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ORIGINAL ARTICLE

Evaluating the Quality and Physicochemical Properties of Honey Commercialized in Iran

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KEYWORDS

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ABSTRACT: This study was intended to evaluate the quality of 30 honey samples, in terms of physicochemical properties such as moisture content, electrical conductivity, ash content, reducing sugars and sucrose, free acidity, pH, diastase activity, and hydroxymethylfurfural (HMF) content. Moreover, three methods recommended by the International Honey Commission for the determination of HMF, including 1) high performance liquid chromatography (HPLC), 2) White spectrophotometry and 3) Winkler spectrophotometry methods, were compared. The average moisture content ranged from 12.08±0.36 to 19.36±0.11%. The Electrical conductivity values (0.43±0.00 to 0.77±0.00 mS/cm), ash content (0.24±0.01 to 0.74±0.03%), pH values (3.37±0.01 to 5.21±0.16), free acidity (29.60±0.36 to 39.66±0.37 meq/kg of honey), total reducing sugar (52.28±0.09 to 88.01±0.63%), sucrose content (2.21±0.07 to 7.55±0.35%), diastase activity (2.07±0.28 to 29.01±0.50), and HMF content (17.33±0.18 to 834.46±0.30 mg/kg) were observed. Thirteen out of 30 samples (43%) showed HMF content higher than standard limits. Results obtained from the current study revealed that except for HMF and diastase activity, all physicochemical properties of samples met the national and international standard limits. Moreover, three methods applied for determination of HMF showed good recovery values and standard deviation. However, Winkler and White methods gave higher HMF value in honey sample than HPLC method.

INTRODUCTION

Food safety has always been a controversial issue in the world. Moreover, because of consumers demand for safe and healthy foods, quality assurance is getting more attention not only for consumers and producers, but also for governments. Various chemical compounds can be used as food quality indicators: (i) some of these indicators are related to natural food composition, (ii)

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some are produced during processing and storage; (iii) and some incorporated in foods as contaminants and adulterants [1].

Honey, the valuable natural foodstuff produced by honeybees (mainly *Apis mellifera* L.), has high nutritious values such as minerals, vitamins, antioxidants and different enzymes [2]. Sugars, especially mono and disaccharides are the main constituent of honey (70-80%). Honey quality is a function of some factors such as season, climate conditions, processing method, storage, and type of flowers used by honeybee [3]. However, due to high nutritional values of honey besides its high price, adulteration of this valuable foodstuff may be practiced by beekeepers to harvest more honey from hives [4]. For example, they may feed honeybees with syrups or sugar instead of flowers, or even mix honey with other sweet products such as sugar and syrups [5].

Therefore, every year scientists from different parts of the world conduct studies to distinguish pure honey from adulterated [6-8]. However, detection of honey adulteration is not always easy and represents a problem to solve, some physicochemical properties of honey are evaluated to verify the honey authenticity and to show the possible presence of artificial components or adulterants [9-10].

In general, raw honey is preferred, but heat processing is applied to delay sugar crystallization, reduce the honey viscosity, and facilitate filling the jars [11]. Researchers well demonstrated that overheating and storage in improper conditions may change the honey constitution or produce undesirable products. Hydroxymethylfurfural (HMF) and diastase activity are the chemical specifications related to the honey quality such as overheating and obsolescence [12]. The HMF is produced in honey because of Mailard reaction or monosaccharides dehydration under acidic condition (pH<5). Therefore, raw honey has HMF in very low concentration or even is HMF-free [13]. There are three methods, proposed by the International Honey Commission [14], for determination of HMF in honey. These methods include spectrophotometric methods (Winkler and White) and high performance liquid chromatography (HPLC).

Other characteristics such as acidity, ash, moisture content, electrical conductivity and sugar content are measured to check the honey quality. The international standards including Codex Alimentarius standard for honey [9] and International Honey Commission [14] present information about the essential composition and quality factors of honey. The articles include standard limits and test methods for determination the honey quality factors.

The aim of the current study was to (i) investigate the quality of honey samples commercialized in Iran through determination of physicochemical properties; (ii) compare the three international standard test methods used for HMF determination in honey.

MATERIALS AND METHODS

Sampling

A total of 30 local multyfloral honey samples were purchased randomly from different supermarkets in Tehran, Iran. A wide range of different brands were collected to make sure that the survey was as comprehensive as possible and representative of the commercial honey products available to consumers in Tehran. Samples were transferred to the lab and prepared for determination the physicochemical specifications, including, moisture content, and electrical conductivity, ash content, reducing sugars, sucrose, free acidity, pH, diastase activity and HMF content.

Materials

HPLC-grade water was obtained from a water purifier (Elga, Marlow, and Buckinghamshire, UK). The HMF,

methanol, potassium hexacyanoferrate (II) ($K_4Fe(CN)_6.3H_2O$), zinc acetate ($ZnCH_3COO)_2.2H_2O$, sodium hydroxide, starch, sodium sulfate (SO4 $Cu.5H_2O$), potassium, sodium tartrate, hydrochloric acid and disodium hydrogen phosphate (Na_2HPO_4), were supplied by Merck (Darmstadt, Germany). Filter paper (Whatman) was purchased from Schleicher & Schuell Microscience GmbH (Dassel, Germany). A Stock solution of 2 mg/ ml HMF was prepared and the working standards at lower HMF concentrations were prepared by diluting appropriate volumes of the stock standard solution with de-ionized water. Carrez solution I and II were prepared by dissolving 15 g of potassium hexacyanoferrate(II), $K_4Fe(CN)6\_3H_2O$ and 30 g of zinc acetate, $Zn(CH_3\ COO)_2\_2H_2O$ in 100 ml of distilled water respectively.

Physicochemical analysis

All of the physicochemical specifications were determined according to the Iranian national standard for honey [10]. An Abbe™ 2 WAJ (Shangai Optical Instrument Co. Ltd., Shangai, China) was used to measure the electrical conductivity in a 20% (w/v) solution of honey at 20°C in terms of mS/cm. A digital pH-meter (Hanna Instruments, Switzerland) was applied to determine the pH in a solution containing 10 g of honey in 75 ml of CO_2 free distilled water. For determination the free acidity 75 mL carbon dioxide-free water was added to 10 g honey sample and titrated by 0.1 M sodium hydroxide (NaOH) to pH 8.3. To calculate water content, refractive index was measured by an Abbe refractometer (ATAGO Co. Ltd., Japan) at 20°C, then the water content of honey samples was calculated based on refractive index table cited in the Iranian standard for honey [10]. To determine the ash content about 5 g of honey samples was placed in a crucible and heated at 600 °C. The reducing sugars (fructose and glucose) and non-reducing sugars (sucrose) were determined according to the Lane Aynon method [10].

Diastase activity was measured according to the Iranian national standard (10). Five mL acetate buffer (pH 5.3) and 3 mL of NaCl (2.9%) was added to 10 g of honey sample. The mixture was warmed up for 15 min at 40 °C. Then 5 mL of starch solution 4% (w/v) and 5 mL diluted iodine solution (0.088 mg/L) was added and absorbance of the mixture was measured at 660 nm.

Determination of HMF content

The White method

To determine the HMF content by white method, 5 g of g honey sample and 25 ml of distilled water was transferred into a 50 ml volumetric flask. Then, 0.5 ml of each Carrez solution (I and II) was added and the mixture was made up to 50 ml with distilled water. After filtration using filter paper (the first 10 ml of the filtrate solution was rejected), 5 ml of the solution was transferred into two test tubes. In one of them, 5 ml of distilled water (sample solution); and to the second tube, 5 ml of sodium bisulphate solution 0.2% (reference solution) was added. The absorbance of the solutions at 284 and 336 nm was determined using a spectrophotometer. The quantitative value of HMF was calculated using the related formula proposed by the International Honey Commission [14].

The Winkler method

To determine HMF value by Winkler method, 10 g of honey sample was transferred to a 50 ml volumetric flask and dissolved in 20 ml of distilled water. After adding 0.5 ml of each Carrez solution (I and II), the mixture was made up to 50 ml with distilled water and filtered. Then 2 ml of the solution and 5 ml of p-toluidine put into two different test tubes. Then, 1 ml of distilled water was added to the first test tubes (reference solution) and 1ml of barbituric acid (0.5%)

was added to the second test tube (sample solution). The absorbance of the solutions was measured at 550 nm using UV/VIS spectrophotometer. The quantitative value of HMF was calculated using the related formula proposed by the International Honey Commission [14].

HPLC method

To evaluate the linearity, a six point calibration of the HMF curve (1, 5, 10, 20, 50, 100 ng/ ml) was constructed using the linear least squares regression procedure of the peak area versus the concentration. The concentrations of HMF in the honey samples were calculated by using the calibration curves.

The HMF content was determined by applying high performance liquid chromatography (HPLC) method, based on the method published by Iranian national standard (10). In brief, after dilution of 10 g of honey with 50 ml distilled water, 1 ml of a 15% (w/v) Carrez I (potassium hexacyanoferrate) solution and 1 ml of a 30% (w/v) Carrez II (zinc acetate dehydrate) solution were added. The solution was filtered through Whatman filter paper (No. 41). After filtration through a 0.45 µm nylon membrane filter, a total of 50 µl of the filtered honey elutes was injected to HPLC. The HPLC system (Varian 9010, Creek, California, USA) equipped with a Knauer degasser (Berlin, Germany); a variable wavelength UV-VIS Detector (Varian 9050, Creek, California, USA) was applied. The HPLC column was from Agilent Bondesil, RP-C18, (4.6 mm, 5 µm, and 25cm). The mobile phase was consisted of 95% water (0.01 Mol/L) and 5% methanol with flow rate of 1.0 ml/min. The HMF content of the sample was calculated by comparing the corresponding peak areas of the sample and those of the standard calibration curve of HMF. The HMF content and all the physicochemical specifications were measured in duplicate.

To determine the repeatability of the methods (Winkler, White and HPLC) applied for determination of HMF, honey samples were spiked with HMF at levels of 10, 100 and 500 mg/kg. Then recovery and relative standard deviation (RSD) were calculated.

STATISTICAL ANALYSIS

A one-way analysis of variance (ANOVA), followed by Post Hoc Tukey's test at $P<0.05$ was applied to determine significant differences between White, Wrinkle and Chromatography methods and between 30 different brands. A probability value of 0.05 was used to determine the statistical significance. The specification of 30 honey samples (moisture content, electrical conductivity, ash content, reducing sugars and sucrose, free acidity, pH, diastase activity, and HMF) was statistically evaluated using basic statistical variables. To present the basic features of the data in the current study, values of all samples are presented as means, standard deviations, minimum, maximum, and median. Data analysis was performed using the Minitab (Version 17, State College, PA., USA) statistical package.

RESULTS AND DISCUSSION

Methods comparison

The mean, standard deviation and RSD associated with Winkler, White and HPLC method used for determination of HMF are shown in Table 1. The lowest and highest RSD values of methods at all spiking levels (10, 100, 500 mg/kg), was found with HPLC and Winkler method respectively (Table 1). These results are in agreeing with those reported by previous researchers [15].

Table 1. The recovery, standard deviation and RSD obtained for HMF determination in honey by Winklwr, White and HPLC methods at three different spiking levels (n = 6).

Spiking level (mg/kg)	Method					
	Winkler		White		HPLC	
	Mean±SD	RSD	Mean±SD	RSD	Mean±SD	RSD
10	107±12.04	11.19	104.83±6.65	6.34	91.17±3.19	3.50
100	105.40±6.43	6.10	101.22±4.64	4.58	99.80±2.80	2.81
500	104.74±3.78	3.61	102.28±2.21	2.17	96.71±1.71	1.76

SD= Standard deviation
RSD= Relative standard deviation of repeatability

The HMF content in all 30 samples was measured by three methods (Winkler, White and HPLC), proposed by the IHC, and obtained results is presented in Table 2.

Table 2. The HMF content of 30 honey samples determined by Winkler, White and HPLC methods (n=3).

Sample	Winkler (mean±SD)	White (mean±SD)	HPLC (mean±SD)
S1	754.93±2.87	474.68±1.20	744.07±3.32
S2	756.53±4.10	752.1±2.01	744.66±2.83
S3	41.00±1.60	35.77±0.68	34.45±0.67
S4	42.97±1.20	41.26±0.65	38.51±0.56
S5	34.83±1.76	32.20±0.72	28.98±0.57
S6	33.13±0.81	34.53±1.46	30.167±0.96
S7	173.50±2.23	171.33±2.32	167.28±1.05
S8	191.63±1.18	187.82±1.28	185.72±0.73
S9	29.60±1.31	27.53±1.50	23.66±0.89
S10	25.80±0.62	22.97±1.76	24.07±0.22
S11	29.71±1.17	26.63±1.48	24.023±1.14
S12	843.51±1.67	836.80±2.71	829.40±2.96
S13	39.56±0.77	37.10±1.75	37.11±1.23
S14	42.58±1.98	41.43±1.50	34.23±2.71
S15	259.70±1.51	256.43±0.60	250.43±1.55
S16	256.84±1.84	251.97±2.55	248.77±2.59
S17	243.08±1.30	239.97±1.79	236.19±1.05
S18	195.63±2.76	192.10±2.01	191.80±0.72
S19	190.85±2.44	188.30±1.57	183.63±0.60
S20	75.35±1.95	69.00±2.65	56.33±1.53
S21	57.473±1.47	53.87±1.63	52.90±0.85
S22	21.53±1.57	18.21±1.16	16.82±0.74
S23	19.86±1.47	19.97±1.70	17.98±1.54
S24	31.44±1.49	28.81±1.91	25.99±0.99
S25	28.66±1.21	25.80±1.71	22.82±0.75
S26	41.30±1.34	36.77±1.37	35.81±0.74
S27	34.31±1.26	30.67±1.53	30.82±1.02
S28	27.65±0.89	22.90±1.02	18.82±0.74
S29	23.81±0.62	19.63±1.52	16.61±0.67
S30	83.59±1.01	80.40±0.95	77.65±1.50

SD= Standard deviation

Two-way ANOVA was applied to determine the significant differences between three methods, and between 30 different brands. The results showed there were significant differences between applied methods and between different brands (Table 3).

Table 3. Analysis of variance for the evaluation effect of determination methods and brand on total HMF in 30 analyzed honey sample (n=3).

Source	Degree of freedom (DF)	SS	MS	F-value	*p*-value
Brand	29	13451843	463857	175312.27	0.000
Method	2	2000	1000	377.89	0.000
Interaction	58	752	13	4.90	0.000
Error	180	476	3		
Total	269	13455071			

SS= sums of squares
MS= mean squares.

Data analysis demonstrated that the spectrophotometric method described by Winkler showed higher HMF content compared to the White and HPLC methods (Table 1). Moreover, data obtained by HPLC were more reliable than Winkler and White methods, thanks to lower standard deviation. In agreeing with current results, Khalil and coworkers [16] reported that the Winkler method showed higher readings compared to White and HPLC methods. In a related study, for samples with HMF content ranging from 1 to 4 mg/kg, the accuracy of the Winkler and HPLC methods are comparable, whereas as regards precision, the HPLC method gives better results and the HPLC method seems to be more appropriate for determination of HMF in honey in the range 1-4 mg/kg. The authors reported when the honey samples have less than 1 mg/kg HMF, the White and Winkler methods are inaccurate [17]. Winkler method gave higher values than other White and HPLC methods for all honey samples [15]. International Commission of Honey [14] suggested not using the Winkler method for determining HMF in honey, because of carcinogenic of p-toluidine and of the low precision of this method.

In the current study, the data obtained by White methods were higher than those by HPLC. However, in contrast with these results, Zapala et al. [15] and Khalil et al. [16], reported that, HPLC and White methods usually give similar values, except for eucalyptus honey.

Physicochemical specification of Honey

Thirty honey samples were investigated in terms of physicochemical properties (moisture content, electrical conductivity, ash content, reducing sugars and sucrose, free acidity, pH, diastase activity, and HMF content) and results were compared regarding the Iranian standard for honey [10]. The results were expressed as mean±SD (Table 4).

Table 4. Descriptive statistics of some of the physicochemical properties (moisture content, electrical conductivity, ash, reducing sugar and sucrose) of 30 analyzed honey samples.

Honey specification	Mean ± SD	Max	Min	Median
Moisture content (%)	15.44±2.05	19.44	11.82	15.44
Electrical conductivity (mS/cm)	0.61±0.12	0.77	0.43	0.61
Ash (%)	0.44±0.13	0.76	0.23	0.42
Reducing sugars (%)	72.69±9.34	88.45	52.21	72.83
Sucrose (%)	3.85±1.33	7.8	2.16	3.60
Free acidity (meq/ kg)	34.62±3.08	39.92	29.34	35.09
pH	4.17±0.52	5.32	3.36	4.21
Diastase (Gothe units)	10.55±8.36	29.36	1.87	8.70
HMF (by HPLC method) (mg/kg)	147.7±223.4	832.8	16.0	36.7

SD= Standard deviation

Moisture content

The average moisture content ranged from 12.08±0.36 to 19.36±0.11. All of the samples showed the moisture content less than 20%, which is the maximum limit recommended by International Codex Alimentarius standard [9] and Iranian standards for honey [10]. The moisture content of honey is a function of some factors such as season, climate conditions and degree of maturity [18]. Iranian standard for honey [10] and Codex [9] require 20% moisture in honey for safety against fermentation caused by the action of osmotolerant yeasts during storage. The results obtained from current study were consistent with some Indian honeys for which the corresponding moisture values ranged from 17.2 to 21.6% [19].

Electrical conductivity and ash content

Electrical conductivity was, likewise, within limits (below 0.8 mS/cm) and ranged from 0.43±0.00 to 0.77±0.00. All of the samples presented ash content between 0.24±0.01 to 0.74±0.03.

The electrical conductivity closely related to other honey characteristics such as ash content, organic acids, protein and some complex sugars. Moreover, it shows great variability according to the floral origin. Therefore, determination of electrical conductivity showed promising results for differentiating between honeys with different floral origins [20]. In the current study all the samples were within the acceptable limit (less than 0.8 mS/cm).

The ash percentage is an important quality index showing the mineral content of honey [21]. In the current study for all honey samples the mean of ash value (%) was below the maximum limit recommended by Iranian standard [10]. The ash content of blossom honey is less than 0.06%, while, for honeydew honey or blends of honeydew and blossom honey, this value is more than 1.2% [22]. In a related study, for most of the honeydew honeys from Romania, ash values ranged from 1.17% to 1.23%, while, for floral honey samples obtained from acacia, sun-flower and lime, it ranged from 0.03 to 0.40 [23]. Therefore, regard to his specification, the analyzed Iranian honeys in our study could be similar to the blossom honey.

Free acidity and pH

The free acidity and pH values ranged from 29.60±0.36 to 39.66±0.37 meq/ kg and 3.37±0.01 to 5.21±0.16 respectively. Regard to Iranian standard for honey [10], the acidity must be less than 40 meq/kg to show that no undesirable fermentation occurred. In the current study acidity values for all samples were less than the maximum limit (40 meq/kg). Honey naturally,

regardless of geographical origin, is an acidic substance with pH varying from 3.7 to 4.5 for blossom honey and from 4.5 to 6.5 for honeydew honey [24]. However, variation in acidity may be related to the botanical origin or harvesting in different seasons [25].

The pH values of all samples were in accordance with the Iranian standards [10] and also in agree with the results obtained by other researchers [15, 26]. In related studies, the pH values of Iranian, Indian and Turkish honeys have been found to vary between 3.48 to 5.06, 3.7 to 4.4 and 3.67 to 4.57, respectively [4, 19, and 22].

Reducing sugars and sucrose

The total reducing sugar and sucrose content in the honey samples varied from 52.28±0.09 to 88.01±0.63 and from 2.21±0.07 to 7.55±0.35, respectively. Except for one sample, all the reducing sugar (glucose and fructose) and sucrose content obtained in the current study were in agree with those recommended by Iranian standard [10], which is more than 65% for reducing sugars and less than 5% for sucrose content. These results confirm that the honey samples in the current study are at an advanced stage of ripening and are authentic because the sucrose is the most important sugar from a legislative point of view [22]. The sucrose content more than 5% observed in one sample could be attributed to some reasons such as overfeeding of honeybees with sucrose syrup, adulteration, or prematurely harvest of honey, before sucrose conversion into glucose and fructose is completed [19]. A high sucrose concentration of honey may be due to addition of commercial sugar to honey [20]. A range of 79.5-91.1% sucrose was reported for various local and imported honeys sold in Nigeria [27].

Diastase activity and HMF content

The diastase activity was between 2.07±0.28 to 29.01±0.50 units on the Gothe scale. Two samples presented diastase activity lower than 3 Gothe. Iranian standard [10] and international Codex Alimentarius standard for honey [9] recommended diastase activity lower than the level of 8 on the Gothe scale.

The HMF content of honey samples analyzed in the current study, ranged from 17.33±0.18 to 834.46±0.30 mg/kg of honey. Both diastase activity and HMF content may be affected by honey storage in improper conditions and overheating during heat treatment. HMF, a decomposition product of reducing sugars, in fresh honey is present only in trace amount. It is a major honey quality factor that indicates honey freshness and adulteration associated with overheating [20].

In the current study a wide range of HMF was found in examining samples. The HMF content of 13 out of 30 (43%) of the samples was higher than maximum limit recommended by ISIRI (40 mg/kg). There are many reports showing high level of HMF in honey samples (16, 26). In Malaysia, honey samples stored for long periods (12–24 months) contained much higher HMF concentrations ranged from 128.19 to 1131.76 mg/kg (16). HMF in honey samples from Portuguese ranged between 1.7 to 471 mg/kg [26].

CONCLUSIONS

The analytical results indicated that honeys distributed in Tehran presented good quality properties according to international and national standards. Most of the investigated physicochemical properties, including moisture content, electrical conductivity, and ash content, reducing sugars and sucrose, free acidity, pH were in the range of related standards and were similar to those reported by other researchers. However, the HMF content and diastase values in some of the samples were not in the range recommended by related national and international standards, indicating some levels of adulteration and/or overheating. Three methods applied for determination of HMF showed good recovery values and standard deviation. However, Winkler and White

methods gave higher HMF value in honey sample than HPLC method.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

1. Lee T.C., King H.J., Preface. In Lee TC., Kim H.J., (Eds.), Chemical markers for processed and stored foods, 3rd ed., American Chemical Society: Washington D.C., 1996.

2. Aloisi P.V., 2010. Determination of quality chemical parameters of honey from Chubut (Argentinean Patagonia). Chem J Agricl Res. 70(4), 640-645.

3. Al-Mamary M., Al-Meeri A., Al-Habori M., 2002. Antioxidant activities and total phenolics of different types of honey. Nutr Res. 22(9), 1041–1047.

4. Mehryar L., Esmaiili M., Hassanzadeh A., 2013. Evaluation of some physicochemical and rheological properties of Iranian honeys and the effect of temperature on its viscosity. American-Eurasian J Agric Environ Sci. 13(6), 807-819.

5. Kenjeric D., Mandic M.L., Primorac L., Bubalo D., Perl A., 2007. Flavonoid profile of Robinia honeys produced in Croatia. Food Chem. 102(3), 683–690.

6. Montilla A., Ruiz-Matute A.I., Sanz M.L., Martinez-Castro I., Del Castillo M.D., 2006. Difructose anhydrides as quality markers of honey and coffee. Food Res Int. 39, 801–806.

7. Windsor S., Kavazos K., Brooks P., 2013. The quantitation of hydroxymethylfurfural in Australian *Leptospermum* honeys. J Pharmacognosy Phytother. 5(1), 21-25.

8. Zirbes L., Nguyen B.K., Graaf D., Meulenaer B., Reybroeck W., 2013. Hydroxymethylfurfural: a possible emergent cause of honeybee mortality? J Agric Food Chem. 61(49), 11865-11870.

9. Codex Alimentarius Standard, Revised Codex Standard for Honey, Rev.2, 24th Session of the Codex Alimentarius, 2001; CODEX ROME

10. Institute of Standards and Industrial Research of Iran, Honey- Specification and test methods. ISIRI. no 92. 7th revision, [In Persian].

11. Tosi E., Martinet R., Ortega M., Lucero H., Re E., 2008. Honey diastase activity modified by heating. Food Chem. 106(3), 883–887.

12. Rotarescu R., Vidican C., 2010. Impact's assessment of thermal processing and storage conditions on enzymatic activity and HMF content in honey. Carpathian J Food Sci Technol. 2(1), 1–13.

13. Cozmuta A.M., Cozmuta L.M., Varga C., Marian M., Peter A., 2011. Effect of thermal processing on quality of polyfloral honey. Romanian J Food Sci. 1(1), 45–52.

14. IHC. Harmonised Methods of the International Honey Commission. 2009. Available at http://www.bee-hexagon.net/en/network.htm.

15. Zappala M., Fallico B., Arena E., Verzera A., 2005. Methods for the determination of HMF in honey: a comparison. Food Cont. 16(3), 273–277.

16. Khalil M.I., Sulaiman S.A., Gan S.H., 2010. High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. Food & Chem Toxicol. 48(8-9), 2388–2392.

17. Truzzi C., Annibaldi A., Illuminati S., Finale C., Rossetti M., Scarponi G., 2012. Determination of very low levels of 5-(hydroxymethyl)-2-furaldehyde (HMF) in natural honey: comparison between the HPLC technique and the spectrophotometric white method. J Food Sci. 77(7), 784-790.

18. Finola M.S., Lasagno M.C., Marioli J.M., 2007. Microbiological and chemical characterization of honeys from central Argentina. Food Chem. 100(4), 1649–1653.

19. Saxena S., Gautam S., Sharma A., 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118(2). 391–397.

20. Gebremariam T., Brhane G., 2014. Determination of Quality and adulteration effects of honey from Adigrat and its surrounding areas. Int J Technol Emerg Engin Res. 2(10), 71-76.

21. Marchini L.C., Moreti A.C.C., Otsuk I.P., 2007. Physicochemical composition of *Apis Mellifera L.* honey samples from São Paulo state, Brazil. Quim Nova. 30(7), 1653-1657.

22. Ouchemoukh S., Louaileche H., Schweizer P., 2007. Physicochemical characteristics and pollen spectrum of some Algerian honey. Food Cont. 18(1), 52–58.

23. Al M.L., Daniel D., Moise A., Bobis O., Laslo L., Bogdanov S., 2009. Physicochemical and bioactive properties of different floral origin honeys from Romania. Food Chem. 112, 863–867.

24. Bradbear N., 2009. Bees and their role in forest livelihoods (monograph on the Internet). Rome, Italy. FAO (Food and Agriculture Organization of the United Nations).

25. Perez-Arquillue C., Conchello R., Arino A., Juan T., Herrero A., 1994. Quality evaluation of Spanish rosemary (*Rosmarinus officinalis*) honey. Food Chem. 51(2), 207-210.

26. Mendes E., Brojo Proença E., Ferreira IMPLVO., Ferreira M.A., 1998. Quality evaluation of Portuguese honey. Carbohydrate Polymers. 37(3), 219-223.

27. Jimoh W.L.O., Ummi U.A., 2014. Chemical characteristic of selected importd and local honey sold around Kano Metropolice, Nigeria. Bayero J Pure Applied Sci. 7(2), 55-58.