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Nutritional and Functional Properties of Quinoa (*Chenopodium quinoa* Willd.) Chimborazo Ecotype: Insights into Chemical Composition

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Abstract: Quinoa is known for its high nutritional value and adaptability; however, there is a lack of data about the chemical composition of quinoa produced in Ecuador, especially the Chimborazo ecotype. Our research aims to evaluate the nutritional components of Chimborazo quinoa. This knowledge (chemical composition) can help to improve cultivation and farmers' understanding. Samples were collected from 49 plots at four altitude ranges (3000–3200; 3201–3300; 3301–3400; 3401–3533) m.a.s.l. Each sample of 2 kg quinoa was cleaned, dried (32 °C/15 h), and stored at −20 °C before analyzing water activity, proximate composition, mineral content, antioxidant activity, and functional compounds. The data were analyzed using ANOVA and mean comparison, Pearson correlation, and principal component analysis. The Chimborazo ecotype shows protein content comparable to or exceeding other global quinoa cultivars. Statistical analysis revealed that altitude had a minimal influence on quinoa's chemical composition, resulting in overlapping altitude-based clusters. Complex relationships between quinoa variables were identified, which varied with altitude. These findings suggest that cultivation of high-quality quinoa across a range of altitudes is feasible without compromising its intrinsic quality. Moreover, the extensive and diverse results from our study provide a solid foundation for further plant breeding and the development of specialized quinoa varieties.

Keywords: quinoa ecotype Chimborazo; chemical composition; altitude

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1. Introduction

Quinoa is a grain native to the Andean region of South America and has been cultivated for thousands of years by Indigenous communities [1]. Of the original producing countries, only Peru, Bolivia, and Ecuador appear among the 10 main exporting countries of this Andean grain [2]. Although this crop was originally grown in South America, its use has now spread throughout the world. This diversification of cultivation areas in different continents is based on extensive research carried out worldwide on its high nutritional, functional, and nutraceutical value and its adaptability to different climates and soils [3]. The recent surge in popularity of quinoa has made it one of the most sought after crops globally and it is now being used as a substitute for rice or couscous in many dishes around the world.

Quinoa production in Ecuador has increased dramatically over the past decade, with hundreds of thousands of small-scale farmers now growing quinoa on their land. This has allowed them to benefit from rising demand both domestically and internationally, providing additional income that can be used to improve their lives or reinvest into their farms [4]. Finally, quinoa provides environmental benefits as well, as it requires minimal inputs (water and fertilizer) compared to other crops such as corn or rice, and it helps to reduce the impact on natural resources while still providing essential nutrients [5]. Additionally, quinoa's drought tolerance makes it particularly suitable for arid climates like those found throughout much of Ecuador. With all of these advantages combined, quinoa is an important asset to many communities across Ecuador—not just providing nourishment but also offering economic and environmental benefits too.

There is extensive research on the chemical composition of quinoa; these studies have been conducted on the chemical composition of quinoa from different countries. Although the quinoa crop is important in Ecuador, there is not much research on this crop in the country [6]. Research has shown that quinoa is a good source of essential amino acids, dietary fiber, vitamins, minerals, antioxidants, and phytochemicals [7–12]. The grain contains all nine essential amino acids, making it a complete protein source [7,13]. In terms of macronutrients, quinoa provides values, expressed in weight, in the range of 11–19% proteins, as well as 5–9% fat, and 46–77% complex carbohydrates, such as starch and dietary fiber [7,9,12,14]. In terms of micronutrients, quinoa is rich in many vitamins and minerals including iron, magnesium, phosphorus, potassium, and zinc. It also contains B vitamins like folate (B9), niacin (B3), and riboflavin (B2) [15]. Furthermore, research indicates that quinoa may be a good source of antioxidants such as flavonoids and phenolic compounds, which can help to protect against oxidative damage [16–19]. Overall, research suggests that quinoa is an excellent source of nutritive components, with significant amounts of essential fats, proteins, and micronutrients with potential health benefits, while being gluten-free [13,20].

The composition of different types of quinoas can vary widely, depending on the variety, growing conditions, and processing methods used [21]. For example, some ecotypes are higher in protein content than others. In addition, some ecotypes may have higher levels of minerals such as iron or zinc compared to other varieties. The study by García-Parra et al. highlights the influence of altitude on different aspects of quinoa grain production [22]. According to their findings, both altitude and variety acted as important factors that impacted the grains' protein content, with the highest values at 2648 m.a.s.l. The study links the altitudinal gradient to changes in temperature and chlorophyll fluorescence dynamics, revealing a higher level of physiological stress in quinoa plants grown in temperate and warm climates compared to cold climates; however, this research was performed at altitudes much lower than those of our study. González et al. in 2012 observed clear differences in yield and in some grain quality parameters due to environmental factors related to altitude. They considered it is necessary to study quinoa cultivars in relation to the agro-environmental conditions in which they were grown to ensure good yields and quality grains [23].

One of the quinoa ecotypes is Chimborazo, which is a traditional landrace grown on the slopes of the Chimborazo volcano at altitudes ranging from 2780 to more than 3500 m above sea level (m.a.s.l.). This ecotype is considered one of the most important crops of this area; its agronomic and phenotypic traits are detailed following the research guidelines established by FAO [24]. It is known for its long lifecycle. During flowering, the panicle color varies between green and purple, while at physiological maturity it displays a mixture of purple, pink, and yellow colors. The panicle shape can be glomerular, amorphous, or present intermediate forms, with an average length of 21 cm, and the diameter ranges from 13 to 80 cm. The seed yield per plant ranges from 0.6 to 14 g. The grains are small, with a diameter falling within the range of 1.5 to 2.3 mm, and the weight of 1000 grains varies between 3 g and 3.3 g. They have a high saponin content, resulting in a bitter taste. The pericarp is cream-colored, and the episperm is white [6,25].

Research on quinoa Chimborazo has been conducted in order to understand its agronomic characteristics and its potential as an alternative crop for marginal areas. Several studies on this topic were developed in the work of the nongovernmental organizations "European Committee for Training and Agriculture", the Instituto Nacional de Investigaciones Agropecuarias del Ecuador (INIAP), and the Escuela Superior Politécnica de Chimborazo (ESPOCH) with quinoa farmers in Chimborazo province. These experiences are reported in the paper Quinoa in Ecuador: Recent Advances under Global Expansion by Hinojosa [4]. However, there is no information about the chemical composition of quinoa Chimborazo. It is essential to study the chemical variation of this ecotype, as producing and marketing organizations often combine grains from different altitudes to form commercial batches.

In this context, the present research aims to evaluate the main nutritional and functional components of the Chimborazo ecotype quinoa as a quality tool for improving the crop, because it allows farmers to understand the nutritional content and quality of their production. This information is a crucial step towards enhancing the quality and productivity of this ancestral crop. By understanding the nutritional and functional components of this ecotype, farmers can adjust their farming practices and increase the crop's yield without losing its unique genetic traits. This project could be a starting point for a plant breeding program that focuses on improving the Chimborazo ecotype while still preserving its ancestral characteristics. Additionally, the cultivation of Chimborazo quinoa has several environmental benefits, such as erosion control and minimal water and fertilizer inputs, highlighting that a significant proportion of the production of this ecotype is organic. By improving this ecotype, farmers can further enhance its environmental benefits and promote sustainable agriculture practices. Therefore, it is essential to focus on improving the Chimborazo ecotype rather than replacing it with other varieties.

2. Material and Methods

2.1. Plant Material and Sampling

Samples of Chimborazo ecotype quinoa were obtained directly from 49 quinoa fields belonging to organic producer members of the Sumak Tarpuey, MAQUITA, and COPROBICH organizations. These samples were taken from different altitudes, which had been divided into four ranges and came from three different cantons: 80% of the random samples corresponded to Colta, the canton of the province of Chimborazo with the highest production of quinoa, and the remaining 20% to the cantons of Guamote and Riobamba (Table 1). Quinoa samples of the Chimborazo ecotype were collected in August and September 2021. The general climatic conditions during the growing season were 86.24% average relative humidity, average temperatures between 16.09 °C and 7.03 °C, and a daily corrected precipitation rate of 4.88 mm. The sampling plan was developed in accordance with Regulation (EC) No. 401/2006. Each sample, composed of at least 2 kg, was taken using a systematic methodology: samples were collected from different areas of the quinoa field to ensure its representativeness, and the geographic coordinates of each sampling site were recorded.

Table 1. Distribution of 49 quinoa samples.

Altitude (m.a.s.l. *)	Canton			
	Colta	Guamote	Riobamba	Total Samples by Altitude
3000–3200	7	2		9
3201–3300	11	1		12
3301–3400	12		3	15
3401–3533	9	2	2	13
Total samples by Canton	39	5	5	49

* m.a.s.l: meters above sea level.

2.2. Sample Preparation

The quinoa samples were prepared at the laboratory; they were manually threshed and winnowed to remove impurities, and then were dried at 32 °C ± −2.5 °C for 15 h to facilitate polishing. Afterward, the samples were subjected to a milling and sieving process (Ultra Centrifugal mill Rescht ZM 200, Hann, Germany), until a particle size smaller than 1 mm was obtained, and they were stored in Ziploc bags at freezing temperature (−20 °C) for further analysis.

2.3. Water Activity and Proximate Composition

Moisture content was determined in quinoa grains after threshing and winnowing (Mo) and in milled quinoa samples (Mf) by the gravimetric method described in the AOAC 945.38 method [26]. For this determination, 2 g of the sample was weighed in aluminum capsules and immediately subjected to a drying process in a Lab-Line Imperial V convection oven (Vernon Hills, IL, USA) at a temperature of 105 °C for 16 h. Subsequently, it was transferred to a desiccator and cooled for 2 h. Finally, each capsule with the dried sample was weighed, and the moisture content was determined by the difference in weight. The results were expressed as a percentage of moisture in each sample. Water activity (aw) of the quinoa was evaluated using a water activity meter (Aqualab, 3TE, Decagon, Pullman, WA, USA). All analyses were performed in triplicate (n = 3).

All chemical reagents used in the different determinations were of analytical grade, and three analyses were carried out for each sample and determination (n = 3). Crude protein content was determined using the AOAC 922.23 [26] methodology. For this, 1 g of the sample was weighed into a 250 mL digestion tube. Two copper catalyst tablets (3.5 g K_2SO_4 and 0.4 g $CuSO_4$ $5H_2O$) and 15 mL of concentrated sulfuric acid were added to the tube. The tubes were immediately placed in a digestion block and heated to 400 °C for 1 h. Afterwards, the tubes were cooled for 1 h and placed in an automatic Protein Analyzer, FOSS Kjeltec Model 8400 (Hillerod, Denmark), where distillation and titration were carried out. The protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25. The results were expressed as grams of protein per 100 g dry basis (db).

Fat content was measured using the Soxhlet extraction method as specified in AOAC 2003.06 [26]. A 0.5 g sample was weighed into stainless steel cups and covered with cotton. Subsequently, the cups were placed in the FOSS SoxtecTM 2043 equipment (Hillerod, Denmark). Once the heater reached 130 °C, the cups were immersed for 10 min, and immediately refluxing for fat extraction began for 30 min. After this time, a 10-min hexane recovery process was initiated. The cups were then removed from the equipment and placed in a 105 °C oven for 1 h to completely evaporate the hexane. Finally, the cups were cooled, weighed, and placed in a desiccator. The results were expressed as grams of fat per 100 g db.

The crude fiber determination was conducted following the AOAC 978.10 method [26]. For this purpose, 1 g of sample was weighed into porous glass crucibles (100 µm) and placed in the FOSS Fibertec 8000 equipment (Hillerod, Denmark). Once the heater reached 120 °C, the samples underwent acid digestion (1.25% v/v sulfuric acid solution) and alkaline digestion (1.25% p/v sodium hydroxide solution) for 1 h each. After this period, the samples were subjected to a washing process with distilled water. Subsequently, the crucibles with the digested samples were removed from the equipment and placed in a Lab-Line Imperial V convection oven (Vernon Hills, IL, USA) at 105 °C for 1 h, followed by a calcination process at 500 °C for 8 h. Finally, the crucibles were placed in a desiccator, cooled, and the weights of the dried and calcined samples were recorded. The results were expressed in grams of fiber per 100 g db.

The ash content was determined following the AOAC 923.03 [26] method. For this purpose, 1 g of sample was weighed into 25 mL porcelain crucibles and subjected to a calcination process at a temperature of 500 °C in a Thermolyne 48000 muffle furnace (Dubuque, IA, USA) for 12 h. The calcined samples were cooled for 1 h and transferred

to a desiccator. Finally, the weight of each crucible was taken, and the ash content was calculated by the difference in weight. The results were expressed in grams of ash per 100 g db.

Non-nitrogenous extract (NNE) was calculated by subtracting the sum of crude protein, crude fat, crude fiber, and ash from the total dry basis content of the sample. The results were expressed in grams of NNE per 100 g db [27].

2.4. Analysis of Mineral Content

Macro and microminerals were determined by Atomic Absorption Spectrophotometry. After obtaining the ashes, 10 mL of bidistilled water and 5 mL of hydrochloric acid (35% v/v) were added to the crucible. The mixture was digested on a hot plate at 100 °C for 30–60 min until the ash was completely dissolved. It was transferred to a 100 mL volumetric flask and diluted with bidistilled water. Finally, the macronutrient and micronutrient contents, with the exception of phosphorus content, were determined in triplicate (n = 3) in a Shimadzu model 7000 atomic absorption spectrophotometer (Kyoto, Japan) [28]. Phosphorus was determined colorimetrically with ammonium molybdate and ammonium vanadate [29]. The results were reported in mg per 100 g db.

2.5. Antioxidant Activity and Functional Compounds

For the extraction of antioxidant compounds, 0.3 g of dry quinoa sample was weighed into 15 mL polyethylene centrifuge tubes. Then, 5 mL of the extraction solution (methanol/water/formic acid 70/30/0.1 $v/v/v$) was added, and the mixture was homogenized by vortexing using a Mistral 4600 vortex mixer (Melrose Park, IL, USA) for 5 min. Afterward, it was subjected to a 10-min ultrasonic bath in a Cole-Parmer 8892-MTH ultrasonic cleaner (Niles, IL, USA) and finally centrifuged at 5000 rpm for 10 min. The supernatant (extract) was transferred to a 25 mL amber volumetric flask. This process was repeated four times to ensure complete extraction of the antioxidant compounds. The extraction was performed in triplicate (n = 3), and the same extraction process was performed for the quantification of antioxidant capacity [30].

2.5.1. Determination of Total Phenolic Content

Total phenolic content (TPC) was quantified using UV-visible spectrophotometry following the method described by [31]. To perform the analysis, an aliquot of 1 mL of the quinoa sample extract was transferred to a 15 mL test tube, followed by the addition of 6 mL of distilled water and 1 mL of Folin–Ciocalteu reagent. The solution was allowed to rest for 3 min before adding 2 mL of 20% (w/v) sodium carbonate. The resulting solution was heated in a water bath at 40 °C for 2 min to develop a blue chromophore, which was measured for its absorbance using a Shimadzu model 2600 spectrophotometer (Kyoto, Japan) at 760 nm. In each of the three extracts, two measurements were made using the mean value so that there were three replicates of this parameter (n = 3). A calibration line was constructed using the same procedure with gallic acid. The quantification of the total phenolic content was reported in milligrams of gallic acid equivalent per gram of quinoa on a dry basis (mg GAE g^{-1}).

2.5.2. Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined using the method described by [30]. A 1 mL aliquot of the quinoa extract was mixed with 4 mL of distilled water in a 15 mL tube and homogenized. Subsequently, 0.3 mL of 5% (w/v) sodium nitrite and 0.3 mL of 10% (w/v) aluminum chloride were added sequentially, and the sample was allowed to rest for 5 min after each addition. Finally, 2 mL of 1N NaOH was added and made up to 10 mL with distilled water. The resulting, pink-colored chromophore was measured for its absorbance at 490 nm using a Shimadzu model 2600 spectrophotometer (Kyoto, Japan) (n = 3). A calibration curve was generated using different concentrations of catechin. The

data were expressed as mg of catechin equivalents per gram of quinoa on a dry basis (mg of Catechin equivalent g^{-1} db).

2.5.3. Determination of Antioxidant Activity by the ABTS Method

The antioxidant capacity was evaluated in triplicate by the 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) cation decoloration method (ABTS*+), following the methodology described by [32]. First, a solution of ABTS*+ (7 mM) was mixed with potassium persulfate (2.45 mM) in a 1:1 ratio and allowed to rest in an amber bottle overnight. The following day, the absorbance at 734 nm of the prepared ABTS*+ working solution was measured and then diluted with phosphate buffer to obtain an absorbance of 1.1. An aliquot of the quinoa extract was then added to the ABTS*+ working solution in a 15 mL test tube and allowed to rest for 45 min. The final absorbance of the reaction was then determined using a Shimadzu model 2600 spectrophotometer (Kyoto, Japan) at 734 nm. A calibration curve was performed with different concentrations of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and the results were expressed as micromolar Trolox equivalent per gram on a dry basis (μmol Trolox equivalent g^{-1} db).

2.5.4. Determination of Antioxidant Capacity by the FRAP Method

The antioxidant capacity was also determined in triplicate, using the iron reduction/antioxidant power (FRAP) method proposed by [33]. To carry out the analysis, an aliquot of the quinoa extract was added to a 15 mL test tube, followed by the addition of phosphate buffer pH 6.6 and 1% potassium ferrocyanide solution. The solution was then shaken and immediately incubated in a bath at 50 °C for 20 min. After incubation, 10% trichloroacetic acid was added, and the mixture was homogenized in a vortex. Water and 1% $FeCl_3$ were then added. The solution was left to rest for 30 min in the dark. During this time, a green complex (ferrous chloride-potassium ferrocyanide) was formed, and the absorbance was determined using a Shimadzu model 2600 spectrophotometer (Kyoto, Japan) at 700 nm. A calibration curve was performed with different concentrations of Trolox, and the results were expressed as micromolar Trolox equivalent per gram on a dry basis (μmol Trolox equivalent g^{-1} db).

2.6. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA), and when significant differences were detected, for each height and among altitudes, means were compared using HSD Tukey's tests with a 95% confidence level. Pearson correlation was used to determine the relationship between the different variables. The data analysis was carried out with IBM SPSS Statistics 19 software (SSPS Inc., Chicago, IL, USA).

Principal component analysis (PCA) was used to assess the relationships between variables and samples, using MultBiplot software 2023.

3. Results and Discussion

3.1. Water Activity and Proximate Composition

In Table 2, the water activity, proximal components, mineral components, antioxidant compounds, and antioxidant capacity of the quinoa ecotype Chimborazo are shown. The data are the mean value of each parameter within each altitude (3000–3200; 3201–3300; 3301–3400; 3401–3533 m.a.s.l.) Within each specific altitude range, noticeable differences resulted, implying significant variations in the data, as can be seen in Supplementary Tables S1–S3, where the values of all of these parameters for the 49 quinoa samples are shown.

Table 2. Proximal composition, aw, antioxidant activity, antioxidant compounds, and mineral content of quinoa grain samples collected at different locations. (For each altitude range, mean values and Standard Deviation).

Altitude /Components	1 (3000–3200 m.a.s.l)	2 (3201–3300 m.a.s.l)	3 (3301–3400 m.a.s.l)	4 (3401–3533 m.a.s.l)
Initial Moisture *	15.22 ± 0.45 a	15.1 ± 0.7 ab	15.08 ± 0.79 ab	14.75 ± 0.53 b
Final Moisture **	7.16 ± 0.73	7.3 ± 1.2	7.42 ± 1.09	6.98 ± 0.85
Water Activity **	0.36 ± 0.03	0.36 ± 0.04	0.35 ± 0.06	0.34 ± 0.05
Crude Protein **	16.29 ± 1.12	16.1 ± 0.85	15.72 ± 1.0	16.18 ± 0.93
Fat **	4.99 ± 0.86 ab	4.63 ± 0.91 b	5.35 ± 0.78 a	4.91 ± 0.69 ab
Ash **	2.79 ± 0.65	2.93 ± 0.42	2.98 ± 0.34	2.86 ± 0.32
Crude Fiber **	7.32 ± 0.56 ab	7.18 ± 0.45 b	7.53 ± 0.49 a	7.1 ± 0.53 b
Non-Nitrogenous Extract **	68.62 ± 1.64	69.16 ± 1.35	68.42 ± 1.4	68.94 ± 1.03
Ca **	47.79 ± 8.04 b	50.75 ± 7.36 ab	54.19 ± 7.05 a	50.19 ± 6.75 ab
P **	536.06 ± 80.81 b	553.7 ± 51.31 ab	537.32 ± 46.58 ab	570.49 ± 43.49 a
Mg **	195.89 ± 13.77 b	206.62 ± 20.02 a	200.45 ± 16.19 ab	202.03 ± 16.48 ab
K **	779.16 ± 56.04 ab	751.73 ± 76.31 ab	790.82 ± 37.06 a	758.82 ± 53.11 b
Na **	2.05 ± 0.55 b	2.13 ± 0.69 b	2.71 ± 0.79 a	2.34 ± 0.61 ab
Cu **	0.39 ± 0.1	0.36 ± 0.18	0.31 ± 0.12	0.36 ± 0.16
Fe **	4.16 ± 0.58 a	4.38 ± 0.73 a	3.69 ± 0.63 b	4.11 ± 0.57 a
Mn **	0.83 ± 0.25 a	0.72 ± 0.13 ab	0.7 ± 0.19 b	0.66 ± 0.14 b
Zn **	3.13 ± 0.26	3.15 ± 0.47	3.18 ± 0.35	3.3 ± 0.3
TPC **	1.91 ± 0.1	1.89 ± 0.14	1.86 ± 0.1	1.86 ± 0.15
TFC **	1.26 ± 0.25 ab	1.16 ± 0.18 bc	1.29 ± 0.23 a	1.12 ± 0.19 c
AA ABTS **	39.41 ± 2.44	39.95 ± 4.11	39.48 ± 2.01	38.98 ± 2.77
AA FRAP **	19.22 ± 4.98 a	17.49 ± 3.64 ab	18.71 ± 4.1 a	15.85 ± 2.87 b

Mean values ± Standard Deviation (n = 3). Values in the same row followed by different letters differ at a significance level of 0.05, according to Tukey's test. m.a.s.l: meters above sea level. (*) Expressed on a wet basis (wb); (**) Expressed on a dry basis (db). aw: water activity; Ash (g 100 g^{-1}); Fat (g 100 g^{-1}); Crude Protein (g 100 g^{-1}); Crude Fiber (g 100 g^{-1}); NNE: Non-Nitrogenous Extract (g 100 g^{-1}); AA ABTS: Antioxidant Activity (µmol Trolox equivalent g^{-1}); AA FRAP: Antioxidant Activity (µmol Trolox equivalent g^{-1}); TPC: Total Phenolic Compounds (mg of gallic acid equivalent g^{-1}); TFC: Total Flavonoid Compounds (mg of Catechin equivalent g^{-1}); Mineral content expressed on mg 100 g^{-1}.

The results of water activity and proximal composition analyses of the quinoa samples for each altitude are shown in Supplementary Table S1. The consistency between the initial (fresh) and final (dehydrated) moisture content and water activity values indicates that the processing method successfully eliminated moisture from the samples. The final moisture content ranged from 5.34% to 9.40%, which is consistent with other authors' findings [34,35], who reported ranges of 5.27–8.64% and 6.1–8.3%, respectively. However, these values are lower than those reported by [36], ranging from 10.09% to 12.23%, and by [11], ranging from 10.21% to 11.71%. The variation of this parameter in different studies where samples were obtained from commercial sites is not only attributed to the type of thermal treatment, but also to the packaging material used to store the product and the storage duration [37]. As for the moisture content of freshly harvested quinoa dried in the field, only one study reports this parameter [14], which was lower (13.42 ± 0.3%) than the values found in our study, which ranged from 13.71% to 16.14%. Moisture and aw are critical in maintaining the freshness and quality of quinoa, especially for freshly harvested grain that has not been processed or stored. Excess moisture in quinoa (aw > 0.7) can cause spoilage and even pose food safety risks by providing a breeding ground for harmful microorganisms.

Quinoa is renowned for its exceptional protein content, offering a perfect balance of amino acids and enriched with thionic amino acids and lysine, positioning it as a high-quality protein source surpassing other grains with lysine limitations [38]. The present study revealed that the crude protein content of the Chimborazo ecotype quinoa can vary considerably, ranging from 13.86 g 100 g^{-1} to 17.97 g 100 g^{-1} on a dry weight basis. The lowest value corresponds to sample 26 at altitude 2, while the highest value belongs to sample 41 at altitude 4. Out of the samples analyzed, 32% exhibited a high protein content

(between 16.75 g 100 g^{-1} and 17.97 g 100 g^{-1}), 41% had a moderate content (between >15.24 g 100 g^{-1} and <16.75 g 100 g^{-1}), and the remaining 27% had a low content (between >13.86 g 100 g^{-1} and <15.24 g 100 g^{-1}).

The protein content in quinoa grains depends not only on the variety, but also on cultural practices and geographical factors, such as chemical composition of the soil, climate, or water availability [22,23]. These factors can interact in complex ways, making it challenging to identify the exact cause of variations in the protein content of quinoa ecotype Chimborazo.

Studies carried out with other varieties in different countries have found a protein content slightly higher than that of our work. For example, Rodríguez Gómez et al. studied the nutritional characterization of six quinoa cultivars (Pasto, Atlas, Marisma, Jessie, Roja, Pot_4) grown in southern Europe and they obtained values between 17.64% and 21.03% [11]. In a study that analyzed quinoa cultivars from Spain and the Andean region, a protein content ranging from 14.6% to 17.9% was reported [34]. In another investigation that evaluated the impact of agroecological conditions on the nutritional profile of three quinoa cultivars in different locations (Spain, Peru, and Chile), the protein content ranged from 14% to 17% [39].

There are also numerous studies, with various quinoa cultivars, in which protein contents have been found to be in the medium and low ranges, or even lower than those found in our work, for the Chimborazo ecotype. Thus, in a study conducted in the central region of Colombia, the physiological performance of seven quinoa cultivars was evaluated at three altitude gradients (cold, temperate, and warm climates), and protein values ranged from 12% to 17% [22]. In contrast to our results, these studies observed that the reduction in the altitudinal gradient had a positive association with the protein content in the grain ($p < 0.001$). In another preliminary study carried out in Egypt to evaluate new quinoa genotypes in sandy soils, three new accessions had a protein content ranging from 10.83% to 13.77% [12]. Präger et al. studied four quinoa cultivars in southwestern Germany, finding that protein values ranged from 11.9% to 16.1% [21]. In the study conducted by González et al., ten quinoa cultivars from the Andean highlands (Bolivia/Argentina) and northwest Argentina were analyzed, and the protein contents ranged from 9.15% to 15.53% [23].

Fat values found in this study range from 3.43 g 100 g^{-1} to 6.94 g 100 g^{-1}, with an average of 5 g 100 g^{-1}. The minimum value in our samples is below those found by [21,34,35], which reported minimum values of 5.5%, 5%, and 4.54%, respectively. Another study that analyzed samples from the same lot located in Argentina found a value of 2.3% fat [40]. In a review about chemical composition of quinoa with studies prior to the year 2000 [41], it was reported that the fat content in quinoa seeds ranges between 1.8% and 9.5%. When compared to widely consumed cereals internationally, the values of Chimborazo ecotype quinoa are higher than those of wheat [42–44] and rice [45–48]. It is important to highlight that several investigations have reported that the fat in quinoa is mostly composed of unsaturated fatty acids, which are beneficial for health [35,49]. These fatty acids, such as omega-3 and omega-6, are essential for a balanced diet and can help to prevent cardiovascular disease and other health problems. However, it is important to remember that the fat content in quinoa can vary depending on the variety and growing conditions [34].

Regarding the ash content in the Chimborazo ecotype quinoa, values ranged from 1.18 g 100 g^{-1} to 3.55 g 100 g^{-1}, with an average of 2.90 g 100 g^{-1}. The distribution of the values was 5% in the range of 1.18–2.08 g 100 g^{-1}, 46% in the range of >2.08–2.98 g 100 g^{-1}, and 49% in the range of >2.98–3.55 g 100 g^{-1}. Similar results (2.55–3.93%) were reported [11] in six cultivars of quinoa ('Pasto', 'Atlas', 'Marisma', 'Jessie, Roja', 'Pot_4') cultivated in Southern Europe, while in another study [50] the measured ash content values ranged from 4.94% to 18.04% for the cultivars 'Inia431-Altiplano', 'White', 'Titicaca', 'Illpa Inia', and 'Carmen', cultivated in Turkey. The discussion suggests that this variability is due to factors inherent to the quinoa varieties themselves as well as to external factors such as cultivation conditions, soil quality, and environmental influences.

The crude fiber content of quinoa ecotype Chimborazo was found to range from 7.30 to 8.49 g 100 g^{-1}, with an average of 7.30 g 100 g^{-1}. Most of the data (52%) fell within the range of >6.87 g 100 g^{-1} to 7.68 g 100 g^{-1}, while 26% of the data were in the range of >7.68 g 100 g^{-1} to 8.49 g 100 g^{-1}. The remaining 22% of the data fell in the range from 7.30 g 100 g^{-1} to 6.87 g 100 g^{-1}. Significant differences in crude fiber content were observed between altitudes, being higher at altitudes 3 and 1. Values were statistically similar at altitudes 1, 2, and 4, revealing that there is a complex relationship between altitude and quinoa composition.

Finally, the non-nitrogenous extract (NNE) in quinoa refers to digestible carbohydrates, primarily starch and sugars. The NNE content in the samples of quinoa analyzed in this study ranged from 65.42 g 100 g^{-1} to 71.39 g 100 g^{-1}, with an average of 68.78 g 100 g^{-1}. Most of the samples (52%) fell within the range of >67.36 g 100 g^{-1} to 69.41 g 100 g^{-1}, while 33% of the data was between >69.41 g 100 g^{-1} to 71.39 g 100 g^{-1}, and the remaining 14% was in the range from 65.42 g 100 g^{-1} to 67.36 g 100 g^{-1}. Other authors [10] studied the chemical composition of several quinoa grains in different colors (black, red, and white) from different origins, and the carbohydrate content fluctuated between 75.3 and 77.0%, while in a study of six quinoa genotypes ('Ancovinto', 'Cancosa', 'Cáhuil', 'Faro', 'Regalona', and 'Villarica') cultivated in Chile, they found values of carbohydrates between 44.46 and 58.72% [51]. The carbohydrate profile of quinoa, with its high-quality starch and dietary fiber, highlights its nutritional value and its ability to support healthy diets and prevent type 2 diabetes. This combination can help manage postprandial glycemia, positioning quinoa as a key functional food [52]. Additionally, quinoa contains a high amount of essential fatty acids, minerals, vitamins, dietary fibers, and carbohydrates, having beneficial hypoglycemic effects while being gluten-free [20].

3.2. Mineral Content

The content of nine minerals in the Chimborazo quinoa ecotype reported in mg 100 g^{-1} db is shown in Supplementary Table S2. Mineral composition in quinoa grains depends on soil characteristics and agronomical practices [38,53]. Potassium showed the highest values, ranging from 615.42 to 865.01 mg 100 g^{-1} db, followed by phosphorus (415.60 to 645.77 mg 100 g^{-1} db), magnesium (162.39 to 251.61 mg 100 g^{-1} db), calcium (33.42 to 66.37 mg 100 g^{-1} db), iron (2.76 to 5.63 mg 100 g^{-1} db), zinc (2.00 to 4.11 mg 100 g^{-1} db), sodium (1.05 to 3.96 mg 100 g^{-1} db), manganese (0.42 to 1.08 mg 100 g^{-1} db), and copper (0.11 to 0.77 mg 100 g^{-1} db). These results are consistent with other publications that revised several studies conducted on quinoa grains from different countries around the world, which reported a wide range of mineral (K, P, Mg, Ca, Fe, Zn, Na, Mn) content in the grain [38,54]. Similarly, when examining the same minerals in the cultivars 'Regalona', 'Salcedo-INIA', and 'Titicaca', cultivated in three different countries (Spain, Chile, and Peru), these studies found that both location and cultivar influenced mineral composition [39]. With regard to copper, this mineral was the least abundant in quinoa grain from Chile (variety not mentioned) with a value of 0.20 mg 100 g^{-1} db [14], which is similar to our data. In another study, six quinoa ecotypes from three geographical areas of Chile were analyzed, and copper content values ranging from 0.75 to 1.52 mg 100 g^{-1} db were found [55]. The Chimborazo ecotype displayed detectable values in all nine minerals analyzed, in contrast to samples from other studies mentioned above that either showed lower concentrations or no detection. Although the values of some minerals were higher and others were lower, the overall results suggest that the Chimborazo quinoa ecotype is a valuable source of minerals for human consumption.

3.3. Antioxidant Activity and Functional Compounds

The analysis of antioxidant activity and functional compounds (Supplementary Table S3) in quinoa Chimborazo ecotype revealed a total phenolic content (TPC) ranging from 1.64 to 2.14 mg GAE g^{-1} db, and a total flavonoid content (TFC) between 0.85 and 1.71 mg catechin equivalent g^{-1} db. Similar findings were reported by others, such as Cañarejo-

Antama [56] in their comparison of nutritional and nutraceutical properties of quinoa (INIAP-Tunkahuan and red quinoa seeds purchased in Ecuador) cultivated in Mexico and Ecuador. They found TPC values ranging from 1.55 to 2.42 mg GAE g^{-1} db and TFC between 1.18 and 2.15 mg quercetin g^{-1} db. Another study [16] reported a TPC value of 2.18 ± 0.45 mg GAE g^{-1} for 13 quinoa varieties available in the UK, which came from many countries, including Ecuador. In contrast, other authors [55] found lower values, TPC of 0.04 to 0.17 mg GAE g^{-1} and 0.08 to 0.14 mg quercetin g^{-1} db, in six ecotypes of quinoa grains cultivated in Chile. However, another study [57] using the INIAP-Tunkahuan, the same cultivar as [56], reported a higher TPC value (11.10 mg GAE g^{-1} db) and TFC value (0.95 mg catechin g^{-1} db) in the same range as the ecotype Chimborazo quinoa in our study.

The ABTS and FRAP methods were used to measure antioxidant capacity, with values ranging from 34.46 to 46.20 and from 12.45 to 29.39 µmol Trolox equivalent g^{-1} db, respectively. To compare these values with other studies, a conversion was necessary. The quinoa ecotype Chimborazo exhibited higher antioxidant capacity values compared to those reported in other studies. Using the ABTS method, Pellegrini's results ranged from 15.5 to 31.08 µmol Trolox equivalent g^{-1} db [35], and Villacrés reported a value of 31.99 µmol Trolox equivalent g^{-1} db for a variety of quinoa from Ecuador [57]. Using the FRAP method, Reguera reported values of less than 10 µmol Trolox equivalent g^{-1} db [39], and Pellegrini found values ranging from 9.47 to 18.26 µmol Trolox equivalent g^{-1} db [35].

These variations in the antioxidant compounds and antioxidant capacity could be attributed to differences in the genetic makeup, geographical location, and environmental factors of the quinoa plants. Additionally, postharvest processing also affected the quinoa quality; the impact of air-drying temperature on the nutritional and functional properties of quinoa was shown where a decrease in TPC was observed with increased drying temperature [14]. These studies found values of TPC of 0.15 mg GAE g^{-1} db in fresh quinoa and 0.02 mg GAE g^{-1} db in quinoa dried at 80 °C. However, in a study about TPC and antioxidant activity of red and yellow quinoa seeds as affected by baking and cooking conditions, it was found that, in most cases, cooking did not cause any significant changes in these three parameters [58]. The importance of analyzing the antioxidant compounds in quinoa lies in their potential health benefits. Proper post-harvest handling and processing techniques are essential.

3.4. Correlations

Figures 1 and 2 display the Pearson's correlation coefficients for all variables: water activity (aw), humidity, proximal analysis, antioxidant capacity, antioxidant compounds, and mineral content, at four different altitudes. The correlation between the different variables changed with altitude, not following a clear trend. The different quinoa quality parameters were related, but the correlations found were not always the same at the different altitude ranges. Crop factors other than altitude should influence the quality characteristics [59–61], making the understanding of the relationships between these variables complex and intricate.

Figure 1 shows the correlations between the water activity, humidity, and proximal analysis with the other variables. High positive correlation between fat, ABTS, and Na was observed at altitude 1 and 2. Protein was negatively correlated with TPC, TFC, and Mn at altitudes 1, 2, and 3, and positively with Fe at the same altitudes. Some proximal components in quinoa exhibited a strong correlation with antioxidant compounds and antioxidant capacity; this correlation was not consistently observed across all altitudes. Fiber positively correlated with ABTS at altitudes 2, 3, and 4, with FRAP (altitudes 1 and 2), and with TFC (altitudes 1, 2, and 3). Certain proximal components (protein, fat, fiber, ash) were highly correlated with mineral content, particularly Ca, P, Mg, K, Na, and Cu, however, the correlation patterns vary at the different altitudes.

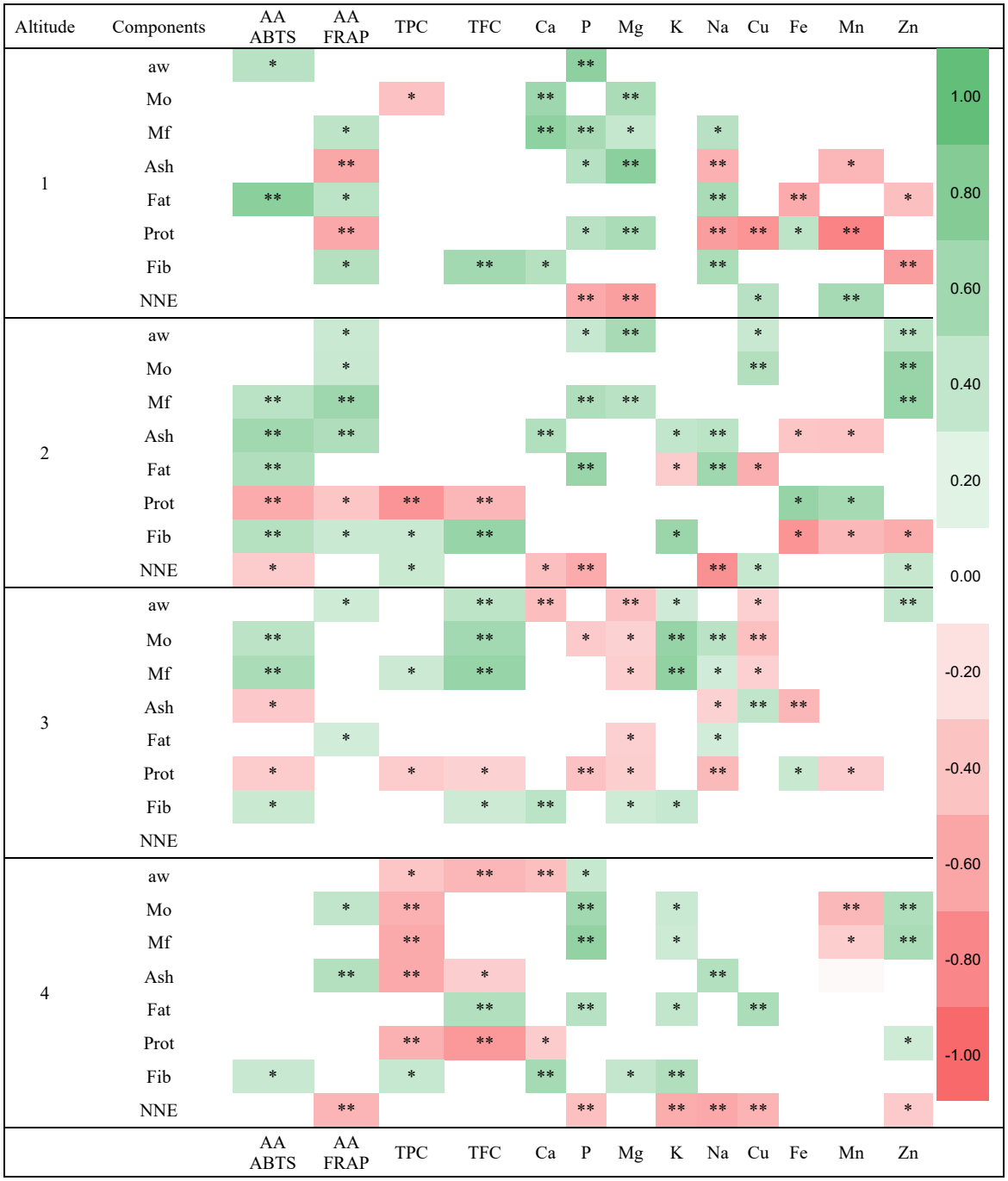

Figure 1. Pearson correlation coefficients between the proximal composition and other chemical composition variables studied of quinoa samples collected from four different altitudes. ** Significant correlation at the 0.01 level (bilateral). * Significant correlation at the 0.05 level (bilateral). Mo: expressed on a wet basis (wb); Mf, Ash, Fat, Prot, Fib, and NNE: expressed on a dry basis (db). aw: water activity. Mo: initial moisture (g 100 g^{-1}). Mf: final moisture (g 100 g^{-1}). Ash (g 100 g^{-1}). Fat (g 100 g^{-1}). Prot: crude protein (g 100 g^{-1}). Fib: crude fiber (g 100 g^{-1}). NNF: non-nitrogenous extract (g 100 g^{-1}). AA ABTS: antioxidant activity (µmol Trolox equivalent g^{-1}). AA FRAP: antioxidant activity (µmol Trolox equivalent g^{-1}). TPC: total phenolic compounds (mg of GAE acid g^{-1}). TFC: total flavonoid compounds (mg of Catechin equivalent g^{-1}). Mineral content expressed on mg 100 g^{-1}.

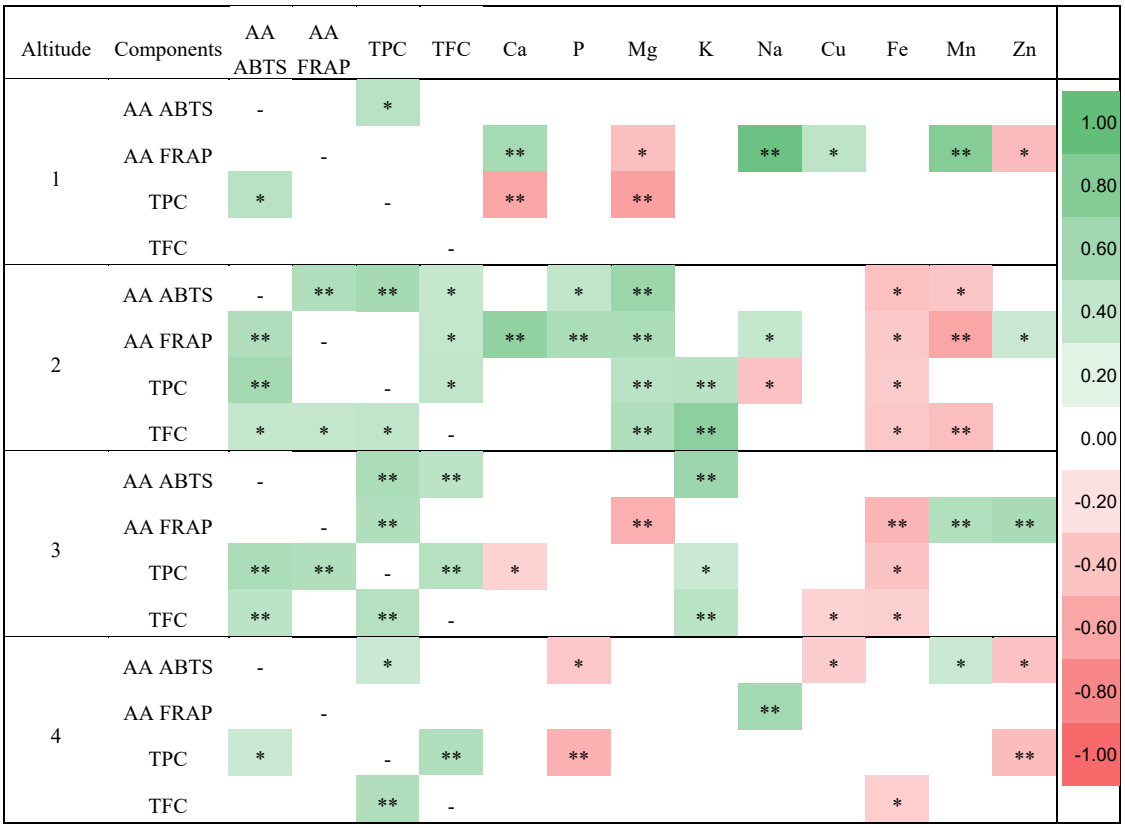

Figure 2. Pearson correlation coefficients between antioxidant activity and antioxidant components and other chemical composition variables studied of quinoa samples collected from four different altitudes. ** Significant correlation at the 0.01 level (bilateral). * Significant correlation at the 0.05 level (bilateral).—Not applied. AA ABTS: antioxidant activity (μmol Trolox equivalent g^{-1}). AA FRAP: antioxidant activity (μmol Trolox equivalent g^{-1}). TPC: total phenolic compounds (mg of GAE acid g^{-1}). TFC: total flavonoid compounds (mg of Catechin equivalent g^{-1}). Mineral content expressed on mg 100 g^{-1}.

In Figure 2, the antioxidant capacity and antioxidant components were compared among themselves and with the mineral content at four different altitudes. The correlations between ABTS and FRAP were not always significant with each other or with phenolic and flavonoid compounds, which could be due to the fact that different compounds are evaluated in each of the methods. The antioxidant capacity measured by ABTS was correlated with TPC at all altitudes, and with TFC at altitudes 2 and 3. When the antioxidant capacity was measured by FRAP, It showed a positive correlation with TFC only at altitude 2. On the other hand, TPC showed a positive correlation with TFC at three of the altitudes (2, 3, and 4). In the study conducted by Park et al., which investigated the antioxidant properties of quinoa cultivated in Korea compared to quinoa imported from the USA and Peru [62], a high correlation between TFC and antioxidant activity was observed, aligning with our findings at altitude 2. They reported a low correlation between TPC and antioxidant activity, which does not agree with our observations. This discrepancy highlights the complex nature of quinoa's antioxidant properties and suggests that environmental factors, such as altitude, may significantly influence these characteristics.

Regarding correlation with minerals, the antioxidant capacity measured by FRAP exhibited more correlations, compared to ABTS method and TPC and TFC.

As can be seen, there are complex relationships between the different variables studied for quinoa, which can vary depending on the altitude. Further research is needed to understand the underlying mechanisms behind these complex relationships. Our findings could be useful in developing strategies to optimize the growth and nutritional content of

quinoa at different altitudes. For example, there seems to be a relationship between protein and Fe content at most altitudes, so it could be interesting to study how iron fertilization affects protein content in quinoa ecotype Chimborazo.

3.5. Principal Component Analysis

Figure 3 presents the principal component biplot generated from the chemical composition data of the 49 samples. Variables with loadings lower than 0.1 (Cu, P, Ca, Ash, Mf, aw, Mo, NNE) were excluded from the analysis. PC1 explains 26.5% of the data variability, and PC2 explains 17.05%. The variables with the loadings > |0.3| in PC1 are crude fiber (−0.76), TFC (−0.73), ABTS (−0.63), K (−0.6), TPC (−0.53), crude protein (0.62), Fe (0.53), and Zn (0.46). Conversely, the variables with the loadings > |0.3| in PC2 are FRAP (−0.75), Na (−0.67), Mn (−0.61), fat (−0.53), Mg (0.47), and Fe (0.33).

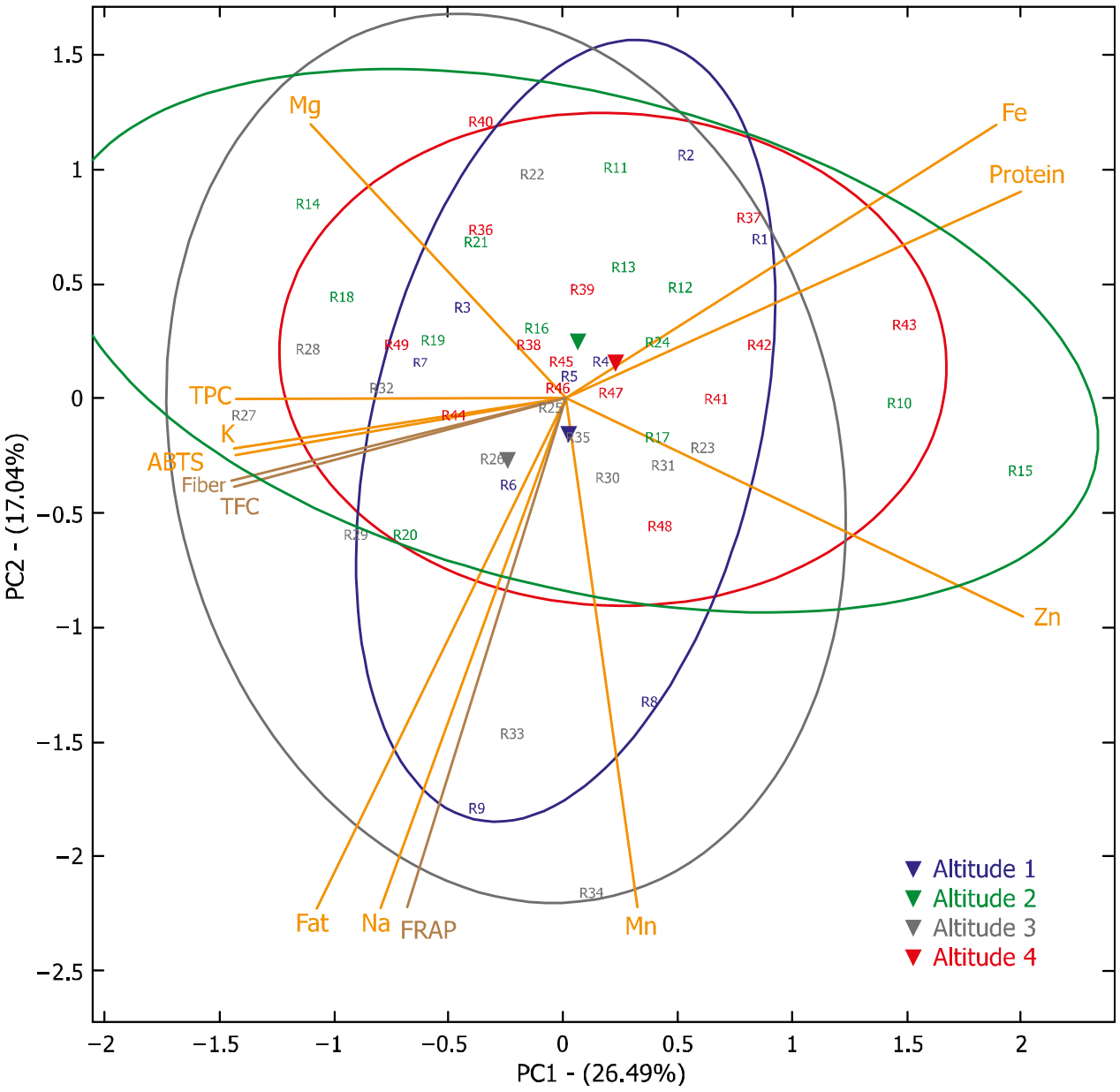

Figure 3. Principal component analysis of chemical composition of quinoa taken from four different altitude ranges. Inverted triangles correspond to the centroids of each altitude range. The colors of the centroids, the circles and the samples for each altitude range are: 1-blue, 2-green, 3-gray and 4-red.

When we observe the plane defined by PC1 and PC2, in which all the samples are included, the variables fiber, ABTS, TPC, TFC, and K are very closely related to each other, since they appear close. On the opposite side of this axis, protein and Fe are also remarkably close to each other, confirming what was mentioned in the correlations section. Finally, fat, FRAP, and Na are closely related variables.

However, there were no differences observed in the chemical composition of the 49 samples collected from four different altitude ranges. The clusters formed for each altitude overlap, indicating a significant variability in the chemical composition of quinoa, which is not dependent on the altitude of cultivation. The ANOVA and Pearson correlation analysis also supports these findings, suggesting that the altitude does not significantly impact the chemical composition of quinoa.

The overlap of altitude-based clusters and the minimal influence of altitude on the chemical composition of Chimborazo quinoa ecotype suggest that cultivating high-quality quinoa at various altitudes is feasible, without compromising its quality. This implies a valuable opportunity for farmers to explore different geographic locations for quinoa cultivation, in areas where other crops are not viable. Furthermore, the fact that the results are so diverse is a strength of the Chimborazo ecotype because it could allow for plant breeding and the development of selected varieties.

4. Conclusions

The results of this study show that quinoa Chimborazo ecotype is a valuable Andean landrace, with proximate composition, mineral content, and antioxidant capacity comparable to other quinoa varieties cultivated in other regions. According to our results, altitude explains only a part of the total variability of the samples. Regardless of the height at which the quinoa crop is grown, similar quality characteristics have been found in this landrace, confirming that even under the harsh growing conditions, this ecotype is capable of producing a good quality grain. Therefore, this study can be a starting point to initiate a process of crop improvement using quinoa samples with higher protein content. Farmers could select seeds for sowing, and/or genetic breeding could also be carried out in order to obtain a Chimborazo quinoa variety with higher quality and crop yield. Cultivation under traditional conditions could improve the economy of local communities, and the high nutritional quality of this ecotype could be the basis for the creation of a protected geographical designation, which would be a guarantee for marketing a product with environmental and social sustainability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14030396/s1, Supplementary Table S1. aw, initial and final moisture, and proximal composition of the studied samples by their altitude. Supplementary Table S2. Mineral composition of the studied samples by altitude range. Supplementary Table S3. Antioxidant activity and antioxidant compounds expressed on a dry basis.

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