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The Correlation of Plant Species and Geographical Regions on Biological Component and Antioxidant Potential of Different Honey

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ABSTRACT: Honey has various attributes according to its plant origin and production area. The aim of present study is to investigate physicochemical and biological functions of astragalus and thyme honey in Damavand and Izeh regions. Honey identification was analyzed on base of their physicochemical activities such as water content, ash, total solids, total soluble solids, pH, acidity, total sugar, reducing sugar, sucrose, fructose to glucose ratio, diastase activity, hydroxymethylfurfural (HMF), viscosity, total phenols, flavonoids and antioxidant behavior. The results of tests such as moisture content (16.16 to 19.08 %), ash (0.49 to 0.59 %), total solids (80.93 to 83.85 %), total soluble solids (80.25 to 81.74 %), pH (4.20 to 4.66), acidity (21.80 to 23.14 mEq / kg), total sugar (72.74 to 73.63 %), reducing sugar (69.26 to 73.07 %), sucrose (0.50 to 3.48 %), fructose to glucose ratio (1.06 to 1.31 %), diastase activity (8.63 to 10.13 DN), HMF (4.7 to 11.06 mg / kg), viscosity (21940 to 24705 cP), total phenolic compounds (23.96 to 30.03 mg gallic acid / 100 g honey), flavonoids (12.31 to 17.24 mg catechin / 100 g honey) and antioxidant activity (44.96 to 52.89 %) were determined. The results of simple correlation analysis illustrated parallel, positive and very strong relationship between 2,2-diphenyl-l-picrylhydrazyl and ash attributes, total phenolic compounds, total sugar, fructose to glucose ratio and also flavonoids.

Keywords: Antioxidant Activity, DPPH, Damavand, Honey, Izeh.

Introduction

According to codex "Honey is a natural and sweet product made by bees from nectar plants, secretions of living parts or excretion of sucking insects" (Shariatifar *et al*., 2017). Honey contains carbohydrates (70 to 80 %), water (10 to 20 %) and minor compounds (1 %) such as organic acids, minerals, proteins, phenolic contents and amino acids (Hossain *et al*., 2018). Vegetation of any region is one of the most prominent phenomena of nature and the best guide to judge ecological status of that region (Ramzi *et al*., 2017). There are complex relationships between plant communities and environmental factors. Therefore, research in ecological studies field is unique importance (Nouri *et al*., 2020).

Damavand reign is representative of semi-steppe vegetation with cold and mountainous climate, therefore temperatures are detected minimum -2.6 °C and maximum 22 °C. Honey is one of the most important products of this region (Eskandari *et al*., 2020). Izeh region is

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located in northeast of Khuzestan province with valuable pastures and consists of two parts: plain and mountain. Cold weather in winter as well as hot or dry in summer with wind are prominent climatic features of Izeh as well as minimum 7.11 °C and maximum 45 °C temperatures are distinguished (Neissi *et al*., 2020).

The physicochemical attributes of honey from stingless bee *Scaptotrigona mexicana* harvested at certain years (2012 to 2015) did not reveal considerable variations in most of their antioxidant and physicochemical functions. A more correlation was detected between antioxidant behavior and total phenolic content of honey (Jimenez *et al*., 2016). Antioxidant activities of single flower honey (23 various samples) from distinct geographical regions in Turkey indicated that phenolic compounds alone are not sufficient to determine antioxidant status. Factors such as geographical characteristics, plant type, and climate with environmental conditions also affect this activity (Hossain *et al*., 2018). All examined honey types from diverse areas in Kosovo demonstrated prominent concentration of phenols, flavonoids, as well as 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant capability, where the smallest concentration/ performance was reported in acacia specimens, while the highest in forest honey (Nouri & Shafaghi Rad, 2021).

Monofloral honey is synthesized in world with inimitable attributes such as nutritional and medicinal fields (Codex, 2001). The aim of present research is to investigate monofloral honey samples in Damavand and Izeh due to nutritional value, several health benefits or worldwide market.

Materials and Methods

- *Materials*

The chemical materials employed in this work included sodium hydroxide, phenolphthalein, sodium bisulfite, sodium thiosulfate, ethylic alcohol, propanol, sulfuric acid, ninhydrine, formic acid, Fehling's solution A and B, concentrated hydrochloric acid, acetic acid, and starch glue solution.

- *Satellite images*

Landsat 8 satellite images related to Damavand and Izeh regions were prepared and selected afterwards, subsequent processing of images was examined such as correction of geometric, atmospheric, altitude and radiometric errors. The processing and reconstruction of images were monitored through classification procedures in order to reveal and determine application types. By examining digitized geological maps, land use, vegetation region and visual interpretation of satellite images, different features and applications were recognized, thus general information was obtained from area. As a result, effects on satellite images were matched to ground reality, vague impacts were identified, thus vegetation distribution was detected in area.

- *Sampling*

The growth cycle of plants in study areas was identified using results of articles in addition consultation with professors and farmers in vegetation area. From each honey, 4 samples in three replicates (Total = 12 samples) with different plant origin of astragalus and thyme in spring of 2020 were completely randomly collected and evaluated from beehives in Damavand and Izeh regions. Each sample was heated in a hot bath at a maximum temperature (45 °C) for 30 min and stirred completely to dissolve any crystals. Samples were poured into sterile glass containers and stored out of light at 2 to 8 °C until experiments were carried out.

- ***Physicochemical performances***

These tests including moisture content, ash, pH and also free acidity were performed through refractometer, burning sample in furnace (600 °C), pH-meter and titration with base NaOH, respectively. After obtaining moisture content, total solids were achieved by subtracting 100 from moisture. The honey total solids were measured using a refractometer at 20 °C (AOAC, 2000).

- ***Calculation of reducing sugars before and after hydrolysis, sucrose percentage and fructose to glucose ratio***

Reducing sugar contains glucose, fructose monosaccharide and some disaccharides such as maltose. This test was carried out based on Lyne-Eynon method using Fehling's solution A and B to measure invert sugar (mg) required for copper reduction. The difference of reducing sugar before and after hydrolysis was multiplied by a coefficient of 0.95 to calculate sucrose percentage. The glucose level was obtained by adding iodine to sample solution, titrating with sodium sulfate to measure fructose to glucose ratio. The fructose value was calculated from difference between reducing sugar and glucose content before hydrolysis, consequently, desired ratio was attained by dividing their percentage (Codex, 2001).

- ***Hydroxymethylfurfural (HMF) determination using spectrophotometric assay (White) and diastatic ability***

Honey (5 g) was stirred in distilled water (25 mL), poured quantitatively into a 50 mL volumetric flask, added using 0.5 mL Carrez I and II solutions and blend to 50 mL with distilled water. The solution was refined using paper rejecting first filtrate (10 mL). Aliquots (5 mL) were placed in two assay tubes; to first tube was added 5 mL water (sample solution); to second was added 5 mL sodium bisulphite solution 0.2 % (reference solution). The absorbance at 284 and 336 nm was measured using spectrophotometry (Thermo Scientific, Madison, WI, USA). HMF content was obtained through subtracting both absorbance readings (Equation 1).

Equation 1

$$\mathrm{HMF} = (\mathrm{A}_{284}) - (\mathrm{A}_{336}) \times 149.7 \times 5 \times \frac{\mathrm{D}}{\mathrm{W}}$$

A starch standard solution can be evaluated with iodine, was added to sample and placed in a water bath at 40 °C to calculate diastase activity. Under standard conditions, enzymes hydrolyze starch and also resulting color change was used as amplitude of reaction intensity detection. Then blue color reduction was measured at specified time intervals using mentioned spectrophotometer at a wavelength of 660 nm. The absorption diagram with a regression equation was used to obtain reaction time ($\mathrm{T_x}$ min) or specific absorbance (0.235). The diastase number (DN) was gained through 300 divided by t_x according to Equation 2 (AOAC, 2000).

Equation 2 $$\mathrm{DN} = \frac{60\ \mathrm{min}}{\mathrm{Tx}} = \frac{0.10}{0.01} \times \frac{1.0}{2.0} = \frac{300}{\mathrm{Tx}}$$

- ***Rheological behavior evaluation***

The viscosity calculations were accomplished at ambient temperature, by a Brookfield disc-type viscometer, an adapter for tiny specimens and spindle number LV, 4 (model programming rheometery DVIII, Brookfield Engineering Labs, INC. Stoughton, MA, USA). The measurement was replayed thrice for each sample (2.5 mL), and average viscosity (centipoise: Cp) was measured (Mooliani and Nouri, 2021). However, device torque must be in range of 10 to 100 % in all these tests to attain reliable answers.

- *Assessment of total polyphenol and flavonoid*

Total phenolic contents were measured using Folin-Ciocalteu techniques. Briefly, honey solutions (0.1 g/mL), 1 mL Folin-Ciocalteu reagent and 4 mL sodium carbonate (Na_2CO_3) were transferred to a target volume (25 mL). The solutions were incubated at room temperature for 1 h; absorbance was calculated at 765 nm against a distilled water blank on a UV–vis spectrophotometer (Thermo Scientific, Madison, WI, USA).

Total flavonoids were determined as honey specimen was diluted in distilled water (0.3 g/ mL), solution (2 mL) was stirred with 0.5 mL aluminum chloride ($AlCl_3$), then distilled water was added to a last volume (25 mL). Solutions improved a yellow color which absorbance was calculated spectrophotometrically at 425 nm after maintaining solutions in dark for 30 min (Zerrouk *et al*., 2017).

- *Measurement of free radical scavenging by DPPH method*

DPPH-free radical scavenging capacity was evaluated by stirring 0.75 ml honey specimen (10 mg / mL) with 0.75 mL DPPH in methanol (0.02 mL) and preserved in dark for 30 min (Basiri and Nouri, 2021). According to Equation 3, (A_c: control absorbance and A_s: sample absorbance) reaction absorbance was estimated at 517 nm by a UV/VIS spectrophotometer (Thermo Scientific, Madison, WI, USA).

Equation 3 Free radical trapping (%) = $\frac{A_c - A_s}{A_c} \times 100$

- *Statistical analysis*

Statistical model of combined variance analysis across locations was applied, therefore from two regions (Damavand and Izeh) and in each region, two vegetation types (thyme and astragalus) were considered. In order to determine the difference between mean numbers, Duncan's multiple range test at 0.05 level was utilized. Correlation analysis technique, regression and also principal components were applied for additional analyzes and SAS software was employed in this research.

Results and Discussion

- *Investigation of vegetation and meteorological statistics of Izeh and Damavand regions*

In order to ensure correct naming of astragalus and thyme honey, satellite images and land maps were evaluated. According to Figure. 1 (a), land area with a height of more than 2000 m is very high in Damavand, which is a suitable habitat for growth of astragalus and thyme. In addition to cultivated lands, rest of lands are relatively sparse pastures that have a unique place in cover of their native plants, astragalus and thyme. The height of Izeh region is from about 800 m to a little more than 2000 m. In this region, land area with a height of more than 2000 m is not very high, therefore astragalus and thyme are dominant in a specific land area and this area was applied to produce honey (Figure. 1b).

According to vegetation map and meteorological statistics of Damavand and Izeh regions, it was distinguished that Damavand has a colder climate than Izeh. On the other hand, area with a height more than 2000 m was significant in Damavand, thus there was enough space and time for growth and flowering of astragalus and thyme. In Izeh region, the altitudes of more than 2000 m were not very large, therefore honey of this area were prepared more carefully compared to areas covered with astragalus and thyme.

- Physicochemical parameters

The results of comparing mean humidity revealed the highest rates for thyme (19.08 %) and astragalus (18.92 %) of Damavand and also the lowest for astragalus (16.16 %) and thyme of Izeh (16.71 %).

The results represented that moisture content of all samples is close to codex alimentarius commission (17.5 to 18.5 %) which is effective on increasing honey shelf life (Bogdanov *et al*., 1999). Regarding to meteorological statistics of two regions, rainfall rate in Izeh region has been less than Damavand region during last ten years and this has led to an increase in nectar moisture of honey in Damavand. The moisture level in various Polish and Manuka honey samples ranged from 16.33 to 20.75 %, which was similar to present study (Jimenez *et al*., 2016).

The maximum total solids were observed in astragalus (83.85 %) and thyme (83.25 %) of Izeh and minimum in thyme (80.93 %) and astragalus (81.08 %) of Damavand (Table. 1). Since the amount of total solids is obtained from difference between 100 % and moisture percentage, thus the result is quite logical and honey of Damavand had less total solids than Izeh region. The region, plant type (p <0.01) and interaction (p <0.05) were significant on moisture content and total solids, as illustrated in results of variance analysis.

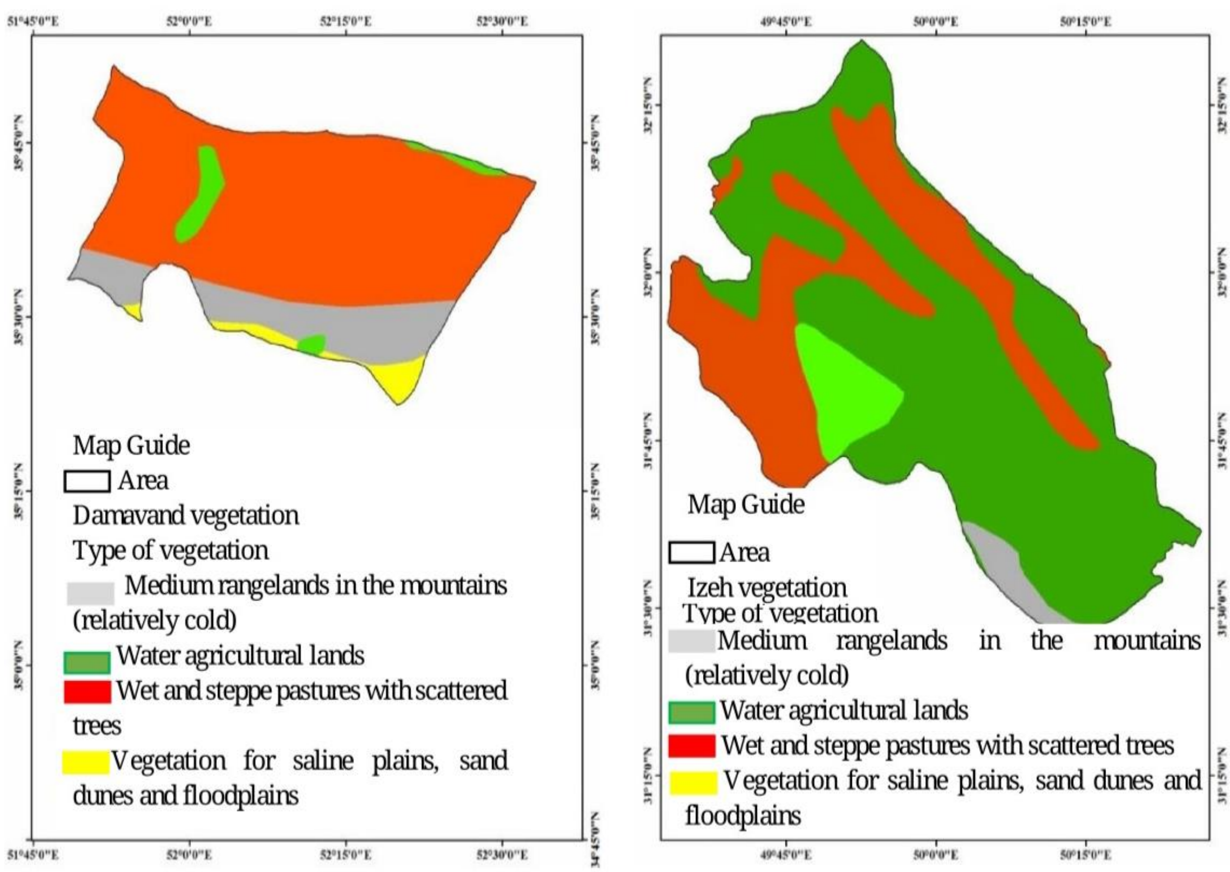

Fig. 1. Vegetation of Damavand (a), Vegetation of Izeh (b).

Table 1. Variance analysis of physicochemical and antioxidant tests of honey samples (mean ± SD)

	Thyme Damavand	Astragalus Damavand	Thyme Izeh	Astragalus Izeh
Moisture (%)	19.08[a] ± 0.74	18.92[a] ± 0.81	16.71[b] ± 0.92	16.16[c] ± 1.10
Total solids (%)	80.93[a] ± 0.65	81/08[a] ± 0.70	83.25[b] ± 0.43	83/85[b] ± 0.52
Total soluble solids (%)	79.09[a] ± 0.50	80/58[a] ± 0.44	80.25[a] ± 0.25	81/74[a] ± 0.40
Ash (%)	0.57[a] ± 0.02	0.59[a] ± 0.01	0.49[b] ± 0.05	0.50[b] ± 0.02
pH	4.66[a] ± 0.01	4.63[a] ± 0.04	4.32[b] ± 0.06	4.20[b] ± 0.03
Acidity (mEq/kg)	23.14[a] ± 0.22	22.32[b] ± 0.13	22.62[b] ± 0.18	21.80[c] ± 0.22
Total sugar (%)	73.63[a] ± 0.43	72.94[b] ± 0.25	72.78[b] ± 0.33	72.74[b] ± 0.34
Reducing sugar (%)	73.07[a] ± 1.20	69.79[b] ± 0.62	72.28[a] ± 1.10	69.26[b] ± 0.81
Sucrose (%)	0.56[b] ± 0.03	3.15[a] ± 0.09	0.50[b] ± 0.02	3.48[a] ± 0.06
Fructose to glucose ratio (%)	1.31[a] ± 0.05	1.06[a] ± 0.03	1.25[a] ± 0.07	1.09[a] ± 0.04
HMF (mg / Kg)	4.7[d] ± 0.72	6.3[c] ± 0.85	10.12[b] ± 0.91	11.06[a] ± 1.03
Diastase (DN)	8.63[b] ± 1.40	10.02[a] ± 1.21	9.27[b] ± 1.61	10.13[a] ± 1.31
Viscosity	21940[d] ± 15.16	22620[c] ± 18.22	23515[b] ± 23.07	24705[a] ± 20.41
Total phenolic content (mg gallic acid / 100 g honey)	30.03[a] ± 1.23	24.01[c] ± 2.10	27.44[b] ± 1.08	23.96[c] ± 1.33
Flavonoids (mg quercetin / 100 g honey)	16.51[a] ± 0.47	12.27[b] ± 0.21	17.24[a] ± 0.76	12.31[b] ± 0.19
DPPH (%)	52.89[a] ± 1.23	45.26[b] ± 1.25	51.34[a] ± 1.15	44.96[b] ± 1.07

The results of comparing means displayed the most water soluble solids for astragalus of Izeh (81.74 %) and the lowest for thyme of Damavand (79.09 %).

Plant species, regional humidity and geography had a significant impact on total amount of water-soluble solids. Damavand honey had more moisture, which indicated lower water-soluble solids and a negative correlation was observed between these two factors ($Y = 1.8335X + 113.91$, $R^2 = 0.87$). Total soluble solids of *Ziziphus lotus* (jujube) honey produced in Algeria were 84.32 and 83.19 %, which is close to our results (Zerrouk *et al*., 2017). The analyzed Nigerian honey had total solids in range of 82.10 to 84.31 % and soluble solids from 80.96 to 82.00 % (Ndife *et al*., 2014).

The highest ash was distinguished in Damavand (0.59 %) and the lowest in Izeh (0.49 %) (Table. 1). Variance analysis for water soluble solids and ash exhibited that land area and plant type were significant ($p < 0.01$).

In all samples, low level of this factor revealed plant origin and naturalness, which was in accordance with international standards 0.6 g / 100 g honey (Bogdanov *et al*., 1999). Thyme honey of Damavand with a darker color had more ash content than other samples (0.59 %) and astragalus honey of Izeh with lighter color had the lowest ash. The honey ash from *Scaptotrigona mexicana* bee was varied from 0.436 to 0.560 %, which was in line with present review (Jimenez *et al*., 2016).

The results of comparing means demonstrated maximum pH for astragalus (4.63) and thyme (4.66) of Damavand and minimum for astragalus (4.20) and thyme (4.32) of Izeh. The highest acidity was detected for honey of Damavand (23.14 mEq / kg) and the lowest for Izeh (21.80 mEq / kg). The results also indicated the greatest acidity (22.62 mEq / kg) in thyme honey and the lowest (22.32 mEq / kg) in astragalus honey (Table. 1). The results of variance analysis showed that growth area and plant type at 1% level were significant on pH and acidity.

The pH level and acidity value of all samples were distinguished in the standard range. The enhancement of honey shelf life leads to fermentation and increases its acidity. In codex alimentarius commission, standard acidity level is expressed as 50 (mEq / kg). In general, honey acidity varies according to the differences in honey origin, harvest season, geographical area, nectar type and moisture (Mesele, 2020). Acidity had a significant negative relationship with pH and a positive correlation was observed between ash and acidity of samples (Y = 1.723X + 22.243, R^2 = 0.97). The more honey acidity acts a prominent role in transformation of sugars into organic acids, which donates to honey flavour and stability against microbial deterioration.[13] It was represented that mean pH 3.5 and acidity 34.10 (mEq / kg) of honey from *Scaptotrigona mexicana* bee were detected (Jimenez *et al*., 2016). Mean pH 5.5 and acidity 12.5 mEq / kg of *Ziziphus lotus* (jujube) honey were reported (Zerrouk *et al*., 2017). Honey of Tamil Nadu region had a pH 4.75 and an acidity of 17.53 mEq / kg (Begum *et al*., 2015).

- *Sugar parameters*

The results of comparing means (Table. 1) represented the most total sugar content for thyme (73.63 %) and astragalus (72.94 %) of Damavand and the least for astragalus (72.74 %) and thyme (72.78 %) of Izeh. The highest reducing sugar is detected in thyme of Damavand (73.07 %) and Izeh (72.28 %), and the lowest in astragalus of Damavand (69.79 %) and Izeh (69.26 %). The results of variance analysis exposed that growth area, plant type and interaction at 1 % level were significant on total sugar and reducing sugar.

The sucrose content for astragalus and thyme honey in Damavand and Izeh was 3.48, 3.15, 0.56 and 0.50 %, respectively (Table. 1).

The results of variance analysis expressed that plant type or interaction at 1% level and growth area at 5 % level were significant. Among samples, the least sucrose belonged to thyme honey of Izeh.

The results of comparing means revealed the highest fructose to glucose ratio for thyme of Damavand (1.31 %) and Izeh (1.25 %), and the lowest for astragalus of Damavand (1.06 %) and Izeh (1.09 %) as presented in Table. 1. The results of variance analysis illustrated that growth area, plant type and interaction were significant (p <0.01).

According to codex alimentarius commission, minimum reducing sugar is 65 %. The data of present examination exhibited that most of soluble sugars in samples were consisted of reducing sugar and their amount is higher than standard (65 %). The maximum reducing sugars before hydrolysis (total sugar) belonged to thyme honey of Damavand. The essential reducing sugar of honey in Eastern Africa accounts 65 to 75 % of total sugar, which was in accordance with results of present study (Mesele, 2020).

The difference between reducing sugar before and after hydrolysis measures the amount of sucrose, and makes it up about 1 % of honey dry matter. Sucrose and invert sugar are crucial parameters for separating honey from each other (Liu *et al*., 2020). In present investigation, the amount of this factor in all samples was less than standard of 5 % (Codex, 2001).

The percentage of fructose to glucose ratio refers to variety of flowers from which honey samples originated. The major monosaccharaides are glucose, fructose and actual fructose to glucose ratio depends to a large extent on nectar source (Zerrouk *et al*., 2017). The standard rate for this factor is at least 0.9 % (Codex, 2001). Honey with a high ratio of fructose to glucose will stay liquid for a longer time. The fructose to glucose ratio also affects taste of honey because fructose is much sweeter than glucose (Ndife *et al*., 2014). The fructose to glucose ratio illustrates honey ability to crystallize. When this ratio exceeds 1.3 and increases or less than 1, crystallization rate decreases (Mesele, 2020). Fructose to glucose ratio and sucrose concentration were identified as adequate evidences for differentiation between monofloral honeys (Liu *et al*., 2020). In present study, the scope of this factor was not large, which was evidence of single flower honeys.

Total sugar, reducing sugar and also sucrose content of honey samples were 78.79, 71.79 and 7.54 g / 100 g, respectively (Begum *et al*., 2015). Honey from Tamil Nadu region contained total sugar 78.79 g / 100 g, reducing sugar 71.79 g / 100 g and sucrose 7.543 g / 100 g (Begum *et al*., 2015). In sugar content of *Ziziphus lotus* (jujube) honey, the percentage of fructose, glucose, sucrose and fructose to glucose ratio were 40.8, 30.7, 2.1 and 1.30 g / 100 g, respectively, which numbers were similar to results of present study (Zerrouk *et al*., 2017).

- *Qualitative factors*

The results of comparing means displayed the highest HMF in astragalus (11.06 mg / kg) and thyme (10.12 mg / kg) honey of Izeh and the lowest in thyme (4.7 mg / kg) and astragalus (6.3 mg / kg) honey of Damavand (Table. 1). The results of variance analysis exhibited that growth area and plant type (p <0.01) and also interaction (p <0.05) were significant.

All samples had allowable limit of HMF content and the reason for increasing the amount of this factor in samples of Izeh region was warmer and drier climatic conditions of that region compared to Damavand. According to codex and European Union, maximum permissible level of HMF in honey is 40 (mg / kg), but there are exceptions, such as 80 (mg / kg) for honey from tropical countries and 15 (mg / kg) for honey with low enzymatic activity. Acidity, pH, plant type, storage time and temperature affect HMF content. Thus, for honey with a pH less than 4, a range of HMF lower than 40 (mg / kg) and for honey with a pH greater than 4, a limit less than 20 to 25 (mg / kg) should be considered (Subramanian *et al*., 2007). The 33 samples of honey from western Nepal revealed that HMF level increases with high heat and honey shelf life (Muli *et al*., 2007). The result of current study was similar to studies obtained by other researches (Begum *et al*., 2015; Jimenez *et al*., 2016; El-Kazafy *et al*., 2020).

Diastase activity was found in astragalus honey of Damavand (10.02 DN) and Izeh (10.13 DN) and in thyme honey of Damavand (8.63 DN) and Izeh (9.27 DN), as depicted in Table. 1. Analysis of variance demonstrated that growth area, plant type and interaction were significant (p <0.01).

According to other researchers, plant type used by bees significantly affects diastase content of honey (Da Silva *et al*., 2016). In present study, diastase content of various honey types obtained from thyme and astragalus species were significantly different. A positive correlation was distinguished between sucrose sugar and diastase number so that samples of honey in Izeh with more sucrose had higher diastase number ($Y= 0.5375X + 0.9984$, $R^2 = 0.98$), which was similar to results gained by researchers (Blidi *et al*., 2017; Tomczyk *et al*., 2019).

Since there were no samples that had high HMF and low diastasis, which were both indicators of honey heating, so honey storage under appropriate temperature conditions was confirmed.

- *Viscosity*

The most viscosity characterized in astragalus (24705 cP) and thyme (23515 cP) of Izeh, and also the least in thyme (21940 cP) and astragalus (22620 cP) of Damavand (Table. 1). Growth area, plant type and interaction (p <0.01) were significant on viscosity.

Regarding to decreasing water content in samples, honey viscosity increased. A negative correlation was recognized between moisture and viscosity ($Y = 0.0012X + 24.10$, $R^2 = 0.83$). The result of present research was similar to studies achieved by pervious researchers (Begum *et al*., 2015; Jimenez *et al*., 2016). Honey viscosity from *Scaptotrigona mexicana* bee (Jimenez *et al*., 2016) was less than this factor in our study (23.82 %), because moisture content of sample in mentioned study was higher than moisture level of Damavand thyme honey (19.08 %).

- *Total polyphenol, flavonoid content and antioxidant activity of honeys*

The results of variance analysis exhibited that growth area and plant type at 1% level were significant on total phenolic content and antioxidant activity, but plant type and interaction at 1 % level and growth area at 5 % level were significant on flavonoids.

In Table. 1, the results of comparing means revealed the highest total phenolic compounds for thyme honey of Damavand (30.03 mg gallic acid / 100 g honey) and the lowest for astragalus honey of Izeh (23.96 mg gallic acid / 100 g honey). Moreover, this Table showed maximum flavonoid compounds in thyme honey of Damavand (16.51 mg catechin / 100 g honey) and thyme honey of Izeh (17.24 mg catechin / 100 g honey). The minimum flavonoid compounds are detected in astragalus honey of Damavand (12.27 mg catechin / 100 g honey) and astragalus honey of Izeh (12.12 mg catechin / 100 g honey).

The results of comparing means indicated that the highest DPPH was related to thyme of Damavand (52.89 %) and Izeh (51.34 %) and the lowest was related to astragalus of Damavand (45.26 %) and Izeh (44.96 %) as depicted in Table. 1.

Antioxidant compositions are among the most active physiological combinations in products (Hematian *et al*., 2020). Honey contains a variety of enzymatic and non-enzymatic antioxidants including glucose oxidase, catalase, L-ascorbic acid, flavonoids (quercetin, kaempferol, galangin, naringin, luteolin and pinocembrin), phenolic acids (caffeic acid, cinnamic acid, gallic acid, coumaric and chlorogenic), carotenoids, organic acids, amino acids and proteins (Hossain *et al*., 2018). Honey obtained from thyme in both regions had a darker color and more phenolic compounds were detected in these two specimens. However, Damavand thyme honey with the darkest color had the most phenolic contents. Honey specimens of astragalus with lower phenolic and flavonoid levels had less antioxidant activities than thyme, which was in line with previous researchers (Sagdic *et al*., 2013; Zarei *et al*., 2019; Babashpour-Asl & Piryaei, 2022). The reason for difference between phenolic and flavonoid compounds to region conditions and various plants from which honey was prepared, thus in current study we also expressed this issue ((Nouri & Shafaghi Rad, 2021). The mean of total polyphenols and DPPH radical scavenging activity (%) of honey from *Scaptotrigona mexicana* bee were 38.5 and 16.20 % (mg gallic acid / 100 g), respectively (Jimenez *et al*., 2016). A study conducted in Malaysia displayed that total phenolic content of studied honeys was 65.67 to 114.38 (mg gallic acid / 100 g honey), the amount of flavonoids was 12.41 to 17.67 (mg Catechin / g honey) and antioxidant activity ranged from 27.12 to 67.66 % (Tuksitha *et al*., 2018).

Since, main purpose of this study was to calculate amount and factors affecting antioxidant features of astragalus and thyme honey samples in Damavand and Izeh regions, in this section, we investigated correlation and regression relationships of DPPH. According to results of correlation, regression and decomposition into principal components, ash, total phenolic, total sugar and flavonoids variables were reliable and had positive effects on DPPH. Furthermore, factors such as sucrose, reducing sugars and also diastase had a negative effect on DPPH.

- *Correlation and regression*

The correlation of DPPH variable with ash, total phenolic content, total sugar, fructose to glucose ratio and flavonoid was a parallel, positive and very strong relationship according to results of simple correlation analysis, but there was a non-parallel, negative and strong relationship between diastase activities, sucrose and reducing sugar (Table. 2).

In stepwise regression analysis, DPPH variable was taken as a function variable and other measured traits were considered as independent factors. The results of variance analysis exhibited that regression relationship was significant at 1 % level (Table. 3). The efficient properties on DPPH including HMF, ash, total phenolic compounds and flavonoid had a positive effect and traits of sucrose and fructose to glucose ratio had a negative effect (Table. 4).

The main and independent parameters identified total variance (97 %) in decomposition into principal components. The initial factor determined changes (64 %) for variables including DPPH, ash, total phenolic content and total sugar (positive effects), diastase and rheological traits (negative effects). The second factor was assigned to changes (33 %) of variables such as HMF, water soluble solids, total solids and flavonoids (positive effect) as well as pH, acidity, moisture, sucrose and also reducing sugar (negative effect, Table. 5).

Conclusion

It was concluded that physicochemical properties of honey can be attributed to plant species from which bees fed. They can be distinguished between honeys produced by two target regions with different geographical conditions. The results showed that astragalus and thyme honey harvested from both Izeh and Damavand regions had a quality in accordance with standards of codex alimentarius commission. The positive, parallel and very strong relationship between DPPH variable and properties of total phenolic contents, total sugar, ash, flavonoids and fructose to glucose ratio was revealed by conclusions of simple correlation analysis. The effective features on DPPH containing ash, HMF, flavonoids and total phenolic levels had a positive impact and traits of sucrose and fructose to glucose ratio had a negative impact.

References

AOAC. (2000). Official methods of analysis (17th Ed.). Association of Official Analytical Chemists. Maryland, USA.

Babashpour-Asl, M. & Piryaei, M. (2022). Antioxidant activities and several bioactive substances of different extracts of *Vitis vinifera L. Journal of Food Biosciences and Technology*, 12(2), 49-60.

Basiri, M. & Nouri, M. (2021). Enrichment of strawberry frozen yogurt by chia (Salvia hispanica L.) seeds. *Food & Health*, 4(4), 28-34

Table 2. correlation analysis results of DPPH with physicochemical and antioxidant activities of honey samples

Variables	DPPH	HMF	PH	Acidity (mEq/kg)	Ash%	Diastase	Viscosity	Moisture%	Sucrose	Total phenolic content	Reducing sugar	Total sugar	Total soluble solids%	Total solids	Fructose to glucose ratio	Flavonoids
DPPH	1	-0.371	0.297	0.344	0.994**	-0.974**	-0.550	0.257	-0.983**	0.996**	-0.916**	0.636*	-0.253	-0.257	0.991**	0.962**
HMF		1	-0.981**	-0.996**	-0.450	0.535	0.956**	-0.982**	0.282	-0.380	0.091	-0.857**	0.990**	0.982**	-0.329	-0.156
pH			1	0.991**	0.386	-0.443	-0.959**	0.997**	-0.236	0.323	-0.074	0.750**	-0.990**	-0.997**	0.235	0.109
Acidity (mEq/kg)				1	0.426	-0.503	-0.960**	0.990**	-0.265	0.360	-0.083	0.825**	-0.994**	-0.990**	0.296	0.137
Ash%					1	-0.978**	-0.627*	0.346	-0.976**	0.994**	-0.901**	0.672*	-0.339	-0.346	0.977**	0.946**
Diastase						1	0.663*	-0.413	0.922**	-0.961**	0.805**	-0.793**	0.422	0.413	-0.971**	-0.877**
Viscosity							1	-0.942**	0.500	-0.576*	0.347	-0.818**	0.932**	0.942**	-0.488	-0.383
Moisture %								1	-0.188	0.297	-0.019	0.753**	-0.996**	-0.996**	0.201	0.060
Sucrose									1	-0.992**	0.971**	-0.507	0.171	0.188	-0.961**	-0.989**
Total phenolic content										1	-0.936**	0.607*	-0.268	-0.279	0.974**	0.969**
Reducing sugar											1	-0.290	-0.011	0.019	-0.885**	-0.985**
Total sugar												1	-0.789**	-0.753**	0.645*	0.407
Total soluble solids%													1	0.996**	-0.205	-0.042
Total solids%														1	-0.201	-0.060
Fructose to glucose ratio															1	0.946**
Flavonoids																1

**Significant at the level of 1% and *Significant at the level of 5%

Table 3. Stepwise regression to select the variables that determine DPPH changes

Source of change	Degrees of freedom	Sum of squares	Average of squares	F
Regression	6	0.061	0.01	18655.27**
Residual	5	0.000003	0.000001	
Total	11	0.06	-	

**Significant at the level of 1% and *Significant at the level of 5%

Table 4. Stepwise regression results for DPPH

Fixed variable	Symbol	Regression coefficients
Width of origin	a	-48.90
HMF	X_1	0.030
Ash	X_2	0.428
Sucrose	X_3	-0.116
Total phenolic content	X_4	0.180
Flavonoids	X_5	0.048
Fructose to glucose ratio	X_6	-0.19
Step	Regression equation	Corrected R^2
Step 5	$YPP=-9.48+0.03X_1+0.428X_2-0.116X_3+0.048X_4+3.35X_5-0.19X_6$	4.99

Table 5. Results of decomposition into principal components for the studied traits

Traits	Coefficients of common factors	
	The first factor	The second factor
DPPH	0.264	0.230
HMF	-0.25	0.259
pH	0.235	-0.279
Acidity (mEq/kg)	0.245	-0.268
Ash	0.278	0.196
Diastase	-0.286	-0.154
Viscosity	-0.282	0.174
Moisture	0.227	-0.295
Sucrose	-0.248	-0.259
Total phenolic content	0.266	0.224
Reducing sugar	-0.204	-0.310
Total sugar	0.271	-0.103
Total soluble solids	-0.226	0.300
Total solids	-0.227	0.295
Fructose to glucose ratio	-0.253	0.243
Flavonoids	0.221	0.302
Eigenvalues	10.32	5.26
Variance	0.64	0.33
Cumulative variance	0.64	0.97

Begum, S.B., Roobia, R.R., Karthikeyan, M. & Murugappan, R.M. (2015). Validation of nutraceutical properties of honey and probiotic potential of its innate microflora. *LWT - Food Science and Technology*, 60(2), 743-750. https://doi.org/10.1016/j.lwt.2021.112087

Blidi, S., Gotsiou, P., Loupassaki, S., Grigorakis, S. & Calokerinos, A.C. (2017). Effect of Thermal Treatment on the Quality of Honey Samples from Crete. *Advance Journal of Food Science and Engineering*, 1(1), 1-8. https://doi.org/10.22606/afse.2017.11001

Bogdanov, S., Lüllmann, C., Martin, P., Von der Ohe, W., Russmann, H., Vorwohl, G., Oddo, L.P., Sabatini, A.G., Marcazzan, G.L., Piro, R., Flamini, C., Morlot, M., Lhéritier, J., Borneck, R., Marioleas, P., Tsigouri, A., Kerkvliet, J., Ortiz, A., Ivanov, T., D'Arcy, B.R., Mossel, B. & Vit, P. (1999). Honey quality and international regulatory standards: Review by the International Honey Commission. *Bee World*, 80(2), 61-69. https://doi.org/10.1080/0005772X.1999.11099428

Codex. (2001). Codex Alimentarius standard for honey 12-1981. Revised Codex standard for honey. Standards and standard methods (Vol. 11). Retrieved December, 2014, from http://www.codexalimentarius.net

Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O. & Fett, R.J. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, 196(3), 309-323. https://doi.org/10.1016/j.foodchem.2015.09.051

El-Kazafy, A., Al-Kahtani, S. & Taha, R. (2020). Comparison of the physicochemical characteristics of sidr (Ziziphus spp.) honey produced by Apis florea F. and Apis mellifera L. *Journal of Apicultural Research*, 60(3), 470-477. https://doi.org/10.1080/00218839.2020.1746036

Eskandari, A., Deevsalar, R., De Rosa, R., Shinjo, R., Donato, P. & Neill, I. (2020). Geochemical and isotopic constraints on the evolution of magma plumbing system at Damavand volcano, N Iran. *Lithos*, 354-355(11), 105274. https://doi.org/10.1016/j.lithos.2019.105274

Hematian, A., Nouri, M. & Safari, S. (2020). Effects of *Capparis spinosa* extract on kashk quality during storage. *Foods Raw Material*, 8(2), 402-410. https://doi.org/10.21603/2308-4057-2020-2-402-410

Hossain, M.D.I., Rahman, M.D.M., Hossain, M.S., Chakraborty, R. & Amin, R. (2018). Role of ways of insect visitors foraging for pollination in yield contributing traits of mustard. *Journal of Biological Research*, 91(2), 63-69. https://doi.org/10.4081/jbr.2018.6982

Jimenez, M., Beristain, C.I., Azuara, E., Mendoza, M.R. & Pascual, L.A. (2016). Physicochemical and antioxidant properties of honey from Scaptotrigona mexicana bee. *Journal of Apicultural Research*, 55(2), 151-160. https://doi.org/10.1080/00218839.2016.1205294

Liu, W., Zhang, Y., Li, M., Han, D. & Liu, W. (2020). Determination of invert syrup adulterated in Acacia honey by Terahertz spectroscopy with different spectral features. *Journal of the Science of Food and Agriculture*, 100(5), 1913-1921. https://doi.org/10.1002/jsfa.10202

Mesele, T. L. (2020). Review on physico-chemical properties of honey in Eastern Africa. *Journal of Apicultural Research*, 60(1), 33-45. https://doi.org/10.1080/00218839.2020.1754566

Mooliani, H. & Nouri, M. (2021). Optimization of oxidative, physical stability and microbial characteristics of salad dressing emulsions based on avocado and whey protein combined with mint (Mentha spicata L) extract. *Journal of Food Measurement and Characterization*, 15:5713–5724. https://doi.org/10.1007/s11694-021-01131-1

Muli, E., Munguti, A. & Raina, S.J. (2007). Quality of honey harvested and processed using traditional methods in rural areas of Kenya. *Acta Veterinaria Scandinavica*, 76(2), 315-320. https://doi.org/10.1002/jsfa.10202

Ndife, J., Abioye, L. & Dandago, M. (2014). Quality assessment of Nigerian honey sourced from different floral locations. *Nigerian Food Journal*, 32(2), 48-55. https://doi.org/10.1016/S0189-7241(15)30117-X

Neissi, L., Albaji, M. & Boroomand Nasab, S. (2020). Combination of GIS and AHP for site selection of pressurized irrigation systems in the Izeh plain, Iran. *Agricultural Water Management*, 231(1), 1-8. https://doi.org/10.1016/j.agwat.2020.106004

Nouri, M. & Shafaghi Rad, M. (2021). Encapsulation of Tribulus terrestris and Valeriana officinalis extracts in nanoliposomes and evaluation of its antibacterial and antioxidant properties. Journal of Food Biosciences and Technology, 11 (2), 59-63.

Nouri, M. (2020). Preparation of nanoliposomes containing Hyssopus officinalis L. and Eryngium caeruleum M.Bieb extracts and investigate their antimicrobial and antimicrobial effects. *Journal of Medicinal Plants*, 19(75), 118-131. https://doi.org/10.29252/jmp.19.75.118

Ramzi, M., Kashaninejad, M., Salehic, F., Sadeghi Mahoonakd A. R. & Razavi S. M. A. (2017). Rheological and physicochemical properties of honeys as a function of temperature, concentration and moisture content. *Journal of Food Biosciences and Technology*, 7(2), 35-48.

Sagdic, O., Silici, S. & Ekici, L. (2013). Evaluation of the phenolic content, antiradical, antioxidant, and antimicrobial activity of different floral sources of honey. *International Journal of Food Properties*, 16(3), 658-666. https://doi.org/10.1080/10942912.2011.561463

Shariatifar, N., Janghorban, A., Rahimnia, R. & Mozaffari Nejad, A.S. (2017). Antibacterial, antifungal and antioxidant activities of bee glue ethanol and aqueous extracts. *Journal of Biological Research*, 90(2), 77-80. https://doi.org/10.4081/jbr.2017.6452

Subramanian, R., Umesh Hebbar, H. & Rastogi, N.J. (2007). Processing of honey: A review. *International Journal of Food Properties*, 10(1), 127-143. https://doi.org/10.1080/10942910600981708

Tomczyk, M., Tarapatsky, M. & Dżugan, M. (2019). The influence of geographical origin on honey composition studied by Polish and Slovak honey. *Czech Journal of Food Sciences*, 37(4), 232-238. https://doi.org/10.17221/40/2019-CJFS

Tuksitha, L., Chen, Y.L.S., Chen, Y.L. & Wong, K.Y. (2018). Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). *Journal of Asia-Pacific Entomology*, 21(2), 563-570. https://doi.org/10.1016/j.aspen.2018.03.007

Zarei, M., Fazlara, A. & Alijani, N. (2019). Evaluation of the changes in physicochemical and antioxidant properties of honey during storage. *Journal Functional Foods in Health and Disease*, 9(9), 593-605. https://doi.org/10.31989/ffhd.v9i9.616

Zerrouk, S., Seijo, M.C., Escuredo, O. & Rodríguez-Flores, M.S. (2017). Characterization of Ziziphus lotus (jujube) honey produced in Algeria. *Journal of Apicultural Research*, 57(1), 166-174. https://doi.org/10.1080/00218839.2017.1399663