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Determination of Phenolic Acids in Natural and Farms Honey of Khyber Pakhtunkhwa, Pakistan

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This study was focused to evaluate the phenolic acids in natural combs honey, branded and unbranded honey of Khyber Pakhtunkhwa, Pakistan by HPLC technique, using UV-visible detector. Five phenolic acids (chloroganic acid, gallic acid, vanallic acid, benzoic acid and syringic acid) were identified and quantified in 18 honey samples. The phenolic acids contents were found higher in all natural honey's samples as compared to branded and unbranded honey. Among the natural honey's sample, the maximum concentration (4.26 mg/100 g) of phenolic acids was found in palosa (Acacia) honey while minimum 1.93 mg/100 g) in bekerr (Justicia) honey sample. Similarly the maximum concentration (2.78 mg/100 g) was found in langanese honey, while minimum (0.71 mg/100 g) in varsatile honey sample. In unbranded honey maximum concentration (2.46 mg/100 g) was found in Beera (Ziziphus) honey, while minimum (0.62 mg/100 g) in Palosa (Acacia) honey sample. It is evident from this study that, processing of honey may effects the phenolic acid contents of honey.

Keywords: Honey, Evaluation, Phenolic acids, HPLC.

INTRODUCTION

Honey is a natural fluid produced by (*Apis mellifera)* honeybees from nectar of flowers and exudates of plants. It is a supersaturated solution of sugars mostly composed of glucose (31 %) and fructose (38 %) and some other ingredients such as proteins, minerals enzymes, vitamins and free amino acids in traces[1,2]. It has been used as a food since the earliest times[3,4]. It is part of conventional medicine for treating certain infections and disease as well promoting overall health because of more than 181 substances[5]. It has been reported to be effective in cough, healing of wounds, burns and gastrointestinal disorders[6]. Due to preventing deteriorative oxidation reaction in foods like lipids oxidation in meats and enzymatic browning of vegetables and fruits so also used as food stabilizer[7]. Besides this honey also have biological activities like antifungal, antibacterial, antioxidants which help in gastric protection against chronic and acute lesions[8].

Honey has improved blood sugar level and controlled insulin sensitivity as compared to other sweeteners. During sleep and exercise, honey providing proper fuelling to the liver is central to optimal glucose metabolism[9].

The antioxidant property of honey is due to the presence of vitamins, glycosides, phenolic acids, flavonoids and their derivatives[10]. The phenolic acids are divided in two subclasses: the substituted benzoic acids and cinnamic acids[11]. These are important due to their contribution to honey colour, taste and flavour and also due to their beneficial effects on health. Moreover, honey phenolic compounds composition and consequently antioxidant capacity depends on their floral sources which predominance are dependent of seasonal and environmental factors[12].

The phenolic acids and flavonoids in honey are actually the secondary metabolite of plants which diverse to honey by bees[13]. They are divided into several subclasses, they include the anthocyanidins, catechins, flavanone, glycosides, flavons, flavonols and isoflavon, whereas, phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic acids and hydroxycinnamic acids[14]. It was reported by that the composition and antioxidant activity of honey affect due to processing technique, storage, climatic condition and environmental stress factor such as temperature, humidity and soil composition[15]. Honeys have a rich phenolic profile consisting of benzoic acids and there esters, cinnamic acids and their esters and flavonoids aglycones[16]. Keeping in view the importance of honey the present study was aimed to determine different types of phenolic acids in natural honey and farms honey samples which collected from honeycombs and local market of Khyber Pakhtunkhwa, Pakistan.

EXPERIMENTAL

Eighteen samples of honey were collected from different farms natural, branded and unbranded from local market of

Khyber Pakhtunkhwa and bring to Pakistan Council of Scientific and Industrial Research for analysis.

Sample extraction: Determination of phenolic acids were carried out by Khan *et al.*[17] method. Each sample was mixed with 100 mL of acidic solution pH 2 with hydrochloric acid and stirred using magnetic stirrer for 15 min. The resultant solutions were extracted with ethyl acetate. The final solution were then dried under nitrogen steam at 40 °C and stored for further analysis.

The phenolic acid standards (chloroganic acid, gallic acid, vanallic acid, benzoic acid and syringic acids) were obtained from (Sigma Aldrich Germany), HPLC grade solvents (ethyl acetate, acetic acid, acetonitrile, methanol, hydrochloric acid) from (Scharlau Spain). All the solvents were filtered through 0.45 µm filter membrane and degassed using ultrasonic cleaner prior to HPLC injection.

The presence of phenolic acids in honey samples were carried by HPLC (Hitachi D-2000 Elite system manager), equipped with dual pumps (L-2130) and auto injector/auto sampler (L-2200). The Chromatographic separation was achieved using Column oven (L-2300) and analytical column Intersil ODS-3 C18 (GL Sciences Inc. Tokyo Japan 5 µm, 250 × 4.6 mm). The present phenolic compounds were monitored and determined by using UV-visible detector (L-2420). The filtration assembly (Rocker-300 Taiwan) and ultrasonic cleaner Celia (CP-104 Italy) were used for solvents filtration and degassing.

Standard preparation: The standards solution were prepared by dissolving 0.01 g of each phenolic acid such as chloroganic acid, gallic acid, vanallic acid, benzoic acid and syringic acid in 10 mL acetonitrile (1 mg/mL).

Chromatographic conditions: The separation of selected phenolic acid were achieved by gradient elution of two mobile phases A and B. Acetonitrile HPLC grade solvent as a mobile phase A and aqueous acidic solution of acetic acid (pH 3.0) as a mobile phase B. the gradient program was set at a flow rate of 1 mL/min, 0 min 10 % A – 90 % B; 5 min 20 % A – 80 % B; 10 min 40 % A – 60 % B; 15 min 60 % A – 40 % B and 20 min 80 % A – 20 % B. The chromatograms were recorded at wavelength of 220 nm by using UV-visible detector. The identification and quantification of phenolic acids were done by external standard method (Fig. 1).

RESULTS AND DISCUSSION

Honey serves as a natural food having great potency of scavenging free radicals, which provide protection against many infectious diseases like atherosclerosis and cancer. The antioxidant activity/phenolic compounds greatly depend on floral source. These phenolic acid content determined by different techniques[18,19].

This study was focused to evaluate the concentration of phenolic acids (mg/100 g) in natural combs, branded and unbranded honey of Khyber Pakhtunkhwa. In Table-1, natural combs honey shows that the maximum concentrations (0.90 ± 0.01) of chloroganic acid were observed in Sperkay (Trachyspermum) honey. While minimum (0.57 ± 0.02) in Small bee's honey, moderate concentration (0.77 ± 0.03) in big bees' honey, Beera (Ziziphus) honey (0.63 ± 0.01) and

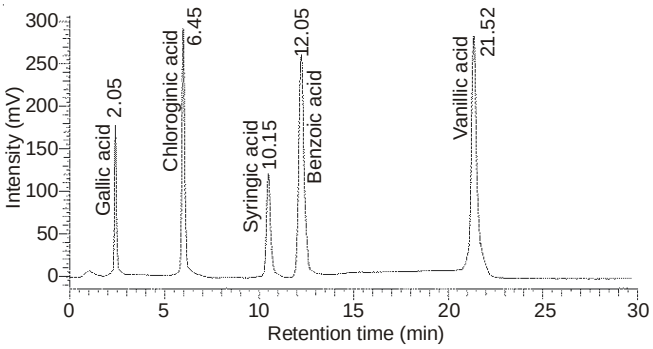

Fig. 1. HPLC chromatogram of phenolic acids standard, 2.05 gallic acid, 6.45 chloroginic acid, 10.15 syringic acid, 12.05 benzoic acid, 21.52 vanillic acid were identified

Palosa (Acacia) honey (0.79 ± 0.02) respectively. Maximum concentrations (0.98 ± 0.03) of gallic acid were observed in small bees' honey. Minimum concentration (0.47 ± 0.03) in big bees honey, whereas moderate concentration (0.92 ± 0.02) in Palosa (Acacia) honey, Beera honey (0.61 ± 0.02) and Bekerr (Justicia) honey (0.76 ± 0.02) respectively. Maximum concentration (0.71 ± 0.03) of vanallic acid were observed in Palosa (Acacia) honey, while minimum concentration (0.22 ± 0.02) in big bees honey, whereas moderate concentration (0.66 ± 0.02) in Sperkay (Trachyspermum) honey, small bees honey (0.59 ± 0.02) Beera (Ziziphus) honey (0.55 ± 0.01) and Bekerr (Justicia) honey (0.52 ± 0.01) respectively. The maximum concentrations (1.73 ± 0.02) of benzoic acid were observed in small bees' honey. Minimum concentration (1.02 ± 0.02) in Beera (Ziziphus) honey, whereas moderate concentration (1.70 ± 0.01) in Palosa (Acacia) honey, Sperkay (Trachyspermum) honey (1.69 ± 0.02) and big bees honey (1.66 ± 0.01). Maximum concentration (0.31 ± 0.02) of syringic acid was observed in Sperkay (Trachyspermum) honey. Minimum concentration (0.07 ± 0.02), in Bekerr (Justicia) honey, whereas moderate concentration (0.23 ± 0.01) in small bees honey, Palosa (Acacia) honey (0.14 ± 0.03), Beera (Ziziphus) honey (0.09 ± 0.01) and Bekerr (Justicia) honey (0.07 ± 0.02) respectively.

In Table-2, branded honey samples shows that the maximum concentration (0.42 ± 0.02) of chloroganic acid were observed in Marhaba honey, minimum concentration (0.16 ± 0.03) in Al-hayat honey, whereas moderate concentration (0.20 ± 0.02) in Versatile honey and Langnese honey (0.32 ± 0.02). It is investigated that phenolic compound in Clover honey was (128 ± 11 mg/1 kg) or (1.28 ± 11 mg/100 g), for honey expressed as milligrams of gallic acid equivalent, Clover honey shows (1.87 mg/100 g) equal to (18.7 mg/kg) honey (1.1 *p*-hydroxybenzoic acid)[20]. The maximum concentration (0.23 ± 0.03) of gallic acid were observed in versatile honey, minimum (0.11 ± 0.02) in Langnese honey, whereas moderate (0.19 ± 0.02) level in Qarshi honey and (0.16 ± 0.03) in Pak-salman. The maximum concentration (0.33 ± 0.01) of vanallic acid was found in Langnese honey, minimum concentration (0.28 ± 0.02) in versatile, whereas moderate (0.29 ± 0.02) level in Marhaba honey, while not detected in Pak-salman, Qarshi and Al-hayat honey. The benzoic acid was found maximum (1.81 ± 0.02) in Langnese honey, minimum (1.21 ± 0.02) in Qarshi, while moderate (1.26 ± 0.02) in Pak-salman honey, Al-hayat

TABLE-1
PHENOLIC ACIDS CONTENTS OF NATURAL COMB HONEY SAMPLES

Acids	Concentration of phenolic acids (mg/100 g)					
	Big bees honey	Small bees honey	Beera (Ziziphus)	Palosa (Acacia)	Sperkay Trachyspermum)	Bekerr (Justicia)
Chloroganic acid	0.77 ± 0.03*	0.57 ± 0.02	0.63 ± 0.01	0.79 ± 0.02	0.90 ± 0.01	0.58 ± 0.01
Gallic acid	0.47 ± 0.03	0.98 ± 0.03	0.61 ± 0.02	0.92 ± 0.02	ND	0.76 ± 0.02
Vanallic acid	0.22 ± 0.02	0.59 ± 0.02	0.55 ± 0.01	0.71 ± 0.03	0.66 ± 0.02	0.52 ± 0.01
Benzoic acid	1.66 ± 0.01	1.73 ± 0.02	1.02 ± 0.02	1.70 ± 0.01	1.69 ± 0.02	ND
Syringic acid	0.11 ± 0.02	0.23 ± 0.01	0.09 ± 0.01	0.14 ± 0.03	0.31 ± 0.02	0.07 ± 0.02
Total	3.23	4.10	2.90	4.26	3.56	1.93

ND: Not detected; *Mean ± Standard deviation

TABLE-2
PHENOLIC ACIDS CONTENTS OF BRANDED HONEY SAMPLES

Acids	Concentration of phenolic acids (mg/100 g)					
	Marhaba honey	Qarshi honey	Versatile honey	Al-hayat honey	Langnese honey	Pak-salman honey
Chloroganic acid	0.42 ± 0.02	ND	0.20 ± 0.02	0.16 ± 0.03	0.32 ± 0.02	ND
Gallic acid	ND	0.19 ± 0.02	0.23 ± 0.03	ND	0.11 ± 0.02	0.16 ± 0.03
Vanallic acid	0.29 ± 0.02	ND	0.28 ± 0.02	ND	0.33 ± 0.01	ND
Benzoic acid	1.40 ± 0.03	1.21 ± 0.02	ND	1.34 ± 0.01	1.81 ± 0.02	1.26 ± 0.02
Syringic acid	ND	ND	ND	ND	0.21 ± 0.03	ND
Total	2.11	1.40	0.71	1.50	2.78	1.42

ND: Not detected; *Mean ± Standard deviation

(1.34 ± 0.01) and Marhaba honey (1.40 ± 0.03). The syringic acid was only found in Langnese honey (0.21 ± 0.03). Several studies found that in different floral honeys, the quercitin is common flavonoids. The mean quercitin contents in lophostemon, bankasia (heath), helianthus, melaleuca (tea tree) and guioa, honeys collected from Australia were of (0.33 ± 0.03 mg/100 g honey)[21].

In Table-3. Among unbranded honey's sample the maximum concentration (0.43 ± 0.02) of chloroganic acid were observed in Beera (Ziziphus) honey, minimum (0.14 ± 0.02) in Sperkay (Trachyspermum) honey, while moderate (0.34 ± 0.03) in big bees honey, small bees honey (0.26 ± 0.010). The vanallic acid was found maximum (0.24 ± 0.01) in small bee's honey, minimum (0.15 ± 0.01) in Beera (Ziziphus) honey, whereas moderate (0.23 ± 0.02) in big bees honey and Palosa (Acacia) honey (0.16 ± 0.01). The maximum concentration (1.77 ± 0.02) of benzoic acid were observed in Beera (Ziziphus) honey, minimum (1.02 ± 0.01) in small bee's honey, whereas moderate (1.64 ± 0.03) in Bekerr (Justicia) honey and Sperkay honey (1.16 ± 0.02), but not detected in Palosa (Acacia) and big bee's honey.

It was reported that the mean content of phenolic acids (*p*-hydroxybenzoic acid and cinnamic acid) were found in citrus honey samples (1.08 ± 0.36) mg/100 g honey. Also, the mean content in citrus honey of cinnamic acid (3.5 ± 0.21 mg/100 g) and quercitin (0.60 ± 0.01 mg/100 g)[22]. The maximum concentration (0.44 ± 0.03) of syringic acid were observed in small bee's honey, minimum concentration (0.17 ± 0.03) in Palosa (Acacia) honey, while moderate concentration (0.31 ± 0.04) in Sperkay (Trachyspermum) honey, but not detected in big bee's honey, Beera (Ziziphus) and Bekerr (Justicia) honey.

Conclusion

It is concluded that most of the honey's samples of Khyber Pakhtunkhwa contains phenolic acids such as chloroganic acid, gallic acid, vanallic acid, benzoic acid and syringic acid. Natural combs honey presented higher concentration of phenolidc acids as compared to branded and unbranded honey's sample. So these sources of honey may be utilized as such, in different food and herbal products; in turn will enhance the export.

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TABLE-3
PHENOLIC ACIDS CONTENTS OF UNBRANDED HONEY SAMPLES

Acids	Concentration of phenolic acids (mg/100 g)					
	Big bees honey	Small bees honey	Beera (Ziziphus)	Palosa (Acacia)	Sperkay Trachyspermum)	Bekerr (Justicia)
Chloroganic acid	0.34 ± 0.03*	0.26 ± 0.01	0.43 ± 0.02	ND	0.14 ± 0.02	ND
Gallic acid	0.24 ± 0.02	0.16 ± 0.03	0.11 ± 0.03	0.29 ± 0.02	0.12 ± 0.02	0.22 ± 0.01
Vanallic acid	0.23 ± 0.02	0.24 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	ND	ND
Benzoic acid	ND	1.02 ± 0.01	1.77 ± 0.02	ND	1.16 ± 0.02	1.64 ± 0.03
Syringic acid	ND	0.44 ± 0.03	ND	0.17 ± 0.03	0.31 ± 0.04	ND
Total	0.81	2.12	2.46	0.62	1.73	1.86

ND: Not detected; *Mean ± Standard deviation

REFERENCES

1. R.A. Pérez, C. Sánchez-Brunete, R.M. Calvo and J.L. Tadeo, *J. Agric. Food Chem.*, **50**, 2633 (2002).
2. A. Terrab, A.G. González, M.J. Díez and F.J. Heredia, *Eur. Food Res. Technol.*, **218**, 88 (2003).
3. T. Cherbuliez and R. Domerego, L'apithérapie: médecine des abeilles, Amyris. (2003).
4. S. Antony, J.R. Rieck and P.L. Dawson, *Poult. Sci.*, **79**, 1846 (2000).
5. K. Inoue, S. Murayama, F. Seshimo, K. Takeba, Y. Yoshimura and H. Nakazawa, *J. Sci. Food Agric.*, **85**, 872 (2005).
6. P.C. Molan, *Gen. Dent*., **49**, 584 (2001).
7. J. McKibben and N.J. Engeseth, *J. Agric. Food Chem.*, **50**, 592 (2002).
8. L. Movileanu, I. Neagoe and M.L. Flonta, *Int. J. Pharm.*, **205**, 135 (2000).
9. T.M. Nemoseck, E.G. Carmody, A. Furchner-Evanson, M. Gleason, A. Li, H. Potter, L.M. Rezende, K.J. Lane and M. Kern, *Nutr. Res*., **31**, 55 (2011).
10. M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López and J. Pérez-Álvarez, *J. Food Sci.*, **73**, 117 (2008).
11. S.R. Joshi, H. Pechhacker, A. Willam and W. von der Ohe, *Apidologie (Celle)*, **31**, 367 (2000).
12. M. Al-Mamary, A. Al-Meeri and M. Al-Habori, *Nutr. Res.*, **22**, 1041 (2002).
13. A. Coqueiro, L.O. Regasini, S.C.G. Skrzek, M.M.F. Queiroz, D.H.S. Silva and V. da Silva Bolzani, *Molecules*, **18**, 2376 (2013).
14. L. Estevinho, A.P. Pereira, L. Moreira, L.G. Dias and E. Pereira, *Food Chem. Toxicol.*, **46**, 3774 (2008).
15. N. Gheldof and N.J. Engeseth, *J. Agric. Food Chem.*, **50**, 3050 (2002).
16. G. Beretta, P. Granata, M. Ferrero, M. Orioli and R. Maffei Facino, *Anal. Chim. Acta*, **533**, 185 (2005).
17. R. Khan, W. Hassan, A. Hussain, K. Rehman and J. Ali, *Pak. J. Pharm. Sci.*, **27**, 1271 (2014).
18. Y. Velioglu, G. Mazza, L. Gao and B. Oomah, *J. Agric. Food Chem.*, **46**, 4113 (1998).
19. M. Kücük, S. Kolayli, S. Karaoglu, E. Ulusoy, C. Baltaci and F. Candan, *Food Chem.*, **100**, 526 (2007).
20. O.I. Aruoma and S.L. Cuppett, Antioxidant methodology: *in vivo* and *in vitro* Concepts: The American Oil Chemists Society (1997).
21. L. Yao, Y. Jiang, R. Singanusong, B. D'Arcy, N. Datta, N. Caffin and K. Raymont, *Food Res. Int.*, **37**, 166 (2004).
22. J.C. Serem, An Exploratory Investigation into the Physicochemical, Antioxidant and Cellular Effects of a Selection of Honey Samples from the Southern African Region, University of Pretoria (2011).