

Impact of altitude and season on the nutritional profile of honey from various *Apis* species in Pakistan

Aqsa Saleem¹, Arshad Javid*¹, Ali Hussain², Shahid Mehmood³

¹Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan

²Institute of Zoology, University of Punjab, Lahore, Pakistan.

³Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan

*Email: arshadjavid@uvas.edu.pk

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Abstract

The quality and composition of honey can be influenced by geographic location, altitude, honeybee species, and season. Honey samples were collected from four areas of different altitudes, viz., Bahawalpur (118 m), Kasur (218 m), Kallar Kahar (679 m), and Murree (2291 m), during all four seasons in Pakistan. The collected honey samples were subjected to proximate analysis to evaluate their nutritional composition, including moisture, ash, protein, fat, fiber, and carbohydrate content. The results indicated that honey produced at higher altitudes (Murree) exhibited higher levels of protein, fat, fiber, and ash content, suggesting that environmental factors at these locations contribute to a richer nutrient profile. In terms of species, honey produced by *Apis mellifera* and *Apis cerana* shows higher fructose values as compared to *Apis florea* and *Apis dorsata*. The fructose content (30.45%) in summer was also the highest compared to other seasons. However, other parameters, such as protein, fat, fiber, and carbohydrate values, remained relatively stable across seasons. The study revealed that *Apis mellifera* and *Apis cerana* produced honey with higher fructose concentrations as compared to *Apis florea* and *Apis dorsata*. However, other nutritional components such as protein, fat, fiber, and ash content remained relatively stable across species, suggesting that these traits are more influenced by climatic conditions and geographic location rather than the species of the honeybee. Future studies should focus on the specific floral diversity and climatic conditions to have more refined methods of honey production, enhancing its nutritional and medicinal benefits.

Keywords: *Apis mellifera*, *Apis cerana*, *Apis florea*, *Apis dorsata*, Honey

Introduction

The chemical composition of honey varies significantly due to many factors such as honeybee species, type of vegetation, geographic location, and climatic conditions. Four species in the genus *Apis*, including *A. mellifera*, *A. florea*, *A. dorsata*, and *A. cerana* reported from Pakistan so far (Singh, 2020). The quality of honey can be checked through proximate analysis, such as carbohydrates, proteins, enzymes, amino acids, vitamins, minerals, and other bioactive compounds (Ranneh et al., 2021). However, many other factors such as season and altitude gradient, affect nectar availability and quality (Plos et al., 2023). During spring and summer, when there is availability of flowers, honeybees can collect a wide range of nectars, which leads to better quality of honey produced (Al-Kahtani & Taha, 2021). However, during winter and autumn, when floral resources are scarce, the nutritional content of honey may decline. In addition, the quality of honey is also influenced by altitude gradient as the types of vegetation may vary (Harris et al., 2024). Honey produced in high-altitude areas often exhibits higher antioxidant activity (Wang et al., 2024).

A. mellifera (European honeybee) is widely distributed and known for significant honey producer. *A. mellifera* is a dominant pollinator in many habitats and is highly adaptable to diverse weather conditions (Beaurepaire et al., 2020). *A. florea* (dwarf honeybee) has its distribution in tropical areas and prefers a warm climate (Nagaraja, 2020). *A. florea* have a small colony size and produce a limited quantity of honey, which has medicinal values. *A. dorsata* (giant honeybee) is native to South and Southeast Asia countries and prefers forest and high altitude areas (Nagaraja, 2023). However, *A. dorsata* is known for its large colony and migratory behavior. Migration helps them to explore a wide range of floral resources throughout the year (Vijayan et al., 2023). Honey produced by *A. dorsata* often contains high levels of bioactive compounds, which are linked to strong antioxidant properties (Wu et al., 2022). Similarly, *Apis cerana* (Asian honeybee) is also a native species that plays a crucial role in honey production across Asia. *A. cerana* is adapted to mountainous regions, and its honey has therapeutic values (Nannan et al., 2022). Recent studies have shown that honey produced by *A. dorsata* and *A. cerana* at higher altitudes has higher antioxidant activity and mineral content compared to honey from lower altitudes (Muhammad & Sarbon, 2023).

Altitude significantly influences the nutritional composition of honey due to temperature, humidity, and the type of vegetation. High-altitude regions are characterized by cooler temperatures and unique vegetation (Mohammed, 2022; Siraj et al., 2022). In addition, seasonal

changes further influenced the quality of honey. During warmer months, the nectar collected by honeybees contains higher sugar content can result in honey with a high level of carbohydrate. However, winter months are associated with reduced nectar availability, leading to honey with lower carbohydrate content but potentially higher concentrations of secondary metabolites (Knoll et al., 2020). The seasonal and altitudinal effects can create a dynamic interplay that determines the quality and composition of honey (Vincze et al., 2024). In recent years, there has been a growing interest in exploring the nutritional and medicinal values of honey in many regions. This interest is increased due to the high demand for natural foods and considering honey as a valuable source for promoting health (Bogdanov, 2012; Majtan et al., 2021). Recent studies on the seasonal and altitudinal variations in honey composition provided vital insights into the environmental factors that influence its quality (Prodanović et al., 2024).

The primary aim of this study was to investigate the impact of altitude and season on the nutritional composition of honey produced by different *Apis* species in Pakistan. Specifically, the study aimed to evaluate how altitude influences the nutritional profile of honey, with a particular focus on factors such as protein, fat, fiber, and ash content. Additionally, the research sought to assess the seasonal variations in honey's nutritional content, particularly fructose levels, and to examine how these variations might be linked to nectar availability during different times of the year.

Material and methods

Sample collection and storage

Honey samples were collected from natural hives of all four honeybee species from districts Bahawalpur (118 m), Kasur (218 m), Kallar Kahar (679 m), and Murree (2291 m) in Pakistan (Figure 1). Honey sampling was done in all four seasons, including winter, spring, summer, and autumn. Five honey samples (n=5) were taken from each sampling site during all four seasons. At the time of honey sample collection, qualitative parameters, viz., pH, color, and taste of the honey were recorded. The honey samples were then placed in sterile plastic containers to avoid any contamination and were stored in a cool and dark place for further analysis (DeGrandi-Hoffman et al., 2021).

Proximate analysis

Proximate analysis of honey samples was performed according to standard AOAC methods (AOAC 999.10, 2005), which are well-established for nutritional analysis of honey. The parameters assessed included moisture, ash, protein, fat, fiber, and carbohydrate content.

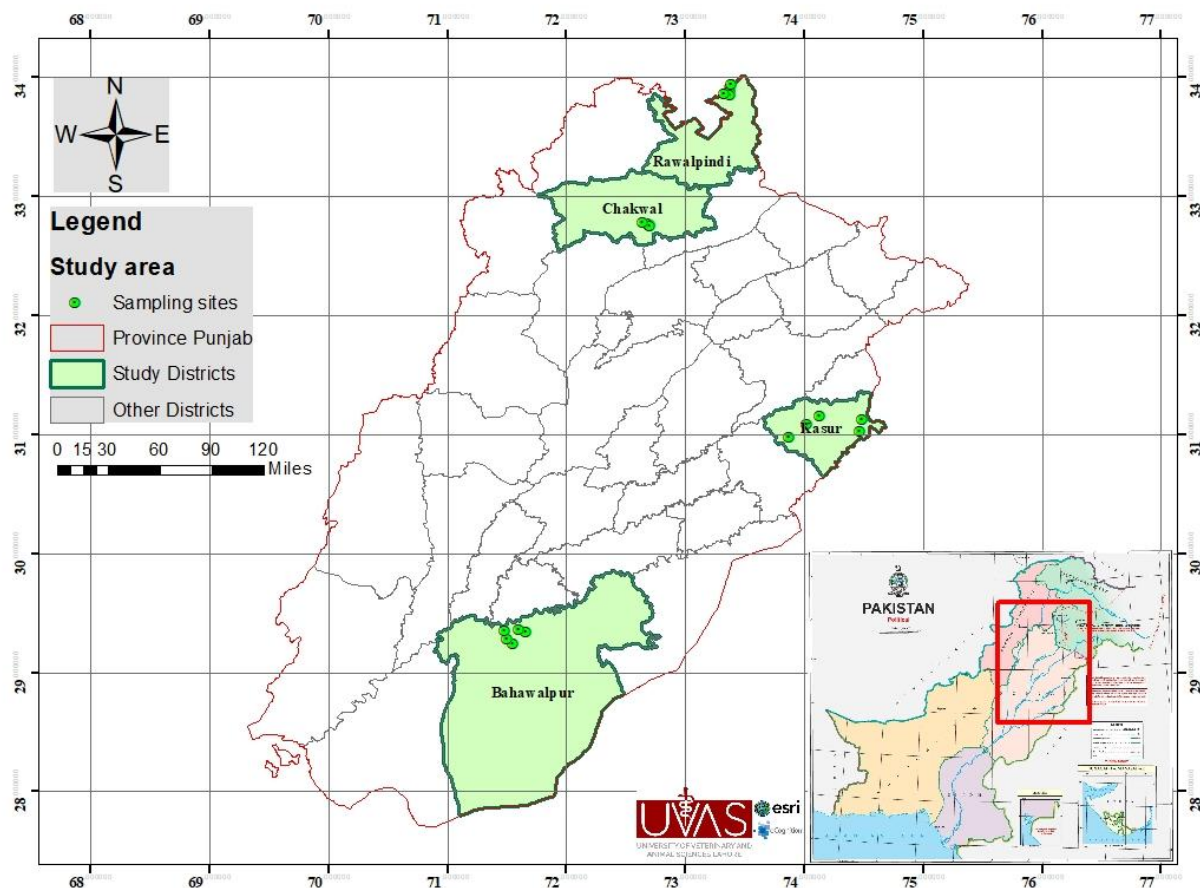


Figure 1. GIS-based map and GPS pinpoints of honey sample collection

Moisture content

Moisture content was determined by the gravimetric method. A 5 g portion of honey was placed in a pre-weighed petri dish and dried at 105°C for 18 hours until a constant weight was achieved (Chua et al., 2004). The percentage moisture was calculated based on the weight loss during drying using the following formula;

$$\% \text{Moisture} = \frac{W1 - W2}{Wt \text{ of sample}} \times 100$$

Ash content: Ash contents of honey samples were 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 the formula (AOAC 999.10, 2005);

$$\% \text{Ash} = \frac{\text{Difference in Wt of Ash} \times 100}{\text{Wt of Sample}}$$

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 distilled, and its concentration was determined by titration. The protein content was calculated using a nitrogen-to-protein conversion factor using the following formula;

$$\% \text{ Crude Protein} = 6.25 \times \% \text{N} \text{ (* . Correction factor)}$$

$$\% \text{N} = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt of the sample} \times V}$$

Where:

S = Sample titration reading

B = Blank titration reading

N = Normality of the sample after digestion

D = Dilution of the sample after digestion

V = Volume taken for distillation

0.014 = Milliequivalent 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 nonpolar solvent. The extracted fat was then weighed to calculate the percentage.

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

Fiber content

Soluble and insoluble dietary fibers were analyzed using enzymatic digestion. The remaining undigested fiber was measured and expressed as a percentage of the total sample weight (AOAC 999.10, 2005).

$$\% \text{Crude Fiber} = \frac{W_1 - W_2 \times 100}{W_0}$$

Carbohydrate and energy calculation

Carbohydrate and energy were estimated using the following formulas (Charrondiere et al., 2004).

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

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

Sugar analysis by HPLC

Major sugar components such as glucose, fructose, sucrose, and maltose were quantified using High-Performance Liquid Chromatography (HPLC). Honey samples were diluted with ultrapure water 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 μ m, 4.6 $\times$ 150 mm) was used for separation and kept at 25°C throughout the analysis.

Statistical analysis

Data were analyzed using Factorial ANOVA under the General Linear Model (GLM) procedures in SAS software (version 9.1). The effects of location, species, and season were considered as main factors. Significant means were compared using Duncan's multiple range test (DMRT) at a significant level of $p \leq 0.05$, following the methodology described by (Steel et al., 1997).

Results

The present one-year study was conducted from January to December 2024 across four different locations in Pakistan.

Effect of location on the nutritional profile of honey

The location of honey collection had a significant effect on the nutritional composition of honey ($p < 0.0001$) for most of the traits. Statistical analysis revealed that there are differences in nutritional composition across the four locations, such as Murree, Kallar Kahar, Kasur, and Bahawalpur (Table 1). Murree produced honey with the highest pH (4.42) and protein content (6.45%), while Bahawalpur recorded the lowest values, 3.93, 5.89% for pH and protein, respectively. Honey from Murree also had the highest fat content (1.07%) and fiber content (3.10%), while Bahawalpur samples exhibited the lowest fat (0.57%) and fiber (2.48%) values. The difference in fat and fiber content indicates that the nutritional density of honey decreases at lower altitudes. Moisture content was higher in Murree (9.42%) compared to Bahawalpur (8.89%), suggesting that honey from higher altitudes tends to have more moisture. The ash content followed a similar pattern, with Murree having the highest ash percentage (1.72%) and Bahawalpur the lowest (1.09%). Fructose content was highest in Murree (30.70%), followed by Kallar Kahar

(30.50%), Kasur (30.37%), and Bahawalpur (30.11%). Similarly, glucose content showed a decreasing trend from Murree (24.23%) to Bahawalpur (23.66%). Sucrose and maltose content showed a consistent decrease from Murree to Bahawalpur. Carbohydrate content remained relatively stable across all locations, and values range between 55.67% and 56.24%. Similarly, energy values were also quite consistent, ranging from 306.05 Kcal in Bahawalpur to 306.65 Kcal in Murree (Figure 2). The nutritional profile of honey in terms of protein, fat, fiber, and sugar content seems to be influenced by the altitude, with higher-altitude honey generally containing more nutrients. However, carbohydrate and energy values remained relatively stable across all locations. This suggests that environmental factors such as altitude, floral diversity, and local climatic conditions play a significant role in determining the nutritional composition of honey.

Table 1. Nutritional profile of honey produced by different *Apis* species at various locations in Pakistan

Traits	Murree	Kallar Kahar	Kasur	Bahawalpur	SEM
pH	4.42 ^a	4.30 ^b	4.03 ^c	3.93 ^d	0.11
Protein	6.45 ^a	6.26 ^b	6.04 ^c	5.89 ^d	0.12
Fats	1.07 ^a	0.95 ^b	0.72 ^c	0.57 ^d	0.11
Fiber	3.10 ^a	2.80 ^b	2.68 ^c	2.48 ^d	0.13
Moisture	9.42 ^a	9.20 ^b	9.02 ^c	8.89 ^d	0.11
Ash %	1.72 ^a	1.54 ^b	1.28 ^c	1.09 ^d	0.14
Carbohydrates	56.24 ^a	56.04 ^b	55.78 ^c	55.67 ^d	0.13
Energy values	306.65 ^a	306.42 ^b	306.21 ^c	306.05 ^d	0.13
Fructose	30.70 ^a	30.50 ^b	30.37 ^c	30.11 ^d	0.12
Glucose	24.23 ^a	24.05 ^b	23.86 ^c	23.66 ^d	0.12
Sucrose	2.25 ^a	2.03 ^b	1.89 ^c	1.67 ^d	0.12
Maltose	9.07 ^a	8.84 ^b	8.57 ^c	8.41 ^d	0.15

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

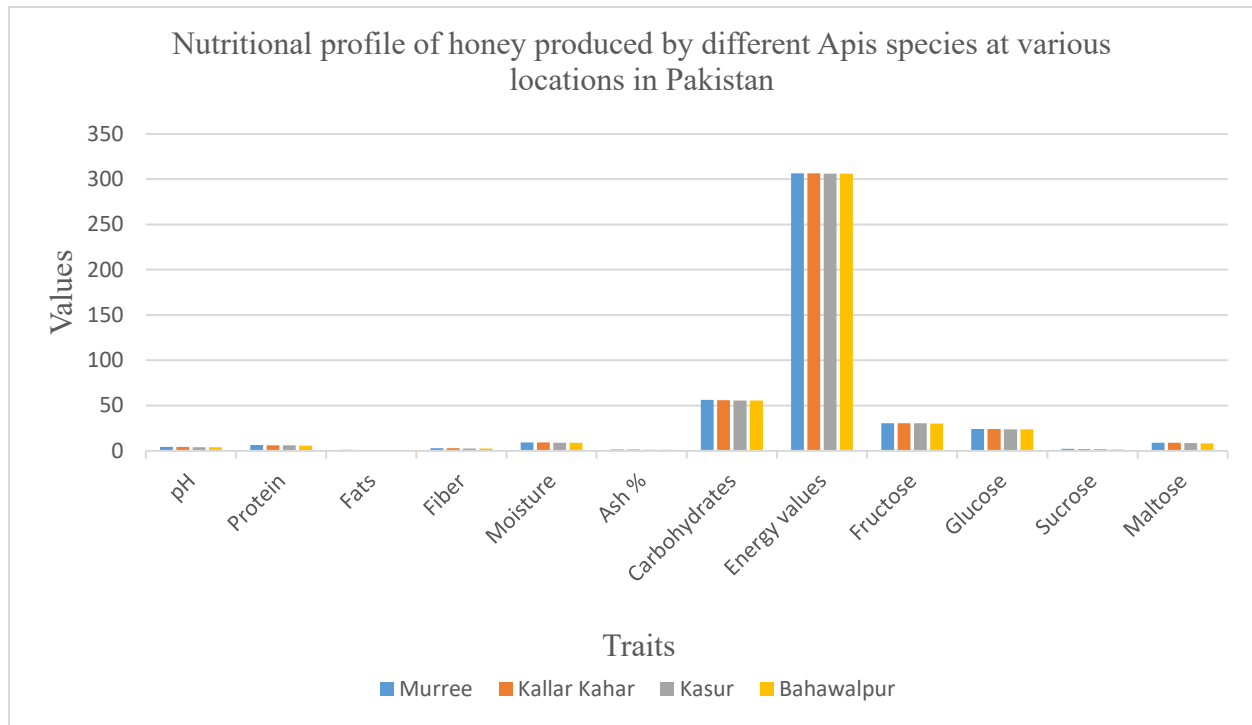


Figure 2. Comparison of the nutritional profile of honey produced by different *Apis* species across various locations in Pakistan

Effect of species on the nutritional profile of honey

The *Apis* species also influenced the nutritional composition of honey, while there were some species-specific differences in fructose content was observed but most of the other nutritional traits did not vary significantly between species. Statistical analysis of the nutritional profile of honey produced by different *Apis* species is presented in Table 2. *A. mellifera* produced honey with the highest fructose content (30.44%) followed by *A. cerana* (30.50%). *A. florea* and *A. dorsata* had slightly lower fructose levels with values of 30.38% and 30.36%, respectively. The difference in fructose levels between species suggests that certain *Apis* species may produce honey with slightly sweeter characteristics. The pH values were similar across all species, ranging from 4.15 to 4.19, indicating minimal species-specific differences in honey acidity (Figure 3). Protein content, fats, fiber, moisture, ash content, carbohydrates, energy values, glucose, sucrose, and maltose showed no significant variation among the species, suggesting that these traits are less influenced by the species of the *Apis* involved.

Table 2. Nutritional profile of honey produced by different *Apis* species in Pakistan

Traits	<i>A. mellifera</i>	<i>A. florea</i>	<i>A. dorsata</i>	<i>A. cerana</i>	SEM
pH	4.18	4.16	4.15	4.19	0.01
Protein	6.13	6.15	6.20	6.17	0.01

Fats	0.78	0.82	0.86	0.85	0.02
Fiber	2.82	2.75	2.73	2.77	0.02
Moisture	9.13	9.09	9.14	9.17	0.02
Ash %	1.35	1.45	1.42	1.40	0.02
Carbohydrates	55.95	55.94	55.89	55.95	0.01
Energy values	306.35	306.34	306.33	306.32	0.01
Fructose	30.44 ^{ab}	30.38 ^b	30.36 ^b	30.50 ^a	0.03
Glucose	23.96	23.97	23.97	23.90	0.02
Sucrose	1.95	1.96	1.95	1.99	0.01
Maltose	8.73	8.68	8.76	8.72	0.02

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

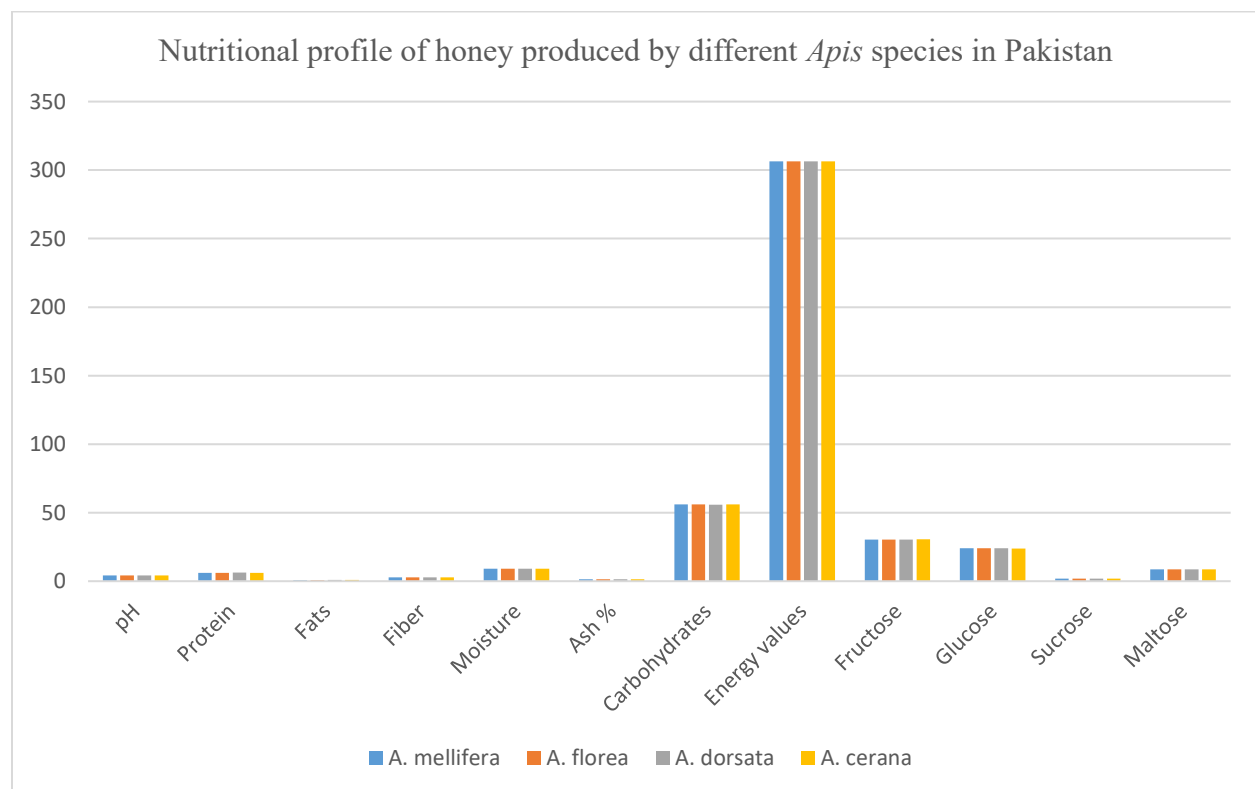


Figure 3. Comparison of the nutritional profile of honey produced by different *Apis* species in Pakistan

Effect of season on the nutritional profile of honey

Seasonality had a significant effect on certain nutritional traits, particularly ash content and fructose levels. The nutritional profile of honey produced by different *Apis* species during different seasons is presented in Table 3. Fructose levels varied significantly across seasons ($p = 0.034$).

The highest fructose content was observed in summer (30.45%) followed by spring (30.40%), winter (30.44%), and autumn (30.38%). This indicates that honey produced during warmer months may be slightly sweeter due to higher fructose concentrations. Ash content was significantly higher in winter (1.48%) as compared to other seasons, which ranged between 1.37% and 1.39% (Figure 4). This suggests that the mineral content in honey may increase during colder months, possibly due to changes in the floral composition available for nectar collection. No significant seasonal differences were observed for pH, protein, fats, fiber, moisture, carbohydrates, energy values, glucose, sucrose, and maltose. These traits remained relatively stable across the four seasons.

Table 3. Nutritional profile of honey produced by different *Apis* species during different seasons in Pakistan

Traits	Summer	Spring	Winter	Autumn	SEM
pH	4.23	4.16	4.16	4.12	0.02
Protein	6.11	6.19	6.15	6.19	0.02
Fats	0.80	0.85	0.83	0.84	0.01
Fiber	2.80	2.76	2.74	2.76	0.01
Moisture	9.13	9.14	9.15	9.11	0.01
Ash %	1.37 ^b	1.37 ^b	1.48 ^a	1.39 ^b	0.03
Carbohydrates	55.94	55.93	55.89	55.98	0.02
Energy values	306.29	306.37	306.36	306.31	0.02
Fructose	30.45	30.40	30.44	30.38	0.02
Glucose	23.91	23.93	24.01	23.95	0.02
Sucrose	1.93	2.01	1.95	1.96	0.02
Maltose	8.72	8.74	8.70	8.73	0.01

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

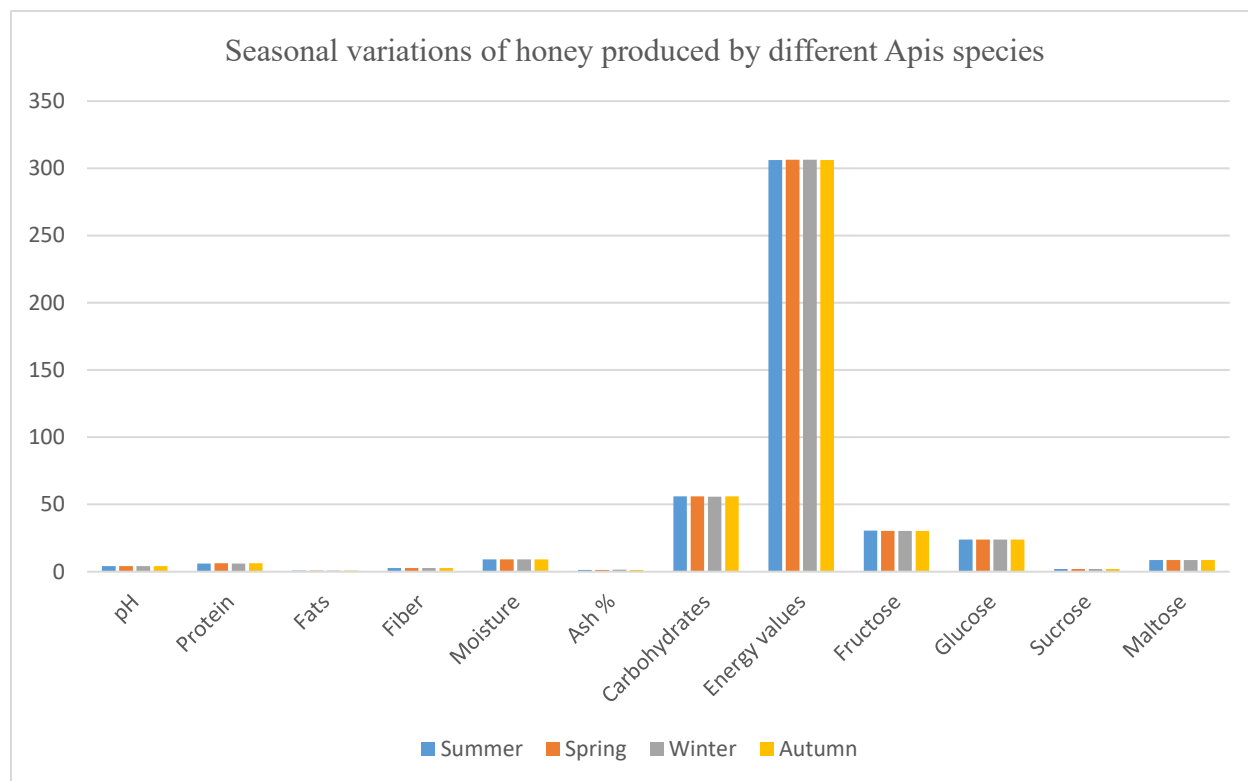


Figure 4. Comparison of the nutritional profile of honey produced by different *Apis* species during different seasons in Pakistan

Interactions between location, species, and season

The interaction effects between location, species, and season were investigated for all traits, and the results are presented in Table 4. Location had a highly significant effect on nearly all traits ($p < 0.0001$). This is especially true for pH, protein content, fats, fiber, moisture, ash (%), carbohydrates, energy values, and sugar content (fructose, glucose, sucrose, and maltose). These results suggested that the geographical location plays a crucial role in determining the nutritional composition of honey. Factors, viz., climate, altitude, and floral diversity at different locations, significantly influence honey's characteristics.

The species factor had a mixed effect, with some traits showing significant differences and others not. The only trait that showed a significant effect due to species was fructose. This indicates that different *Apis* species may produce honey with varying fructose concentrations. Other traits, such as protein, fats, fiber, moisture, ash content, carbohydrates, energy values, glucose, sucrose, and maltose showed no significant differences between species ($p > 0.05$). This suggests that while species may influence some components (like fructose), they have little impact on many other nutritional traits.

Season had a significant effect observed for ash content ($p = 0.045$) and fructose content ($p = 0.034$). This implies that seasonal variations can alter certain nutritional components of honey. The remaining traits (pH, protein, fats, fiber, moisture, carbohydrates, energy values, glucose, sucrose, and maltose) showed no significant seasonal variation ($p > 0.05$). This suggests that the seasonal impact on honey's nutritional profile may be less pronounced for most of the traits assessed.

Interaction effects between location, species, and season were generally non-significant for most of the traits. Several other interactions, including LC $\times$ SP (location $\times$ species), LC $\times$ SN (location $\times$ season), and SP $\times$ SN (species $\times$ season), had p-values greater than 0.05, indicating no significant impact on the traits of interest. These results further suggest that the main factors (location, species, and season) have independent effects on the nutritional profile of honey, with limited influence from their interactions. The only significant interaction was observed for sucrose content ($p = 0.01$), suggesting that the effect of location and season on sucrose content was dependent on the species of *Apis* involved.

Table 4. Analysis of variance of different treatment and their interaction

Traits	LC	SP	SN	LC $\times$ SP	LC $\times$ SN	SP $\times$ SN	LC $\times$ SP $\times$ SN
pH	<0.0001	0.846	0.131	0.991	0.414	0.338	0.508
Protein	<0.0001	0.492	0.317	0.092	0.783	0.408	0.34
Fats	<0.0001	0.342	0.694	0.402	0.258	0.496	0.15
Fiber	<0.0001	0.225	0.697	0.686	0.054	0.141	0.57
Moisture	<0.0001	0.486	0.895	0.219	0.806	0.333	0.555
Ash %	<0.0001	0.151	0.045	0.292	0.416	0.108	0.356
Carbohydrates	<0.0001	0.546	0.38	0.182	0.868	0.614	0.523
Energy values	<0.0001	0.915	0.246	0.526	0.916	0.203	0.274
Fructose	<0.0001	0.007	0.354	0.941	0.828	0.685	0.034
Glucose	<0.0001	0.382	0.136	0.416	0.168	0.974	0.713
Sucrose	<0.0001	0.76	0.395	0.694	0.01	0.51	0.281
Maltose	<0.0001	0.378	0.822	0.319	0.247	0.111	0.275

LC = location; SP = species; SN = Season.

Discussion

The present study assessed the impact of altitude and season on the nutritional profile of honey produced by various *Apis* species in Pakistan. The findings of our study revealed that both the location and season significantly influence the composition of honey. Honey samples collected from higher altitudes, viz., Murree, showed significantly higher levels of nutrients like pH, protein, fat, fiber, and ash content as well as moisture. The findings are in line with Wang et al. (2024), who documented that environmental factors and altitude have a significant impact on the

biochemical composition of honey. Low altitude regions are often warmer and with less floral diversity, which might result in less nutrient-rich nectar. On the other hand, the cooler regions have a varied flora of higher altitudes that supports the production of honey with a richer nutrient profile. The protein and fat contents were highest in honey samples collected from Murree, which could be due to the variety of plants that provide more nutrients and pollen sources. However, honey samples collected from Bahawalpur have lower levels of moisture, fructose, and protein. These findings suggested that honey produced in arid areas is more concentrated in sugars and has lower levels of nutrients, viz., proteins and fats. Aqueel et al. (2023) reported that climatic conditions and altitude have a significant effect on the chemical composition of honey. Moreover, the ash content was also highest in Murree (1.72%) and lowest in Bahawalpur (1.09%). This could be due to the mineral-rich nectar of certain floral species at higher altitudes. Wu et al. (2020) documented that higher altitude areas often have plants with different mineral profiles, which could explain the higher mineral content of honey produced. Interestingly, the altitude clearly affected certain traits of honey, but the carbohydrate and energy values remained stable across all the sampling locations. Honey from all four locations showed similar energy values (ranging from 306.05 Kcal in Bahawalpur to 306.65 Kcal in Murree). The carbohydrates in honey is mainly composed of glucose, fructose, and sucrose contents and despite variations in sugar contents, the overall carbohydrate contents did not show any significant variations across all locations. The results are in line with Ranneh et al. (2021), who reported that the carbohydrate content of honey is primarily determined by the balance of sugars, and it does not fluctuate significantly across different geographic locations. Pakistan is represented by four species in the genus *Apis*, namely *A. mellifera*, *A. florea*, *A. dorsata*, and *A. cerana*. The nutritional composition of honey produced by various *Apis* species varies (Mohammed MEA, 2022). The results of our study revealed that there were some species-specific differences in honey composition. The honey produced by *A. mellifera* and *A. cerana* had the highest fructose content at 30.44% and 30.50% respectively, as compared to the honey produced by *A. florea* and *A. dorsata*, which had 30.38% and 30.36% respectively. These results indicated that various honeybee species have different preferences for nectar and pollens. The quality of nectar and pollens ultimately influences the sweetness and sugar profile of honey (Nagaraja N. 2020). Fructose is the primary sugar content in the honey, and it is the main factor in determining its sweetness. The variations in sugar composition of honey is due to foraging behaviour and the specific floral resources (Ali et al., 2021). Other parameters, viz., pH, protein content, fat, fiber, moisture, ash, and glucose, remained non-significant among honey samples of

different *Apis* species. This finding suggested that these traits are more influenced by climatic conditions than by the honey bee species themselves. These findings are in line with previous studies as reported by Geană et al. (2020), who documented that honey bee species can only influence the flavor and sweetness of the honey they produce, but the overall nutritional composition tends to be more strongly affected by geographical location and seasons.

Nutritional composition of honey based on season remained non-significant during the present study. The results of our study showed that fructose content was highest in honey samples collected during summer and spring, with the maximum concentration reported in summer (30.45%). This might be due to increased availability of nectar during warmer months, which may provide honeybees with a higher quantity of nectar. On the other hand, during autumn and winter, there is limited availability of nectar. The honey produced during colder months had lower fructose. Knoll et al. (2020) reported that the seasonal variation in fructose content reveals that different types of nectar are available to honeybees throughout the year as flowers bloom and die off with the changing seasons. Ash contents were also high in honey samples collected during winter (1.48%) as compared to other seasons. The seasonal increase in mineral contents is consistent with a previous study by Lebedev et al. (2023), who reported that the mineral contents of honey can vary with the season. The decrease in moisture level during colder months could also contribute to the high ash content, as less water is available, leading to a more concentrated mineral profile. However, all other traits, such as protein, fats, fiber, glucose, sucrose, and maltose, did not show any significant seasonal variations. As reported by Quinlan & Grozinger (2023) that these factors are more stable and less influenced by seasons. This consistency is might be due to the overall nutrient composition of the nectar, which may not vary significantly in different seasons for many plants (Vincze et al., 2024).

During the present research, the location, species, and season effects were significant, but overall generally their interactions were not. The only significant interaction was found on the sucrose concentrations, in which the location by season effect depended on the *Apis* species. The finding indicated that the fluctuation of sucrose content could relate to the foraging manner of bees and the nectar source, which is also supported by the previous investigations. The interaction between environmental and honeybee behavior influences honey nutritional properties, and our results are concordant with supported finding previously reported.

Conclusions

The study provides valuable insights into the nutritional composition of honey produced by various *Apis* species across different altitudes and seasons in Pakistan. It was observed that honey produced at higher altitudes, such as Murree, showed higher levels of protein, fat, fiber, and ash contents. Additionally, the seasonal variations were observed in fructose content, with the highest levels in summer. These findings highlight the importance of nectar availability during warmer months of the year that influence the honey composition. The results revealed that *Apis mellifera* and *Apis cerana*, produced honey with higher fructose concentrations as compared to *Apis florea* and *Apis dorsata*. However, nutritional components such as protein, fat, fiber, and ash content remained relatively stable across species, suggesting that these traits are more influenced by climatic factors and geographic location rather than the species of the honeybee. It is recommended that future studies should focus on the specific floral diversity and climatic conditions to have more refined methods of honey production, enhancing its nutritional and medicinal benefits.

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