PAPER

HONEY-BASED "ÁGUA-MEL" CHEMICAL CHARACTERIZATION AND MICROBIOLOGICAL QUALITY

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ABSTRACT

In Mediterranean countries such as Italy and Portugal an ancient practice among beekepers is the production of a honey-based product that is called "água-mel" (Portuguese designation) or "abbamele" (Italian designation) that have not only food applications but also medicinal purposes. However, the characterization of such foodstuff is completely absent in Portugal. In our study the main goal was to provide the general chemical characterization and the microbiological quality of samples of "água-mel". The chemical characterization showed a great variability of the ash percentage (0.167-0.474); electrical conductivity (407-1067 μS/cm); free acidity (33.2-91.2 meq/kg); lactone acidity (14.60-20.50 meq/kg); total acidity (53.7-122.72 meq/kg); glucose (185.57-258.52 g/kg); fructose (218.49-315.36 g/kg); total polyphenols (1780.0-4963.8 mg/kg); flavonoids (188.8-1702.4 mg/kg) and 5-(hydroxymethyl)-2-furaldehyde (HMF) (1812.6-8428.9 mg/kg), depending on the beekeeper and production year. The microbiological quality included the counts of aerobic mesophilic bacteria, yeasts and moulds, Enterobacteriaceae, sulphite-reducing *Clostridium* spp. and the presence of *Salmonella* spp. The results showed that from all "água-mel" samples analyzed only one sample was contaminated with *Clostridium* spp. and aerobic mesophilic bacteria. Taken together both chemical and microbiological data indicates a safe consumption of "água-mel".

- Keywords: "água-mel", honey, chemical characterization, microbiological quality -

INTRODUCTION

For a long time beekeepers from Portugal, mainly in the South (Algarve and Alentejo regions), obtain a honey-based product known as "água-mel". Such product is obtained according to that already reported for a traditional honey-based Sardinian product called "abbamele" (SPANO *et al.*, 2008) with little differences. For "abbamele", after the extraction of honey from the honeycombs, the latter are crumbled and dipped into warm water (40°C). The emerging wax separates and the remaining liquid (water, some honey and pollen) is heat-treated (up to 100°C) until a brown, honey-like product. In Portugal, honeycombs are also crumbled and dipped into warm water but at 70°C. The remaining liquid constituted by washing water, some honey, propolis and pollen is, then, heat-treated until a brown, honey-like product with 70°-77° Brix (Fig. 1). This procedure is time-consuming (9-12 hours).

Since ancient times, "água-mel" is used in Portugal as sweetener in cakes, tea, and of great importance as natural medicine on the alleviation of simple symptoms of upper respiratory tract. More recently, the haute cuisine started to use this product in salads and cakes.

In Portugal no information about the physico-chemical characteristics of "água-mel" exists, in contrast to the Italian "abbamele" which has been under research since a few years ago (SPANO *et al.*, 2008; JERKOVIĆ *et al.*, 2011). Thus, the general characterization of the "água-mel" from the South of Portugal is our main goal.

MATERIAL AND METHODS

Samples

Samples of "água-mel" were given by the following producers; through the beekeepers Association "Associação dos Apicultores do Sudoeste Alentejano e Costa Vicentina" (AASACV), Portugal:

- 1A/2008: Producer 1A/year of production 2008;
- 1A/2010: Producer 1A/year of production 2010;
- 1A/2011: Producer 1A/year of production 2011;
- 1B/2010: Producer 1B/year of production 2010;
- 1B/2011: Producer 1B/year of production 2011;
- 1H/2011: Producer 1H/year of production 2011;
- 1I/2011: Producer 1I/year of production 2011;
- 1M/2011: Producer 1M/year of production 2011;
- 2A/2011: Producer 2A/year of production 2011;
- 2B/2011: Producer 2B/year of production 2011.

Samples were kept at room temperature and flasks were opened at aseptic conditions, in a laminar flow chamber (BIOHAZARD, Bio II A, Telstar, Madrid, Spain). For each sample of "água-mel", the producers provided 3 bottles. For each bottle, 3 determinations were done. Thus, data are the mean of 9 determinations (n=9).

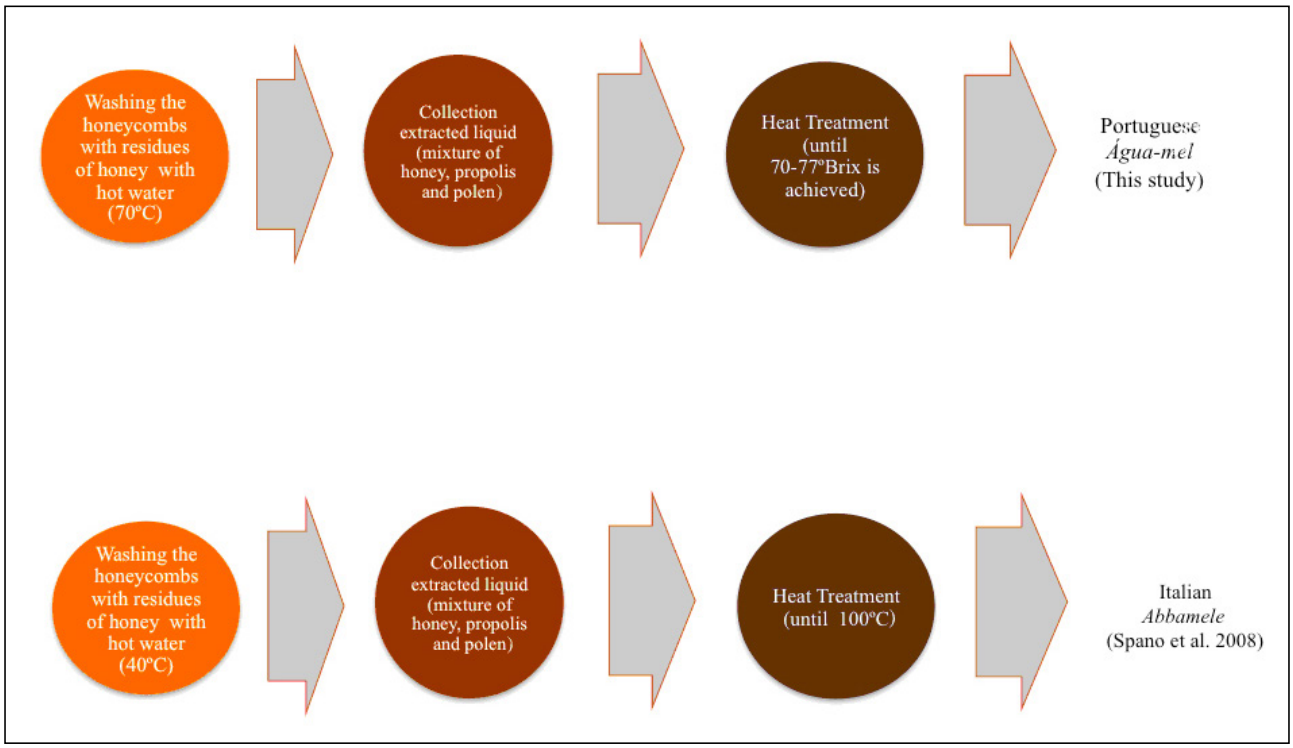

Fig. 1 - Flow chart of "água-mel" and "abbamele" production.

Microbiological analysis

To evaluate the microbiological quality of "água-mel", counts of aerobic mesophilic bacteria (NP-4405:2002), yeasts and moulds (ISO 21527-2:2008), Enterobacteriaceae (ISO 21528-2:2004) and sulphite-reducing *Clostridium* spp. (ISO 15213:2003) were determined. Culture media were purchased from Oxoid (Basingstoke, Hampshire, UK) and Biokar (Paris, France). Ten gram of "água-mel" samples were transferred to 90 mL of peptone water (Oxoid) and homogenized. Decimal dilutions were prepared using the same diluent. The detection procedure for *Salmonella* spp. was done according to the international standard ISO 6579:2002. The microbiological determinations were done in triplicate. Microbial counts were expressed in Log_{10} CFU/g.

Ash

The samples were submitted to 550°C in an electric furnace (Cassel, Portugal) and the residue weighed in an analytical balance (Shimadzu, Aux 220, Philippines), after cooling in a desiccator, according to the harmonized method for honey developed by the International Honey Commission IHC (2002).

Electrical conductivity

Electrical conductivity was measured according to the International Honey Commission IHC (2002) and using a (Thermo Electron corporation, Orion 3 STAR, USA) conductivity meter equipped with a conductivity probe (Orion, 013005MD, USA). The sample solution was prepared using MilliQ water (MQ Integral 5, 2RXP-005TO, Portugal). The cell constant value was checked with 0.1 M aqueous solution of KCl (BHD Prolabo, Leuven, Belgium).

Water content

The water content of the samples was determined by measuring the refractive index at 20°C according to the International Honey Commission IHC (2002) for honey. This determination was conducted using an Abbe Refractometer (HANNA, HI968601, Romania).

pH, free acidity, lactone acidity and total acidity

The measurement of pH and determination of free acidity was performed according to the International Honey Commission IHC (2002), after obtaining a solution of 10 g of sample dissolved in 75 mL of carbon dioxide-free water. The pH was measured using a potentiometer combined with glass electrode (Thermo Electron Corporation, Orion 3 STAR, USA). After the measurement of pH, free acidity was determined by titration with 0.1 M NaOH (Pronalab, Madalena, Portugal) to pH 8.30 (free acidity). Immediately a volume of 10 mL 0.05 M NaOH was added and, without delay, back-titrated with 0.05 M HCl (Fisher Scientific UK Ltd, Loughborough, UK) to pH 8.30 (lactone acidity).

HMF content

The HMF concentration was determined according to the harmonized method for honey. One gram of "água-mel" samples was diluted up to 50 mL with distilled water, filtered on 0.45 μm filter and immediately injected in a HPLC (Hitachi, LaChrom Elite, Japan) equipped with a Diode Array Detector (L-2455), Autosampler (L-2200) and Pump (2100/2130). The HPLC column was a Merck KGaA, Lichrosorb RP-18, 10 μm, Hibar 250-4. The HPLC conditions were the following: isocratic mobile phase, 90% water and 10% methanol HPLC grade (Labscan, Dublin, Ireland); flow rate, 1 mL/min; injection volume, 20 μL. The wavelength was 285 nm. HMF was identified by splitting the peak in "água-mel" with a standard HMF (Acros Organis, New Jersey, USA), and by comparison of the spectra of the HMF standard with that of an "água-mel" sample. The amount of HMF was determined using an external calibration curve (8-500 mg/L). Data were elaborated using EZChrom Elite (VWR International, Carnaxide, Portugal). Each sample was analyzed in triplicate.

Fructose and glucose

Fructose and glucose were determined according to the International Honey Commission IHC (2002). About 0.5 g of "água-mel" was weighed directly into polypropylene tubes and mixed with 10 mL 25% methanol. Afterwards, 1 mL of the solution was filtered through a 0.45 μm filter (VWR International, USA) prior to HPLC analysis. The determination of sugars was performed with the same high-performance liquid chromatograph equipped with a refractive index (RI) detector (Hitachi model L-2490, Japan). The separation was performed by using a Merck NH_2-bonded column for Carbohydrate Analysis (LiChroCART 250-4) with a particle size diameter of 5 μm, equipped with a guard column (Merck LiChroCART 4-4).

The column was kept at 30°C throughout the analysis. The HPLC pumps, autosampler, column oven and RI detector were monitored and controlled using EZChrom Elite system. The mobile phase was composed of 80% acetonitrile HPLC Grade (Panreac, Barcelona, Spain) in water. The injection volumes of the samples were 20 μL, with a flow rate of 1 mL/min.

The HPLC sample peaks were identified by comparing the retention times obtained from standards. The "água-mel" samples were also spiked with standards in order to verify the iden-

tity of the chromatographic peaks. Triplicate injections were performed and average peak areas were used for the peak quantification. The standard of fructose (3-20 g/L) and glucose (4-20 g/L) were from Sigma (S. Louis, MO, USA). The amount of the monosaccharides was determined using an external calibration curve.

Estimation of total polyphenols

The total polyphenol content was determined by a modification of the Folin-Ciocalteu method and the results are expressed as mg gallic acid (Acros Organics, New Jersey, USA)/kg. The method was that followed by some authors for honey (AL *et al.*, 2009). Five grams of "água-mel" were treated with 50 mL of distilled water, mixed and filtered using a qualitative filter. Five hundred microlitres of this solution was mixed with 2.5 mL Folin-Ciocalteu reagent (0.2 N) (Merck KGaA, Darmstadt, Germany) for 5 min and then 2 mL of a Na_2CO_3 (PRONALAB, Lisboa, Portugal) solution were added (75 g/L). All samples were incubated at room temperature in the dark conditions for 2 h, and the absorbance was read at 760 nm.

The blank solution contained water instead of "água-mel". For calibration curve, a stock solution of gallic acid (1 g/L) was prepared for further dilutions (4-500 mg/L).

Estimation of total flavonoids

A method described by ISLA *et al.* (2011) was used for total flavonoids determination. Briefly, 0.5 mL $AlCl_3$ (Carlo ERBA reagents, Val de Reuil, France) (20 g/100 mL) was added to 0.5 mL of "água-mel" samples. After 1 h at room temperature, absorbance was measured. Total flavonoid contents were expressed as mg quercetin (Alfa Aesar GmbH & CoKG, Carsluhe, Germany)/kg of "água-mel" (mg/kg of "água-mel"), using a calibration curve over the range of 15.6-125 mg quercetin/L.

Estimation of proline content

The proline content was determined by using a colour comparison after applying ninhydrin, with a proline standard. The content was expressed as a proportion to the mass of "água-mel" tested. The proline content was determined according to the harmonized method for honey developed by the International Honey Commission IHC (2002). A solution (0.5 mL) of "água-mel" (0.05 g/mL) was mixed with 1 mL of formic acid (80%) (Acros Organis, New Jersey, USA), 1 mL of ninhydrin (Acros Organis, New Jersey, USA) solution [3% in ethylene glycol monomethylether, from (Panreac Quimica, Barcelona, Spain)] and shaken vigorously for 15 min. The mixture was placed in a boiling water bath for 15 min and transferred to a 70°C bath for 10 min. Five mL of 50% 2-propanol (Riedel-de-Haën, Seelze, Germany) in water was then added to the mixture and was left to cool. The absorbance was read at 510 nm, 45 min after removal from the 70°C water bath. Water was used as blank and 0.032 mg/mL solution of proline (Acros Organis, New Jersey, USA) was used as standard solution.

Proline concentration in mg/kg of honey was calculated as follows:

Proline (mg/kg) = (Es/Ea) x (E1/E2) x 80, where Es is the absorbance of the sample solution; Ea is the absorbance of the proline standard solution (average of 3 readings); E1 is the mg of proline used for the standard solution; E2 is the weight of "água-mel" in grams; 80 is the dilution factor. The mean of three readings was used.

Diastase activity

Two different methods are used to determine honey diastase. The traditional Schade method uses starch as a substrate. The diastase activity of "água-mel" was evaluated by the methodology previously reported in the International Honey Commission IHC (2002).

RESULTS AND DISCUSSION

All tested samples were negative for all microbiological indicators, except sample 1M/2011 that was contaminated with 3,41±0,09 Log_{10} CFU/g of aerobic mesophilic bacteria and 4,05±0,11 Log_{10} CFU/g of sulphite-reducing *Clostridium* spp.

The consumption of the tested "água-mel" samples poses no risk to human health, except sample 1M/2011 that evidences a contamination with *Clostridium* spp. The consumption of honey or honey derivatives contaminated with *C. botulinum* poses a particular risk to children, elderly and immunocompromised individuals (EUROPEAN COMMISSION, 2002; ANONYMOUS, 2005). The sources of contamination of honey or its derivatives with *Clostridium* spp. may occur through bee's digestive tract, pollen, soil, dust and from not properly cleaned equipment and processing areas (NEVAS *et al.*, 2006). In Portugal botulism cases are rare: between 2003-2006 the mean of notified cases were 8 cases (Direcção Geral de Saúde, 2007) and the first infant botulism case was just reported (SARAIVA *et al.*, 2012). The case involved a 1-month-old infant that was breastfed but his parents used to give him chamomile tea and occasionally honey (chamomile tea and honey brought from Moldavia). In the case reported *C. botulinum* type B was isolated from infant faeces sample and as well from chamomile tea herbs and honey (SARAIVA *et al.*, 2012). In what concerns "água-mel" sample 1M/2011, the identification of the *Clostridium* spp. contamination source is under investigation and the produc-

er will be instructed with actions to eliminate the source of contamination.

The physicochemical parameters of "água-mel" of beekeepers from Portugal are depicted in Tables 1 and 2. The values found for ash (%) ranged from a minimal 0.167% to a maximal of 0.474%. A great variability was even detected for "água-mel" produced by the same beekeeper but in different years, such as observed for 1A/2010 and 1A/2011, and 1B/2008 and 1B/2011 (Table 1). Ash represents the direct measure of inorganic residues after "água-mel" carbonization. In honey, the ash percentage expresses its richness in mineral content and constitutes a quality parameter, which depends mainly from floral origin of honey (MARCHINI *et al.*, 2007; BOGDANOV *et al.*, 2009; ALOISI, 2010). The diversity of ash percentages found in "água-mel" samples in the same producer but in different years may reveal the utilization of honeys from different floral origins.

Electric conductivity is a quality parameter which is closely related to the concentration of mineral salts, organic acids and proteins and shows a great variability according to the floral origin (ACQUARONE *et al.*, 2007; ZERROUK *et al.*, 2011). The values of electric conductivity found are within the range reported by SPANO *et al.* (2008) for "abbamele" samples from Sardinia. The sample 1M/2011 (1,067 μS/cm) was the sole exception which may be partly explained by the presence of aerobic mesophilic bacteria and *Clostridium* spp.. A linear relationship has been reported between ash and electric conductivity of different types of honey (SANCHO *et al.*, 1991; MALIKA *et al.*, 2005), although some authors consider that such relationship may depend on the floral origin of honeys (THRASYVOULOU and MANIKIS, 1995). In our samples of "água-mel" a direct correlation was found between those two parameters (r=0.980, $P<0.01$). Fig. 2 depicts such correlation.

The water content found in one sample of "água-mel" (1M/2011) exceeded the maximum moisture content of 25% established by European legislation (EU Council, 2002) for water content in honey. The remaining samples had lower levels, nevertheless the majority showed percentages superior to 20%, which is generally found in unifloral honeys from Europe (MATEO and BOSCH-REIG, 1998; PERSANO and PIRO, 2004), and our sample values are within the range found for "abbamele" from Sardinia (SPANO *et al.*, 2008). The only exclusion was samples 1B/2011 and 1H/2011 that showed lower 20% water content. This variability of water content in "água-mel" samples may be attributed to three main factors: the residual moisture

Table 1 - Physico-chemical results obtained from "água-mel" samples of Portugal.

Beekeeper/ Year	Ash (%, w/w)	Electrical conductivity (μS/cm±SD)	Water content (%, ±SD)	pH±SD	Free acidity (meq/kg ±SD)	Lactone acidity (meq/kg ±SD)	Total acidity (meq/kg ±SD)
1A/2010	0.185±0.005	407.89±0.32	22.0±0.1	3.25±0.00	53.6±0.6	19.84±1.14	73.45±1,35
1A/2011	0.425±0.022	715.22±3.05	23.0±0.0	3.35±0.00	83.3±1.6	17.22±1.14	100.51±2.68
1B/2008	0.202±0.019	487.89±3.87	24.2±0.0	3.44±0.01	52.6±0.5	18.53±3.41	71.15±3.08
1B/2010	0.284±0.008	563.33±2.64	25.0±0.0	3.49±0.00	57.3±0.6	18.53±1.97	75.81±2.24
1B/2011	0.425±0.022	751.89±0.27	19.2±0.0	3.35±0.00	102.2±0.5	20.50±0.00	122.72±3.64
1H/2011	0.209±0.013	418.44±0.43	18.3±0.1	3.56±0.01	40.7±0.5	14.60±0.00	55.27±3.67
1I/2011	0.167±0.017	439.44±2.12	22.8±0.0	3.49±0.01	33.2±0.6	20.50±0.00	53.72±0.51
1M/2011	0.474±0.093	1067.00±1.68	>25.0	3.91±0.01	74.8±0.7	15.26±1.14	90.03±1.15
2A/2011	0.442±0.013	765.56±3.20	20.1±0.1	3.35±0.00	91.2±0.2	18.53±3.41	109.76±0.79
2B/2011	0.392±0.013	696.44±3.15	23.4±0.1	3.35±0.00	77.5±0.9	15.91±1.14	93.42±2.07

Table 2 - Monosaccharide, phenols and HMF results obtained from "água-mel"samples of Portugal.

Sample	Glucose (g/kg±SD)	Fructose (g/kg±SD)	Total polyphenols (mg/kg±SD)	Flavonoids (mg/kg±SD)	HMF (mg/kg±SD)
1A/2010	241.16±1.20	306.15±2.03	2718.7±75.45	603.2±6.92	4370.7±71.0
1A/2011	258.52±3.05	266.34±3.79	4508.5±116.36	1316.6±16,14	6620.0±79.8
1B/2008	241.01±3.88	299.19±5,01	2403.1±67.51	582.6±6.57	3327.9±29.9
1B/2010	229.63±6.11	288.02±1.72	2733.9±46,71	698.6±5.04	3938.6±128.2
1B/2011	250.65±0.64	269.22±0.96	4963.8±93.96	1702.4±13.89	6884.2±57.4
1H/2011	252.41±14.76	315.36±20.01	4125.8±96.21	355.6±9.34	1812.6±13.6
1I/2011	243.46±3.24	289.75±3.87	1780.0±40.48	188.8±6.47	1969.5±4.5
1M/2011	185.57±0.33	218.49±0.72	2777.9±23.32	278.3±2.53	4352.9±157.8
2A/2011	246.12±14.53	252.92±0.93	2718.7±81.97	1579.7±11.97	8428.9±42.9
2B/2011	237.94±8.33	271.94±11.45	2611.0±20.42	1155.7±24.98	6506.5±31.1

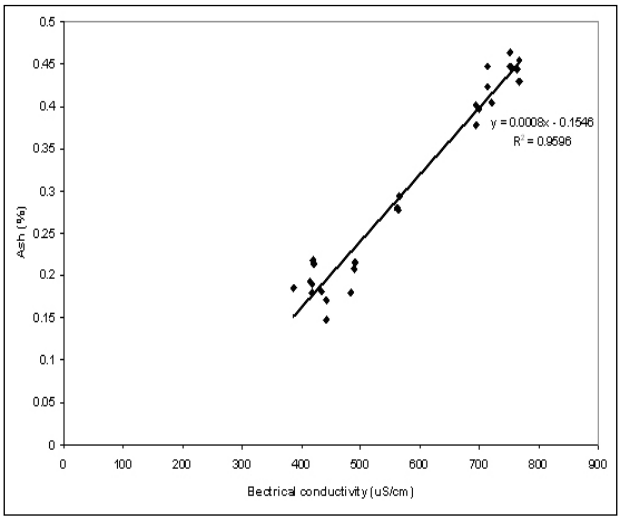

Fig. 2 - Correlation found between ash and electrical conductivity in "água-mel" samples.

in the honeycombs, the amount of water initially added for treating honeycombs, the temperature used and the length of the heating process (SPANO *et al.*, 2008).

The parameter pH is within the ranges reported for honey (3.5-5.5) and "abbamel" from Sardinia (Italy) (3.21-3.92) (SPANO *et al.*, 2008). Nevertheless the free acidity parameter of some samples was superior to that reported for honey (not more than 80 meq/kg for baker's honey) (EU Council, 2002). In our case, a great range of values was found: 33.2 meq/kg for sample 1I/2011 and 102.2 meq/kg for IB/2011 (Table 1).

The acid content of honey is due to the acids added by the bees, being the main one gluconic acid, a product of glucose oxidation by glucose oxidase. This acid is generally present in honey as its internal ester, a lactone, which does not contribute to honey's active acidity (BOGDANOV *et al.*, 2004). Lactone acidity is determined by adding an excess of alkali to a neutralized honey and back-titrating with acid (WHITE *et al.*, 1958). The sum of free acidity and lactone acidity gives the total acidity of honey samples. Lactone acidity in "água-mel" ranged from 14.60 to 20.50 meq/kg. Total acidity exceeding 100 meq/kg were found in samples 1A/2011, 2A/2011 and 1B/2011 (Table 1).

Glucose and fructose were the main sugars present in "água-mel", predominating fructose in all samples. The amounts found in our samples were similar to those already reported for some "abbamele" samples from Sardinia (JERKOVIĆ *et al.*, 2011). The variability found and according to the same authors may be attributed to the preparation procedure, rather than with different types of honey used. In our samples such is not so evident because for the same producer but in different years of production the amounts of the two monosaccharides changed (Table 2). It is our opinion that both factors may be responsible for the observed differences.

The absence of diastase activity in all samples is understandable since "água-mel" is a product obtained by heating the raw material for several hours, inactivating enzymes. The same would be expected for proline as also reported for Italian "abbamele" (SPANO *et al.*, 2008). In fact, only one sample had a significant content of this amino acid (462.4 mg/kg): 1M/2011. This value may be associated to the presence of *Clostridium* ssp. which can synthesize this amino acid by the Stickland reaction (NISMAN, 1954). The absence of proline in all samples, excepting 1M/2011, may be attributed to an increased rate of Maillard reaction, which is initiated by the reaction of a carbohydrate with a compound possessing a free amino group (PAÄT-ZOLD and BRÜCKNER, 2006; CZIPA, 2010). The levels of sugars slightly inferior to those reported for honey samples may also be attributed to their consumption in the Maillard reactions, as reported for OOSTERVELD *et al.* (2003) in roasted *Coffea arabica* beans. These authors reported that during roasting, oligosaccharides hydrolyse to monosaccharides and both are able to react with protein/peptides/amino acids originating Maillard products which are responsible for the formation of volatile compounds and organic acids (OOSTERVELD *et al.*, 2003). The relative high free and total acidity found in "água-mel" samples may be, therefore, endorsed to the liberation of organic acids. Such may explain the inverse correlation obtained between fructose and total acidity ($r=-0.796$; $p<0.01$). Fig. 3 represents this inverse relation between fructose and total acidity, which was not detected for glucose.

At same time, the thermal treatment is also responsible for the degradation of monosaccharides to hydroxymethylfurfural (HMF), and therefore to the decrease of sugars, particularly fructose, because an inverse correlation was obtained between the fructose concentration and

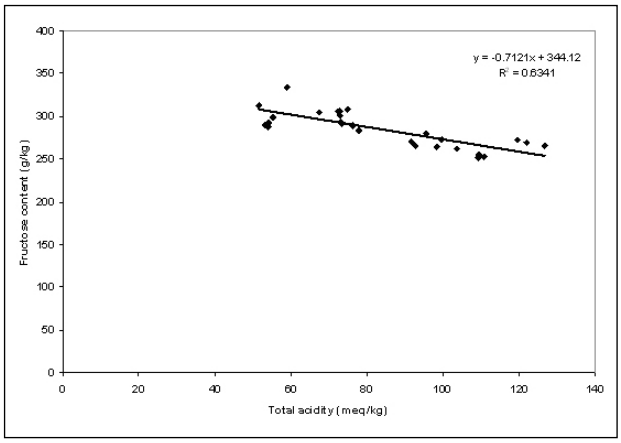

Fig. 3 - Correlation found between total acidity and fructose content in "água-mel" samples.

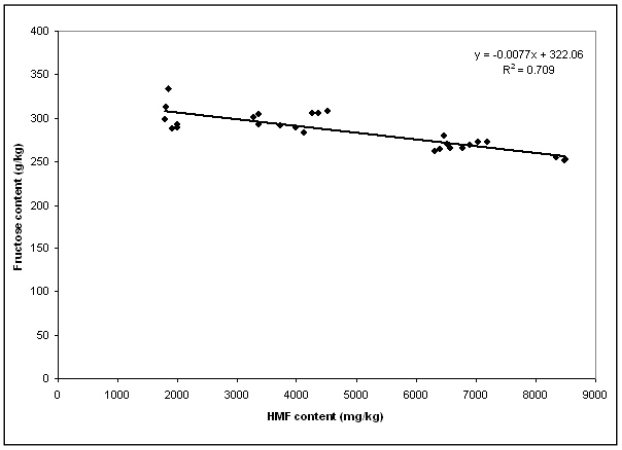

Fig. 4 - Correlation found between HMF content and fructose content in "água-mel" samples.

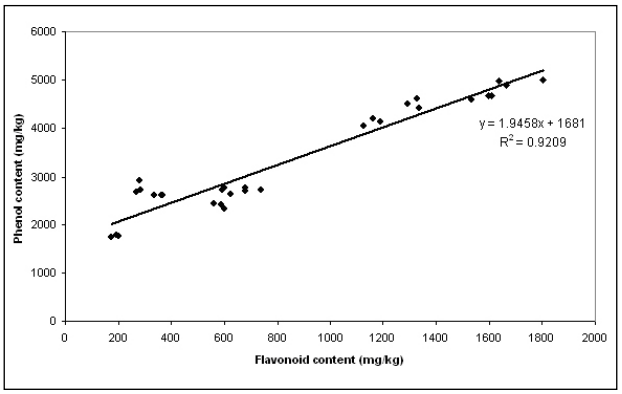

Fig. 5 - Correlation found between flavonoid content and phenol content in "água-mel" samples.

HMF content (r=-0.842, *p<0.01*) as can be seen in the Fig. 4.

As expected, HMF content (Table 2) is largely superior to that of honey samples, nevertheless comparable to those found for "abbamele" (SPANO *et al.*, 2008; JERKOVIĆ *et al.*, 2011). Nevertheless some samples (2A/2011) showed higher concentrations (8428.9 mg/kg) in comparison to the sample with the highest concentration reported by SPANO *et al.* (2008) (4,476 mg/kg), still within the ranges found for liquid caramel as reported by those authors. In order to have reference HMF values from thermally treated foodstuffs the HMF value of liquid caramel, instant coffee powder and coffee substitutes was determined. These foodstuffs showed higher HMF values in comparison to our "água-mel" samples: liquid caramel (9,700 mg/kg), instant coffee powder (11,500 mg/kg), and coffee substitutes (9,700 mg/kg).

Polyphenol concentration ranged from 1780.0 mg/kg in sample 1I/2011 to 4963.8 mg/kg in sample 1B/2011 (Table 2), amounts significantly superior to those reported by SPANO *et al.* (2008), but within the ranges reported by JERKOVIĆ *et al.* (2011) As reported by these authors, such differences may be explained by different sample preparation and data expression.

It is remarkable the great variability of flavonoid content in "água-mel" samples, even in the same producer but in different years of production. For example, for samples 1A (2010 and 2011) and 1B (2008, 2010 and 2011) a large variability in flavonoids content was observed (Table 2). This variability may result from diverse floral origin of honeys used in different years for the production of "água-mel". The levels of flavonoids go along with the amounts of total polypehenols, there is even a significant correlation between these two parameters (r=0.960, *p<0.01*) as can be observed in the Fig. 5.

Our study shows that "água-mel" obtained from honey after thermal treatment continue to possess some of the parameters similar to those of honey, including pH, glucose and fructose as main constituents, but generally relative higher percentages of water content, free acidity and HMF, and low levels or even absence of proline and diastase activity, as expected due to the thermal processing, but the values of HMF in our samples are comparable to other foodstuffs. The presence of phenols and flavonoids in "água-mel" was as predicted due to their invariable presence in honey. Nevertheless higher quantities were detected which must be enlightened because some interference may be responsible for such data. The determination of the microbiological quality of "água-mel" showed the presence of *Clostridium* spp. in one of the samples. This result shows that as for honey the use of "água-mel" is not recommended for infant feed.

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