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SEASONAL VARIATION IN THE PHYSICO-CHEMICAL QUALITY OF HONEY PRODUCED BY APIS DORSATA IN TEHSIL PATTOKI, PUNJAB, PAKISTAN

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ABSTRACT
The present study evaluated the seasonal variation in the physico-chemical quality and sugar composition of Apis dorsata's honey across different seasons collected from Tehsil Pattoki, Punjab, Pakistan. A total of 30 honey samples were collected from active hives during winter, spring, and summer seasons, with ten samples collected per season from 10 different hives. The results revealed significant seasonal variations in several quality parameters of honey. Moisture content was significantly higher during spring (9.15 ± 0.03%) and winter (9.14 ± 0.02%) as compared to summer (6.15 ± 0.01%). In contrast, carbohydrate content recorded highest during summer (82.79 ± 0.13%), while lower values were recorded during winter (79.69 ± 0.05%) and spring (79.72 ± 0.03%). Similarly, the energy value was significantly higher in summer honey (363 ± 0.26 kcal/g) than in winter (351.03 ± 0.18 kcal/g) and spring (351.19 ± 0.20 kcal/g). The pH values remained relatively stable throughout the seasons, ranging from 4.16 ± 0.01 to 4.22 ± 0.02. Protein content varied slightly between 6.09 ± 0.04% and 6.15 ± 0.02%, whereas fat, fiber, and ash contents showed no significant seasonal differences. Among sugars, fructose concentration was highest during summer (30.59 ± 0.09 g/100 g), followed by spring (27.49 ± 0.11 g/100 g) and winter (26.50 ± 0.10 g/100 g). Glucose content peaked during winter (24.32 ± 0.18 g/100 g), while sucrose was highest in spring (2.12 ± 0.06 g/100 g). Maltose content remained comparatively constant across all seasons. These findings revealed that seasonal floral availability and environmental conditions significantly influence the nutritional quality and sugar profile of Apis dorsata's honey. Future studies should focus on detailed melissopalynological analysis to identify the specific floral sources contributing to seasonal changes in honey composition. Furthermore, evaluating antioxidant activity, phenolic compounds, enzyme activity, mineral profile, and antimicrobial properties is recommended to better understand the therapeutic potential of Apis dorsata honey. Long-term monitoring of climatic factors and habitat changes should be conducted to evaluate their effects on honey quality and bee ecology. Additionally, sustainable and non-destructive honey harvesting techniques should be explored to support conservation of wild bee populations and maintain ecological balance.

Keywords: Apis dorsata, Honey quality, Seasonal variation, Physico-chemical properties, High-Performance Liquid Chromatography.

INTRODUCTION

Honey is one of the major bee products that has high nutritional and economic value (Bhalchandra & Joshi, 2021). It has a complex composition and various therapeutic properties due to the influence of floral sources and environmental conditions. In particular, the physico-chemical parameters of honey such as moisture content, pH, electrical conductivity, and sugar profile are critical parameters for indicating the quality, authenticity, and potential bioactivity of honey. These parameters are important in assessing the shelf life of honey, its tendency towards granulation, its suitability for medicinal and nutritional purposes. The quality of honey produced by the giant honeybee (Apis dorsata) is extremely variable and is determined by a combination of floral sources, geographical location, and seasonal environmental conditions (Rivera-Mondragony et al., 2023). Apis dorsata is a wild, migratory species (Kastberger et al., 2024) and its honey is often physically and chemically different from that of managed species, such as Apis mellifera (Joshi et al., 2000).

Apis dorsata honey has various unique parameters that differentiate it from other bee species. The honey produced by these bees usually has a higher moisture content, often varying from 17.20% to 21.5% (Joshi et al., 2000; Thakur et al., 2023). High levels of moisture in honey can impact its shelf life and risk of fermentation if not properly managed (Moniruzzaman et al., 2013). The honey produced by Apis dorsata has significantly higher levels of invertase (approximately 373.4 U/kg) and proline (approx. 875.8 mg/kg) compared with A. mellifera and A. cerana (Joshi et al., 2000; Sharma et al., 2024). The pH of A. dorsata honey differs depending on the region

(Sharma et al., 2024). Some multifloral types have pH as low as 3.73-3.95 while others have as high as 6.25 (Thakur et al., 2023; Wu et al., 2020),). Free acidity levels frequently correlate with the content of amino acids and vitamin C in honey (Thakur et al., 2023). It typically has a higher EC (around 0.96 mS/cm) than A. mellifera (0.31 mS/cm) and consequently a richer mineral content (Joshi et al., 2000; Sharma et al., 2024). Apis dorsata honey usually has high fructose to glucose (F:G) ratio around 1.98 (Thakur et al., 2023). It also has more oligosaccharides than other species of Apis (Sharma et al., 2024).

Seasonal variations strongly affect the amount and composition of the nutritional content of the honey. In Pakistan, the fructose content of honey has been found to be maximum during the summer season (approximately 30.45%) compared to other seasons of the year. Seasonal changes, often coupled with altitude, affect the levels of protein, fat, fiber and ash in honey. Honey collected in various seasons exhibits variations in these proximate components because of the changing availability of floral resources. Apis dorsata colonies migrate seasonally between highland and lowland locations to "surf the sweet wave" of blooming flora (Robinson, 2021). Their foraging behavior is greatly affected by seasonal changes, resource availability, temperature and humidity (Raffiudin et al., 2024; Vincze et al., 2024). Bee activity is optimal between 20°C and 30°C (Vincze et al., 2024). Seasonal extremes in temperature, wind (comfort > 1.6-6.7 m/s) or precipitation (heavy rain) can hinder the foraging process and decrease the nectar production, thus affecting the final honey composition (Vincze et al., 2024). Autumn honey samples from Khyber Pakhtunkhwa, Pakistan, mostly composed of both unifloral and multifloral honey types, depending on the availability of nectar and pollen sources (Ahmad et al., 2023). Predominant pollens for A. dorsata honey are S. cumini, Eucalyptus, and P. pinnata (Georgiadou, 2021). These plant sources give the honey unique taste, smell and health benefits and require detailed analysis of its physical and chemical properties across different seasons.

Assessing the quality of honey in a systematic way links traditional honey hunting and modern food safety standards. Apis dorsata is a migratory species and its honey quality evaluation has several critical purposes (Pallujam et al., 2025). As the commercial value of honey rises, intensive quality management becomes necessary to ensure that healthier products are provided and consumer expectations are met (Ayvaz, 2017). Furthermore, the identification of plant origin through melissopalynological analysis provides high added value and enables differentiation of the product in the competitive global market (Garcia et al., 2020). One of the major reasons that makes quality assessment necessary is preventing food adulteration. Practices such as mixing honey with water and sugar syrups not only compromise the reliability of the product but can also pose serious public health risks (Nascimento et al., 2020; Żak & Wilczyńska, 2023). Adulterated honey could contain toxic or allergenic ingredients that are banned for human consumption (Nascimento et al., 2020). Analytical testing helps to make sure that honey is free from artificial tastes, fermentation or excessive heating, which destroys natural enzymes (Bhalchandra & Joshi, 2021; Daugaliyeva et al., 2025; Krishnasree & Ukkuru, 2017). In the context of South Asia, quality parameters are often compared to quality standards such as the Food Safety Standards Authority of India or authorities of Pakistan to ensure compliance for both domestic and export markets (Thakur et al., 2023; Yasmeen et al., 2023). Physico-chemical parameters are used to indicate the maturity and stability of honey (Salazar et al., 2017). For example, moisture content is one of the most important parameters for wild honey of A. dorsata, which has a natural tendency toward higher levels (up to 21.5%) and prone to fast fermentation and spoilage without proper management (Joshi et al., 2000; Kharkamni, 2021). Similarly, monitoring Hydroxymethylfurfural and diastase activity is very important in order to detect improper storage or excessive heat treatment that may lead to deterioration of the nutritional and therapeutic integrity of the honey (Eshete & Eshete, 2019). In the case of Apis dorsata, quality measurement is directly associated with conservation (Sihag, 2025). Conventionally used honey-hunting techniques that usually require smoking or burning colonies are not only harmful to the populations of bees but are also regarded as unsuitable for producing high-quality honey (Sihag, 2025). Through the evaluation of the physico-chemical profile of A. dorsata honey, scientists can promote more eco-friendly and non-destructive harvesting methods that would help preserve the function of bees as pollinators of crops (Sihag, 2025).

Altitude is a key factor in the nutritional variability of honey. Research from various altitudinal zones of Pakistan from the plains of Bahawalpur (118 m) to the highlands of Murree (2291 m) has revealed that the honey from higher altitude has significantly higher contents of protein, fat, fiber, and ash. Furthermore, increasing altitude is related to an increasing percentage of water, pH, and acidity in honey which may be due to environmental stressors such as hypoxia and low barometric pressures on bee physiology and pollen characteristics (Mohammed et al., 2017). In addition, honey production is extremely sensitive to weather conditions because both nectar secretion and bee activity are affected by these factors (Vincze et al., 2024). Optimal foraging efficiency for honeybees occurs within a temperature range of 20°C to 30°C, while extremes in temperature, high wind speeds (above 1.6–6.7 m/s), or heavy precipitation can obstruct flight and reduce yield (Vincze et al., 2024; Shakoori et al., 2023). There is a significant positive correlation between the relative humidity and the level of moisture in honey samples (Adalina et al., 2024; Shakoori et al., 2023; Wu et al., 2020).

The mineral profile and the electrical conductivity of honey are largely determined by the geographical location and soil types where the floral sources are located (Edo et al, 2022; Rivera-Mondragon et al, 2023).

Minerals such as potassium, the most abundant element of many honey samples, can be found in different concentrations in different seasons based on the specific floral resources used by the bees (Rivera-Mondragón et al., 2023). Additionally, the foraging behavior of bees enables them to gather materials from their environment, which means that soil or air contamination in a particular area can be transferred into the final honey product as heavy metals such as lead or cadmium (Nascimento et al., 2020; Salama et al., 2019).

Punjab province of Pakistan has a diverse ecological landscape that affects floral resources available to Apis dorsata, influences its honey's unique characteristics (Kousar, 2017). The province's diverse agro-climatic zones from the arid plain to sub-mountainous regions provide dynamic flowering plants for nectar and pollen which directly impact seasonal variations in honey production. These geological and climatic factors combined with human impact such as agriculture intensification and urbanization directly impact the quality of honey produced by A. dorsata (Munir et al., 2024). Therefore, detailed spatiotemporal studies are necessary to determine the distinctive biochemical markers of the floral biodiversity of Punjab.

In the agricultural and forest landscapes of the Punjab, peak abundance of Apis dorsata bees and their floral resources is observed from early March to late May with a subsequent decline until December (Ali et al., 2017). During the spring and early summer, giant honey bees are dependent on a wide range of plant species. The most common and frequently visited plants are Helianthus annuus (sunflower), Citrus reticulata, Trifolium alexandrinum (clover), Moringa oleifera, and Calotropis procera (Ali et al., 2017). In southern Punjab (e.g., Bahawalpur) the flowering season of canola (Brassica napus) from February to mid-April provides an important nectar source. The foraging efficiency of A. dorsata is very sensitive to short-term changes in the weather. Diurnal patterns also differ by crop, for example, on Brassica species, peak foraging rates are often found around noontime (12:00 h) with a sharp decline in late afternoon (16:00 h) (Islam et al., 2024). Furthermore, the concentration of sugar in the nectar itself is also not constant and can commonly be positively correlated with temperature and luminosity, as an evolutionary strategy to signal attractiveness to pollinators at specific times of the day (Puentes et al., 2019). Unlike many other honeybee species, Apis dorsata is cathemeral (active during daytime and night-time, provided environmental illumination is sufficient, e.g., a lunar cycle with at least a half-full moon) (Young et al., 2021). This nocturnal activity is especially prominent during January and February, the winter months, correlating with the highest availability of night-blooming floral resources (Young et al., 2021). This flexibility helps the species to maximize resource collection under different seasonal and light conditions (Robinson, 2021; Cui & Corlett, 2016). The survival and productivity of these colonies are therefore inextricably linked to the seasonal succession of plants such as Eucalyptus in the summer and Brassica or Borassus species in the winter and spring (Raffiudin et al., 2024).

Recent research in Pakistan has put an emphasis on the importance of environmental gradients in the determination of honey quality (Yasmeen et al., 2023). There are distinct seasonal variations in climatic conditions within Punjab, Pakistan such as variations in temperature, rainfall, humidity, wind speed, photoperiod and soil moisture. These factors directly influence the nature of floral resources and physico-chemical characteristics of honey produced by Apis dorsata. The climatic variations can impact the secretion of nectar and on the phenology of flowers with subsequent variation in sugar profiles, enzyme activity, mineral content and secondary metabolites reflecting seasonal flora and foraging behavior of bees (Descamps et al., 2021). This variability emphasizes the importance of detailed study of honey composition on a year-round basis. This in turn inform quality standards, sustainable harvesting practices, robust seasonal profiling and quality control measures on the basis of unique organoleptic, therapeutic and economic characteristics. Previous studies on Apis dorsata honey have mostly centered on comparing its unique chemical signature with other honeybee species and the importantly, high levels of moisture and enzymes (Sihag, 2025). Climatic conditions in Pakistan suggest that up to 79% of appropriate habitat for Apis dorsata might be lost by 2080 under the extreme conditions (Khan et al., 2025). The habitat destruction, especially in northern high-altitude areas, poses a threat to the ecological niche of these pollinators and the constancy of honey profiles currently produced in the areas (Khan et al., 2025).

The existing literature on the physico-chemical properties of Apis dorsata honey from Punjab, Pakistan, is notably scarce, particularly regarding seasonal variations. While some studies have focused on general characteristics of A. dorsata honey from other regions. A comprehensive analysis linking specific Punjab’s seasonal climatic patterns to distinct honey profiles remains largely unexplored. This gap in the literature highlights a critical need for detailed investigations into the impact of Punjab's diverse agro-climatic conditions on the biochemical composition of A. dorsata honey throughout the year. The present study was therefore planned to provide baseline data on the quality of honey produced by Apis dorsata during various seasons in tehsil Pattoki, Punjab, Pakistan.

MATERIALS AND METHODS

Study Area

Tehsil Pattoki, in the Kasur District of Punjab, Pakistan located about 60 kilometers to the south of Lahore. It is described by subtropical climate and three pronounced seasons including winter, spring and summer, which impact greatly on local flora and fauna. The winter season (December to February) is not very cold with an average temperature of 7 °C to 20 °C. The spring (March to May) is mild with temperatures of between 18 °C to 30 °C, whereas the summer (June to August) is hot with temperatures of between 30 °C to 45 °C. July- September is the monsoon season with an average precipitation of between 700 mm and 900 mm (Mustafa et al., 2022). The farmland in Pattoki is fertile; it is irrigated with the river Ravi canals. Some crops that grow in the region are wheat (Triticum aestivum), rice (Oryza sativa), sugarcane (Saccharum officinarum), cotton (Gossypium spp.), and maize (Zea mays). The other vital vegetations are acacia (Acacia nilotica), eucalyptus (Eucalyptus camaldulensis), and neem (Azadirachta indica). These plants provide shelter and food to the pollinators. Pattoki boasts of very many vegetation which can feed the various pollinators of the wildflowers and bushes with supplementary nectar and pollen. They also provide high amount of nectar that is caused by other fruit trees such as the guava (Psidium guajava), citrus (Citrus sinensis), pomegranate (Punica granatum), and the ber (Ziziphus mauritiana). The supply of flowers increases during the monsoon months and spring months to the benefit of the honeybees as it offers them easy source of food (Mustafa et al., 2022).

Samples Collection

Honey samples were collected from Apis dorsata's hives located in the study area during the winter, spring and summer seasons. A total of 10 active hives (n=10) were randomly selected in each season to ensure that the samples are representative of the broader bee population. During each season, approximately 100-150 grams of honey was collected from each hive. The collection was done by carefully removing the honeycombs containing ripe honey. To avoid contamination, the honey was collected directly into sterile containers under field conditions, ensuring that only the honey produced by Apis dorsata is included in the sample. The collected honey samples were transported to the laboratory at the University of Okara and stored at room temperature until further analysis (Mustafa et al., 2021).

Proximate analysis

Proximate analysis of honey samples was performed according to standard AOAC methods (Charrondiere et al., 2004) which are well-established for nutritional analysis of honey. The parameters assessed included pH, moisture, ash, protein, fat, fiber, and carbohydrate content.

pH

The pH of honey samples was measured using a digital pH meter. A small portion of honey was dissolved in distilled water (1:1 ratio) and the pH was recorded.

Moisture content

Moisture content was determined by the gravimetric method. A 5g of honey was placed in a pre-weighed petri dish and dried at 105 °C for 18 hours until a constant weight was achieved (Mustafa et al., 2022). The percentage moisture was calculated based on the weight loss during drying using following formula;

%Moisture = W1 − W2 ×100

Weight of sample

Where;

W1: Initial weight

W2: Final Weight

Ash content

Ash contents of honey samples was determined by incinerating the samples in a muffle furnace at 550 °C. The residual ash weight was expressed as a percentage of the original sample weight following formula (Yasmeen et al., 2025)

%Ash = Difference in weight of Ash ×100

Weight of Sample

Protein content

Protein content was quantified using the Kjeldahl method, which measures total nitrogen. The honey samples were digested in sulfuric acid with a catalyst to convert nitrogen into ammonium sulfate. The ammonia was then be distilled, and its concentration was determined by titration (Mustafa et al., 2021). The protein content was calculated using a nitrogen-to-protein conversion factor using following formula;

% Crude Protein = 6.25* × %N (*. Correction factor)

%N = (S− B)× N×0.014× D×100

Weight of the sample× V

Where:

S = Sample titration reading

B = Blank titration reading
N = Normality of sample after digestion
D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 = Milli equivalent weight of Nitrogen

Fat content

The fat contents of honey samples were determined using acid hydrolysis. Each honey sample was treated with hydrochloric acid and the fat was extracted using a non-polar solvent. The extracted fat was weighed to calculate the percentage.

$$\%\text{Crude Fat} = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100$$

Fiber content

Soluble and insoluble dietary fibers was analyzed using enzymatic digestion. The remaining undigested fiber was measured and expressed as a percentage of the total sample weight (Yasmeen et al., 2025).

$$\%\text{Crude Fiber} = \frac{W1 - W2}{W_0} \times 100$$

Carbohydrate's calculation

Carbohydrate was estimated using following formulas following Shugaba et al., (2012).

%Total Carbohydrate=100%–(%Moisture+%Crude Fat+%Crude Protein+%Ash+%Fiber).

Energy calculation

The energy values of the samples were determined by calculation as follows (Mustafa et al., 2022; Shugaba, 2012).

Energy (kcal/g) = [(%Crude Protein x 4)+(%Crude Fat x9)+(%Carbohydrate x 4)]

Sugar analysis by HPLC

Major sugar components such as glucose, fructose, sucrose, and maltose were quantified using High-Performance Liquid Chromatography (HPLC). Honey samples was diluted with DDW and filtered to remove particulates. The prepared samples were injected into an HPLC system equipped with a refractive index detector. An amide column (3.5 µm, 4.6 × 150 mm) was used for separation and kept at 25 °C throughout the analysiss.

Statistical Analysis

Collected data were analyzed through one-way ANOVA using PROC GLM in SAS software (version 9.1). For the comparison of significant treatment means Fisher's least significant difference test was applied considering $p \leq 0.05$ (Steel et al., 1997).

RESULTS

Sampling and Collection

The sampling was conducted through a series of systematic field visits to Tehsil Pattoki over two primary flowering seasons. A total of 30 samples were collected during field visits during the spring season (March–April), summer season (May–July) and winter season(December–January). During each visit, active Apis dorsata colonies were identified in diverse habitats, including agricultural areas, urban and peri-urban areas. Honey was harvested manually from the upper storage section of the combs using the traditional squeezing method. For each sample, approximately 100g to 150g of honey was extracted and immediately stored in sterilized, airtight glass containers. These samples were then transported to the laboratory for for subsequent physico-chemical analysis.

Seasonal Variation in Physico-chemical Parameters

The table 1 presents the seasonal variations in the physico-chemical properties of Apis dorsata honey collected from Tehsil Pattoki across three distinct flowering seasons: Spring, Summer, and Winter. The statistical analysis reveals that while several parameters remained stable, others were highly influenced by the seasonal changes. The pH values of the honey samples showed a slight increase during the Winter (4.22 ± 0.02) compared to Spring and Summer, which both recorded a mean value of 4.16 ± 0.01. However, this variation was not statistically significant ($p = 0.083$). Similarly, the ash content, indicating the mineral residue, showed no significant seasonal fluctuations ($p = 0.474$), with mean values ranging from 1.36 ± 0.02% in Summer to 1.42 ± 0.04% in Spring.

The proximate analysis, encompassing protein, fat, and fiber content, also exhibited seasonal stability with no significant differences observed. Protein levels were recorded at 6.10 ± 0.03% in Spring, 6.15 ± 0.02% in Summer, and 6.09 ± 0.04% in Winter ($p = 0.427$). Fat content remained low and consistent, ranging from 0.83 ± 0.01% in Winter to 0.87 ± 0.02% in Spring ($p = 0.441$). Crude fiber followed a similar trend of stability, with values maintained between 2.76 ± 0.04% and 2.78 ± 0.02% throughout the study period ($p = 0.898$). In contrast, moisture content, carbohydrate concentration, and total energy values were significantly impacted by the seasons ($p < 0.0001$). The moisture content was significantly higher during the Summer (9.15 ± 0.03%) and Spring (9.14 ± 0.02%) but decreased sharply to 6.15 ± 0.01% during the Winter. Carbohydrate levels showed an inverse relationship to moisture, reaching a significant peak in the Winter at 82.79 ± 0.13%, while Spring and Summer values were notably lower at 79.69 ± 0.05% and 79.72 ± 0.03%, respectively. Correspondingly, the energy value of the honey was significantly higher in the Winter season, reaching 363 ± 0.26 kcal/g, whereas the energy values for Spring (351.03 ± 0.18 kcal/g) and Summer (351.19 ± 0.20 kcal/g) were significantly lower and comparable to each other.

Table 1: Proximate Composition of Apis dorsata honey samples collected from Tehsil Pattoki. All values are expressed as Mean ± S.D.

Parameters	Winter	Spring	Summer	p-value
pH	4.16 ± 0.01	4.16 ± 0.01	4.22 ± 0.02	0.083
Moisture (%)	9.14a ± 0.02	9.15a ± 0.03	6.15b ± 0.01	<0.0001
Protein (%)	6.10 ± 0.03	6.15 ± 0.02	6.09 ± 0.04	0.427
Fat (%)	0.87 ± 0.02	0.86 ± 0.03	0.83 ± 0.01	0.441
Fiber (%)	2.78 ± 0.02	2.77 ± 0.01	2.76 ± 0.04	0.898
Ash (%)	1.42 ± 0.04	1.36 ± 0.02	1.38 ± 0.03	0.474
Carbohydrates (%)	79.69b ± 0.05	79.72b ± 0.03	82.79a ± 0.13	<0.0001
Energy (kcal/g)	351.03b ± 0.18	351.19b ± 0.20	363a ± 0.26	<0.0001

Superscripts on different means within row differ significantly at $p \leq 0.05$.

Seasonal Variation in Sugar Contents

The sugar composition of Apis dorsata honey samples showed distinct seasonal patterns, with most major sugars exhibiting statistically significant variations across the three studied seasons. Fructose, the primary sugar found in the samples, showed highly significant seasonal differences ($p < 0.0001$). The highest fructose concentration was recorded during the Summer season at 30.59 ± 0.09 g/100g, followed by the Spring season at 27.49 ± 0.11 g/100g, while the lowest concentration was observed in Winter at 26.50 ± 0.10 g/100g. Glucose levels also varied significantly ($p = 0.039$), reaching their peak during the Winter season at 24.32 ± 0.18 g/100g. In contrast, the glucose concentrations during Spring (23.82 ± 0.09 g/100g) and Summer (23.84 ± 0.04 g/100g) were statistically lower and comparable to each other. Sucrose content followed a different seasonal trend, with a significant peak observed during the Spring season (2.12 ± 0.06 g/100g) compared to the Summer (1.79 ± 0.02 g/100g) and Winter (1.87 ± 0.04 g/100g) seasons ($p = 0.003$). Maltose was the only sugar analyzed that did not show significant seasonal fluctuations ($p = 0.399$), maintaining a consistent range between 8.54 ± 0.12 g/100g in Spring and 8.72 ± 0.03 g/100g in Summer. Overall, the results indicated that the fructose and glucose ratios are influenced by the prevailing flowering seasons in Tehsil Pattoki, with the Summer season producing the most fructose-rich honey.

Table 2: HPLC Quantitative Data of Sugars (g/100g) of Apis dorsata honey samples collected from Tehsil Pattoki. All values are expressed as Mean ± S.D.

Sugar Type	Winter	Spring	Summer	p-value
Fructose	26.50^{c} ± 0.10	27.49^{b} ± 0.11	30.59^{a} ± 0.09	<0.0001
Glucose	24.32^{a} ± 0.18	23.82^{b} ± 0.09	23.84^{b} ± 0.04	0.039
Sucrose	1.87^{b} ± 0.04	2.12^{a} ± 0.06	1.79^{b} ± 0.02	0.003
Maltose	8.60 ± 0.09	8.54 ± 0.12	8.72 ± 0.03	0.399

Superscripts on different means within row differ significantly at $p \leq 0.05$.

DISCUSSION

Physico-chemical analysis of Apis dorsata honeys from Tehsil Pattoki, Punjab, shows that the chemical composition of honey and its seasonal variations is unique and closely associated with the floricultural geography of the area. This study provides an overview of how the "City of Flowers" influences the quality and nutritional profile of honey produce by Apis dorsata.

The Tehsil Pattoki, Kasur District, Punjab is a worldwide renowned center for nurseries and floriculture. The flora is dominated by a high density of ornamental plants, fruit orchards and agricultural crops with a continuous but variable supply of nectar for Apis dorsata (Alam et al., 2025). The situation is different in other parts of Punjab where the bees suffer a great shortage period, but in Pattoki the intensive practices adopted in the nurseries provide floral resources to the bees throughout the year with different predominant species in each season (Qamer et al., 2023). The crops grown around Pattoki during the winter season are mostly those that produce oil such as Brassica campestris and Eruca sativa (Ahmad et al., 2023). These species contribute to the huge inflow of nectar and pollen to the colonies which is crucial for their maintenance and honey production during the cold season of the year (Jaswal, Kumar, & Sharma, 2022). Furthermore, the availability of Eucalyptus species in the nurseries and road sides is also responsible for the high amount of carbohydrates in the winter honey (Alam et al., 2025; Qamer et al., 2023). Plants that are present during the winter bloom have adapted to lower temperatures in the Punjab plains, and the nectar they produce is frequently sugar rich, as reflected in current research, exceptionally low moisture and high carbohydrate levels during this time were recorded (Oyedokun et al., 2025).

Pattoki has the highest floral diversity during the springtime. The nurseries are in full bloom but the primary melliferous flora for Apis dorsata include Citrus limon and various citrus cultivars (Qamer et al., 2023). Citrus nectar is very aromatic and provides unique aroma and chemical components to the honey. This is also the flowering time for Morus species, Moringa oleifera and Acacia nilotica (Ahmad et al., 2023). Moringa is particularly rich in minerals and proteins, which may explain the consistency of protein levels across the seasons noted in this study (Ahmad et al., 2023; Thakur et al 2023). The spring bloom offers a phase between the abundant oilseed nectars and a wider spectrum of multifloral flowers. Pattoki's summer weather is dominated by heat-tolerant species. The most important resources are the Ziziphus mauritiana (Ber/Sidr) and Ziziphus jujuba (Ahmad et al., 2019; Taha, Al-Kahtani, & Taha, 2020). Ziziphus honey is well-known in the world for its medicinal value and low moisture content (Taha et al., 2020). Moreover, Apis dorsata is a frequent visitor of summer crops like Helianthus annuus and trees like Syzygium cumini and Prosopis juliflora (Kikar) (Ahmad et al., 2023; Qamer et al., 2023). The summer bloom demands bees to forage in the high ambient temperatures (usually more than 40°C), the nectar from these heat adapted plants may be of high mineral and phenolic density that is why the nectar of the plant recorded high ash content in the present study (Oyedokun et al., 2020).

The findings of present study revealed the exceptional concentration and quality of the honey from study area. The most significant result is the moisture content that varied from 6.15% during winter season and 9.15% during summer. These values are extremely lower when compared to the values normally reported for Apis dorsata. The Apis dorsata honey is traditionally known as high moisture content honey, often with moisture content above 21%, due to the fact that the Apis dorsata honey combs are built in the open air and are exposed to environmental humidity (Buawangpong & Burgett, 2019; Sajid, 2023). For example, moisture found in Apis dorsata was reported as 22.7% in northern Thailand (Buawangpong & Burgett, 2019), and 17.20% to 21.51% in India (Thakur et al., 2023 & Prajapati & Patel, 2025), respectively. The very low moisture content of honey (<10%) is more similar to the honey collected from very arid areas such as Balochistan (with the lowest moisture content of 7.68%) rather than the typical wild honey collected from the subtropical region (Yasmeen et al., 2023). This extreme dryness is justified from the unique microclimate of the Pattoki nurseries that features the presence of dry wind and high evaporation rates during the ripening phase, coupled with the high efficiency of the local Apis dorsata bee population at collecting the nectar (Oyedokun et al., 2025).

Moisture and carbohydrate had inverse trends, with the highest concentration of carbohydrates being found in the winter at 82.79%. This is in line with the high prevalence of Brassica campestris in the winter flora with Brassica nectar being known for its high sugar density and quick ripening (Ahmad et al., 2023). In recent years, various Pakistani honeys were tested for export quality, where the carbohydrates range from 12% to 89% with Pattoki honey being at the higher end. The high sugar content and low moisture level leads to an elevated energy density (351–363 kcal/g), substantiating the fact that honey is a nutrient-dense natural food from Pattoki (Firdous et al., 2025).

Moreover, the protein content was very stable at about 6.1%. This is much higher than the other reported average percentage of crude protein in many other Pakistani honeys (0.01 – 1.43%) (Firdous et al., 2025). The high protein content is generally related to high pollen content or high enzymatic secretions of the bees (Sajid, 2023; Taha et al., 2020). This high protein signature is likely due to the bees' abundant supply of protein-rich pollen from sources such as Moringa and Ziziphus, which are commonly found in Pattoki's floricultural business. (Qamer et al., 2023; Thakur et al., 2023). The ash content (1.36%–1.42%) was also consistently high in all seasons. The results indicate that the wild flora of Pattoki are efficient in transferring soil minerals to the nectar (Ajmal et al., 2025; Haider et al., 2022). High levels of minerals have been reported in the honey of Apis dorsata honey in previous studies in Punjab, where the level of minerals was always found more in Apis dorsata honey than in the honey of domesticated Apis mellifera (Ajmal et al., 2025).

The pH values observed (4.16–4.22) were within the acidic range typical of high-quality honey. It is important to consider the acidic pH as it is a critical quality parameter and it hinders the growth of most bacteria and fungi (Muzaffar et al., 2025). The result of the present study is in accordance with the recent studies conducted all over Pakistan and India, in which the pH of the honey of Apis dorsata ranges from 3.72 to 6.61 (Tariq et al., 2021; Thakur et al., 2023). The organic acids such as gluconic acid present in the honey gives it its bio-stability even after long storage (Muzaffar et al., 2025; Qamer et al., 2023).

The statistical analysis carried out in this study showed that the seasonal changes had a significant effect on a number of physico-chemical parameters, such as moisture and carbohydrate content ($p < 0.0001$). Three main factors explain this seasonal effect: climatic variability, floral phenology, and bee foraging behavior (Oyedokun et al., 2025; Rivera-Mondragón et al., 2023; Santana et al., 2020).

This was due to the higher moisture in the summer (9.15%) when compared with winter (6.15%), which is directly related with the humidity levels in Punjab. During the summer, the air's relative humidity rises, making it more difficult for Apis dorsata to deplete the moisture content of the honey through fanning (Oyedokun et al., 2025; Santana et al., 2020). The dry, cold winter, on the other hand, helps the bees to obtain maximum nectar concentration. In other tropical and subtropical areas, climatic conditions at harvest time have also been found to be a key factor in the quality of honey produced (Alaerjani & Mohammed, 2024; Rivera-Mondragón et al., 2023).

An increase in carbohydrate and energy values in the winter can be attributed to the flowering of Brassica campestris. Due to high sugar content, the nectar of Brassica is produced in huge quantity and the nectar is rapidly filled and capped by the bees (Ahmad et al., 2023; Jaswal et al., 2022). The change in source (Ziziphus to Helianthus) in summer alters the sugar profile of the nectar and results in reduced (but still high) carbohydrate concentrations (Santana et al., 2020; Taha et al., 2020). The intermediate values observed throughout the spring bloom period (Citrus and Moringa) may be attributed to the variety of spring blooms.

Interestingly, no significant seasonal variation for parameters like protein, fat and ash was found ($p > 0.05$). This indicates that these components rely more on the internal physiology of the bees, as well as on the mineral composition of the soil where the bees live than on the type of flowers that they are visiting (Rivera-Mondragón et al., 2023). Intensive nursery rearing system in Pattoki creates a stable mineral rich environment where they are able to be continuously introduced into the honey all year round (Ajmal et al., 2025). The stability is crucial for the geographical characterisation of Pattoki honey (Alaerjani & Mohammed, 2024).

The quality of Pattoki honey is assessed in comparison to the international standards (including Codex Alimentarius (CODEX STAN 12-1981) and the European Union Honey Directive (2001/110/EC)) and the results indicate that this honey is of exceptional quality (Bugárová & Majtán, 2020, Thrasyvoulou et al., 2018). Moisture is the most important international quality standard in the honey, with a maximum limit of 20% - 21% that would exclude fermentation (Bugárová & Majtán, 2020; Thrasyvoulou et al., 2018). The moisture content in honey produced by Apis dorsata in pattoki is very low (below 10%) which is way below these safety levels. The study is of great importance because several wild Apis dorsata honeys in Southeast Asia are failing to achieve the quality of 21% under the standards established by International Agencies (Buawangpong & Burgett, 2019; Sajid, 2023). Pattoki honey is highly suitable for exportation due to its very low moisture content which guarantees a good shelf-life without the use of high heat processing (Qamer et al., 2023).

The carbohydrate content (up to 82.79%) is well above the minimum requirement for reducing sugars ($\geq$ 60g/100g) specified by most international regulatory bodies (Firdous et al., 2025; Raweh et al., 2023). The ash content (1.42%) is slightly above the limit (0.6%) that is frequently used for blossom honeys, but is within the range of mineral and honeydew levels permitted in forest or honeydew honeys (up to 1.2% or more) and are expected in wild harvested honeys that naturally contain higher mineral and honeydew levels (Ajmal et al., 2025; Thrasyvoulou et al., 2018). The natural deviations from these limits by wild honey produced by different bee species and in different geographical areas are increasingly acknowledged in new legislation (Editor, 2019; Thrasyvoulou et al., 2018).

The pH and acidity values are in line with international standards established by the International Honey Commission which are indicators of the freshness and natural purity of samples (Raweh et al., 2023; Thrasyvoulou et al., 2018). Also, its nutritional properties such as high energy and protein content indicate that Pattoki honey may have better nutritional properties than many commercially processed honeys (Firdous et al., 2019). The sugars found in natural honey during all seasons were mainly fructose and glucose, in accordance with previous investigations that revealed fructose and glucose as the major sugars that account for the sweetness, viscosity and physicochemical properties of natural honey (Choi et al., 2024; Oyedokun, William, & Mable, 2025; Noor et al., 2021; Sadaf et al., 2021; Hussain et al., 2023; Ali et al., 2017; Khan et al., 2014; Ali et al., 2018; Masood et al., 2021; Azmat et al., 2016; Khalid et al., 2023; Laraib et al., 2022).

The fructose showed a high seasonal variability, being the maximum concentration in the Summer and the minimum in the Winter. The rise of fructose could be related to changes in the composition of floral nectar during different seasons and to environmental conditions during summer flowering seasons. Seeing the sugar profile of the different sources and the effect of the sugar profile through the enzymatic activity during the maturation of the honey. The higher fructose concentration in Summer may indicate the dominance of nectar-producing floral species with high fructose content or good environmental conditions for nectar concentration and ripening (Mushtaq et al., 2021; Hussain et al., 2020; Masood et al., 2021; Ali et al., 2021; Hussain et al., 2025; Muneeb et al., 2020). The changes in fructose content were also seen in earlier studies, which correlated increased fructose levels with warmer seasons and prolific floral resources in honey (Sajid, 2023; Sana et al., 2025).

Glucose concentration was also found to differ considerably between the seasons, but with not such marked differences as were found for fructose. All the other samples (Spring and Summer) had a similar concentration of glucose, which was comparatively low but statistically different from that of Winter honey. Honey composition and its content of glucose is one of the factors that determines the behavior of honey crystallization and granulation, honey with higher glucose content crystallizes quickly. It is likely, therefore, that the relatively high level of glucose in Winter honey suggests that it is more likely to crystallise than honey collected at other times. Changes in botanical origin and bee foraging habits throughout the flowering season can also be reflected in fluctuations in the amount of glucose present in a season (Choi et al., 2024; Oyedokun, William, & Mable, 2025; Ali et al., 2020 ; Batool et al., 2018; Laraib et al., 2021 ; Mustafa et al., 2021; Ali et al., 2017).

Sucrose showed a very clear seasonal trend with significantly higher concentration in the Spring. The presence of sucrose in honey is often used as an indicator of the source of nectar and degree of honey maturation because the sucrose is converted to fructose and glucose through the activity of the enzyme, invertase (Ali et al., 2021; Ali et al., 2020; Masood et al., 2024; Fatima et al., 2023; Hussain et al., 2025; Shafique et al., 2026; Hussain et al., 2025). Honey processed in the summer, with an increased sucrose content in Spring honey, may therefore reflect nectar sources naturally higher in sucrose, or perhaps the relative absence of enzymatic hydrolysis during honey processing. However, the sucrose values in all the samples studied were relatively low, indicating good maturity of the honey and thus, confirming authenticity. Similar findings were found in previous research that correlated sucrose variability with the seasonal changes of flower type and nectar characteristics (Mustafa et al., 2021; Oyedokun, William, & Mable, 2025).

In contrast, fructose, glucose and sucrose did not show significant change throughout each season, suggesting a relative stability in response to the environmental changes in each season and/or botanical diversity. The second disaccharide, maltose, is formed during the transformation of nectar and maturation of honey; and its concentration does not fluctuate within the season to a great extent compared with other enzymatic pathways, implying that it is not very sensitive to seasonal changes. This is also in line with earlier studies on the relative constancy in the concentration of maltose in honey (Mustafa et al., 2021).

In general, the results show that the sugar composition of the honey of Apis dorsata is highly affected by seasonal flowering pattern and in relation with the botanical resources in Tehsil Pattoki. The fructose content was very high in summer honey, but relatively high in Winter honey, and relatively high in Spring honey. The seasonal differences are not only due to the variation in the food source in the form of nectar, but may also influence the quality properties of the honey, including sweetness, crystallization properties and consumer acceptance. The present results corroborate with previous reports highlighting the importance of botanical & environmental factors in influencing the sugar profile of honey (Sajid, 2023; Sana et al., 2025; Rikwentishe et al., 2024; Rode et al., 2026; Safi et al., 2023; Khan et al., 2025; Ullah et al., 2025). Additional research using the combined analysis of floral diversity and melissopalynological data would give more insight into the relationship between the seasonal flora and the composition of honey carbohydrates.

This study provides some ideas regarding the quality of honey samples collected from Pattoki area but this study has some drawbacks. This study was restricted to the geographical area of Tehsil Pattoki. The results may not be representative of honey of Apis dorsata from other ecological zones in Pakistan like the northern mountains or the southern deserts due to the fact that the composition of honey is highly dependent on ecological zone, soil and flora. In this study the following important quality markers, which are the key indicators of freshness and heating of the honey, were not examined: critical quality markers (CQMs): Hydroxymethylfurfural (HMF); Diastase activity. These parameters should be added in future studies to obtain a comprehensive quality assessment as per International standards. Lastly, a concurrent melissopalynological (pollen) examination of the actual honey samples would be needed to establish the botanical sources of the pollen grains of each of the seasonal honey samples. It is also advised that a multi-year study be conducted, to consider the influence of inter-annual climate variability on floral cycles and honey quality.

Conclusions

The study concludes that the honey of Apis dorsata of Tehsil Pattoki is a special and good natural product, having very low moisture content and very high carbohydrate content. The year-round supply of a variety of nectar sources (from winter oilseed crops to summer fruit orchards) and the particular dry climatic conditions in the region are key to these superior physico-chemical characteristics. The detailed sugar profiling and enzymatic analysis should be done in the future to fully characterize the medicinal potential of Pattoki honey. It is recommended that advanced testing for antioxidant capacity and antimicrobial properties be done to compare its efficacy with globally accepted therapeutic honeys. Further, routine pesticide residue and environmental contamination testing are important to ensure sustained quality in the region's heavy agricultural production. It is also important to create standard and sustainable harvesting guidelines for wild bee colonies to both minimize impact on the local ecosystem and to maximize economic value. Finally, for future investigations, the inclusion of full pollen analysis will enable botanical certification of each seasonal harvest.

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