey produced by stingless bees derives from its composition, which has antiseptic, antimicrobial, anticancer, anti-inflammatory, and wound-healing properties. Furthermore, stingless bee honey has its own characteristics, such as its distinct taste and aroma, more fluid texture, and slow crystallisation (Alves, Alfredo, Souza, Sodré, & Marchini, 2005; Oliveira et al., 2012). The honey's fluidity derives from its high water content, which can undergo undesirable fermentation either caused by the yeast of nectar microflora or introduced by apiary management. This fermentation results in the formation of organic

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compounds that can affect taste and colour, as well as decrease shelf life (Abramovič, Jamnik, Burkan, & Kač, 2008).

With the advantages of stingless bee honey in mind, it is clearly necessary to extend the product's shelf life without altering its nutritional and medicinal properties. To this end, some techniques previously applied include dehumidification, thermal treatment, and refrigeration (Kowalski, 2013; Sodré et al., 2008; Tosi, Ciappini, Re, & Lucero, 2002; Turhan, Tetik, Karhan, Gurel, & Reyhan Tavukcuoglu, 2008), among which thermal treatment has proven simple and effective.

The thermal treatment of honey is a practical method for preventing or postponing crystallisation, destroying micro-organisms, and facilitating filling by viscosity reduction (Tosi et al., 2002; Turhan et al., 2008). However, regarding honey characteristics, thermal treatment favour 5-hydroxymethylfurfural (5-HMF) formation, which consequently decreases sugar content.

5-HMF is a furanic compound that can be formed by Maillard reaction or hexose dehydration in acid media (Tosi et al., 2002). 5-HMF also can be formed in low temperatures over long storage period of storage, but in lower concentrations once Maillard reaction slows in these conditions. Aside from temperature, 5-HMF concentration formation depends on the kind of sugar, pH, water activity, and divalent cations concentrated in the media. *In vitro* studies indicate that 5-HMF can be cytotoxic, mutagenic, carcinogenic, and genotoxic (Capuano & Fogliano, 2011), and due this toxicity is important to monitor its concentrations in food products as honey.

To contribute with scientific research related to stingless bee honey, due the lack of information about this product, the aim of this study was to evaluate 5-HMF and carbohydrates in stingless bee honey. The work included monitoring the compounds of *in natura* honey, proposing a thermal treatment through factorial design, and examined the effects of thermal treatment on the 5-HMF formation and sugar content. Compound content was determined via capillary electrophoresis methods that were validated for the parameters of linearity, matrix effects, precision, and accuracy for a stingless bee honey matrix, since this method was previously validated for *Apis mellifera* honey samples.

2. Materials and methods

2.1. Honey samples

Thirteen multifloral stingless bee honey samples were obtained directly from producers in the three municipalities of Florianópolis, Santo Amaro da Imperatriz, and São Miguel do Oeste in Santa Catarina, Brazil. The *Apis mellifera* honey was obtained from Itaiópolis in Santa Catarina, Brazil. Samples were harvested between March and May of 2013 and stored at −18 ± 2 °C in the dark until the experiment.

2.2. Sample preparation

For 5-HMF analysis, samples were prepared by following the method proposed by Rizelio et al. (2012a) with some modifications, in which *in natura* and thermally treated honey samples were weighed (0.5 g) and dissolved in 0.5 g deionised water. Caffeine (internal standard, I.S.) was added and volume recorded until obtaining a final concentration of 200 mg L^{-1}. For carbohydrates analysis honey samples were prepared according to Rizelio et al. (2012b) for *in natura* and thermally treated samples, by which they weighed approximately 2.5 g and were dissolved in deionized water in a 50 mL volumetric flask. Volume was properly completed, and the honey sample solution was dissolved 1:10 (v/v) in deionized water. Both solutions were filtered through 0.45 μm membrane filters (Millipore, Bedford, MA, USA) and directly injected into CE equipment for analysis. All experiments were performed in triplicate.

2.3. Reagents and solutions

All reagents were of analytical grade, solvents were of chromatographic purity, and water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA). 5-HMF, caffeine, sodium tetraborate (STB), methanol (MeOH), sodium dodecylsulfate (SDS), sorbic acid, and cetyltrimethylammonium bromide (CTAB) were obtained from Sigma–Aldrich (Santa Ana, CA, USA). Sodium hydroxide (NaOH), D-(+)-glucose monohydrate, D-fructose, and sucrose were obtained from Merck (Rio de Janeiro, RJ, Brazil).

Standard solutions were prepared daily, stored at 4 °C, and diluted with ultra pure water to obtain the concentrations required for CE experiments. In 5-HMF analysis the background electrolyte (BGE) was composed of 5 mmol L^{-1} STB and 120 mmol L^{-1} SDS at pH 9.3 and stored at ambient temperature until analysis, as per Rizelio et al. (2012a). In the carbohydrate analysis the BGE was composed of 20 mmol L^{-1} sorbic acid, 0.2 mmol L^{-1} CTAB, and 40 mmol L^{-1} NaOH at pH 12.2, also as per Rizelio et al. (2012b).

2.4. Capillary electrophoresis system

CE assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector and a temperature control device maintained at 25 °C. Acquisition and data treatment software were supplied by the manufacturer (HP ChemStation®). At the beginning of each day, the capillary was conditioned by flushing with 1 mol L^{-1} NaOH (10 min) followed by a 10 min flush with deionised water and BGE solution (15 min). In between runs, the capillary was reconditioned with the background solution (2 min flush). At the end of each working day, the capillary was rinsed with 1 mol L^{-1} NaOH (5 min) and water (10 min) and then dried with air (2 min). In both analyses, standard solutions and samples were introduced at the extremity of the capillary nearest to the detector and injected hydrodynamically at 50 mbar for 3 s (1 mbar = 100 Pa) with negative pressure (Rizelio et al., 2012a, 2012b).

5-HMF separation was conducted in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 32.0 cm total length (8.5 cm effective length × 50 μm I. D. × 375 μm O. D.). The applied separation voltage was 30 kV with positive polarity on the injection end and the detector set at 284 nm (Rizelio et al., 2012a).

Table 1
Experimental design applied for thermal treatment in stingless bee honey.

Variable	Level		
	−1	0	+1
Temperature (°C)	75	85	95
Time (s)	20	40	60

Carbohydrates analyses were performed in an uncoated fused-silica capillary of 60.0 cm total length (8.5 cm effective length × 50 μm I. D. × 375 μm O. D.). The applied separation voltage was 25 kV with negative polarity at the injection end and the detector set at 254 nm (i.e., indirect detection with a reference at 360 nm for peak inversion) (Rizelio et al., 2012b).

2.5. Experimental design to optimise the conditions of thermal treatment

In order to provide more confidence to the results and reduce the number of experiments, the thermal treatment conditions were conducted using a 2^2 factorial design with central point. An overview of the experimental designs is shown in Table 1, and the response was 5-HMF formation.

To apply the thermal treatment, a stingless honey sample with higher sugar content was chosen. The sample was heated in water bath according to the conditions established by factorial design. A thermometer was inserted into the geometric centre of a sample tube in the bath to monitor sample temperature, the tube were kept uncovered and after reaching the condition of time and temperature, the sample was returned to room temperature.

To compare data of stingless bee honey, two other extreme conditions were tested to assess 5-HMF formation in *Apis mellifera* honey. In the first condition, both honey samples were heated in a water bath at 75 °C for 15 min, and in the second condition, at 75 °C for 24 h in the oven. After thermal treatment, the samples were subjected to CE analysis.

2.6. Validation parameters for stingless bee honey

Rizelio et al. (2012a, 2012b) methods were validated for *Apis mellifera* honey and due the differences between this honey and the stingless bee honey is necessary to assess some validation parameters again. The parameters chosen were linearity, matrix effects, precision and accuracy.

The fitness-for-purpose of this method was assessed based on results obtained for established performance characteristics (Eurachem, 1998; Thompson, Ellison & Wood, 2002).

2.6.1. Linearity and matrix effect

Linearity was established in standard solutions and matrix calibration curves due to the unavailability of blank samples. Five concentration levels were prepared in the range of 10–80 mg L^{-1} for 5-HMF. For the carbohydrates curves were created for the fructose and glucose that ranged from 180 to 3600 mg L^{-1} and for the sucrose that ranged from 342 to 5130 mg L^{-1}, each with equidistant increments. Matrix calibration curves were obtained using the standard addition method in the same concentrations previously reported. These curves were prepared on 3 different days to obtain independent replicates. Matrix effect was assessed by comparing the slopes obtained for each standard solution and with a matrix calibration curves *t*-test with a confidence level of 95% (Thompson et al., 2002).

2.6.2. Precision and accuracy

To assess the repeatability, intra-day precision for 5-HMF and carbohydrates were determined by three independent replicates of each standard used to obtain the calibration curves (at five concentration levels). A dilution with I.S. at 200 mg L^{-1} was used for 5-HMF. The intermediate precision (i.e., inter-day precision) was assessed by analysing three preparations of standard solutions over a 3-day period with six consecutive injections. The precision results were expressed in terms of the relative standard deviation (% RSD) for the replicates of standard solutions at each concentration level, calculated as t_m(analyte) and t_m(analyte)/t_m(I.S.) and area(analyte) and area(analyte/area(I.S.) for carbohydrates and 5-HMF, respectively.

Accuracy was assessed according to the apparent recovery obtained from the mean for the three independent replicates of a spiked sample at three levels in the range used for the calibration curves for 5-HMF and carbohydrates.

2.7. Statistical analysis

The statistical analyses were performed in Statistica software 7.0 (Statsoft Inc., Tulsa, USA).

3. Results and discussion

3.1. Validation parameters for stingless bee honey

3.1.1. Linearity and matrix effect

The assumptions of linearity were confirmed in the curves of both the standard solution and standard addition solutions. The matrix effect was assessed by comparing the slopes of standard and standard addition by F and *t*-tests for the sample containing the analytes, 5-HMF, and carbohydrates. The method did not include an observed matrix effect, thus quantification was accomplished using the standard solution curve for both carbohydrates and 5-HMF. All regression assumptions were tested and confirmed

Table 2
Analytical performance of the proposed CZE methods.

Parameter	*n*	5-HMF	Fructose	Glucose	Sucrose
Intra-day precision — peak area (RSD, %)	3	6.15	7.81	8.08	6.81
Intra-day precision — migration time (RSD, %)	3	1.21	7.26	7.63	8.98
Inter-day precision — peak area (RSD, %)	9	0.79	6.22	3.52	3.86
Inter-day precision — migration time (RSD, %)	9	5.44	2.79	2.89	3.44
Linearity — linear range (mg L^{-1})		10–80	180–3600	342–5130	
Linearity — slope		0.0229	0.0598	0.0646	0.0443
Linearity — intercept		−0.0925	−4.1315	−4.6321	−4.8186
Linearity — coefficient of regression, R^2		0.995	0.999	0.998	0.997
F[a]	15	2337	10197	6943	5097
LOQ (mg L^{-1})		0.31	0.088	0.097	0.074
LOD (mg L^{-1})		0.09	0.026	0.026	0.022
Solvent[b]					
t	6	5.42	0.7809	1.0112	0.633
p		0.116	0.516	0.496	0.591

n: number of observations, *t*: t-statistic, *p*: significance.
[a] *F* critical: 1.13.
[b] Residual homoscedasticity evaluation by test *t* for solvent and matrix-matched calibration curves (*t* critical: 12.70 for HMF and 4.30 for carbohydrates).

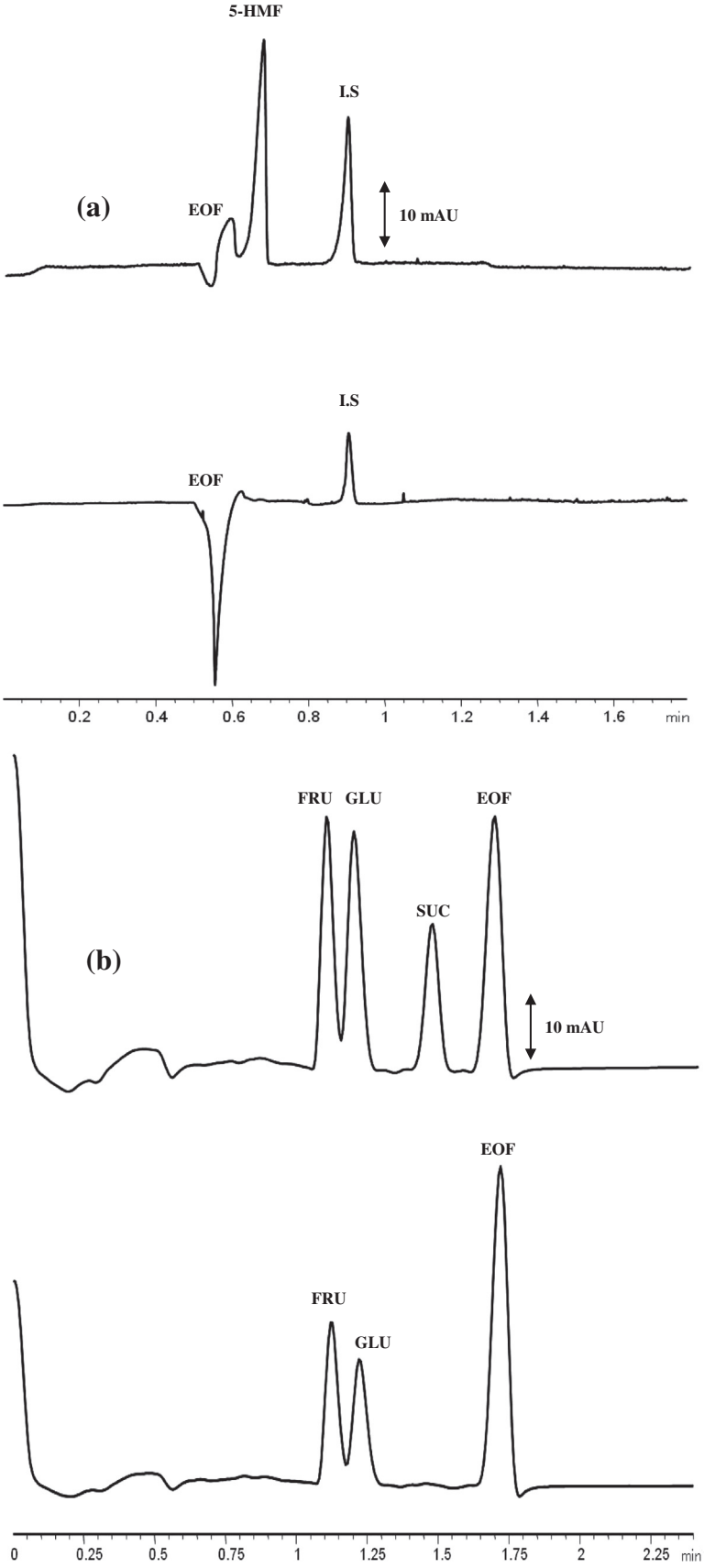

Fig. 1. Electropherograms in standard solution and stingless bee honey sample of (a) 5-HMF and (b) carbohydrates.

for the ranges evaluated, and the curves demonstrated linearity in this range (Table 2).

3.1.2. Precision and accuracy

Intra-day precision was determined by consecutive injections carried out randomly at each level of the calibration curves for 5-MF and carbohydrates. Each point (i.e., each of the 5 levels) was prepared with three replicates of independent standard solutions. For 5-HMF, the repeatability (i.e., intra-day precision) for the migration time and area corrected (% RSD) better than 1.21 and 6.15%, respectively. Values of % RSD for migration time below 8.98% and area exceeding 8.08% were reached for carbohydrates repeatability. The % RSD results for the intermediate precision (i.e., inter-day precision) for 3 consecutive days were also obtained by using three replicates of independent standard solutions. For the corrected migration time and area, values exceeded 0.79% and 5.44% for 5-HMF, as well as 3.44% for migration time and 6.22% for area of carbohydrates (Table 2). In a study of *Apis mellifera* honey samples, Rizelio et al. (2012a, 2012b) found values for intra-day and inter-day that ranged from 0.60% to 5.41% for 5-HMF and from 0.62% to 3.87% for carbohydrates.

The recovery for 5-HMF varies from 90.44% to 110.37%, while carbohydrates recoveries ranged from 104.83% to 121.85% for fructose, 110.14% to 126.21% for glucose, and 90.15% to 102.33% for sucrose. Results of the validation parameters indicate the viability of the method to determine 5-HMF and carbohydrates in samples of stingless bee honey. For *Apis mellifera* honey samples, Rizelio et al. (2012a, 2012b) obtained recoveries varying from 96.37% to 99.56% for 5-HMF and 96.20% to 109.2%, showing values similar to those obtained in this present study.

3.2. Determination of 5-HMF and carbohydrates of in natura stingless bee honey

The proposed method of Rizelio et al. (2012a) was successfully applied for 5-HMF determination in stingless bee honey (Fig. 1a), and in all thirteen samples 5-HMF was under the limit of quantification (LOQ), achieving the result expected once samples were rightly harvested and stored. Brazilian legislation (Brasil, 2000) has determined that 5-HMF content must not exceed 60 mg kg^{-1} for *Apis mellifera* honey, while the Codex Alimentarius of the World Health Organization and the European Union have established a maximum quality level for 5-HMF content in honey at 40 mg kg^{-1} (Alinorm 01/25, 2001; Directive 2001/110/EC, 2001). In this study all samples tested were in accordance with the legislation. Silva et al. (2013) also investigated the 5-HMF content in nine samples of stingless bee honey (Paraíba, Brazil), for which 5-HMF concentrations ranged from 10.80 to 15.76 mg kg^{-1}. Guerrini et al. (2009) analysed Ecuadorian stingless bee honey and found 5-HMF content of 15 ± 1.91 mg kg^{-1}. The 5-HMF content can be influenced by the climate, thus in both situations in which 5-HMF was found, stingless bee honey samples were obtained from tropical regions that are more favourable for its formation.

Rizelio et al.'s (2012b) method was successfully applied to analyse carbohydrates in 13 stingless bee honey (Fig. 1b). Quantification results are shown in Table 3. Since fructose and glucose are the major carbohydrates of honey, the total content of reduced sugar (i.e., fructose and glucose) in the samples ranged from 48.59% to 69.36% (m/m). Sample E presented the highest sugar content of all samples tested. The significant difference between the results can be justified by the variability of species, flowering, and climate once the samples were collected from different regions of the state. Five samples presented values below the limits stipulated by Brazilian legislation, which stipulates minimum values of 60% (m/m). Evidence has shown that stingless bee honey contains lower sugar content, is sweeter, and has higher moisture compared to *Apis mellifera* (Rizelio et al., 2012b; Silva et al., 2013). In all samples tested, sucrose content was under LOQ. Silva et al. (2013) also evaluated the carbohydrate content in stingless bee honey samples, in which reduced sugar concentrations ranged from 50.50 ± 0.28 to 72.77 ± 0.14 mg kg^{-1}. Guerrini et al. (2009) found similar results in a study of Ecuadorian stingless bee honey by obtaining 44.9 ± 5.74 mg kg^{-1}.

Table 3
Quantification of fructose, glucose, sucrose, and 5-HMF of *in natura* stingless bee honey samples.

Sample	Fructose (% m/m)	Glucose (% m/m)	Fructose and Glucose (% m/m)	Sucrose (% m/m)	5-HMF (mg kg^{-1})
A	34.97 ± 0.80	27.25 ± 0.09	62.21 ± 0.20	<LOQ	<LOQ
B	36.37 ± 0.15	29.45 ± 0.13	65.81 ± 0.29	<LOQ	<LOQ
C	32.46 ± 0.22	29.46 ± 0.16	61.92 ± 0.37	<LOQ	<LOQ
D	34.68 ± 0.15	26.41 ± 0.15	61.09 ± 0.29	<LOQ	<LOQ
E	38.38 ± 0.12	30.98 ± 0.06	69.36 ± 0.17	<LOQ	<LOQ
F	33.45 ± 0.25	28.20 ± 0.14	61.65 ± 0.39	<LOQ	<LOQ
G	40.20 ± 0.64	8.20 ± 0.20	48.59 ± 0.84	<LOQ	<LOQ
H	36.11 ± 0.29	21.3 ± 0.20	57.34 ± 0.49	<LOQ	<LOQ
I	31.54 ± 0.20	26.18 ± 0.33	57.72 ± 0.51	<LOQ	<LOQ
J	36.38 ± 0.36	26.32 ± 0.28	62.70 ± 0.64	<LOQ	<LOQ
K	31.11 ± 0.24	26.96 ± 0.13	58.07 ± 0.37	<LOQ	<LOQ
L	35.59 ± 0.12	29.96 ± 0.08	65.55 ± 0.15	<LOQ	<LOQ
M	31.88 ± 0.19	26.46 ± 0.17	58.34 ± 0.63	<LOQ	<LOQ

3.3. Determination of 5-HMF and carbohydrates after thermal treatment

Currently no studies of thermal treatment for stingless bee honey appear in the literature, so literature for *Apis mellifera* honey has been consulted to chose the variables (time and temperature) and the range applied in the study (Kowalski, 2013; Tosi et al., 2002; Turhan et al., 2008). The chosen conditions are suitable for the pasteurisation process, and aiming a practical condition for conservation stingless bee honey by honey producers. In all conditions tested regarding factorial design, 5-HMF content was under LOQ, so it was not possible to build a response surface or a mathematical model of the thermal treatment conditions. Furthermore, thermal treatment did not affect the sugar content in the stingless bee honey sample (Table 4). Observing a possible resistance to 5-HMF formation in the stingless bee honey sample, thermal treatment was performed in two extreme conditions (see Section 2.5) in order to compare 5-HMF formation in *Apis mellifera* and stingless bee honey, both samples not presenting this compound.

When submitted to 75 °C temperatures for 15 min, *Apis mellifera* honey formed 8.05 mg kg^{-1} of 5-HMF, while the stingless bee honey did not exceed LOQ. When the sample was submitted to temperatures of 75 °C for 24 h, 5-HMF content of *Apis mellifera* honey was 695.40 mg kg^{-1} while the stingless bee honey was only 238.18 mg kg^{-1} (Table 5). These results suggest a possible resistance to HMF formation in stingless bee honey that can be justified by its type of carbohydrates, in which fructose content predominates. It is known that since honey's major sugar is glucose, the speed of Maillard reaction is higher, thus results are higher regarding 5-HMF formation. Other factors that can be observed include water activity (Aw) and acidity; when Aw is higher, Maillard reaction slows and 5-HMF formation thus decreases (Fennema, 2000; Ribeiro & Seravalli, 2004). When acidity is high, Maillard reaction slows, and by contrast, 5-HMF formation is inhibited. Studies have indicated that stingless bee honey has higher Aw and acidity compared to *Apis mellifera*, which justifies the reduced HMF content of stingless bee honey (Guerrini et al., 2009; Silva et al. 2013). Table 5 shows the possible relationship between sugar reduction and 5-HMF formation, for once the sugar content (i.e., fructose and glu-

Table 4
Results obtained for experimental design after heat treatment.

Run	Time (s)	Temperature (°C)	Fructose and Glucose (% m/m)	5-HMF (mg kg^{-1})
1	20 (−1)	75 (−1)	65.25 ± 0.70	<LOQ
2	60 (+1)	75 (−1)	68.38 ± 0.79	<LOQ
3	20 (−1)	95 (+1)	66.00 ± 0.85	<LOQ
4	60 (+1)	95 (+1)	61.80 ± 2.35	<LOQ
5	40 (0)	85 (−1)	66.47 ± 1.61	<LOQ
6	40 (0)	85 (−1)	59.80 ± 0.32	<LOQ
7	40 (0)	85 (+1)	71.72 ± 0.73	<LOQ

Table 5
Quantification of reducing sugars (fructose and glucose) and 5-HMF in honey samples of stingless bee and *Apis mellifera* before and after extreme thermal treatment.

Honey samples	5-HMF (mg kg^{-1})	Fructose and glucose (% m/m)
Stingless bee *in natura*	<LOQ	69.36 ± 0.17
Stingless bee 15 min/75°C	<LOQ	61.86 ± 1.24
Stingless bee 24 h/75 °C	238.18 ± 8.75	58.31 ± 0.70
Apis mellifera in natura	<LOQ	70.35 ± 1.13
Apis mellifera 15 min/75 °C	8.05 ± 1.30	68.64 ± 2.18
Apis mellifera 24 h/75 °C	695.40 ± 26.49	67.41 ± 7.41

cose) is decreased upon applying thermal treatment, results include 5-HMF formation and other Maillard byproducts.

Other studies have investigated the formation of 5-HMF after thermal treatment in *Apis mellifera* honey. Kowalski (2013) evaluated the effect of thermal processing on the formation of 5-HMF in *Apis mellifera* honey, and results reached 94.33 mg kg^{-1} of 5-HMF after a heat treatment of 90 °C during 60 min. Turhan et al. (2008) assessed the quality of honey samples and quantified 5-HMF by RP-HPLC as a consequence of thermal treatment in which the maximum value observed was 73.78 ± 1.01 mg kg^{-1} at 100 °C for 90 min.

4. Conclusion

In all thirteen samples *in natura* evaluated, 5-HMF was under LOQ, and the carbohydrates ranged from 48.59% to 69.36%, presenting fructose content higher than glucose, and sucrose was under LOQ. In the factorial design conditions tested was not observed 5-HMF formation, it was confirmed when applied extreme time and temperature conditions. The methods shown good results for linearity, precision and accuracy, when applied for the stingless bee honey samples. Other studies must be performed to corroborate with a possible resistance of stingless bee honey to 5-HMF formation, what can extend the shelf life of the product.

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