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Scientific Research

Investigating the antioxidant and physicochemical properties honeys from Mazandaran province in comparison to commercial samples

Razie Razavi[1], Reza Esmaeilzadeh Kenari[2*]

1– Postdoctoral researcher of food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Department of food science and technology, Faculty of agricultural engineering, Sari, Iran

2–* professor of Sari Agricultural Sciences and Natural Resources University, Department of food science and technology, Faculty of agricultural engineering, Sari, Iran

ABSTRACT

The reputation, price and high demand for honey as a complex matrix of nutrients with antioxidant properties have increased the possibility of adulteration. In this study, 12 samples of natural honey were collected from the farms of Mazandaran province, and their physicochemical and antioxidant properties were compared with 12 commercial honey samples purchased from market in Sari. Honey samples were compared for pH, acidity, moisture, and ash content, electrical conductivity, hydroxymethyl furfural, sucrose content, glucose, fructose, fructose to glucose ratio, refractive index, viscosity, content of total phenolic compounds and antioxidant activity. The results showed that average pH, acidity, total phenol, moisture, electrical conductivity and ash, almost all natural and commercial samples were within the standard range. The sucrose content and hydroxymethylfurfural in none of the commercial samples was within the standard range. Only three natural honey samples had sucrose content less than 5%. Ten samples of natural honey had less than 40 % hydroxymethylfurfural. Although total reducing sugars in natural and commercial honey samples had no statistically significant difference ($p<0.05$), all of the natural honey samples and three commercial honey samples had fructose to glucose ratio higher than 0.9. Considering the lower quality of commercial honeys, it is suggested that the inspection organizations pay more attention to the production units of this product.

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*Corresponding Author E-Mail:
reza_kenari@yahoo.com

1- Introduction

Honey as a natural sweet substance produced by bees*Apis mellifera* It is produced from the nectar of plants or the secretions of living parts of plants. In this way, bees collect them and combine them with special ingredients and turn them into honey [1]. Honey contains about 80% carbohydrates (35% glucose, 40% fructose and 5% sucrose) and 20% water, and is considered a great source of energy. Honey also contains more than 200 types of compounds including amino acids, vitamins, minerals, enzymes, organic acids and phenolic compounds [2, 3].

The nutritional value, increasing demand and high price of honey have made this product the main target of frauds with commercial purposes. Honey adulteration can occur by adding foreign substances such as molasses, starch, glucose syrup, sucrose, water and invert sugar or by changing the physicochemical parameters. The evaluation of the authenticity of honey is mainly focused on the detection of added sugars, as this type of adulteration has been proven to be one of the most common cases [4]. The authenticity of honey is very important in terms of business and health aspects. It is difficult to detect fraud in honey due to the natural diversity of honey, which is caused by differences in plant species, maturity, environment, processing and storage methods.

Counterfeit honey has less nutritional and medicinal benefits compared to pure honey, and its physical and chemical properties such as refractive index, moisture content, total dissolved solids, density, specific gravity, capillarity effect, surface tension and quantity may be altered.pH be different from pure honey. For example, pure honey has Newtonian behavior, while adulterated honey exhibits non-Newtonian pseudoplastic behavior that fits well with the power law model. The study of physical and chemical properties of honey has increased in recent years, because these parameters are important for the certification process of honey quality assurance [5]. Today, profiteers are trying to sell fake products and earn huge profits by misleading consumers, so it is necessary to make efforts to inform consumers about the quality of honey. So far, no study has been done on the physicochemical and antioxidant properties of the honeys of Mazandaran province that were collected from farms and compared with commercial samples. Therefore, this study will be conducted with the aim of comparing the quality of commercial and natural honeys of Mazandaran province.

2- materials and methods

1-2- Materials

Natural honeys were collected in spring from different regions of Mazandaran province. Commercial honeys were purchased from the store (Table 1). All chemicals were obtained from Sigma Aldrich, USA.

2-2- Methods

1-2-2- MeasurementpH and acidity

pH Different samples of honey by potentiometric method and at temperatureC° 20 usingpHMeter (Model 654, Metrohm, Switzerland) was determined. Measuring acidity by neutralizing honey solution (mL 75 distilled water without carbon dioxide containing 10 grams of honey) against 0.1 normal sodium hydroxide [6].

Table 1. The origin of the studied samples

The origin of the commercial sample	Sample code	The origin of the natural sample	Sample code
Isfahan province	C1	Sari city	N1
Isfahan province	C2	Sari city	N2
Hamedan Province	C3	Sari city	N3
Isfahan province	C4	Sari city	N4
Isfahan province	C5	Noor city	N5
The center is a province	C6	Savadkuh city	N6
Khorasan-Razavi province	C7	Let the city	N7
Alborz province	C8	Qaemshahe city	N8
Isfahan province	C9	Amol city	N9
Khorasan-Razavi province	Q10	Ramsar city	N10
Tehra province	C11	Savadkuh city	N11
Alborz province	C12	Behshahr city	N12

2-2-2- Refractive index and viscosity

Refractive index of different honey samples using a desktop refractometer (Atago NAR-Series, Tokyo, Japan) in temperature C° 25 were measured [7]. Viscosity of different honey samples at temperature C° 20 and spindle number 18 using a Brookfield viscometer (RVDV-II+ pro, Brookfield, Inc, USA) At rpm 100 readings were done [8].

2-3-2- Total amount of phenolic compounds and antioxidant properties

To measure the total amount of phenolic compounds, µL 100 of a 10% solution w/v Honey was prepared and with µL 100 of a 50% solution of folin ciocaltio and mL 2 sodium carbonate solutions of 2% weight/volume were combined. After 30 minutes, their absorbance was read at a wavelength of 750 nm. Results as mg GA/100 g Asal was reported. To measure antioxidant activity, mL 0.5% honey solution (2.5% weight/volume) with mL 1 solution DPPH Methanolic mmol 0.06 was combined. After 15 minutes, the absorbance of the solution was read at a wavelength of 517 nm and the antioxidant activity was calculated based on equation 1 [9]:

(1) $100 \times ([A_c-A_s]/A_c)$ = inhibition percentage

In this regard A_c And A_s It is the absorption of the witness and the sample, respectively.

4-2-2- Measurement of humidity, electrical conductivity and ash

The moisture content of different honey samples using the number obtained from a desktop refractometer (Atago NAR-Series, Tokyo, Japan) in temperature C° 25 and using the method AOAC It was measured as 969.38 [10]. To measure the electrical conductivity, a 20% solution of honey was prepared according to the dry matter and using a conductivity meter (Oakton, TDS 6 Acorn, Vernon Hills, IL, USA) electrical conductivity at temperature C° 25 readings [7]. The amount of ash was calculated based on electrical conductivity and according to the national standard of Iran number 92 [11].

5-2-2- Hydroxymethylfurfural measurement

To measure the amount of hydroxymethylfurfural, first the solution of honey samples was clarified using caries 1 and 2. Then, sodium bisulfite and distilled water were added to the control and sample solutions, respectively. The absorbance of the samples was read at 284 and 336 nm. The amount of hydroxymethylfurfural was calculated from Equation 2 [11]:

(2) IN/D×5×7/149×(Difference in absorbance number at 284 and 336 nm wavelengths) = Hydroxymethylfurfural

In this regardD Dilution coefficient (volume of the final solution divided by ten) andIN The weight of the sample is in grams.

6-2-2- Measurement of sugar level

To measure the amount of total reducing sugars, Fehling's method was used in accordance with Iran's national standard No. 92. The total amount of reducing sugars was calculated. Glucose percentage was obtained using iodometry method and titration of honey sample solution prepared from test of reducing sugars. The percentage of fructose was calculated from the difference in the amount of reducing sugars minus the amount of glucose. Sucrose percentage was calculated by multiplying the difference of reducing sugars before and after hydrolysis by a factor of 0.95 [11].

3-2- Statistical analysis

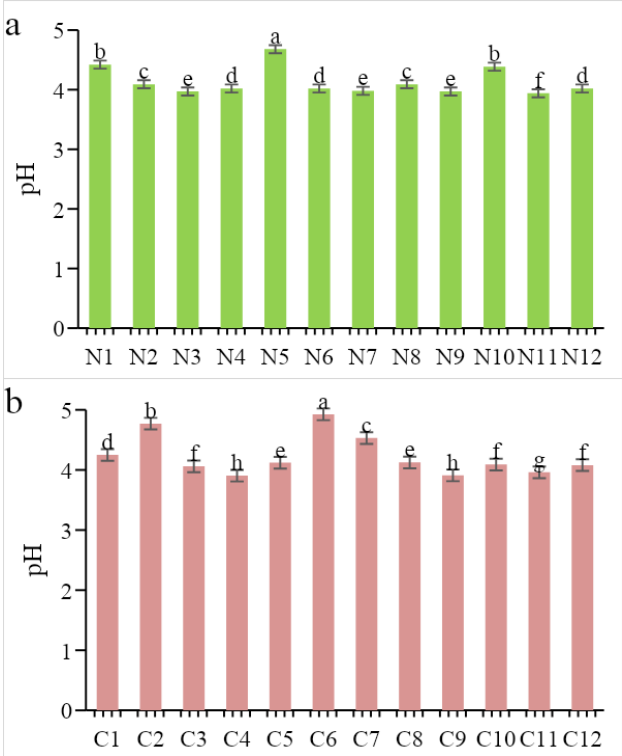
Statistical analysis of data related to different tests was done using Duncan's test and one-way ANOVA method. Data using softwareSPSS 20 versions were compared with each other and Excel software was used to draw graphs.

3. Results and Discussion

1-3- pH and acidity

Honey contains amino acids and organic acids that give it an acidic nature [6]. Results related topH Samples of natural and commercial honey, respectively, in Figa1 andb1 is shown.pH Natural honey samples in the range of 3.94 to 4.68 andpH Commercial samples ranged from 3.91 to 4.92. In accordance with the national standard of Iran, at leastpH The standard of honey is 3.5. Based on this, all natural and commercial samples were within the standard range. Gruposila-Constantinescu et al. (2020) quantpH reported honey in the range of 3.5 to 2.4 [6].

Acidity is a parameter to show the freshness of honey. Acidity can increase in case of fermentation. In case of adulteration by adding sugar, if the acid used is not neutralized, honey can become highly acidic. In case of excessive neutralization, honey becomes neutral to alkaline [6]. The results related to the acidity of natural and commercial honey samples, respectively, in Figc1 andd1 is shown. Acidity of natural honey samples in the rangemeq/kg 8/85 tomeq/kg 14/93 and acidity of commercial samples in the rangemeq/kg 13/68 tomeq/kg 23/91 was variable. According to the national standard of Iran, the maximum acidity of honey standardmeq/kg It is 40. Based on this, all commercial and natural samples were within the standard range in terms of acidity.



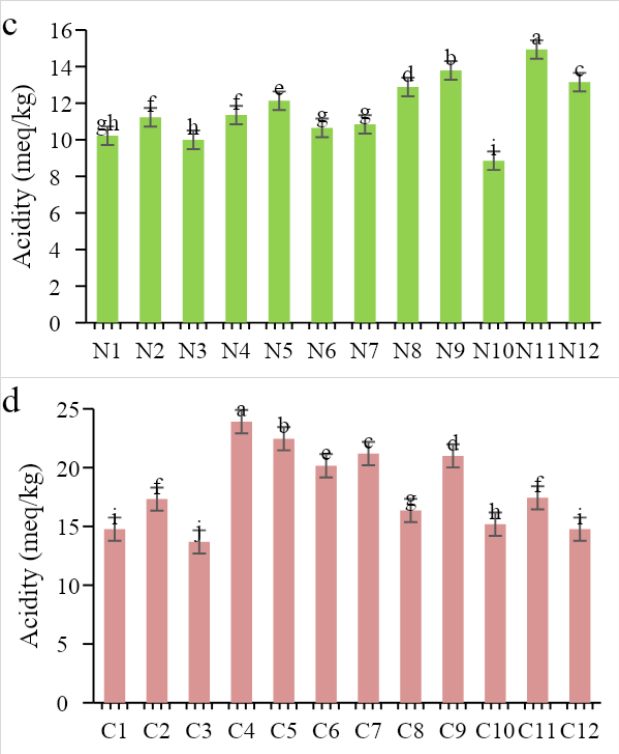


Fig. 1. pH and acidity of different honey samples. a) and b) pH of natural and commercial honey samples, respectively. c) and d) acidity of natural and commercial honey samples, respectively.

2-3- Refractive index

The refract method is the most widely used method because it is easy to perform and can be easily repeated. Refractive index is a parameter whose value is related to the values of other parameters such as water content, solids, total dissolved solids and specific gravity. The higher the refractive index, the lower the humidity. Low levels of moisture prevent honey from fermenting and increase shelf life as well as storage time [7]. Refractive index of natural honey samples ranged from 1.4865 to 1.4880 and for commercial honeys from 1.4860 to 1.4920. Ramzi et al. (2015) have reported the refractive index value of natural honeys between 1.4867 and 1.4895, which is in line with the results obtained from the present study [8]. Satihi et al. (2016) reported the refractive index value of Romanian honeys from 1.4840 to 1.4990. The refractive index depends on the content of solids and the amount of honey water [12].

3-3- Viscosity

The results related to the viscosity of different honey samples are shown in Table 2. The viscosity of natural honey samples is from 348 to 1117 and the viscosity of commercial samples is fromcP° 19400 tocP° There were 86088 variables. Gruposila-Constantinescu et al. (2020) measured the viscosity of different honey samples in the rangecP° 240 tocP° 8860 reported [6]. Ramzi et al. (2015) the viscosity of different honeys cP° 5900 tocP° reported 45,500 [8]. There was a negative correlation between the moisture content and the viscosity of the honey samples, so that the viscosity decreased with the increase of the honey moisture content. Blay et al. (2017) investigated the effects of moisture and water activity on the viscosity of honey and stated that with the increase in the moisture content of honey, its viscosity decreases. This can be due to the tendency of solid components attached to water molecules, which reduces the liquid phase of honey. As a result, the viscosity of honey increases with the decrease of moisture in different cutting rates [10].

Table 2- Viscosity, a total phenolic content and antioxidant activity of the studied samples

DPPH radical scavenging (%)	Total phenolic (mg/100g)	Viscosity (cP)	Sample code	DPPH radical scavenging (%)	Total phenolic (mg/100g)	Viscosity (cP)	Sample code
12.94^{a}	10.36^{a}	19400^{k}	C1	15.02^{a}	21.17^{ab}	1117^{a}	N1

12.36^{a}	10.12^{a}	24700^{j}	C2	13.02^{d}	12.47^{h}	875^{d}	N2
10.38^{c}	6.32 It is	55668^{d}	C3	13.66^{c}	13.51^{g}	920^{c}	N3
11.51^{b}	8.66^{c}	66810^{c}	C4	13.86^{c}	14.36^{f}	1120^{a}	N4
11.74^{b}	9.04^{b}	39600^{g}	C5	14.88^{b}	20.65^{bc}	1112^{a}	N5
12.05^{a}	10.01^{a}	31793^{i}	C6	13.25^{c}	13.03^{g}	905^{c}	N6
11.89^{b}	9.35^{b}	35400^{h}	C7	13.75^{c}	14.02^{f}	1000^{b}	N7
9.15^{d}	3.21^{h}	48190^{f}	C8	13.92^{c}	16.25 It is	949^{bc}	N8
11.25^{b}	8.31^{c}	86088^{a}	C9	14.12^{b}	18.17^{d}	348^{g}	N9
10.11^{c}	4.54^{g}	53180^{of}	Q10	15.13^{a}	22.35^{a}	980^{b}	N10
11.03^{b}	7.86^{d}	73220^{b}	C11	14.35^{b}	19.32^{cd}	520^{f}	N11
10.25^{c}	5.19^{f}	50250^{if}	C12	14.05^{b}	17.13^{of}	690^{I} t is	N12

Different letters in each column indicate statistically significant differences at p<0.05.

4-3- Total phenol and antioxidant properties

The results related to the amount of phenolic compounds of different honeys are shown in Table 2. As can be seen, the amount of total phenolic compounds in natural honeys ranges from 12.47 to 22.35 and in commercial honeys.mg/100g It was from 3/21 to 10/36. According to the national standard of Iran, the minimum total phenolic compoundsmg/mL It is 0.03 that all natural and commercial samples were within the standard range. Ken et al. (2015) measured the amount of phenolic compounds in honeymg E GA/100 g 16 to 120 reported that it corresponds to the amount of phenolic compounds of natural honey samples [13]. The difference in the amount of phenolic compounds in honey is related to their geographical and botanical regions [8, 14]. The rate of inhibition of free radicalsDPPH In natural honey samples (13.02 to 15.13 percent) it was higher than commercial honey samples (96.15 to 12.96 percent) (Table 2). There was a positive correlation between the amount of phenolic compounds and the antioxidant property of honeys, and with the increase in the amount of phenolic compounds, the antioxidant property of honey increased. The antioxidant properties of honey depend on free amino acids and the total amount of phenolic compounds in honey [15]. Atmani et al. (2021) the amount of phenolic compounds of honeys collected in Algeria in the rangemg E GA/100 g 64 to 200 reported. Also, they increase the antioxidant activity of honeys with free radical inhibition methodsDPPH ،ABTS, iron chelation and reducing power were measured. Their results showed an increase in the antioxidant properties of honey with an increase in the total amount of phenolic compounds [9]. A significant positive relationship between the amount of phenolic compounds and antioxidant properties has also been reported by other researchers [16-18].

5-3- Humidity

The results related to the moisture content of natural and commercial honey samples are shown in Figa2 andb2 is shown. The moisture content of natural honey samples ranged from 19.5 to 20.1 and the moisture content of commercial samples ranged from 17.9 to 20.3. According to the national standard of Iran, the maximum moisture standard of honey is 20 percent by weight. Based on this, two commercial samples and three natural samples were not within the standard range in terms of moisture content. Ramzi et al. (2015) reported the moisture content of four honey samples with the origin of forest, mountain, sunflower and Eshghe in the range of 15.3 to 19.9 percent. The moisture content of honey depends on several parameters, such as the harvest

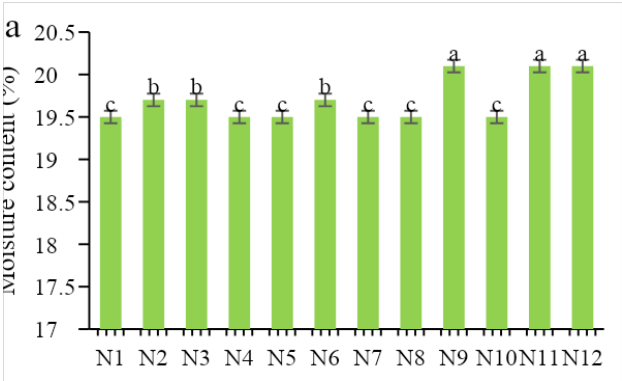
season, the maturity level of the hive, the moisture content of the main plant, and weather factors. The minimum and maximum humidity reported for honey is 10.6 and 29%, respectively, which is related to domestic and acacia honey [8]. Blay et al. (2017) reported the moisture content of different honey samples in the range of 14.79 to 20.54 percent [10].

6-3- Electrical conductivity

Electrical conductivity refers to the property of a general material to conduct electric current. In honey, it is directly influenced by the plant origin of the nectar as well as other inorganic or organic molecules such as ions, organic acids and proteins that can act as electrolytes [19]. The results related to electrical conductivity of natural and commercial honey samples are shown in Figc2 andd2 is shown. Electrical conductivity of natural honey samples in the range of 0.30 to 0.77 and electrical conductivity of commercial samples in the rangem$_s$/cm It varied from 0.36 to 0.87. According to the national standard of Iran, the maximum electrical conductivity of the honey standardm$_s$/cm It is 0.8. Based on this, all natural honey samples were in the standard range in terms of electrical conductivity, and only four commercial samples were not in the standard range. Albo et al. (2021) measured the electrical conductivity of honeys from East Indonesia in the rangem$_s$/cm reported 0.13 to 0.50 [7].

7-3- ash

Ash and electrical conductivity are the characteristics used to distinguish natural honey from fake honey. The amount of ash is obtained by the macro and micro elements of honey, which are influenced by the composition of collected nectar. Minerals are important nutritional compounds that play a role in the health and well-being of consumers [7]. The results related to the ash of natural and commercial honey samples, respectively, in FigIt is2 andf2 is shown. The ash of natural honey samples ranged from 0.09 to 0.36 and the ash of commercial samples ranged from 0.13 to 0.42. According to the national standard of Iran, the maximum ash of honey standard is 0.6 percent by weight. Based on this, all natural and commercial honey samples were within the standard range. Albu et al. (2021) reported the amount of ash in honey samples collected from eastern Indonesia in the range of 0.03 to 0.22 percent [7], which is in accordance with the present study.



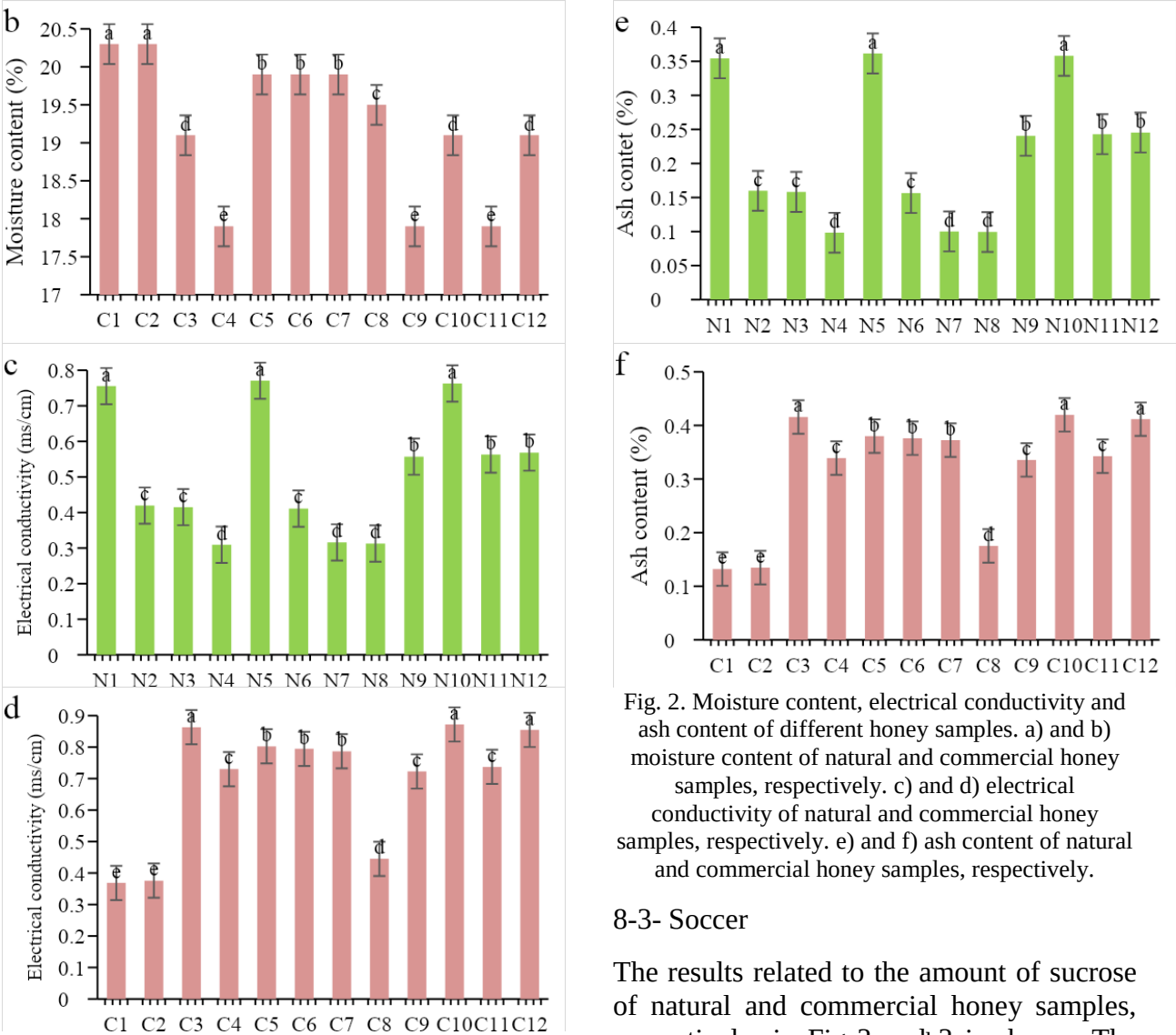


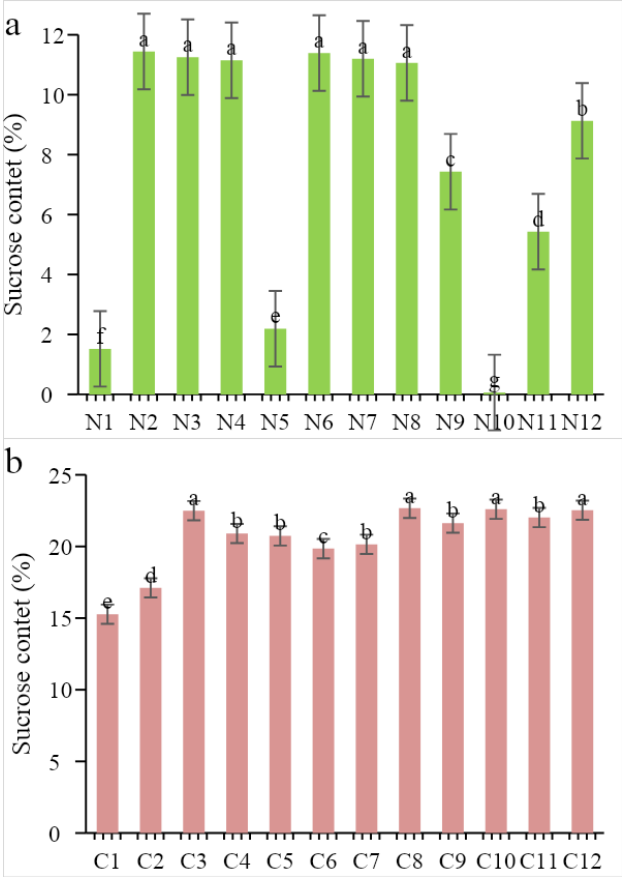
Fig. 2. Moisture content, electrical conductivity and ash content of different honey samples. a) and b) moisture content of natural and commercial honey samples, respectively. c) and d) electrical conductivity of natural and commercial honey samples, respectively. e) and f) ash content of natural and commercial honey samples, respectively.

8-3- Soccer

The results related to the amount of sucrose of natural and commercial honey samples, respectively, in Figa3 andb3 is shown. The amount of sucrose in natural honey samples varied from 0.06 to 11.44 and the amount of sucrose in commercial samples varied from 15.27 to 22.66%. According to the national standard of Iran, the maximum amount of sucrose in standard honey is 5% by weight. Based on this, only three samples of natural honey were within the standard range of sucrose in terms of sucrose content. Sucrose content of all commercial honey samples was outside the standard range. Bonta et al. (2020) reported the amount of sucrose in different honey samples between 0.0 and 14.84% [20].

3-9- Hydroxymethylfurfural

measurementHMF It is used to evaluate the quality of honey. In general, it is not present in fresh honey, its content increases during preparation and storage. Then, it can be useful to estimate the freshness of honey and since sucrose is formed during acid hydrolysis. The presence of high levels of this compound raises the possibility of adulteration in honey with invert syrup [21]. The results related to the amount of hydroxymethylfurfural in natural and commercial honey samples, respectively, in Figc3 andd3 is shown. The amount of hydroxymethylfurfural in natural honey samples ranged from 25.11 to 43.25 and the amount of hydroxymethylfurfural in commercial samples ranged from 95.32 to 183.5. According to the national standard of Iran, the maximum amount of hydroxymethyl furfural in honey standardmg/kg It is 40. Based on this, only two samples of natural honey were not within the standard range in terms of hydroxymethylfurfural content. The amount of hydroxymethylfurfural in all commercial honey samples was outside the standard range. Mohoubi-Tafinieh et al. (2018) found the amount of hydroxymethylfurfural in Algerian honey samples in the rangemg/Kg reported 04/11 to 0/82 [21]. In another study, Makhloufi et al. (2020) compared the amount of hydroxymethylfurfuralmg/Kg reported 0.5 to 123.98 [22] and the natural honeys in the present study were in this range.



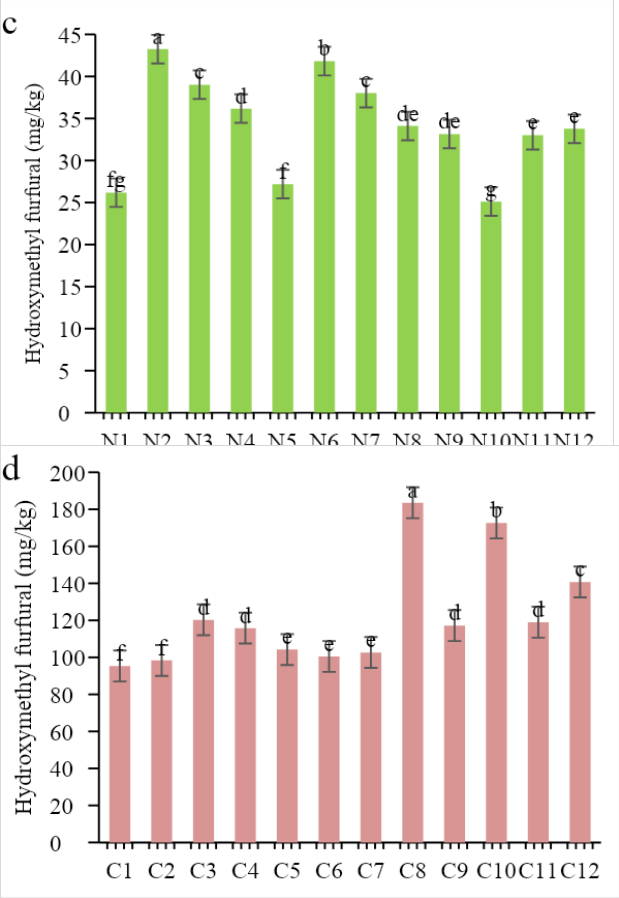
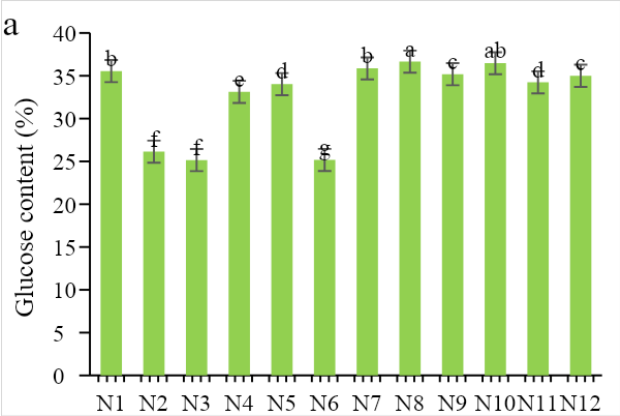
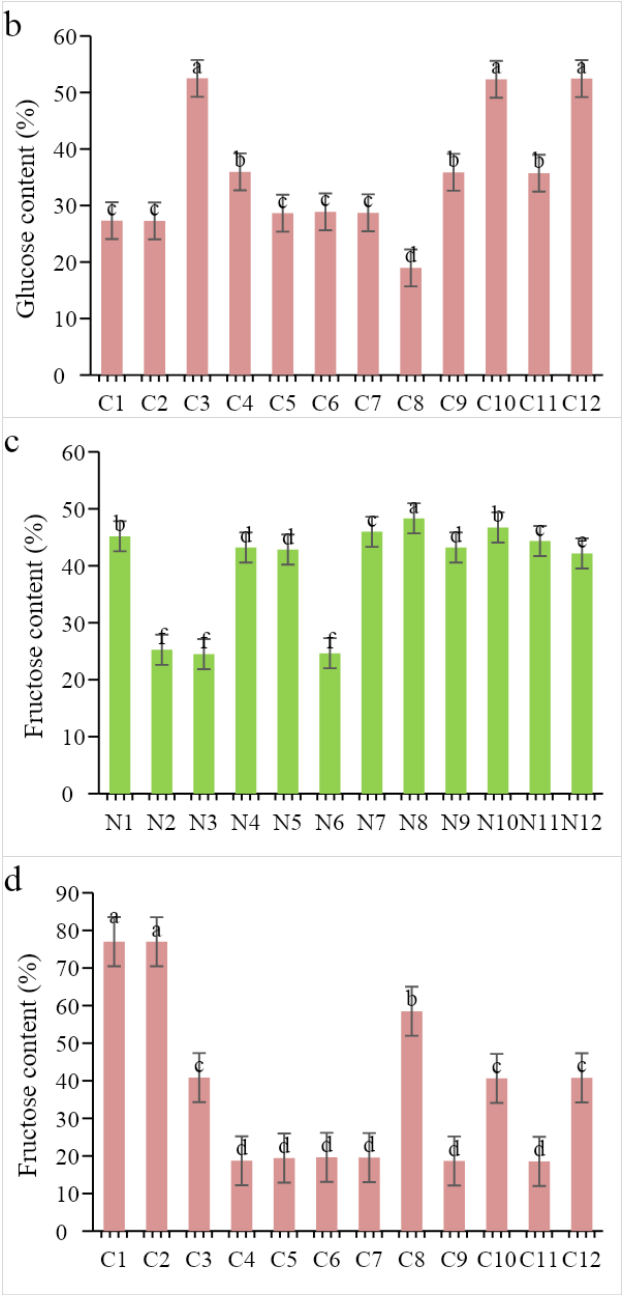


Fig. 3. Sucrose content, and hydroxymethyl furfural of different honey samples. a) and b) Sucrose content of natural and commercial honey samples, respectively. c) and d) hydroxymethyl furfural of natural and commercial honey samples, respectively.

10-3- Glucose, fructose and the ratio of fructose to glucose

The results related to the amount of glucose of natural and commercial honey samples, respectively, in Figa4 andb4 is shown. The glucose content of natural honey samples ranged from 25.17 to 36.65% and the glucose content of commercial samples ranged from 18.95% to 52.49%. The results related to the amount of fructose of natural and commercial honey samples, respectively, in Figc4 andd4 is shown. The amount of fructose of natural honey samples was in the range of 24.50 to 43.22 and the amount of fructose of commercial samples was in the range of 18.54 to 77.01%. The results related to the ratio of fructose to glucose of natural and commercial honey samples, respectively, in FigIt is4 andf4 is shown. The ratio of fructose to glucose of natural honey samples varied from 0.96 to 1.31 and the ratio of fructose to glucose of commercial samples varied from 0.51 to 3.08% [20]. According to the national standard of Iran, the minimum ratio of fructose to glucose in standard honey is 0.9. Based on this, all natural honey samples and three commercial honey samples were in the standard range in terms of fructose to glucose ratio. Bonta et al. (2020) reported the amount of fructose, glucose and the ratio of fructose to glucose in different honey samples in the range of 30.74 to 44.16 percent, 24.84 to 42.30 percent, and 0.87 to 1.68 percent, respectively. [20] which is in accordance with the values obtained for natural honeys in the present study.





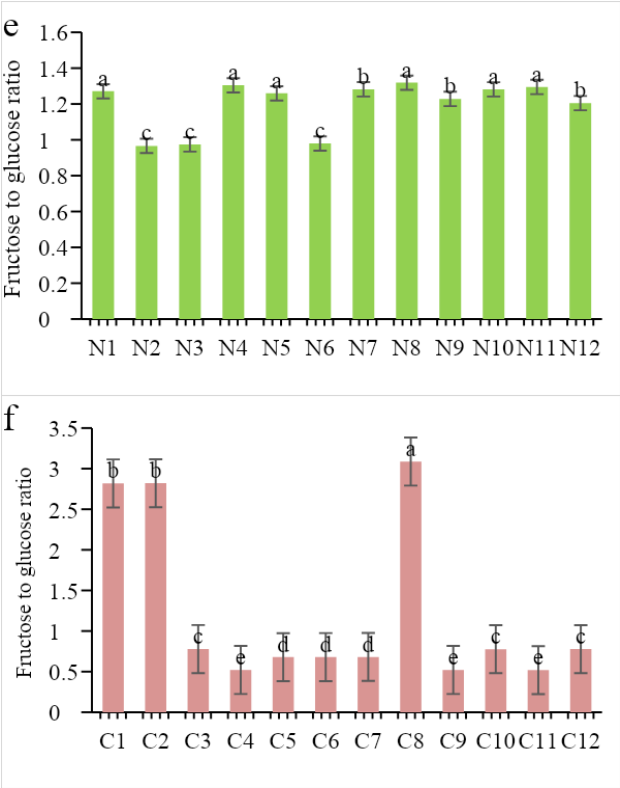


Fig. 4. Glucose, fructose and fructose to glucose ratio of different honey samples. a) and b) glucose content of natural and commercial honey samples, respectively. c) and d) fructose content of natural and commercial honey samples, respectively. e) and f) fructose to glucose ratio of natural and commercial honey samples, respectively.

3-Conclusion

In this research, the physicochemical and antioxidant properties of honeys collected from Mazandaran province were compared with commercial honey samples available in the market. Unfortunately, commercial honeys were not within the standard range in terms of sucrose and hydroxymethyl furfural. The natural honeys collected from the farms of Mazandaran province were favorable in terms of physicochemical and antioxidant properties, which increases the tendency of consumers to use non-commercial traditional honeys. It is necessary for relevant organizations to monitor the quality of honey products more so that people can safely use commercial honey products and benefit from the health

benefits of honey. Considering that the amount of sucrose and hydroxymethylfurfural can be considered as a quality indicator in honey products, future researches can be focused on finding quick tests to identify honey frauds.

5- Resources

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بررسی خصوصیات آنتی اکسیدانی و فیزیکوشیمیایی عسل‌های استان مازندران در مقایسه با نمونه‌های تجاری

راضیه رضوی[۱]، رضا اسماعیل زاده کناری[۲*]

۱-محقق پسادکتری گروه علوم و مهندسی صنایع غذایی، دانشکده مهندسی زراعی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

۲*- استاد گروه علوم و مهندسی صنایع غذایی، دانشکده مهندسی زراعی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

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* مسئول مکاتبات:

reza_kenari@yahoo.com

چکیده

شهرت، قیمت و تقاضای بالا برای عسل به عنوان یک ماتریکس پیچیده از مواد مغذی با خاصیت آنتی اکسیدانی، احتمال تقلب در آن را افزایش داده است. در این پژوهش ۱۲ نمونه عسل طبیعی از مزارع عسل استان مازندران جمع آوری شدند و خصوصیات فیزیکوشیمیایی و آنتی اکسیدانی آن‌ها با ۱۲ نمونه عسل تجاری خریداری شده از فروشگاه‌های شهر ساری مقایسه گردید. نمونه‌های عسل از نظر میزان pH، اسیدیته، رطوبت، خاکستر، هدایت الکتریکی، هیدروکسی متیل فورفورال، محتوای ساکارز، گلوکز، فروکتوز، نسبت فروکتوز به گلوکز، اندیس رفراکت، ویسکوزیته، مقدار ترکیبات فنولی کل و خاصیت آنتی اکسیدانی با هم مقایسه شدند. نتایج نشان داد از نظر میانگین pH، اسیدیته، فنول کل، رطوبت، هدایت الکتریکی و خاکستر تقریبا تمام نمونه‌های طبیعی و تجاری در محدوده استاندارد قرار داشت. مقدار ساکارز و هیدروکسی متیل فورفورال در هیچ یک از نمونه های تجاری در محدوده استاندارد قرار نداشت. تنها سه نمونه عسل طبیعی مقدار ساکارز کمتر از ۵ درصد داشتند. ۱۰ نمونه عسل طبیعی هیدروکسی متیل فورفورال کمتر از ۴۰ داشتند. اگرچه مجموع قندهای احیاءکننده در نمونه‌های عسل طبیعی و تجاری تفاوت معنی دار آماری ($p<۰/۰۵$) نداشت اما تمام نمونه‌های عسل طبیعی و سه نمونه عسل تجاری نسبت فروکتوز به گلوکز بالاتر از ۰/۹ داشتند. با توجه به کیفیت پائین تر عسل‌های تجاری، پیشنهاد می‌شود سازمان‌های نظارتی، توجه بیشتری به واحدهای تولید کننده این محصول داشته باشند.