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ORIGINAL ARTICLE

Physico-chemical, rheological and sugar profile of different unifloral honeys from Kashmir valley of India

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Abstract The aim of the study was to characterize four unique varieties of honey (saffron, apple, cherry and *Plectranthus rugosus*) from Kashmir valley of India on the basis of physico-chemical parameters (moisture content, pH, hydroxymethylfurfural, water activity, proline content, invertase and diastase activity) and carbohydrate profile. Also the effect of temperature (0, 5, 10, 20 and 30 °C) on rheological properties of all unique honey varieties was studied. All the physicochemical parameters studied were significantly different ($P < 0.05$) among all honey varieties. Eleven types of sugars were identified and quantified by HPLC which include three monosaccharides, four disaccharides and four trisaccharides. The presence of carbohydrates in all analyzed honey varieties was further confirmed by obtaining a band from 1400 to 750 cm^{-1} which corresponds to the most sensitive absorption region of the sugars by using FTIR–ATR. All the honey samples displayed a Newtonian fluid behavior, with loss modulus (G″) very much greater than storage modulus (G′) which confirmed the dominancy of viscous nature in all honey varieties.

1. Introduction

Honey is unique, natural rich source of amino acids, vitamins, minerals, biologically active compounds and sweet concentrated solution of readily available sugars produced by honey bees (Ouchemoukh et al., 2007; Lachman et al., 2010). Honey composition not only depends on botanical and geographical origin but also on processing and storage conditions (Lazaridou et al., 2004; Nayik and Nanda, 2015b). Honey carbohydrates are complex mixture made up of about 70% monosaccharides (mainly glucose and fructose), and 10–15%

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disaccharides (De La Fuente et al., 2006). In addition, blossom honey is characterized by small concentration of trisaccharides such as melezitose and raffinose, which are normally found in very high concentration in honeydew honeys (De La Fuente et al., 2011; Bentabol Manzanares et al., 2011). The sugar composition of honey affects physicochemical properties such as hygroscopicity, viscosity and crystallization (Kang and Yoo, 2008). Sugar profile of different types of honey has been reported by many scientists (Ouchemoukh et al., 2010; De La Fuente et al., 2011) and such profile of a particular honey depends highly on the type of flowers visited by the bees as well as regional and climatic conditions (Gomez-Barez et al., 2000). In order to evaluate the sugar content in honey, various techniques and methods viz. HPLC, GCMS, and high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC–IPAD) have been used in the last decade. But there has been a demand for new and rapid analytical methods and Fourier transform infrared (FTIR) spectroscopy is one of the well-accepted and accurate methods (Anjos et al., 2015; Gallardo-Velazquez et al., 2009; Ozbalci et al., 2013).

Rheology of fluids has been considered as a vital parameter that plays an important role in fluid heat transfer (Ahmed et al., 2007). The rheological knowledge of honey is necessary in equipment design for transport, pumping, processing, quality control, sensory analysis and storage (Yoo, 2004). The rheological property of honey depends not only on moisture content but also on temperature as well as on its chemical composition (Gomez-Diaz et al., 2009). During processing and storage, the honey experiences a wide range of temperature change; thus, the effect of temperature on rheological properties of honey needs to be analyzed (Rao, 1999). The moisture content and its relation with ratio of fructose to glucose (F/G) is one of the factors which determines the crystallization rate which affects the rheological properties of honeys (Lazaridou et al., 2004; Witczak et al., 2011). Honey has been reported as Newtonian fluid by most of the authors (Juszczak and Fortuna, 2006; Lazaridou et al., 2004) except heather, buckwheat, white clover and eucalyptus which show non-Newtonian behavior that may be due to presence of some proteins or dextran (Witczak et al., 2011). Although studies on physicochemical parameters, rheological behavior and mineral content have been reported on different honey varieties viz. berseem clover (*Trifolium alexandrinum*), mustard (*Brassica*), litchi (*Litchi chinensis*), sweet orange (*Citrus sinensis*), ber (*Ziziphus mauritiana*), peach (*Prunus persica*), sunflower (*Helianthus annuus*), eucalyptus (*Eucalyptus globulus*), cotton (*Gossypium hirsutum*), coriander (*Coriandrum sativum*), curry (*Murraya koenigii*), and Indian rosewood (*Dalbergia sissoo*) which are available in northern parts (Punjab &Haryana) of India (Nanda et al., 2003, 2009; Ahmed et al., 2007; Saxena et al., 2010, 2014; Kamboj et al., 2013) the honey varieties available in mountainous region (Kashmir Valley) viz. saffron (*Crocus sativus*), cherry (*Prunus avium*) apple (*Malus domestica*) and *Plectranthus rugosus* have never been studied before. Moreover, the sugar profile of any of Indian honey variety using HPLC and FTIR–ATR and rheology using compact Rheometer has also never been reported before. The uniqueness and authenticity of saffron (*C. sativus*), cherry (*P. avium*) and apple (*M. domestica*) honey was established by identification and quantification of forty two different volatile compounds using SPME–GCMS (Nayik and Nanda, 2015a) which encouraged the authors to further characterize physico-chemical characteristics, rheological properties and sugar profile of these three unique honey varieties along with the honey from *P. rugosus* (a wild bush). So the aim of the present research work was to characterize the honeys from four unifloral varieties belonging to Kashmir valley (apple, cherry, saffron, *P. rugosus*) on the basis of the physico-chemical characteristics and sugar profile (HPLC and FTIR–ATR) and also to study the effect of temperature on their rheological properties.

2. Materials and methods

2.1. Chemicals for estimation of sugar profile

Standards of sugars: maltose, isomaltose, trehalose, raffinose, melezitose, glucose, maltotriose, maltotetraose, sucrose, fructose, xylose, ultrapure water and organic solvents (HPLC grade) (Sigma–Aldrich Co., Mumbai, India).

2.2. Sample preparation and pollen analysis

Thirty seven samples from four varieties of raw and fresh honey (saffron honey, apple honey, cherry honey and *P. rugosus* honey) were collected from local bee-keepers during March 2013 to September 2014 and packed in glass bottles before storing at 4 °C under refrigeration condition. The origins of each honey sample were confirmed by melissopalynology. Honey samples were classified according to their botanical origin using the method described by Von der Ohe et al. (2004). The following terms were used for frequency classes: predominant pollen (>45% of pollen grains counted), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen (<3%).

2.3. Physico-chemical and enzymatic analysis

2.3.1. Moisture content

Moisture content was determined with an Abbe refractometer (Atago Co., Ltd., Tokyo, Japan) at 20 °C. The corresponding moisture content values were obtained from the Chatway table (AOAC (2012).

2.3.2. pH

The pH was measured using a pH meter (Eutech Instruments Pvt Ltd., Singapore) for a 10% (w/v) solution of honey prepared in distilled water (AOAC (2012)).

2.3.3. Hydroxymethylfurfural (HMF)

The HMF content in honey was determined using the White spectrophotometric method based on the determination of difference between the absorbance at 284 and 336 nm of a honey solution and the same solution after addition of bisulfate using Hach Lange DR6000 UV–VIS Spectrophotometer (Germany). The results were expressed in mg/kg (Codex Alimentarius Commission, 2001).

2.3.4. Water activity

A small aliquot of honey (1 g) sample was used for determining water activity (a_w) using a water activity meter (Aqualab

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CX2T, Decagon Devices, USA) which measures the water activity of the sample based on its equilibrium relative humidity (ERH). The relationship between a_w and ERH is a_w = ERH (in percentage)/100 (Acquarone et al., 2007).

2.3.5. Proline content

The proline content was determined based on its reaction with ninhydrin which forms a colored complex. After adding 2-propanol, the absorbance of the sample solution and a reference solution at 510 nm using Hach Lange DR6000 UV–VIS Spectrophotometer (Germany) was determined. Results were expressed in proline milligrams per kilograms of honey (Codex Alimentarius Commission, 2001).

2.3.6. Diastase activity

Diastase activity was based on the rate of starch hydrolysis by diastase present in a honey buffer solution at 40 °C. The end point for this reaction was established by measuring the absorbance at 660 nm with a Hach Lange DR6000 UV–VIS Spectrophotometer (Germany) until it was less than 0.235. The results were expressed in diastase number (DN) (Codex Alimentarius Commission, 2001).

2.3.7. Invertase activity

Invertase activity was determined according to the method of International Honey Commission, (2009), which is based on the spectrophotometric measurement of decomposition of p-nitrophenyl-α-d-glucopyranoside (p-NPG) in p nitrophenol and is determined spectrophotometrically at 400 nm. The honey invertase activity was calculated from the measured absorbency multiplying by the factor of 158.94 and calculated to a kilogram of honey. Then the value was expressed as invertase number (IN).

2.4. Determination of different sugars by HPLC–Refractive Index

The sugar profile was determined using high-performance liquid chromatography. 0.5 g of honey was weighed into polypropylene tubes and mixed with 10 ml of 60% methanol. Afterward, a milliliter of the dissolution was filtered through a 0.45 μm filter prior to HPLC analysis. The determination of sugar was performed with HPLC system equipped with refractive index (RI) detector (Waters isocratic (USA)). The separation was performed by using Waters X-bridge Amide HPLC Column, 5 μm (250 × 4.66 mm), maintained at 85 °C throughout the analysis. The injection volume of the sample was 20 μl, with a flow rate of 0.6 ml/min, using as mobile phase prepared by dissolving 80% of acetonitrile in ultrapure water. The freshly prepared mobile phase (ACN to water proportion, 80:20) was filtered and degassed by vacuum filtration through a 0.45 μm PTFE membrane filter. Before injection onto the column all syringes were fitted with syringe filters to remove particle/impurities from a sample so that the column should not be blocked. The separated carbohydrate peaks were identified by comparing the retention times obtained from standards. The honey samples were also spiked with standards in order to verify the identity of the chromatographic peaks. Duplicate injections were performed and average peak areas were used for the peak quantification (Mora and Marioli, 2001).

2.5. Sugar quantification by FTIR–ATR

Spectra from all samples were recorded in the one-bounce ATR mode using an Agilent Technologies Cary 660 FTIR spectrometer equipped with a Universal ATR accessory. Samples were placed on Diamond/ZnSe crystal plate (Perkin–Elmer) and scanned between 4000 cm^{-1} and 400 cm^{-1} representing the average of 64 scans. All spectra were recorded at room temperature (25 °C) under ambient conditions (Anjos et al. (2015)).

2.6. Rheological measurement

The dynamic rheological characteristics of honey samples were obtained by using Modular Compact Rheometer (MCR-102, M/s. Anton Paar, Austria), equipped with parallel plate system (50 mm diameter) at a gap of 0.5 mm at different temperatures (0, 5, 10, 20, and 30 °C). In order to determine the rheological data, frequency sweeps over the range of 0.63–63 rad/s at 3% strain were performed. A Rheoplus data analysis software (32 V3.40.) was used to obtain the experimental data to calculate storage modulus (G′) and loss modulus (G″). Results were obtained as an average of two measurements. The honey samples were heated to 50 °C for 1 h in a water bath to dissolve crystals, and then kept in 30 °C to remove air bubbles (Yoo, 2004).

3. Statistical analysis

All analytical determinations were performed in triplicate. One-way analysis of variance (ANOVA) was used to find the significant differences among the means followed by Duncan's multiple range test (DMRT) ($P < 0.05$).

4. Results and discussion

4.1. Pollen analysis

The percentages of pollen spectra are related to pollens of nectar producing plants. Apple honey (*M. domestica*) contained 54–60% pollen of *M. domestica* sp., Cherry honey (*P. avium*) possessed 46–57% pollen of *P. avium, P. rugosus* contained 42–54% of *Plectranthus* sp. pollens while saffron honey (*C. sativus*) contained 47–49% pollen of *C. sativus*.

4.2. Physico-chemical and enzymatic analysis

The moisture content of all analyzed samples was within the range of 17.5–19.1% recommended by Codex Alimentarius (< 20%) as shown in Table 1. Chirife et al. (2006) stated that moisture content was affected by climate, season, and moisture content of original plant nectar and was considered unripened at the moisture content higher than 20%. All the honey samples tested were found to be acidic in nature (3.01–4.35) and statistical significant difference ($P < 0.05$) was observed among the tested varieties (Table 1). In general, honey is acidic in nature irrespective of its variable geographical origin. The pH values were in agreement with the results of Algerian, Brazilian, Spanish and Turkish honeys (Azeredo et al., 2003; Ouchemoukh et al., 2007; Ozcan and Olmez, 2014).

Table 1 Physico-chemical and sugar analysis of honeys assessed by Duncan's test.

Parameters	Saffron (n = 8)	Apple (n = 11)	Cherry (n = 8)	*Plectranthus rugosus* (n = 10)
Moisture content	19.10 ± 0.65^{a}	18.82 ± 0.95^{ab}	17.8 ± 0.87^{bc}	17.5 ± 1.52^{c}
pH	3.57 ± 0.32^{b}	4.35 ± 0.22^{a}	3.01 ± 0.18^{c}	3.42 ± 0.28^{b}
HMF (mg/kg)	7.13 ± 1.10^{c}	10.37 ± 1.35^{b}	8.62 ± 1.60^{bc}	22.66 ± 3.01^{a}
Water activity	0.597 ± 0.01^{a}	0.559 ± 0.01^{a}	0.518 ± 0.01^{c}	0.514 ± 0.01^{a}
Proline (mg/kg)	205.31 ± 6.11^{d}	551.74 ± 6.11^{a}	488.31 ± 5.52^{b}	377.3 ± 6.28^{c}
Diastase activity (DN)	12.18 ± 2.49^{c}	22.52 ± 3.61^{a}	18.72 ± 4.01^{b}	15.58 ± 4.13^{bc}
Invertase activity (IN)	9.71 ± 2.45^{c}	19.69 ± 2.43^{a}	14.63 ± 3.09^{b}	13.07 ± 2.91^{b}
Fructose (%)	37.56 ± 1.51^{b}	40.61 ± 1.48^{a}	36.42 ± 0.48^{b}	38.87 ± 0.17^{b}
Glucose (%)	30.56 ± 0.35^{c}	32.44 ± 0.28^{b}	33.98 ± 0.45^{a}	31.21 ± 0.57^{c}
Xylose (%)	0.21 ± 0.02^{b}	0.29 ± 0.05^{a}	0.18 ± 0.06^{b}	0.16 ± 0.02^{b}
Maltose (%)	1.28 ± 0.05^{a}	1.07 ± 0.02^{b}	0.95 ± 0.03^{c}	1.36 ± 0.09^{a}
Isomaltose (%)	0.92 ± 0.03^{a}	0.66 ± 0.12^{c}	0.81 ± 0.03^{ab}	0.70 ± 0.03^{bc}
Trehalose (%)	0.06 ± 0.01^{b}	0.17 ± 0.03^{a}	0.07 ± 0.03^{a}	0.02 ± 0.01^{b}
Sucrose (%)	1.11 ± 0.05^{c}	1.40 ± 0.03^{a}	1.23 ± 0.04^{b}	1.05 ± 0.04^{c}
Raffinose (%)	0.08 ± 0.02^{ab}	0.04 ± 0.02^{c}	0.10 ± 0.03^{a}	0.06 ± 0.01^{bc}
Maltotriose (%)	0.90 ± 0.06^{a}	0.85 ± 0.04^{a}	0.68 ± 0.03^{b}	0.48 ± 0.01^{c}
Melezitose (%)	0.46 ± 0.06^{a}	0.29 ± 0.03^{b}	0.33 ± 0.04^{b}	0.19 ± 0.03^{c}
maltotetraose (%)	0.75 ± 0.03^{a}	0.63 ± 0.06^{b}	0.86 ± 0.10^{a}	0.49 ± 0.04^{c}
Total MS (%)	68.33	73.34	70.58	70.24
Total DS (%)	3.37	3.30	3.05	3.12
Total TS (%)	2.19	1.81	1.97	1.22
Total sugars (%)	73.89	78.45	75.60	74.58
Total reducing sugars (%)	71.43	75.92	73.01	72.77
Total non-reducing sugars (%)	2.46	2.53	2.59	1.81
F/G ratio	1.23 ± 0.06^{a}	1.25 ± 0.05^{a}	1.07 ± 0.02^{b}	1.24 ± 0.03^{a}
G/M ratio	1.6 ± 0.02^{c}	1.71 ± 0.03^{b}	1.91 ± 0.11^{a}	1.78 ± 0.03^{b}

Results are expressed as mean values ± standard deviations, n = number of honey samples. Means in a row with same superscripts (a,b,c,d) are not significantly different ($P < 0.05$).
IN: Invertase Number; DN: Diastase Number; HMF: Hydroxymethylfurfural; MS: Monosaccharides; DS: Disaccharides; TS: Trisaccharides; F/G: Fructose/Glucose; G/M: Glucose/Moisture.

Hydroxymethylfurfural (HMF) content is widely used as an indicator of honey freshness. All the tested samples showed lower HMF level than the limit (40 mg/kg), recommended by the Codex Alimentarius and statistical analysis revealed significant difference among all the four varieties ($P < 0.05$). The maximum content of HMF was found in *P. rugosus* (22.66 mg/kg) followed by apple (10.37 mg/kg), cherry (8.62 mg/kg) and saffron honey (7.13 mg/kg) as shown in Table 1. These values suggested that the samples were raw and unprocessed. Our results were in agreement with the values reported by Ozcan and Olmez, (2014).

The water activity is an important factor, which governs the food stability by preventing or limiting microbial growth. The water activity of all the honey samples varied from 0.514 to 0.597 (Table 1). Our results were quite similar to those of Greek honeys for which the a_w values ranged from 0.530 to 0.670 and Indian honeys whose a_w values ranged from 0.570 to 0.700 (Lazaridou et al., 2004; Saxena et al., 2010). A high positive correlation exhibited between water activity and water content (r = 0.93) indicated high water activity due to high moisture content.

The proline content of honey is also a criterion for its ripeness as well as adulteration with sugar (Von der Ohe et al., 1991). A minimum value of 180 mg/kg for genuine honey has been accepted in codex standards. The proline content values were significantly different among all the samples ($P < 0.05$) and the proline content ranged from 205.3 to 551.74 mg/kg (Table 1). Therefore, all the honey samples analyzed contain higher proline limit than the standard limit and thus can be considered as ripened and unadulterated ones. Our results were consistent with Algerian honey, Malaysian honey and Indian honey (Saxena et al., 2010; Ouchemoukh et al., 2007).

4.3. Enzymatic activity

Diastase activity is one of the main criteria utilized in the determination of the intensity of heating of honey during processing and storage. The diastase activity of the tested samples ranged from 12.18 DN (saffron honey) to 22.52 DN (apple honey) (Table 1), and all the samples showed the values within the Codex Standard (>8 DN) which indicated that all the samples were unprocessed and properly stored. Our results were in agreement with those reported by Saric et al. (2008) in Croatian honeys. By comparing our results with those reported by Cantarelli et al. (2008), it can be concluded that the Kashmir valley being a moderate temperature region of India; thus, the honey available in valley will have high diastase activity. Invertase number (IN) indicator of honey freshness was found within the Codex standard (>4 IN) and it ranged from 9.71 IN (saffron) to 19.69 IN (apple). Both IN > 4 and DN > 8 determined that all honey samples were fresh and unprocessed. Our results were consistent with the reporting of Saric et al. (2008).

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4.4. Sugar analysis

Sugars are the main components of honey which depend mostly on floral and geographical origins and less on seasonal, processing and storage conditions (Dobre et al., 2012; Ouchemoukh et al., 2010). Sugar composition has been used to discriminate honey samples on the basis of floral as well as geographical origin (Gomez-Barez et al., 2000). However, many authors have concluded that sugar composition alone is not enough to discriminate honeys (Foldhazi, 1994). In this study, eleven types of sugars were identified and quantified, including three monosaccharides, four disaccharides and four trisaccharides (Table 1). Fig. 1 shows the graphical representation of typical HPLC chromatographic profile of sugars in saffron honey. The HPLC-RI detection proved to be very responsive as reflected from the values of average recovery (94–98%), limits of detection (LOD) (0.05–0.19 g/100 g) and limits of quantification (LOQ) (0.08–0.23 g/100 g). The total sugar content of honey samples varies between 73.89% and 78.45%. The result of total sugar content of our tested unifloral honeys is in agreement with that reported by Bentabol Manzanares et al. (2011) in blossom honey. The results showed that the monosaccharide glucose and fructose are the main sugars in all samples which confirmed that all honey varieties are genuine honeys. Statistical differences were observed in identified sugars and moisture content from all honey types ($P < 0.05$). The fructose values of all the samples ranged between 36.42% and 40.61% (Table 1) with highest percentage in apple and lowest in cherry. The range of amounts quantified in our study is same as reported for some Spanish unifloral honeys (De La Fuente et al., 2011), but higher ranges for fructose 41.3–43.30% (acacia honey) and 39.70–49.10% (black locust honey) were reported by Escuredo et al. (2014) and Primorac et al. (2011), respectively.

The glucose content of all analyzed samples from four varieties ranged from 30.56% to 33.98% (Table 1). Manikis and Thrasivoulou (2001), showed the quicker crystallization of honey samples with glucose content more than 30%. The glucose values obtained in present study were similar to lime honey as reported by Escuredo et al. (2014) in their study on honey from Northwest of Spain and different regions of Romania. All the honeys presented a value of glucose plus fructose higher than 60 g/100 g, which is the value, required for all the kinds of honey in the European and Codex standards. In accordance with Soria et al. (2004) the sum of glucose plus fructose was a discriminatory variable used to distinguish between blossom and suspected honeydew honeys. Xylose is one of the rare reducing sugars found in honeys. The xylose content estimated in four different honey varieties ranged between 0.16% and 0.29% which was lower as compared to Croatians unifloral honeys (0.1–0.7%) reported by Primorac et al. (2011), which may be due to different geographical origins or different climatic variations. All the honey samples had sucrose content lower than the limit (5 g/100 g), prescribed by European Community Directive (European Economic Community, 2002) which confirmed that these honeys are at an advanced stage of ripening.

4.4.1. Minor sugars

The percentage of sucrose varied from 1.05% to 1.40%, and other identified disaccharides include maltose, trehalose and isomaltose. The variation in sucrose concentration among the different honey varieties could be due to transglucosylation reaction which is initiated by transference of α-D-glucopyranosyl unit from sucrose to an acceptor molecule (Da Costa Leite et al., 2000). Serrano et al., 2007 found similar results for sucrose content in Andalusian honeys. The concentration of Maltose ranged between 0.95% and 1.36% which agrees with results reported by Juszczak et al. (2009). The isomaltose concentrations were found between 0.66% and 0.92%, which were somewhat similar as reported by Bentabol Manzanares et al. (2011) in several Spanish honeys. In this study, a low concentration of trehalose was found which does not exceed 0.20% (0.02–0.17%) as shown in Table 1. Similar results for trehalose were found

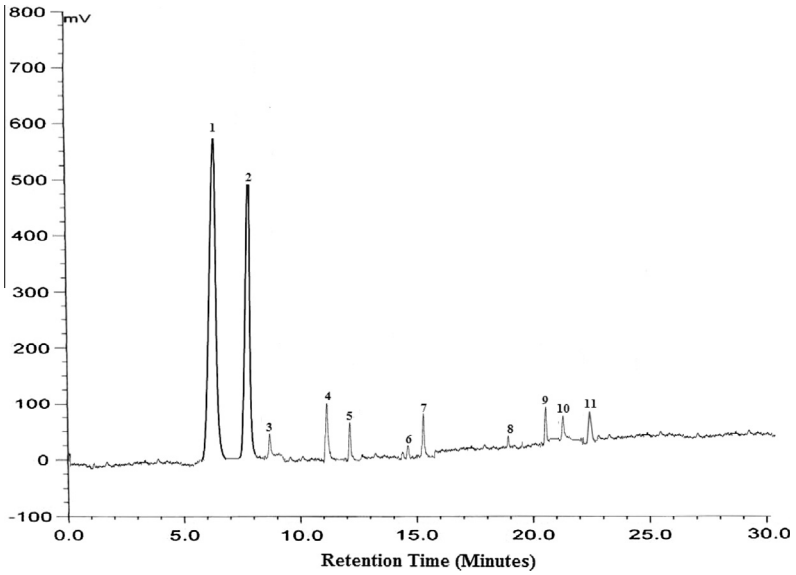

Figure 1 HPLC chromatographic profile of sugars in Saffron honey (**1**: Fructose, **2**: Glucose, **3**: Xylose, **4**: Maltose, **5**: Isomaltose, **6**: Trehalose, **7**: Sucrose, **8**: Raffinose, **9**: Maltotriose, **10**: Melezitose, **11**: Maltotetraose).

by Ouchemoukh et al. (2010) in Multifloral and Eucalyptus and Pasini et al., 2013 in Buckwheat honey. Among the trisaccharides, raffinose displayed a range of 0.04–0.10% which is quite similar to 0.02–0.08% in Brazilian honeys (Da Costa Leite et al., 2000) and 0.02–0.08% in Spanish honey samples (De La Fuente et al., 2011). Raffinose could be nectar constituent or it may arise due to honeydew contamination (Da Costa Leite et al., 2000). The maltotriose (0.48–0.90%), followed by maltotetraose (0.49–0.86%) and melezitose (0.19–0.46%) was the most abundant trisaccharides registered in the present study and content in various honey samples was statistically ($P < 0.05$) different. The average concentration of melezitose which is generally an indicative of honeydew honey was within those found by De La Fuente et al. (2011) in Spanish honeys. Sanz et al. (2004) obtained a high concentration of melezitose (6.57%) in Spanish honeydew samples which were described as specific markers for such honeys. The low percentage of melezitose is indicating that these are nectar honeys.

4.4.2. Crystallization ratios

Sugar ratios were calculated and evaluated to study their contribution to the crystallization tendencies according to honey type. The tendency of honey to granulate is explained by (F/G) ratio because glucose is less water soluble than fructose, and therefore this makes it an important parameter to predict the crystallization tendency of honey (Laos et al., 2011). In the studied honey types; cherry, saffron, *P. rugosus* and apple had an F/G ratio of 1.07, 1.23, 1.24 and 1.25, respectively (Table 1). Except cherry variety, all the other three varieties displayed a mean value of around 1.2 which is common F/G ratio found in most of the honey varieties around the world (Bentabol Manzanares et al., 2011; Dobre et al., 2012). Our values were significantly lower than those found for chestnut, eucalyptus, heather, acacia and honeydew honeys (>1.4). The F/G ratio of 1.14 or less would indicate a faster crystallization; thus, cherry honey crystallizes faster than other three varieties while values over 1.58 are associated with no tendency to crystallize (Venir et al., 2010). Moisture content is another important parameter that affects the crystallization of honey. According to the National Honey Board (2010), crystallization time depends mostly on the F/G and the glucose/moisture (G/M) ratios as well. Some researchers have explained that the G/M ratio can be a better indicator for prediction of honey crystallization (Dobre et al., 2012; Manikis and Thrasivoulou, 2001). Lower the moisture content and higher the glucose content of honey, the faster the crystallization will take place. According to the literature, honey crystallization is slow or null when the G/M ratio is less than 1.7, and is complete and rapid when the ratio is greater than 2 (Dobre et al., 2012). All the four honey samples analyzed except saffron honey showed G/M ratio higher than 1.7, which is consistent with the aspect of the honeys (Table 1).

4.5. FTIR–ATR spectroscopy

ATR–FTIR spectroscopy was used to compare honey samples based on their spectral differences in the 4000 cm^{-1} to 400 cm^{-1} spectral region. A representative ATR-FTIR spectrum of saffron honey is shown in Fig. 2. The band from about 1400 to 750 cm^{-1} (Fig. 2) corresponds to the most sensitive absorption region of the honey sugars. The characteristic bands of major monosaccharides absorption (glucose,

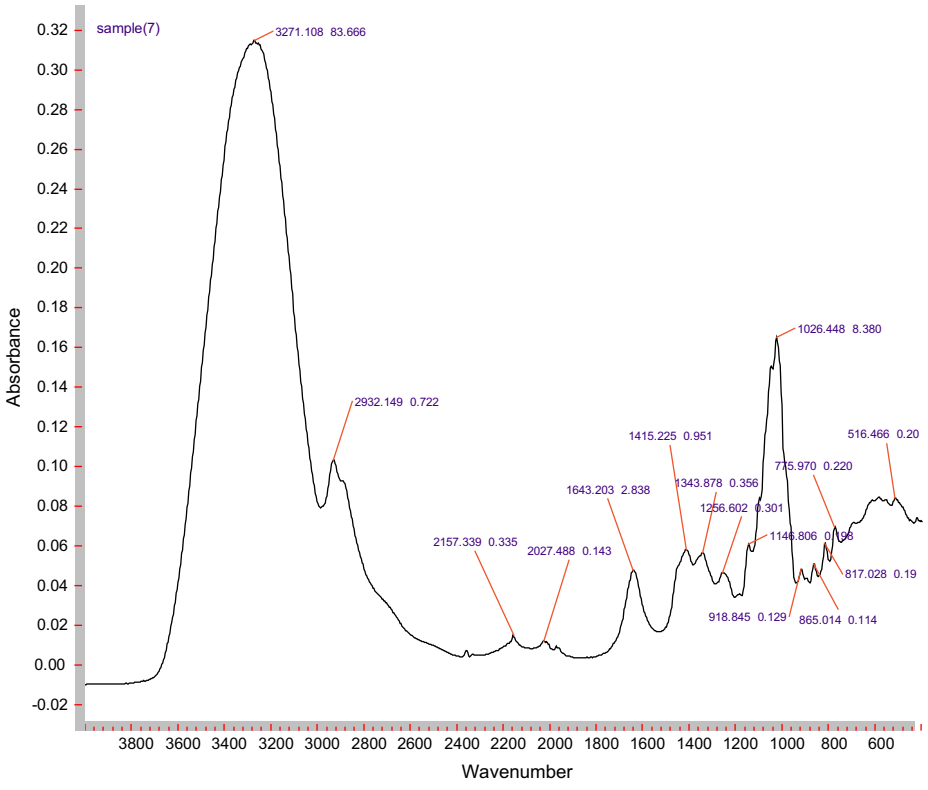

Figure 2 Representative FTIR–ATR spectrum of saffron honey in the 4000–400 cm^{-1} spectral region.

fructose) and disaccharide (sucrose) were present in the region between 1400 and 900 cm^{-1} and major infrared bands of water were located at 3330 cm^{-1} for O–H stretching and 1645 cm^{-1} for H–O–H bending vibrations (Fig. 2). According to some authors, peak at 918 cm^{-1}, 1043 cm^{-1} and 1254 cm^{-1} corresponds to the C–H bending of the carbohydrate, C–O stretch in the C–OH group as well as the C–C stretch in the carbohydrate structure, respectively (Subari et al., 2012; Tewari and Irudayaraj, 2005). Similarly, Anjos et al. (2015) reported that spectral region located between 1500 and 750 cm^{-1} was characteristic of the carbohydrate configuration. As shown in Fig. 2, the small peak located at 1110 cm^{-1} corresponds to stretching of the C–O band of the C–O–C linkage while the peak that appeared at 1321 cm^{-1} was due to O–H bending of the C–OH group. The broad band located between 3200 and 3000 corresponds to the C–H stretching of carboxylic acids and NH_3^+ stretching band of free amino acids (Fig. 2). The similar spectral band corresponding to carboxylic acids and NH_3^+ was reported by Zhbankov et al. (1998), Tewari and Irudayaraj (2004), Bureau et al. (2009), Gallardo-Velazquez et al. (2009), Gok et al. (2015).

4.6. Rheology

Dynamic frequency sweep tests were carried out to determine the rheological parameters (G′ and G″) and their dependence on angular frequency. The frequency dependent magnitude obtained for loss modulus (G″) was much greater and significant than storage modulus (G′) which determined that all honey samples show more viscous behavior than elastic (G″ ≫ G′). Similar behaviors for different types of honey were predicted by Yoo (2004), Gomez-Diaz et al. (2009) and Witczak et al. (2011). Both magnitudes G′ and G″ increased with increase in angular frequency. Fig. 3a–d shows the typical diagram of loss modulus (G″) as a function of angular frequency (ω) for all honey samples at various temperatures from 0 °C to 30 °C. Therefore in this study the effect on rheological properties of honey varieties by temperature was characterized by G″ only. As mentioned earlier G″ is much informative than G′; thus, the data obtained for both parameters at different temperatures (0–30 °C) were also subjected to linear regression, as suggested by Rao and Cooley (1992), using the power law equation (Eqs. (1 and 2)) as follows:

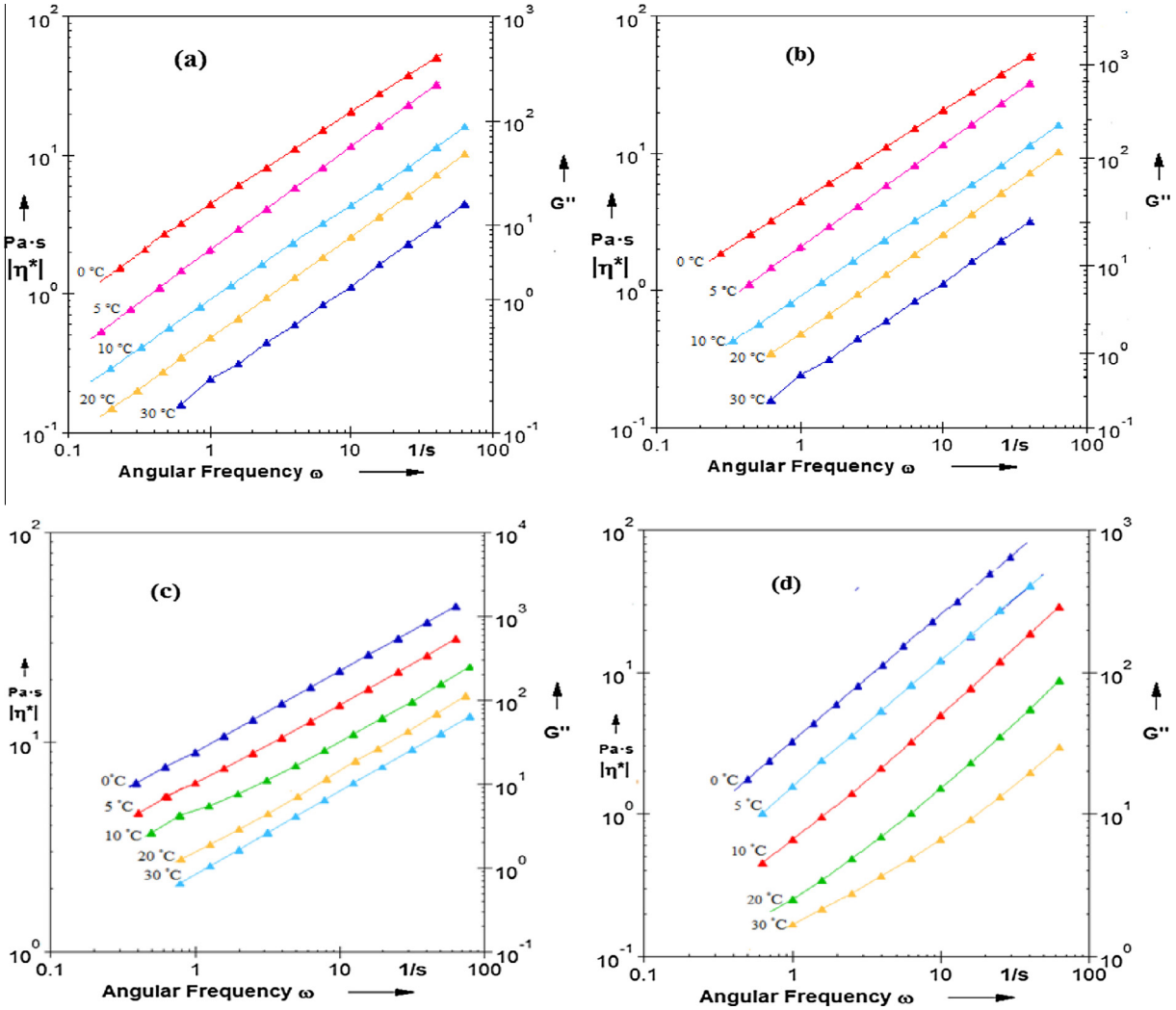

Figure 3 **(a–d)** Loss modulus (G″) as a function of angular frequency (ω) for honey samples of (a) apple, (b) cherry, (c) *Plectranthus rugosus,* and (d) saffron at various temperatures from 0 °C to 30 °C.

Table 2 Slopes and intercepts of loss modulus (G′), complex modulus (G″) versus angular frequency (ω) of different varieties of honeys.

Honey	Moisture content (%)	Temperature (°C)	G′			G″		
			Slope	Intercept	R^2	Slope	Intercept	R^2
Saffron	19.10	0	1.00	0.48	0.97	1.00	13.80	0.99
		5	0.68	0.83	0.99	1.00	8.50	0.99
		10	0.31	1.98	0.99	1.00	4.26	0.99
		20	0.60	0.41	0.99	0.99	1.20	0.99
		30	0.86	0.06	0.98	0.96	0.37	0.97
Apple	18.82	0	0.73	0.52	0.97	1.00	15.85	0.99
		5	0.84	0.17	0.99	1.00	10.09	0.99
		10	1.07	0.05	0.99	1.00	5.30	0.99
		20	0.88	0.06	0.99	0.99	1.49	0.98
		30	1.29	0.01	0.99	0.97	0.47	0.99
Cherry	17.8	0	0.99	1.45	0.99	1.00	19.20	0.98
		5	1.21	0.38	0.93	1.00	11.70	0.99
		10	0.51	3.37	0.99	1.00	6.68	0.99
		20	0.46	2.36	0.99	0.99	1.84	0.99
		30	0.68	0.26	0.99	0.96	0.57	0.99
Plectranthus rugosus	17.05	0	0.27	7.86	0.97	1.00	22.02	0.99
		5	0.38	3.51	0.99	1.00	12.26	0.99
		10	0.22	5.42	0.99	1.00	7.27	0.99
		20	0.65	0.45	0.99	0.99	2.22	0.99
		30	0.11	0.75	0.99	0.98	0.81	0.99

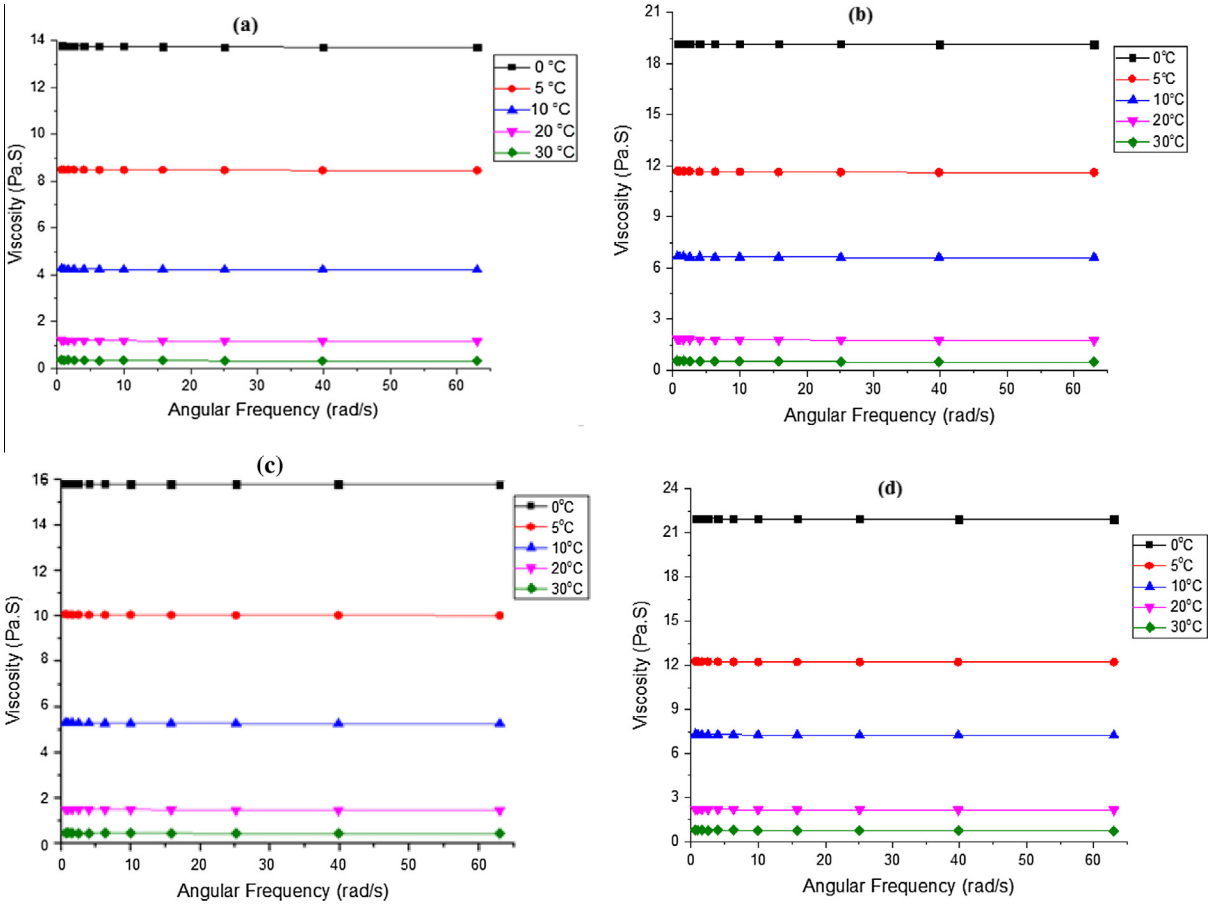

Figure 4 **(a–d)** Viscosity as a function of angular frequency of different unifloral honeys: (a) saffron, (b) *Plectranthus rugosus*, (c) apple and (d) cherry at different temperatures (0, 5, 10, 10, 20 and 30 °C).

Table 3 Viscosity of different honey types as a function of moisture content and temperature.

Honey	Moisture content (%)	Temperature (°C)	Viscosity (Pa s)
Saffron	19.10	0	13.76
		5	8.48
		10	4.24
		20	1.19
		30	0.35
Apple	18.82	0	15.82
		5	10.06
		10	5.28
		20	1.47
		30	0.43
Cherry	17.8	0	19.16
		5	11.66
		10	6.64
		20	1.79
		30	0.53
Plectranthus rugosus	17.05	0	21.97
		5	12.24
		10	7.26
		20	2.19
		30	0.77

$$G'' = K''(\omega)^{n''} \quad (1)$$

$$G' = K'(\omega)^{n'} \quad (2)$$

where K′ and K″ are elastic and viscous intercepts, respectively while n' and n'' represent elastic and viscous slopes, respectively. The magnitudes of intercepts, slopes and the coefficient of regression (R^2) for Eqs. (1 and 2) are presented in Table 2. Based on the rheological data obtained from equations 1 and 2, honey displayed a liquid like behavior because the magnitudes of K″ (0.37–22.02), are much greater than those of K′ (0.01–7.86), with a high dependence (n' = 0.11–1.29, n'' = 0.96–1.0) on frequency. Similar results for such magnitudes were found in Spanish honeys (Oroian et al., 2013b). However the viscosity was not influenced by frequency i.e. viscosity is independent on share rate or frequency, exhibiting a Newtonian behavior as shown in Fig. 4. The Newtonian behavior of different honeys from different parts of world has been observed by many authors Oroian et al. (2014), Gomez-Diaz et al. (2009), Saxena et al. (2014). The viscosity of different honeys as a function of moisture content and temperature is shown in Table 3. The viscosity decreases with increase in temperature (Fig. 5) and moisture content because when temperature increases average intermolecular forces decrease i.e. kinetic energy increases and molecules become more mobile (Patil and Muskan, 2009). The viscosity of all samples ranged from 0.35 Pa s at 30 °C in saffron honey to 21.97 Pa s at 0 °C in *P. rugosus*. Similar results for combined effect of temperature and moisture on viscosity have been observed in Chinese Rape honey, Greece pine honey, Greece cotton honey and in various Spanish unifloral honeys (Junzheng and Changying, 1998; Oroian et al., 2013a). The diverse variation observed in honey viscosities around the globe can be described due to the fact that viscosity is greatly

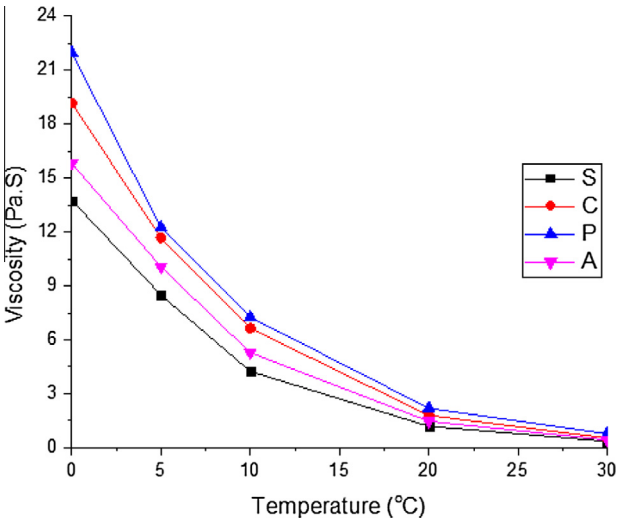

Figure 5 Effect of temperature on viscosity of different honey types (S = saffron; C = cherry; P = *Plectranthus rugosus*; A = apple).

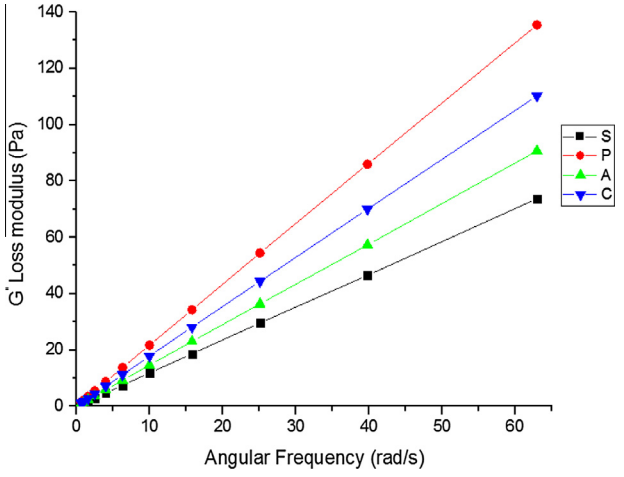

Figure 6 Loss modulus (G″) at 20 °C in dynamic oscillatory procedure for all honey samples (S = saffron; C = cherry; P = *Plectranthus rugosus*; A = apple).

affected by chemical composition parameters, such as moisture content, sugars and protein contents, which change with the geographical and floral origins of each honey. It should be noted that a low deformation stress occurs when the shear is applied due to the presence of large amounts of crystallized glucose in honey. During the rheological characterization of honeys, glucose, fructose and F/G ratio are one of the most important parameters. In order to understand the flow mechanism at a structural and molecular level during the rheological characterization of honeys, glucose, fructose and F/G ratio are the most important parameters that should be taken into consideration (Dobre et al., 2012). As earlier mentioned the values for G″ and G′ increase with increase in angular frequency. The values for G″ ranged from 0.23 Pa s in saffron honey at 30 °C to 1382 Pa s in *P. rugosus* at 0 °C

Table 4 Effect of temperature on honey viscosity and loss modulus (G″).

	Viscosity		Loss modulus (G″)		
Honey	E_a (kJ/mol)	η_o (mPa s)	E_a (kJ/mol)	G″ (mPa s)	R^2
Saffron	85.59	6.43×10^{-16}	86.88	5.64×10^{-15}	0.99
Cherry	83.51	2.27×10^{-15}	84.88	1.92×10^{-14}	0.99
Plectranthus rugosus	77.18	1.77×10^{-12}	77.80	4.54×10^{-13}	0.99
Apple	84.21	1.38×10^{-15}	84.76	1.68×10^{-14}	0.99

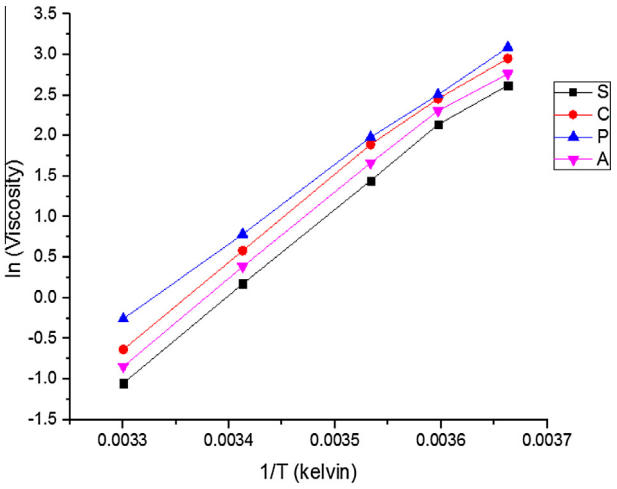

Figure 7 Arrhenius model fit for different types of unifloral honey (S = saffron; C = cherry; P = *Plectranthus rugosus*; A = apple).

while value of G′, ranged from 0.009 Pa s in apple honey at 30 °C to 85.95 Pa s in cherry honey at 0 °C. The differences among honey varieties could be due to variations in chemical composition of sugars, pollens and moisture content (Lazaridou et al., 2004). The descending order of loss modulus (G″) for all studied samples is shown in Fig. 6: *P. rugosus* honey, cherry honey, apple honey and saffron honey.

4.7. Effect of temperature on honey viscosity and loss modulus (G″)

The effect of temperature on viscosity and loss modulus (G″) was described using the Arrhenius model as follows:

$$\mu = \mu_o \exp[E_a/RT]$$

where μ is viscosity (Pa s), μ_o is constant, E_a is activation energy (kJ/mol), R is gas constant (8.314 kJ/mol/K) and T is absolute temperature in Kelvin. The pre-exponential factor (μ_o) of Arrhenius model and activation energies (E_a) were estimated, respectively, from the intercept and slope of straight lines obtained by the least square regression. The significant effect of temperature was found to be more up to 20 °C. However, at temperatures beyond 20 °C, the differences in viscosity are very small in all honey samples (Table 3). The values of activation energy of the analyzed honey samples ranged from 77.18 kJ/mol in *P. rugosus* to 85.59 kJ/mol in saffron honey (Table 4). The sensitivity of the viscosity to the temperature change is indicated by activation energy. Higher E_a value means that the viscosity of honey is relatively more sensitive to temperature change. Table 4 shows that saffron honey is the most sensitive (highest E_a value), whereas *P. rugosus* is the least sensitive (least E_a value) among the honey varieties analyzed. Fig. 7 shows the linear relationship of $\ln(\eta)$ vs $(1/T)$ obtained from the linearization of Arrhenius equation and fitted to experimental data. Similar values for activation energy were reported for Greek honeys (Lazaridou et al., 2004) and Polish honeys (Juszczak and Fortuna, 2006). Honey with less moisture content possessed less activation energy, so the highest one was achieved by saffron honey and the smallest one by *P. rugosus*. According to Al-Malah et al. (2001), the material constant (pre-exponential factor in the Arrhenius equation), represents viscosity at a temperature approaching infinity. Higher the values of material constant, higher the viscosity of the samples. Thus *P. rugosus* was more viscous and saffron honey least viscous of all samples analyzed. A similar effect of temperature on loss modulus (G″) was described using the Arrhenius model and the values of activation energy of the analyzed honey samples ranged from 77.80 kJ/mol in *P. rugosus* to 86.88 kJ/mol in saffron honey (Table 4) which was quite similar to the values reported by Oroian et al. (2014) in Spanish honeys.

5. Conclusion

The results of physico-chemical analysis of all the honey varieties were within the limits recommended by European Commission and the Codex Alimentarius. The carbohydrate profile of studied honey revealed that all the unique honey varieties possessed reducing sugars, mainly fructose and glucose in largest portion and also small quantities of disaccharides and trisaccharides. Vibrational spectra recorded by using FTIR–ATR showed to be a good methodology in evaluation of sugars in honey. All the honey samples analyzed showed Newtonian behavior (G″ ≫ G′) irrespective of their different floral origins within a temperature range of 0–30 °C. The results also showed that honey with the highest activation energy is more sensitive toward temperature gradient and honey with highest material constant displayed the highest viscosity value.

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