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Physicochemical parameters and antibiotics residuals in Algerian honey

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The aim of the present study was to evaluate the quality of 36 samples of different honey type supplied by local producers from Algeria in order to verify its compliance with the standards of Codex Alimentarius and European Union (EU). For that, five physicochemical parameters were analyzed using the HPLC method: hydroxyl-methyl furfural (HMF), sugars, diastase activity and search of antibiotic contamination with streptomycin and tetracycline. The physicochemical analyses of the Algerian honeys show that 56% of samples correspond to Codex standards and 44% not in conformity with the standards required by the Codex Alimentarius and EU, because part of the samples had one or more defects. The percentage not in conformity was due to the high rates of hydroxyl-methyl furfural, sucrose and also to the low enzyme level. Analysis performed by the laboratory to detect residues of tetracycline and streptomycin in honey have revealed insignificant traces of oxytetracycline in two samples of honey (0.03 ppb). From the present study, it is observed that the Algerian honey samples is not completely in agreement with the requirements of international honey standards which could be caused by inappropriate actions during processing and storage steps.

Key words: Honey quality, sugar, diastase activity, hydroxymethylfurfural, antibiotic residues.

INTRODUCTION

The Codex Alimentarius (2001) define honey as a natural sweet substance, produced by honeybees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living

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parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature. Honey composition basically depends on the nectar composition of each producing plant species, conferring specific characteristics to it (Marchini et al., 2007). Honey is a complex mixture which presents very great variations in composition and characteristics due to its geographical and botanical origin, its main features depend on the floral origin or the nectar foraged by bees (Kebede et al., 2012; Saxena et al., 2010).

The composition and quality of honey also depend on several environmental factors during production such as weather and humidity inside the hive, nectar conditions and treatment of honey during extraction and storage. However, quality and composition of honey are negatively affected by other factors such as overfeeding with sucrose and other sucrose variants, harvesting prior to maturity, unhealthy storage conditions and overused veterinary drugs (Sahinler et al., 2004).

Honey is composed primarily of the sugar: glucose and fructose; its third greatest component is water (Singh et al., 2012). Honey also is composed of a complex mixture of carbohydrates and other less frequent substances, such as organic acids, amino acids, proteins, minerals, vitamins, lipids (Blasa et al., 2006; Ball, 2007; Zerrouk et al., 2011), aromatic compounds, flavonoids, vitamins, pigments, waxes, pollen grains, several enzymes and other phytochemicals (Gomes et al., 2010; Lazarevic et al., 2010; de Almeida-Muradian et al., 2013).

According to the Codex Alimentarius and Council Directive of the European Union (EU), honey is a natural product and should be exempt of contaminant. On the other hand, contamination of honey may occur through the common use of antibiotics such as the streptomycin and its derivative dihydrostreptomycin (DHSTR) which often combined with tetracycline. These antibiotics are used as veterinary drugs or crop-protection agents in broad-spectrum anti infection formulation (Michel et al. 2004) because they are against both Gram-positive and few Gram-negative bacteria (Kwapong et al., 2013).

In order to guaranties the nomination of honey and also protect human health, the use of antimicrobials in apiculture is usually strictly regulated or banned. According to Regulation (EC) No 470/2009 and Regulation (EU) No 37/2010, in the European Union, no maximum residue level (MRL) for tetracycline and any other antibacterial substance residues in honey are allowed (Cara et al., 2012).

Considering the nutritional properties of honey and its scarcity in the Algerian market, it is exposed to fraud. To check its quality, various international institutions, such as the International Honey Commission (IHC), the Codex Alimentarius and the European Commission propose methods of analyses to ensure that honey is authentic in respect to the legislative requirements.

Algerian honey production is estimated to average 33,000 quintals in 2011 with a yield of 4-8 Kg/hive (Oudjet, 2012), which is less than the needs of local consumption while it is supposed to be at the origin of an important export outlet. This low production affects the price and makes it remain high. Therefore, consumption remains as low as production. This lack of production is the result of multiple causes such as absence of national regulation, lack of a professional organization and insufficient quality control laboratory (Bendeddouche and Dahmani, 2011). Nevertheless, Algerians researchers and scientists try to establish correct denominations to assure a minimum marketing level of the product.

The aim of the present study was to evaluate the quality of 36 samples belonging to different honey type supplied from local producers from Algeria in order to verify its compliance with the standards of Codex Alimentarius, 2001 and the Council of the European Union (EU), 2002. Five physicochemical parameters were analysed using the HPLC method: hydroxyl-methyl furfural, sugars, diastase activity and search of antibiotic contamination with streptomycin and tetracycline.

MATERIALS AND METHODS

Sampling

Thirty six (36) honey samples produced in various regions of Algeria (Figure 1) were collected directly from beekeepers or from apicultural corporations or from beekeepers vendors of trade fairs (Local open air markets) between July and September 2012. All honey samples were labeled either according to their botanical and geographical origin as suggested by the beekeepers; sampling was accompanied by an interview with the beekeeper for information on these honeys as date of harvest and mode of extraction, or according to the testimonies of the vendors. The samples were stored in a refrigerator at 4-6°C in airtight plastic containers until analysis.

Technical analysis

Determination of diastase activity

The diastase activity was measured using the Phadebas amylase test tablets purchased from (Megazyme) (Phadebas method), insoluble blue-dyed, cross-linked starch was used as the substrate for the degradation reaction (Sak-Bosnar and Sakac, 2012), according to the International Honey Commission (Bogdanov, 2002). This is hydrolysed by the enzyme, yielding blue water-soluble fragments, determined photometrically at 620 nm with molecular Devices Spectramax 340 Microplate Reader. The absorbance of the solution is directly proportional to the diastase activity of the sample. The diastase activity, expressed as DN or diastase number, was calculated from the absorbance measurements using Equations (1) and (2), respectively:

$$DN = (28.2 \cdot \Delta A_{620}) + 2.64 \quad (1)$$

$$DN = (35.2 \cdot \Delta A_{620}) - 0.46 \quad (2)$$

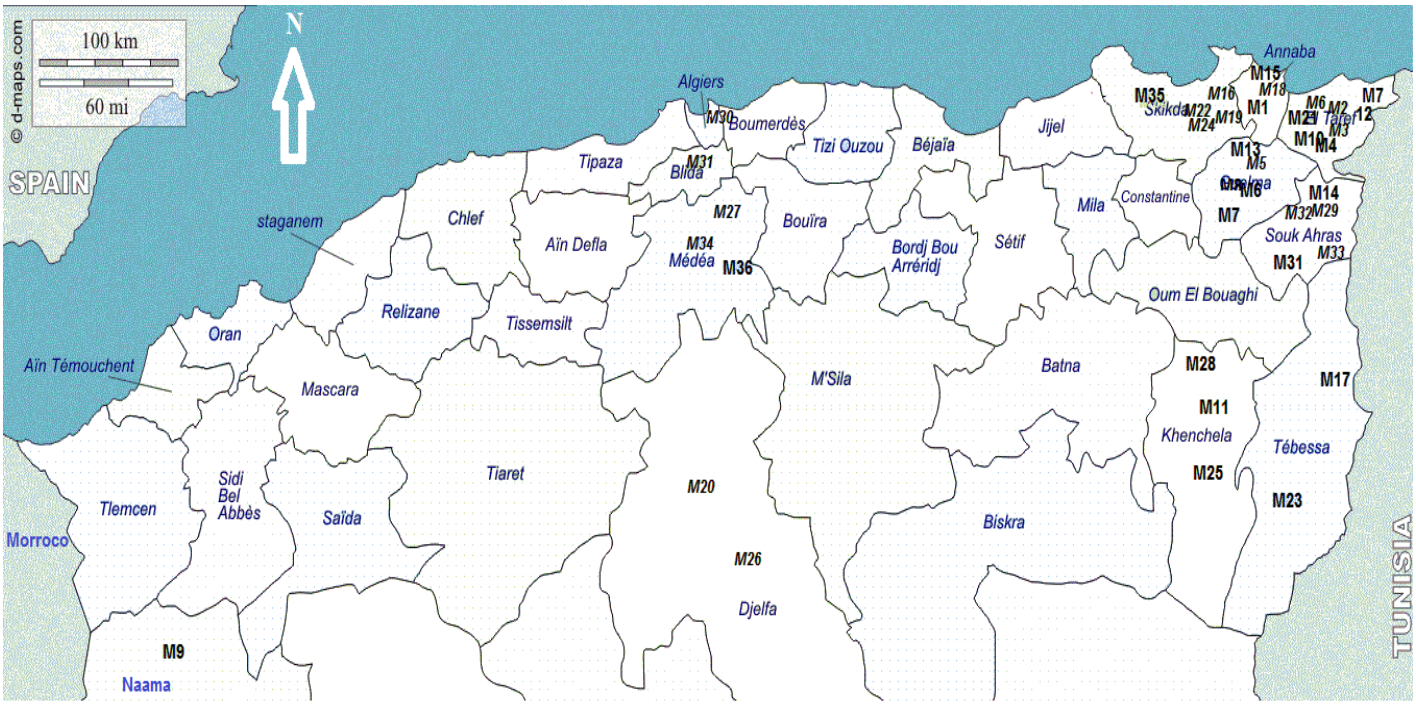


Figure 1. Geographical origins of Algerian honey samples.

For either high (8 to 40 diastase units) or low (up to 8 diastase units), respectively, Equations 1 and 2 are suggested by the International Honey Commission (Bogdanov, 2002). Diastase activity was referred to as diastase number (DN) in the Schade scale, which corresponds to the Gothe scale number, or to gram of starch hydrolysed per hour at 40ºC per 100 g of honey.

Determination of sugars

Determination of sugars in honey was established in the current study by the use of a chromatographic system (HPLC SHIMADZU LC-20 AD) with RI detector (IOP) and Column chromatography apHera NH_2 150 mm x 4.60 mm x 5 microns (or equivalent). Fructose, glucose and sucrose were measured, according to the analytical methods harmonized by the European Honey Commission (Bogdanov, 2002).

The chemical reagents used for HPLC analysis were acetonitrile and methanol (HPLC grade) from Sigma- Aldrich (Milan, Italy). The sugar standards used were fructose, glucose, sucrose of 99.5% purity from Sigma- Aldrich (Milan, Italy).

10.00 ± 0.01 g of honey was weighed in a beaker of 100 ml and dissolved with distilled water (40 ml) which was quantitatively transferred in graduated flask. After that, 25 ml of methanol was added to the solution and completed to 100 ml with distilled water. The solution was filtered with a nylon filter (0.45 µm), and the first 2 ml was discarded and further chromatographic analysis under the following operating conditions was done: Flow rate of 1.5 ml / min, injection volume of 20 µl, and detector temperature of 30 ± 1°C. The acquisition time was 10 min. Identification of the sugars was carried out by comparing the retention times of the peaks of the sample solution with that of the reference solution. The sugar concentration was calculated by comparing the peak area of the sample corresponding to the peak of the reference solution.

Determination of HMF

The method determines the concentration of 5-(hydroxymethyl-) furan-2-carbaldehyde (HMF) (Fallico et al., 2004). The determination of HMF was carried out in solutions of honey samples (5.00 ± 0.01 g of honey) and diluted to 50 ml with distilled water, filtered on 0.45 µm filter and injected into an HPLC (HPLC SHIMADZU LC-20 AD) equipped with: pump UV detector, auto sampler, column thermostating system, data acquisition and processing system. The HPLC was chromatography on reverse phase column with dimensions of 150 x 3 mm, packed with octadecylsilane (C18), containing particles of a diameter of 2.7 microns. The HPLC conditions were the following: isocratic elution with 85% water and 15% methyl alcohol. All the solvents were of HPLC grade (Sigma-Aldrich). The column was thermostated at 34°C; at flow rate of 1.0 ml / min and injection volume of 4 µl. The wavelength range was 285 nm, and acquisition time was 3 min. HMF was identified from the peak in honey with a standard HMF from Sigma-Aldrich (Milan, Italy), and by comparison of the spectra of the HMF standard with that of one honey samples. The amount of HMF was determined using an external calibration curve, measuring the signal at λ=285 nm.

Analysis of residues

The quantitative analysis of residues of streptomycin was performed with HPLC technique according to Albino et al. (2005). Sample (5 g) was mixed with 20 ml of extracting solution (sodium heptasulphonate (0.05 M) and sodium hydrogen phosphate (0.08 M, pH=2). The solution was then vortexed for 30 min and centrifuged for 10 min. Streptomycin was eluted with methanol and evaporated by a Rota vapor (60°C, 250 mbar). The residues were recovered with 1 ml of sodium dodecylsulfate (70 mM). The mixture

was vortexed and then put in ultrasonic bath for 5 min (Gallina et al., 2005; Baggio et al., 2009). Streptomycin was detected and quantified by the external standard calibration. HPLC analyses were performed, using post-column derivatization, performed in 1 ml of reaction coil placed in a column heater (55°C), and fluorimetric detection system (SHIMADZU 20 AD HPLC) with fluorescence detection (SHIMADZU, Japan) and chromatographic column Alltech (Alltech, Italia) C18 Platinum 5 µM 250 mm x 4.60 mm. After filtration, a 100 ml aliquot of residue solution was injected into the chromatographic system. The streptomycin was analyzed at 1 ml /min of flow with an isocratic elution of mobile phase 40% of sodium dodecyl sulfate (0.1 M) + sodium 1,2-naphthoquinone-4-sulfonate (0.5 mM) and 60% of acetonitrile for 15 min. Post-column, a sodium hydroxide solution 0.2 M was added (0.4 ml /min) in flow. Excitation and emission wavelengths of 263 and 435 nm, respectively, were used to detect the streptomycin. Samples quantification was performed using the external standards. This approach allows determining the streptomycin in honey in the range of concentrations ranging from 5 to 200 ng/kg. The positive results were confirmed by co-chromatographic method according to Decision 657/2002/CE.

This method allows the determination of tetracyclines (tetracycline, oxytetracycline, chlortetracycline and doxycycline) in honey in the range of concentrations ranging from 3 to 30 ng/g. A honey sample (5 g) was dissolved with 25 ml extraction buffer (succinic acid 0.1 M, pH 4). The sample solution was vortexed and then centrifuged for 10 min at 4000 *g*. The supernatant layer was recovered and purged in a metal-chelating affinity column (MCAC) cartridge. Tetracyclines were eluted with McIlvaine EDTA solution (citric acid, disodium hydrogen phosphate, EDTA and sodium chloride). The MCAC cartridge was prepared by fulfilling with chelating sepharose fast-flow resin (1.5 ml) and conditioned with copper sulfide solution (0.01 M), the quantitative analysis of any residues of tetracycline was performed with HPLC-MS technique according to Gallina et al. (2005), Cristofani et al. (2009) and Baggio et al. (2009) with some modification, using HPLC SHIMADZU coupled to LCMS-2010 EV rivelatore and chromatographic column C18MS (100 mm x 2,1) 5 µm X TERRA (Waters). The flow rate was 0.6 ml/min; Injection volume was 20 µl, at room temperature with a gradient for elution of mobile phase consisting of 0.5% formic acid + methanol +acetonitrile (50/50). The wavelength used to detect the TCs was 365 nm. The positive results were confirmed by co-chromatographic method according to Decision 657/2002/CE.

The standard solutions were obtained by Sigma-Aldrich. The solvents used were HPLC grade (99.9%) and the other chemicals were all of analytical grade from Sigma-Aldrich (Milan, Italy). Before being applied for HPLC analyses, all solutions were filtered by micro-filter (4.5 µm).

Statistical analyses

Physic-chemical results were compared with International Regulatory Standards. Means, standard deviations and the correlation coefficient (HMF-Diastase index) were calculated by using the software (Statistica version 10).

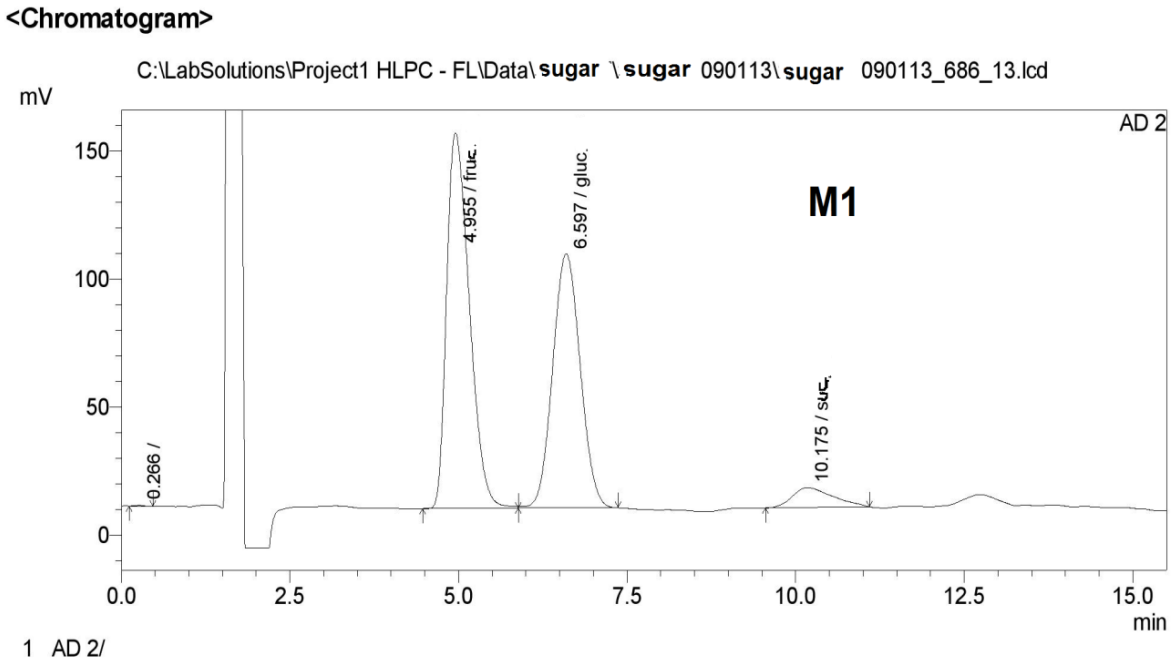
RESULTS AND DISCUSSION

The physicochemical analyses carried out on Algerian honey showed that 56% of samples met Codex standards and 44% did not, because a part of them have one or more defects (Table 1). The latter was due to the high rates of hydroxyl-methyl furfural, sucrose and also to the low enzyme level.

The HMF for the 36 honey samples analyzed in the present work ranged from 0.170 to 571.9 mg/kg and revealed that 26 of them (72%) had levels below the limits of HMF Codex acceptable standard (≤40 mg/kg) according to Codex Alimentarius (2001) and Council Directive of EU (2002), indicating the use of good practices by beekeepers. Eleven (11%) had a rate of HMF between 40 and 80 mg/kg and showed inadequate HMF content. According to White (1992), honey samples from subtropical countries may have naturally high HMF values regardless of the fact that the honey was not overheated or adulterated, due to high temperatures (Silva et al., 2013). However, nine samples were considered of unacceptable quality with very high values of HMF. These values relate to samples M5, M19, M27, M29, M34, and M36 and can be explained by inadequate treatment of these honeys probably overheating (Singh and Bath, 1998; Kubis and Ingr, 1998; Zappalà et al., 2005; Zerrouk et al., 2011), poor storage conditions and old honey (Khalil et al., 2010). Besides that, it is reported that extremely high >500 mg/kg HMF values demonstrate an adulteration with invert syrup (Coco et al., 1996; Popa et al., 2009; Ajlouni and Sujirapinyokul, 2010), also it is reported that the use of high fructose corn syrup as sweetener can lead to high HMF content reaching 100-1000 mg/kg (Makawi et al., 2009). Thus, there is a very high possibility that the honeys bought from the open-air markets are adulterated, since their HMF values are higher than 500 mg/kg.

These studies have yielded similar results to previous results (Bendeddouche and Dahmani, 2011; Zerrouk et al., 2013; Ouchemoukh et al., 2007). Jilani et al. (2008) reported for Tunisian multifloral honeys HMF values which also showed heat influence (HMF ranged between 3.0-39.6 mg/kg). In Morroco, Chakir et al. (2011) have obtained values of HMF limited between 0.09 and 53.38 mg/kg, but four honey samples contained a high HMF value which included between 90.76 and 783 mg/kg. The hot Algerian climate may also be the origin of this phenomenon as similar heat damage was also observed in the mean diastase number (Makhloufi et al., 2010). However, it is essential to quantify this component in order to check on product quality (Marchini et al., 2007), because HMF is a compound that may be mutagenic (Sommer et al., 2003; Glatt et al., 2005). Furthermore, it may also be carcinogenic (Kowalski et al., 2013) and cytotoxic (Islam et al., 2014).

Diastase is a natural enzyme of honey. The diastase activity measured the combined activities of both α-amylases and β-amylases which were secreted from bee salivary (Vit and Pulcini, 1996; Chua et al., 2014). Enzyme activity in honey depends on the intensity of the



Quantitative Results

detector

ID#	Name	Ret. Time	Area	Height	Conc.
1	Fructose (Fruc)	4.955	3433078	146574	0.000
2	Glucose (Gluc)	6.597	2815284	99243	0.000
3	Sucrose(Suc)	10.175	324092	7793	0.000

Figure 2. Sugar chromatograms of some Algerian honey (example of sample M 1).

nectar flow and the amount of nectar the bee processes in each period (Escuredo et al., 2011). In addition, the diastase activity and the diastase content varies according to floral source (White et al., 1962). Diastase and invertase activities are commonly used in Europe as an indicator for honey freshness (Manzanares et al., 2011). This is because the enzyme activities decrease in heated or old honey.

Nine samples (M5, M6, M16, M19, M21, M27, M29, M34 and M36) have a lower diastase index than the minimum standard value (superior than 8) from 00 - 45.30; the scale with an average value of 17.58 ± 12.27. The result can be due to either, overheating by beekeeper, or to the natural poor levels of amylase in the sample because the diastase activities in honey vary in wide limits depending on botanical origin of honey (Persano-Oddo et al., 1990) and thus, have a limited freshness indicating power; HMF is regarded as better quality criterion in this respect (Buba et al., 2013). Both HMF and diastase activity are the international parameters used to control the limit for thermal treatment to honey (Chua and Adnan, 2014). The correlation test between HMF content and diastase activity showed strong negative correlation (r = -0.605436). This confirms that, those two parameters are inversely proportionate to each other.

Glucose and fructose are the main sugars in honey and their actual proportion depends largely on the source of the nectar (Anklam, 1998). The sugars of honey were determined by HPLC and an example of chromatogram is presented in Figure 2. The results of the sugar analysis of all the 36 honey samples (Table 1) show that the fructose contents varied between 29.33 and 42.39% with an average of 37.61 ± 3.11%. The glucose contents of the samples were within a range of 25.38 -37.65% with a mean value of 31.88 ± 3.39%. In our study, 85.5% of the honey samples analyzed had fructose as the dominating sugar. In respect to reducing sugars (fructose and glucose), the EC Directive 2001/110 imposes reducing sugars ≥60 g/100 g, except for honeydew honey, which is ≥45 g/100 g. The reducing sugar contents varied between 60.18 and 79.29 g/100 g with an average of 69.50± 4.40 g/100 g. Our results met this standard and are similar to other published levels for reducing sugars. Furthermore, the reducing sugar content of the honey tested was similar with the findings of other previously

Table 1. Results of physicochemical analysis of 36 samples (Mean values ± standard deviations).

Sample	ID (Gothe scale)	HMF (ppm)	Sucrose (%)	Fructose (%)	Glucose (%)	Reducing sugar (%)	G/F ratio
M1	17.1±0.32	12.4±0.54	3.4±0.15	38.72±0.53	32.47±0.25	71.49±0.007	1.19±0.006
M2	20.81±0.02	5.6±0.04	2.09±0.09	42.00±0.16	27.15±0.27	69.18±0.02	1.54±0.01
M3	25.57 ±0.54	13.9±0.15	2.99±0.01	38.79±0.24	32.82±0.28	71.61±0.01	1.18±0.001
M4	12.95±0.32	30.9±0.97	2.50±0.61	38.78±0.28	32.34±0.96	71.11±0.02	1.20±0.02
M5	0.091±0.009	571.9±0.94	5.91±0.05	34.17±0.27	36.89±0.16	71.08±0.02	0.92±0.002
M6	6.46±0.06	73.5±0.1	1.20±0.08	40.43±0.44	34.60±0.13	75.02±0.02	1.16±0.004
M7	27.50±0.39	10.4±0.39	2.71±0.11	38.07±0.38	32.20±0.31	70.29±0.01	1.18±0.01
M8	31.25±0.35	9.7±0.25	2.47±0.02	38.63±0.009	32.36±0.08	70.97±0.04	1.19±0.005
M9	24.6±0.1	5.1±0.05	2.72±0.18	37.14±0.37	33.88±3.23	72.07±3.92	1.1±0.09
M10	14.15±0.49	78.8±0.06	3.67±0.02	37.82±5.19	30.71±0.64	74.52±0.02	1.23±0.13
M11	40.24±0.23	0.1±0.008	11.89±0.04	34.78±0.22	25.54±0.29	60.18±0.02	1.36±0.009
M12	18.25±0.05	40.3±0.13	2.44±0.08	37.93±0.14	32.72±0.08	70.63±0.04	1.15±0.00
M13	22.57±0.04	18.2±0.05	5.63±0.25	38.14±0.68	31.16±0.12	69.31±0.01	1.22±0.01
M14	36.651±0.47	21.5±0.5	2.92±0.17	38.25±0.09	31.36±0.11	66.61±4.26	1.21±0.001
M15	33.17±0.23	1.2±0.2	5.51±0.04	37.65±0.49	25.38±0.06	63.01±0.02	1.48±0.008
M16	4.36±0.15	41.6±0.07	2.02±0.76	37.39±0.27	31.00±0.31	68.09±0.007	1.20±0.004
M17	27.51±0.64	10.7±0.25	2.22±0.19	40.68±0.39	30.06±0,08	71.04±0.05	1.35±0.006
M18	26.54±0.66	5.6±0.12	2.19±1.18	37.98±0.23	30.36±0.07	68.32±0.03	1.25±0.006
M19	5.52±0.072	554.6±0.2	2.72±0.14	37.39±0.12	36.44±0.21	73.81±0.02	1.02±0.001
M20	26.73±0.05	0.6±0.05	5.00±0.01	36.65±0.067	29.56±0.99	66.21±0.01	1.24±0.02
M21	4.59±0.17	7,81±0.06	3.70±0.24	37.88±0.017	29.37±0.04	67.22±0.03	1.28±0.00
M22	9.25±0.50	29,7±0.08	1.57±0.14	42.39±2.35	36.89±0.42	79.29±0.007	1.14±0.04
M23	8.88±0.06	21,8±0.16	2.68±0.14	37.55±0.04	32.28±0.04	69.82±0.03	1.16±0.00
M24	14.33±0.16	11,6±0.18	1.20±0.23	40.93±0.05	32.64±0.04	73.54±0.05	1.25±0.001
M25	33.4±0.2	21,5±0.22	3.91±0.17	37.37±0.50	30.80±0.93	68.18±0.02	1.21±0.01
M26	45.3±10.78	1,12±0.16	6.27±0.10	39.41±0.48	26.48±0.26	65.90±0.007	1.48±0.002
M27	00	303,5±0.45	14.93±0.28	29.33±0.02	31.98±0.07	61.36±0.05	0.91±0.001
M28	10.72±1.94	5,7±0.05	5.90±0.32	31.94±0.01	30.71±0.06	62.43±0.3	1.04±0.001
M29	0.86±0.02	263,4±0.06	12.39±0.25	31.22±0.15	31.67±0.12	62.74±0.2	0.98±0.000
M30	15.80±0.29	28,8±0.85	2.98±0.11	36.23±0.01	29.56±0.10	65.78±0.02	1.22±0.001
M31	10.85±0.22	8,3±0.05	4.00±0.17	36.21±2.99	30.91±0.58	68.66±0.65	1.17±0.07
M32	14.87±0.17	16,8±0.09	2.49±0.16	36.61±0.02	28.05±0.21	64.88±0.31	1.30±0.006
M33	22.18±0.65	15,2±0.05	7.11±0.78	38.40±1.34	31.17±0.12	69.59±0.01	1.23±0.02
M34	0.11±0.005	514,3±0.26	6.02±0.24	33.69±0.79	37.079±0.03	70.79±0.01	0.90±0.01
M35	14.97±0.22	18,5±0.16	2.51±0.18	41.99±0.13	31.68±0.36	73.68±0.02	1.32±0.007
M36	0.12± 0.10	517,03±0.05	6.30±0.14	33.33±0.06	37.65±0.00	71±0.00	0.88±0.001
Min	00	0.170	1,20	29.33	25.38	60.18	0.88
Max	45.3	571.9	14.93	42.39	37.65	79.29	1.54
Limits of international standards (Codex, EU)							
	≥8	40	≤5	No fixed limit	No fixed limit	≥60	No fixed limit

studied Algerian honeys (Ouchemoukh et al., 2007; Khalil et al., 2012).

The fructose/glucose ratio ranged between 0.88 and 1.54 with an average of 1.19± 0.15, indicating their floral origin because it is known that flower honeys have a fructose/glucose ratio of about 1 while in honeydew honeys the ratio ranges between 1.5 and 2.0 (Gleiter et al., 2006; Kivima et al., 2014).

In addition, the fructose/glucose ratio was calculated for all the 36 honey samples. This ratio tells about the

Table 2. Results of antibacterial substances.

Parameter	Number of samples	Results	Limits of international standards (Codex)
Search of Tetracycline	34	No residues of tetracyclin	No fixed limit
Search of oxytetracycline	2	0.03 ppb	No fixed limit
Search of Streptomycin	36	No residues of streptomycin	No fixed limit

crystallization state of honey, that is, when fructose is higher than glucose the honey is fluid (Ouchemoukh et al., 2010). Eventually, 30 examined Algerian honey samples were fluid (ratio great than 1).

The sucrose content of the honey samples analyzed in this study varied between 1.18 - 14.9 g/100 g with an average value of 4.55± 0.12. However 11 samples are not in conformity with international standard (≤5 g/100 g). The high content of this sugar means most of the time, an early harvest of the honey, that is, a product in which the sucrose has not been fully transformed into glucose and fructose by the action of invertase. This value indicates probably that the beekeeper use sucrose syrup to over feeding the bees in the winter season (Chefrour et al., 2009).

Honey is generally considered a natural and healthy food without additives or other foreign substances according to Directive 2001/110/CE. However, in the last decade the results of residue analyses carried out on presently marketed honey have changed the situation revealing what was known by many (Bogdanov, 2006).

The aim of this study was to clarify the situation concerning antibiotic residues in some Algerian honey (Table 2). Analysis performed by the laboratory to detect residues of tetracycline by LC/MS and streptomycin by HPLC in honey have revealed only insignificant traces of oxytetracycline (Figure 3a & b) in two samples of honey (0.03 ppb). All the other samples were negative for both antibiotics (Table 2, Figure 3).

Accordingly, in Europe a lack of harmonization among different countries on this matter is registered. For instance, in Belgium the action limit for antibacterial substances is fixed at 20 ng/ g. In Switzerland and the UK, the action limit applied for tetracycline (TCs) is 20 ng/g, respectively (Bogdanov, 2006; Baggio et al., 2009). Given the absence of maximum residue limits (MRLs) set for honey, the detection threshold was considered the threshold of positivity: 3 - 30 ng/g for tetracycline and from 5 - 200 ng/kg for streptomycin. Antibiotics are used in apiculture as anti-bacterial foulbrood diseases, like American Foulbrood (AFB).

Antimicrobial drugs are effective against foulbrood diseases; however, antibiotic drug residues in honey pose a potential risk to human health. These antibiotic residues have toxic acute and chronic effects on human health and also reduce the efficacy and quality of honey (Zai et al., 2013; Ajibola et al., 2012). On the other hand, to protect the image of honey as a healthy natural product, these bactericides are banned from honey (Michel et al., 2004).

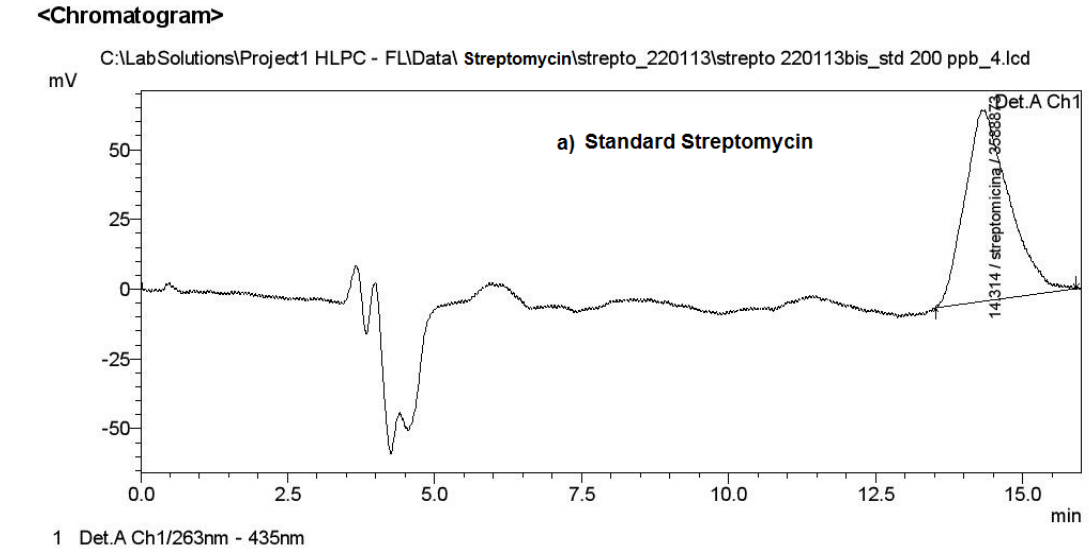
Conclusions

Thirty six Algerian honey samples were investigated for their physicochemical properties and research of streptomycin and tetracycline antibiotics. The study assessed the quality of honey samples analysed. In this study only 56% of samples were in agreement with the requirements of European Union and Codex Alimentarius Standards, while about 44% of them did not fit within European and Codex standards relative to the sucrose content, diastase activity and HMF reflecting inadequate sample manufacture and/or storage and adulteration. On the other hand, it was also concluded from this study that streptomycin and tetracycline were not used by the Algerian beekeepers for curing bacterial honeybee diseases.

According to the results some consideration may be given to Algerian beekeepers:

(i) It will be necessary to create more effective extension service to improve the beekeepers knowledge on honey harvesting techniques, honey processing and storage technologies.
(ii) Also, there is the need to increase the educational activity addressed to the beekeepers about taking care of Algerian honey production with special reference to physico-chemical characteristics, and have more responsibility for the quality of honey to be placed on the market with the appropriate label comprising the floral origin and chemical composition of honey.
(iii) There is the need to use reliable methods of control in order to ensure the conformity of honey product to avoid any risk of falsification and adulteration of Algerian honeys.

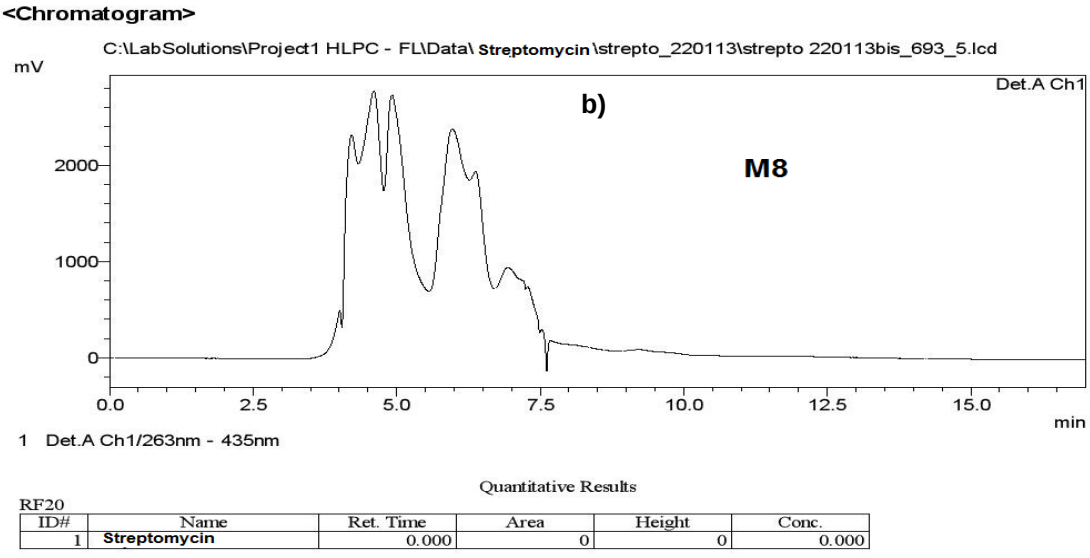
Moreover, a good knowledge of the Algerian product would provide the scientific support for the introduction of a national norm for honey.



Quantitative Results

RF20

ID#	Name	Ret. Time	Area	Height	Conc.
1	**Streptomycin**	14.314	3588873	68584	280.276



Quantitative Results

RF20

ID#	Name	Ret. Time	Area	Height	Conc.
1	**Streptomycin**	0.000	0	0	0.000

Figure 3. Chromatograms of Streptomycin by HPLC in Algerian honey (**a**) standard of streptomycin. (**b**) Example of an Algerian honey exempt of streptomycin.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Ajibola AW, Joseph PC, Kennedy HE (2012). Nutraceutical values of natural honey and its contribution to human health and wealth. J.

Nutr. Metab. 61(9): 1743-7075.
Ajlouni S, Sujirapinyokul P (2010). Hydroxymethyl furfuraldehyde and amylase contents in Australian honey. Food Chem. 119(3):1000-1005.
Albino G, Cristiana B, Biancotto G, Baggio A, Dainese N, Manzinello C, Mutinelli F (2005). Antibiotic residue in honey: alidation procedure. Honey analytical methods validation. *Apiacta*. 40:45-49.
Anklam E (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chem. 63: 549-562.
Baggio A, Gallina A, Benetti C, Mutinelli F (2009). Residues of antibacterial drugs in honey from the Italian market. Food Additives and Contaminants: Part B. 2(1):52-58.
Ball WD (2007). The chemical composition of honey. J. Chem. Edu. 84:1643.
Bendeddouche B, Dahmani K (2011). Physical properties of honey products in Algeria. J. Stored Prod. Postharvest Res. 2:237-244.
Blasa M, Candiracci M, Accorsi A, Piacentini MP, Albertini MC, Piatt E (2006). Raw millefiori honey is packed full of antioxidants. Food Chem. 97: 217-222.
Bogdanov S (2002). Harmonised Methods of the International Honey Commission. International Honey Commission, 1-62 pp.
Bogdanov S (2006). Contaminants of bee product. Apidologie 37: 1-18.
Buba F, Gidado A, Shugaba A (2013). Physicochemical and Microbiological Properties of Honey from North East Nigeria. Biochem Anal. Biochem. 2(142):1-7.
Cara CM, Dumitrel GA, Glevitzky M, Perju D (2012). Stability of tetracycline residues in honey. J. Serb. Chem. Soc. 77:879-886.
Chakir A, Romane A, Marcazzan GL, Ferrazzi P (2011). Physicochemical properties of some honeys produced from different plants in Morocco. Arabian J. Chem. doi:10.1016/j.arabjc.2011.10.013
Chefrour A, Draiaia R, Tahar A, Ait Kaki Y, Bennadja S, Battesti M J (2009). Physicochemical characteristics and pollen spectrum of some North-east Algerian honeys. Afr. J. Food Agric. Nutr. Dev. 9:1276-1293.
Chua LS, Adnan NA, Abdul-Rahaman NL, Sarmidi MR (2014). Effect of thermal treatment on the biochemical composition of tropical honey samples. Inter. Food Res. J. 21(2):773-778.
Chua LS, Adnan NA (2014). Biochemical and nutritional components of selected honey samples. Acta Sci. Pol. Technol. Aliment. 13(2):169-179.
Coco FL, Valentini C, Novelli V, Ceccon L (1996). High-performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in honey. J. Chromatogr. A. 95-102.
Codex Alimentarius. Commission Standards, Codex Standards for Honey, (1981/ revised 1987/revised 2001) (2001). FAO-Rome. pp. 1-7.
Council Directive of the European Union. Council Directive 2001/110/EC of 20 December 2001 relating to honey (2002). Official J. Eur. Communit. L10:47-52.
Cristofani E, Antonini C, Tovo GR, Fioroni L, Piersanti A, Galarini R (2009). A confirmatory method for the determination of tetracyclines in muscle using high-performance liquid chromatography with diode-array detection. Analytica Chimica Acta. 637:40-46.
Escuredo O, Seijo MC, Fernandez-Gonzalez M (2011). Descriptive analysis of *Rubus* honey from the north-west of Spain. Int. J. Food Sci. Tech. 46:2329-2336.
Fallico B, Zappala M, Arena E, Verzera A (2004). Effects of conditioning on HMF content in unifloral honeys. Food Chem.. 85: 305-313.
Glatt H, Schneider H, Liu Y (2005). V79-hCYP2E1-hSULT1A1, a cell line for the sensitive detection of genotoxic effects induced by carbohydrate pyrolysis products and other food-borne chemicals. Mutation Res. 580:41-52.
Islam MN, Khalil MI, Asiful MI, Gan SH (2014). Toxic compounds in honey. J. Appl. Toxicol. 34: 733-742.
Jilani IBH, Schweizer P, Khouja ML, Zouaghi M, Ghrabi Z (2008). Physicochemical spectra of honeys produced in Tunisia (Southwest of Kef). Apiacta 43:38-48.
Kebede N, Subramanian PA, Gebrekidan M (2012). Physico-chemical analysis of Tigray honey: An attempt to determine major quality markers of honey. Bull. Chem. Soc. Ethiop. 26: 127-133.
Khalil MI, Moniruzzaman M, Boukraâ L , Benhanifia M, Asiful MI, Nazmul IM, Amrah SS, Siew Gan H (2012). Physicochemical and Antioxidant Properties of Algerian Honey. Molecules**.** 17(9): 11199-11215.
Khalil MI, Sulaiman SA, Gan S H (2010). High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. Food Chem. Toxicol. 48: 2388-2392.
Kivima E, Seimanb A, Palla R, Sarapuua E, Martverka K, Laos K (2014). Characterization of Estonian honeys by botanical origin. Proceedings of the Estonian Academy of Sciences. 63(2): 183-192.
Kowalski S, Lukasiewicz M, Aleksandra DC, Gabriela Z (2013). 5-Hydroxymethyl-2-Furfural (HMF) – Heat-Induced Formation, Occurrence in Food and Biotransformation – a Review. Pol. J. Food Nutr. Sci. 63: 207-225.
Kubis I, Ingr I (1998). Effects inducing changes in hydroxylmethylfurfural content in honey. Czech J Animal Sci. 43: 379-383.
Kwapong PK, Ilechie AA, Kusi R (2013). Comparative antibacterial activity of stingless bee honey and standard antibiotics against common eye pathogens. J. Microbiol. Biotechnol. Res. 3: 9-15.
Makawi SZA ,Taha MI, Zakaria BA, Siddig B, Mahmod H, Elhussein AR, Elrasheed AGK (2009). Identification and Quantification of 5-Hydroxymethyl Furfural HMF in Some Sugar-Containing Food Products by HPLC. *Pak* . J. Nutr. 8:1391-1396.
Makhloufi C, Kerkvliet JD, D'albore RG, Choukri A, Samar R (2010). Characterization of Algerian honeys by palynological and physico-chemical methods. Apidologie, 41: 509-521.
Manzanares AB, Garcia ZH, Galdon BR, Rodriguez ER, Romero CD (2011). Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. Food Chem. 126:664-672.
Marchini LC, Moreti ACCC, Ivani PO, Sodré GS (2007). Physicochemical composition of *Apis mellifera* honey samples from São Paulo State, Brazil. Quim. Nova. 30: 1653-1657.
Michel VB, Stef JMO, Klaas MJ, Enne DB (2004). Determination of streptomycin and dihydrostreptomycin in milk and honey by liquid chromatography with tadem mass spectrometry. J. of chromatography A, 1058: 137-142.
Ouchemoukh S, Louaileche H, Schweitzer P (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Cont. 18: 52-58.
Ouchemoukh S, Schweitzer P, Bey MB, Djoudad-Kadji, H, Louaileche H (2010). HPLC sugar profiles of Algerian honeys. Food Chem. 121:561-568.
Oudjet k (2012). Le miel: une denrée a promouvoir .Infos-CACQE 00:1-3.
Persano-Oddo L, Baldi E, Accorti M (1990). Diastatic activity in some unifloral honeys. Apidologie 21:17-24.
Popa M, Roxana B, Simona V, Mirel G, Elena B (2009). Study regarding the quality of honey-changes in hydroxymethylfurfuraldehyde (HMF) content of honey from Transylvania (Romania). *Annals.* Food Sci. Technol. 10:506-509.
Sahinler N, Sahinler S, Gul A (2004). Biochemical Composite ion of Honeys Produced in Turkey. *J.* Apic. Res. 43:53-56.
Sak-Bosnar M, Sakac N (2012). Direct potentiometric determination of diastase activity in honey. Food Chem.135: 827-831
Saxena S, Gautam S, Sharma A (2010). Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118:391-397.
Silva ADS, Alves CN, Fernandes KDG, Müller RCS (2013). Classification of honeys from Pará State (Amazon region, Brazil) produced by three different species of bees. J. Braz. Chem. Soc. 24:1135-1145.
Singh N, Bath PK (1998). Relationship between heating and
Singh MP, Hemant RC, Manish A, AKHIL M , Mukesh S, Deepak S, Sheeba K (2012). Honey as complementary medicine: - A review. Int. J. Pharma Biol. Sci. 3:12-31.

hydroxymethylfurfural formation in different honey types. J. Food Sci. Technol. 35: 154-156.
Sommer Y, Hollnagel H, Schneider H, Glatt H (2003). Metabolism of 5-hydroxymethyl-2-furfural (HMF) to the mutagen 5-sulfoxymethyl-2-furfural (SMF) by individual sulfotransferases. Naunyn-Schmiedeberg's. Arch. Pharmacol. 367: 166.
Vit P, Pulcini P (1996). Diastase and invertase activities in Meliponini and Trigonini honeys from Venezuala. J. Apicul. Res. 35 (2): 57-62.
White JW (1992). Quality Evaluation of Honey. Role of HMF and Diastase Assay. *Am. Bee J.* 132: 737-743, 792-794.
White JW, Riethof M, Subers M, Kushmir I (1962). Composition of American honey. USDA Technol. Bull. 1261:1-124.
Zai I U M, Khaliqur R, Arshad H, Shafqatullah (2013). Detection and quantification of residues in honey samples by chromatographic Tech. *Middle- East J. of scientific Research*. 14: 683-687.
Zerrouk S, Boughediri L, Carmen MS , Fallico B, Arena E, Ballistreri G (2013). Pollen spectrum and physicochemical attributes of sulla (*Hedysarum coronarium*) honeys of Médéa region (Algeria). Albanian J. Agric. Sci. 12 (3): 511-517.