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Full Length Research Paper

Quality assessment and antimicrobial activity of various honey types of Pakistan

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Forty samples of different honey types (Acacia, Ziziphus, Brassica and Citrus) were collected from different areas of Pakistan and analyzed for moisture, pH, total acidity, ash, electrical conductivity, hydroxymethylfurfural (HMF), sucrose, total sugars, invert sugar, protein, proline contents as well as macro and micro elements. The variation in composition of honey samples was observed due to different types of flora. Higher pH (6.56 ± 0.05) was observed for *Ziziphus* honey, acidity (45.0 ± 2.35 mg/kg) for Citrus, moisture (36.8 ± 1.8%) for Brasica and HMF (32.7 ± 0.49 mg/kg) for Acacia. Whereas, higher concentrations of proline (2.1 ± 0.04 mg/kg) and invert sugar (0.38 ± 0.1%) for Citrus honey and protein (16.5 ± 1.5 g/100g) for Acacia honey were observed. Likewise, a significant level (P < 0.05) of ash, electric conductivity, sucrose, total sugar as well as macro and micro elements was also found in these honey types. Different formulations of honey has significantly inhibited growth of pathogenic microorganisms, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* when compared to control group, which is an evidence that honey is a therapeutic agent being used since ancient time throughout the world.

Key words: Honey types, flora, sugar, nutritional food, therapeutic agent.

INTRODUCTION

Honey is a sweet natural substance that is produced by honeybees from the nectar (secretion) of living parts of plants. Honeybees collect this material, transform and combine it with specific substances of their own, store and leave in the honey comb to ripen and mature (White and Landis, 1980). Honey is a strong concentrated aqueous solution of invert sugar, but also contains a very complex mixture of other carbohydrates (mostly glucose and fructose), amino acids proline, minerals, aromatic substances, pigments waxes and pollen grains (Bogdanov et al., 1998; Qiu et al., 1999). However, the presence of unstable compounds, such as enzymes, vitamins and other compounds in honey was also reported by different workers (Coco et al., 1996).

The variation in physicochemical properties of the honey samples, such as ash contents, the spectrum of saccharides, the activity of enzymes, hydroxymethylfurfural (HMF), electrical conductivity, pH and optical rotation are due to regional and floral diffe-rences (Serra and Ventura, 1995; Singh and Bath, 1997; Terrab et al., 2003). As Honey depends on biotic and abiotic factors around the beehives, therefore, the presence of heavy metals could be related to its geographical and botanical origin. Preliminary studies also confirmed a correlation between the elemental content of honey and the status of the environment (Kump et al., 1996; Caroli et al., 1999). It has been observed to have therapeutic properties (exhibiting healing power) in the treatment of digestive, respiratory, cardiac and rheumatic disorders (Abdullah and Abdulaziz, 1998). However, the presence of enzy-mes such as glucose oxidase produces acids like gluconic acid and hydrogen peroxide. While the lowered pH and oxidizing agent serve as antimicrobials to preserve the honey, the low pH tends to impact a sour taste which makes the honey unpalatable if its level is too high (low pH). In addition, it takes advantage of its three antimicrobial components (hyperosmotic, low pH and presence of hydrogen peroxide). Since ancient times,

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Table 1. Analysis of physicochemical parameters of honey samples.

Types of honey	pH	Acidity (meg/kg)	Moisture (%)	Ash (g/100 g)	Electrical conductivity (mS/cm)
Acacia	3.32 ± 0.14*	33.9 ± 1.94*	18.7 ± 0.18	0.17 ± 0.09	0.28 ± 0.00
Brassica	4.01 ± 0.02	27.6 ± 0.19	36.8 ± 1.8*	0.14 ± 0.00	0.34 ± 0.00*
Citrus	3.33 ± 0.04	45.0 ± 2.35*	19.2 ± 0.08	0.13 ± 0.10	0.22 ± 0.00
Ziziphus	6.56 ± 0.05*	14.2 ± 0.59	18.0 ± 0.12	0.44 ± 0.01*	0.67 ± 0.00*

Values obtained after triplicate analysis; *Significant at $P < 0.05$; $n = 10$.

natural unprocessed honey was used to prevent microbial infections and aid wound healing.

Pakistan's Ziziphus honey is regarded as the most valuable in the world. The purpose of the present study was to assess antimicrobial activity as well as to evaluate the quality of various honey types from different flora, according to the International honey standards of the Codex Alimentarius (2001) and EU (2002).

MATERIALS AND METHODS

Collection of honey samples

A total of 40 honey samples (10 each) of Ziziphus, Acacia, Citrus and Brassica were collected from the local beekeepers of different areas of Pakistan. The samples were stored in half litre plastic containers duly labeled with numbers, names and date of collection. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth before analyzing their physicochemical properties. All chemicals used in this study were of analytical grade and analysis was carried out in triplicate. Determination of pH of acidity, moisture, ash, electrical conductivity, HMF, sucrose and total sugars from honey samples were according to the method of AOAC (2000) and EU Council (2002). Proline and invert sugar contents were determined by using method described by Sidney (1984), where as, total protein contents were determined following methods described by Bradford (1976) and AOAC (2000).

The concentration of macro and micro nutrients (Na, K, Ca, Fe Zn, Mn, Cu and B) was determined by using flame atomic absorption spectrophotometer (FAAS). (GBC, 932 plus; shimadzu).

Antimicrobial assay

Antimicrobial activity of honey was assessed by using the agar-well diffusion method which was evaluated as described by Perez et al. (1990). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 were grown in Luria Bertani (LB) media, while, the yeasts *Candida albicans* ATCC 10231 and the fungi *Aspergillus niger* ATCC 16404 were grown in potato dextrose broth (PDB) at 130 rpm at 30°C and 37°C for bacteria. Bacterial inoculum was adjusted to 0.5 McFarland turbidity standard (10^8 CFU/ml) and then diluted 1:10 while, the concentration of fungi and yeast was adjusted to 3.4×10^7 cells/ml. Honey was diluted in LB or PDB, as appropriate, to yield solutions of 0 (control), 25, 50 and 100% (v/v). After agar medium (LB agar for bacteria and potato dextrose agar (PDA) for yeast and fungi) in a plate was spread with 100 ml of the test microbe culture, a hole in the center was made using a cork borer (# 6) and 200 ml of the selected honey dilution was filled in the hole. Assays were performed in triplicate for each honey dilution-test. The bacterial samples were incubated at 37°C for three days, while the yeast and fungi samples were incubated at 30°C for three days. The diameter of the inhibition zone (mm) on the plates was measured every day.

Statistical analysis

The results obtained were analyzed statistically. Comparisons between means were made using the least significant difference (LSD) at 0.05 probabilities (SPSS).

RESULTS AND DISCUSSION

The quality parameters include ash, sugar, moisture, electrical conductivity, acidity and hydroxymethylfurfural (HMF) contents. The pH of different honey samples was found to be significantly different from each others (Table 1). The pH of Acacia (3.32 ± 0.14), Citrus (3.33 ± 0.04), Brassica (4.01 ± 0.02) and Ziziphus (6.56 ± 0.05) honey was observed. The results of pH obtained in present study were within the ranges specified by EU Council (2002) and the Codex Alimentarius (2001). The acidic pH of honey might be due to the varieties of different acids and minerals present in the honey samples (Williams et al., 2009; Kamal et al., 2002). There was a higher acidity (45.0 ± 2.35 meg/kg) for Citrus honey, whereas, lower acidity contents (14.25 ± 0.59 meq/kg) of Ziziphus honey was found (Table 1). However, 23.55 - 58.52 meq/kg of acidity was reported by Nasiruddin et al. (2006) for local honey, whereas, Kamal et al. (2002) reported only 6.73 - 22.9 meq/kg of acidity in different honey samples. The variations found in acidity contents of honey samples might be due to different sources of nectar from different areas of Pakistan. The moisture contents of various honey types ranged 18.04 ± 0.18 to 18.0 ± 0.12%, which is in agreement with results reported by Nasiruddin et al. (2006). No variation was found in moisture and sucrose contents of honey samples used in the present study. The moisture content of honey is widely related to the harvest season and the level of maturity released in the hive. This parameter is highly important for the shelf life of honey.

Ash (0.13 ± 0.10 to 0.44 ± 0.1 g/100 g) and electrical conductivity (0.22 - 0.67 m.S/cm) was observed in various honey samples (Table 1). Ash represents the direct measure of the inorganic residues after honey carbonization

Table 2. Analysis of selected parameters from honey samples.

Types of honey	Proline (mg/kg)	Protein (g /100 g)	Invert sugar (%)	Sucrose (g/100 g)	Total sugar (g/ 100 g)	HMF (mg/kg)
Acacia	1.25 ± 0.06	16.5 ± 1.5	0.21 ± 0.01	6.4 ± 0.16	81.4 ± 0.34 *	32.7 ± 0.49*
Brassica	1.82 ± 0.03*	14.7 ± 1.3	0.35 ± 0.12	7.1 ± 0.08*	78.4 ± 0.16	24.9 ± 0.42
Citrus	2.1 ± 0.04*	15.1 ± 0.05*	0.38 ± 0.1*	6.5 ± 0.19	79.0 ± 0.22	17.8 ±0.44
Ziziphus	1.96 ± 0.05*	15.8 ± 1.4*	0.31 ± 0.02	6.7 ± 0.26	81.6 ± 0.24 *	21.1 ± 0.37

HMF, Hydroxymethylfurfural.
Values obtained after triplicate analysis, *Significant at $P < 0.05$; n =10.

Table 3. Concentration of micro nutrients (µg/g) analyzed from honey.

Types of honey	Fe	Zn	Mn	Cu	B
Acacia	166.7 ± 1.23	32.1 ± 0.53	2.35 ± 0.08	14.33 ± 0.33	1.40 ± 0.19
Brassica	182.7 ± 1.62	33.0 ± 0.54*	3.70 ± 0.10	18.33 ± 0.29*	0.31 ± 0.19
Citrus	128.7 ± 2.92	30.9 ± 0.69	3.76 ± 0.11*	17.47 ± 0.51	0.50 ± 0.17
Ziziphus	224.4 ± 2.49*	24.5 ± 0.60	2.31 ± 0.03	12.42 ± 0.25	1.45 ± 0.08*

Values obtained after triplicate analysis; *Significant at $P < 0.05$; n = 10.

Table 4. Concentration of macro nutrients (µg/g) analyzed from honey.

Types of honey	Na	K	Ca
Acacia	412.6± 3.54	688.0 ± 16.70	586.7± 5.53
Brassica	525.8± 6.37	525.8± 15.43	1035.5± 61.49*
Citrus	813.6± 15.32	813.6 ± 29.35	1005.9± 6.68
Ziziphus	856.6± 33.43*	1506.4± 10.79*	848.1± 13.65

Values obtained after triplicate analysis; *Significant at $P < 0.05$; n = 10.

(Malika et al., 2005), while electrical conductivity of honey depends on its mineral contents. The electrical conductivity is a good criterion related to botanical origin of honey and thus is very often used in routine honey control instead of the ash contents. The relationship between the two parameters has been shown by several authors (Accorti et al., 1983; Piazza et al., 1991; Serrano et al., 2004). The results of proline, protein, invert sugar, total sugar, sucrose and HMF contents analyzed from different honey samples are shown in Table 2. Significant ($P < 0.05$) variation in level of these parameters was observed in various honey types. The presence of such parameters in these honey types indicates their adulteration as well as nutritional importance. The level of HMF was within the acceptable limit and proline is an indicator of honey adulteration which confirmed that the honey was genuine.

The higher concentration of iron was found in Ziziphus honey 224.4 ± 2.49 µg/g, while lowest 128.7 µg/g of Fe was found in Citrus honey (Table 3). The levels of Zn (24.5 ± 0.6 - 33.0 ± 0.54 µg/g) found in different honeys samples were significantly different from one another ($P < 0.05$). Mn (2.31 ± 0.03 to 3.76 ± 0.11 µg/g), Cu (12.42 ± 0.25 to 18.33 ± 0.29 µg/g) and B (0. 31 ± 0.19 to 1.45 ± 0.8 µg/g) were observed ($P < 0.05$) (Table 3). The level of boron found in these samples was in much lower amounts as compared to the other elements. Boron is a light trace element that is turning out to be essential to human health and behavior. Today, it has been scientifically demonstrated that boron is important to brain function, especially in enhancing memory, cognitive function and hand-eye coordination (Saif et al., 2008).

Macro nutrients concentrations: Ca (586.7 ± 5.53 to 1035.5 ± 61.49 µg/g), K (525.08 ± 15.43 to 1506.4 ±10.79 µg/g) and Na (525. 8 ± 6.37 to 856.6 ± 33.43 µg/g) were found to be significantly different from one another ($P < 0.05$) (Table 4). The nutrients found in Pakistani honey are considered very important for the total composition of honey. This is due to the high level of these nutrients in plant tissues. Nutritionally, the presence of these metals makes honey an excellent food for humans, especially for children.

Data regarding antimicrobial activity of various honey types are given in Table 5. According to our results, Acacia and Citrus honey inhibited growth of bacteria, yeast and fungi significantly as compared to other honey

Table 5. Antimicrobial activity of various honey types against different microorganisms. Zone of inhibition was measured in mm.

Honey type	Formulation (%)	*C. albicans*	*A. niger*	*S. aureus*	*E. coli*
Control	0%	0.51±0.1	0.72± 0.1	1.1±0.4	0.92 ± 0.5
	25	1.92±0.1	1.21±0.5	1.23±0.3	1.21±0.6
Acacia	50	1.89±0.2	1.92±0.8*	2.43±0.7*	2.47±0.5*
	100	2.91±0.5*	3.12±0.9*	3.22±0.6*	2.98±0.3*
	25	1.78±0.2	1.16±0.3	1.22±0.4	1.16±0.5
Brassica	50	1.81±0.3	1.32±0.4	2.41±0.5	2.45±0.6
	100	2.38±0.6	2.48±0.5	2.82±0.3	2.73±0.8
	25	1.76±0.3	1.36±0.6	1.26±0.4	1.36±0.7
Citrus	50	1.82±0.4	1.41±0.5	2.54±0.5	2.54±0.6
	100	2.83±0.8*	2.87±0.4*	3.51±0.6*	3.45±0.7*
	25	1.90±0.7	1.24±0.4	1.24±0.5	1.22±0.6
Ziziphus	50	1.82±0.4	1.32±0.6	2.63±0.6	2.29±0.5
	100	2.44±0.5*	2.41±0.3	2.91±0.5*	2.72*±0.6*

Mean values of triplicate analysis zone of inhibition (mm).

types, which might be combination or synergistic effects of low pH (Table 5), hydrogen peroxide, antimicrobial peptides or other volatile substances. It may be better to use this honey in traditional medicine, especially for the topical treatment of antibiotic-resistant pathogenic strains. It was noted that 100% honey was the most suitable for the inhibition of growth of pathogenic microorganisms, although this may simply represent the hyperosmotic effects rather than the concentration of bioactive components.

The levels of total sugar, acidity, pH, HMF invert sugar, proline, protein and essential nutrients found in honey samples indicate its high quality. The quality of most natural raw honey of Pakistan fulfills all the requirements of international standards. Furthermore, the antimicrobial activity of honey against pathogenic bacteria, yeast and fungi confirmed that honey is a broad spectrum antimicrobial agent and has been used for the healing of various types of wounds since ancient times.

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