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Quality Assessment and Authentication of Raw Honey-Available at Local Markets of Punjab

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ABSTRACT

Honey have delightful sweetness, propitious nutrition, antioxidant, bioactive, viscous golden yellow substances and used widely in food, beverages, medicinal research against treatment of a number of infections caused by microbes (bacteria, viruses, and parasites) due to antifungal and antibacterial properties and its complex carbohydrates, vitamins, minerals, organic acids, phenolic compounds and anti-oxidants nature. Aim of this study was to identify adulteration in honey samples collected from local stores of different regions of Punjab, Pakistan. Seven honey samples were procured and analyzed for hydroxyl methyl furfural (HMF) contents, moisture content, protein contents, pollens, ash contents, total sugar contents (sucrose, non-reducing and reducing), viscosity, total soluble solids, pH, acidity and electrical conductivity. Results ranged for moisture contents from 19.97 to 25.6%, total soluble solids (TSS) ranged from 74.4 to 80, pH 2.4-4, protein contents from 0.29 to 0.44%, ash contents from 0.15 to 0.45%, pollens from 22.4 to 74.7, viscosity from 376.7 to 1030cP/s, total acidity from 17 to 41.9 meq/kg, total sugars from 66.2 to 78.2%, reducing sugars from 58.4 to 72.3%, non-reducing sugars from 4.6 to 8.8% and HMF contents ranged from 15.7-35mg/kg. Higher amount of HMF present in honey is the indication of heating and lower quality honey. The results were associated with standards and specifications of "Pakistan Pure Food Rules". 40% samples did not meet criteria. The accurate quantification should employ for production of high quality honey with strict national legislations and monitoring on apiculture to avoid mixing of adulterants in honey.

INTRODUCTION

Honey is a sweet substance, a golden liquid, a nice gift from God, made by Apis mellifera (honey bees) from flowers' nectar or secretions of plants. Nectar is mixed with enzymes that are present in the saliva of bees and changes into sweet liquid "honey" (Pasanen et al., 2011). The taste and smell of honey can vary from dark colour (amber) to light yellow. The color of liquid honey changes due to many factors, including mineral content (Al et al., 2009). Honey can be sorted into different types based on the flowers and bee size. Honeybees create floral honey by collecting nectar from flowers, while honeydew honey comes from plant secretions or from insects that suck on plants (Sanz et al., 2005).

Nearly sixty substances are noted for causing

honey smell. These include aromatic alcohols, aliphatic acids, aldehydes, and esters. Raw honey contains extraneous elements like wax, pollens, and yeast that tolerate sugar, as well as crystals formed from hydrated dextrose. Yeasts cause fermentation. The levels of acidity influence not only the taste but also the stability of honey against various microorganisms. The honey composition relies on several factors, including climate, types of pollen, processing methods, and environmental conditions. These traits are linked to honey's high osmolarity, antioxidant capacity, and antibacterial features (Alvarez et al., 2010).

Honey has wide range of antibacterial property (Green et al., 2022) and this is due to the hydrogen peroxide content and other such compounds as phenolic acids, lysozyme and flavonoids. Flavonoids and ascorbic acid has capability of acting as an antioxidant, anti-browning and antimicrobial agents (Salama and Chennaoui, 2024). Considering all these factors, it is possible to state that the mentioned above features make honey a unique wound dressing: it causes a very fast removal of infections, very fast removal of devitalized tissues from wounds, very fast suppression of inflammation process, minimization of scars formation, and stimulation of angiogenesis as well as tissue granulation and epithelium growth (Almasaudi, 2021). This is because it has high sugar content which limits the water activity to check microbes. It is taken in the management of gastrointestinal infections or heart burns, ulcer, asthma, and wounds (McArdle et al., 2023; Orhan et al., 2003). According to Gheldof et al. (2003), using honey as a food increases the reducing power, the concentration of anti-oxidants and the amount of total plasma in humans. Two honey component affects properties of starch include amylase and sugar.

Confectionery, baking, drinks, fruit goods, medications, and food preservation are all areas with growing application potential. Pakistan ranked at 20th for honey production and 34th in world for honey export with more than 11,000 tons (50 g/capita) domestic consumption. The 4,000 tons is the main export of worth US$ 23 million to Arab countries (Munir et al., 2024). Because of its nutritional benefits and therapeutic qualities, honey consumption has increased substantially during the past 25 years (Simsek et al., 2012). Japan consumes over 40,000 tons of honey annually. Argentina is the second-largest producer of honey, after China.

There are over two hundred different sensory and physicochemical criteria that can be used to classify and characterize honey types. Pure honey is a supersaturated sugar solution, contain 31% glucose, 38% fructose, 17.7% moisture, 0.18% ash, and 0.08% total acidity. The primary sugars that give energy, sweetness and physical properties to honey are fructose and glucose sugars. Flavonoids, phenolic acids (Meda et al., 2005), proteins, amino acids, carotenoids, α-tocopherol and organic acids (Alvarez et al., 2010), catalase, and glucose oxidase (Molan and Betts, 2004) are examples of minor constituents. Honey's mineral and trace element concentration indicates its geological origin. The market price of honey varies according on its nutritional value and organic status (Simsek et al., 2012).

The conditions under which honey is stored after it is extracted from the hive have a significant impact on its moisture content, a crucial variable that affects product quality, granulation, and texture. Harvesting honey before maturity, excessive heat treatment, and poor storage conditions have a negative impact on honey moisture content (Jdayil et al., 2002). Many varieties of honey naturally crystallize or granulate when stored. Liquid honey is favored by the retail honey market. Therefore, certain processing methods, such as heating, filtering, or straining, are required to keep honey in its liquid state.

The remarkable advancements in transdermal drug delivery systems, such as transferosomes, offer innovative solutions for the effective delivery of bioactive compounds. Similarly, honey, with its delightful sweetness, antioxidant properties, and bioactive components, holds significant potential for medicinal applications, particularly against infections caused by microbes. However, challenges such as adulteration impact its physicochemical properties and therapeutic value, emphasizing the need for stringent quality control and innovative delivery strategies to maximize its benefits (Patel et al., 2024).

Unfortunately, honey adulteration is common due to the honey market's high popularity. Corn syrup, invert sugars and corn sugar syrup are the most common adulterants found in honey. Among the many acids present in pure honey, the gluconic

acid is the most prevalent. Other acids include citric acid, α-ketoglutaric, pyroglutamic, glycollic, malic, butyric, oxalic, lactic, maleic, tartaric, pyruvic, succinic, acetic, formic, butyric, and G-6-phosphate. A study was carried out to evaluate the quality of honey that is sold in local markets across several regions of Punjab, Pakistan.

MATERIAL AND METHODS

Procurement of Material

Seven honey samples were purchased directly from honey beekeepers from seven districts of Punjab, including Okara, Sahiwal, Lahore, Chichawatni, Burewala, Faisalabad, and Multan. They were then stored at room temperature (25–30ºC) at dark place.

Preparation of Raw Samples

Unwanted materials, like wax sticks, comb fragments, and dead bees were eliminated from all samples by passing them through cheesecloth prior to physicochemical property analysis. Samples of granulation-free honey were combined by shaking or stirring. For 30 minutes, granulated honey was heated to 60°C in a closed container in a water bath. If more heating was required to liquefy it, it was done at 65°C. Analytical-grade substances from the National Institute of Food Science and Technology were used in this investigation. The analysis was done three different times.

Physiochemical Parameters

Following parameters were studied according to the prescribed methods as described in AOAC (2019):

Moisture Content

The moisture content of all samples were determined by following method as described in AOAC (2019).

Determination of sugar contents

Sugars in collected honey samples were measured by procedure of Lane and Eynon as described in AOAC (2000).

Pollen contents

A mixture of the Louveaux et al. 1978 and Erdtman 1960 and 1969 procedures was used to analyse pollen, as suggested by Iwana and Melhem (1969).

Viscosity

A Brookfield, DV-E viscometer was used to measure viscosity. A fluid's viscosity is a measurement of its internal friction. At room temperature, the viscosity measurements were carried out. S-4 was the spindle type, while 50 rpm was the spindle speed (shear rate in rpm).

Hydroxymethyl Furfural (HMF)

Hydroxymethyl furfural (HMF) contents were determined through spectrophotometeric method.

pH.

A digital pH meter was used to calculate the pH of all collected honey samples. A solution comprising 10g of honey in 75mL of distilled water was used to measure the pH using an Orion 420 A pH meter (AOAC, 2000).

Titratable Acidity

The AOAC (2000) method was used to determine the acidity of honey.

Ash Content

Five grams of honey samples were put into a crucible that had already been burned and set on fire to ignite in order to measure the amount of ash present. Until it went black, the sample was placed on fire and then heated for six hours at 500–550ºC in a muffle furnace.

Crude Protein

Kjeldhal's method, as outlined in AOAC (2000), was used to calculate the sample's nitrogen %.

Total Soluble Solids

The Atago hand held refractometer was used to measure the honey samples' total soluble solids, and the findings were reported in degree brix.

Sensory Evaluation

Using the methodology described by Piana et al. (2004), NIFSAT personnel and postgraduate students evaluated the colour, aroma, taste, texture, aftertaste, and overall acceptability of honey samples in comparison to standard honey samples.

Statistical Analysis

The data obtained through testing was analyzed through the "Analysis of Variance" (ANOVA) approach, with a significance level set at $p < 0.05$. Then mean values were compared by using methods as described by Steel and Torry (1997).

RESULTS AND DISCUSSIONS

The Pakistan Pure Food Rules of 1965 state that honey must meet certain requirements and specifications in order to be considered a natural

product that is well-ripened and free of any undesirable flavours that may have been caused by fermentation, overheating, or smoking. Moisture content should not exceed 20%, ash content should not exceed 0.5 %, sucrose should not exceed 6 %, reducing sugars should not fall below 65 %, acidity should not exceed 40 meq/kg and HMF should not exceed 40 mg/kg.

Samples taken from various districts had moisture contents of 19.97 (S_1), 21.97(S_2), 25.6 (S_3), 21.4 (S_4), 21.14 (S_5), 24.87 (S_6) and 20.2 (S_7) percent in the collected honey samples. The values of samples S_1 and S_7 were standard. According to Chakir et al. (2011), moisture levels varied from year to year and were influenced by temperature, ambient factors, beekeeper input during harvest, and maturity level. The highest moisture content was found in samples S_6 and S_3. During storage, the increased moisture content may cause honey to granulate and ferment, according to Kahraman et al. (2010) and Al et al. (2009). High moisture content promoted crystallization in some honey types and raised water activity to levels where yeasts may grow, according to Gomes et al. (2010). Based on their moisture content, samples S_6 and S_3 were not high-quality honey, and crystallization was seen in them.

There was a strong correlation between moisture and sugar levels. Thus, it served as a trustworthy indicator of adulteration. The honey's sugar content and other components were mostly indicated by total soluble solids (TSS). The honey samples' mean TSS values varied from 74.4 to 80^0Brix. The TSS content was highest in sample S1 and lowest in sample S2. The current findings corroborated the results. In their investigation, Guo et al. (2010) found a negative linear association between water contents and total soluble solids. Numerous factors, including temperature and source flora, influence variations in total soluble solids. Sample S_1 contained the highest TSS and lower water content. Similarly, sample S_3 contained the lowest sugar contents and the highest moisture contents.

The pH values of collected honey samples were ranged from 2.4 to 4. Sample S_1 showed lowest pH values, while samples S_5 and S_7 had the highest pH values. As samples S_3 and S_6 had the lowest pH values, highest moisture, highest sucrose content, so these samples were not of good quality. Terrab et al. (2004) found that the sugar content in relation to pH is an index of the adulteration, and samples with the higher sugar (sucrose) contents and the lower pH values are an indication of adulteration.

According to Kamal et al. (2002), the material collected by honey bees during foraging and the floral origin of the samples were the causes of the variations in ash concentrations. Seasonal honey was extracted from many plants. As a result, the ash contents of the various samples varied because of the variations in the floral source. There was no discernible difference between S_3 and S_7's ash contents. Likewise, the ash contents of samples S_2 and S_6 were nearly identical. S_1 had the highest amount of ash and was substantially different from the other samples. Samples S_1 through S_7 had mean ash values of 0.45%, S2 0.34%, S_3 0.21%, S_4 0.17%, S_5 0.15%, S_6 0.31%, and S7 0.25%. The amount of ash in each sample was less than 0.5%. The ash concentration of every sample was less than 0.5%. It indicates that all have typical amounts of ash.

According to Mohammed and Babiker's (2009) analysis, honey's crude protein concentration was a novel way to assess its quality. The samples' mean crude protein content complied with requirements and criteria. For honey samples, the average crude protein content varied between 0.29 and 0.44%. The amount of protein was within normal limits.

According to Saxena et al. (2010), the water content of honey samples had a significant impact on viscosity as well. As honey's water content rose, its viscosity dropped. The honey samples had mean viscosity ranges between 376.7 and 1030 cP/s. Sample S_3 showed signs of adulteration due to its low pH, high moisture content, and sugar content. Similar case was seen in case of sample S_6.

According to research by Kahraman et al. (2010), the presence of organic acids—especially gluconic acid-in equilibrium with their lactones or esters, as well as inorganic ions like phosphate and chloride, was the reason of the honey's overall acidity. Higher acidity was also thought to be a sign of honey purity, according to Qamer et al. (2008). For S_1, S_2, S_3, S_4, S_5, S_6, and S_7, the mean total acidity values were 17 meq/kg, 37.5 meq/kg, 33.9 meq/kg, 29.3 meq/kg, 26.3 meq/kg, 41.9 meq/kg, and 25.7 meq/kg, respectively. All samples' total acidity was within acceptable bounds (less than 40

meq/kg of honey), indicating that the unwanted fermentation had not occurred.

According to the European Commission's honey rules and requirements from 2002, it was also prohibited to separate pollen or any other particular component from honey. Filtration was used to separate the pollens (fine filtration). Therefore, the honey that was used to extract pollen was not regular honey. For honey samples, the average number of pollens varied between 22.4 and 74.67. The amount of pollens was lowest in sample S_3 and greatest in sample S_5. The quantity of pollen in honey was a measure of its richness.

One metric that proved a good indicator of adulteration was sugar concentration (Terrab et al., 2004). According to Anklam (1998) and Guler et al. (2007), there are a number of reasons why honey samples have greater sucrose levels, such as early harvest, honeybees feeding on sucrose syrup, adulterated honey, or sucrose that hasn't been fully converted by bees into fructose and glucose. Gomes et al. (2010) stated that a number of variables, including the floral source, pH, temperature, and heating time, affect the amounts of hydroxymethyl furfural. As a result, it gives information regarding overheating and unfavourable storage conditions.

The honey samples' mean total sugar contents varied between 66.2 and 78.2%. S_1 had the largest total sugar content, whereas S_3 had the lowest. For honey samples, the average reducing sugar values varied from 58.4 to 72.3%. The reducing sugar content was lowest in sample S_3 and greatest in sample S_7. The guidelines state that sugar content reduction should not fall below 65%. With the exception of sample S_3, all samples had standard values for decreasing sugar levels. 6.7% for S_1, 5.8% for S_2, 7.4% for S_3, 6.1% for S_4, 5.1% for S_5, 8.8% for S_6, and 4.6% for S_7 were the average sucrose levels for the samples. Higher sucrose concentrations were found in the honey samples for a variety of causes, such as early harvest, honeybees feeding on sucrose syrup, adulterated honey, or sucrose not being completely converted by bees into fructose and glucose.

For honey samples, the average HMF level ranged from 15.7 to 35 mg/kg. The remaining samples satisfy the standards, whereas samples S_3 and S_6 did not, according to physicochemical data. In terms of hue, Sample S_2 was neither preferred nor hated. In terms of texture, samples S_2 and S_3 were neither preferred nor hated. Samples S_3 and S_6 had lower scores overall and were deemed less acceptable in terms of taste, colour, and flavour, according to sensory evaluation. According to Zafar et al. (2008), sensory evaluation is a suitable method for authenticating honey types and can get over the drawbacks of chemical and pollen analysis. According to a study by Esti et al. (1997), the most crucial method for assessing the quality of honey was sensory evaluation.

Samples S_3 and S_6 were less acceptable to the panelists based on sensory characteristics and had lower viscosity, pollen content, reducing sugars, total sugars, and pH in accordance with standards. They also had higher reducing sugars and moisture content. Accordingly, S_3 and S_6 were of inferior grade. The low pH, high moisture level, and sugar content of samples S_3 and S_6 were signs of adulteration. According to standards, S_1 and S_7 were the best samples out of all of them because they had standard values for every parameter and were favored by the panelists the most. Standard values, or values close to them, were present in S_2 and S_5. Thus, the quality of these samples was medium. S_2 and S_5 contained standard values or values that were similar to them. As a result, these samples were average quality. During the sensory examination, S_4 received the highest score from the majority of panelists and had standard values for all criteria. Therefore, based on criteria and sensory evaluation, it was of superior quality.

Table 1

Physiochemical Analysis of Honey Samples

Parameter	Samples						
	S_1	S_2	S_3	S_4	S_5	S_6	S_7
Moisture (%)	19.97±1.01^{G}	21.97±0.55^{C}	25.6±1.23^{A}	21.4±0.75^{D}	21.1±0.05^{E}	24.87b±0.05	20.2f±0
Viscosity (cP/s)	1030±80.3^{A}	590e±29.7	376.7f±25.6	756.7c±29.3	690d±33.23	590e±22.11	930b±30.5
HMF (mg/kg)	15.7±3.52^{F}	31.4bc±1.15	35a±1	27.67d±2.77	30.17c±2.38	34ab±2	20e±1.73
Ph	2.4±1.09^{E}	3.36b±0.66	2.87d±0.17	3.98a±0.35	4a±1.50	3c±0.51	4a±0.19
Acidity Lactonic	6.6±1.21	15a±0.86	12b±1	9.4c±0.404	8.5c±1.33	11.5b±1.28	7.8cd±0.76

(meq/k)	Free	10.5±1.32[F]	22.5b±1.5	21.97b±2.55	19.9d±3.36	21cd±1.86	30.5a±3.5	17.8e±0.95
	Total	17f±2	37.5b±0.5	33.9c±0.45	29.3d±0.25	26.5d±1.32	41.9a±0.83	25.7e±1.04
Ash (%)		0.45a±0.14	0.34b±0.07	0.21cd±0.03	0.17de±0.08	0.15e±0.09	0.31b±0.12	0.25c±0.07
Sugars	Total	78.2a±5.28	70f±4.5	66.2g±3.17	75.4c±5.07	74.4d±3.40	73.1e±3.15	77b±6.21
(%)	Reducing	71b±2.43	64d±4.39	58.4e±2.88	69.1c±3.16	68.9c±2.54	63.9d±1.05	72.3a±3.23
	Non-reducing	6.7c±0.173	5.8e±0.07	7.4b±0.02	6.1d±0.059	5.1f±0.17	8.8a±0.05	4.6g±0.35
TSS (^{0}Brix)		80a±5.05	78e±3.6	74.4g±1.97	78.6d±2.76	78.9c±4.12	75.1f±	79.8b±0
Number of pollens		67ab±4.55	69ab±3.94	22.4d±2.67	49.4c±5.51	74.7a±5.84	24d±2.64	54.4bc±3.8
Protein (%)		0.37bc±0.16	0.35bc±0.10	0.29d±0.17	0.44a±0.15	0.4ab±0.08	0.32cd±0.15	0.44a±0.12

S_1=Okara, S_2=Sahiwal, S_3=Lahore, S_4=Chichawatni, S_5=Burewala, S_6=Faisalabad and S_7=Multan

Table 2

Sensory Analysis of Honey Samples

Parameter	Samples						
	S_1	**S_2**	**S_3**	**S_4**	**S_5**	**S_6**	**S_7**
Color	7.7ab±0.57	5.7c±0.57	7.7ab±0.57	8a±0	8.4a±0.57	6.7bc±0.57	8.0a±1
Aroma	6.7bcd±0.57	5.4d±0.57	6.4cd±1.15	8ab±1	8.4a±0.57	7.4abc±1.52	7.7abc±0.57
Texture	6.4b±1.15	5.7b±1.57	5.3b±0.57	8a±0	8a±1	6b±1	8a±1
Taste	7.7ab±1.57	5.3c±0.57	5.3c±0.57	8.7a±0.57	8ab±1	6.7bc±2.57	8ab±1
Overall acceptability	7bcd±0.57	5.4e±0.57	6.4de±0.57	8.4a± 0.57	8ab±1	6.7cd±1.15	7.7abc±0.57

Where S_1=Okara, S_2=Sahiwal, S_3=Lahore, S_4=Chichawatni, S_5=Burewala, S_6=Faisalabad and S_7=Multan

CONCLUSION

This study evaluated the physicochemical properties, sugar content, and sensory attributes of honey samples collected from various regions of Punjab, Pakistan, with the aim of identifying adulteration and assessing quality in accordance with the Pakistan Pure Food Rules (1965). Among the analyzed samples, moisture content, ash content, reducing sugars, and sucrose levels served as key indicators of honey quality and adulteration. Samples S3 and S6 displayed characteristics such as high moisture content, low pH, higher sucrose levels, and reduced sugar content, marking them as substandard and adulterated. These samples also exhibited poor sensory attributes, such as lower acceptability in terms of taste, color, and texture, confirming their inferior quality.In contrast, samples S1 and S7 met the standard parameters, including reducing sugars above 65%, sucrose levels below 6%, and HMF below 40 mg/kg, and were preferred during sensory evaluation. This positions them as high-quality honey. Samples S2, S4, and S5 demonstrated medium quality with values close to the acceptable standards. Importantly, the study highlighted the correlation between moisture content, sugar levels, and viscosity, identifying them as reliable markers for honey adulteration. The findings also emphasize the impact of environmental factors, floral sources, and harvesting practices on the physicochemical properties of honey.The study underscores the importance of regular monitoring and stringent quality control in the honey industry to ensure consumer safety and maintain the nutritional and medicinal value of honey. Standardization of testing methods and adherence to regulatory guidelines are essential to curtail adulteration and enhance honey quality.

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