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NUTRIENT COMPOSITION OF SEASONAL POLLENS AND ITS RELATIONSHIP WITH HONEY PRODUCED BY VARIOUS *APIS* SPECIES

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Abstract:
The current study was conducted at selected sites of Punjab, Pakistan. These sites were Lahore, Kasur and Okara. Available pollens were harvested during spring and summer seasons and 5 honey samples from the natural hives and farms were collected. The proximate constituents like moisture, ash, carbohydrate, protein, dietary fiber and fat were analyzed by (AOAC). Moisture content of natural honey samples of *Apis florea* and *Apis dorsata* were 12.10±0.93% and 7.50±0.66% while in farmed honey of *Apis mellifera* was 14.40±0.69%. Ash and protein contents of natural honey samples of *A.florea* and *A.dorsata* were 1.40±0.26%, 6.28±0.31%, 1.27±0.17% and 5.44±0.39% while in farmed honey samples of *A.mellifera* was 0.32±0.08 and 4.94±0.27%. Fat and fiber contents of natural honey samples from *A.florea* and *A.dorsata* were 0.69±0.02%, 3.40±0.12%, 0.30±0.06% and 1.83±0.13% while the fat and fiber contents from farmed honey sample of *A.mellifera* were 0.14±0.03% and 1.84±0.09%. Carbohydrate contents of natural honey samples of *A.dorsata* and *A.florea* were 57.02±0.37% and 53.06±0.30% while the farmed honey sample of *A.mellifera* was 62.06±1.30%. In the same way, glucose and fructose contents of natural honey samples from *A.dorsata* and *A.florea* were 23.73±0.22%, 30.75±0.17%, 20.55±0.51% and 25.63±0.43% while in farmed honey samples of *A.mellifera* were 26.52±0.43% and 33.65±0.56%. Sucrose and maltose contents from natural honey samples of *A.dorsata* and *A.florea* were 1.65±0.05%, 8.9±0.25%, 0.99±0.14% and 6.66±0.41% and farmed honey samples from *A.mellifera* were 2.30±0.21 and 11.76±0.46%. The main sugar components of honey and pollen samples were measured through HPLC.

KEYWORDS: Carbohydrates, fructose, honey, pollens

1. Introduction

Pakistan has gained a good status in the Middle East regarding the production of honey because of its specific quality and taste. In Pakistan beekeeping industry mainly found in the province Khyber Pakhtunkhwa (KPK) (1). Honeybees belong to genus *Apis* are well known for the ability to produce

honey. About eight species of honeybees exist in the world i.e. *Apis mellifera, A. dorsata, A. florea, A. koschevnikovi, A. andreniformis, A. nuluensis, A. cerana* and *A. nigrocincta.* Four species of genus *Apis* exist in Pakistan including three endemic species i.e. *A. florea, A. dorsata* and *A. cerana* and one exotic species *A. mellifera* (2).

Natural honey produced by honeybees is a viscous sweetener that is obtained from the flowering plants secretions and nectar (3). Honey contains major components like 80% of carbohydrates (5% sucrose, 35% glucose and 40% fructose) and 20% of water while its minor constituents are proteins, vitamins, minerals, organic acids, enzymes, phenolic acids, flavonoids and also phytochemicals. Proximate analysis shows that ash, moisture, nitrogen-free extract (NFE), protein, fat and fiber are included in honey (4). Honey is used in food cosmetics and pharmaceutical industries due to several nutrients that are very useful for humans.The chemical constituents such as texture, therapeutic and nutritional efficiency, granules and keeping quality of honey have great importance (5). The main components found in the honey samples are almost similar, whereas honey varies in physical properties and chemical composition reliant on the species of the plant foraged by honeybees (6). Floral and climatic conditions are the other factors on which the natural honey varies. Natural honey is the abundantly required product as it contains several nutritional and therapeutic properties along with different group of the substances. Bees obtain nectar, pollen and water in order to nourish the larvae (7). For the honeybees pollen is considered as a significant and unique source of the protein, minerals, lipids and vitamins where the content of the protein use to measure the pollen quality (8).

Depending upon the species of the plant the pollen grains show variability among size, color, shape, weight, flavor and aroma as well. Although depending on the origin of plants it's biological, chemical and physico-chemical activities also varies (9). About 200 components have been observed from the chemical composition which consist of carbohydrates, vitamins, elements, fatty acids, proteins, amino acids, enzymes-coenzymes and phenolic compounds as well. Honeybee farms are considered integral part of normal agricultural enterprise in many countries while production of honey largely remained at a subsistence level in Pakistan. Similarly, the nutritional profile of honey and its relationship with that of seasonal availability of pollens in different seasons need exploration. Present study is therefore planned to record seasonal availability of pollens and their influence on nutrient profile from various *Apis* species during different seasons.

2. Materials and Methods

2.1 Collection of samples

Available pollens were harvested during two seasons i.e. spring and summer from district Okara, Kasur and Lahore through pollen traps fixed at entry points of honey bee colonies. Collected pollens were dried in shady places and were identified. Similarly, honey samples were collected from natural hives and honeybee farms from the same districts. The physical properties of pollens and honey samples viz. color and smell were recorded. For the further processing the pollens and honey samples were stored in plastic bottles.

2.2 Proximate analysis of honey and pollens

The proximate composition such as moisture, ash, carbohydrates, dietary fiber, fat and protein of pollens and honey samples were examined following Association of Official Analytical Chemists (10). These analyses were performed in triplicate. To analyze moisture content, 2 g of the samples were placed in an oven for 5 hours at the temperature of 105°C based on the recommendations of AOAC Official Method 925.10, 2002. For the analysis of the ash content same samples were placed in furnace at 550°C till the weight becomes constant (AOAC Official method 923.03, 1998). The dietary fibers dependent on the total soluble and insoluble fibers of pollens and honey samples were measured by the recommendation of AOAC Official Method 991.43, 1991. The fat was analyzed by acid hydrolysis method recommended by AOAC Official Method 14.019, 1984. Crude protein was measured by the Kjeldahl method. To analyze 1 g of pollen and 1 ml of honey sample was taken separately and heated with 20 ml of sulfuric acid (95–97%) using potassium sulphate as catalyst for 4 h until blue-green color appears. It was neutralized with 90 ml NaOH (30%). Ammonia was

distilled and collected in boric acid solution. It was tittered with standard solution of hydrochloric acid following AOAC (2005). Carbohydrates were calculated by following equation (11).

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber)

The energy values for the honey samples were calculated as following (11).

Energy (kcal/g) = 9 (fat) + 4 (protein) + 4 (carbohydrate)

2.3 Glucose oxidase activity with peroxide test

Glucose oxidase activity in the samples of the pollens and honey were screened for peroxide accumulation by Merckoquant test strip (no. 10011) from Merck, Germany defined by Kerkvliet (1996) (12). The results were given in milligram of hydrogen peroxide stock in a liter of sample solution for an hour at 20°C.

2.4 Sugar analysis by HPLC

The main sugar components of honey samples like maltose, glucose, sucrose and fructose were measured through HPLC system attached to a refractive index detector. For the separation, amide column with dimension of 3.5 µm, 4.6 × 150 mm was used. During the analysis the column was retained at 25°C. Then 0.5 g of sample was dissolved in deionized water and was vortexed strongly prior to filter for injection. The volume of the injection was 20 µl. The standard sugar solution comprised of maltose, glucose, sucrose and fructose were arranged at different concentrations from 5 to 20 g/kg range for the construction of the calibration curve.

Statistical Analysis

Data regarding the nutritional profile of pollens and honey was subjected to SAS 9.1 and Analysis of Variance (ANOVA) was applied to find out interspecific variations.

3. Results

Nutritional profile of natural and farmed honey samples produced by *Apis dorsata*, *A. florea* and *A. mellifera* is mentioned in Table 1. Statistically significant ($p<0.05$) variations in moisture, protein, fat contents, carbohydrates, glucose, fructose, sucrose and maltose was recorded during present study. Overall, the data highlights significant variations in multiple nutritional parameters among the natural and farmed honey samples produced by *A. dorsata*, *A. florea*, and *A. mellifera*, indicating distinct characteristics and compositions of the honey from each bee species.

Parameters	**Species**		
	A. dorsata **(Natural sample)**	***A. florea*** **(Natural sample)**	***A. mellifera*** **(Farmed sample)**
Moisture	7.50 ± 0.66^{c}	12.10 ± 0.93^{b}	14.40 ± 0.69^{a}
Ash	1.27 ± 0.17^{a}	1.40 ± 0.26^{a}	0.32 ± 0.08^{b}
Protein	5.44 ± 0.39^{b}	6.28 ± 0.31^{a}	4.94 ± 0.27^{c}
Fat	0.30 ± 0.06^{b}	0.69 ± 0.02^{a}	0.14 ± 0.03^{c}
Dietary fiber	1.83 ± 0.13^{b}	3.40 ± 0.12^{a}	1.84 ± 0.09^{b}
Carbohydrate	57.02 ± 0.37^{b}	53.06 ± 0.30^{c}	62.06 ± 1.30^{a}
Glucose	23.73 ± 0.22^{b}	20.55 ± 0.51^{c}	26.52 ± 0.43^{a}
Fructose	30.75 ± 0.17^{b}	25.63 ± 0.43^{c}	33.65 ± 0.56^{a}
Sucrose	1.65 ± 0.05^{b}	0.99 ± 0.14^{c}	2.30 ± 0.21^{a}
Maltose	8.9 ± 0.25^{b}	6.66 ± 0.41^{c}	11.76 ± 0.46^{a}

Table 1: Proximate analysis of natural and farmed honey samples

Proximate analysis of honey samples of *A. dorsata* and *A. florea* from three districts of the Punjab is mentioned in Table 2. Statistically no significant variations in moisture, ash, fiber, carbohydrate, glucose and fructose were recorded between the locations.

Table 2: Proximate analysis of honey samples from three districts of the Punjab

Parameters	*A. Dorsata*			*A. florea*		
	Lahore	**Kasur**	**Okara**	**Lahore**	**Kasur**	**Okara**
Moisture	7.50± 0.65[b]	7.30 ± 0.54[b]	7.02 ± 0.72[b]	12.10±0.93[a]	12.20 ± 0.82[a]	12.00 ± 0.82[a]
Ash	1.27±0.17[a]	1.27 ± 0.11[a]	1.29 ± 0.12[a]	1.40± 0.26[a]	1.36 ± 0.09[a]	1.34± 0.05[a]
Protein	5.44± 0.39[c]	6.00± 0.80[b]	4.70 ± 0.47[d]	6.28±0.31[b]	7.30 ± 0.48[a]	7.44 ± 0.58[a]
Fat	0.30±0.06[c]	0.43 ± 0.26[b]	0.43± 0.25[c]	0.69±0.02[a]	0.63 ± 0.06[a]	0.59 ± 0.06[b]
Dietary fiber	1.83±0.13[b]	1.79 ± 0.16[b]	1.86± 0.22[b]	3.40±0.12[a]	3.14± 0.64[a]	3.15 ± 0.66[a]
Carbohydrate	57.02±0.37[a]	56.62 ± 0.48[a]	56.74 ± 0.44[a]	53.06± 0.30[b]	52.62 ± 1.03[b]	52.56 ± 1.08[b]
Glucose	23.73±0.22[a]	23.06 ± 0.72[a]	23.54 ± 0.48[a]	20.55±0.51[b]	20.11 ± 0.84[b]	20.11 ± 0.84[b]
Fructose	30.75±0.17[a]	31.27± 0.59[a]	31.45 ± 0.70[a]	25.63±0.43[b]	24.95 ± 1.22[b]	24.70± 1.09[b]
Sucrose	1.65±0.05[a]	1.58 ± 0.09[a]	1.59 ± 0.10[a]	0.99±0.14[b]	0.98± 0.21[b]	1.01 ± 0.32[b]
Maltose	8.90±0.25[a]	8.10 ± 0.52[b]	8.30 ± 0.52[a]	6.66±0.41[c]	6.49± 0.64[c]	6.66 ± 0.53[c]

The proximate analysis of pollen samples during spring and summer seasons are mentioned in Table 3.

Table 3: Nutritional profile of pollen samples

Season	Plant species	Moisture	Ash	Protein	Fat	Dietary fiber	Carbohydrate
Spring	Eucalyptus (*Eucalyptus amaldulensis*)	4.2	2	18.6	0.6	13.5	61.1
	Date palm (*Phoenix dactylifera*)	20.8	2.56	28.11	15.54	0.37	32.62
	Corn (*Zea mays*)	30.84	2	17.54	1.12	3.54	44.96
Summer	Sunflower (*Helianthus annuus*)	8.1	1.02	16	4.48	1.56	68.84
	Rape (*Brassica napus*)	7.75	3.5	20.04	3	0.36	65.35

4. Discussion

The results of current study determined that moisture content of natural honey samples of *A. florea* and *A. dorsata* were 12.10±0.93% and 7.50±0.66% while the moisture contents of farmed honey of *A. mellifera* was 14.40±0.69%. Same contents were determined in a research that moisture content of natural and artificial honey samples were 17.85±0.74% and 21.65±0.94% (13). The findings of ash contents of natural honey samples of *A. florea* and *A. dorsata* were 1.4±0.26% and 1.27±0.17% while in farmed honey samples of *A. mellifera* was 0.32±0.08 of the current study which is consistent to the findings of the research were 0.29±0.05%, 0.15±0.09%, 0.37% and 1.70±1.98% (13,14).

The protein contents of the natural honey samples of *A. florea* and *A. dorsata* were 6.28±0.31% and 5.44±0.39% and the farmed honey sample from *A. mellifera* was 4.94±0.27 in the current study. Fat contents of natural honey samples from *A. florea* and *A. dorsata* were 0.69±0.02% and 0.30±0.06% while from farmed honey sample of *A. mellifera* was 0.14±0.03% in present study which is consistent to the findings of the research was 0.29 ± 0.11% (15). In the present study dietary fiber in natural honey samples of *A. florea* and *A. dorsata* were recorded 3.40±0.12% and 1.83±0.13% while in farmed honey sample of *A. mellifera* was 1.84±0.09%. Same content was conducted in a research were 0.19±0.02%, 0.12±0.01%, 2.76±1.07% and 1.99% (13, 14). The findings of carbohydrate contents of natural honey samples of *A. dorsata* and *A. florea* were 57.02±0.37% and 53.06±0.30% while the farmed honey sample of *A. mellifera* was 62.06±1.30% in present study. These contents were also recorded (16) were 82.71 ± 3.1%. Same as glucose and fructose contents of natural honey samples from *A. dorsata* and *A. florea* were 23.73±0.22%, 30.75±0.17%, 20.55±0.51% and 25.63±0.43% while in farmed honey samples of *A. mellifera* were 26.52±0.43% and 33.65±0.56%. These contents were recorded as 31.0% and 38.5% (16). The present study revealed sucrose and maltose contents from natural honey samples of *A. dorsata* and *A. florea* were 1.65±0.05%, 8.9±0.25%, 0.99±0.14% and 6.66±0.41% while the same contents of farmed honey samples from *A. mellifera* were 2.30±0.21% and 11.76±0.46%. Same contents were determined as 1.34% and 7.2% respectively (17). During present study moisture, ash, crude protein, crude fat, crude fiber and carbohydrate contents of the honey bee collected pollens of eucalyptus was recorded as follows; 4.2% , 2%, 18.6%, 0.6% 13.5% and 61.1% respectively. Same contents of pollen also

recorded as 5.1±0.5%, 2.3±0.1%, 27.9±0.3, 1.0±0.1%, 6.9±0.3% and 56.8% (18). Proximate constituents of the date palm pollen grains were determined in present study. The moisture content of the date palm pollen grains was 20.8%. Same content was also determined in another studies were 3.6-4.8% and 28.80%. Values of 2.56% and 28.11% were estimated for ash and protein respectively. These values are comparable to those reported by Human and Nicolson who found that the ash of the pollen was 4.57% and crude protein was 31.11%. The crude fat content value was 15.54% (19). The proximate constituents of the sunflower pollen grains were also recorded. The moisture content was 8.1%, ash content 1.02%, crude protein 16%, crude fat 4.48%, crude fiber 1.56% and carbohydrate 68.84% respectively. Similarly, the proximate constituents of the rape pollen grains were also recorded. The moisture content was 7.75%, ash content 3.5%, crude protein 20.04%, crude fat 3%, crude fiber 0.36% and carbohydrate 65.35% respectively. These values are comparable to those reported by Taha (20).

5. Conclusion

Current study revealed that constituents like carbohydrates, moisture, sucrose and maltose were recorded high in farmed honey as compared to the natural honey. So it is suggested that natural honey is better than farmed honey.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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