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Nutritional and antinutritional compounds in leaves of quinoa

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ABSTRACT

In the context of climate change, especially for the temperate continental climate, new potential crop species are emerging, originating from the tropics. One of them is quinoa (*Chenopodium quinoa* Willd.) with multiple benefits for seed and leaf production. Quinoa is native to South America, grown mainly for seeds, with a high ecological plasticity. Little is known about the potential of using quinoa plants as a leafy vegetable for food diversification. In this study, the nutritional and antinutritional content of quinoa leaves was evaluated in three cultivars (Titicaca, Puno, Vikinga), considering different densities and times of sowing. Puno cultivar had a higher total content of carbohydrates, lipids, proteins and dietary fibers, and lower mineral contents in leaves. Low levels of antinutrient compounds were found in Vikinga leaves. Regarding the time of the crop establishment, the highest content of primary metabolic compounds (carbohydrates, lipids, proteins, dietary fibers) was achieved by April 17, the sowing date. Crop densities of 7.7, 3.2, and 1.6 mil. plants/ha did not significantly influence the content of compounds with antinutritive role, such as oxalates, saponins or trypsin inhibitors. The content of mineral elements such as: Fe, Zn, Na and K were significantly influenced by the cultivar, compared to Mg and Ca whose values were insignificant regardless of the treatment.

1. Introduction

The performance of the agricultural system in many countries is currently poor in terms of economic, social and environmental sustainability. A way of promoting the sustainability of the local food system is through diversification, by encouraging the cultivation and consumption of lesser-known sustainable food, especially non-traditional leafy vegetables. The advantages of these types of vegetables are multiple: usually they are highly nutritious, require low natural and agricultural resources and, also, are suitable for the current context of climate change (FAO/INFOODS, 2013; Rampa & Kanaepen, 2019). An example of such a plant species is quinoa (*Chenopodium quinoa* Willd.). According to the UN Food and Agriculture Organization (FAO) quinoa can assure the world food security due to its high nutritional qualities, also to its high tolerance to various abiotic stresses including salinity (Orsini et al., 2011; Ruiz et al., 2016; Stoleru et al., 2019). Due to the fact that it can be grown in the fields, tunnels or greenhouses, quinoa ensures also a sustainable production throughout the year.

Quinoa is a species grown especially for grains (Jacobsen et al., 2015), due to the high contents of proteins, minerals, vitamins or antioxidants (Vitanescu et al., 2019). The integration of highly nutritious grains such as quinoa in the daily diet has boosted the local economies by consumers of developed countries. In recent years, quinoa has gained global popularity, with Peru becoming a global player in the quinoa market (Bedoya-Perales et al., 2018). Peru, Bolivia and Ecuador produce more than three-quarters of the total world production of quinoa seeds (FAOSTAT, 2018).

Recently, the possibility of consuming quinoa for its leaves started to be taken more into consideration. Quinoa for leaves can be a serious alternative to other leafy vegetables, such as spinach, lettuce, rucola or

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lupine, which are less resistant to drought and saline stresses. In addition, lettuce and spinach under high temperatures develop flowering stems and make the leaf consumption inadequate during summer (Stan et al., 2003). Studies regarding the use of quinoa as a leafy vegetable for food diversification are still scarce (Pathan et al., 2019; Vasquez-Luna et al., 2019). Only a few researches have focused on the nutritional value of quinoa leaves. Pathan et al. (2019) reported that the content of protein, amino acids and potassium was higher than that found in amaranth or spinach. Bhargava et al. (2007), El-Naggar et al. (2018) or Khater et al. (2019) analyzed the effect of different nitrogen fertilizers and sources, the effect of salinity or the effect of sowing dates and row spacing on the quality of quinoa leaves. They concluded that quinoa has the potential to produce good quality leaves. Vasquez-Luna et al. (2019) stated that quinoa leaves can be introduced in the human diet as a green vegetable due to the good nutritional profile and the polyphenol and flavonoid contents. Regarding the antinutritional factors in quinoa leaves little is known about the possible antinutrients that might alter the leaves' nutritional quality. Few studies were done just on the saponin content of quinoa leaves suggesting a linear increase from the onset of flowers, until the seed ripening and a very small amount in the young leaves. More studies are needed in order to establish the benefits of consuming quinoa leaves. Moreover, studies regarding the integration of a new leafy vegetable in a new area and the attitude of people towards this new species have to be taken into consideration, taking into account the reluctance that usually appears (Dibsdall et al., 2003).

In this regard, we carried out a study to evaluate the influence of three agronomic factors (cultivar, seeding time and crop density) on the nutritional (lipids, proteins, carbohydrates, fibers, minerals) and antinutritional (phytic acid, oxalates, saponins, trypsin and α–amylase inhibitors) composition of quinoa leaves in order to improve the diversity of this leafy species.

2. Materials and methods

2.1. Experimental site

The study conducted between the years 2017–2018 aimed to establish the effect of some agronomic factors (cultivar type, seeding time and crop density) on the content of the nutritional compounds and the content of antinutrients in quinoa leaves. The experimental study was carried out in a stationary farm from Cudalbi village, Galati County, with the following characteristics: 46°15′ north latitude and 27°30′ east longitude, on a cambic chernozem type soil. The experiment was organized in a split plot design with three replications, each experimental plot having 7.5 sq, and a total harvestable area of 607.5 m^2. The agronomic factors studied were represented by cultivar (Titicaca, Puno, Vikinga), seeding time (April 03 (St_1), April 10 (St_2) and April 17 (St_3)) and crop density (7.7 mil. pl·ha^{-1} (12.5 × 1 cm) (D_1), 3.2 pl ha^{-1} (12.5 × 2.5 cm) (D_2) and 1.6 mil. pl·ha^{-1} (12.5 × 5 cm) (D_3) (pl = plants)). Quinoa seeds were kindly provided by Quinoa Quality ApS (Denmark). The growing practices were applied according to the technological norms described by Garcia-Parra et al. (2020). Plants were harvested at 42 days after seeding and all the leaves were collected for further analysis.

2.2. Determination of nutritional composition of quinoa leaves

The nutritional composition of quinoa leaves (ash content, total lipids, total proteins, carbohydrates, caloric value, total fiber and digestive fiber) was determined in triplicate. The ash content was evaluated by using the ashing technique; 20 g of quinoa leaves were heated in a muffle furnace at 550 °C for 8 h (AOAC, 2005). For the determination of total lipids, total proteins, carbohydrates, total and digestive fibers, the quinoa leaves were oven dried at 70 °C until the weight remained constant (AOAC, 2000). Then, the total lipids were obtained by petroleum ether extraction, using the Soxhlet method. The extraction was done for 8 h. The total amount of lipids was calculated with the formula: % Lipid = Weight of lipid extracted × 100/Weight of dry sample (1) (AOAC, 2000).

The total proteins were determined by using the Kjeldhal method and the conversion factor of 6.25 (AOAC, 2000). The content of dietary fiber was obtained by enzymatic digestion of defatted and gelatinized quinoa leaves (AOAC, 2000). The carbohydrates were determined by subtracting from 100% the total percentage of moisture, protein, lipid, ash and fiber. The caloric value of quinoa leaves was calculated using the relation: Caloric value (kcal) = Protein (g) × 4 kcal/g + Carbohydrates (g) × 4 kcal/g + Lipids (g) × 9 kcal/g + Fibers (g) × 2 kcal/g (2) (AOAC, 2005). The final results of the content of nutritional compounds, except for the ash content, were reported in fresh weight (fw).

2.3. Mineral content

The content of zinc (Zn), iron (Fe), calcium (Ca) and magnesium (Mg), was determined by atomic absorption spectrophotometry, with an AAS contrAA® 300 system (Analytikjena Contra AA 300). The potassium content (K) was analyzed by using a FP910 Flame Photometer instrument (PG Instruments) according to the method described in AOAC (1990).

2.4. Determination of antinutritional compounds

The antinutritional compounds were determined from quinoa leaves dried at 70 °C until constant weight (AOAC, 2000). The results were reported in fresh weight (fw).

Phytic acid content. The method consisted in adding 100 mL 2% HCl over 2 g of ground quinoa leaves; the mixture was left to react in the dark for 3 h and then filtered. 50 mL of filtrate was then collected and 100 mL of distilled water was added. To the mixture, a volume of 10 mL of 0.3% ammonium thiocyanate was added and then it was titrated with $FeCl_3$ as standard solution (1.95 mg Fe/mL) until a persistent (5 min) brown color was observed. The percentage of the phytic acid was obtained with the formula: Phytic acid (mg/100 g) = Y × 1.19 × 100/Sample weight (3), where, Y = titer value × 1.95 mg (Lucas & Markaka, 1975).

Tannin content. 50 mL of distilled water was added over 2 g of ground quinoa leaves and heated to 60 °C. The solution obtained was then filtered and a volume of 10 mL of 4% copper acetate solution was placed over the hot extract and boiled again for 10 min. The resulted residue was dried on filter paper, weighted, and then incinerated in a muffle furnace at 550 °C, cooled in a desiccator, and then weighed again. The tannin content was obtained by calculating the difference between the sample weight before incineration and the ash residue after incineration (Ogawa & Yazaki, 2018).

Oxalate content. The amount of oxalate was determined as follows: 2 g of ground quinoa leaves were added in 190 mL distilled water and 10 mL 6 M HCl. The mixture was digested at 100 °C for 1 h and then left to cool. After cooling, the sample was made up to 250 mL by using distilled water and then was filtered. Four drops of methyl red indicator were added to the filtrate, which was titrated with concentrated NH_4OH until the solution turned pale yellow (Chai & Liebman, 2005). The resulted filtrate was heated to 90 °C and 10 mL of 5% $CaCl_2$ solution were added under constant stirring. After that, the sample was left to cool and kept in the dark for 12 h. Then it was filtered and the precipitate obtained was washed with distilled water and dissolved in 5 mL 25% H_2SO_4. The solution obtained was maintained at 80 °C and titrated with 0.5% $KMnO_4$ until the pink persisted for 1 min (Umogbai et al., 2016). The amount of oxalate in quinoa leaves was related to the volume of $KMnO_4$ used for titration, where 1 mL $KMnO_4$ = 2.24 mg oxalate (Attalla et al., 2014).

Saponin content. The amount of saponins was determined by using the AOAC (1990) method: 2 g of finely ground quinoa leaves were extracted by refluxing in a Soxhlet extractor. Initially the extraction

operation was done with 200 mL of acetone for 3 h, and then with 200 mL CH_3OH for another 3 h. After the second extraction, the sample was dried in an oven, then cooled to room temperature and weighted. The amount of saponin was determined by the formula: Saponin mg/100 g = A − B × 100/Sm (4), where, A = mass of flask and extract; B = mass of empty flask; Sm = sample mass (AOAC, 1990).

Alpha–amylase inhibitors. 250 μL of 0.02 M sodium phosphate buffer, pH 6.9, containing α-amylase solution was added to a 250 μL of extract. The mixture obtained was pre-incubated for 10 min at 25 °C, and then 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer, pH 6.9, was added and then further incubated for another 10 min at 25 °C. After incubation, the reaction was stopped by adding 500 μL of dinitrosalicylic acid reagent (DNS) and the sample was incubated again for 5 min at 100 °C and then left to cool at room temperature. The obtained mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm, using T80 series of UV–Visible Spectrophotometer. The control was obtained by using the same method, replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as: % Inhibition = $[(A_{control} - A_{samples})/A_{control}] \times 100$ (5), where, A control = absorbance of each control and A samples = the net absorbance of each sample. The net absorbance of each sample was calculated using the following equation: A samples = A test − A blank (6), where A test = absorbance of each test and A blank = absorbance of each blank. The half maximal inhibitory concentration (IC50) was determined and expressed as mg·ml^{-1} fw (Barrett & Udani, 2011).

Trypsin inhibitors. 1 g of finely ground quinoa leaves was added in 50 mL of 0.5 M NaCl solution and the mixture was stirred for 30 min at room temperature. After stirring, the sample was centrifuged at 10.000×*g* rpm in a Beckman JA-20 rotor, Beckman Coulter, Indianapolis, IN, USA and the supernatant was filtered; the filtrate was used for the assay. 1 mL of trypsin inhibitor (1 mg/mL of trypsin in 0.1 M HCl) was added to 2 mL of trypsin standard solution and incubated for 10 min at 37 °C. After incubation, the reaction was stopped by adding 3 mL of 5% trichloroacetic acid (TCA). Then the solution was filtered and the absorbance was measured at 410 nm. The blank consisted of 5 mL substrate (1% casein substrate in 0.1 M phosphate buffer pH 7.7) (Korsinczky et al., 2004). The trypsin inhibitor activity was reported as the content of trypsin unit inhibited (TUI) per unit weight of the sample analyzed: TUI/mg = (B - A)/0.1 × F (7), where B = absorbance of test sample solution, A = absorbance of the blank, F = (1 × Vf/Va × D)/W (W = weight of sample, Vf = total volume of extract used in the assay, D = dilution factor, Va = volume of standard) (Lukanc et al., 2017).

2.5. Mineral bioavailability

The mineral bioavailability was represented by the molar ratios of antinutrient (e.g., phytic acid and oxalate)/mineral or mineral (e.g., calcium)/antinutrient. The critical values used to anticipate a favorable bioavailability of minerals were as follows: phytate [Phy]/zinc [Zn] < 15; calcium [Ca]/phytate [Phy] < 6; phytate [Phy]/iron [Fe] < 1; phytate × calcium [Phy × Ca]/zinc [Zn] < 0.5; oxalate [OX]/calcium [Ca] < 2.5 (Hailu & Addis, 2016; Mihalache et al., 2020).

2.6. Statistical analyses

The data are expressed as the means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to see the influence of cultivar, sowing time and crop density on the nutritional and antinutrient composition of quinoa leaves. The significant differences between treatments were established by using Tukey's post hoc test with a degree of confidence of 95% ($p < 0.05$), using a SPSS ver. 21.

3. Results and discussion

3.1. Primary metabolic compounds from quinoa leaves

The results of the primary metabolic compounds from quinoa leaves are presented in Table 1. The amount of ash varied with the cultivar from 3.97 g·100 g^{-1} dw for Titicaca to 5.32 g·100 g^{-1} dw for Puno; the difference of 34.01% between cultivars can be attributed to the higher adaptation of Puno to the experimental conditions. This cannot be said about the influence of the seeding time and crop density because these factors did not significantly influence the amount of ash (Table 1). In studies done on quinoa seeds or flour, which are considered a richer source of minerals as compared to rice, wheat or corn (Filho et al., 2015), the ash content was lower as compared to that registered in leaves, the data ranging from 1.2 g·100 g^{-1} fw for flour (Ogungbenle et al., 2003) up to 3.2 g·100 g^{-1} fw (Wright et al., 2002) or 3.7 g·100 g^{-1} fw for seeds (Dini et al., 1992).

The total lipids concentration in the leaves of quinoa was the lowest (between 0.76 and 1.25 g·100 g^{-1} fw) as compared with the rest of the primary compounds analyzed. This is normal taking into account that in general leafy vegetables have low lipid contents in the leaves, except for the seeds where the content is high (Dini et al., 1992; Wright et al., 2002). For instance, in amaranth or in spinach, the fat content can reach 2.02 or 5.39 g·100 g^{-1} dw (Pathan et al., 2019). In quinoa leaves the lipids content was significantly influenced by both the cultivar and the seeding time. The values recorded for Titicaca and Puno cultivars were significantly higher (on average 1.17 g·100 g^{-1} fw) as compared to that registered for Vikinga cultivar (0.76 g·100 g^{-1} fw). Regarding the seeding time, the total lipids concentration in the quinoa leaves of the crop sown on April 3 (St_1) and on April 10 (St_2) was significantly higher compared to that of the crops sown at St_3 (April 17). This can be attributed to the temperature which was more favorable for the production and accumulation of primary metabolism compounds when the plants were sown on April 3 (St1) than on April 17 (St3). Similar observations were done by Bhargava et al. (2007) who reported a decrease in the quality of quinoa seeds along with the temperature increase.

The total proteins were significantly influenced by the cultivar, the

Table 1
Primary metabolic compounds from quinoa leaves under agronomic factors.

Treatment	Ash (g·100 g^{-1} dw)	Total lipids (g·100 g^{-1} fw)	Total proteins (g·100 g^{-1} fw)	Carbohydrate (g·100 g^{-1} fw)	Caloric value (cal·100 g^{-1} fw)
Cultivar					
Titicaca	3.97 ± 0.16b	1.25 ± 0.05a	2.43 ± 0.09b	4.27 ± 0.17b	441.76 ± 16.77ns
Puno	5.32 ± 0.21a	1.10 ± 0.04a	2.83 ± 0.10a	5.10 ± 0.19a	426.00 ± 16.11ns
Vikinga	4.60 ± 0.18 ab	0.76 ± 0.03b	1.61 ± 0.06c	2.90 ± 0.11c	436.24 ± 16.52ns
Seeding time					
St_1	4.70 ± 0.15ns	1.18 ± 0.03a	2.64 ± 0.08a	4.73 ± 0.14a	433.90 ± 13.23ns
St_2	4.63 ± 0.42ns	1.05 ± 0.09 ab	2.31 ± 0.20 ab	4.14 ± 0.36 ab	434.65 ± 39.94ns
St_3	4.56 ± 0.03ns	0.87 ± 0.00b	1.91 ± 0.01b	3.41 ± 0.01b	435.46 ± 1.12ns
Crop density					
D_1	4.38 ± 0.22ns	1.06 ± 0.06ns	2.24 ± 0.11ns	3.97 ± 0.20ns	437.17 ± 21.36ns
D_2	4.60 ± 0.16ns	1.09 ± 0.04ns	2.40 ± 0.10ns	4.30 ± 0.17ns	434.72 ± 15.07ns
D_3	4.90 ± 0.33ns	0.96 ± 0.06ns	2.23 ± 0.14ns	4.01 ± 0.26ns	432.11 ± 29.58ns

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant; St_1 – April 3; St_2 – April 10; St_3 – April 17; D_1-7.7 mil. pl·ha^{-1}; D_2 – 3.2 mil. pl·ha^{-1}; D_3 – 1.6 mil. pl·ha^{-1}).

highest value being recorded for Puno (2.43 g·100 g^{-1} fw), which was 5.9% higher than that registered for Vikinga. The effect of the seeding time on the protein content highlighted that the highest values were registered in St_1, respectively 2.64 g·100 g^{-1} fw and the lowest in St_3 (1.91 g·100 g^{-1} fw), between which the differences were significant. Researches done on the total proteins in quinoa showed that the leaves can contain on average 20% high quality proteins (Abd El-Samad et al., 2018), while in the seeds, the amount can be of approximatively 14 g·100 g^{-1} fw (Garcia-Parra et al., 2020; Repo-Carrasco et al., 2003). The values recorded in leaves for the tested quinoa cultivars were comparable with those reported in spinach, chard or broccoli (Vasquez-Luna et al., 2019).

Carbohydrates are complex substances of organic nature and the source of glucose, fructose and galactose for the human body (Blanco & Blanco, 2017). The cultivars and the seeding time significantly influenced the amount of carbohydrates in quinoa leaves. Significant differences were registered between all the tested cultivars. The highest value was recorded for Puno (5.10 g·100 g^{-1} fw), followed by Titicaca (4.27 g·100 g^{-1} fw) and Vikinga (2.90 g·100 g^{-1} fw). The results recorded for the cultivars were in agreement with previous studies which stated that the carbohydrate content in quinoa leaves is usually low as compared with other leafy vegetables such as spinach or amaranth or with the content of the seeds, being suitable for the diet with a low glycemic index (Repo-Carrasco et al., 2003; Vasquez-Luna et al., 2019). For the seeding time, the carbohydrate concentration in the quinoa leaves of the crop sown on April 3 (St_1) was significantly higher than that recorded in the quinoa leaves of the crop sown on April 17 (St_3).

The data registered for the caloric value of the quinoa leaves, showed that there were not significantly influenced by the tested agronomic factors.

The values recorded for the total fiber and digestive fiber in quinoa leaves were much lower compared to the quinoa seeds or flour, where the fiber content can reach a value of 7.0–9.6% (Garcia-Parra et al., 2020; Ogungbenle, 2003; Wright et al., 2002). Furthermore, it was observed that their contents were significantly influenced by the cultivar and seeding time, but not by the crop density. Puno cultivar registered the highest amount of total fiber and digestive fiber, followed by Titicaca and Vikinga cultivars between which significant differences were registered (Fig. 1). Also, for this cultivar, the total percent of digestive fiber from the total fiber was of 51.7%. This might be attributed to the cell wall type, which differs depending on the species and cultivar (Widaningrum et al., 2020). Regarding the seeding time, significant differences were registered between St_1 and St_3, for both total fiber and digestive fiber. St_1 had the highest influence on the fiber contents, recording the highest amounts, whereas St_3 the lowest, registering the lowest values (Fig. 1).

The crop density did not influence any of the primary metabolic compounds analyzed, as compared to the data recorded by Bhargava et al., 2007 who stated that the maximum protein and carotenoid contents were recorded when the row spacing was of 15 cm.

3.2. The influence of cultivar, sowing time and crop density on mineral contents

The consumption of raw leafy vegetables is important for the health not only due to the content of nutritional compounds, but also due to the richness in minerals (Natesh et al., 2017).

The content of macro and microelements of quinoa leaves under the influence of cultivar, seeding time and crop density is presented in Table 2. The values registered showed that the mineral content in quinoa leaves was significantly influenced by the cultivar, the highest values being recorded by Titicaca cultivar regarding Fe (4.73 mg·kg^{-1} fw), Zn (0.73 mg·kg^{-1} fw) and Na (0.62 mg·kg^{-1} fw), while K (9.39 g·100 g^{-1} dw) was the highest registered in the leaves of Puno cultivar.

The content of Mg and Ca had almost the same value for all the cultivar tested, no significant differences being recorded. As far as we know, this is the first study reporting the magnesium content in the leaves of quinoa, under stress-free conditions; previous studies were done on the seeds where the content reached high values like 270 g·100 g^{-1} dw (Repo-Carrasco et al., 2003) or in saline conditions where the content varied between 50.74 mg·g^{-1} dw for the control plants and 106.12 mg·g^{-1} dw for the plants treated with NaCl (Wada et al., 2020).

In another leafy vegetable highly preferred by consumers, spinach, the magnesium content can range between 117.2 mg·100 g^{-1} dw to 429.2 mg·100 g^{-1} dw (Ferreira et al., 2016). Regarding the calcium content, in a study done on quinoa sprouts and seeds, the amount registered was of 48.35 mg·100 g^{-1} fw and 32.44 mg·100 g^{-1} fw respectively (Darwish et al., 2020), while in spinach it can reach 99 mg·100 g^{-1} (Butnariu & Butu, 2015).

The seeding time influenced only the Fe content, the highest values being recorded for St_1 (3.92 mg·kg^{-1} fw) and St_2 (3.58 mg·kg^{-1} fw). As

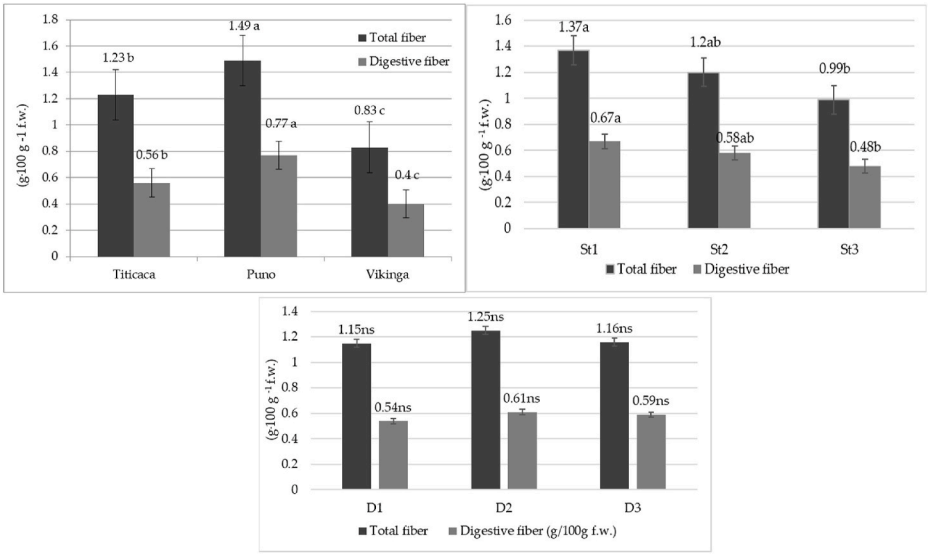

Fig. 1. Total fiber and digestive fiber content from quinoa leaves under the influence of cultivar, seeding time and crop density. The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant; St_1 – April 03; St_2 – April 10; St_3 – April 17; D_1-7.7 mil. pl·ha^{-1}; D_2 – 3.2 mil. pl·ha^{-1}; D_3 – 1.6 mil. pl·ha^{-1}).

Table 2
Mineral contents of quinoa leaves under agronomic factors.

Treatment	Potassium (K) (g·100 g^{-1} dw)	Magnesium (Mg) (g·100 g^{-1} dw)	Calcium (Ca) (g·100 g^{-1} dw)	Iron (Fe) (mg·kg^{-1} fw)	Zinc (Zn) (mg·kg^{-1} fw)	Sodium (Na) (mg·kg^{-1} fw)
Cultivar						
Titicaca	5.42 ± 0.22c	3.23 ± 0.12ns	6.54 ± 0.25ns	4.73 ± 0.18a	0.73 ± 0.03a	0.62 ± 0.02a
Puno	9.39 ± 0.36a	2.80 ± 0.11ns	5.80 ± 0.22ns	3.44 ± 0.13b	0.42 ± 0.02c	0.17 ± 0.01c
Vikinga	7.67 ± 0.31b	2.96 ± 0.11ns	5.96 ± 0.23ns	2.30 ± 0.09c	0.57 ± 0.02b	0.31 ± 0.01b
Seeding time						
St_1	7.60 ± 0.26ns	2.97 ± 0.09ns	6.05 ± 0.18ns	3.92 ± 0.10a	0.55 ± 0.02ns	0.36 ± 0.01ns
St_2	7.54 ± 0.68ns	3.00 ± 0.27ns	6.12 ± 0.56ns	3.58 ± 0.32 ab	0.58 ± 0.05ns	0.37 ± 0.04ns
St_3	7.33 ± 0.06ns	3.01 ± 0.01ns	6.14 ± 0.01ns	2.97 ± 0.01b	0.59 ± 0.00ns	0.38 ± 0.01ns
Crop density						
D_1	6.74 ± 0.34ns	3.05 ± 0.15ns	6.17 ± 0.30ns	3.72 ± 0.19ns	0.63 ± 0.03ns	0.43 ± 0.02a
D_2	7.64 ± 0.27ns	3.00 ± 0.10ns	6.13 ± 0.21ns	3.66 ± 0.14ns	0.58 ± 0.02ns	0.37 ± 0.01 ab
D_3	8.09 ± 0.55ns	2.93 ± 0.20ns	6.00 ± 0.41ns	3.09 ± 0.20ns	0.51 ± 0.04ns	0.31 ± 0.02b

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant; St_1 – April 3; St_2 – April 10; St_3 – April 17; D_1-7.7 mil. pl·ha^{-1}; D_2 – 3.2 mil. pl·ha^{-1}; D_3 – 1.6 mil. pl·ha^{-1}).

in the case of nutritional components, a decreasing trend in the iron content was observed from the first decade until the end of the second decade of April.

The crop density influenced only the Na content; the highest content was recorded in the plants where the density was of 7.7 mil. pl·ha^{-1} (D_1) (0.43 mg·kg^{-1} fw) and of 3.2 mil. pl·ha^{-1} (D_2) (0.37 mg·kg^{-1} fw). As in the case of seeding time for Fe, a decreasing trend of the Na content was observed from D_1 to D_3, suggesting that with the decrease of the plant density the contents of Fe or Na in quinoa leaves increase.

3.3. Influence of cultivar, seeding time and crop density on the antinutritive composition of quinoa leaves

The importance of this leafy vegetable in the human diet is equally given by its nutritional composition and mineral content, and also by the antinutritional factors. In general, plants are rich sources of bioactive compounds, essential for the health, but at the same time contain phytochemicals which can have a negative impact on human health (Natesh et al., 2017). The antinutritive components found in leafy vegetables such as phytic acid, oxalates, saponins, the trypsin inhibitor or α-amylase can affect the intestinal absorption of minerals (Mihalache et al., 2020; Oyetayo & Ibitoye, 2012).

The antinutritive composition of quinoa leaves under the influence of different agronomic factors is shown in Table 3.

Phytic acid can have positive effects on human health by enhancing the phosphorus storage, but at the same time, in high concentration, it can inhibit the absorption of different essential minerals such as Fe, Zn, or Ca (Mihalache et al., 2020; Oyetayo & Ibitoye, 2012).

In quinoa, the content of phytic acid was influenced only by the cultivar, the amount registered varying between 0.03 g·100 g^{-1} fw for Vikinga cultivar and 0.06 g·100 g^{-1} fw for Titicaca. No significant differences were registered between the seeding times or the crop densities. As far as we know, studies on the phytic acid content of quinoa leaves are not found in the literature, but in a research on the phytic acid content of the leaves of other five different species (*Taraxacum officinale* L., *Rumex acetosa* L., *Alliaria petiolata* (M. Bieb.), *Galium aparine* L. and *Vicia sativa* L.), the recorded values varied from 0.26 mg·g^{-1} for *V. sativa*, up to 1.29 mg·g^{-1} for *G. aparine* (Alkarawi & Zotz, 2014). Higher amounts of phytic acid were found in ground chickpea seeds, with values ranging from 8.94 mg·g^{-1} to 21.97 mg·g^{-1} (Joshi-Saha & Reddy, 2015). In our study, the values recorded for the phytic acid were lower compared with the contents mentioned above.

Oxalates are another example of antinutritive factors which can bind Ca^{2+}, Fe^{2+} or Mg^{2+} ions, leading to nutritional deficiencies and different irritations to the gut lining. In plants the oxalates content can vary with the species. For instance, in legumes it can reach 8 mg·kg^{-1}, in grains between 35 and 270 mg·100 g^{-1} or in tubers between 0.4 and 2.3 mg·100 g^{-1} (Mihalache et al., 2020).

Table 3
Antinutritive compounds from quinoa leaves under agronomic factors.

Treatment	Phytic acid (g·100 g^{-1} fw)	Oxalates (g·100 g^{-1} fw)	Saponins (g·100 g^{-1} fw)	Trypsin inhibitor (TUI·mg^{-1} fw)	α–amilase IC 50 (mg·ml^{-1} fw)
Cultivar					
Titicaca	0.06 ± 0.00a	0.14 ± 0.01b	0.15 ± 0.01a	0.43 ± 0.02b	0.95 ± 0.04b
Puno	0.04 ± 0.00b	0.25 ± 0.01a	0.10 ± 0.00b	0.62 ± 0.03a	1.11 ± 0.04a
Vikinga	0.03 ± 0.00b	0.11 ± 0.01b	0.07 ± 0.00c	0.34 ± 0.01c	0.63 ± 0.02c
Seeding time					
St_1	0.05 ± 0.00ns	0.20 ± 0.01a	0.12 ± 0.00a	0.54 ± 0.02a	1.03 ± 0.03a
St_2	0.05 ± 0.01ns	0.17 ± 0.01 ab	0.11 ± 0.01 ab	0.47 ± 0.04 ab	0.90 ± 0.08 ab
St_3	0.04 ± 0.00ns	0.14 ± 0.00b	0.09 ± 0.00b	0.39 ± 0.00b	0.74 ± 0.00b
Crop density					
D_1	0.05 ± 0.00ns	0.20 ± 0.01ns	0.12 ± 0.01ns	0.44 ± 0.02ns	0.87 ± 0.05ns
D_2	0.05 ± 0.00ns	0.17 ± 0.01ns	0.11 ± 0.00ns	0.49 ± 0.02ns	0.93 ± 0.04ns
D_3	0.04 ± 0.00ns	0.14 ± 0.01ns	0.09 ± 0.01ns	0.46 ± 0.03ns	0.87 ± 0.06ns

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant; St_1 – April 03; St_2 – April 10; St_3 – April 17; D_1-7.7 mil. pl·ha^{-1}; D_2 – 3.2 mil. pl·ha^{-1}; D_3 – 1.6 mil. pl·ha^{-1}).

In quinoa leaves, the oxalates content was influenced by the cultivar and the seeding time. The smallest amount of oxalates was recorded for Vikinga (0.11 g·100 g^{-1} fw) and Titicaca (0.14 g·100 g^{-1} fw) while the highest for Puno cultivar (0.25 g·100 g^{-1} fw). Regarding the seeding time, the results showed that oxalates accumulated less when quinoa seeds were sown on April 17 (St_3) and more when sown on April 3 (St_1) or on April 10 (St_2), between which significant differences were registered. The crop density did not significantly influence the oxalates concentration in quinoa leaves. In another research done on *Chenopodium album* L. the values registered were between 682.79 mg·100 g^{-1} dw for boiled leaves and 1112.40 mg·100 g^{-1} dw for fresh leaves (Savage et al., 2019). Studies done on other leafy species, like spinach, showed that the oxalates concentration can vary between 2.38 mg·g^{-1} fw and 34.72 mg·g^{-1} fw (Wang et al., 2018) or between 53.4 mg·g^{-1} dw and 79.9 mg·g^{-1} dw (Shi et al., 2016).

Saponins as an antinutritive factor interfere in the vitamins and minerals uptake, also in the protein digestion, by reducing these processes (Mihalache et al., 2020; Vitanescu et al., 2019). As in the case of oxalates, the saponin content was influenced by only the cultivar and the

seeding time, for which significant differences were registered. The lowest amount of saponins was recorded for Vikinga cultivar (0.07 g·100 g^{-1} fw), followed by Puno (0.10 g·100 g^{-1} fw) and Titicaca (0.15 g·100 g^{-1} fw). Regarding the seeding time, the smallest value of saponins was registered at St_3 (0.09 g·100 g^{-1} fw), while the highest at St_2 and St_1 (0.11 g·100 g^{-1} fw and 0.12 g·100 g^{-1} fw respectively). No significant differences were registered for the crop density, the amount of saponins being on average of 0.10 g·100 g^{-1} fw. Compared to the saponin content in the seeds of quinoa (Vitanescu et al., 2019), the content found in leaves was smaller. The content is smaller also compared to the content found in the red and yellow quinoa seeds (0.50 mg•g^{-1} and 6.53 mg•g^{-1}) (Al-Qabba et al., 2020). Other studies done on the saponins content in quinoa leaves reported concentrations of 0.04%–0.07% (Fiallos-Jurado et al., 2016).

Another antinutrient factor analyzed was the trypsin's inhibitor, known for its inhibitory action on the activity of some enzymes, such as trypsin or chemotrypsin, preventing the digestion of proteins. In plants, trypsin's inhibitors are more common in *Fabaceae*, *Solanaceae* or *Gramineae* families (Gemede & Ratta, 2014). The trypsin inhibitor in quinoa leaves was influenced by the cultivar and the seeding time, but not by the crop density. The lowest concentration was recorded for Vikinga cultivar (0.34 TUI·mg^{-1} fw) and when the seeding was done on April 17 (St_3 = 0.39 TUI·mg^{-1} fw), while the highest was registered for Puno cultivar (0.62 TUI·mg^{-1} fw) and for the leaves of the plants whose seeds were sown on April 3 (St_1 = 0.54 TUI·mg^{-1} fw), the trypsin inhibitor concentration – depending on the crop density – was on average of 0.10 TUI·mg^{-1} fw. As far as we know, studies on the trypsin inhibitor in quinoa leaves are not found in the literature, but it has been examined in quinoa flour, where the values recorded ranged from 0 TUI·ml^{-1} to 6.5 TUI·ml^{-1} (Chauhan et al., 1992). The values are very high compared with those registered in the leaves of quinoa. In other plant species, like sweet potato, the trypsin inhibitor concentration found in leaves was between 369.3 TUI· 100 g^{-1} fw and 488.2 TUI· 100 g^{-1} fw (Hou & Lin, 1998).

The presence of alpha-amylase inhibitors in the human nutrition can impair the carbohydrate digestion and can cause digestive diseases (Choi et al., 2019). In quinoa leaves, only two treatments had a significant influence: the cultivar and the seeding time. Significant differences were registered between all the cultivars tested. The values varied between 0.63 mg·ml^{-1} fw for Vikinga and 1.11 mg·ml^{-1} fw for Puno. Regarding the seeding time, significant differences were recorded between St_1 and St_2, for which the alpha-amylase inhibitors concentration was the highest (1.03 mg·ml^{-1} fw and 0.90 mg·ml^{-1} fw respectively), and St_3, for which the lowest value of alpha-amylase inhibitors was registered. No significant differences were recorded between the crop densities, the average amount being of 0.89 mg·ml^{-1} fw. As far as we know, studies on the α-amylase content of quinoa leaves are not found in the literature, but in the case of quinoa seeds, the value recorded ranged from 0.22 g/100 g db to 5.89 g/100 g db, 48 h after the seeds germinated (Suarez-Estrella et al., 2020). In other plant species, like basil, the α-amylase inhibitors content in the essential oil was between 1.32 and 13.34 μL mL−1 (Zlotek et al., 2020).

Tannins are another group of antinutritive components which can impair the digestion of proteins, the activities of enzymes (e.g. lipase, trypsin or amylase) or the absorption of minerals (e.g. iron). In plants, the concentration of tannins can vary with the species: in legumes it can be between 1.8 and 18 mg·g^{-1} while in tubers between 4.18 and 6.72 mg·100 g^{-1} (Popova & Mihaylova, 2019). The tannin content in the quinoa leaves is showed in Fig. 2. The tannin concentration varied along with the cultivar and the seeding time, for which significant differences were registered. The values recorded depending on the cultivar varied between 0.13 g·100 g^{-1} fw for Vikinga and 0.31 g·100 g^{-1} fw for Puno, significant differences being registered only between Puno cultivar and Titicaca or Vikinga. The sowing of the quinoa seeds on April 17 (St_3) resulted in leaves with the lowest tannin concentration (0.16 g·100 g^{-1} fw), while the sowing on April 10 or April 3 gave the highest concentrations of tannins (0.2 g·100 g^{-1} fw and 0.23 g·100 g^{-1} fw respectively). No differences were recorded between the tannin concentration resulted from the three different crop densities used, the average amount being of 0.19 g·100 g^{-1} fw. As far as we know, studies on the tannin content of quinoa leaves are not found in the literature, but this component has been identified in quinoa seeds, with concentrations between 0.23% and 0.92% (Chauhan et al., 1992).

Taking into account the phytic acid and oxalate concentration, the bioavailability of zinc, calcium, and iron in the quinoa leaves was calculated (Table 4).

The zinc bioavailability from [Phy]/[Zn] molar ratio varied depending on the treatments as follows: between 5.26 and 9.52 mg·100

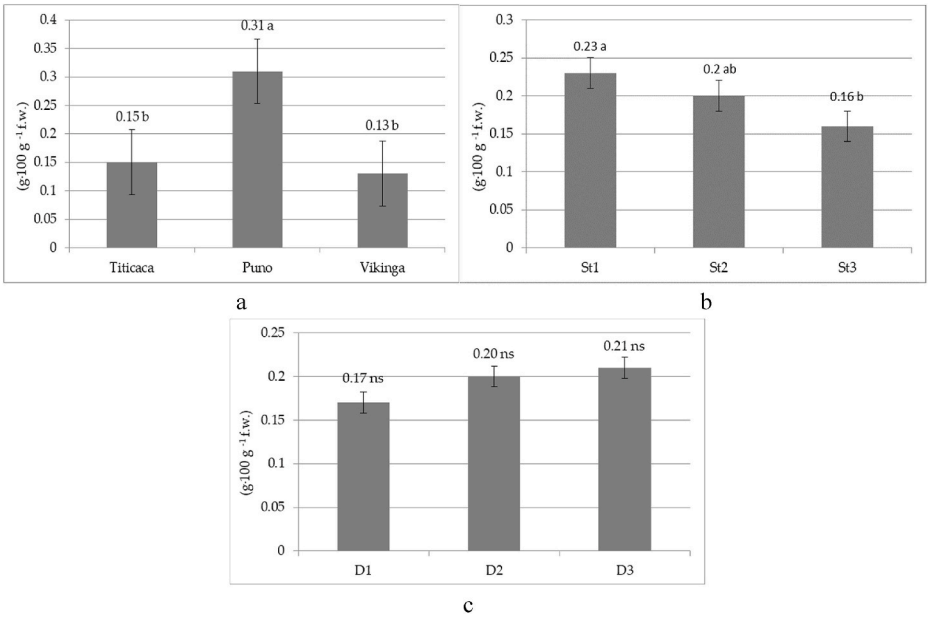

Fig. 2. Tannin content from quinoa leaves under the influence of cultivar (a), seeding time (b) and crop density (c). The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant; St_1 – April 3; St_2 – April 10; St_3 – April 17; St3 –17.04; D1-7.7 mil. pl·ha-1; D2 – 3.2 mil. pl·ha-1; D3 – 1.6 mil. pl·ha-1).

Table 4
Bioavailability of macro and micronutrients.

Treatment	[Phy]/[Zn] (mg·100 g^{-1}fw)	[Ca]/[Phy] (mg·100 g^{-1}fw)	[Phy]/[Fe] (mg·100 g^{-1}fw)	[Phy × Ca]/[Zn] (mol·kg^{-1})	[OX]/[Ca] (mg·100 g^{-1}fw)
Cultivar					
Titicaca	8.22	1.09	0.127	0.538	0.214
Puno	9.52	1.45	0.116	0.552	0.431
Vikinga	5.26	1.99	0.130	0.314	0.185
Seeding time					
St_1	9.09	1.21	0.128	0.550	0.331
St_2	8.62	1.22	0.140	0.528	0.278
St_3	6.78	1.54	0.135	0.416	0.228
Crop density					
D_1	7.94	1.23	0.134	0.490	0.324
D_2	8.62	1.23	0.137	0.528	0.277
D_3	7.84	1.50	0.129	0.471	0.233
Critical value	<15	<6	<1	<0.5	<2.5
Bioavailability	*Favorable for Zn absorption*	*Favorable for Ca absorption*	*Favorable for Fe absorption*	*Favorable for Zn absorption*	*Favorable for Ca absorption*

g^{-1} in the case of cultivar; between 6.78 and 9.09 mg·100 g^{-1} in the case of seeding time and between 7.84 and 8.6 mg·100 g^{-1} in the case of crop density, which suggest a moderate zinc availability to human nutrition. The best values were registered for Vikinga cultivar (5.26 mg·100 g^{-1} fw), St3 (April 17) seeding time (6.78 mg·100 g^{-1} fw) and D3 crop density (1.6. mil. pl·ha^{-1}).

Zinc bioavailability can also be impaired when the phytic acid is present along with calcium ions, because of the low solubility complex that results between them (Ca:Zn:Phy).

Zinc bioavailability from the [Phy]/[Zn] complex can also be affected by calcium ions, which interact with zinc ions, creating a synergism between them, resulting in the Ca:Zn:Phy complex with low solubility (Hailu & Addis, 2016). In leaves of quinoa, the [Phy × Ca]/[Zn] molar ratio was below the critical value (>0.5 low zinc availability), for Vikinga cultivar, St3 seeding time and D1 crop density, suggesting that the calcium does not affect the zinc availability (Table 4).

The phytic acid can also affect the calcium bioavailability. In our study, the results recorded for [Ca]/[Phy] molar ratio varied between 1.09 and 1.99 mg·100 g^{-1} fw in the case of cultivar; 1.21–1.54 mg·100 g^{-1} fw in the case of seeding time and 1.23–1.50 mg·100 g^{-1} fw in the case of crop density, suggesting a favorable calcium absorption.

The availability of Ca can result, also, from the molar ratio between oxalate and calcium. For a good bioavailability the ratio value should be under 2.5. In the quinoa leaves, the critical values registered, regardless of the treatments, were below 2.5, suggesting a good availability of this element.

The phytic acid can also have a negative impact on the iron absorption if the molar ratio is less than 1 (Umeta et al., 2005). In quinoa leaves the values registered regardless of the treatment were less than 1, suggesting a low Fe absorbtion.

4. Conclusions

The cultivation of quinoa as a leafy species depends on the nutritional composition and antinutritive components. Only the cultivar and seeding time influenced the primary metabolic and antinutritive compounds. The primary metabolic compounds (lipids, proteins, carbohydrates) were the highest in Puno cultivar, while the antinutritive compounds (tannins, phytic acid, saponins, oxalates, trypsin inhibitor and alpha-amylase) were the lowest in Vikinga cultivar. Regarding the time of crop establishment, the highest content of primary metabolic compounds (carbohydrates, lipids and total proteins) was achieved by the sowing on April 17. The same sowing dates resided with the lowest content of antinutritive compounds. The interaction T x St_2 x D_2 was the best for the total lipids content, while the interaction P x St_1 x D_2 gave the highest values for total proteins, carbohydrates, total fiber and digestive fibers. Regarding the antinutritive compounds, the lowest content was recorded for Vikinga cultivar, sown on April 17, at a crop density of 7.7 mil. pl·ha^{-1} for tannins and trypsin inhibitors, 3.2 mil. pl·ha^{-1} for phytic acid, tannins, trypsin inhibitors or α-amylase and 1.6 mil. pl·ha^{-1} for phytic acid, saponins and trypsin inhibitors. The content of mineral elements such as Fe, Zn, Na and K was significantly influenced by the cultivar, which was not the case for Mg and Ca. For the interactions: P x St_1 x D_3 and P x St_2 x D_3 the highest content of K, for T x St_1 x D_1 the highest content of Fe, for T x St_3 x D_1 the highest content of Zn, while for T x St_2 x D_1 and T x St_3 x D_2 the highest content of Na were registered. Regarding the bioavailability of minerals for the human body, zinc and calcium were favorable for absorption, while iron had a low bioavailability. The results obtained in this study demonstrated that the tested cultivars of quinoa have a good adaptation to the temperate continental climate.

Author contributions

V.S., S.E.J. and M.V. conceived and planned the experimental protocol, and performed the research supervision; M.V., N.M. and G.J. were involved in laboratory analyses; A.C., M.V. and G.C.T. conducted the field experiment and biometrical determinations; M.B. involved for biochemical analyses; T.S., V.S. and G.C.T. contributed to data statistical processing and interpretation; N.M., T.S. and A.C. were involved in bibliographic research; V.S., G.M. and S.E.J., wrote the draft and final manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

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