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Comparative nutritional evaluation of different varieties of unifloral honeys

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Abstract

Honey positions among one of the most nutritive and beneficial natural viscous food material, having diverse nutritional profile including total phenolic contents, total flavonoid contents, antioxidant potential, water-soluble vitamins, sugars as well as macro and micro minerals, which play a crucial role in providing numerous health benefits. For this reason, nutritional evaluation of different unifloral honeys (*Acacia nilotica* and *Citrus limetta*) was conducted by variant analytical techniques (RP-HPLC & Spectrophotometer) to find its beneficial contents quantitatively. Results revealed that in *Acacia* honey, both DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assay presented higher values i.e. 81.4±0.19% and 593.25±0.04µmol/100g respectively, as compared with Citrus. From the major sugar contents of honey, composition of fructose sugar was also observed greater (42.03±0.31g/100g) in *Acacia* honey. With reference to water-soluble vitamins; pantothenic acid, riboflavin and niacin, all were observed significantly ($p<0.05$) higher in *Acacia* honey. As far as the mineral composition of both the varieties of honey is concerned, results also showed the significant ($p<0.05$) difference between them. Among the mineral profile of honey varieties, Na, Ca, K and Cd were higher in *Acacia* honey i.e. 178.16±3.25, 85.26±2.25, 411.20±5.75 and 0.79±0.05ppm respectively, as compared to Citrus honey. The results of present study conclude that owing to the presence of strong water-soluble vitamins, antioxidant potential and mineral contents, *Acacia* honey has proved more effective against metabolic disorders.

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Introduction

Honey is a sweet, viscous and nutritionally diverse natural product comprising of carbohydrates (sugars), amino acids, phenolic and flavonoid compounds, organic acids, vitamins, aromatic substances, minerals and enzymes. It is being prepared by honey bees, by sucking the sugar rich liquid (nectar) from various flowering plants, using their tube like tongue and convert it into honey, which can then be stored in the cells or "honey comb" of bee hives for further ripening (Ahmad *et al.,* 2017). Owing to its diverse nutritional composition, it helps to perform several clinical and biologically significant roles for the health of human beings including wound healing, antioxidant, anti-cancer, anti-inflammatory, anti-microbial, anti-hyperlipidemic and cardio-protective agent, fertility complications and also for the treatment of eye diseases as well as neurological issues (Rao *et al.,* 2016).

Sugars are the most significant component of honey, which are responsible for its energy value, hygroscopicity and mouth feel. Around 70% of the composition of honey belongs to sugars including glucose, fructose while sucrose, maltose, iso-maltose, trehalose, raffinose and others present in minor quantities (Da Silva *et al.,* 2016). The wide range of antioxidant compounds in honey, aid it to defuse the effect of free fatty acids in the human body, which protect the consumers from various maladies. Nutritive profile of honey gets strengthened by the presence of water soluble vitamins including vitamin C, B-complex vitamins i.e. B_1 (thiamine), B_2 (riboflavin), B_3 (nicotinic acid), B_5 (pantothenic acid), B_6 (pyridoxine), B_8 (biotin) and B_9 (folic acid) (Almasaudi *et al.*, 2017). In addition, there are numerous micro and macro-mineral contents in honey including; potassium, calcium, phosphorus, manganese, magnesium, iodine, zinc as well as iron, which make it more beneficial against the onset of mineral deficiency diseases (Kadri *et al.,* 2017).

Several varieties of honey are available based on its plant source, including basswood honey, sunflower, buckwheat honey, honey from Ziziphus (Beri) plant but the most popular ones are the honey procured from the plants of *Acacia nilotica* (Desi Kikar) and *Citrus limetta* (Delgado *et al.,* 2012). Owing to the strong nutritional profile, both the plants have secured medicinal properties to struggle against number of illnesses, which can further be transferred to their honeys as well (Meo *et al.,* 2017). Keeping in view the therapeutic potential and strong nutritional content of honey, the current project was designed for the comparative nutritional evaluation of unifloral honeys including *Acacia* and Citrus through advanced analytical techniques.

Materials and methods

Procurement of raw material

The honey samples of *Acacia nilotica* and *Citrus limetta* commonly known as "Desi Kikar" and "Mosambi" respectively were procured in their relevant seasons, from the local farms, which were located at District Sargodha, in the Punjab province, Pakistan. These were transported to the Institute of Home and Food Sciences, Government College University, Faisalabad in proper air-tight jars at ambient conditions. Each sample was of 1000 grams, packed in glass bottle, and none of samples exceeded the storage period of three months. All the samples were stored at 4°C and before analysis kept overnight at 25 ± 2°C.

Preparation of honey extract

Methanolic extract of honey was prepared by adding 25mL of methanol (99%) and 7.5g of honey in 50mL falcon tube. This mixture was uninterruptedly stirred at room temperature; with a shaker for the period of 24h. In this method, filter paper was used to remove the particles. The final volume of the solution was adjusted by the addition of 25mL of methanol. This methanolic extract was then used in the upcoming trials (Ruiz-Ruiz *et al.,* 2017).

Physicochemical composition

In both of the honey varieties, nine parameters were determined for physicochemical studies. Water mass fraction (moisture) was measured by refractometer using AOAC Official Methods, (AOAC Int, 2006), as well as the sucrose, total reducing sugar, ash and free acidity were also measured by following the same methods.

Electrical conductivity was estimated by Mettler conductivity meter according to the methods described in the International Honey Commission (Stefan Bogdanov *et al.,* 2002). Invertase, diastase and proline mass were also determined according to the methods proposed by the International Honey Commission (Stefan Bogdanov *et al.*, 2002).

Antioxidant potential

Total phenolic contents

Total phenolic contents of honey samples were analyzed by Folin-Ciocalteu reagent, using the protocol of Singleton *et al.* (1999) with few modifications. The absorbance of the honey solution was measured at 765nm by UV-Visible spectrophotometer (Perkin-Elmer Lambda 25, Waltham, MA, USA).

Total flavonoid contents

Total flavonoid contents of honey were analyzed by the method of Isla *et al.* (2011). The absorbance of sample was measured at 415nm with the help of an UV-Visible spectrophotometer.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power of extracts was estimated according to the protocol of Yuan (2003). The absorbance was measured at 700nm. During the analysis, an increase in the absorbance (A) of the reaction mixture indicated the reducing power.

Free radical scavenging activity (DPPH)

Free radical scavenging activity was determined by 1,1-Diphenyl-2-Picryl hydrazyl (DPPH) assay as reported by the method of Isla *et al.* (2011). The absorbance was measured at 520nm using UV/Visible Spectrophotometer.

Sugar content determination

Different sugars i.e. glucose, fructose, sucrose, maltose and isomaltose were determined through HPLC by following the method given in IHC (International Honey Commission) report of Bogdanov & Baumann (1988).

Estimation of water soluble vitamins

Water soluble vitamins were determined from both the honeys through RP-HPLC by following the protocol of (Ciulu *et al.*, 2011), in which separation was performed on an Alltima C18 column having 250mm×4.6mm and 5µm particle size (Alltech, Sedriano, Italy) fixed with a guard cartridge and packed with the same stationary phase.

Mineral analysis

Mineral contents of honey samples were determined by following the method of Qadir *et al.* (2015) through atomic absorption spectrophotometer (PG-990).

Results and discussion

Physicochemical composition

Means for moisture content (table 1) showed higher value in citrus honey (18.39%) followed by the *acacia* honey (16.58%) while the mean value of electrical conductivity was ranged from 0.27 to 0.15mS/cm. *Citrus* honey showed highest value for free acidity concentration i.e. 29.32mEq/kg, followed by 27.79mEq/kg as it is observed in *Acacia*. Likewise, ash content ranged from 0.11 to 0.17g/100g in Citrus and *Acacia* honey, respectively. Diastase and invertase activities were higher in *Acacia* i.e. 12.08±1.74 DN and 13.45±0.28 IN, respectively, as compared to Citrus honey. Reducing sugar concentration was reported 76.43% in Citrus as compared to *Acacia* honey i.e. 73.87%. Likewise, sucrose content was also observed with more concentration in *Citrus* honey (6.51g/100g) whereas, *Acacia* honey (6.46g/100g) showed lower value in this regard. Gulfraz *et al.* (2010) also studied the physicochemical properties of *Acacia* and *Citrus* varieties and their outcomes are in accordance with the current research.

Antioxidant potential

Antioxidant parameters including total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assay were analyzed in honey varieties as depicted in Table 2. Both the varieties of honey showed significant effect regarding antioxidant potential. The TPC ranged from 11.21±0.01 to 56.31±0.12mg GAE/g with maximum value observed in *Acacia* and minimum in *Citrus* honey. Likewise, DPPH radical scavenging assay and ferric reducing antioxidant power assay were also higher

(81.4±0.19% and 593.25±0.04µmol/100g) in *Acacia* as compared to that of *Citrus* honey. On the other hand, in term of total flavonoid content, citrus honey exhibited higher value 5.05±0.07mg RE/g as compared to that of *Acacia* honey 4.63±0.01mg RE/g.

Table 1. Physicochemical composition of different honey varieties.

Honey variety	*Acacia*	*Citrus*
Moisture (%)	16.58±0.41^{b}	18.39±0.46^{a}
Electrical conductivity (mS/cm)	0.15±0.04^{a}	0.27±0.03^{a}
Reducing sugar (%)	73.87±2.32^{b}	76.43±1.54^{a}
Free acidity (mEq/kg)	27.79±2.67^{b}	29.32±2.32^{a}
Diastase activity (DN)	12.08±1.74^{a}	9.56±0.33^{b}
Invertase activity (IN)	13.45±0.28^{a}	10.56±1.11^{b}
Sucrose (g/100g)	6.46±0.03^{a}	6.51±0.03^{a}
Proline content (mg/kg)	293.22±2.67^{a}	357.53±2.5^{b}
Ash (g/100g)	0.17±0.05^{a}	0.11±0.02^{a}

Means carrying same letters do not differ significantly (p>0.05)

These results are parallel with the findings of Ahmed *et al.* (2016) who revealed that different honey varieties showed significant effect regarding total flavonoids, phenols, DPPH and FRAP assay. By keeping in view all the parameters, *Acacia* honey is considered as the best variety regarding its overall antioxidant potential than Citrus honey.

Table 2. Antioxidant potential of honey varieties.

Varieties	TPC (mg GAE/g)	TFC (mg RE/g)	DPPH (%)	FRAP (µmol/100g)
Acacia	56.31±0.12^{a}	4.63±0.01^{b}	81.4±0.19^{a}	593.25±0.04^{a}
Citrus	11.21±0.01^{b}	5.05±0.07^{a}	55.14±0.04^{b}	390.62±0.02^{b}

TPC= Total phenolic content; TFC= Total flavonoid content; DPPH=DPPH radical scavenging assay; FRAP=Ferric reducing antioxidant power assay.
Means carrying same letters do not differ significantly (p>0.05)

Sugar profile

In table 3, the results regarding sugar content of honey varieties indicated that the range of fructose, glucose, sucrose, maltose and isomaltose was 39.42±0.02 to 42.03±0.31g/100g, 30.42±0.07 to 31.33±0.11g/100g, 1.73±0.01 to 2.83±0.05g, 2.90±0.04 to 3.42±0.01g/100g and 0.42±0.23 to 0.44±0.03g/100g, respectively. *Acacia* honey exhibited higher fructose content (42.03±0.31g/100g) than Citrus honey. The already reported values of fructose (43.55g/100g), glucose (30.56g/100g) and sucrose (1.27g/100g) were also in-line with the current study, according to the research findings of Madas *et al.* (2014).

Table 3. Sugar profile (g/100g) of honey varieties.

Varieties	Fructose	Glucose	Sucrose	Maltose	Isomaltose
Acacia	42.03±0.31a	31.33±0.11a	1.73±0.01a	2.90±0.04a	0.42±0.23a
Citrus	39.42±0.02b	30.42±0.07a	2.83±0.05a	3.42±0.01a	0.44±0.03a

Means carrying same letters do not differ significantly (p>0.05)

Water soluble vitamins

Mean values regarding water-soluble vitamins (riboflavin, pantothenic acid and niacin) analyzed in different varieties of honey are illustrated in Table 4. The results revealed that both the varieties showed significant differences for niacin, riboflavin and pantothenic acid. *Acacia* honey contained more water-soluble vitamins than Citrus honey. Riboflavin, pantothenic acid and niacin were ranged from (2.24±0.60 to 9.73±1.20mg/kg), (7.55±1.10 to 16.33±1.15mg/kg) and (25.32±1.75 to 130.23±2.70mg/kg) in *Citrus* and *Acacia* honey. Abano and Dadzie (2014) evaluated water-soluble vitamins like thiamine, riboflavin, niacin, biotin and pantothenic acid in honey by using high performance liquid chromatography and proved that variation in *Citrus* and *Acacia* honey vitamin contents is because of the variety of flowering plant from which honey bees collect the nectar, and *Acacia* honey gave its best regarding water-soluble vitamins.

Mineral profile

Different macro and micro-minerals were assessed in *Acacia* and *Citrus* honey as depicted in Table 5 and 6, respectively. Both honey varieties showed significant difference regarding macro and micro-mineral composition except for nickel (non-significant). The results indicated that *Acacia* honey exhibited higher contents of sodium (178.16±3.25ppm), calcium (85.26±2.25ppm) and potassium (411.20±5.75ppm) as compared to Citrus honey. Whereas, Citrus honey was higher in magnesium (77.16±2.23ppm) content as compared to *Acacia* honey. The results regarding micro-mineral composition revealed that cadmium (0.79±0.05ppm) contents were higher in *Acacia* honey, whereas Citrus honey showed higher contents of iron (39.32±2.30ppm) among the micro minerals.

Table 4. Water soluble vitamins (mg/kg) of honey varieties.

Varieties	Riboflavin	Pantothenic acid	Niacin
Acacia	9.73±1.20a	16.33±1.15a	130.23±2.70a
Citrus	2.24±0.60b	7.55±1.10b	25.32±1.75b

Means carrying same letters do not differ significantly (p>0.05).

Table 5. Macro-mineral (ppm) composition of honey varieties.

Varieties	Na	Ca	K	Mg
Acacia	$178.16±3.25^a$	$85.26±2.25^a$	$411.20±5.75^a$	$46.38±1.92^b$
Citrus	$15.86±1.15^b$	$61.75±1.75^b$	$299.56±3.91^b$	$77.16±2.23^a$

Means carrying same letters do not differ significantly (p>0.05)

Table 6. Micro-mineral (ppm) composition of honey varieties.

Varieties	Fe	Mn	Co	Ni	Pb	Zn	Cd	Cr
Acacia	$19.10±1.25^b$	$0.79±0.18^b$	$0.017±0.003^b$	$0.17±0.02^a$	$0.05±0.004^b$	$1.93±0.04^b$	$0.79±0.05^a$	$0.032±0.002^b$
Citrus	$39.32±2.30^a$	$3.95±0.50^a$	$0.49±0.05^a$	$0.19±0.03^a$	$0.2±0.06^a$	$5.94±0.70^a$	$0.02±0.001^b$	$0.72±0.06^a$

Means carrying same letters do not differ significantly (p>0.05).

Similar trend, same as in current study was reported regarding mineral composition of local and imported honey samples which detected highest concentration of potassium among macro-minerals followed by magnesium, calcium, sodium and phosphorus (Alqarni *et al.,* 2014). Whilst, among micro-minerals, iron level showed highest concentration than iodine, manganese, zinc, cobalt, nickel, lead and cadmium. There was most frequent effect observed on mineral composition due to climate change, type and variety of flowering plant, type of soil, soil condition and harvesting time in addition to the genetic type.

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