

COMPARATIVE NUTRITIONAL PROFILING OF CYDONIA OBLONGA LEAVES AND FRUITS IN KHANOZAI AREA, BALOCHSITAN

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خلاصہ

Abstract

For domestic consumption, the quince (Cydonia oblonga) is grown throughout Pakistan's temperate regions, particularly the upland areas of Balochistan. Despite its importance, the province has notably lacked nutritional composition profile for quince. This study investigates the mineral and proximate compositions of quince fruit and leaves. Matured quince fruits and leaves were systematically collected from Khanozai (Kn) and Churmian (Ch) area in the Pishin district of Balochistan. Leaf sampling was conducted in July 15, 2021, where leaves from twelve shoots per tree and four leaves per shoot were collected from six randomly selected quince trees. While, 4-8 fresh yellow Cydonia oblonga fruits per tree were collected In October 2021 and analyzed for proximate and nutrient analysis. The results revealed disparities in mineral concentrations, with Churmian area exhibiting higher levels of Nitrogen (0.077%), Potassium (1.66%), Iron (872.32 mg kg⁻¹), and Zinc (30.423 mg kg⁻¹) compared to Khanozai. However, Manganese was not detected in the fruits from either location. The analysis of Cydonia oblonga leaves displayed statistically significant differences in Potassium, Sodium, Copper, Iron, Zinc, and Manganese content, while Nitrogen and Phosphorus showed no significant variation. Proximate analysis of both fruit and leaves exhibited statistically significant differences across locations, with Khanozai recording the highest ash content in both fruit (2.62%) and leaves (9.59%), whereas Churmian area showcased the maximum protein content in the fruit (0.47%). Interestingly, crude fiber showed non-significant differences. This exploration provides fundamental insights into the Proximate and nutritional characteristics of quince fruit and leaves cultivated in Khanozai and Churmian, offering a crucial foundation for further research in this field.

Keywords: Cydonia oblonga; Fruit; Leaf; Khanozai, Nutrient; Quality

Introduction

Cydonia oblonga Miller, commonly referred to as Quince, is a member of the Rosacea family, specifically the sub-family Maloideae, which also includes apples and pears. It is a type of pome fruit known by various names such as Bahee in Urdu, Beh in Persian, Bihi in Hindi, Strythion in Greek, and Bamchont in the Kashmir valley (Raja *et al.*, 2018). The quince, which is native to the Caucasus, has progressively spread throughout Central Europe and Mediterranean countries due to its temperature resilience (Abdollahi, 2019). Despite losing its historical renown, quince cultivation persists globally including Pakistan. Over the past decade, global quince

production has seen a notable increase, reaching 666,589 tons in 2019 across a harvested area of 93,699 hectares (FAOSTAT, 2021). Turkey and China jointly account for approximately 41% of the world's total quince production, with significant contributions also coming from Uzbekistan, Iran, Morocco, and Azerbaijan (FAOSTAT, 2021). Production is primarily distributed across home gardens and commercial orchards. While quinces are not favored in their fresh state due to their tough flesh, bitterness, and astringency, their ripe form is in high demand for the production of various processed goods such as marmalade, jams, jelly, and cakes.

The quince tree is a deciduous tree that produces large, hairy fruits resembling pears or apples. These fruits have a distinctive yellow color, a classic flavor, and a fragrant aroma (Wagener, 2011). Quince trees are characterized by having monoecious flowers, with the androecium consisting of about 15-20 fiber-like structures with slightly yellowish anthers arranged in three circular lines. The carpal structure comprises five productive leaves that include the stigma, style, and ovary. Quince also contains yellowish microspores, and the pollen structure, including shape and size, can vary depending on factors like temperature, humidity, agrochemical composition of the soil (Bystrická *et al.*, 2017) and other environmental influences (Mratinic, 2011; Nagy-Deri, 2011).

Quince stands out as a highly significant fruit due to its nutritional components and bioactive compounds with health-promoting properties (Sut *et al.*, 2019). The fruit is characterized by a low fat content, making it an effective source of essential acids, fiber, carbohydrates, carbolic acid, and vital nutrients such as calcium (Ca), potassium (K), and phosphorus (P) (Iqbal *et al.*, 2019). It is also rich in essential phenolic and flavonoids compounds (Silva *et al.*, 2002; Silva *et al.*, 2005; Magalhães *et al.*, 2009). However, Fresh quince is not palatable due to its hard texture, sour flavor, and mouth-drying astringency. Consequently, quince is typically consumed after being processed into various products, including jam, jelly, marmalade, juice, nectar, and snacks (de Ferreira *et al.*, 2004; de Almeida Lopes *et al.*, 2018; Yıkmış *et al.*, 2019; Torres *et al.*, 2019).

In traditional medicine, quince has been historically employed to address various health concerns. It was commonly utilized in beverages for treating sore throats and also served as a remedy for both diarrhea and stomach distress. Additionally, a beverage made from quince seeds could be used as a breath freshener, or when mixed with glycerol, it was applied to alleviate skin problems. The research findings indicate that numerous components of quince are recognized for their therapeutic properties in managing respiratory issues, lung diseases, coughs, digestive tract disorders, diarrhea, constipation, anti-peristalsis, inflammation, urinary system distension, kidney and bladder conditions, as well as cardiac and metabolic ailments such as hypercholesterolemia, hyperlipidemia, and diabetes (Khoubnasabjafari and Jouyban, 2011). Investigating the comparative nutritional profiles of quince leaves and fruits, this research aims to contribute valuable insights that can inform future applications in the fields of nutrition, medicine, and food science. Such knowledge may also pave the way for sustainable utilization of various components of the *Cydonia oblonga* plant, thereby promoting its inclusion in a diversified and health-conscious diet.

Materials and Methods

Study Area: Karezat is a very large sub tehsil of district Pishin of Balochistan province, situated in the north east of Pishin. Khanozai and Churmian are main union councils in Karezat sub tehsil recognized as pivotal deciduous fruit-growing areas in Balochistan's Pishin district, successfully cultivate a range of fruits, including apple, apricot, peach, pear, and grapes. Despite the prosperity of these fruits, quince (*Cydonia oblonga* Miller) is not a major focus in the region, being grown on a limited scale within deciduous orchards primarily for domestic use. Six quince trees of uniform size and age in Khanozai and Churmian, were selected for the sampling fruit and leaves as marked on the district map of Pishin (Figure 1).

Plant Tissue Sampling and Processing: After July 15, 2021, leaf sampling was undertaken on six quince trees by selecting twelve newly emerged shoots that covered the entire circumference of the tree at shoulder height, following the recommendation of Kenworthy (1964). These trees were randomly chosen from each location. Four leaves from the middle of each shoot, as suggested by Kelling *et al.* (2000), were collected. This process resulted in the composite of 60 leaves from each tree, appropriately labeled and placed in paper envelopes. The samples were then delivered to the Soil and Water Testing Laboratory at the Agriculture Research Institute (ARI) in Sariab Quetta. Additionally, fruit sampling occurred in late October 2021, where 4-8 yellow-colored fresh fruits of *Cydonia oblonga* were randomly selected and harvested from each of the six trees. These fruits were also placed in paper envelopes, labeled, and delivered to the same laboratory.

Following the collection process, the leaves underwent a thorough cleaning with tap water and were subsequently immersed in 0.1 N HCl to remove any impurities from the tissue surface. Two additional rinses were performed using double-distilled water. The leaves, once air-dried, were laid out on filter paper and

subjected to oven drying at temperatures ranging from 60 to 65 degrees Celsius for a duration of 24 hours, in accordance with Chapman (1964). Dryness was confirmed by ensuring consistent weight across randomly selected samples at different intervals. Upon complete drying, the leaves were finely ground using a stainless-steel grinding machine (NM-8300, Nima-Japan), mixed to achieve homogeneity, and stored in airtight plastic jars. Similarly, fruit samples were also processed the same way as leaf. Average monthly temperature ($^{\circ}$ C) and precipitation (mm) of Khanozai area district Pishin, Balochistan (i.e. the data is the monthly average of three years spanning from 2020-22) is presented in Figure 2.

Mineral Analysis: The plant sample digestion utilized the Modified Kjeldahl method. A quantity of 0.5 grams of dry plant material was weighed and placed into a 100-mL digestion tube. Subsequently, 3-4 boiling granules and 5 mL of concentrated H2SO4 were added, thoroughly mixed, and left to stand overnight. The tubes were then heated on a block digester at a moderate temperature of 100-150°C. After cooling, 2 mL of 30% H_2O_2 was added and heated for 10 minutes at a high temperature of 280°C. This process was repeated until the white color fumes stopped and the solution became clear. Distilled water was added, and the solution was filtered and made to the mark in 100 mL volumetric flasks. The clear filtrate was used to determine the total nitrogen, phosphorus, and potassium, copper, iron, manganese and zinc using standard procedures (Wolf, 1982). In the clear digest, N was determined in two steps i.e. distillation and titration as described for soil N (Bremner, 1965; Jones, 1991). Calculated the N concentration in samples including a blank sample (B) using the following formula.

$N(\%) = (T-B) \ge 0.280134 \ge 50/10 \ge 100/sample weight in mg.$

For the determination of P contents in the clear digest, first mix reagent was prepared by dissolving 22.5 g of Ammonium Heptamolybdate in 400 ml of distilled water separately, and 1.25 g of Ammonium Vanadate in 300 ml distilled water distillate and mixing both solutions in 1L volumetric flasks, added 250 concentrated HNO₃ and make the volume up to the mark. The working standards containing 2.5, 5.0, 7.5, 10.0, and 12.5 mg kg-1 P was prepared from P stock solution. In 25 ml test tubes, added 1 ml of each of the samples, standards, and blank, added 2.5 ml Molybdovanadate Ammonium reagent and 2.5 ml distilled water. Kept them for 30 minutes to develop color and stirred intermittently on the vortex mixture and then run the working standards with blanks followed by the samples on a JENWAY 6100 spectrophotometer at 410 nm wavelength (Cottenie, 1980; Estefan *et al.*, 2013). In the digests, the quantification of K and Na was directly performed using the emission spectroscopy mode of the JENWAY PFP7 flame photometer, as outlined by Knudsen *et al.* (1982). Similarly, the determination of micronutrients, namely Cu, Fe, Mn, and Zn in the digests, followed the identical method employed for soil determination, utilizing the AA-7000 atomic absorption spectrophotometer, SHIMADZU model (Baker and Amacher, 1982).

Proximate Analysis: Standard procedures, as outlined by AOAC (2005) and AOAC (1990), were employed for the proximate analysis to assess the diverse nutritional parameters of *Cydonia oblonga* obtained from two distinct locations. The following methods were adhered to:

Moisture Content (%): Clean aluminum dishes were utilized, having been initially cleansed and dried in an oven at 105°C for one hour. The unoccupied aluminum dish was weighed (W_1), followed by the weighing of the aluminum dish containing a 2.0 g sample (W_2), which was subsequently dried in the oven at 105°C. After removal, the dish was reweighed (W_3). The moisture content was determined using the equation specified in ACOC (1990).

Moisture content (%) = $\frac{W_2 - W_1}{W_3 - W_1} \times 100\%$

Ash Content: The crucible was first weighed (W_1). Subsequently, two grams of pretreated sample powder were added, and the crucible was reweighed (W_2). The contents in the crucible were then subjected to heating in a furnace at 600 °C for ten hours, yielding whitish-grey ash (W_3). Following cooling, the crucible was reweighed. The percentage of ash content was calculated using the provided equation in AOAC (1990).

Percentage of ash (Dry basis) =
$$\frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

Crude Protein (%): To determine crude protein, the total nitrogen percentage in a sample is measured. This is often done using the Kjeldahl method or other nitrogen determination techniques. The crude protein content is then calculated by multiplying the total nitrogen percentage by a factor (usually 6.25), which is based on the assumption that protein typically contains about 16% nitrogen (AOAC, 1984).

Crude Lipid (%): Crude lipid content in a plant sample was determined using Soxhlet extractor. Weighed 2 g of the material and place it in a thimble within a flask. Added 200ml of petroleum ether to the flask and heat at

45°C for three hours to extract crude lipids. After extraction, allow the flask to cool for 15 minutes. Weigh the extracted crude lipids and used the following formula (AOAC, 2005).

Crude lipid (%) =
$$\frac{\text{Weight of fat}}{\text{weight of the sample}} \times 100\%$$

Crude Fiber (%): To analyze crude fiber, the AOAC (1990) method was applied, involving the digestion of samples with petroleum ether to obtain a fat-free material. The processed materials were then placed in a container, and 200 mL of preheated 1.25% sulfuric acid was added. Boiling the mixture for approximately 30 minutes, with consistent water volume maintained by adding water as needed, was carried out. Filtration was performed using a suction pump through a preheated Buckner flask funnel. Residues were thoroughly washed with boiled water multiple times and transferred into a beaker. Subsequently, 200 mL of preheated 1.25% Na₂SO₄ was combined, followed by boiling for 30 minutes. After straining, the residue was washed appropriately with ethanol (2 times) and hot water. The residue was then dried for 24 hours at 65° C and weighed. Using a crucible container, the residue was placed in a muffle furnace for 4 hours at 400-600°C. The resulting ash was cooled in a desiccator and weighed using the provided formula.

Crude fiber (%) =
$$\frac{\text{Weight before ashing} - \text{weight after ashing}}{\text{weight of the sample}} \times 100$$

Carbohydrates (%): The calculation for the percentage of carbohydrate content in the sample was determined using the subsequent formula (AOAC, 1990):

Carbohydrate (%) = 100 - (Crude lipid % + Ash content % + Crude fiber % + Crude protein %)

Statistical Analysis: The data were statistically analyzed in one-way ANOVA based on a randomized complete block design and the pairwise comparison of the treatments was performed using LSD test at the Probability level of 0.05. All the statistical analysis was computed on statistics 8.1 software (Math soft inc., Cambridge, MA, USA).



Fig. 1: Mape of district Pishin showing sampling point in Tehsil Karezat (Khanozai and Churmian), Balochistan.



Fig. 2: Average monthly temperature (°C) and precipitation (mm) of Khanozai area district Pishin, Balochistan (i.e. the data is the monthly average of three years spanning from 2020-22).

Results and Discussion

Mineral Analysis: The mineral composition of fruits and leaves can vary, but generally, they contain a range of essential minerals that contribute to their nutritional value. Quince (Cydonia oblonga) is a fruit-bearing plant, and the mineral composition of its fruits and leaves were first time evaluated in the Khanozai region Balochistan. The analysis of variance for fruit tissue nutrient concentration revealed statistically significant (p<0.05) differences across the locations except sodium and phosphorus. Whereas, leaf tissue mineral concentration such as sodium (Na), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) manifested significant (p < 0.05) variation across the location while the macronutrients including nitrogen (N), phosphorus (P) and potassium (K) expressed at par variation. The results showed higher fruit N concentration (0.077%) of Cydonia oblonga grown in Churmian area indicating 92.21% increase over Khanozai area (Table 1). In addition to that higher concentration of K (1.66%), Fe (872.32 mg kg⁻¹) and Zn (30.423 mg kg⁻¹) were found in fruit tissues of Cydonia oblonga in Churmian manifesting 9.21, 28.67, and 62.72% K, Fe and Zn increase as compared to fruit Cydonia oblonga in Khanozai. However, manganese concentration was not detected in fruit tissues of both locations. These results are supported by the statement of USDA that approximately 79% edible part of quince fruit is comprised of a nutritious component that serves as a valuable reservoir of macronutrients, minerals, and vitamins essential for the health and well-being of consumers (USDA, 2019). As per the USDA Nutrient Database, that 100g uncooked quince pulp contain 0.70, 0.13 and 0.04 mg iron, copper and zinc (USDA, 2019). Among the fruit tissue micronutrients, a group of researchers found Cu, Fe, Mn and Zn concentration in varying levels and reported that Cu and Zn were quantified in lower ranges and Mn in the lowest detection level (Otten et al., 2006). In contrast, we could not detect Mn concentration in fruit tissue of Cydonia oblonga from both locations. A group of researchers in Iran investigated the influence of soil lime stress on mineral composition and storage quality of Quince fruit. Their results revealed statistically significant changes in mineral contents and quality variables (Mirabdulbaghi et al., 2023). The influence of location on fruit mineral concentration of quince fluctuates that might be due to the differences in soil properties and climatic factors as noted in this study which is supported by the investigation of Rasheed et al. (2018) who examined the nutritional composition of quince fruits collected from various areas in Poonch, AJ&K, Pakistan and observed statistically significant variations (p < 0.05) in its organic and inorganic components.

In case of leaf tissue nutrient concentration, the macronutrients (N, P and K) revealed non-significant differences across locations but micronutrients (Na, Cu, Fe, Mn and Zn) showed statistically significant (p<0.05) variations for locations. The higher concentration of Na (0.357 mg kg⁻¹) and Mn (21.0 mg kg⁻¹) were found in leaf tissue of *Cydonia oblonga* in Churmian area indicating 17.93 and 38.10% increase over leaf tissue of *Cydonia oblonga* in Khanozai area (Table 1). But higher concentration of Cu (7.26 mg kg⁻¹), Fe (945.86 mg

kg⁻¹) and Zn (22.84 mg kg⁻¹) in leaf tissue of *Cydonia oblonga* were found in Khanozai area which were increased by 6.88, 11.09 and 8.54% Cu, Fe and Zn level over those found in leaf tissue of Cydonia *oblonga* in Churmian area respectively (Table 1). These results are in line with findings of Ferreira *et al.* (2022) who conducted study by sampling quince leaves from the Pinhel region, Portugal and performed quantitative and qualitative analyses to assess the levels of various macro- and micro-elements through mineral analysis. Their results revealed leaf tissue concentration of P (0.76 ± 0.003 g kg⁻¹), K (2.20 ± 0.03 g kg⁻¹), Na 0.10 ± 0.03 g kg⁻¹), Cu (3.90 ± 0.003 mg kg⁻¹), Fe ($119, 0\pm0.05$ mg kg⁻¹), Mn (52.0 ± 0.003 mg kg⁻¹) and Zn (27.0 ± 0.05 mg kg⁻¹).

Parameters	Khanozai		Churmian	
	Fruit	Leaf	Fruit	Leaf
Total nitrogen (%)	0.006±0.001 ^b	0.017±0.001 ^a	0.077 ± 0.005^{a}	0.018±0.002 ^a
Phosphorus (%)	0.332±0.001 ^a	0.399±0.01ª	0.33±0.031 ^a	0.413±0.005 ^a
Potassium (%)	1.507±0.022 ^b	1.413±0.009 ^a	1.66±0.006 ^a	1.38±0.006 ^a
Sodium (mg kg ⁻¹)	0.137±0.032 ^a	0.293±0.003 ^b	0.23±0.006 ^a	0.357±0.007 ^a
Copper (mg kg ⁻¹)	6.83±0.25 ^a	7.26±0.16 ^a	5.567±0.23 ^b	6.76±0.17 ^b
Iron (mg kg ⁻¹)	622.153±1.13 ^b	945.86±0.52 ^a	872.32±0.06 ^a	840.93±0.49 ^b
Manganese (mg kg ⁻¹)	0.0±0.0	13.0±0.001 ^b	0.0±0.0	21.0±0.116 ^a
Zinc (mg kg ⁻¹)	11.343±0.6 ^b	22.84±0.35 ^a	30.423±0.26 ^a	20.89±0.46b

 Table-1: Comparative minerals composition in the fruit and leaf of Cydonia oblonga planted in Khanozai and Churmain area of Balochistan.

Proximate Analysis: Proximate analysis of quince fruit and leaves is essential for assessing their nutritional value which plays a significant role in optimizing agricultural practices for quince cultivation. Overall, proximate analysis serves as a foundational tool for understanding the nutritional and functional aspects of quince, contributing to its utilization in various field. The analysis of variance pertaining to moisture content, ash content, crude fat, crude protein, crude fiber, and carbohydrate concentration of fruit and leaves of Cydonia oblonga revealed statistically significant (p<0.05) differences across locations. The findings indicated that leaf ash content surpassed that of the fruit in both locations, as illustrated in Figure 3a. Notably, the Khanozai area exhibited the highest recorded ash content for both fruit (2.62%) and leaves (9.59%), marking an increase of 17.20% and 17.38%, respectively, compared to the levels observed in the Churmian area. Even though the moisture content of the fruit was observed to be higher than that of the leaves in both locations, in accordance with the predictions in Figure 3b, there was a notable difference of 5.17% more moisture content in the fruit of the Churmian area compared to Khanozai. However, the moisture content in the leaves appeared to be relatively similar in both locations. Ouince fruit serves as a notable source of protein, fat, fiber, and carbohydrates. Across both locations, the fruit exhibited higher fat and fiber contents compared to the leaves, but interestingly, the maximum carbohydrate content was recorded in the leaves rather than the fruit. Although the fat content in the fruit was statistically non-significant between the two locations, a significant difference (p<0.05) was observed in the leaf composition, as depicted in Figure 3c. Regarding protein, the fruit in the Churmian area displayed greater protein content (0.47%), surpassing that of both the Khanozai area and the leaves in both locations. Notably, the protein contents in the leaves were similar in both locations, as illustrated in Figure 3d. Regarding fiber content, quince fruit in both locations exhibited a non-significant but higher level of fiber compared to the leaves, and these levels were statistically at par to each other as depicted in Figure 3e. These results are in line with findings of a group of researchers who studied proximate analysis of quince fruit pulp and recorded moisture content (82%), protein (0.45%), fat (0.07%), fiber (5.7%) and ash content (2.05%) (Sood et al., 2015). Similar results were also reported by many researchers' groups which are in line with this study (Rasheed et al., 2018; Najman et al., 2023). Concerning carbohydrates, the Khanozai area exhibited a notably higher mean fruit carbohydrate content (18.98%), surpassing that of the Churmian area by 14.903%. Conversely, the Churmian area recorded the maximum leaf carbohydrate content (23.71%), indicating a 13.41% increase compared to the Khanozai area (Figure 3f). These results align with research findings of Rasheed et al. (2018), where they observed the highest carbohydrate content in quince fruit of 13.38% from one location and the lowest of 12.98% from the fifth location. Corroborating these findings, Ferreira et al. (2022) also reported similar results. The variations in the examined proximate parameters of both fruit and leaves of Cydonia oblonga may be attributed to factors such as soil quality and environmental conditions. This observation is consistent with the studies conducted by Rasheed et al. (2018) and Kader (1998), who also highlighted the influence of these factors on plant characteristics.



Fig. 3: Proximate analysis of *Cydonia oblonga* leaves and fruits in Khanozai and Churmian area of Baluchistan that encompassing ash contents (a), moisture contents (b), fat contents (c), protein contents (d), fiber contents (e) and carbohydrate contents (f). Error bar indicates standard error of mean.

Conclusion

This study examined mineral and proximate analyses of Cydonia oblonga fruit and leaf from Khanozai and Churmian areas, Pishin District, Balochistan, revealing significant regional variations. Variations in fruit included Nitrogen, Potassium, Copper, Iron, and Zinc; leaves showed differences in Potassium, Sodium, Iron, Copper, Zinc, and Manganese. Proximate analysis highlighted distinctions in Ash, Moisture, Crude Lipid, Crude Fiber, Crude Protein, and Carbohydrate content. These findings underscore geographical influences on *Cydonia oblonga* nutritional profile, emphasizing the importance of region-specific considerations for cultivation and consumption practices in Khanozai and Churmian, Balochistan.

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