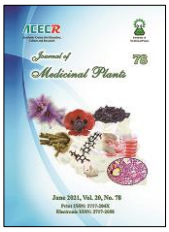
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Research Article

Evaluating the quality of commercial Iranian honeys

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ABSTRACT

Background: Honey is one of the most valuable foods that used in human societies to treat many diseases due to its healing properties for centuries. The lack of an objective way to distinguish natural honey from counterfeit honey has strengthened the field of profiteering in this area and has led to the supply of counterfeit honey instead of natural honey. So honey quality must be controlled analytically with the aim of guaranteeing the reality and preserving the consumer from commercial speculation. **Objective:** The present study was conducted to evaluate 24 samples of 9 commercial brands of honey in Tehran's markets with number 1 to 9 (1-AB, 2-TF, 3-DP, 4-JK, 5-SN, 6-SK, 7-IM, 8-MD, and 9-MH). A number of physical, chemical, and biological parameters of the samples were evaluated. **Methods:** Carbohydrate composition was determined by GC/Mass to evaluate the contents of fructose, glucose, and sucrose. 5-hydroxymethyl-2-furfuraldehyde (HMF) was quantified by HPLC-UV and other physicochemical quality parameters including moisture, pH, proline content; and diastase activity were also evaluated according to the Codex Alimentarius. **Results:** Only 2 brands met all major international specifications. Although all the parameters of the other brands were conformed, the diastase activity was not in a specific range. **Conclusion:** The diastasis activity is considered as the most important factor to evaluate honey quality based on the results of this study.

1. Introduction

Honey is a sweet sticky yellow substance which is made from the nectar by honeybees [1]. It is an important edible which is used as a complementary medicine [2, 3]. From a long time ago; honey is used as a nutritional supplement and in some religious beliefs, it is an effective remedy for many diseases[4]. Many scientific researches confirm this claim too. Nowadays, based on literature review, honey is used widely in treatment of wound [5]. It has the antimicrobial and antioxidant properties [6-8].

Abbreviations: GC/Mass, Gas Chromatography/Mass Spectrometry; HMF, Hydroxymethylfurfural; HPLC-UV, High Performance Liquid Chromatography-Ultraviolet

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Recent studies have shown the anti-cancer properties of the honey [9]. The unique properties of honey depend on its complex substances [10]. Natural honey contains carbohydrate (glucose, fructose, sucrose, and maltose), water, vitamins and biomolecules [11].

Biomolecules which are detected in natural honey are proteins [12], amino acids, enzymes (diastase, invertase, glucose oxidase, catalase, acid phosphatase, protease, and esterase) [13-15] and other substance like phenolic and flavonoid combinations [16].

Honey's combinations are affected by region, climatic conditions and vegetation cover [17-25]. The International Honey Commission (IHC) was established in 1990, due to great variety of types of honey in all over the world, to create a harmonized world honey standard which includes physical, chemical and biological parameters [26]. Since the measurement of biological parameters needs exact laboratory experiments, the types of honey quality have been solely compared based on physical and chemical parameters, in some researches [27]. While the nutritional value of honey is mainly due to the presence of biological combinations which pull out from herbs or honeybees, this neglect may leads to wrong results.

The present study investigated the chemical and biological factors of 9 commercial brands of Iranian honey based on international honey standards to find which parameter plays a significant role to control the quality of honey.

2. Materials and Methods

2.1 Reagent and standards

Hexamethyldisilazane (HMDS), hydroxylamine hydrochloride, trifluoroacetic acid (TFA), pyridine, ninhydrin, starch, formic acid, ethylene glycol monomethyl ether, potassium iodide, iodide, glucose, sucrose, fructose, proline, mannitol and hydroxymethylfurfural (HMF) are considered as standards which were obtained from Fluka.

2.2. Sample collection

Nine commercial brands of honey (3 from each brand in 2018) in the Tehran province and 24 samples were purchased from different supermarkets and used for the study. Since it is not morally possible to name brands, they are denoted by the numbers 1 to 9 with a two-letter abbreviation (1-AB, 2-TF, 3-DP, 4-JK, 5-SN, 6-SK, 7-IM, 8-MD, and 9-MH).

2.3. Determination of pH

The pH was measured with a pH meter (Metrohm 914) in a solution containing 10 g honey in 75 mL of distilled water.

2.4. Moisture content

Moisture was measured using the refractometry method. The refractive indices of honey samples were measured at ambient temperature using refractometer and all measurements were done at 20ºC by adding a correction factor to obtain the corresponding percentage of moisture from the refractive index by referring to a standard table of AOAC, 1990.

2.5. Diastase activity assay

Diastase activity was measured using a buffered solution of soluble starch, which fulfils the requirements of the method, and honey which was incubated in a bath at 40 ºC. Reducing blue color absorbance which was formed in the

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presence of iodine solution was followed by the use of a Human crap XMA 2000 UV/VIS spectrophotometer at 660 nm. Lines were fitted to the absorption data by the use of regression, and the diastase number was calculated from the time taken for the absorbance to reach 0.235.

2.6. Determination of HMF

A C_{18} reversed phase column, and isocratic elution with methanol/water (10:90) was used to determine the HMF, at a flow rate of 1.0 ml/min and UV detection at 280 nm. The sample preparation involved only dissolution in deionized water and filtration through W42 paper and 0.2 mm filter paper. Calibration curve was obtained for HMF and used for quantification (Fig. 1).

2.7. Determination of proline content

The proline content was determined by use of the AOAC standard method. 5.0 g honey was placed in a beaker and dissolved in 50 mL distilled water. Then, the solution was quantitatively transferred to a 100 mL volumetric flask was diluted using distilled water and shaken. Afterward, 0.5 mL of the sample solution was poured into a tube with 1.0 mL of formic acid (98-100 %) and 1.0 mL ninhydrin solution (3 % in ethylene glycol monomethyl ether). The tubes were capped and shaken vigorously. The tubes were placed in a boiling water bath for 15 min and they were transferred to a water bath at 70 ºC for 10 min. Then, 5 mL of 2-propanol was added to each tube. The tubes were removed for 45 min and the absorbance was determined at 520 nm at the room temperature. Strict control of the timing of each stage was critical. The honey color was corrected by determining the absorbance of the solution containing 0.5 mL of sample solution, 2.0 mL distilled water and 5 mL 2-prppanol. Calibration curve was obtained for proline and used for quantification (Fig. 2).

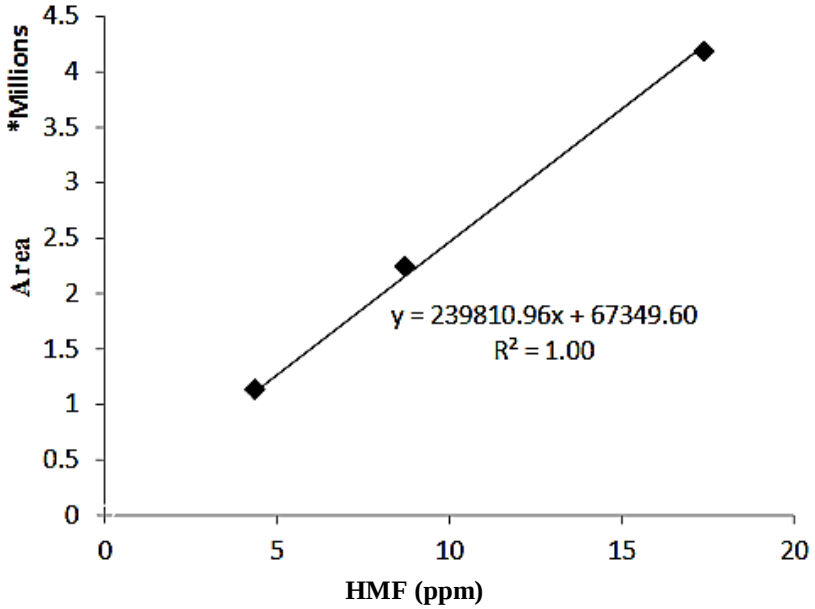


Fig. 1. HMF calibration curve

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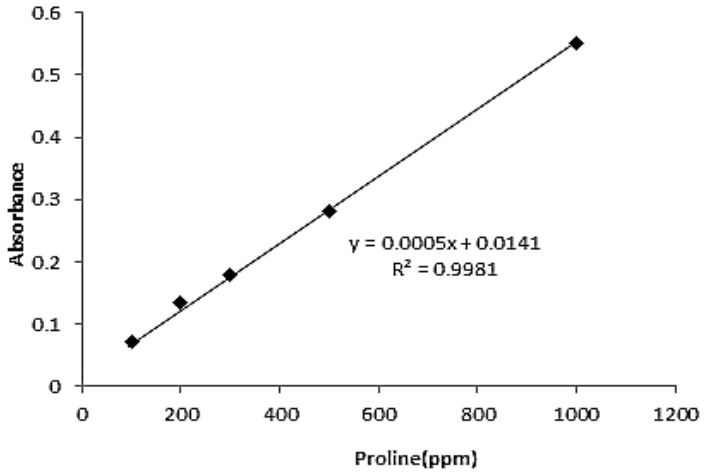


Fig. 2. Proline calibration curve

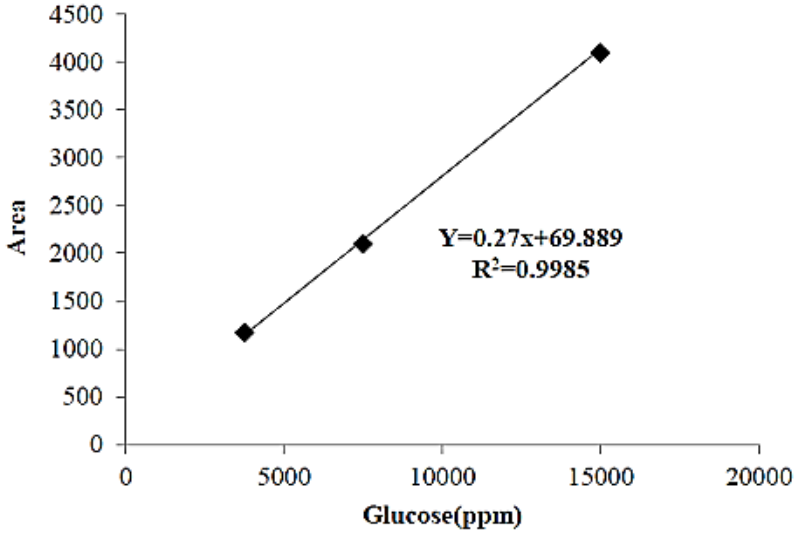


Fig. 3. Glucose calibration curve

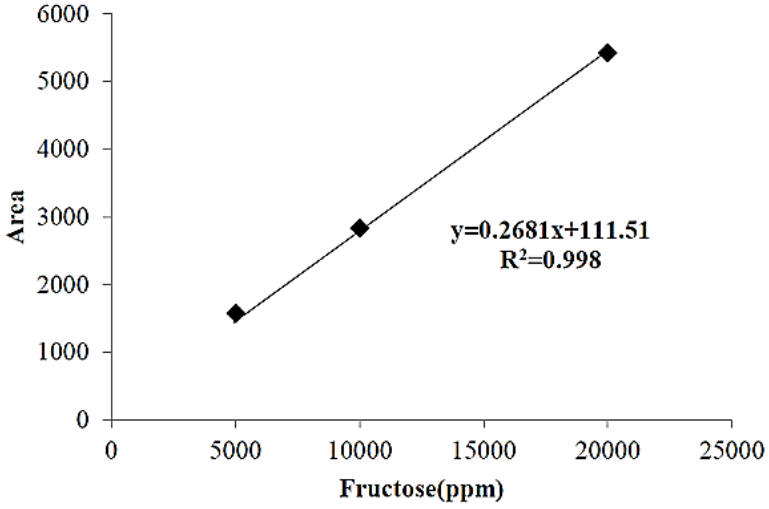


Fig. 4. Fructose calibration curve

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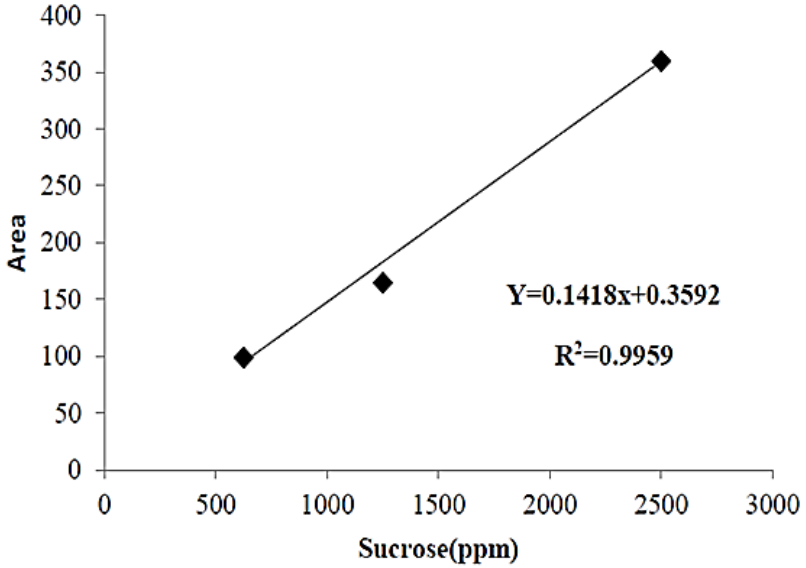


Fig. 5. Sucrose calibration curve

2.8. Determination of sugars content

Sugars were determined by gas chromatography as their trimethylsilyl-oxime derivatives. The Sugar standards were put (1.5 g glucose, 2 g fructose, and 0.25 g sucrose) in a beaker and were dissolved in about 40 mL methanol. Then, the solutions were quantitatively transferred to a 100 mL volumetric flask, were diluted using distilled water and shaken. Honey sample (0.6 g) was dissolved in distilled water and transferred to a volumetric flask, then; 1 mL of 10 % (W/V) mannitol solution was added as an internal standard and adjusted 100 mL with distilled water.

The solution (100 µL) was transferred to a test tube and was dried out in a current of air. 200 µL oxime reagents (pyridine solution containing 12 mg/mL hydroxylamine hydrochloride) was added and then sealed, mixed and heated at 70-75 ºC for 30 min. The sample was cooled at the room temperature then; trifluoroacetic acid (10 µl) was added and remained for 30 min. The prepared sample (1 µL) was injected into the BP5 capillary column (30 m and 0.25 mm id). Helium was used as a carrier gas at a flow-rate of 1 mL/min-1. Injector and detectors were set at 250 °C. The oven temperature was programmed to raise from70 °C to 140 °C at 50 °C/min and to 300 °C at 6 °C/min. Calibration curves were obtained for glucose, fructose and sucrose and were used for quantification (Fig. 3-5).

3. Results

Table 1 shows the analysis results of 24 samples from 9 commercial brands of Iranian honeys (Tehran).

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Table 1. Physicochemical parameters of honey samples (mean ± standard deviation (SD), n=3)

Brands	pH	Moisture	Diastase	HMF**	Proline	Fructose	Glucose	Sucrose	F/G***
Unit	_	%	DN*	ppm	ppm	%	%	%	_
1 (AB)	4.27	16.00 ± 0.20	0.9	74.32 ± 0.67	177 ± 9	34.53	32.91	2.3	1.0
2 (TF)	4.58	16.50 ± 0.96	0.2	12.35 ± 0.47	308 ± 3	26.65	21.11	4.2	1.3
3 (DP)	4.65	15.50 ± 0.06	2.7	1.37 ± 0.25	330 ± 8	32.92	26.48	4.7	1.2
4 (JK)	4.60	15.70 ± 0.21	6.3	3.76 ± 0.30	646 ± 1	35.64	28.93	2.1	1.2
5 (SN)	4.60	15.00 ± 0.06	5.2	18.02 ± 0.13	381 ± 6	41.93	34.32	2.1	1.2
6 (SK)	4.58	14.80 ± 0.20	4.3	5.43 ± 0.16	676 ± 33	34.6	27.55	0.7	1.3
7 (IM)	**4.74**	**15.80 ± 0.06**	**10.5**	**4.88 ± 0.03**	**482 ± 20**	**35.63**	**31.19**	**0.3**	**1.1**
8 (MD)	**4.51**	**15.80 ± 0.06**	**12.2**	**5.01 ± 0.10**	**1233 ± 37**	**39.13**	**29.64**	**0.4**	**1.3**
9 (MH)	4.40	15.30 ± 0.32	2.3	8.52 ± 0.44	270 ± 7	38.07	33.87	3.6	1.1
Satisfactory Limit by EU	≥ 3.5	≤ 20	≥ 8	≤ 40	≥ 180	-	-	≤ 5	≥ 0.9

DN*: Diastase number
HMF**: Hydroxymethylfurfural
F/G***: Fructose/Glucose ratio

4. Discussion

According to the honey standard, the pH value of the honey solution (10 %) is defined as at least 5.3 and the range of pH value of honey samples is between 4.27 (honey sample 1/AB) and 4.74 (honey sample 7/IM). Therefore, all samples were in standard condition for pH value (Fig. 6).

The maximum moisture content of honey is 20 % based on the honey standard. Honey sample 2/TF had the highest moisture content while honey sample 6/SK had the lowest content and then the moisture content of all samples was in the standard range (Fig. 7).

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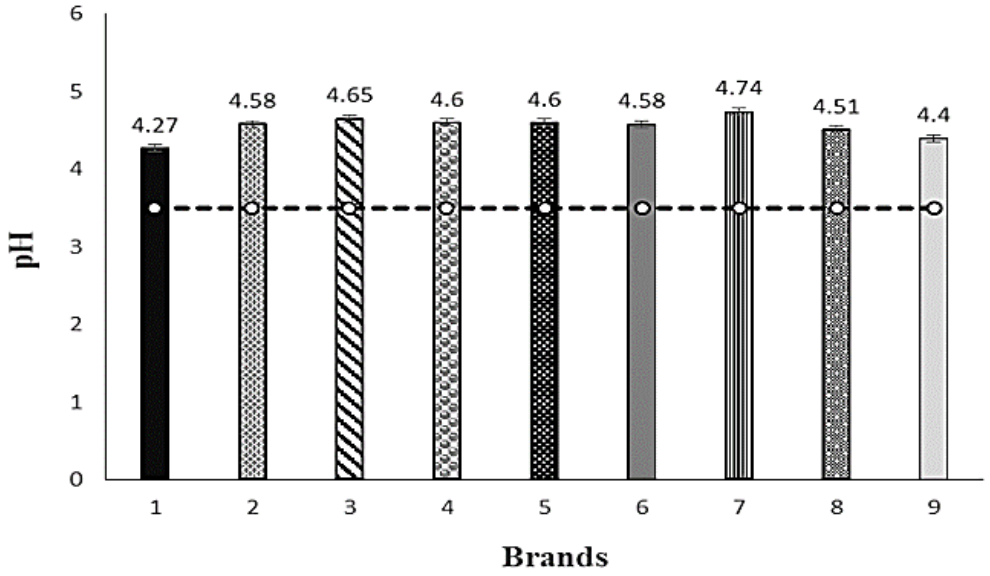


Fig. 6. pH value comparison of honey samples (Acceptable value ≥ 3.5)

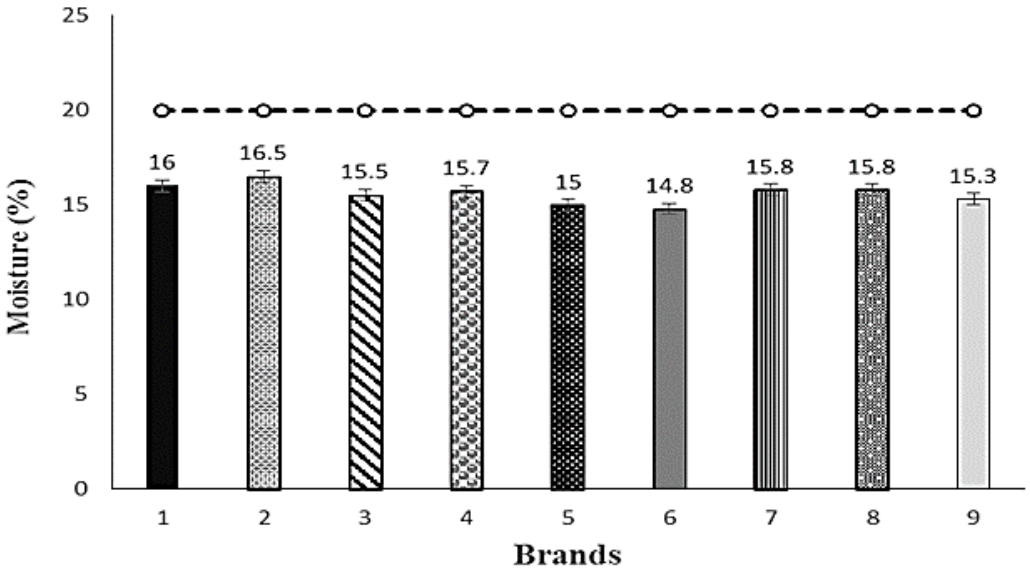


Fig. 7. Moisture content comparison of honey samples (Acceptable value ≤ 20 %)

The diastase enzyme is the only biomolecule that has been considered in the standard honey to evaluate its quality. The range of diastase activity for the honey samples in this study is from 0.2 up to 12.2 to Schade units. Since the minimum amount of diastase number defined in the honey standard is 8 Schade units, only two samples (7/IM and 8/MD) had acceptable enzymatic activity (Fig. 8).

HMF is considered as a hazard chemical compound which is produced by the heat from sugars. The amount of this compound was increases by heating, because of high percentage of sugar in the honey. According to the honey standard, the amount of this compound should not exceed 40 ppm. The results of this study show that, the amount of this compound in the other samples is lower than 40 ppm, except honey sample 1/AB which is 74 ppm (Fig. 9).

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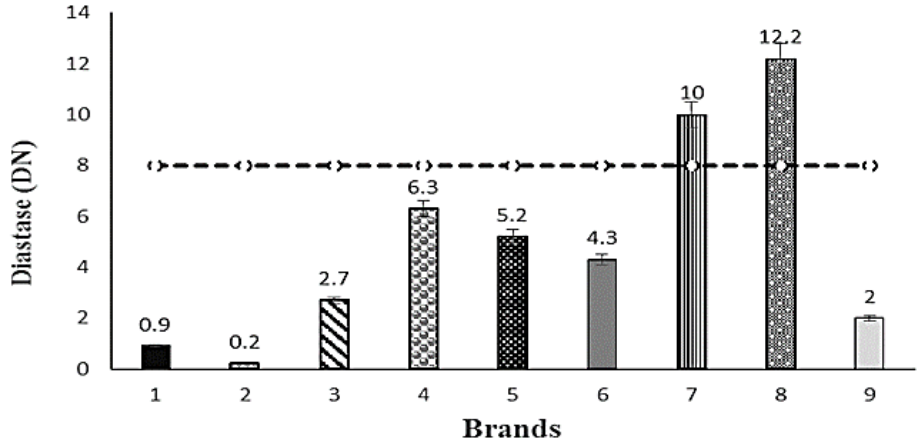


Fig. 8. Diastase content comparison of honey samples (Acceptable value ≥ 8 DN)

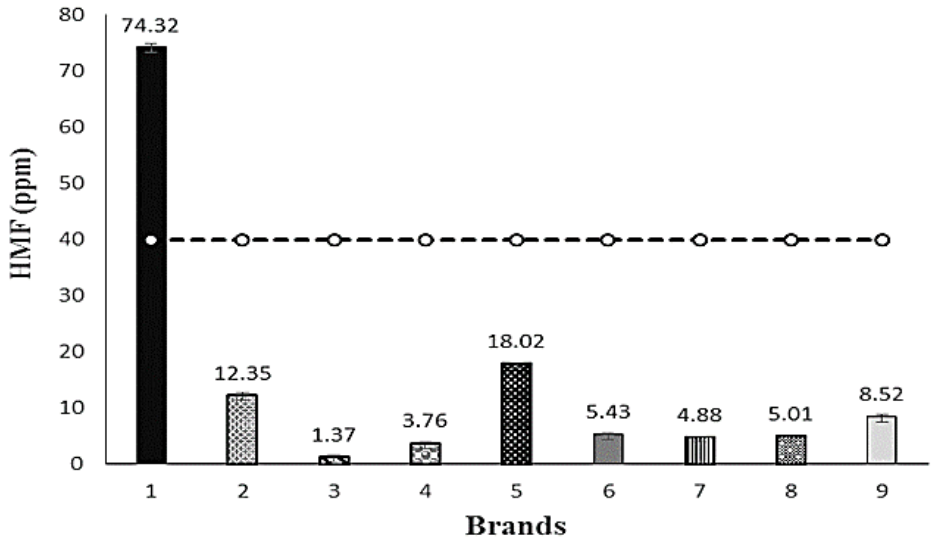


Fig. 9. HMF content comparison of honey samples (Acceptable value ≤ 40 ppm)

There are approximately 27 free amino acids in honey and proline is the major amino acid in honey (50-85 %). Proline content varies in different honeys according to its floral type. Also, proline comes mainly from honey bee during the conversion of nectar into honey, which leads to high variability of the proline content within honeys from the same botanical source. The natural honey should have a proline content of more than 180 ppm. The results show that all samples had appropriate proline content, except honey sample 1/AB, with proline content at least acceptable limit, and honey sample 8/MD with 1233 ppm had the highest proline content (Fig. 10).

Honey is considered as a sugar-rich food. Honey sugar profile plays an important role in determining the quality of honey. Sucrose is considered as one of the most important sugar in honey and the maximum amount of sucrose in the honey standard is 5 %. In this study, the sucrose contents of the honey samples was detected from 0.3 up to 4.7 (g/100 g) and fructose glucose ratio were more than 0.9 for all samples (from 1.0 up to 1.3). The sucrose content of samples was between 0.3 and 4.7 % (Fig. 11).

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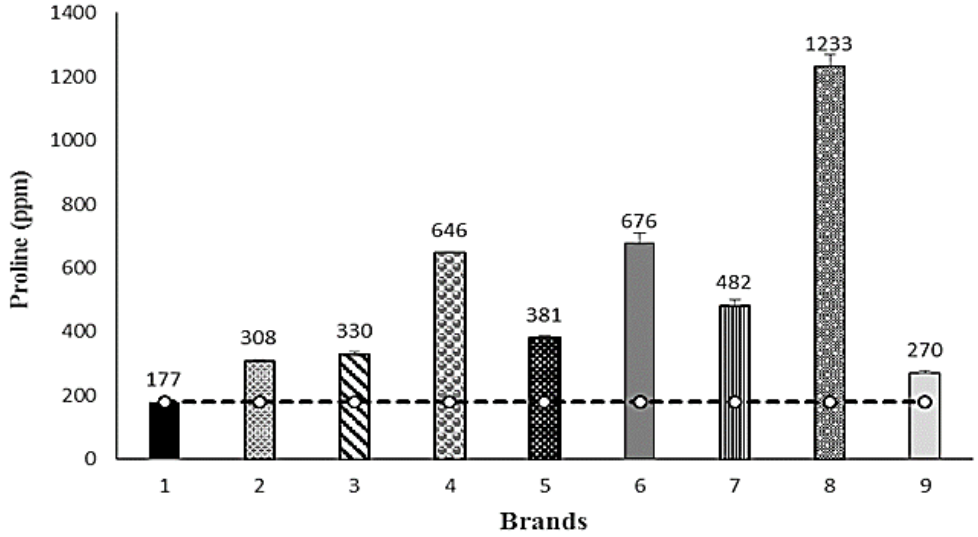


Fig. 10. Proline content comparison of honey samples (Acceptable value ≥ 180 ppm)

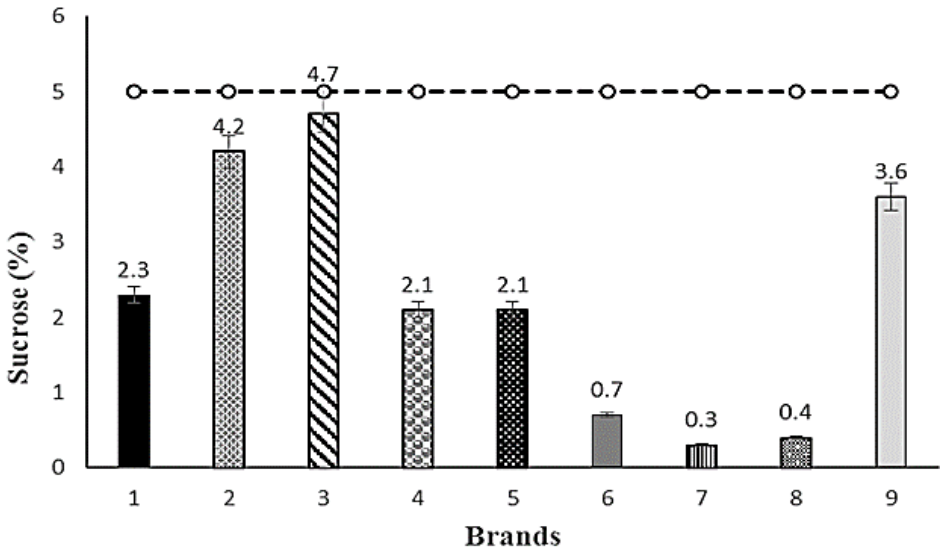


Fig. 11. Sucrose content comparison of honey samples

Considering all the factors together can lead to a true judgment about the quality of honey. In honey sample 1/AB, high HMF levels and low activity of diastase indicate inadequate conditions of honey processing. The use of heat in the process of separating honey from the wax can increase the amount of HMF and decrease enzyme activity. Despite the low-level of enzyme activity in honey samples of 2/TF, 3/DP, 4/JK, 5/SN, 6/SK and 9/MH, the amount of HMF and sugar profiles are in full compliance with standard values. Since the only source of honey diastase enzyme is by bee processing, so it seems that adulteration was acquired with cheaper sweeteners such as sugar syrups and molasses. However, the amount of the diastase activity in the honey samples 7/IM and 8/MD was in the defined range; and the low HMF and high proline levels confirm the high quality of them (Fig. 12.).

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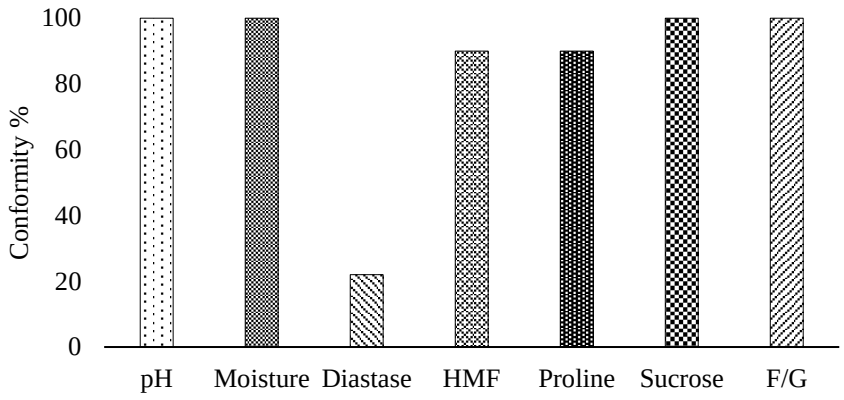


Fig. 12. The conformity to the CAC standards between honey brands

5. Conclusion

Honey is considered as a valuable food so control of its quality is important. According to the results of this study, it seems that among the physical, chemical and biomolecular factors, the biomolecules are the most important index to evaluate honey. There is a positive relation between biomolecules content, activity of the bees and the plants which is used by the bees. The amino acids, phenolic combination and total flavonoids are biomarkers by plant origin. While the enzyme such as diastase, glucose oxidase, invertase and catalase are biomolecules by bee origin. Since the diastase activity is only the biomolecular factor which is measured in CAC standard and recommends other biological factors added to the international honey standard.

Author contributions

F. N.: Investigation, Data curation, Supervision, F. Kh-S.: Scientific adviser, M. K.: GC/Mass operator, SV. Gh.: HPLC operator, H. S.: Data analysis, F. T.: Spectrophotometer operator.

Conflict of Interest

Authors declare that there is no conflict of interest.

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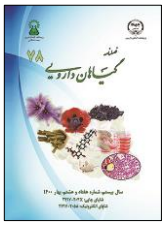
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مقاله تحقیقاتی

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دیاستاز

کدکس الیمنتاریس

چکیده

مقدمه: عسل یکی از مواد غذایی با ارزش است که از قرن‌ها پیش در جوامع بشری مورد استفاده بوده و به واسطه ویژگی شفابخش خود به عنوان دارو در درمان بسیاری از بیماری‌ها به کار می‌رود. عدم وجود روشی عینی برای تشخیص عسل طبیعی از عسل تقلبی زمینه سوء استفاده سودجویان در این عرصه را قوت بخشیده و موجب عرضه عسل تقلبی به جای عسل طبیعی شده است. بنابراین کیفیت عسل باید با هدف تضمین و حفظ حقوق مصرف‌کنندگان کنترل شود. **هدف:** مطالعه حاضر جهت ارزیابی ۲۴ نمونه از برندهای تجاری عسل با کدهای ۱ تا ۹ (AB-۱، TF-۲، DP-۳، JK-۴، SN-۵، SK-۶، IM-۷، MD-۸ و MH-۹) در بازارهای تهران انجام شده است و در آن تعدادی از پارامترهای فیزیکی، شیمیایی و بیولوژیکی نمونه‌ها مورد ارزیابی قرار گرفت. **روش بررسی:** برای ارزیابی محتوای فروکتوز، گلوکز و ساکارز، و ترکیب کربوهیدرات از روش کروماتوگرافی گازی متصل به طیف‌سنج جرمی استفاده شد. ارزیابی ترکیب هیدروکسی متیل فورفورال با استفاده از دستگاه کروماتوگرافی مایع با کارآیی بالا با شناساگر ماورای بنفش و سایر پارامترهای کیفیت فیزیکوشیمیایی از جمله رطوبت، pH، مقدار پرولین و فعالیت دیاستاز با توجه به روش‌های استاندارد کدکس الیمنتاریس ارزیابی شد. **نتایج:** از بین ۹ برند انتخاب شده فقط ۲ برند با کلیه مشخصات اصلی بین‌المللی مطابقت داشتند. اگرچه تمام پارامترهای برندهای دیگر با مقادیر استاندارد مطابقت داشتند ولی میزان فعالیت دیاستاز در محدوده مورد قبول نبود. **نتیجه‌گیری:** بر اساس نتایج این مطالعه به نظر فعالیت دیاستاز عامل تعیین کننده جهت ارزیابی کیفیت عسل می‌باشد.

مخففها: GC/MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی؛ HMF، هیدروکسی متیل فورفورال؛ HPLC-UV، کروماتوگرافی مایع با کارآیی بالا با شناساگر ماورای بنفش

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