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Phytochemical Analysis and Chemical Composition of Different Branded and Unbranded Honey Samples

[1]Khaliqur Rahman, [2]Arshad Hussain, [2]Shafqat Ullah and [1]Imdad Ullah Muhammad Zai

[1]Institute of Chemical Sciences, University of Peshawar, KPK, Pakistan
[2]Pakistan Council of Scientific and Industrial Research, Peshawar, KPK, Pakistan

Abstract: This study was undertaken to evaluate the nutritional significance and phytochemicals of branded and unbranded honey samples. Thirteen samples were collected or purchased from different area of Khyber Pakhtun Khawa (KPK) Pakistan. The branded and unbranded honeys samples under study were Marhaba, Qarshi, Versatile, Al-hayat, Young's, Pak-salman, Langness, Big bees honey, Small bees honey, Beera, Palosa, Sperkay, Bekerr and Granda. The chosen parameter included such as; total ash, pH, moisture, total acidity, electrical conductivity and total sugars were analyzed by standard methods of AOAC. The phytochemical such as tannins, phlobatanin, flavonoides, terpenoids, glycosides, saponins, alkaloids and fluorides of branded and unbranded honey samples were carried out by UV-Spectrophotometer. The Hydroxy methyl furfural (HMF) content was determined by Winkler's method. The result showed that unbranded honey samples are good source of nutrients and valuable phytochemical as compared to branded samples. Due to lack of information available on chemical composition and phytochemical in these honeys and their role in diet, the assessment was carried out on the basis of nutritional quality. So these available honeys can be utilized in various food products as well as in herbal formulations

Key words: Phytochemicals · Chemical composition · Evaluation · Honey

INTRODUCTION

Honey was defined as "the sweets substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transforms and store in honey combs". Honey bee produces dense and stable energy food called as nectar which ripened into honey [1]. Traditionally honey is good source of energy produced by *Apis mellifera* (honeybees). it also has antioxidant and antimicrobial properties. It is a complex mixture of invert sugar concentrated solution that also contains some other carbohydrates, aromatic substances, waxes, minerals, pollen grains, pigments, organic and amino acids [2].

Honey can be characterized according to its geographical origin. It has been reported that honey samples showed regional variation in the physicochemical parameters such as pH, enzymes activities, ash contents, electrical conductivity and hydroxyl methyl furfural [3]. The variations in honey colors are entirely due to different plant sources and form a continuous range from pale yellow through amber to a darkish red to black. The darkening of honey may be due to change in heat or temperature [4]. Honey has the tendency to form granules, due to which make it different from other sweeteners. In its compositions, sugars are the main components of its dry matter. The physical properties of honeys such as high density, high viscosity and moisture absorbance from air and immunity from spoilage are due to concentrated solution of sugars [5].

More than 22 sugars have been found in honey but dextrose and laevulose are the major sugars. Most of these sugars are more complex than laevulose, dextrose and monosaccharides. Ten disaccharides have been identified includes maltose, sucrose, maltulose, turanose, isomaltose, laminaribiose, nigerose, kojibiose, gentiobiose and *B*-trehalose. The trisaccharides are also found consist of maltotriose, erlose, melezitose, centose 3-*a*5 isomaltosylglucose,l-kestose, isomaltotriose, panose, isopanose and theanderose. All these sugars are present in very small quantities [6].

Corresponding Author: Arshad Hussain, Pakistan Council of Scientific and Industrial Research, Peshawar, KPK, Pakistan.

Many organic acids are also present in honey. These are lactic, formic, butyric, tartaric, pyruvic, acetic, citric, oxalic, succinic, malic, maleic, *a*-ketoglutaric G- 6-phosphate, pyroglutamic and glycolic acid etc. Among these the major one is gluconic acid. It produces from the action of an enzyme glucose oxidase on the dextrose [7]. The presence of enzymes in honey is a unique characteristic that present it different from all other sweetening agents. These enzymes originate from the yeasts, pollen, bee, nectar and micro-organisms present in the honey. Enzymes are complex protein materials that under mild conditions bring about chemical changes. Some of the most important honey enzymes are acid phosphatase, diastase, glucose oxidase, catalase and invertase. Heating of honey can be weakened or destroyed all these enzymes [8].

The mineral content or ash in floral honey varies from 0.02 to 01%. Due to its richer mineral content is said to be less suitable for storage in the winter [9]. The physicochemical parameters have great importance to the honey for industry. These constituents such as minerals, moisture content, reducing sugars, electrical conductivity, free acidity, sucrose content and HMF have influence on nutritional quality, granulation, the storage quality, flavor and texture of the honey. The medicinal value of honeys is also due to these constituents. Therefore, the International Honey Commission (IHC) has proposed certain constituents as quality criteria for honey [10]. Honey has a long history of use as an effective medicine since ancient civilization for a wide range of disease conditions [11]. Keeping in view the importance of honey the present study was therefore aimed to evaluate chemical composition and phytochemicals for different branded and unbranded honey samples collected from different areas of Peshawar, KPK, Pakistan.

MATERIALS AND METHODS

Collection of Samples: A total of 13 samples were collected from market including six samples were branded while other seven samples unbranded and bring to the PCSIR Labs Complex Peshawar for chemical composition and phytochemical analysis.

Phytochemical Analysis: The qualitative and quantitative screening test for phytochemicals such as tannins, phlobatanins, flavonoids, terpenoids, glycosie, saponins, alkaloids, flourides of branded and unbranded honey samples were carried out by Official Methods of Analysis [12].

Chemical Composition: The chemical composition such as ash, pH, moisture, total acidity, electrical conductivity and total sugars were analyzed by standard methods of AOAC [13].

Hydroxy Methyl Furfural (HMF) Content: HMF was analyzed following Winkler's method. Five grams of every sample were treated with a clarifying agent (Carrez solution), transferred to 50 mL volumetric flasks and made up to volume with deionized water. The absorbance of the filtered solution was measured at 284 and 336 nm using a blank produced with an aliquot of the solution treated with $NaHSO_3$ 0.1 % [14].

RESULTS AND DISCUSSION

The study was under taken to evaluate the phytochemical analysis and chemical composition of branded and unbranded honey samples. The qualitative study of phytochemicals showed that the branded and unbranded honey samples were composed of tannins, phlobatanins, flavonoids, terpenoids, glycosides, saponins, alkaloids and flourides (Table 1 and 2). The concentration of saponin, flavonoids and tannins confirms the astringent property of honey. This compound can also be effective in protecting the kidneys [15].

The quantitative analysis of phytochemicals is presented in Table 3. It showed that in branded honey samples the maximum concentration of tannin (0.35±0.07) was found in versatile sample, while minimum concentration (0.25±0.06) in Young's honey. Tannins have also shown potential antibacterial and antiviral effects [16]. The maximum concentration of phlobatanin (0.61±0.05) was found in Qarshi, while minimum concentration (0.39±0.08) in Young's honey. The maximum concentration of flavonoids (0.33±0.01) was found in Pak-salman, while minimum concentration (0.18±0.04) in Qarshi. Earlier reported that flavonoids have antibacterial, anti-inflammatory, ant allergic, antimultagenic, antiviral, antineoplatic, anti-thrombotic and vasodilatory activities. However, the low amount of alkaloid present is also indicative of its harmless effect based on its content [17].

The maximum concentration of terpenoids (0.41±0.06) was found in Langness, while minimum concentration (0.24±0.03) in Marhaba. The maximum concentration of glycoside (0.32±0.06) was found in Langness, while minimum concentration (0.14±0.04) in Al-hayat.

Table 1: Qualitative test for Phytochemicals in branded honey samples

Parameter	Marhaba	Qarshi	Versatile	Al-hayat	Young's	Pak-salman	Langness
Tannins	–	–	+	–	+	–	–
Phlobatanins	–	+	–	–	+	–	+
Flavonoids	–	+	+	–	–	+	–
Terpenoids	+	–	–	–	–	+	+
Glycosides	+	–	–	+	+	–	+
Saponins	+	–	+	–	+	+	–
Alkaloids	–	+	–	–	+	–	+
Flourides	+	–	+	+	–	+	–

+ = Present - = absent

Table 2: Qualitative test for Phytochemicals in unbranded honey samples

Parameter	Big bees honey	Small bees honey	Beera	Palosa	Sperkay	Bekerr	Granda
Tannins	+	+	+	+	+	+	+
Phlobatanins	–	+	+	–	+	–	+
Flavonoids	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	–	+	+
Glycosides	+	+	+	+	+	+	–
Saponins	+	+	+	+	–	–	+
Alkaloids	+	–	–	–	+	+	+
Flourides	+	+	+	+	–	–	+

+ = Present - = absent

Table 3: Quantitative analysis for Phytochemicals in branded honey samples

Parameter (mg/g)	Marhaba	Qarshi	Versatile	Al-hayat	Young's	Paksalman	Langness
Tannins	-----	-----	0.35±0.07	-----	0.25±0.06	-----	-----
Phlobatanins	-----	0.61±0.05	-----	0.59±0.07	0.39±0.08	-----	0.61±0.01
Flavonoids	-----	0.18±0.04	0.21±0.06	-----	-----	0.33±0.01	-----
Terpenoids	-----	-----	-----	-----	-----	0.39±0.08	0.41±0.06
Glycosides	0.21±0.05	-----	-----	0.14±0.04	0.29±0.02		0.32±0.06
Saponins	2.13±0.02	-----	1.43±0.01	-----	2.35±0.09	2.44±0.03	-----
Alkaloids	-----	0.25±0.03	-----	0.27±0.01	0.46±0.06	-----	0.27±0.02
Flourides	0.14±0.09	-----	0.23±0.07	0.34±0.04	-----	0.10±0.02	-----

* Mean ± S.D

Table 4: Quantitative test for Phytochemicals in unbranded honey samples

Parameters (mg/g)	Big bees honey	Small bees honey	Beera	Palosa	Sperkay	Bekerr	Granda
Tannins	0.43±0.03	0.51±0.05	0.54±0.04	0.41±0.06	0.49±0.07	0.34±0.03	0.39±0.09
Phlobatanins	--------	0.72±0.06	0.65±0.09	--------	0.76±0.05	--------	0.66±0.06
Flavonoids	0.28±0.09	0.30±0.06	0.27±0.05	0.36±0.08	0.29±0.04	0.33±0.07	0.37±0.03
Terpenoids	0.33±0.05	0.38±0.04	0.41±0.07	0.39±0.09	--------	0.42±0.08	0.45±0.06
Glycosides	0.31±0.08	0.33±0.08	0.44±0.09	0.39±0.05	0.47±0.06	0.37±0.07	--------
Saponins	3.24±0.06	3.22±0.07	2.72±0.03	3.49±0.07	--------	--------	2.11±0.04
Alkaloids	0.14±0.02	--------	--------	--------	0.18±0.07	0.12±0.06	0.17±0.08
Flourides	0.11±0.03	0.23±0.06	0.25±0.04	0.21±0.03	--------	--------	0.14±0.06

* Mean ± S.D.

Table 5: Chemical composition of branded honey samples

Parameters	Marhaba	Qarshi	Versatile	Al-hayat	Young's honey	Paksalman
Crude fiber (%)	0.3±0.03*	0.5±0.01	0.8±0.02	0.7±0.09	0.9±0.05	0.7±0.06
Vitamin C (%)	0.17±0.03	0.32±0.08	0.56±0.04	1.77±0.07	0.10±0.01	0.44±0.03
Moisture (%)	26.0±2.0	21.4±4.0	27.6±3.0	24.5±2.0	29.1±2.0	24.6±3.0
Fats (%)	0.7±0.03	0.4±0.04	0.9±0.06	0.3±0.01	0.6±0.07	0.1±0.02
Nitrogen (%)	51.7±2.0	55.4±6.0	54.6±3.0	42.9±3.0	57.4±1.0	50.3±2.0
pH	4.1±0.2	3.9±0.1	3.8±0.3	4.0±0.1	3.1±0.5	3.7±0.4
Acidity (meq/kg)	42.2±2.0	36.5±4.0	41.3±3.0	40.1±6.0	38.4±1.0	43.7±2.0
Ash (%)	0.23±0.01	0.35±0.08	0.61±0.07	0.16±0.04	0.36±0.09	0.14±0.03
Electrical conductivity (mS/cm)	3.43±0.04	3.26±0.03	2.66±0.01	4.36±0.06	6.33±0.07	1.24±0.02
HMF (mg/kg)	13.3±0.2	13.0±0.5	10.8±0.7	3.4±0.1	5.2±0.6	8.1±0.4
Reducing sugar (%)	55.8±2.4	39.2±3.7	60.6±1.8	57.9±3.9	44.5±2.7	56.4±4.1
Sucrose (%)	3.2±0.5	3.1±0.1	1.6±0.8	3.7±0.3	2.6±0.4	3.9±0.6

* Mean ± S.D.

Table 6: Chemical composition of unbranded honey samples

Honey samples	Big bees honey	Small bees honey	Beera	Palosa	Sperkay	Bekerr
Crude fiber (%)	0.1±0.06	0.9±0.03	0.2±0.08	0.5±0.01	0.3±0.05	0.2±0.04
Vitamin C (%)	0.14±0.02	1.28±0.07	0.16±0.01	0.41±0.03	1.36±0.04	0.29±0.08
Moisture (%)	24.1±3.0	26.4±3.0	29.3±2.0	22.2±2.0	23.9±1.0	27.3±3.0
Fats (%)	0.6±0.09	0.2±0.01	0.4±0.06	0.8±0.04	0.6±0.07	0.3±0.05
Nitrogen (%)	55.1±4.0	51.4±3.0	47.2±2.0	44.6±2.0	56.7±3.0	43.1±1.0
pH	3.2±0.4	2.9±0.8	4.7±0.1	3.5±0.9	2.4±0.2	2.9±0.3
Acidity (meq/kg)	41.4±3.0	32.2±4.0	39.1±2.0	36.2±3.0	37.6±1.0	31.4±2.0
Ash (%)	0.12±0.05	0.25±0.01	0.53±0.07	0.22±0.02	0.17±0.09	0.32±0.03
Electrical conductivity (mS/cm)	2.33±0.01	2.71±0.03	3.11±0.07	3.53±0.02	2.56±0.06	3.28±0.09
HMF (mg/kg)	14.5±0.4	12.2±0.7	16.4±0.1	15.8±0.6	11.6±0.5	13.1±0.2
Reducing sugar (%)	60.1±3.0	42.5±4.0	76.2±6.0	81.7±9.0	62.3±3.0	59.2±4.0
Sucrose (%)	1.4±0.7	2.5±0.1	3.6±0.4	1.8±0.8	2.0±0.7	1.7±0.1

* Mean ± S.D.

The saponin contents is important source of detergents, surface active agents used in industrial applications and also possesses beneficial health effects [18]. The maximum concentration of saponine (2.44±0.03) was found in Pak-salman, while minimum concentration (1.43±0.01) in Versatile. The maximum concentration of Alkaloid (0.46±0.06) was found in Young's honey, while minimum concentration (0.25±0.03) in Qarshi. It has been reported that honey containing alkaloids do not feature strongly in herbal medicine because they are extremely toxic, so low as to be harmless [17]. The maximum concentration of flouride (0.34±0.04) was found in Al-hayat, while minimum concentration (0.10±0.02) in Pak-Salman.

In unbranded honey samples (Table 4) showed that the maximum concentration of tannin (0.54±0.04) was found in Beera, while minimum concentration (0.34±0.03) in Bekerr honey. The maximum concentration of phlobatanin (0.76±0.05) was found in Sperkay while minimum concentration (0.65±0.09) in Beera. The maximum concentration of flavonoids (0.36±0.08) was found in Palosa, while minimum concentration (0.27±0.05) in Beera. The maximum concentration of terpenoids (0.45±0.06) was found in Granda, while minimum concentration (0.33±0.05) in big bees honey. The maximum concentration of glycoside (0.47±0.06) was found in Sperkay while minimum concentration (0.31±0.08) in Big bees honey. The maximum concentration of saponin (3.49±0.07) was found in Palosa, while minimum concentration (2.11±0.04) in Granda. The maximum concentration of Alkaloid (0.34±0.02) was found in Big bees honey, while minimum concentration (0.22±0.06) in Bekerr. The maximum concentration of flourides (0.25±0.04) was found in Beera, while minimum concentration (0.11±0.03) in big bees honey.

The chemical composition such as moisture, ash, acidity, fiber, fats, sugar, nitrogen, pH, electrical conductivity, HMF, sucrose content and vitamin C for branded honey samples (Table 5) and for unbranded honey samples (Table 6) were reported. The result showed slight variation among the concentration of different parameters. On the basis of overall chemical composition in branded honey samples the maximum concentration of crude fiber (0.9±0.05) was found in Young's honey while minimum (0.3±0.03) in Marhaba. The maximum concentration of vitamin C (1.77±0.07) was found in Al-hayat, while minimum (0.10±0.01) in Young's honey. The maximum concentration of moisture (29.1±2.0) was found in Young's honey, while minimum (21.4±4.0) in Qarshi. The maximum concentration of fats (0.9±0.06) was found in Versatile, while minimum (0.1±0.02) in Paksalman. The maximum concentration of nitrogen (57.4±1.0) was found in Young's honey, while minimum (42.9±3.0) in Al-hayat. The maximum concentration of pH (4.1±0.2) was found in Marhaba, while minimum (3.1±0.5) in Young's honey. It has reported that pH values in the range of 3.34 to 4.70, which are normally accepted [19]. The maximum concentration of acidity (43.7±2.0) was found in Paksalman while minimum concentration (36.5±4.0) in Qarshi. Free acidity shows a mean value of (27.2 meq kg^{-1} one reported in Spain [20].

The maximum concentration of ash (0.61±0.07) was found in Paksalman while minimum concentration (0.36±4.0) in Qarshi. Ashes mean concentration reported in samples from Turkey is (0.25 to 0.45%) [19]. The maximum concentration of electrical conductivity (6.33±0.07) was found in Young's honey while minimum (1.24±0.02) in Paksalman. The maximum concentration of HMF (13.3±0.2) was found in Marhaba while minimum (3.4±0.1) in Al-hayat. The maximum concentration of reducing sugar (60.6±1.8) was found in Versatile while minimum (39.2±3.7) in Qarshi. The maximum concentration of sucrose (3.9±0.6) was found in Paksalman while minimum (1.6±0.8) in Versatile.

In case of unbranded samples, the maximum concentration of crude fiber (0.9±0.03) was found in Small bees honey while minimum (0.1±0.06) in big bees honey. The maximum concentration of vitamin C (1.36±0.04) was found in Sperkay while minimum (0.14±0.02) in big bees honey. The maximum concentration of moisture (29.3±2.0) was found in Beera, while minimum (22.2±2.0) in Palosa. The maximum concentration of fats (0.8±0.04) was found in Palosa, while minimum (0.2±0.01) in Small bees honey. The maximum concentration of nitrogen (56.7±3.0) was found in Sperkay while minimum (43.1±1.0) in Bekerr. The maximum value of pH (4.7±0.1) was found in Beera, while minimum (2.4±0.2) in Sperkay. The maximum concentration of acidity (41.4±3.0) was found in Big bees honey while minimum (31.4±2.0) in Bekerr.

The maximum concentration of ash (0.53±0.07) was found in Beera, while minimum (0.12±0.05) in big bees honey. The maximum concentration of electrical conductivity (3.53±0.02) was found in Palosa, while minimum (2.33±0.01) in Big bees honey. HMF represents the freshness of honey and depends on adequate beehives and harvest practice. The value was reported in Turkey (4.52+40 mg kg^{-1} [20]. In our samples, the maximum concentration of HMF (16.4±0.1) was found in Beera, while minimum (11.6±0.5) in Sperkay. The maximum concentration of reducing sugar (81.7±9.0) was found in Palosa, while minimum (42.5±4.0) in Small bees honey. Reducing sugar has reported in samples from Turkey (71.32 %) [19]. The maximum concentration of sucrose (3.6±0.4) was found in Beera, while minimum (1.4±0.7) in big bees honey.

CONCLUSIONS

All these verities of honey are easily available and contains nutrients especially as energy provider sugar, vitamin C and phenolic compounds which having medicinal importance. In branded honey the concentration and quantity of ash, pH, moisture, total acidity, electrical conductivity and total sugars contents are more as compare to unbranded honey. The phenolic and antioxidant compound concentration in branded honey is also more than unbranded honey. but as a whole these available honey can be utilized in various food products and herbal formulations

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