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NUTRITIONAL PROFILING OF PAKISTANI ACORN

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Abstract

This work provides a comprehensive nutritional characterization of 4 acorn species native to Pakistan, including *Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana*, examining their physicochemical properties, mineral composition, fatty acid profile, bioactive compounds, and antioxidant activity. Results revealed that the targeted acorns were rich in carbohydrates (39.79–42.68%) and contained adequate levels of protein (5.52–8.32%) and lipids, along with substantial amounts of essential minerals, particularly potassium, calcium, and iron. These characteristics highlight their potential as a micronutrient-rich dietary resource, while the absence of gluten-containing proteins suggests suitability for gluten-free food formulations. Bioactive profiling confirmed the presence of phenolic and flavonoid compounds, with *Q. dilatata* showing the highest total phenolic and flavonoid content. DPPH assays indicated moderate to high radical-scavenging activity across all species. GC-MS analysis identified oleic, linoleic, and palmitic acids as the predominant fatty acids among the potential phytochemicals, and correlation analysis revealed notable relationships among nutritional, mineral, fatty acid, and bioactive parameters. Collectively, these findings position Pakistani acorns as an underutilized, nutritionally valuable, and potentially gluten-free resource with promising applications in functional food and nutraceutical development, while establishing a foundation for further exploration of these indigenous plant resources.

Key words: *Quercus baloot*; *Quercus dilatata*; *Quercus glauca*; and *Quercus incana*; Nutritional composition; Antioxidant activity; Functional foods

Introduction

Quercus (oak), a genus in the family Fagaceae, comprises approximately 600 species distributed across the temperate and subtropical regions of the world. Oaks are important components of forest ecosystems, contributing to soil stabilization and carbon sequestration (Szabłowska & Tańska, 2024; Tizemmour *et al.,* 2024). Their fruits, commonly known as acorns, have historically served as food for humans and animals owing to their nutritional value and resilience under diverse environmental conditions (Suo *et al.,* 2025; Bhunia, 2026). In recent years, scientific interest in acorns has grown due to their potential as sustainable, nutrient-rich food resources, particularly in the context of global food insecurity and the need to utilize underexploited crops (Gil-Martín *et al*., 2022; Farooq & Yu, 2024; Stoin *et al.,* 2021).

Acorns contain substantial amounts of carbohydrates and dietary fiber, along with essential fatty acids and bioactive compounds such as phenolic and flavonoids, which contribute to their antioxidant properties. Previous studies report that acorns are notable sources of unsaturated fatty acids, particularly oleic and linoleic acids as well as minerals such as potassium, calcium, and magnesium (Vinha *et al.,* 2016; Szabłowska & Tańska, 2024). These characteristics support their potential use as functional foods and as ingredients in nutraceutical and other value-added applications (Güneş *et al.,* 2025; Farazi *et al.,* 2024). Their traditional use in flour, bread, porridge, and other cereal-based products further illustrates their versatility and suitability for incorporation into modern food systems.

Despite their nutritional importance, acorns remain underutilized in many regions of the world, including Pakistan, where 6 indigenous *Quercus* species are widely distributed across the mountainous regions of the norther Pakistan (Gillani *et al.,* 2023; Muhammad *et al.,* 2023). Species such as *Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana* are fairly common in these areas, yet their nutritional composition, phytochemical characteristics, and potential applications remain insufficiently investigated. This gap is particularly significant in relevance of such resources to sustainable food systems and strategies for addressing nutritional deficiencies in these area. Although acorns are globally recognized as sustainable and nutritious food resources, comprehensive comparative studies integrating the physicochemical, nutritional, bioactive, and functional characteristics of native Pakistani *Quercus* species remain scarce, particularly for evaluating nutritional profiles and identifying species with superior functional properties.

Studies on European and American oak species, including *Q. robur*, *Q. ilex*, and *Q. alba*, have demonstrated the nutritional and bioactive potential of acorns, with variation in proximate composition, fatty acid profiles, and antioxidant activity attributed to geographic origin, environmental conditions, and genetic factors (Stanković *et al.,* 2022).

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Beyond conventional nutritional and phytochemical analysis, recent studies have increasingly examined associations between compositional characteristics and biological activity (Romanić *et al.,* 2021; Radojičić *et al.,* 2021). Correlation and multivariate approaches, including principal component analysis, can help elucidate relationships between nutritional constituents such as phenolic compounds and fatty acids and functional properties like antioxidant activity, offering further insight into compositional patterns linked to bioactivity and guiding future research on health-promoting food resources. This knowledge gap is particularly relevant given the diversity of indigenous *Quercus* species in Pakistan and their potential contribution to food security and improved dietary quality.

Seven species of Quercus are reported from Pakistan, among these, 4 species (*Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana*) were selected for the present investigation based on their relative abundance, wide geographical distribution, local ecological dominance, and traditional dietary usage in the mountainous northern regions of Pakistan. In view of the above, and to the best of our knowledge, the present study is the first to comprehensively evaluate the nutritional and bioactive profiles of these selected indigenous acorn species. Specifically, the study aimed to (i) determine the proximate composition and mineral content of the acorns; (ii) characterize their fatty acid profiles using GC-MS; (iii) evaluate total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using the DPPH assay; and (iv) assess relationships and patterns among nutritional, mineral, fatty acid, and bioactive parameters through correlation analysis, heatmap visualization, and principal component analysis (PCA). This study aims to provide comparative evidence on the nutritional and functional potential of these underexplored Pakistani *Quercus* species and to identify species with promising nutritional and bioactive characteristics for potential food and nutraceutical applications.

Materials and Methods

Geographical distribution of Oak species: Seven species of Quercus are reported from Pakistan, namely *Q. baloot*, *Q. dilatata*, *Q. glauca*, *Q. incana*, *Q. robur*, *Q. semecarpifolia*, and *Q. floribunda* (Muhammad *et al.,* 2023; Gillani *et al.,* 2023). Oak species in Pakistan exhibit distinct distribution patterns in relation to climatic conditions and elevation. *Q. baloot* and *Q. incana* are predominantly associated with dry temperate environments, whereas *Q. dilatata* is more commonly found in moist temperate habitats influenced by summer monsoon precipitation. In contrast, *Q. glauca* occurs less frequently, being restricted to limited moist temperate regions of Pakistan. The geographical distribution of the reported oak species in Pakistan is presented in Figs. 1 and 2.

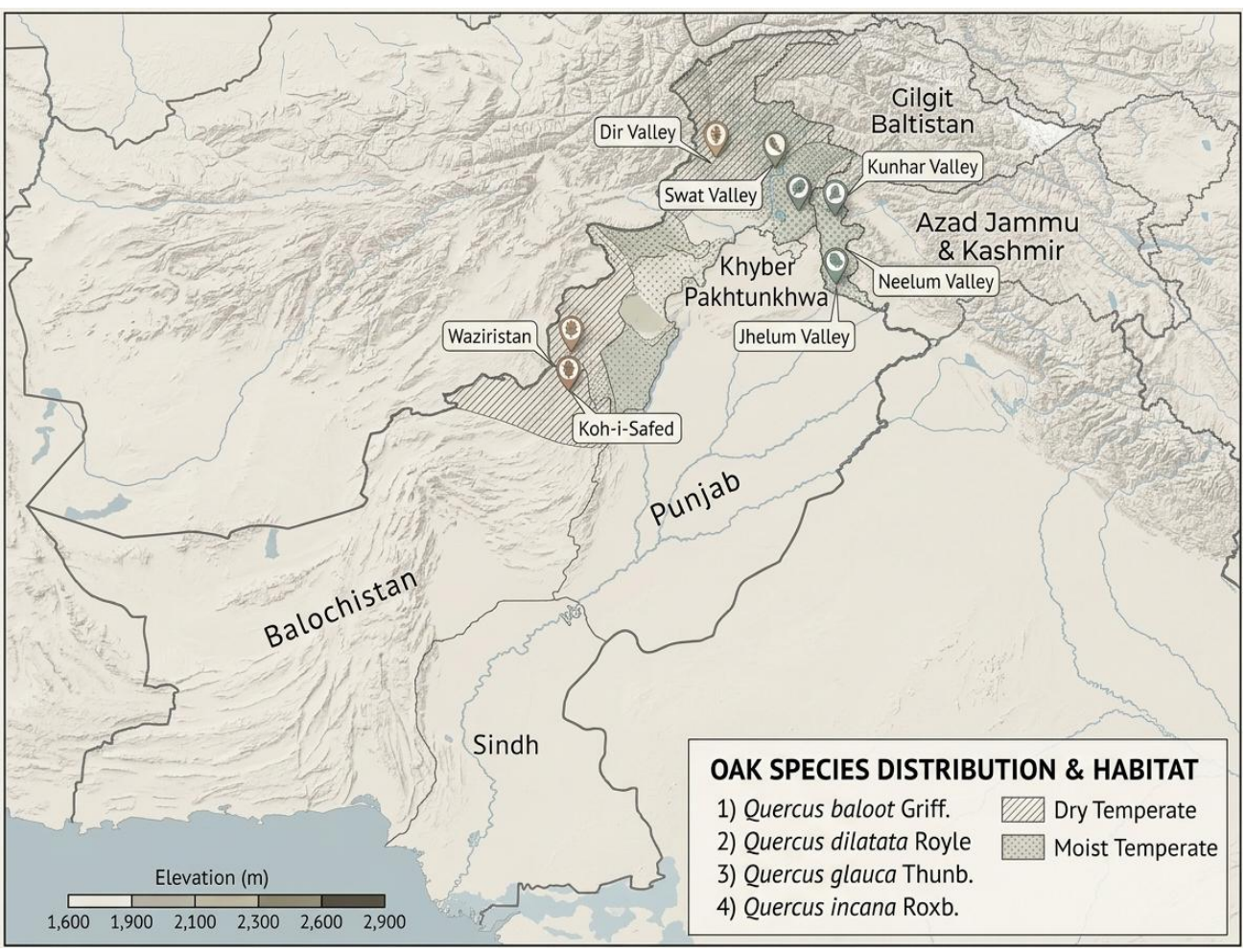


Fig.1. Map showing the location of *Quercus* species in Pakistan along with the location of sampling and collection across northern and western Pakistan. Map highlights key sampling regions across major valleys (Neelum, Kunhar, Jhelum, Dir, Swat, Waziristan, and Koh-i-Safed) spanning elevations from 1,600 to 2,900 m.

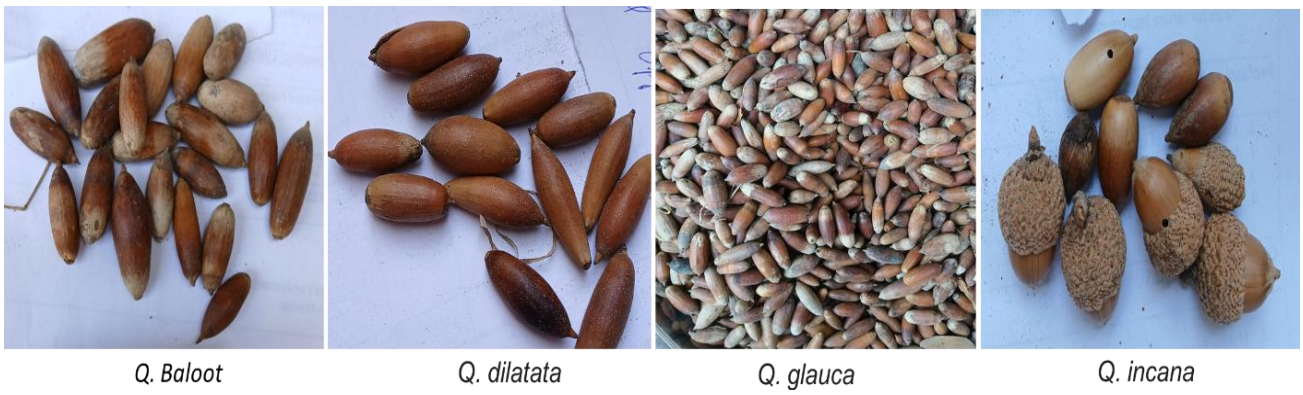


Fig. 2. Illustration of acorn nuts from selected oak species found in Pakistan: (a) *Quercus baloot* Griff, (b) *Quercus dilatata* Royle, (c) *Quercus glauca* Thunb, and (d) *Quercus incana* Roxb.

Sample collection, identification, and preparation: Acorns of 4 *Quercus* species including *Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana* were collected from their respective natural habitats in Pakistan. *Q. baloot* acorns were collected from South Waziristan, *Q. dilatata* and *Q. glauca* from Upper Swat, and *Q. incana* from Upper Dir. Sampling was conducted during the natural acorn harvesting season (September to November 2024), when the fruits had reached physiological maturity. Mature, intact, and apparently healthy acorns were selected, while damaged, diseased, insect-infested, or visibly immature acorns were excluded. Maturity was assessed based on external morphological characteristics, particularly the development of the characteristic mature brown coloration and natural detachment from the cupule or tree. Samples were transported to the laboratory and processed according to the procedures described below in preparation for subsequent nutritional, mineral, fatty acid, and bioactive analyses.

Sampling design and biological replication: For each *Quercus* species, mature and apparently healthy trees were selected within the respective sampling locations. Five individual trees were sampled per species, with approximately 10-20 mature acorns collected from each tree. Acorns were collected from different portions of each selected tree to ensure a representative sample. Fruits showing visible damage, insect infestation, disease symptoms, or signs of immaturity were excluded. Acorns from individual trees were kept separate during sampling and subsequently processed according to the analytical requirements of the study. For each analytical determination, laboratory measurements were performed in triplicate, and results are expressed as mean ± standard deviation. The individual tree was considered the biological sampling unit, while repeated measurements of the same sample were treated as analytical replicates.

Sample preparation and storage: The collected acorns were cleaned to remove adhering debris and foreign material and transferred to a clean laboratory environment. Samples were roasted at 200°C for 15 min in a laboratory oven to facilitate shell removal, then allowed to cool to room temperature before further processing. The kernels were manually separated, dried in a hot-air oven at 60°C to constant weight, ground into a fine flour, and stored in airtight containers until analysis. All subsequent proximate, mineral, fatty acid, total phenolic, total flavonoid, and antioxidant analyses were performed using roasted acorn flour rather than raw acorn flour. Storage temperature and duration were not explicitly recorded in the experimental documentation and are therefore not reported.

Proximate composition analysis: Proximate composition including moisture, crude ash, crude protein, crude fat, and total dietary fiber was evaluated according to standard methods (Anon., 2019; Ajo *et al.,* 2020).

Moisture content: Moisture content was determined using the oven-drying method (Konjević *et al.,* 2021). Samples were dried at 100°C until a constant weight was obtained.

Ash content: Ash content, representing the total inorganic mineral residue, was determined by incinerating the samples in a muffle furnace (Branković *et al.,* 2021) at 550–650°C until a constant weight was obtained.

Protein content: Crude protein content was determined using the Kjeldahl method (Moravčević *et al.,* 2021). Nitrogen content was measured and converted to crude protein using a factor of 6.25.

Fat content: Crude fat content was determined by Soxhlet extraction using hexane as the extraction solvent (Stamenov *et al.,* 2021). The samples were extracted for 6–8 h, after which the solvent was evaporated and the extracted fat was weighed.

Crude fiber content: Crude fiber content was determined by acid and alkaline digestion (Castro *et al.,* 2022). This method estimates the indigestible fraction of plant material, including cellulose and lignin.

Carbohydrate content: Carbohydrate content was calculated by difference, based on the proportions of moisture, protein, fat, ash, and crude fiber, and expressed as a percentage. Carbohydrate content was determined by difference using the following equation:

$$\text{Carbohydrate (\%)} = 100 - [\text{moisture (\%)} + \text{protein (\%)} + \text{fat (\%)} + \text{ash (\%)} + \text{crude fiber (\%)}]$$

Mineral composition analysis: Atomic absorption spectrophotometry (AAS) was used to determine the concentrations of microelements (Fe, Zn, Cu, and Mn) following standard analytical procedures (Anon., 2019; Ajo *et al.,* 2020). Flame photometry and spectrophotometric methods were used to determine the macroelements Ca, Mg, Na, K, and P (Gillani *et al.,* 2023). Samples were treated with nitric acid (HNO_3), filtered, and diluted to a standard volume. Calibration curves were prepared using appropriate standard solutions.

GC–MS analysis (Fatty Acid Profiling): The fatty acid composition of the *Quercus* species was determined by GC–MS following lipid extraction and derivatization of the fatty acids into their corresponding fatty acid methyl esters (FAMEs). Analysis was performed using a GC-MS-QP2010 Plus system (Shimadzu, Japan) installed at the Medicinal Botanic Centre, PCSIR Laboratories, Peshawar. Samples were introduced in split injection mode at a split ratio of 20:1, with the injector temperature maintained at 240°C. Helium was used as the carrier gas under linear velocity control, with a column flow rate of 3.29 mL/min, total flow of 77.1 mL/min, linear velocity of 65.8 cm/s, and purge flow of 8.0 mL/min (Ilyas *et al.,* 2024). The oven temperature program began at 50°C for 1 min, increased at 5°C/min to 150°C, followed by a further increase at 2°C/min to 175°C (held for 4 min), and finally increased at 10°C/min to 220°C, with a final hold of 5 min. The ion source and interface temperatures were maintained at 250°C and 240°C, respectively. Electron ionization (EI) was employed, with a solvent cut time of 2.20 min; mass spectra were acquired in scan mode over an m/z range of 85–380, with a scan speed of 625 and an event time of 0.50s. The total chromatographic run time was approximately 46.8 min. Fatty acid methyl esters were identified by comparing retention times and characteristic mass spectral fragmentation patterns with those in the NIST mass spectral library and relevant published literature (Ilyas *et al.,* 2025).

Determination of bioactive compounds

Total phenolic content (TPC): Total phenolic content (TPC) was determined using the Folin–Ciocalteu method (Burlacu *et al.,* 2020). Absorbance was measured using a spectrophotometer, and TPC was expressed as milligrams of gallic acid equivalents (GAE).

Total flavonoid content (TFC): Total flavonoid content (TFC) was determined using an aluminum chloride colorimetric assay (Paniagua-Zambrana *et al.,* 2024) and expressed as milligrams of quercetin equivalents (QE).

Antioxidant activity (DPPH assay): Antioxidant activity was evaluated using the DPPH radical-scavenging assay (Bussmann *et al.,* 2025). Sample solutions at concentrations of 25–100 µg/mL were prepared, and absorbance was measured at 517 nm. DPPH radical-scavenging activity was calculated as a percentage of inhibition.

Heatmap analysis of nutritional composition: A heatmap was generated to visualize variation in the proximate nutritional composition of the 4 Quercus species using MetaboAnalyst 6.0. The data for carbohydrate, crude fiber, protein, fat, ash, and moisture were standardized using Z-score transformation [$Z=(X-\bar{X})/SD$] to facilitate comparison among variables measured on different scales. Positive and negative Z-scores represented values above and below the respective parameter mean, respectively. The standardized data were visualized as a color-gradient heatmap, while the original measured values were displayed within the individual cells. This approach has been used for comparative visualization of nutritional and plant-composition datasets (Saha *et al.,* 2026).

Where X represents the observed value for a species, $\bar{X}$ represents the mean value of that parameter across the 4 species, and SD represents its standard deviation.

Principal component analysis (PCA): Principal component analysis was performed to explore the multivariate variation in proximate nutritional composition among the 4 Pakistani *Quercus* species (*Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana*) using XLSTAT 2020. The analysis included carbohydrate, crude fiber, protein, fat, ash, and moisture contents obtained from Table 1. Prior to PCA, the variables were standardized using Z-score transformation [$Z=(X-\bar{X})/SD$] to place variables measured on different scales on a comparable scale. PCA was then performed using the standardized data, and the first two principal components (PC1 and PC2) were used to construct a two-dimensional biplot. Species scores were used to visualize the relative positioning of the 4 species, while variable loadings were used to identify the nutritional parameters contributing most strongly to species variation. PCA was used as an exploratory multivariate approach to summarize the major patterns of variation in the proximate composition (Brereton, 2025).

Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to determine significant differences among the 4 *Quercus* species, with statistical significance set at $p<0.05$.

Pearson correlation analysis was performed as an exploratory assessment of co-variation among selected nutritional, mineral, fatty acid, and bioactive parameters. Correlation coefficients were calculated using species-level mean values from the 4 *Quercus* species (n = 4 species). Because the analysis was based on only 4 species, the resulting estimates have limited statistical power and generalizability. Accordingly, the observed correlations were treated as descriptive, hypothesis-generating patterns rather than robust estimates of population-level associations, and were not interpreted as evidence of causal, mechanistic, or direct biological relationships. The correlation analysis was intended to identify compositional patterns that may warrant further investigation using a larger number of independent biological samples and species.

One-way ANOVA revealed significant differences ($p<0.05$) among the 4 *Quercus* species for crude fiber, protein, fat, and ash contents. In contrast, carbohydrate and moisture contents did not differ significantly among the species ($p>0.05$), indicating relatively comparable levels of these parameters across the studied acorn species.

Results

Proximate composition: Significant differences in proximate composition among species were observed for carbohydrates, protein, fat, crude fiber, and ash contents (Table 1).

Carbohydrate content differed among the studied species, with the highest value observed in *Q. baloot* (42.68%), followed by *Q. dilatata* (41.46%) and *Q. incana* (40.49%), while *Q. glauca* had the lowest value (39.79%). High percentage of carbohydrates constituted the major proximate component of the studied acorns. This finding aligns with published studies, which have similarly identified carbohydrates particularly starch as major constituents of acorns, although considerable variation has been reported among *Quercus* species and geographical origins. For instance, carbohydrate and starch values vary substantially among different *Quercus species*, highlighting the influence of species characteristics and growing conditions on acorn composition (Vinha *et al.,* 2016).

Protein content ranged from 5.52% to 8.32% among the Pakistani acorn species (Table 1). *Q. baloot* had the highest protein content (8.32%), followed by *Q. incana* (7.65%), *Q. dilatata* (6.67%), and *Q. glauca* (5.52%). Although acorns generally contain less protein than conventional cereals, their reported absence of gluten-containing proteins supports their potential use in gluten-free food products (Thadani and Singh, 2025). This characteristic may be particularly relevant for the development of specialized food products.

Crude fat content ranged from 5.45% to 8.37% among the Pakistani acorns (Table 1). The highest value was observed in *Q. dilatata* (8.37%), followed by *Q. incana* (7.58%), *Q. glauca* (6.78%), and *Q. baloot* (5.45%). These values are lower than those reported for some European species, which may contain approximately 10–20% fat, while American species have generally been reported to contain intermediate levels of about 8–12%. The observed fat content contributes to the overall macronutrient profile and nutritional value of the studied acorns.

Crude fiber content ranged from 4.54% to 9.63% (Table 1), with the highest value in *Q. baloot* (9.63%), followed by *Q. dilatata* (7.23%), *Q. incana* (6.38%), and *Q. glauca* (4.54%). These values are within or above the ranges reported for some European (3–8%) and American (3–6%) acorn species, suggesting that Pakistani acorns may provide a useful source of dietary fiber. Dietary fiber is an important component of a balanced diet and may contribute to the nutritional value of foods formulated from acorn flour (Castro *et al.,* 2022).

Ash content ranged from 1.25% to 2.46% among the Pakistani acorn species (Table 1). *Q. dilatata* had the highest ash content (2.46%), followed by *Q. glauca* (2.12%), *Q. baloot* (1.78%), and *Q. incana* (1.25%). These values are within the range reported for some other acorn species and are consistent with previous observations that acorns can provide mineral nutrients (Taib *et al.,* 2020; Taib & Bouyazza, 2021). The mineral content reflected by the ash fraction supports the potential use of acorn flour as an ingredient for nutritionally enriched food formulations.

Overall, the proximate composition indicates that the Pakistani acorns have a balanced macronutrient profile characterized by relatively high carbohydrate content and moderate levels of protein and fat. Their relatively high fiber content and potential suitability for gluten-free applications further support their possible incorporation into nutritionally enriched and functional food products.

Fatty acid profile: Fatty acids are important determinants of the nutritional, functional, and industrial value of acorn oils. Table 2 represent the fatty acid composition of the Pakistani acorn species. The profiles were characterized by the presence of unsaturated fatty acids, particularly oleic and linoleic acids, together with varying proportions of palmitic and stearic acids. Oleic acid (C18:1) was the predominant monounsaturated fatty acid, with the highest concentration in *Q. baloot* (36.97%), followed by *Q. glauca* (36.72%), *Q. dilatata* (35.45%), and *Q. incana* (34.70%). Linoleic acid (C18:2) ranged from 7.33% in *Q. glauca* to 15.10% in *Q. dilatata*. Palmitic acid (C16:0) was particularly high in *Q. dilatata* (39.12%), whereas lower values were observed in *Q. incana* (4.07%) and *Q. baloot* (4.22%). Stearic acid (C18:0) ranged from 0.89% in *Q. incana* to 9.17% in *Q. dilatata*. Several minor fatty acids including myristic, pentadecanoic, palmitoleic, heptadecenoic, elaidic, docosanoic, and eicosadienoic acids were also detected as trace elements in most species.

The 4 Pakistani acorn species exhibited a nutritionally relevant macronutrient composition, supporting their potential as underutilized *Quercus* resources for functional food applications. Carbohydrates were the predominant macronutrient, ranging from 39.79% to 42.68%, along with moderate protein (5.52% to 8.32%), fat (5.45% to 8.37%), crude fiber (4.54% to 9.63%), and ash contents (1.25% to 2.46%), reflecting differences in mineral residues among species. Overall, these findings indicate that Pakistani acorns provide carbohydrates, moderate amounts of protein and fat, and appreciable fiber and mineral fractions, supporting their potential use as raw materials for nutritionally enriched, functional food products.

These findings are consistent with previous reports identifying oleic acid as a major fatty acid in acorn oils, followed by linoleic and palmitic acids (Kiaei *et al.,* 2024). Oleic acid has also been reported at relatively high concentrations in European acorn species, particularly *Q. ilex*, where it can exceed 50% in some samples. American species such as *Q. alba* shows broadly similar fatty acid patterns, although the proportions of individual unsaturated fatty acids may differ. Thus, the lipid profiles observed in the Pakistani acorns are broadly consistent with those reported for the genus *Quercus*, while quantitative differences may reflect genetic background, environmental conditions, fruit maturity, harvesting time, and processing methods (Umar *et al.,* 2026).

From a nutritional perspective, the predominance of oleic acid is noteworthy. Oleic acid is the major monounsaturated fatty acid in many plant oils and has been associated with favorable effects on cardiovascular and metabolic health. Linoleic acid also contributes to the nutritional value of acorns, as it is an essential fatty acid required in the human diet. However, the physiological significance of these fatty acids depends on the overall dietary context and the amount consumed.

The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) is commonly considered an indicator of lipid nutritional quality. In the present study, the PUFA/SFA ratio ranged from 1.24-1.63. Atherogenic index (AI),

thrombogenic index (TI), and the hypocholesterolemic and hypercholesterolemic ratio (HH) were used to further characterize the nutritional quality of the fatty acid profiles. Lower AI and TI values are generally considered favorable, whereas higher HH values indicate a greater proportion of hypocholesterolemic relative to hypercholesterolemic fatty acids (Zocchi *et al.,* 2022). In the present study, AI and TI ranged from 0.15 to 0.16 and 0.17 to 0.22, respectively, while HH ranged from 6.17 to 6.36. These values are comparable to those reported for some nutritionally valued plant oils, including olive, linseed, and sesame oils (Szabłowska & Tańska, 2024).

In *Q. glauca*, palmitoleic acid (C16:1) was the most abundant fatty acid, accounting for 44.09%, whereas palmitic acid (C16:0) was present at only 0.10%. This distinction is noted explicitly to avoid confusion between the two structurally similar fatty acids. The fatty acid values reported in Table 2 were verified against the underlying GC–MS data and are presented as percentages of the total detected fatty acid composition.

The quantitative differences in fatty acid composition among the 4 species may arise from interactions between species-specific lipid metabolism and environmental conditions. Fatty acid desaturation and elongation are influenced by plant developmental stage and temperature, while differences in genotype can affect the relative accumulation of saturated and unsaturated fatty acids. The distinct profiles observed here may therefore reflect both inherent species characteristics and adaptation to the environmental conditions of the collection regions. In particular, *Q. glauca* displayed an unusual predominance of palmitoleic acid (44.09%), whereas *Q. dilatata* contained a markedly higher proportion of palmitic acid (39.12%). These differences suggest that lipid metabolism may vary substantially among species, although the present observational design does not allow genetic effects to be separated from geographic or environmental influences. Differences in fruit maturity and post-harvest processing may also contribute to quantitative variation and should be controlled in future comparative studies.

Mineral composition: Mineral composition is an important indicator of the nutritional value of acorns and is relevant to their potential use in functional foods and micronutrient-fortified products. Table 3 presents the mineral composition of the 4 Pakistani acorn species. Significant differences were observed among species for several macro- and micro-elements, as indicated by the different superscript letters in the table. Potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg) were the predominant macro-minerals, whereas iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were the principal micro-minerals.

Among the macro-minerals, potassium showed the highest concentrations, ranging from 2,650 mg/kg in *Q. baloot* to 13,460 mg/kg in *Q. glauca*. Phosphorus ranged from 457.20 to 545.80 mg/kg, calcium from 896 to 2112 mg/kg, magnesium from 402.02 to 525.22 mg/kg, and sodium from 103.80 to 127.50 mg/kg. *Q. glauca* had the highest potassium and phosphorus concentrations, whereas *Q. incana* had the highest calcium and magnesium concentrations. Among the micro-minerals, iron ranged from 270.90 to 305.50 mg/kg, with the highest concentration in *Q. incana*. Zinc varied from 27.52 to 37.30 mg/kg and was highest in *Q. baloot*, while manganese ranged from 245.14 to 254.50 mg/kg, and copper from 23.26 to 29.52 mg/kg.

Compared with ranges reported in literature, the Pakistani acorns analyzed in the present study showed substantial mineral concentrations. Potassium ranged from 2,650 to 13,460 mg/kg, compared with approximately 3,000–8,000 mg/kg reported for European species and 2,500–7,000 mg/kg for American species (Vinha *et al.,* 2016). Calcium ranged from 896 to 2,112 mg/kg, compared with reported ranges of approximately 500–1,500 mg/kg for European and 400–1,200 mg/kg for American species (Szabłowska and Tańska, 2024). Magnesium (402.02–525.22 mg/kg), iron (270.90–305.50 mg/kg), and zinc (27.52–37.30 mg/kg) also occurred at substantial concentrations. These comparisons suggest that Pakistani acorns represent valuable mineral-rich plant resources, although differences in analytical methods, growing conditions, and sample preparation should be considered when comparing values across studies.

These findings are consistent with previous reports describing acorns as useful sources of minerals, although the concentrations of individual elements vary among *Quercus* species. Similar patterns have been reported for *Q. ithaburensis*, *Q. calliprinos*, and *Q. glandulifera*, which contain substantial amounts of potassium, calcium, iron, and magnesium, although concentrations differ among species (Suo *et al.,* 2025). Acorn flour has also been reported to provide iron, manganese, and copper, highlighting its potential contribution to dietary mineral intake when incorporated into food products (Bhunia, 2026).

The pronounced interspecific variation in mineral concentrations may reflect differences in both soil nutrient availability and species-specific nutrient acquisition mechanisms. Plants growing in geographically distinct areas encounter different soil mineral pools, pH conditions, organic matter contents, moisture regimes, and parent materials, all of which influence nutrient solubility and root uptake. In addition, *Quercus* species may differ in their capacity to absorb, transport, and accumulate particularly high potassium concentration in *Q. glauca*, and the higher calcium, magnesium, and iron concentrations in *Q. incana*, may therefore reflect differences in soil nutrient availability and/or species-specific mineral homeostasis. However, because soil chemistry and plant nutrient-use traits were not measured in this study, these mechanisms remain plausible explanations rather than demonstrated causal factors. Direct measurements of soil and plant mineral status across replicated populations would be necessary to establish these relationships.

Antioxidant and bioactive profile: Phenolic compounds are widely recognized for their antioxidant and other biological activities (Gil-Martín *et al.,* 2022; Farooq and Yu, 2024). In the present study, the antioxidant and bioactive characteristics of Pakistani acorns were evaluated using total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical-scavenging activity. Table 4 summarizes the measured antioxidant and phenolic characteristics of the 4 species and demonstrates variation among the samples.

Table 1. Proximate composition of Pakistani Acorn species (% dry weight).

Species	Carbohydrates	Crude Fiber	Protein	Fat	Ash	Moisture
Q. baloot	42.68 ± 0.39	9.63^{b} ± 1.89	8.32^{a} ± 0.07	5.45^{a} ± 2.16	1.78^{ab} ± 0.38	41.38 ± 0.22
Q. dilatata	41.46 ± 1.24	7.23^{b} ± 0.05	6.67^{b} ± 0.45	8.37^{b} ± 0.06	2.46^{b} ± 0.79	39.01 ± 1.23
Q. glauca	39.79 ± 1.16	4.54^{a} ± 0.34	5.52^{c} ± 1.39	6.78^{a} ± 0.56	2.12^{b} ± 0.31	40.32 ± 0.49
Q. incana	40.49 ± 1.27	6.38^{a} ± 0.04	7.65^{a} ± 0.21	7.58^{a} ± 0.09	1.25^{a} ± 0.08	41.00 ± 1.24

*Values are presented as mean ± SD (n = 3). Different superscript lowercase letters within a column indicate significant differences among species according to one-way ANOVA followed by Tukey's post hoc test ($p<0.05$). NS = Non-significant ($p>0.05$). The carbohydrate and moisture contents did not differ significantly among the studied *Quercus* species ($p>0.05$), whereas significant interspecific differences were observed in crude fiber, protein, fat, and ash contents ($p<0.05$)

Table 2. Fatty acids composition of Pakistani Acorns (%).

Phytochemical	*Q. Baloot*	*Q. Incana*	*Q. Glauca*	*Q. Dilatata*
Oleic acid, methyl ester	36.97	34.70	36.72	35.45
Linoleic acid, methyl ester	13.64	14.18	7.33	15.10
Palmitic acid, methyl ester	4.22	4.07	0.10	39.12
Stearic acid, methyl ester	8.58	0.89	1.14	9.17
Myristic acid, methyl ester	0.08	0.22	0.20	0.15
Pentadecanoic acid, methyl ester	0.01	0.05	0.09	0.05
Palmitoleic acid, methyl ester	0.09	0.06	44.09	0.08
Heptadecenoic acid, methyl ester	0.21	0.30	0.38	0.32
Elaidic acid, methyl ester	1.05	0.02	0.02	0.52
Docosanoic acid, methyl ester	0.01	0.01	0.04	0.04

*Values represent the relative concentration (%) of individual fatty acid methyl esters detected by GC–MS

Table 3. Mineral composition of Pakistani Acorns (mg/kg).

Mineral (mg/kg)	Q. Dilatata Thunb (Swat Upper)	Q. Glauca (Swat Upper)	Q. Incana Roxb (Dir Upper)	Q. Baloot Griff (South Waziristan)
	Microminerals			
Mn	253.02 ± 2.01^{b}	245.14 ± 1.78^{d}	248.60 ± 1.02^{c}	254.50 ± 1.34^{a}
Zn	27.52 ± 0.81^{d}	33.42 ± 1.03^{b}	30.72 ± 0.94^{c}	37.30 ± 1.23^{a}
Cu	25.86 ± 0.89^{c}	29.52 ± 0.87^{a}	23.26 ± 0.78^{d}	26.92 ± 0.84^{b}
Fe	279.20 ± 1.24^{b}	274.80 ± 2.01^{c}	305.50 ± 1.45^{a}	270.90 ± 1.07^{d}
	Macro-minerals			
P	481.60 ± 2.31^{c}	545.80 ± 1.89^{a}	515.40 ± 1.63^{b}	457.20 ± 2.01^{d}
Ca	1426 ± 2.47^{b}	1240 ± 1.26^{c}	2112 ± 3.05^{a}	896 ± 2.62^{d}
Mg	418.16 ± 2.46^{c}	459.60 ± 2.43^{b}	525.22 ± 3.04^{a}	402.02 ± 1.47^{d}
Na	113.20 ± 1.69^{c}	127.50 ± 2.46^{a}	118.60 ± 1.73^{b}	103.80 ± 1.08^{d}
K	5481 ± 2.45^{b}	13460 ± 3.68^{a}	4116 ± 2.15^{c}	2650 ± 3.79^{d}

*Values are expressed as mean ± standard deviation (n = 3). Different superscripts indicate significant differences at $p<0.05$

Table 4. Antioxidant and phenolic content of *Quercus* species.

Specie	DPPH radical-scavenging activity (%)	TPC (mg GAE/mL)	TFC (mg QE/mL)
Q. Baloot	73.32 ± 0.54^{b}	0.294 ± 0.008^{c}	2.931 ± 0.08^{c}
Q. Dilatata	69.81 ± 0.61^{c}	0.742 ± 0.012^{a}	5.078 ± 0.12^{a}
Q. Glauca	72.36 ± 0.48^{b}	0.635 ± 0.010^{b}	4.282 ± 0.10^{b}
Q. Incana	68.71 ± 0.66^{c}	0.096 ± 0.005^{c}	2.205 ± 0.07^{d}

Values are expressed as mean ± standard deviation (n = 3). Different superscripts indicate significant differences at $p<0.05$

The measured TPC values ranged from −0.096 to 0.742 mg GAE/mL, with the highest value observed in *Q. dilatata* (0.742 mg GAE/mL), followed by *Q. glauca* (0.635 mg GAE/mL) and *Q. baloot* (0.294 mg GAE/mL), whereas *Q. incana* showed a value of 0.096 mg GAE/mL. TFC ranged from 2.205 to 5.078 mg QE/mL, with the highest value in *Q. dilatata* (5.078 mg QE/mL), followed by *Q. glauca* (4.282 mg QE/mL), *Q. baloot* (2.931 mg QE/mL), and *Q. incana* (2.205 mg QE/mL). Overall, *Q. dilatata* showed the highest measured TPC and TFC among the studied species, indicating a comparatively greater abundance of the phenolic and flavonoid compounds measured by these assays.

DPPH radical-scavenging activity was evaluated at 100 µg/mL and ranged from 68.71% to 73.32%. The highest activity was observed for *Q. baloot* (73.32%), followed by *Q. glauca* (72.36%), *Q. dilatata* (69.81%), and *Q. incana* (68.71%). The variation in DPPH activity indicates that antioxidant capacity was not directly proportional to total phenolic or flavonoid content alone and may reflect the combined contribution of multiple chemical constituents.

The phenolic and flavonoid contents measured in the Pakistani acorns were within ranges reported for other *Quercus* species (Vinha *et al.,* 2016; Szabłowska & Tańska, 2024), although direct comparisons should be made cautiously because extraction procedures, assay conditions, and units of expression may differ among studies. Reported European acorns have shown approximately 0.2–0.5 mg GAE/mL for TPC and 2–4 mg QE/mL for TFC, while American species have generally shown somewhat lower values (Burlacu *et al.,* 2020; Tizemmour *et al.,* 2024). DPPH activity in the Pakistani species (68.7–73.3%) was also within or above the ranges

reported in some European and American species. These findings support further investigation of Pakistani acorns as potential sources of bioactive compounds for functional food and nutraceutical applications.

Previous studies have reported relatively high concentrations of phenolic compounds in acorn extracts and have associated these compounds with antioxidant activity (Tizemmour *et al.,* 2024). Other investigations have also reported positive relationships between total phenolic content and antioxidant capacity in plant-based foods (Kowalska *et al.,* 2026). However, direct comparison among studies is challenging because antioxidant assays, extraction procedures, and units of measurement are not standardized across all investigations (Khan *et al.,* 2022). The present findings nevertheless indicate that Pakistani acorns contain measurable antioxidant-related constituents and warrant further investigation as potential ingredients for functional foods, natural additives, and nutraceutical products.

The interspecific differences in TPC, TFC, and DPPH activity may also reflect variation in secondary-metabolite biosynthesis associated with species identity and environmental conditions. Phenolic and flavonoid accumulation can be influenced by developmental stage, irradiance, temperature, water availability, and other forms of environmental stress, in addition to genetic differences in biosynthetic capacity. The relatively high TPC and TFC observed in *Q. dilatata* may therefore reflect species-specific metabolic characteristics and/or the environmental conditions experienced at its collection site. However, *Q. baloot* exhibited the highest DPPH radical-scavenging activity despite having lower TPC and TFC than *Q. dilatata*. This observation indicates that antioxidant activity cannot be attributed exclusively to total phenolic or flavonoid concentration and may involve the combined effects of individual phenolic compounds, other reducing constituents, and synergistic interactions. Because individual phenolic compounds and environmental variables were not characterized in the present study, the mechanisms underlying these interspecific differences remain to be established.

Correlation interpretation: Correlation analysis was used to explore patterns of co-variation among proximate composition, mineral content, fatty acid profiles, and antioxidant-related parameters across the 4 Pakistani *Quercus* species. The analysis integrated nutritional, phytochemical, and antioxidant measurements to explore whether specific compositional characteristics were associated with antioxidant activity or other bioactive parameters. Pearson correlation coefficients were calculated using species-level mean values for selected nutritional, mineral, fatty acid, and bioactive variables. Because only 4 species were included in this analysis, each correlation coefficient was derived from four observations and should therefore be interpreted with considerable caution. The magnitude and direction of these coefficients describe numerical patterns within the present dataset but do not establish reproducible biological associations, causality, or mechanistic relationships. Consequently, the correlation findings are considered exploratory and hypothesis-generating rather than confirmatory and should be validated using larger datasets comprising multiple independent biological samples and, where appropriate, additional *Quercus* species.

Q. dilatata showed the highest measured total phenolic content (0.742 mg GAE/mL) and total flavonoid content (5.078 mg QE/mL), whereas *Q. incana* showed the lowest values for both parameters. The correlation matrix (Table 5) showed a strong numerical correlation between TPC and TFC (r = 0.971) within the four-species dataset. However, this coefficient should not be interpreted as evidence that higher phenolic content directly causes higher flavonoid content. Similarly, the relatively weak numerical correlations between TPC and DPPH activity (r = 0.327) and between TFC and DPPH activity (r = 0.094) should be regarded as descriptive patterns rather than evidence for or against specific biological mechanisms.

Relationship between phenolic, flavonoids, and antioxidant activity: *Q. dilatata* showed the highest measured total phenolic content (0.742 mg GAE/mL) and total flavonoid content (5.078 mg QE/mL), whereas *Q. incana* showed the lowest values for both parameters. These findings suggest that the differences in antioxidant activity among the acorn species cannot be explained solely by total phenolic or flavonoid content and may instead reflect interactions among multiple chemical constituents. The correlation matrix (Table 5) showed a strong positive association between TPC and TFC (r = 0.971), indicating that species with higher measured phenolic content also tended to have higher flavonoid content. In contrast, TPC showed only a weak-to-moderate positive association with DPPH activity (r = 0.327), while TFC showed a very weak association with DPPH activity (r = 0.094). These patterns suggest that other constituents may also contribute to the observed radical-scavenging activity.

Table 5. Pearson correlation matrix among selected nutritional and bioactive variables.

Variable	DPPH (100 µg/mL)	TPC	TFC	Protein	Fat	Fiber	Fe	Ca	K	Oleic acid	Linoleic acid
DPPH	1.000	0.327	0.094	0.001	-0.855	0.296	-0.854	-0.926	0.223	0.993	-0.488
TPC	0.327	1.000	0.971	-0.666	0.208	-0.196	-0.739	-0.565	0.524	0.430	-0.318
TFC	0.094	0.971	1.000	-0.694	0.433	-0.271	-0.567	-0.366	0.487	0.203	-0.203
Protein	0.001	-0.666	-0.694	1.000	-0.396	0.860	0.212	0.012	-0.939	-0.113	0.725
Fat	-0.855	0.208	0.433	-0.396	1.000	-0.640	0.741	0.660	0.093	-0.790	0.294
Fiber	0.296	-0.196	-0.271	0.860	-0.640	1.000	-0.297	-0.420	-0.850	0.214	0.681
Fe	-0.854	-0.739	-0.567	0.212	0.741	-0.297	1.000	0.973	-0.266	-0.892	0.341
Ca	-0.926	-0.565	-0.366	0.012	0.660	-0.420	0.973	1.000	-0.120	-0.937	0.278
K	0.223	0.524	0.487	-0.939	0.093	-0.850	-0.266	-0.120	1.000	0.317	-0.917
Oleic acid	0.993	0.430	0.203	-0.113	-0.790	0.214	-0.892	-0.937	0.317	1.000	-0.547
Linoleic acid	-0.488	-0.318	-0.203	0.725	0.294	0.681	0.341	0.278	-0.917	-0.547	1.000

Note. Correlations were calculated from species-level mean values reported for the 4 Pakistani acorn species. These values should be interpreted cautiously because of the limited sample size

Heatmap analysis of nutritional composition: The heatmap (Fig. 3) summarizes the proximate composition of *Quercus* species and reveals clear species-specific nutritional studied. Carbohydrates dominated the composition profile throughout, peaking in *Q. baloot* (42.68%) and decreasing progressively in *Q. dilatata* (41.46%), *Q. incana* (40.49%), and *Q. glauca* (39.79%). This carbohydrate dominance aligns with established knowledge that starch serves as the primary storage reserve in acorns, reinforcing their recognized potential as starch-based food resources.

Protein and crude fiber followed a similar ranking, both peaking in *Q. baloot* (8.32% and 9.63%, respectively) and reaching their lowest points in *Q. glauca* (5.52% and 4.54%). *Q. dilatata* occupied a middle ground for protein (6.67%) while standing out for its fat content (8.37%). Such divergence points to a genuinely species-dependent nutritional identity rather than uniform composition across the genus, a pattern consistent with prior work (Vinha *et al.,* 2016; Ajo *et al.,* 2020; Kowalska *et al.,* 2026) attributing compositional variation to genetics, geography, environmental conditions, maturity stage, and post-harvest handling.

Fat content followed a distinct ordering altogether, topping out in *Q. dilatata* (8.37%) and declining through *Q. incana* (7.58%), *Q. glauca* (6.78%), and *Q. baloot* (5.45%). Given that acorn lipids typically consist largely of unsaturated fatty acids chiefly oleic and linoleic acid the elevated fat fraction in *Q. dilatata* signals a meaningful energy and lipid-nutrient contribution, best interpreted alongside its fatty acid profile reported elsewhere in this study.

Ash content, a proxy for total mineral load, was greatest in *Q. dilatata* (2.46%) and lowest in *Q. incana* (1.25%), with *Q. glauca* (2.12%) and *Q. baloot* (1.78%) falling between. While this hints at a stronger overall mineral contribution from *Q. dilatata*, ash values cannot distinguish individual elements; the detailed mineral analysis reported separately offers a more precise nutritional picture, particularly given that acorns are known to supply potassium, phosphorus, calcium, and magnesium in variable amounts.

Moisture varied comparatively little from 39.01% (*Q. dilatata*) to 41.38% (*Q. baloot*) making it the most stable of the measured parameters. Even this narrow range matters practically, since moisture level influences storage stability and spoilage risk, and can shift how other proximate values appear when expressed on a fresh-weight basis.

Taken together, no single species led across all parameters: *Q. baloot* excelled in carbohydrate, protein, and fiber; *Q. dilatata* in fat and ash; *Q. glauca* trailed on most fronts; and *Q. incana* sat consistently in between. This diversity underscores that Pakistani acorns should not be treated as a nutritionally uniform group, and each specie should be separately evaluated. The interspecies differences likely trace back to genetic and environmental factors tied to geographic origin, though since these variables were not directly assessed here this remains a plausible explanation rather than a confirmed one.

PCA analysis of species variation: Principal component analysis (PCA) further demonstrated distinct variation in the proximate nutritional profiles of all the 4 *Quercus* species (Fig. 4). PC1 and PC2 explained 59.4% and 27.7% of the total variance, respectively, accounting for 87.1% of the cumulative variation. *Q. baloot* was positively associated with carbohydrate, protein, and crude fiber contents, whereas *Q. dilatata* showed a stronger association with fat and ash. *Q. glauca* occupied a distinct position characterized by comparatively lower protein and fiber contents, while *Q. incana* showed an intermediate profile with a positive association with moisture. Overall, the PCA confirms species-specific differences in proximate composition and complements the individual comparisons (Table 1).

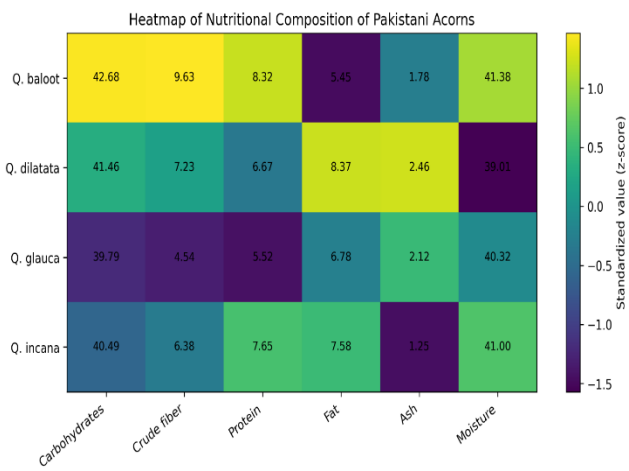


Fig. 3. Heatmap illustrating the proximate nutritional composition of 4 Pakistani Quercus species. Values within the cells represent the measured percentage of carbohydrates, crude fiber, protein, fat, ash, and moisture, while the color gradient represents standardized values (z-scores) to facilitate comparison among species.

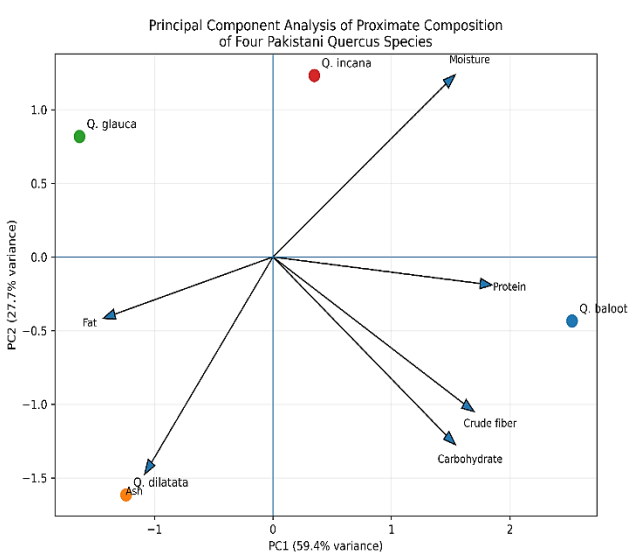


Fig. 4. Principal component analysis (PCA) biplot showing variation in the proximate nutritional composition of 4 Pakistani Quercus species. PCA was performed using standardized values of carbohydrate, crude fiber, protein, fat, ash, and moisture contents. The first two principal components (PC1 and PC2) explained 59.4% and 27.7% of the total variance, respectively, accounting for 87.1% of the cumulative variance.

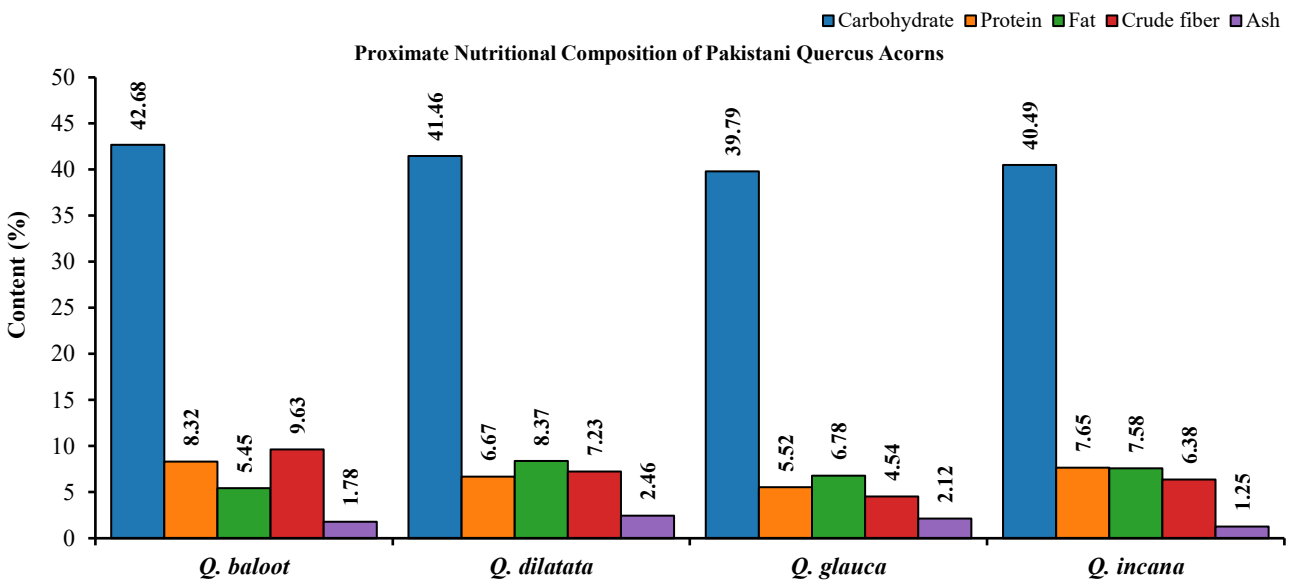


Fig. 5. Comparative nutritional composition of 4 Pakistani acorn species. The grouped bar chart compares carbohydrate, protein, fat, crude fiber, and ash contents of *Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana*. Values are expressed as percentages on a dry-weight basis. *Q. baloot* shows the highest carbohydrate, protein, and crude-fiber contents. *Q. dilatata* has the highest fat and ash contents. *Q. glauca* has the lowest carbohydrate and protein contents among the 4 species. *Q. incana* shows relatively high protein and fat but the lowest ash content.

Nutritional value of *Quercus* species: The comparative profile showed clear differences in the proximate composition of the 4 studied species. Carbohydrates was the predominant component in all species, with the highest value observed in *Q. baloot* (42.68%). *Q. baloot* also exhibited the highest protein (8.32%) and crude fiber (9.63%) contents, indicating a comparatively favorable nutritional profile. In contrast, *Q. dilatata* contained the highest fat (8.37%) and ash (2.46%), suggesting greater contributions from lipid and mineral fractions. *Q. glauca* showed comparatively lower protein and fiber contents, whereas *Q. incana* displayed intermediate values for most parameters. Overall, these species-specific differences highlight the nutritional diversity of Pakistani acorns and support their potential as locally available food resources, as depicted in Fig. 5.

Discussion

Association of proximate composition with functional properties: Protein content ranged from 5.52% to 8.32%, while crude fiber ranged from 4.54% to 9.63% among the studied species. Protein and fiber showed a strong positive correlation (r = 0.860). Protein content was also positively correlated with linoleic acid (r = 0.725) and negatively correlated with TPC (r = −0.666) and TFC (r = −0.694). These associations indicate differences in the distribution of macronutrients and bioactive compounds among the 4 species. DPPH activity showed a strong negative correlation with crude fat content (r = −0.855). Thus, within the limited species-level dataset, higher crude fat content was not associated with greater DPPH radical-scavenging activity. *Q. dilatata* had the highest crude fat content (8.37%), whereas *Q. baloot* had the highest DPPH activity (73.32%) despite having the lowest fat content (5.45%). These observations suggest that antioxidant activity may depend on the overall chemical composition rather than on crude fat content alone.

Mineral-bioactivity interactions: Mineral composition varied considerably among the 4 acorn species. *Q. glauca* showed the highest potassium concentration (13,460 mg/kg), whereas *Q. incana* had the highest calcium (2,112 mg/kg) and iron (305.50 mg/kg) concentrations. Iron and calcium showed a strong positive correlation (r = 0.973), indicating that species with higher iron concentrations also tended to have higher calcium concentrations in this dataset. In contrast, iron and calcium were negatively correlated with DPPH activity (r = −0.854 and −0.926, respectively), indicating that higher mineral concentrations were not accompanied by higher antioxidant activity across the 4 species. Potassium showed positive correlations with TPC (r = 0.524) and TFC (r = 0.487), while strong negative correlations were observed between potassium and protein (r = −0.939) and between potassium and linoleic acid (r = −0.917). These relationships describe compositional patterns within the studied species but should not be interpreted as evidence of causation.

Fatty acid correlation and functional interpretation: GC–MS analysis identified oleic acid as a major fatty acid across the 4 species, with concentrations ranging from 34.70% to 36.97%, while linoleic acid ranged from 7.33% to 15.10%. Oleic acid showed a very strong positive correlation with DPPH activity (r = 0.993). However, because the correlation was calculated from only 4 species, this relationship should be interpreted cautiously and should not be considered evidence of a direct mechanistic effect. Linoleic acid showed a moderate negative correlation with DPPH activity (r = −0.488) and positive correlations with protein (r = 0.725) and crude fiber (r = 0.681). These patterns indicate compositional differences among species and may be useful for generating hypotheses for future experimental studies.

Environmental, geographic, climatic, and genetic factors underlying species variation: The differences observed among the 4 Pakistani acorn species likely reflect the combined influence of species-specific genetic characteristics and environmental conditions associated with their respective geographic distributions. In the present study, *Q. baloot* was collected from South Waziristan, *Q. dilatata* and *Q. glauca* from Upper Swat, and *Q. incana* from Upper Dir. These geographically separated populations occur under different ecological conditions, and variation in altitude, temperature, precipitation, solar radiation, water availability, and growing-season characteristics may influence plant growth, resource allocation, nutrient acquisition, and secondary metabolism. Previous studies on *Quercus* acorns have similarly indicated that nutritional and phytochemical composition can vary according to species, geographic origin, climatic conditions, fruit maturity, and environmental factors (Vinha *et al.,* 2016; Ajo *et al.,* 2020; Kowalska *et al.,* 2026). Throughout this discussion, the proposed genetic, environmental, and soil-related mechanisms are interpreted as plausible hypotheses rather than demonstrated causal effects, since the present study did not include molecular characterization, controlled environmental experiments, or soil analysis, and therefore cannot separate these factors from one another.

The observed differences in proximate composition may represent species-specific responses to contrasting ecological conditions. Genetic background can influence the allocation of assimilated carbon and nitrogen to carbohydrates, proteins, lipids, and structural components, whereas environmental conditions can modify these metabolic processes during fruit development. Recent literature on *Quercus* acorns has demonstrated considerable variation in nutritional composition among species and populations, supporting the possibility that both genetic and geographic factors contribute to compositional differences (Vinha *et al.,* 2016; Kowalska *et al.,* 2026).

Soil characteristics may also contribute to the pronounced differences observed in mineral composition. Soil pH, parent material, organic matter, moisture status, fertility, and the chemical availability of individual elements can influence mineral solubility and plant uptake, while species may additionally differ in root characteristics, nutrient-use efficiency, transport, and mineral homeostasis (Pant *et al.,* 2021). These mechanisms could contribute to the particularly high potassium concentration observed in *Q. glauca* and the comparatively high calcium, magnesium, and iron concentrations observed in *Q. incana*.

Climatic conditions may similarly influence fatty acid composition, as temperature and other environmental factors can affect lipid biosynthesis and fatty acid desaturation during seed development. Recent reviews of *Quercus* acorn lipids indicate that fatty acid profiles vary among species and geographic regions, and that genetic characteristics, maturity, geographic origin, and climatic conditions may influence the relative abundance of individual fatty acids. In the present study, *Q. glauca* showed a markedly high proportion of palmitoleic acid (44.09%), whereas *Q. dilatata* showed a high proportion of palmitic acid (39.12%), differences that may reflect species-specific lipid metabolism interacting with local environmental conditions.

Genetic differences among *Quercus* species provide a further plausible explanation for the observed compositional variation. Species-specific genetic backgrounds can influence pathways responsible for carbohydrate storage, protein accumulation, fatty acid biosynthesis, mineral transport, and phenolic metabolism. The fact that the 4 studied species displayed distinct nutritional, mineral, fatty acid, and bioactive profiles despite belonging to the same genus is consistent with the possibility of species-specific metabolic characteristics (Zhang *et al.,* 2018).

Environmental conditions may also contribute to differences in phenolic and flavonoid accumulation. Plant secondary metabolites are strongly influenced by genetic background as well as environmental factors such as light intensity, temperature, water availability, soil fertility, and salinity, and environmental stress can modify phenylpropanoid and related metabolic pathways, altering the accumulation of phenolic and flavonoid compounds (Yang *et al.,* 2018; Zhang *et al.,* 2018). This may partly explain the comparatively high TPC and TFC observed in *Q. dilatata*. Notably, *Q. baloot* exhibited the highest DPPH radical-scavenging activity despite having lower TPC and TFC than *Q. dilatata*, indicating that antioxidant activity is not determined by total phenolic or flavonoid concentration alone and may involve the combined contribution of individual phenolic constituents and other reducing compounds.

The variation in protein and mineral contents among the *Quercus* species may also contribute to differences in their nutritional quality. Dietary proteins provide amino acids required for tissue maintenance, enzyme synthesis, and other physiological processes, whereas minerals such as potassium, calcium, magnesium, iron, and phosphorus participate in electrolyte balance, bone metabolism, energy metabolism, and cellular function. The observed interspecific variation therefore suggests that the nutritional contribution of acorns may differ according to species (Vinha *et al.,* 2016; Castro *et al.,* 2022).

The fatty acid profiles further highlight the nutritional importance of the studied acorns. Acorn lipids are generally characterized by a relatively high proportion of unsaturated fatty acids, particularly oleic and linoleic acids, which are important structural components of biological membranes and participate in lipid metabolism and cellular signaling. Oleic acid is commonly associated with favorable lipid-related nutritional profiles, whereas linoleic acid is an essential polyunsaturated fatty acid that must be obtained from the diet. The predominance of unsaturated fatty acids observed in several of the studied species therefore supports the potential nutritional value of their lipid fraction; however, these compositional findings should not be interpreted as direct evidence of cardioprotective and/or therapeutic effects, as such

physiological outcomes require validation through appropriate biological and clinical studies (Vinha *et al.,* 2016; Lončarić *et al.,* 2026).

The differences in total phenolic content, total flavonoid content, and DPPH radical-scavenging activity among the species may relate to variation in their secondary metabolite profiles. Phenolic acids, flavonoids, and tannins are important phytochemical constituents of *Quercus* acorns and can contribute to antioxidant activity through radical-scavenging and reducing mechanisms. Dietary polyphenols may participate in the modulation of oxidative processes in vivo; however, antioxidant activity measured by chemical assays such as DPPH represents in vitro radical-scavenging capacity and cannot be directly equated with antioxidant effects in humans. Consequently, the higher TPC, TFC, or DPPH activity observed in a particular species should be considered an indicator of greater in vitro antioxidant potential rather than proof of a clinical health benefit (Vinha *et al.,* 2016; Castro *et al.,* 2022).

The heatmap and PCA jointly illustrate the nutritional differentiation among the 4 Pakistani *Quercus* species, with the PCA providing a multivariate confirmation of the patterns visible in the heatmap. Although carbohydrates were the predominant proximate component in all species, substantial species-specific variation was evident in protein, crude fiber, fat, and ash contents. *Q. baloot* was distinguished by a comparatively favorable combination of carbohydrate, protein, and fiber contents consistent with its separation along the principal components most strongly associated with these variables while *Q. dilatata* was characterized by relatively higher fat and ash contents, reflecting greater contributions from lipid and mineral fractions. *Q. glauca* generally exhibited lower protein and fiber concentrations, and *Q. incana* occupied an intermediate position across most nutritional parameters. Such interspecific variability is consistent with the known compositional diversity of acorns and may be associated with species-specific genetic characteristics as well as differences in geographical origin, environmental conditions, and maturity. Collectively, these multivariate analyses reinforce the nutritional diversity of Pakistani acorns and support further investigation of species-specific characteristics for their potential utilization as food and functional-food resources (Brereton, 2025; Saha *et al.,* 2026).

Overall, the interspecific variation observed in this investigation should be regarded as the likely result of interactions among species, geographic origin, environmental conditions, soil characteristics, and climatic factors, rather than being attributed to a single determinant. This interpretation is consistent with recent evidence demonstrating substantial nutritional and phytochemical variation both among *Quercus* species and among populations of the same species. Importantly, the present study was not designed to partition genetic effects from environmental plasticity. Future investigations should therefore incorporate replicated populations across multiple geographic locations and years, together with measurements of altitude, temperature, precipitation, soil pH, organic matter, and soil mineral availability. Common-garden or controlled-environment experiments and molecular approaches would further help distinguish genetic effects from environmental influences, providing a stronger mechanistic understanding of nutritional and phytochemical variation among Pakistani *Quercus* species.

Conclusion

This study provides an integrated assessment of 4 native Pakistani *Quercus* species namely *Q. baloot*, *Q. dilatata*, *Q. glauca*, and *Q. incana* with respect to their proximate composition, mineral profile, fatty acid composition, phenolic and flavonoid contents, and antioxidant activity. The findings revealed substantial variation among species in nutritional and bioactive characteristics, positioning Pakistani acorns as promising underutilized plant resources with potential applications in food and nutraceutical development. Further research, including validation with larger sample sets and appropriate functional and safety assessments, is needed to establish their suitability for practical food applications.

A notable limitation of this study was that the correlation analysis was based on only 4 *Quercus* species, providing limited statistical power for establishing generalizable relationships. The reported correlations should therefore be regarded as exploratory, hypothesis-generating patterns rather than evidence of biological, mechanistic, or causal relationships. Future studies incorporating larger numbers of independent biological samples, populations, and *Quercus* species are needed to determine whether these compositional associations are reproducible and biologically meaningful.

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Declaration of Interest: All the authors declare no conflict of interest.

Author's Contribution: M.A. Dilawar planned the study, conducted the experiments, curated and analyzed the data, wrote the manuscript, and secured funding. M.M. Hashim supervised the research work and handled correspondence. M. Munir contributed to data curation, experimental execution, and statistical analysis. M.S. Baloch and Q.U. Khan critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Data Availability: Data will be made available upon reasonable request to the corresponding author.

Abbreviations

Abbreviation	Full Term
AAS	Atomic Absorption Spectrophotometry
AI	Atherogenic Index
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
Ca	Calcium
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
Cu	Copper
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FAMEs	Fatty Acid Methyl Esters
Fe	Iron
GAE	Gallic Acid Equivalent
GC–MS	Gas Chromatography–Mass Spectrometry
HH	Hypo-cholesterolemic/Hypercholesterolemic Ratio
HNO_3	Nitric Acid
K	Potassium
Mg	Magnesium
Mn	Manganese
n	Sample size (number of replicates)
Na	Sodium
NIST	National Institute of Standards and Technology
P	Phosphorus
PCA	Principal Component Analysis
PUFAs	Polyunsaturated Fatty Acids
Q. baloot	*Quercus baloot*
Q. dilatata	*Quercus dilatata*
Q. glauca	*Quercus glauca*
Q. incana	*Quercus incana*
QE	Quercetin Equivalent
r	Pearson correlation coefficient
SD	Standard Deviation
SFAs	Saturated Fatty Acids
SPSS	Statistical Package for the Social Sciences
TFC	Total Flavonoid Content
TI	Thrombogenic Index
TPC	Total Phenolic Content
Zn	Zinc

References

Ajo, R.Y., W.M. Al-Rousan, T. Rababah, S. Maghaydah, M.M. Angor, D.Z. Alomari and V. Bartkute-Norkūniene. 2020. Physiochemical properties and nutritional profile of Mediterranean oak acorn. *Afr. J. Food Agric. Nutr. Dev.,* 20: 16371-16385.

Anonymous. 2019. Official Methods of Analysis of AOAC International. 21st Edition, Association of Official Agricultural Chemists, Washington, DC, USA.

Bhunia, A.K. 2026. Abstracts of the 6th International Electronic Conference on Foods. Biol. *Life Sci. Forum,* 56: 15.

Branković, G., D. Dodig, D. Knežević, N. Đurić, S. Vasiljević, I. Radinović and J. Pavlov. 2021. Variability of Zeleny sedimentation volume in bread wheat and durum wheat from multi-environment trial. In: Book of Abstracts, 12th International Scientific Agriculture Symposium "AGROSYM 2021", Jahorina: 203.

Brereton, R.G. 2025. Principal component analysis: *Standardisation J. Chemom.,* 39: e3607.

Burlacu, E., A. Nisca and C. Tanase. 2020. A comprehensive review of phytochemistry and biological activities of *Quercus* species. *Forests,* 11: 904.

Bussmann, R.W., N.Y. Paniagua-Zambrana, N.P. Mehdiyeva, V.M. Alizade, N. Mursal, Z. Kikvidze, K. Batsatsashvili, G. Fayvush, A. Aleksanyan, M. Khutsishvili, I. Maisaia, S. Sikharulidze and D. Tchelidze. 2025. Ethnobotany of *Quercus castaneifolia* C.A. Mey., *Quercus dshorochensis* C.A. Mey., *Quercus ilex* L., *Quercus petraea* subsp. *iberica* (Steven ex M. Bieb.) Krassiln. and *Quercus pontica* K. Koch (Fagaceae). In: *Ethnobotany of the Caucasus* (Eds.): Bussmann, R.W., N.Y. Paniagua-Zambrana and Z. Kikvidze. Springer, Cham, pp. 4657-4697.

Castro, L.M.G., T.B. Ribeiro, M. Machado, E.M.C. Alexandre, J.A. Saraiva and M. Pintado. 2022. Unraveling the effect of dehulling methods on the nutritional composition of acorn Quercus spp. *J. Food Compos. Anal*., 106: 104354.

Gil-Martín, E., T. Forbes-Hernández, A. Romero, D. Cianciosi, F. Giampieri and M. Battino. 2022. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chem.*, 378: 131918.

Farazi, M., M.J. Houghton, B.R. Cardoso, M. Murray and G. Williamson. 2024. Inhibitory effect of extracts from edible parts of nuts on α-amylase activity: A systematic review. *Food Funct.,* 15: 5209-5223.

Farooq, M.A. and J. Yu. 2024. Recent advances in physical processing techniques to enhance the resistant starch content in foods: A review. *Foods,* 13: 2770.

Gillani, N.A., M.Q. Khan, K. Hussain and S. Sabir. 2023. Agro-morphological diversity of evergreen oaks (*Quercus*) in Western Himalayas, Azad Kashmir, Pakistan. *Pure Appl. Biol.,* 13: 156-174.

Güneş, Ş.N., Ş. Öncü Glaue and T. Akcan. 2025. The effect of acorn flour on the sensory properties of *baklava. J. Inst. Sci. Technol.,* 15: 960-974.

Ilyas, M., A.A. Shad, A. Orakzai, Z.A. Shah, B.S. Alotaibi and M.S. Maashi. 2025. Novel targets for treating cancer and inflammatory disorders: A disintegrin–metalloprotease-10 and cyclooxygenase-2 feedback loop modulated by *Aconitum heterophyllum* leaf biomarkers with computational biology. *Biomed. Pharmacother.,* 193: 118752.

Ilyas, M., A.A. Shad, J. Bakht, P. Villalta and W.T. Shier. 2024. Insights into metabolites profiling and pharmacological investigation of *Aconitum heterophyllum* Wall ex. Royle stem through experimental and bioinformatics techniques. *ACS Omega,* 9: 26922-26940.

Khan, N.M., M. Saeed, F.A. Khan, S. Ahmad, M.A. Nawaz, Z.U. Khan, M. Shafique, M. Almehmadi, O. Abdulaziz and A. Ullah. 2022. Effects of pH and ionic salts on the emulsifying and rheological properties of acorn protein isolate. *Molecules,* 27: 3646.

Kiaei, F., M.R. Delnavazi, S. Mojtabavi, A.R. Shahverdi and M.A. Faramarzi. 2024. Exploring the nutritional benefits of acorns: Phytochemical profiles and biological activity. Ann. Univ. Dunarea de Jos Galati Fasc. *VI Food Technol.,* 48: 174-191.

Konjević, A., Ž. Milovac, S. Kontsedalov, S. Kanakala, M. Ghanim, T. Gariepy and D. Musolin. 2021. Invasive insect species importance and possible pathways of their spreading in Serbia. In: Book of Abstracts, 12th International Scientific Agriculture Symposium "AGROSYM 2021", Jahorina, 352.

Kowalska, D., Z. Kołowrocka, E. Gruczyńska-Sękowska, K. Tarnowska and B. Zieniuk. 2026. Evaluation of the quality and composition of the lipid fraction obtained from acorns. *Appl. Sci.,* 16: 2564.

Lončarić, P., M. Jukić, T. Moslavac and J. Lukinac. 2026. Lipids of Quercus acorns: Health implications, oxidative stability, and food applications. *Foods,* 15: 2747.

Moravčević, Đ., S. Vuković, A. Vujošević, Ž. Dolijanović, J. Gvozdanović-Varga, S. Gordanić and S. Kilibarda. 2021. Impact of substrate volume on the cucumber seedling quality. In: Book of Abstracts, 12th International Scientific Agriculture Symposium "AGROSYM 2021", Jahorina: 194.

Muhammad, N., M.Á. Castillejo, M.D. Rey and J.V. Jorrín-Novo. 2023. An overview of oak species in Pakistan: Past, present, and future research perspectives. *Forests,* 14: 777.

Paniagua-Zambrana, N.Y., R.W. Bussmann and Z. Kikvidze. 2024. *Quercus cerris* L., *Quercus frainetto* Ten., *Quercus robur* L., *Quercus rubra* L., Fagaceae. In: Ethnobotany of the Mountain Regions of Eastern Europe: *Carpathians, Springer Nature Switzerland Cham.,* 1-33.

Pant, P., S. Pandey and S. Dall'Acqua. 2021. The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review *Chem. Biodivers.,* 18: e2100345.

Radojičić, V., L. Pejić and Ž. Dolijanović. 2021. The influence of agroecological conditions of growing on the smoke sensory properties of burley tobacco. In: Book of Abstracts, XII International Scientific Agriculture Symposium "AGROSYM 2021", Jahorina, 250.

Romanić, R., T. Lužaić, N. Grahovac, N. Hladni, S. Kravić and Z. Stojanović. 2021. Color characteristic of non-refined oils obtained by cold pressing of the seeds oils obtained from confectionary sunflower hybrids. In: Book of Abstracts, 12th International Scientific Agriculture Symposium "AGROSYM 2021", Jahorina, 366.

Saha, S., R. Roy, M.A.H. Chowdhury, B.K. Saha and P. Jha. 2026. Assessment of nutritional, medicinal, and antioxidant properties in Moringa oleifera Lam. leaves: Source-dependent patterns and health implications. *J. Food Process. Preserv.,* 2026: 9201854.

Stamenov, D., S. Đurić, T. Hajnal Jafari, B. Kiprovski and V. Seman. 2021. Azotobacter, Pseudomonas and Bacillus isolates stimulate the germination and seedling growth of *Melissa officinalis*. In: Book of Abstracts, 12th International Scientific Agriculture Symposium "AGROSYM 2021", 200.

Stanković, S., A. Obradović, M. Nikolić, A. Nikolić and V. Krnjaja. 2022. Novel toxigenic species on maize kernels in Southeastern Europe. In: Book of Abstracts of the XIII International Scientific Agriculture Symposium "AGROSYM 2022", Jahorina, Bosnia and Herzegovina, 194.

Stoin, D., C. Jianu, M.A. Poiana, E.C. Alexa and A.B. Velciov. 2021. Current trends in the use of unconventional raw materials for the development of gluten-free bakery and pastry products with high nutritional value: A review. *J. Agroaliment. Process. Technol.,* 27: 378-391.

Suo, X., C. Mannozzi, L. Moriconi, D. Piatti, G. Caprioli, G. Falistocco, G. Sagratini, A. La Terza and E. Vittadini. 2025. Acorn flour and bread-making application: Technological and chemical insights. *ACS Food Sci. Technol.,* 5: 2306-2316.

Szabłowska, E. and M. Tańska. 2024. Acorns as a source of valuable compounds for food and medical applications: A review of *Quercus* species diversity and laboratory studies. *Appl. Sci.,* 14: 2799.

Taib, M. and L. Bouyazza. 2021. Composition, physicochemical properties, and uses of acorn starch. *J. Chem.,* 2021: 9988570.

Taib, M., Y. Rezzak, L. Bouyazza and B. Lyoussi. 2020. Medicinal uses, phytochemistry, and pharmacological activities of *Quercus* species. Evid. -Based *Complement. Altern. Med.,* 2020: 1920683.

Thadani, R. and S.P. Singh. 2025. Major Forest Types and Associations in the Himalayas. *Himalayan Forests in a Changing World*. I: 95-148.

Tizemmour, Z., M. Mechmeche, N. Messadi, M. Hamdi and F. Kachouri. 2024. Evaluation of the physicochemical, nutritional, and antioxidant activities of acorn from *Quercus* spp.: A comparative study between Algerian and Tunisian varieties. *Chem. Afr.,* 7: 4273–4284.

Umar, M., M. Ahmad, M. Sadeeq, H. Ali, A. Khan, A.A. Chaudry, M.A.M. Ali and M.U. Naeem. 2026. Green synthesis and characterizations of zinc oxide nanoparticles using acorn fruit extract for antimicrobial, larvicidal and *In silico* activities. *Sci. Rep.,* 16: 7072.

Vinha, A.F., J.C.M. Barreira, A.S.G. Costa and M.B.P.P. Oliveira. 2016. A new age for Quercus spp. fruits: Review on nutritional and phytochemical composition and related biological activities of acorns. *Compr. Rev. Food Sci. Food Saf.,* 15: 947-981.

Yang, L., K.S. Wen, X. Ruan, Y.X. Wang, F. Wang and S. Wei. 2018. Response of plant secondary metabolites to environmental factors. *Molecules,* 23: 762.

Zocchi, D.M., C. Bondioli, S.H. Hosseini, M.D. Miara, C.M. Musarella, D. Mohammadi and A.K. Manduzai. 2022. Food security beyond cereals: A cross-geographical comparative study on acorn bread heritage in the Mediterranean and the Middle East. *Foods,* 11: 3898.

Zhang, L., X. Ruan, Y.X. Wang, F. Wang, K.S. Wen and S. Wei. 2018. Genetic and environmental regulation of plant secondary metabolite pathways. *Molecules*, 23: 762-778.