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Original research

Rheological, antioxidant, physicochemical, and biochemical characterization of Iranian monofloral honeys

Ali Jafari[a], Mohsen Esmaiili[b], Saber Amiri[b,*], Reza Heidari[a]

[a] *Department of Food Science and Technology, Saba Institute of Higher Education, Urmia, Iran*
[b] *Department of Food Science and Technology, Faculty of Agriculture and Natural Resources, Urmia University, Urmia, Iran*

ABSTRACT

The present study provides an analysis of physicochemical, biochemical, and rheological properties of four types of monofloral honey: *Ziziphus*, *Thymus*, *Astragalus*, and *Alfalfa*. Physicochemical (palynology, moisture, pH, free acidity, insoluble solid, ash, conductivity, hue, and minerals), biochemical (sucrose, Hydroxymethylfurfural, diastase activity, antioxidant properties, total flavonoid, and total phenol) and rheological parameters were measured. The results of the palynology (pollen) test confirmed that the honey samples were monofloral. Hydroxymethylfurfural was 0.64±0.34 mg/kg for *Ziziphus*, 1.09±0.37 mg/kg for *Thymus*, 4.98±0.37 mg/kg for *Astragalus*, and 2.94±0.52 mg/kg for *Alfalfa*. The results showed that sucrose content for *Ziziphus*, *Thymus*, *Astragalus*, and *Alfalfa* was 0.89±0.34, 3.66±1.79, 2.17±1.10, and 4.14±0.97%, respectively. Diastase activity was 18.06±0.17 DN for *Ziziphus*, 16.36±2.08 DN for *Thymus*, 15.21±0.31 DN for *Astragalus*, and 2.94±0.09 DN for *Alfalfa*. Antioxidant activity was 13.64±3.34% for *Ziziphus*, 29.52±2.52% for *Thymus*, 29.51±3.30% for *Astragalus*, and 57.77±4.79% for *Alfalfa*. The results of the present study showed that monofloral honey samples in Iran have an appropriate level of sucrose and can be a good dietary option for people with diabetes. Moreover, total phenol and total flavonoid contents in our samples were lower than other types in other countries. DPPH free radical scavenging activity of our samples was comparable to other types and can be exported to other countries. The results showed that Iranian monofloral honey has the potential to compete with other countries' honey in terms of quality and nutritional value.

Keywords: Monofloral honey; Antioxidant activity; Rheological properties; Total phenol content; Total flavonoid content

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1. Introduction

Honey defines as "the natural sweet substance produced by honey bees from the nectar of plants or secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature" (Amiry et al., 2017; Nasrolahi et al., 2013). Honey is tasty and viscose and has long been consumed by humans for its high nutritional value and positive impacts. It is consumed almost invariably across the world. However, it is classified as nectar, honeydew, or a mixed nectar-honeydew, depending on the raw material from which the bee produces it (Amiry et al., 2017). Honey is not a complete food on its own but is considered a high potent nutritional supplement that can be easily digested, making it an appropriate dietary choice for children and the elderly (Silva et al., 2009). This natural product is rich with sugar and minerals, proteins, enzymes, and volatile compounds with varying degrees, the quality of which is determined by a host of factors such as plant, environmental condition, harvesting season, and extraction mechanism. Processing, storage and transportation also impact its composition (Akbari et al., 2020; Moniruzzaman et al., 2014). Honey is composed mainly of carbohydrates (sucrose, fructose, and maltose), 0.3% protein, 17% water, 0.7 minerals, vitamins, and antioxidants (Dżugan et al., 2020). It is classified as monofloral, collected from a single plant, or polyfloral, collected from various

*Corresponding author.
E-mail address: St_sa.amiri@urmia.ac.ir (S. Amiri).
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plants. Monofloral honey is more expensive and is favored for its unique taste and medicinal properties. Honey has been extensively used in traditional medicine to treat burns, digestive disorders, asthma, skin injuries, and infectious injuries (Farooq et al., 2020).

Monofloral honey is produced worldwide and has unique properties, nutritional, and medicinal properties. Due to the nutritional value and many health characteristics of monofloral honey as well as the worldwide market for this type of honey, the purpose of this study was to investigate the characteristics of four monofloral honey produced in Iran.

2. Material and Methods

2.1. Materials

Four honey types (*Ziziphus*, *Thymus*, *Astragalus*, and *Alfalfa*) were bought from West Azerbaijan province of Iran. Chemical materials used in the study included phenolphthalein, sodium hydroxide, iodine, sodium thiosulfate, sodium bisulfite, nine hydrine, formic acid, copper(II) sulfate, potassium sodium tartrate, hydrochloric acid, acetic acid, starch glue solution, buffers 4 and 7, ethanol, propanol, sulfuric acid, and formic acid which were purchased from Merck (Darmstadt, Germany). 2, 2-diphenyl-1-picryl-hydrazyl-hydrate, Folin–ciocalteu reagent, Gallic acid, catechin, proline, and ninhydrin were purchased from Sigma-Aldrich (St Louis. MO, USA).

2.2. Palynology

Pollen grains were acetolyzed and dyed, and the identified by Erdtman method (1960) (Diez et al., 2004).

2.3. Physicochemical properties

2.3.1. Moisture content

The sample was measured according to the White method by a refractometer at 20 ºC (AOAC, 2000).

2.3.2. pH

Accurately 10 g of the honey sample was solved with distilled water with no carbon dioxide (75 mg) in a beaker. The device was calibrated with buffer 4 and 7. Then, pH was measured at 20 ºC (AOAC, 2000).

2.3.3. Free acidity

Accurately 10 g of the honey sample was weighed in a beaker and solved with distilled water. The solution was tethered in the proximity of phenolphthalein to reach pH of 8.3 at 0.1 N (AOAC, 2000).

$$\text{Free acidity (eq/kg)} = \frac{1000 \times \text{N}(\text{V} - \text{V1})}{\text{W}} \quad (1)$$

2.3.4. Ash

Accurately 5 g honey sample was loaded on platinum or Chinese furnace. A few drops of oil were added to prevent foaming. We stirred the furnace until it was black and then burn honey in an oven at 600 ºC to turn to ash. Then, we compare the weight of an empty furnace with the one containing ash. The bush containing ash is divided by the sample weight and then multiplied at 100 to get ash rate, as follows (AOAC, 2000).

$$Ash\ (\%) = \frac{weight\ of\ empty\ \ furnace - weight\ of\ furnace\ containing\ ash}{sample\ weight} \times 100 \quad (2)$$

2.3.5. Insoluble solids

Accurately 10 g of honey sample is solved with distilled water and the solution is centrifuged at 2800 ×g for 15 minutes. The resulting sediment is solved in distilled water and filtered by Whatman filter paper 1. The added weight indicates the amount of insoluble solids (Amiry et al., 2017).

$$\text{Insoluble solids (\%)} = \text{weight of filter paper} - \text{dried sediment} \quad (3)$$

2.3.6. Electrical conductivity

Cell constant electrode is determined and then washed with 40 ml potassium chloride in a small beaker. Electrode conductivity is measured at 20 ºC Sample solution is then prepared. Accurately 20 g of the honey sample was solved in distilled water and added to a 100 ml volumetric flask. The flask was then placed into a water bath at 20 ºC. About 40 ml of the solution was added to a beaker. Conductivity Meter cell was washed with the solution and its conductivity was measured at 20 ºC (AOAC, 2000).

$$\text{Sh} = \text{K} \times \text{G} \quad (4)$$

2.3.7. Color

The honey color was measured using a colorimeter (CR 410, Tokyo, Japan) (Amiry et al., 2017).

2.3.8. Minerals

Minerals were measured using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Emmertz, 2010).

2.4. Biochemical properties

2.4.1. Sugar content

2.4.1.1. Reducing sugar content before hydrolysis

Accurately 1 g honey sample was solved in distilled water and added to a 250 ml volumetric flask, from which 50 ml was added to a burette. Then, 5 ml Fehling's solution A and 5 ml Fehling's solution B were added to an Erlenmeyer flask and 15 ml of the solution was added to the mixture to be tethered with an appropriate assessment procedure (AOAC, 2000).

$$S\ (\%) = \frac{F \times 250 \times 100}{V \times W \times 1000} \quad (5)$$

2.4.1.2. Reducing sugar content after hydrolysis

Accurately 50 ml of the solution from the 250 ml volumetric flask was poured into a 100ml flask and 2 ml containing concentrated hydrochloric acid. The flask was placed into a water bath for 10 min and then cooled. It was then neutralized using phenolphthalein and NaOH 0.1N (AOAC, 2000).

$$S_1\ (\%) = \frac{F \times 250 \times 100 \times 100}{w \times v \times 50 \times 1000} \quad (6)$$

2.4.1.3. Sucrose

It was determined as follows (AOAC, 2000):

$$\text{Sucrose}\ (\%) = (S_1 - S) \times 0.95 \quad (7)$$

2.4.1.4. Fructose-glucose (Fru/Glu) ratio

Accurately 25 ml of sample was added to a 250 ml Erlenmeyer flask. Using pipette filler, 20 ml of iodine 0.1 normal, and then NaOH 0.5 normal, were added. Erlenmeyer flask was kept for 15 minutes in a dark place to be added 5ml sulfuric acid 2 normal. Excess iodine was tethered by sodium thiosulfate 0.1 using starch glue solution (AOAC, 2000).

$$\text{Glucose}\ (\%) = \frac{250 \times 9.01 \times D \times 100}{25 \times w \times 1000} \quad (8)$$

$$\text{Fructose}\ (\%) = \text{Reducing sugar before hydrolysis} - \text{Glucose} \quad (9)$$

2.4.2. Antioxidant properties

2.4.2.1. Total phenol content

Accurately 1ml of the honey extract was mixed with 1ml phenol. After 10 min, a 10% solution was added to the mixture. The resulting solution was regulated with 10ml distilled water and kept in the dark place for 90 min. The absorption of the solution was measured using Ultraviolet–visible (UV-Vis) spectrometry (Model T60, Beijing, China) at 725 nm. Calibration was done by the Gallic acid standard curve at concentrations 12.5, 25, 50, 62.5, 100, and 125 µg/ml, R^2=0.9987 (Amiri et al., 2019b).

2.4.2.2. Total flavonoid content

Accurately 1 ml of the honey extract was mixed with distilled water. First, $NaNo_2$ was added and within 5 and 6 min, $AlCl_3$ (10% w/v, 3 ml) and NaOH (1 M, 2 ml), respectively. Solution volume was increased by 10 ml distilled water and stirred to ensure an appropriate mixture. The absorption of the solution was measured using UV-Vis spectrometry at 725 nm. Calibration was done by catechin standard curve at concentrations 20, 40, 60, 80, and 100, R^2=0.9987 µg/ml (Serem & Bester, 2012).

2.4.2.3. DPPH free radicals scavenging

Accurately 5 ml of the honey extract was mixed with a methanol solution containing DPPH free radicals. The solution was stirred and kept for 5 minutes in a dark place to avoid any variations in absorption the levels. Reduction in level of DPPH radicals was measured by determining absorption at 517 nm (Amiri et al., 2019).

$$\text{DPPH radical scavenging}\ (\%) = 1 - \frac{X1 - X2}{X3} \times 100 \quad (10)$$

X1= Absorbencies of the sample, X2= Sample blank, X3= DPPH blank.

2.4.3. Hydroxymethylfurfural (HMF)

Accurately 5 g of the honey sample was added to a beaker and mixed with distilled water to be poured in a 50ml volumetric flask. Then, 0.5 ml of solutions 1 and 2 were added to the mixture. Two drops of ethanol were added to suppress foam. The initial 10 ml of the product was disposed and the rest was collected. About 5 ml of filtered honey was added to two separate tubes, one containing 5 ml distilled eater (sample tube) and the other containing 5ml sodium bisulfite (to avoid absorption of HMF). The tubes were then mixed. Absorption against the reference tube was measured at wavelengths of 284 and 336 nm with 10 ml cell (AOAC, 2000).

$$\text{HMF}\ (\text{mg/kg}) = (A284 - A336) \times 149.7 \times 5 \times \frac{D}{W} \quad (11)$$

2.4.4. Diastase activity

Accurately 10 ml of the honey sample was added to a 50 ml volumetric flask while 10 ml starch was added to another flask. Both flasks were placed into a water bath at 40 ºC. After 15 minutes, 5 ml of the starch solution was added to the honey solution. Five minutes later, 0.5 ml of the solution was extracted and 5 ml diluted iodine was added. Some water was also added to standardize starch solution. The mixture was stirred. The absorption level of each solution was separately measured at 660 nm (AOAC, 2000).

$$\text{Diastase activity (DN)} = \frac{60\ \text{minutes}}{Tx} = \frac{0.10}{0.01} \times \frac{1.0}{2.0} = \frac{300}{Tx} \quad (12)$$

Tx = Time per minute.

2.4.5. Proline content

Accurately 0.5 ml of the sample solution and 0.5 ml of proline were added to two separate tubes. Also, 0.5 ml of water was added to a blank tube. Then, 1 ml of ninhydrin-formic acid was added to each tube. The tubes were capped and stirred for 15 min and then were placed into a boiling water bath for 5 min. They were then placed into a water bath at 70 ºC. Here, 5 ml, 2-propanol was added to tubes and they were recapped. Then, they were taken out of the bath and let for 45 min in the room temperature to complete the color process. Finally, tube absorption at wavelength 500-520 nm as measured for the blank tube (maximum absorption was 510) (AOAC, 2000).

$$Wp = \frac{Ep}{Es} \times \frac{M1}{M2} \times 80 \quad (13)$$

2.5. Rheological properties

2.5.1. Surface stickiness

Stickiness was measured using a TA. XII plus Texture Analyzer. Using a rod (probe 25 mm), 5 g force was exerted for 2 min on a sample surface. The rod was then separated at 8 mm/s from the sample and kept at a distance of 170 mm above the sample. The maximum force required to separate the rod from the surface was defined as stickiness (Amiry et al., 2017).

2.5.2. Stringiness

Stringiness is the distance the rod travels after separating from the sample surface before the force drops to 2.5 g. Greater distances indicate more stringiness (Amiry et al., 2017).

2.6. Statistical analysis

The present study used a completely randomized and descriptive design at three replications. Analysis of variance (ANOVA) at 95% confidence level (α=0.05) and Tukey's test to compare means were performed using MINITAB Statistical Software Release 19.0 (Minitab Inc., State College, PA, USA) and charts were prepared using Excel 2016 (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Palynology

Palynology was conducted in the Agricultural and Natural Resources Research and Education Center of Urmia, Iran. Results indicate that all four honey samples under study were monofloral and pure (low pollen grains count in the sample) (Fig. 1). Terrab et al. (2003) performed palynology on 20 honey samples in Morocco (such as Lannea, Vitellaria and Combretaceae, Acacia) and reported that most of them were monofloral (> 45% of the total pollen grains counted). Meda et al. (2005) analyzed pollen grains on 27 honey types (including 7 monofloral honeys of Sunflower, Loeflingia, Heathet, Mint, Wood sag, Crucifer, and Carob tree) in Burkina Faso. Results of our study were in agreement with other findings in the literature.

3.2. Physicochemical properties

3.2.1. Moisture

Water is the second most important component of honey. The moisture content of honey depends on the ripening factors of honey, including the harvest season, the degree of ripeness of honey in the hive, climatic conditions, plant origin, harvesting techniques and storage conditions. This parameter is very important in terms of quality, stability against fermentation, shelf life and crystallization of honey during storage and its amount may change due to storage conditions (Akbari et al., 2020). Moisture content was in the range of 16.10-17.48% for *Alfalfa*, *Thymus*, *Astragalus*, and *Ziziphus*. Maximum and minimum moisture were observed for *Alfalfa* (17.48 ±0.81%) and Knoar (16.10 ±0.11%), respectively (Table 1). Silva et al. (2009) reported that moisture content in Portuguese monofloral eucalyptus honey was 16.65%. Ozcan and Olmez (2014) reported 17.1-20% in Turkey, and Chirife et al. (2006) reported 15-21% moisture in Argentina. Moisture content in Iranian samples was in agreement with other studies.

3.2.2. pH

In general, honey is acidic in nature, regardless of its variable geographical origin. pH is an indicator that affects the consistency and texture of honey during storage (Akbari et al., 2020). pH was in the range of 3.6-5.73 for *Ziziphus*, *Thymus*, *Astragalus*, and *Alfalfa* samples. Maximum and minimum pH were observed for *Ziziphus* (5.73±0.03) and *Alfalfa* (3.63 ±0.002), respectively (Table 1). Silva et al. (2009) reported that pH in Portuguese monofloral eucalyptus honey was 3.83. Manu Kumar et al. (2013) found the pH of 3.30-4.13 for Indian samples. Adenekan et al. (2010) reported 3.1-4.5 for Nigerian samples and Hasan (2013) found the pH level of 3.9 in Iraq. These are in agreement with our findings on Iranian samples.

3.2.3. Free acidity

Acidity is one of the important quality indicators of honey, which increases in the case of fermentation in honey. Acids play a major role in the sensory properties of honey, with organic acids responsible for the acidity of honey, most notably gluconic acid in equilibrium with its lactones or esters and inorganic ions such as phosphate and chloride (Akbari et al., 2020). Acidity was found to be in the range of 28-36.33 (eq/kg) for *Thymus*, *Astragalus*, *Alfalfa*, and *Ziziphus*. Maximum and minimum acidity were observed for *Thymus* (36.33±2.51 eq/kg) and *Ziziphus* (28±1 eq/kg), respectively (Table 1). Adenekan et al. (2010) reported acidity of 6.15-41.2 (eq/kg) for Nigerian samples. In Iraq, it was reported by Hasan (2013) as 28.76 (eq/kg). Ozcan and Olmez (2014) reported acidity of 18.2-47.5 (eq/kg) in Turkish samples. Our results are in agreement with these findings.

3.2.4. Ash

Ash content is one of the parameters that are related to the plant and geographical origin of honey samples. The ash content of honey is mainly low and depends on the nectar composition of the dominant plants in its formation (Farooq et al., 2020). Ash was in the range of 0.07-0.18% for *Astragalus*, *Thymus*, *Ziziphus*, and *Alfalfa*. Maximum and minimum ash were observed for *Astragalus* (0.18 ±0.03%) and *Alfalfa* (0.07 ±0.03%), respectively (Table 1). Terrab et al. (2004) studied Spanish monofloral Avishn and reported an ash level of 0.16-0.60%. Similarly, Sancho et al. (1992) reported an ash level of 0.05-0.50%. Ash content in the study by Vit et al. (2009) was 0.03-0.13%. These are in agreement with our findings on Iranian samples.

3.2.5. Insoluble solids

This component represents the suspended wax particles and the remains of insects and plants in honey and is a measure of its cleanliness (Farooq et al., 2020). Insoluble solids content was in the range of 0.11-0.13% for *Thymus*, *Astragalus*, Knoar and *Alfalfa*. Maximum and minimum insoluble solid content were observed *Thymus* 0.13±0.01% and *Ziziphus*, and *Alfalfa* (0.11±0.005%)

(Table 1). Owayss (2005) reported insoluble solid content in Egypt as 0.07%. In Brazil, it was found by Santos et al. (2014) as 0.27-0.95%. However, our results on Iranian samples are in disagreement with these findings and this is because of differences in plant species, climate and soil productivity.

3.2.6. *Electrical conductivity*

Electrical conductivity is one of the important physicochemical indicators used in distinguishing flower honey from honeydew. The electrical conductivity depends on the content of ash and honey acid, so the higher the content, the higher the electrical conductivity. Today, electrical conductivity measurement has replaced the measurement of ash content (Farooq et al., 2020). Conductivity was in the range of 0.15-0.45 ms for *Ziziphus*, *Thymus*, *Alfalfa*, and *Astragalus*. Maximum and minimum conductivity were observed for Knoar (0.45±0.02 ms) and *Astragalus* (0.15-0.001 ms), respectively (Table 1). In the study by Adenekan et al. (2010), conductivity was 0.25-0.64 ms and Hasan (2013) reported conductivity of 0.28 ms in Iraq. The conductivity of Portuguese monofloral eucalyptus honey was 21.5 ms in the study by Silva et al. (2009). Our results are in agreement with these findings.

Table 1. Physicochemical properties of monofloral honeys.

Characteristics		*Ziziphus* Honey	*Thymus* Honey	*Astragalus* Honey	*Alfalfa* Honey
Moisture (%)		16.10±0.11^{A}	16.94±0.28^{A}	16.89±0.59^{A}	17.48±0.81^{A}
pH		5.73±0.030^{A}	3.91±0.005^{B}	3.79±0.005^{C}	3.63±0.002^{D}
Free acidity (eq/kg)		28.00±1.00^{B}	36.33±2.52^{A}	35.00±1.00^{A}	34.00±1.00^{A}
Ash (%)		0.15±0.04^{A}	0.15±0.06^{A}	0.18±0.03^{A}	0.07±0.03^{A}
Insoluble solids (%)		0.11±0.005^{A}	0.13±0.01^{A}	0.12±0.01^{A}	0.11±0.005^{A}
Electrical conductivity (ms)		0.45±0.02^{A}	0.17±0.007^{B}	0.15±0.001^{C}	0.15±0.008^{C}
Color					
	L^*	51	71	91	73
	a^*	34	43	1	21
	b^*	4	31	66	37
	Hue	6.74	41.31	3781.52	100.95
	Chroma	34.23	53.01	66.01	42.54
Mineral content (ppm)					
	Ag	0.51	0.54	0.57	0.69
	Al	28493	39890	25398	30200
	As	2.5	2.5	3	2.5
	Ba	77	119	68	87
	Be	1	1	1	1
	Ca	37111	40089	36447	44229
	Cd	0.19	0.2	0.21	0.2
	Ce	12	15	11	13
	Co	4	7	5	6
	Cr	62	38	16	33
	Cu	227	284	252	248
	Fe	4263	5874	3931	4121
	K	10	10	10	10
	La	5	6	4	5
	Li	1522	1623	885	1857
	Mg	15314	16559	16284	18902
	Mn	320	384	283	253
	Mo	1.77	1.63	1.93	2.27
	Na	32584	20614	17058	51958
	Ni	53	77	76	46
	P	19141	22856	3*	22510
	Pb	20	30	17	63
	S	10447*	2578	3808	5487
	Sb	0.95	1.05	0.98	1.07
	Sc	1.1	1.3	1.1	1.2
	Sr	473	163	166	782
	Th	5.4	6.8	6.2	6.9
	Ti	185	211	190	194
	U	5	5	5	5
	V	6	6	6	7
	Y	7	9	6	7
	Yb	0.8	0.9	0.7	0.8
	Zn	351	305	215	216
	Zr	69	85	65	76

* Mean ± standard deviations in the same raw with different letters are significantly different ($p < 0.05$).

Table 2. Biochemical Properties of monofloral honeys.

Characteristics		*Ziziphus* Honey	*Thymus* Honey	*Astragalus* Honey	*Alfalfa* Honey
Sugar content					
	Reducing sugar (%)	79.42±1.59^{B}	82.42±3.83^{B}	92.47±1.10^{A}	81.36±2.22^{B}
	Total sugar (%)	80.36±1.23^{C}	86.29±2.07^{B}	94.76±1.99^{A}	85.72±1.39^{B}
	Fru/Glu	0.97±0.05^{B}	1.05±0.0 ^{AB}	0.93±0.03^{B}	1.11±0.03^{A}
	Sucrose (%)	0.89±0.34^{B}	3.66±1.79^{AB}	2.17±1.10^{AB}	4.14±0.97^{A}
Antioxidant properties					
	Total phenolic content (mg/kg)	1.257±0.098^{C}	1.399±0.093^{B}	0.784±0.15^{B}	1.217±0.098^{A}
	Total flavenoid content (mg/kg)	0.025±0.0015^{A}	0.017±0.0005^{C}	0.022±0.00^{B}	0.023±0.0015^{AB}
	DPPH radical scavenging (%)	13.64±3.34 ^{A}	29.52±2.52^{A}	29.51±3.30^{B}	57.77±4.79^{A}
Hydroxymethylfurfural (mg/kg)		0.64±0.34^{C}	1.09±0.37^{C}	4.98±0.37^{A}	2.94±0.52^{B}
Diastase activity (DN)		18.06±0.17^{B}	16.36±0.08^{C}	15.21±0.31^{D}	20.94±0.09^{A}
Proline content (mg/kg)		453.82±2.13^{B}	325.52±2.80^{D}	352.58±2.02^{C}	744.54±2.88^{A}

* Mean ± standard deviations in the same raw with different letters are significantly different ($p < 0.05$).

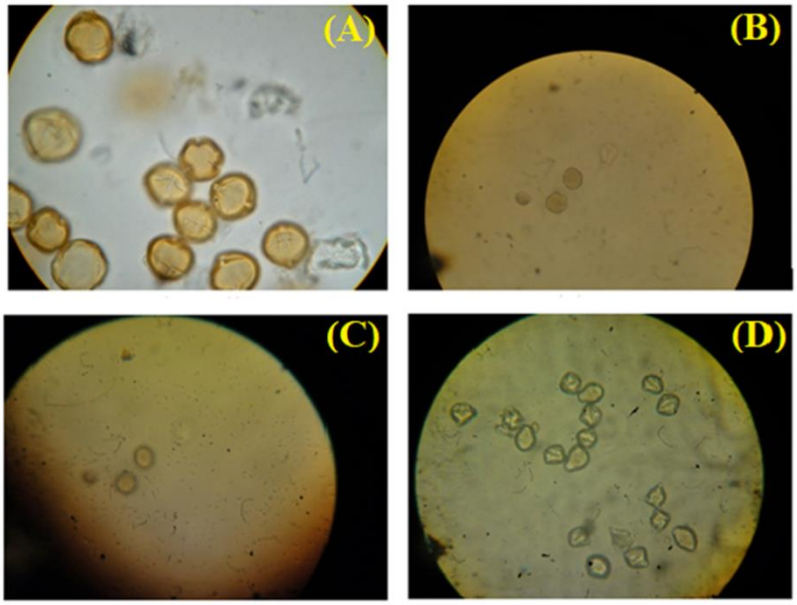

Fig. 1. The results of palynology analysis of monofloral honeys: (A) *Ziziphus*, (B) *Thymus*, (C) *Astragalus* and (D) *Alfalfa*.

3.2.7. Color

Color ranged for L^* (51-91), a^* (1-43), b^* (4-66), *hue* angle (6.74-3781.52), and *chrome* angle (34.23-66.01). Maximum and minimum L^* were observed for *Astragalus* (91) and *Ziziphus* (51). Maximum and minimum a^* were observed for *Thymus* (43) and *Astragalus* (1). Maximum and minimum b^* were observed for *Astragalus* (66) and *Ziziphus* (4). Maximum and minimum *hue* angle were observed for *Astragalus* (3781.52) and *Ziziphus* (6.74). Maximum and minimum *chrome* angle were observed for *Astragalus* (66.1) and *Ziziphus* (34.23). The results are given in Table 1. Popek et al. (2002) reported levels of L^*, a^*, and b^* in Poland as 13±0.47, 0.63±0.17, and 3.48±2.17, respectively. In Turkey, Ozcan and Olmez (2014) reported levels of L^*, a^*, and b^* as 24.56-34.16, 0.08-0.67, and 0.60-5.09, respectively. In a study by Ahmed et al. (2007) in India, 40.96-53.53, 0.1-5.86, and 10.62-22.99 were reported. However, our results on Iranian samples are in disagreement with these findings and this is because of differences in plant species, climate and soil productivity.

3.2.8. Minerals

Minerals content was Ag (0.51-0.69 ppm), Al (25398-39890 ppm), As (2.5-3 ppm), Ba (68-119 ppm), Be (1-1 ppm), Ca (36447-44229 ppm), Cd (0.19-0.21 ppm), Ce (11-15 ppm), Co (4-7 ppm), Cr (16-62 ppm), Cu (227-284 ppm), Fe (3931-5874 ppm), K (10-10 ppm), La (4-6 ppm), Li (885-1857 ppm), Mg (15314-18905 ppm), Mn (253-384 ppm), Mo (1.63-2.27 ppm), Na (17058-51958 ppm),

Ni (46-77 ppm), P (3-22856 ppm), Pb (17-63 ppm), S (2578-10477 ppm), Sb (0.95-1.07 ppm), Sc (1.1-1.3 ppm), Sr (163-782 ppm), Th (5.4-6.9 ppm), Ti (185-211 ppm), U (5-5 ppm), V (6-7 ppm), Y (6-9 ppm),Yb (0.7-0.9 ppm), Zn (215-351 ppm), and Zr (65-85 ppm) (Table 1). Moniruzzaman et al. (2014) reported Malaysian minerals of As (9.41 ppm), Pb (9.17 ppm), Cu (49.48 ppm), Cd (9.89 ppm), Co (19.71 ppm), Na (49.25 ppm), K (49.85 ppm), Fe (49.09 ppm), Mg (49.91 ppm), Ca (19.76 ppm), and Zn (19.81 ppm). Emmertz et al. (2010) found minerals in New Zealand as Al (6.6 ppm), As (0.08 ppm), B (4.42 ppm), Pb (0.017 ppm), Cu (0.25 ppm), Cd (0.149 ppm), Cr (0.37 ppm), Na (23.93 ppm), K (1053.2 ppm), Fe (1.706 ppm), Mg (24.75 ppm), Mn (1.04 ppm), Mo (1.01 ppm), Ca (50.92 ppm), Ni (0.23 ppm), P (46.04 ppm), S (28.34 ppm), and Zn (1.18 ppm). Cavalcanti et al. (2013) reported mineral contents in Brazil as Cu (0.43 ppm), Cr (77.17 ppm), Na (15.06 ppm), K (310.30 ppm), Fe (1.58 ppm), Mg (13.53 ppm), Mn (0.80 ppm), Ca (62.00 ppm), Se (4.31 ppm), and Zn (0.56 ppm). However, our results on Iranian samples are in disagreement with these findings and this is because of differences in plant species, climate and soil productivity.

3.3. Sugar content

3.3.1. Reducing sugar content before hydrolysis

Measuring the amount of reducing sugars is very useful in distinguishing nectar honey from honeydew (Dżugan et al., 2020). Reducing sugar content before hydrolysis was in the range of 92.47-79.42% for *Astragalus*, *Thymus*, *Alfalfa*, and *Ziziphus*. Maximum and minimum sugar content were observed for *Astragalus* (92.47±1.10%) and *Ziziphus* (79.42±1.59%), respectively (Table 2).

3.3.2. Reducing sugar content after hydrolysis

Reducing sugar content after hydrolysis was in the range of 94.76-80.36% for *Astragalus*, *Thymus*, *Alfalfa*, and *Ziziphus*. Maximum and minimum sugar content were observed for *Astragalus* (94.76±1.99%) and *Ziziphus* (80.36±1.23%), respectively (Table 2).

3.3.3. Fructose to glucose ratio

The average ratio of fructose to glucose is 1 up to 1.2 and depends on the plant origin of honey nectar. The higher value of this ratio cause to delay of the honey crystallizes (Dżugan et al., 2020). Fructose-Glucose ratio was in the range of 1.11-0.93 for *Alfalfa*, *Thymus*, *Ziziphus*, and *Astragalus*. Maximum and minimum Fructose-Glucose ratio was observed for *Alfalfa* (1.11±0.03) and *Astragalus* (0.95±0.03), respectively (Table 2). Fructose-Glucose ratio was reported in other studies as follows: Daniel et al. (2009) in Romania (0.81-1.57); Serem and Bester (2012) in South Africa (0.85-1.31); Yardibi and Gumus (2010) in Turkey (0.91-1.42). Our results for Iranian samples are in agreement with these findings.

3.3.4. Sucrose

Sucrose was in the range of 4.14-0.89% for *Alfalfa*, *Thymus*, *Astragalus*, and *Ziziphus*. Maximum and minimum Sucrose were observed for *Alfalfa* (4.14±0.97%) and *Ziziphus* (0.89±0.34%), respectively (Table 2). Sucrose was reported in other studies as follows: Buba et al. (2013) in Nigeria (1.84±0.79%), Martos et al. (2010) in Mexico (2.93%), and Solayman et al. (2016) in Malaysia (3.19±3.81%). This is in agreement with our results.

3.4. Antioxidant properties

3.4.1. Total phenol

Total phenol was in the range of 0.784-1.399 (mg/kg) for *Thymus*, *Ziziphus*, *Alfalfa*, and *Astragalus*. Maximum and minimum phenol were observed for *Thymus* (1.399±0.93 mg/kg) and *Astragalus* (0.784 ±0.151 mg/kg), respectively (Table 2). Total phenol was examined in other studies and was found to be 580.03±0.38 (mg/kg) and in the study by Moniruzzaman et al. (2013) on Gelam, Longan, Rubber tree and Sourwood monofloral honeys in Bangladesh; 31.72±80.11 (mg/kg) in the study by Krpan et al. (2009) on Acacia honey; 199.20±135.23 (mg/kg) in the study by Moniruzzaman et al. (2014) in Bangladesh. Zahir Hussein et al. (2011) also studied Gelam and Nenas monofloral honey in Malaysia and reported total phenol levels of 8.47, 41.76, 3.62, and 21.60 (mg/kg) for concentrations of 0.1-0.4, respectively. Total phenol content in our samples was lower than these studies and this is because of differences in plant species, climate and soil productivity.

3.4.2. Total flavonoid

Total flavonoid was in the range of 0.017-0.025 (mg/kg) for *Ziziphus*, *Alfalfa*, *Astragalus*, and *Thymus*. Maximum and minimum flavonoid contents were observed for *Ziziphus* (0.025±0.0015 mg/kg) and *Thymus* (0.017 ±0.0005 mg/kg), respectively (Table 2). Total flavonoid was examined in other studies and was found to be 156.82±0.47 (mg/kg) and in the study by Moniruzzaman et al. (2013) on Gelam, Longan, Rubber tree and Sourwood monofloral honeys in Bangladesh; 46.73±34.16 (mg/kg) in the study by Moniruzzaman et al. (2014) in Bangladesh. Zahir Hussein et al. (2011) also studied Gelam and Nenas monofloral honey in Malaysia and reported total flavonoid levels of 1.47-4.94 (mg/kg) and 1.23-4.52 (mg/kg) for concentrations of 0.1-0.4, respectively. Total flavonoid content in our samples was lower than these studies and this is because of differences in plant species, climate and soil productivity.

3.4.3. DPPH free radical scavenging

DPPH content was in the range of 13.64-57.77% for *Alfalfa*, *Thymus*, *Astragalus*, and *Ziziphus*. Maximum and minimum DPPH content were observed for *Alfalfa* (57.77±4.79%) and *Ziziphus* (13.64 ±3.34%), respectively (Table 2). DPPH content was examined in other studies and was found to be 59.26±3.77 % and in the study by Moniruzzaman et al. (2013) on Gelam, Longan, Rubber tree and Sourwood monofloral honeys in Bangladesh; 36.95±20.53% in the study by Moniruzzaman et al. (2014) in Bangladesh. Zahir Hussein et al. (2011) also studied Gelam and Nenas monofloral honey in Malaysia and reported DPPH content of 31.46-76.29% and 3.69-28.67% for concentrations of 0.1-0.4, respectively. Our results for DPPH content are in agreement with these findings.

3.5. HMF content

HMF content is used as an indicator to determine the freshness and intensity of the thermal process applied. Hydroxymethylfurfural is the breakdown product of fructose, the production process of which depends on pH and temperature. Because the pH of honey is higher than the pH of honey of flower origin, the rate of HMF formation in honey of flower origin is higher than that of honey. In fresh honey, this substance is present in very small amounts and its concentration increases with honey storage (Dżugan et al., 2020). HMF content was in the range of 0.64-4.98 mg/kg for *Astragalus*, *Alfalfa*, *Thymus*, and *Ziziphus*. Maximum and minimum HMF content were observed for *Astragalus* (4.98±0.37 mg/kg) and *Ziziphus* (0.64±0.34 mg/kg), respectively (Table 2). HMF content in other studies was 9.41 mg/kg in the study by Silva et al. (2009) on Eucalyptus honey in Portugal; 3.91 (mg/kg) in the study by Hasan (2013) in Iraq; 31.28 mg/kg in the study by Ozcan and Olmez (2014) in Turkey. HMF content in our samples was lower than these studies and this is because of differences in plant species, climate and soil productivity.

3.6. Diastase activity

Diastasis is a natural enzyme in honey. Diastasis activity mainly indicates the freshness of honey and therefore the level of diastasis is considered as an indicator to determine the intensity of the applied thermal process. Of course, the amount of honey diastasis depends on the type of flower and can be reduced during storage at normal temperatures (Dżugan et al., 2020). Diastase activity was in the range of 15.21-20.94 DN for *Alfalfa*, *Ziziphus*, *Thymus*, and *Astragalus*. Maximum and minimum diastase activity were observed for *Alfalfa* (20.94±0.09 DN) and *Astragalus* (15.21±0.31 DN), respectively (Table 2). Tosi et al. (2008) reported diastase activity of 11.2-25.8 DN in Argentina. Ahmed et al. (2013) was 7.3-26 DN in Algeria. Our results were in agreement with these findings.

3.7. Proline content

Proline content was in the range of 325.5-744.54 mg/kg for *Alfalfa*, *Ziziphus*, *Astragalus*, and *Thymus*. Maximum and minimum proline contents were observed for *Alfalfa* (744.54±2.88 mg/kg) and *Thymus* (325.52±2.80 mg/kg), respectively (Table 2). Muli et al. (2007) studied proline content in Kenya and reported 20.83-300.6 (mg/kg). Darvishzadeh et al. (2015) found proline content of 324-368.7 mg/kg in Iran. It was also reported by Manikis and Thrasyvoulou (1995) to be 326-790 mg/kg in Greece. Our results were in agreement with these findings.

3.8. Rheological properties

3.8.1. Surface stickiness

Surface stickiness was in the range of 29.143-83.805 g for *Ziziphus*, *Astragalus*, *Thymus* and *Alfalfa*. Maximum and minimum surface stickiness were observed for *Ziziphus* (83.805 g) and *Alfalfa* (29.143 g), respectively (Fig. 2A). Surface stickiness in the study by Amiry et al. (2017) was 0.139-0.159 g in Iran. Our results are not in agreement with other findings. This is explained by quality and moisture content of honey samples.

3.8.2. Stringiness

Stringiness was in the range of 22.86-40.14 mm for *Ziziphus*, *Astragalus*, *Thymus*, and *Alfalfa*. Maximum and minimum stringiness were observed for *Ziziphus* (40.14 mm) and *Alfalfa* (22.86 mm), respectively (Fig. 2B). Stringiness in the study by Amiry et al. (2017) was 21.124-17.430 mm in Iran. Our results are not in agreement with other findings. This is explained by quality and moisture content of honey samples.

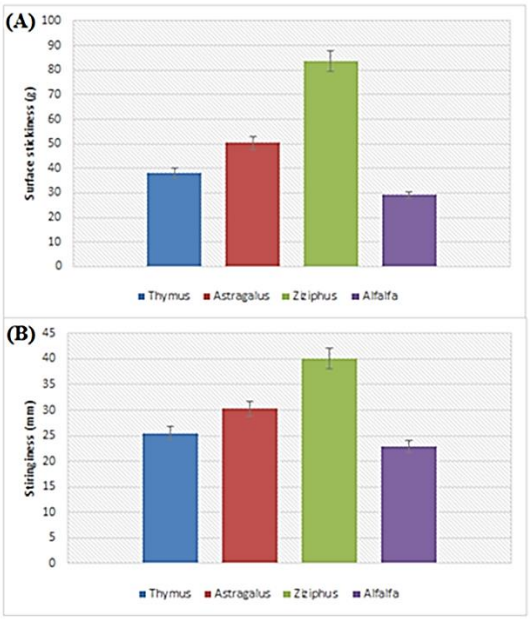

Fig. 2. Rheological properties: (A) Surface stickiness (g) and (B) Stringiness (mm) of monofloral honeys.

4. Conclusion

In this study, physicochemical, biochemical, antioxidant, and rheological properties of Iranian monofloral honey were characterized comprehensively. Results indicated that monofloral honey had high quality and had international standards. Therefore, this valuable product can be exported to different countries. It is also recommended to evaluate the health effects of Iranian monofloral honey for the treatment of various diseases.

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Conflict of interest

Authors declare no conflicts of interest.

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