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ORIGINAL RESEARCH PAPER

CHARACTERIZATION OF DIFFERENT UNIFLORAL INDIAN HONEY VARIETIES BASED ON THE PHYSICO-CHEMICAL AND RHEOLOGICAL PROPERTIES

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Honey is a natural product well known for its nutritive value and being usually used as natural sweetener. India is one of the major honey exporting countries in the world. The present research refers to an investigation of the physico chemical and rheological behavior of four freshly harvested unifloral honey samples collected from different plant sources viz Sunflower (H_1), Eucalyptus (H_2), Mustard (H_3) and Prosopis (H_4). Rheological studies were carried out by varying honey samples temperature from 10 to 50 °C. Three different models (Arrhenius, Ostwald-de Waele Power law and Newtonian-I) were investigated. A continuous fall in apparent viscosity ($p<0.05$) with the increase in temperature was observed for all honey varieties. Moreover no significant changes ($p>0.05$) in apparent viscosity of honey was observed with the variation in shear rate, indicating their Newtonian behavior. The moisture content present in different honey samples (H_1, H_2, H_3 and H_4 was 13.6 %, 17.2 %, 15.5 % and 16.1 % respectively) also had a significant effect ($p <0.05$) on the apparent viscosity of honey following changes in temperature (10-50 °C). The honey samples having higher moisture content exhibited greater decrease in their apparent viscosity. Power law was found to be the most suitable model ($R^2 > 99\%$) in explaining the rheological behavior of honey following the variations in temperature when compared with Arrhenius and Newtonian-I respectively.

Keywords: browning index, color, honey, rheology, viscosity

Introduction

Honey is a supersaturated sugar solution, principally contains mainly fructose and glucose with traces of maltose and sucrose (Jeffrey and Echazarreta, 1996; Omafuvbe and Akanbi, 2009). The ratio of glucose to fructose is mainly responsible for the crystalline nature of honey and ultimately for rheological behaviour. Moisture also plays a key role in deciding the rheological behaviour of honey. In practical terms moisture effects both viscosity as well as rheological behaviour of honey (Sopade *et al*., 2004; Lazaridou *et al*., 2004; Juszczak and Fortuna, 2006; Yanniotis *et al*., 2006; Kang and Yoo, 2008). Sugar types along with water amount in honey are the major factors for the shelf life and storage behaviour of honey (Camara and Laux, 2010). Study of deformation and flow of liquid is together known as rheological behaviour and its knowledge is very important and useful specially in deciding post-harvest processing, handling and storage of honey (Steffe, 1996; Rielly, 1997). Along with concentration and proportion of different sugars present and moisture content, temperature is the third most important factor. Viscosity is highly temperature sensitive and in general terms inversely proportional to the temperature, as molecular friction and hydrodynamic forces decreases with increase in temperature, which ultimately decreases the viscosity or vice versa. Maximum change in honey viscosity is observed up to 30 ^{o}C and change is almost negligible after 45 ^{o}C temperature (Davis, 1995). In most of the honey varieties viscosity is independent of shear rate and depends only upon temperature and composition, which indicates their Newtonian behaviour (Junzheng and Changying, 1998; Bhandari *et al.*, 1999; Mossel *et al.*, 2000; Al-Malah *et al.*, 2001; Zaitoun *et al.*, 2001; Sopada *et al.*, 2002; Lazaridou *et al*., 2004; Juszczak and Fortuna, 2006). However, some authors (Munro, 1943; Pryce-Jones, 1953; Serra Bonvehi and Granados Tarres, 1993; Samanalieva and Senge, 2009) also observed the thixotropic behaviour of few honey varieties. The non-Newtonian thixotropic behaviour of heather, manuka and buckwheat honey may be because of high molecular compounds – protein or crystals of dextran etc. (Bakier and Lewczuk, 2000; Chen *et al*., 2009; Samanalieva and Senge, 2009; Witczak *et al*., 2011). According to Bhandari (1999) on removal of colloidal particles from honey using filtration or any other mode, honey loses its thixotropic properties and behaves like a Newtonian fluid. Many researchers studied and described the rheological behavior of honey using various mathematical models (Mossel, *et al*., 2000; Sopade, *et al*., 2002; Recondo, *et al*., 2006; Witczak, *et al*., 2011). However, rheological research related to unprocessed unifloral honey from different botanical sources is not yet reported. Therefore, current study involves the examination of unprocessed honey samples gathered from different plant sources of India for rheological parameters using three different mathematical models, *i.e*. Arrhenius, Power Law and Newtonian-I.

Material and methods

Material

Four samples of freshly harvested unifloral honey samples were procured directly from the beekeepers of Haryana and Punjab regions of northern India. Immediately after harvesting, the honey was filtered through clean muslin cloth, packed and immediately sealed in 500 ml glass bottles, followed by labelling of varieties and coding i.e. Sunflower (H_1), Eucalyptus (H_2), Mustard (H_3) and Prosopis (H_4). Samples were stored in refrigerator at 5 °C. The storage period did not exceed more than 15 days for any of the honey samples. Before analysis the honey samples were overnight kept at room temperature (25± 2 °C).

Methods

For source identification melissopalynological method of pollen analysis by Louveaux *et al*. (1978) was followed and after examining the slides under optical microscope at X400 and X1000, pollen identification, counting and classification was done.

Moisture content

Moisture content of honey was calculated using protocol given by AOAC 969.38 method (AOAC, 1995), according to which obtained refractive index at 20 °C was compared with given table and converted into moisture.

Ash Content

Ash content of honey samples was calculated using standard method given in Harmonized methods of the international honey commission (IHC, 2009). Thus, ashing was conducted below 600 °C in electric furnace and residues were weighed.

Diastase Activity

The diastase activity of honey samples was reported in Shade Units (SU) after the method of Shade *et al.* (1958) and IHC (2009). One unit corresponds to the enzyme activity of 1 g of honey that can hydrolyse 0.01 g of starch in 1 h at 40 °C (Oddo *et al*., 1999). Decomposition of starch by α-amilase enzyme present in honey results into discoloration of solution from blue to violet or pink, which is directly proportional to the amount of starch decomposed. The degree of decomposition mainly depends upon the intensity of enzymatic activity. The color change was observed through UV-spectrophotometer (UV-1800, Shimadzu, Japan.) at a wavelength of 660 nm against distilled water as blank.

Hydroxy-Methyl Furfural Analysis

HMF content in honey samples was measured using a spectrophotometric method (direct absorption method) (White, 1979), where honey solution was filtered after dissolving in Carrez solution (Merck, Millipore, Darmstadt, Germany) and then absorption was measured at 284 and 336 nm (UV-1800; Shimadzu) against the reference solution of honey and sodium bisulphate 0.2%; (Merck). The final reading (in triplicate for each sample) was obtained using the following equation:

$$HMF\,(mg/kg) = (A_{284} - A_{336}) \times 149.7 \times 5 \times \frac{D}{W} \qquad (1)$$

where: A_{284} - absorbance of sample solution at 284 nm, A_{336} - absorbance of sample solution at 336 nm, 149.7 – constant, 5g - initial weight of honey sample taken, D - dilution factor (in case dilution is necessary), W - weight of honey sample (g).

Color measurement and Browning index calculation

Hunter lab Color Flex EZ, 45/00 color spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) was used to measure color parameters of honey. The three-dimensional color space perceive in L, a* and b*, where L (Luminance) from vertical axis express brightness, between complete black to complete white (i.e. 100 % black to 100 % white), whereas a* axis and b* axis ranging from green (-a) to red (+a) and from blue (-b) to yellow (+b) respectively (Ferrari *et al*., 2010).

Browning index (BI) was calculated using the equation 2 and 3

$$BI = \frac{100(x - 0.31)}{0.172} \tag{2}$$

where: $$x = \frac{a^* + 1.75L}{5.645L + a^* - 3.012b^*} \tag{3}$$

Rheological Analysis

The rheological properties of honey were evaluated by using a molecular compact rheometer (MCR)-52 from ANTON PAAR GmbH, Austria equipped with a fixed lower plate for keeping sample in 50 ml size cuvet and rotating upper plate with spindle. Due to the fact that presence of crystals and air bubbles may affect the results of viscosity analysis all the samples were heated in a water bath at 50-55 °C for one hour to dissolve all the D- glucose monohydrate crystals which may be present in the samples. After de-crystallization the samples were placed in incubator at 30 °C for 48 hours to avoid air sacs. Then the samples were carefully transferred to measuring element of rheometer. Measurements were performed at four different temperatures, *i.e.* 10 °C, 20 °C, 30 °C and 40 °C at increasing shear rate from 0 to 100 s^{-1}. The rheometer data were analyzed by using Rheoplus software. Three different models were applied to study the effect of temperature on apparent viscosity of honey.

Arrhenius Model

Arrhenius parameters were obtained by plotting a linear graph between inverse of temperature (1/T) and observed log viscosity (In η) on x & y-axis respectively (Bhandari *et al*., 1999). A linear equation (y = mx + c) respond to equation-4 in log form i.e. $In\ \eta = In\ \eta_0 + E_a/R\ (1/T)$ was obtained and material constant (η_0) and flow activation energy (E_a) values were calculated (Saxena *et al*., 2014).

$$\eta = \eta_0 e^{\left(E_a/RT\right)} \tag{4}$$

where: η - viscosity (Pa·s), η_0 - material constant (Pa·s), E_a - flow activation energy (J/mol), R - gas constant (R=8.315/mol.K), T - absolute temperature (K).

The mean absolute percentage error (MA%E), was identified to check the deviation between calculated and observed viscosity values through equation-5 (Mayer and Butler, 1993; Mossel *et al*., 2000; Saxena *et al*., 2014).

$$MA\%E = \left(\frac{100}{n}\right)\sum\left(\frac{Y_0 - Y_c}{Y_c}\right) \tag{5}$$

where: Y_0 - observed viscosity value, Y_c - calculated viscosity value, n - number of pairs of samples.

Power law Model

Shear stress *vs.* shear rate plots of many fluids became linear when plotted on double logarithmic coordinates, and the power law model describes the data of shear thinning and shear thickening fluids:

$$\tau = \kappa\dot{\gamma}^{n} \qquad (6)$$

where: κ is the consistency coefficient with the units: Pa s^n is the shear stress at a shear rate of 1.0 s^{-1} and the exponent n, the flow behaviour index, is dimensionless that reflects the closeness to Newtonian flow (Rao, 2014).

Newtonian-I Model:

Newtonian model is described by straight lines in terms of shear rate and shear stress, and is described by one parameter, *i.e.* viscosity:

$$\eta = a.T \qquad (7)$$

where: η - viscosity, a - slope of the line, T - temperature (Vélez-Ruiz and Barbosa-Cánovas, 1998).

All experiments were performed in triplicate and observations were expressed as mean and standard deviation values by taking data points into consideration. Graphs and other correlations were determined using Microsoft excel software.

Results and discussion

Physico-Chemical Properties of Unprocessed Unifloral Honey Samples

Physico-chemical examination involving moisture, ash content, diastase activity and Hydroxy-methyl furfural (HMF) contents of all four unifloral honey samples (H_1 to H_4) obtained from different flower sources are presented in Table 1. Sample H_2 showed significantly higher (p <0.05) moisture content, whereas H_1 sample showed lowest moisture content. The moisture content values varied from 13.6 % to 17.2 %. The moisture content of different unifloral honey may depend on composition due to variable floral sources, temperature, humidity conditions, stage of harvesting etc. Our findings for moisture content of honey are within the range of Lazaridou *et al*. (2004) who found 13 % to 29 % variation in the moisture content of Greek honey and slight deviation was observed from the data reported by Adenekan *et al*. (2012) for honey samples from different regions of Nigeria (16.15 % to 21.41 % MC). Moisture content is directly related to honey stability, higher moisture content of honey have higher water activity and more susceptible for microbial spoilage than honey having lower moisture content. Hence, with the above results it can be concluded that sample H_2 may present lower stability against microorganisms/ microbial spoilage in comparison to sample H_1.

Ash content represents the mineral content of a sample and significant difference (p <0.05) was observed in the ash content of all four honey samples, minimum to maximum ash content range was 0.32 % to 0.98 % found in H_2 and H_3 samples respectively (Table 1). The fluctuation in ash content may be explained by geographical, floral and climatic variation. Our research findings for ash content were almost similar to that of Adenekan *et al*. (2012) who documented 0.32 – 0.96 % ash content in honey from Ogun state, Nigeria.

Table 1. Physico-chemical properties of freshly harvested unifloral honey samples.

Honey Source (Flower)	Code	Moisture content (%)	Ash content (%)	Diastase Activity (SU)	HMF content (mg/kg)
Sunflower	H_1	13.6 ± 0.12^a	0.70 ± 0.13^c	38.09 ± 2.08^a	11.17 ± 0.84^f
Eucalyptus	H_2	17.2 ± 0.60^c	0.32 ± 0.07^a	50.20 ± 2.33^e	06.43 ± 0.79^c
Mustard	H_3	15.5 ± 0.30^b	0.98 ± 0.19^d	38.57 ± 2.13^a	10.31 ± 0.82^e
Prosopis	H_4	16.1 ± 0.96^b	0.38 ± 0.10^a	45.86 ± 0.84^d	07.82 ± 0.61^d

Data are presented as means±SEM (n=3).

$^{a\text{-}b}$Means within columns with different uppercase superscript are significantly different ($p<0.05$) from each other.

Diastase activity in honey is the most important factor of quality which exclusively dependents upon season, biological origin and floral source of honey (Da Silva *et al*., 2016). Significantly high ($p<0.05$) diastase activity was found in H_2 sample followed by H_4, H_3 and least in H_1 as shown in Table 1. Higher diastase activity is indicative of freshness in honey, which decreases with storage and heating during processing (D'Arcy, 2007; Basmaci, 2010). The current results of our study for diastase activity are supported by Janghu *et al*. (2017) who reported 12 to 58 Shade Units (SU) for freshly harvested honey samples from India. In contrast, Chaikham *et al*. (2016) reported only 13.87-15.12 SU of diastase activity in unprocessed Logan, Lychee and Wildflower honeys.

5- Hydroxymethylfurfural (HMF) is a chemically breakdown product of furan group from caramelized sugars in acidic medium (Kroh, 1994) and recent research suggested that its consumption indirectly contribute to cancer or it might metabolize into carcinogenic compounds in human body (Capuano and Fogliano, 2011). Its existence and further increase in honey indicates heating/ improper storage or adulteration (Nozal *et al*., 2001). As per our observations, the highest HMF content presented the sunflower honey sample *i.e*. 11.17 mg/kg. The observations were in agreement with Yilmaz and Kufrevioglu (2001), reported 0.0 to 11.5 mg/kg HMF range in 45 honey samples. HMF content is directly proportional to the color or browning index of honey, as shown in Table 2. In all four honey samples trend of HMF content was inversely proportional to moisture content as shown in Table 1. Thus, honey having higher moisture content has lesser HMF formation or vice versa. Likewise, Zhang *et al*. (2012) showed that presence of organic acids and lower moisture content contributes to high HMF content.

Color of honey is mainly given by pigments from nectar source and pollens present inside honey (Can *et al*., 2015). In the current investigation difference in color parameters were documented in the form of *L* (lightness), *a** (redness) and *b** (blueness) and on its basis browning index was calculated for all four honey samples (Table 2). Among all, sample H_2 showed the most significant brightness followed by H_4, H_3 and H_1 sample. Whereas *a** and *b** values showed the reverse trend than *L* values. These findings are supported by Chaikham *et al.* (2016), which stated that changes in *L*, *a** and *b** are due to HMF formation. Other reasons for difference in color may include climate, nectar source and geographical variations.

Table 2. Color parameters and browning Index of freshly harvested honey samples.

Honey Source	**Code**	**Color Parameters**			**Browning Index**
		L (Lightness)	**a* (redness)**	**b* (yellowness)**	
Sunflower	H_1	42.41 ± 0.37^{a}	7.93 ± 0.39^{d}	38.04 ± 0.41^{f}	179.51 ± 2.78^{f}
Eucalyptus	H_2	58.26 ± 1.81^{d}	5.87 ± 0.07^{b}	31.60 ± 1.28^{c}	81.39 ± 5.71^{c}
Mustard	H_3	47.30 ± 0.58^{b}	7.04 ± 0.14^{c}	36.72 ± 0.14^{e}	$139.26 \pm 4.27_{e}$
Prosopis	H_4	50.08 ± 1.26^{c}	6.39 ± 0.11^{bc}	32.51 ± 0.66^{d}	105.73 ± 2.03^{d}

Data are presented as means±SEM (n=3).

$^{a\text{-}b}$Means within columns with different uppercase superscript are significantly different ($p<0.05$) from each other.

Rheological Behaviour Analysis of Unifloral Honey Samples

Viscosity evolution presented by all four honey samples at increasing shear rates 0-100 s^{-1}, indicated a Newtonian behaviour. As can be seen from Figure 2 to 5, negligble effect of increasing shear rates on viscosity for all four unifloral honey samples was observed when tested at 10 °C, 20 °C, 30 °C and 40 °C but substantial reduction in viscosity was observed as temperature increased. Our findings were supported by Bhandari *et al.* (1999) and Mossel *et al.* (2000), who reported Newtonian behavoiur in all honey samples during their investigation.

Temperature dependence of honey viscosity

The temperature dependent behaviour of all honey samples was studied and viscosity changes are shown in Figure 1. All honey samples showed a uniform trend of decrease in viscosity values between 10 °C and 50 °C temperature range. Decrease in viscosity band was maximum between 10-30 °C; viscosity band at 10 °C for all four honey samples ranged between 32.08 (H_1) – 10.95 (H_2) Pa·s and decreased up to 3.64 (H_1) – 1.58 (H_2) Pa·s at 30 °C. This finding was similar to that of Mossel *et al*. (2000) who stated that heating process resulted in rapid decreases in viscosity with each temperature . Less downfall in viscosity was observed when tempreature raised to 40 °C *i.e*. 1.49 (H_1) to 0.75 (H_2) Pa.s, whereas the changes in viscosity were almost negligible above 40 °C (Figure 1). Our work is supported by Munro (1943) and Saxena *et al.* (2014), who found unsignificant changes in honey viscosity above 30 °C. Moisture content of honey also played a significant role in decideing the viscosity; for instace Eucalyptus honey sample presented the highest moisture content and the lowest viscosity value. Hence moisture content of honey is inversly proportional to viscosity. A similar corelation between viscosity and moisture content of honey was also reported by Steffe (1996) and Yanniotis *et al*. (2006) in four unfloral nectar honeys from Thymus, Orange, Helianthus and cotton plants.

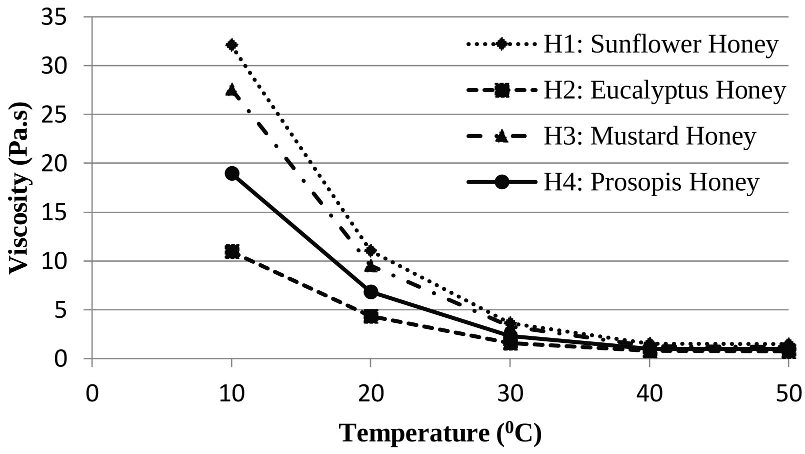

Figure 1. Effect of temperature on viscosity of different honey types.

Temperature dependence of viscosity can be appropriately described by using Arrhenius, Power law and Newtonian model according to Equation 4, 6 and 7. Values obtained for rheological parameters of each model are shown in Table 3. Power law showed the highest R^2 values for all the four honey samples ≥ 0.99, indicating a higher suitablity of Power law model in explaining the temperature dependence behaviour of honey. Thereafter the calculated co-efficients and corelated coefficients values (R^2) of Arrhenius model for all samples were ≥ 0.96, while Newtonian model presented the lowest R^2 values.

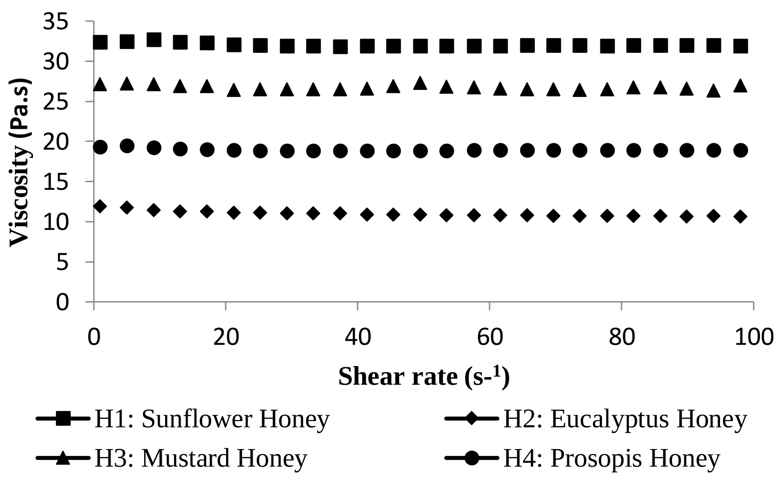

Figure 2. Viscosity-shear rate curve of different honeys at 10 °C.

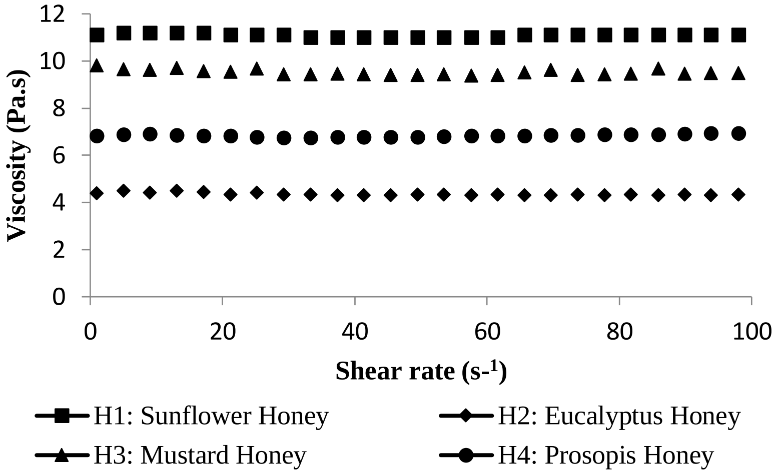

Figure 3. Viscosity-shear rate curve of different honeys at 20 °C.

Table 3. Parameters calculated with Arrhenius, Power law & Newtonian-I models, for four single flora honey samples at different temperatures.

Models	Parameter	Systems			
		H_1	**H_2**	**H_3**	**H_4**
Arrhenius	η_0 (Pa·s)	2.02	0.9	1.62	1.27
	E_a (KJ/mol)	229.92	207.64	241.12	224.55
	R^2	0.97	0.96	0.98	0.97
	RMSE	2.88	1.12	2.44	1.78
	X^2	2.95	1.09	2.52	1.86
Power law	***k*** **(mPa·s)**	1829.72	419.12	1567.64	977.81
	n	-1.75	-1.58	-1.75	-1.71
	R^2	0.99	0.99	0.99	0.99
	RMSE	1.58	0.44	0.97	0.7
	X^2	1.04	0.37	0.94	0.64
Newtonian-I	**a**	-0.97	-0.32	-0.83	-0.57
	R^2	0.58	0.63	0.59	0.59
	RMSE	8.32	2.62	7.12	4.84
	X^2	115.4	11.5	84.41	39.01

The desired constants of Power law (equation 6) i.e. proportionality constant (κ) and power (*m*) ranged from 419.12 mPa s (H_2) to 1829.72 mPa s (H_1) and -1.58 (H_2) to -1.75 (H_1) respectively as shown in Table 3. Our values of κ and *m* are lower than the values obtained by Mehryar *et al.* (2013) who studied the physicochemical and rheological properties in six honey samples from Iran. The difference in the values may be because of compositional variation of honey due to botanical and other environmental factors.

After considering lower values of root mean square error (RMSE) and chi-square (X^2) among all three models for rheological analysis the results suggests that Power law model fits best the temperature dependence of honey viscosity of all the four uniflora honey samples from Haryana & Punjab regions of India.

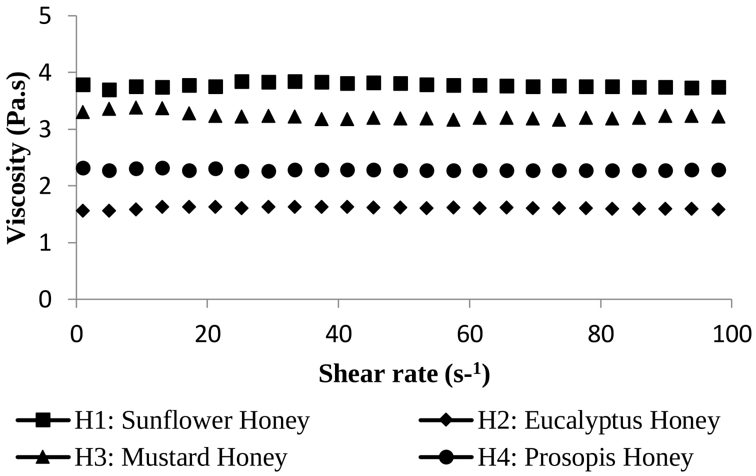

Figure 4. Viscosity-shear rate curve of different honeys at 30 °C.

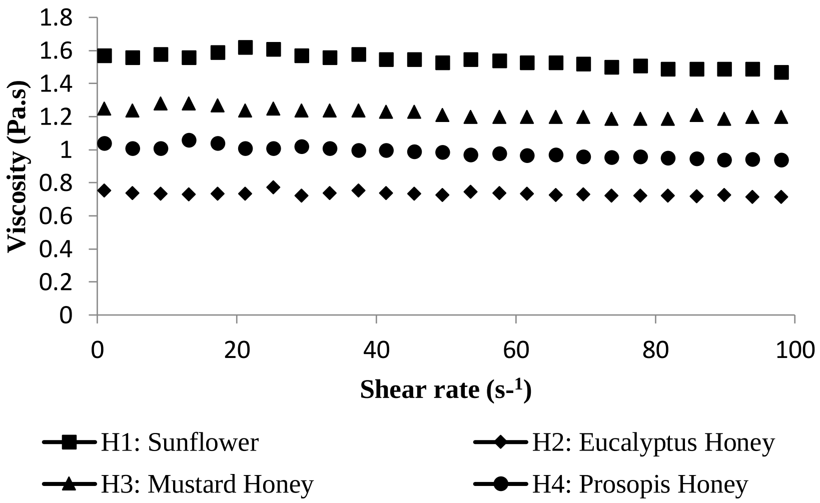

Figure 5. Viscosity-shear rate curve of different honeys at 40 °C.

Conclusions

Physicochemical analysis of all four single flora honey samples showed great variations in composition because of difference in botanical environment and agro climatic diversity. All samples in this study exhibited Newtonian flow behavior index when studied under the temperature range of 10-40 °C and manifested temperature viscosity dependence. Above 40 °C the changes in viscosity was not significant. Power law equation was found most suitable for predicting the temperature dependence behavior of honey as compare to Arrhenius and Newtonian models. Study shows that rheology and colorimetric parameters may be effectively used to differentiate honey from different floral sources, so encouraging its use should help the honey producers and processors economically.

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