OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2016.450.456

# Research Article
# Proximate Chemical Composition and Content of Biologically Active Components in Leaves of Two Quinoa Cultivars (Salcedo and Altiplano) Produced in Peru

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## Abstract

**Objective:** The purpose of the presented study was to describe the proximate chemical composition and content of biologically active components in leaves of two quinoa cultivars (Salcedo and Altiplano) produced in Peru. **Methodology:** This study conducted by means of different analytical methods. It is describe changes of different phenological stages in relation to nutritional composition, pigment contents, polyphenols, flavonoids and antioxidant activity of plant extract evaluated by standard methods of DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2 -azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) assay and the correlation between mean antioxidant index scores were also analyzed. **Results:** The results presented no considerable variations. However, the ash content for Altiplano and Salcedo leaves was 2.8 and 3.6%, respectively. The total pigment content is much higher in the Salcedo leaves (4816.77 μg $g^{-1}$ dry weight) when compared with Altiplano leaves (2662.92 μg $g^{-1}$ dry weight). The total polyphenol and total flavonoid contents is similar in both samples for Salcedo (10.55 mg GAE $g^{-1}$ and 8.69 mg rutin $g^{-1}$) and Altiplano (10.72 mg GAE $g^{-1}$ and 9.14 mg rutin $g^{-1}$), respectively. The antioxidant activity as measured by DPPH and ABTS, expressed as Trolox Equivalent Antioxidant Capacity (TEAC), evaluated in Altiplano was higher than that found in Salcedo. The antioxidant potency composite index was 16.32 and 11.95, respectively. The antioxidant potency showed the positive correlation coefficients with phenolics ($r^2$ = 0.6575), flavonoids ($r^2$ = 0.3896) and the correlation between phenolics and flavonoids ($r^2$ = 0.6744). **Conclusion:** These results indicated promising perspectives for the leaves of two quinoa cultivars (Salcedo and Altiplano) that are excellent resources of bioactive components and can be used in the food industry as infusions.

Key words: Quinoa leaves, chemical composition, active components, antioxidant activity

Received: August 08, 2016 Accepted: September 12, 2016 Published: October 15, 2016

Citation: Lourdes Chacaliaza-Rodríguez, Grace Espinoza-Begazo, Fernando Ramos-Escudero and Karin Servan, 2016. Proximate chemical composition and content of biologically active components in leaves of two quinoa cultivars (Salcedo and Altiplano) produced in Peru. Res. J. Med. Plants, 10: 450-456.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), a native plant to the Andean highlands in South America. For centuries, *Chenopodium* spp., has been cultivated as a leafy vegetable and subsidiary grain crop in different parts of the world[1]. There is extensive literature on the chemical composition of quinoa seed[2-5]. They are rich in protein and essential amino acid, starch, minerals and oils[6]. Recently have been examined the hydrophilic and lipophilic bioactive components of quinoa seeds and have been identified at least 23 phenolic compounds in either free or conjugated forms, namely vanillic acid, ferulic acid and their derivatives as the main phenolic acids and quercetin and kaempferol and their glycosides as the main flavonoids[5]. Other components as fatty acids, tocopherols, carotenoids and their respective antioxidant contributions in quinoa seed samples. It has been found that the lipid yield was 6.03-6.74%, with Unsaturated Fatty Acids (UFAs) being the predominant fatty acids, 81.44-84.49% in quinoa seeds. Carotenoids, mainly β-carotene is reported first in quinoa seed. The predominant tocopherols in quinoa seeds areγ and α-tocopherol[5]. Industrially the following products are obtained: Pearl, flake, precooked, flour, instant quinoa, noodles, grits, biscuits, expanded, etc.[7].

In the Peru, this plant has a great variability and diversity of forms. They can be classified varieties or ecotypes in 5 basic categories according to their adaptation to the geographical characteristics: Valley quinoa, altiplano quinoa, salt flat quinoa, sea level quinoa[8] and subtropical quinoa[7]. In Peru there are more than 3000 varieties of quinoa, yet only 30 varieties are used. The main varieties of quinoa grown in Peru are: Blanca Junín, Rosada Junín, Nariño Amarillo, Marangani, Tahuacol, Kancolla, Cheweca, Chucapaca, Kamiri, Camacan II, Rosada Cusco, Real, Boliviana Jujuy, Sajama, Blanca de Juli, Mantaro and Hualhas, etc. The Instituto de Innovación Agraria (INIA) has been responsible for preserving the genetic material from approximately 2000 varieties of which only 7 have been improved with the aim of optimize performance, grain quality, resistance to diseases and pests. Quinoa varieties present in the INIA are quinoa Quillahuaman INIA, quinoa Salcedo INIA, quinoalllpa INIA, quinoa INIA Pasancalla, quinoa INIA Negra Collana, quinoa INIA Amarilla Sacaca, quinoa INIA Altiplano and quinoa INIA Santa Ana[9].

In contrast to seeds, there are few studies on the chemical composition and bioactive components of quinoa leaves[10]. Some investigations on the functional and potential biological properties of bread fortified with quinoa leaves in the light of protein-phenolic interactions[11] and the effect of fortification with ground *Chenopodium quinoa* leaves on the sensory value and nutraceutical potential of breads and chemical procedure for determination their quality[12].

Other studies on the evaluation of nutraceutical potential of *Chenopodium quinoa* leaves in the context of the bioaccessibility and bioavailability of their phenolic compounds[13]. In particular, the leaves, till now treated as worthless waste, are edible and may be consumed in salad and also used as a valuable supplement for functional food[14]. The quinoa leaves contain an ample amount of ash (3.3%), fiber (1.9%), nitrates (0.4%), vitamin E (2.9 mg α-TE/100 g) and Na (289 mg/100 g), vitamin C (1.2-2.3 g $kg^{-1}$) and 27-30 g $kg^{-1}$ of proteins[15]. Fatty acid composition in leaves ranging of SFA (15-22%), MUFA (15-19%) and PUFA (50-56%). The concentrations of carotenoids rangins between 496-738 μg $g^{-1}$ dry weight, whereas, that the concentration of tocopherols varies between 9-93 μg $g^{-1}$ dry weight[10].

The objectives of this study were to carry out a comprehensive examination on the bioactive components in leaves of quinoa including the proximate chemical composition, pigment contents, polyphenols and flavonoids and to evaluate the possible roles of these compounds on antioxidant activity in leaves of two quinoa cultivars (Salcedo and Altiplano) produced in Peru.

## MATERIALS AND METHODS

**Collection and preparation of leaf materials:** Leaves of two quinoa cultivars (Salcedo and Altiplano) were collected at four phenological stages from the Estación Experimental del Instituto de Innovación Agraria (INIA), Distric La Molina, Lima, Peru, in May, 2015. The leaves was harvested of at least 10 plants per cultivar were hand picked. Each plant were washed thoroughly with deionized water, then were dried under the shade at room temperature, subsequently in oven at 60°C for 12 h. The dried leaves were grinded well into a fine powder with Rotor Mill Pulverisette 14. The fine powder was stored in polyethylene bags at -4°C until extraction for the various analyses. Other samples studied were purchased in the supermarket (Lima, Peru): Green tea (*Camellia sinensis* L.) (Saint-Gottard, Argentina), lemon verbena (*Aloysia triphylla* (L'Her) Britton) (Saint-Gottard, Argentina), cats claw (*Uncaria tomentosa*) (Sunka, Peru) and coca (*Erythroxylum coca*) (Herbi, Peru).

**Proximate chemical composition:** The proximate composition (ash, moisture, protein and fiber) of fresh leaf samples was determined using protocol of the AOAC[16]. Briefly, ash content was determined by dry ashing method i.e., placing the sample in furnace. Kjeldhal apparatus was used for

the estimation of nitrogen content and protein content was calculated as N*6.25. Method for determining moisture content by oven drying at 103°C for 3 h and crude fiber was determined by acid hydrolysis with 1.25% $H_2SO_4$, followed by alkaline hydrolysis with 1.25% NaOH.

**Determination of pigment contents:** Accurately weighted 2.0 g of dried plant leaf sample was taken and homogenized in tissue homogenizer with 50 mL of 80% acetone. Homogenized sample mixture was centrifuge for 10000 rpm for 15 min at room temperature. The supernatant were separated and 200 µL of it is mixed with 800 µL of the respective solvent. The solution was analyzed for chlorophyll-a, chlorophyll-b and total carotenoids content in spectrophotometer[17]. The concentrations for Chl-a, Chl-b and the sum of leaf carotenoids ($C_{x+c}$) can be calculated with the following equations given for acetone with 20% (v/v) water:

$$\text{Chl-a } (\mu g\ mL^{-1}) = 12.25\ A_{663} - 2.79\ A_{647}$$

$$\text{Chl-b } (\mu g\ mL^{-1}) = 21.50\ A_{647} - 5.10\ A_{663}$$

$$C_{(x+c)} = (1000\ A_{470} - 1.82\ \text{Chl-a} - 85.02\ \text{Chl-b})/198$$

**Determination of polyphenols and flavonoids:** Total polyphenols were determined by the Folin–Ciocalteu method. A sample aliquot of 100 µL or standard was reacted with 750 µL of 0.2 N Folin-Ciocalteu reagent, after 5 min of reaction 750 µL of sodium carbonate (7.5%) was added. The calibration curve consisted of the following concentrations of gallic acid: 5, 10, 40, 70 and 100 µg $mL^{-1}$.The reaction was carried out at room temperature for 24 h in darkness and the absorbance[18] was read at 725 nm.

Total flavonoid was determined according to Miliauskas *et al.*[19] using the aluminum chloride colorimetric method. Rutin (quercetin-3-O-rutinoside) was used as the reference standard at the corresponding concentrations for the calibration curve: 2, 5, 10, 25, 50 and 100 µg $mL^{-1}$. Extract (1 mL) was added to distilled water (4 mL) in a flask. Then, 5% $NaNO_2$ (0.3 mL) was added. After 5 min, 10% $AlCl_3$ (0.3 mL) was added and after 6 min, 1 M NaOH (2 mL) was added. The mixture was diluted to 10 mL with distilled water. The absorbance of the solution was measured at 510 nm using a spectrophotometer. The mixture was allowed to react at 25°C for 40 min, after this time the absorbance was read.

**DPPH radical-scavenging activity:** The method used for the DPPH radical scavenging was adapted from that of De Campos *et al.*[20]. The reaction occurred between 50 µL of sample and 950 µL of 100 µmol $L^{-1}$ DPPH. The sample concentrations were in the range of 5-500 µg $mL^{-1}$. The absorbance values were recorded every 1 min for 10 min. The antioxidant activity was expressed as the 50% inhibitory concentration ($IC_{50}$).

**ABTS radical scavenging assay:** For ABTS assay, the procedure followed the method of Arnao *et al.*[21] with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 20 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 0.974±0.01 U at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. The reaction occurred between 50 µL of sample and 950 µL of ABTS solution.

**Determination of antioxidant index score:** An overall antioxidant potency composite index was determined by assigning an index value of 100 to the best score for each test and then calculated an index score for all other samples within the test as follows[22]:

$$\text{Antioxidant index score} = \frac{\text{Sample score}}{\text{Best score}} \times 100$$

The average of all four tests for each mistletoe extract was then taken for the antioxidant potency composite index.

**Statistical analyses:** Results were expressed as the Mean±Standard Deviation (SD) of three independent extractions. Statistical comparison was performed by analysis of variance (one-way ANOVA). Duncan test significant difference was used as *post hoc* test. A comparison of assays was made by correlation and linear regression analysis. Differences were considered significant at $p<0.05$. Statistical analysis was performed using SPSS software for Windows (release 18.0).

## RESULTS AND DISCUSSION

There is increasing interest both in the industry and in scientific study for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants[23]. Many herbs and spices, usually used for flavor, color, aroma and preservation of foods and beverages are an excellent source of phenolic compounds which have been reported to show good antioxidant activity[24].

Table 1: Proximate composition of different phenological stages of two quinoa cultivars Salcedo and Altiplano leaves

| | Salcedo leaves (%) | | | | Altiplano leaves (%) | | | |
|---|---|---|---|---|---|---|---|---|
| Stages | Moisture | Protein | Ash | Fiber | Moisture | Protein | Ash | Fiber |
| First | 88.7±2.1 | 4.4±0.7 | 2.8±0.1 | 0.8±0.0 | 87.9±1.5 | 3.8±0.2 | 2.3±0.1 | 0.8±0.0 |
| Second | 86.1±1.8 | 5.1±0.3 | 3.3±0.1 | 0.9±0.0 | 89.5±2.3 | 4.2±0.1 | 2.6±0.2 | 0.9±0.1 |
| Third | 86.2±2.6 | 4.6±0.2 | 3.4±0.2 | 0.9±0.1 | 84.3±2.8 | 5.9±0.2 | 3.4±0.2 | 1.1±0.0 |
| Fourth | 85.6±2.2 | 3.8±0.2 | 4.9±0.2 | 0.8±0.0 | 83.8±3.4 | 4.7±0.2 | 3.0±0.2 | 0.9±0.1 |
| AV | 86.7 | 4.5 | 3.6 | 0.9 | 86.4 | 4.6 | 2.8 | 0.9 |

First stage: 4 true leaves, Second stage: 6 true leaves, Third stage: Panicle initiation, Fourth stage: Floral initiation

Table 2: Total chlorophyll, total carotenoid and total pigment contents (μg $g^{-1}$ dry weight) in two quinoa cultivars Salcedo and Altiplano leaves and other samples

| Resource | Ch-a | Ch-b | $C_{x+c}$ | Total pigment |
|---|---|---|---|---|
| Salcedo | 3186.01±17.69$^{a}$ | 724.94±9.70$^{a}$ | 905.82±8.30$^{a}$ | 4816.77 |
| Altiplano | 1383.65±23.68$^{d}$ | 636.92±4.41$^{b}$ | 642.35±9.90$^{b}$ | 2662.92 |
| Coca | 83.26±4.82$^{f}$ | 208.64±7.12$^{e}$ | 137.45±11.53$^{f}$ | 429.35 |
| Lemon verbena | 1476.90±3.80$^{c}$ | 497.46±4.63$^{c}$ | 444.63±8.20$^{c}$ | 2418.99 |
| Cats claw | 1538.25±9.90$^{b}$ | 437.0.3±5.50$^{d}$ | 421.66±7.01$^{d}$ | 1959.91 |
| Green tea | 579.55±5.0$^{e}$ | 198.40±4.41$^{e}$ | 214.16±5.24$^{e}$ | 992.11 |

Ch-a: Chlorophyll a, Ch-b: Chlorophyll b, $C_{x+c}$: Carotenoids. Means followed by different letters in the column indicate statistically significant differences by Duncan test at least $p<0.05$

**Proximate chemical composition of phenological stages:** The proximate composition was calculated over fresh weight of the samples are displayed in Table 1.

The two quinoa cultivars no showed variable results regarding the proximate composition. These results are similar to those reported by Bhargava *et al.*[15]. In this case the ash content for Altiplano and Salcedo leaves was 2.8 and 3.6%, respectively, while the fiber content was similar in both samples. Furthermore, the protein content showed a similar variation in both crops. Kalber *et al.*[25] indicated that all plants grow and are different in rates of development. In the case of crude protein and fiber content in buckwheat changed with progressing phenological stage, whereas, this was not the case in chicory and phacelia.

**Determination of pigment contents:** The main natural pigments in fruits and vegetables are chlorophyll, carotenoids; carotenoids are the most widespread of all naturally occurring pigment groups and are responsible for the yellow, orange and red colors of fruits, roots and vegetables. Natural pigments color living biological material and often possess essential functional properties such as anti-oxidative, radical scavenging. Table 2 summarizes the concentrations of chlorophyll-a, chlorophyll-b and carotenoids.

Table 2 shows the chlorophyll a, chlorophyll b, carotenoids and total pigment contents in the two quinoa cultivars Salcedo and Altiplano leaves and other samples investigated in the present study. The total pigment content is much higher in the Salcedo leaves (4816.77 μg $g^{-1}$ dry weigt) when compared with Altiplano leaves (2662.92 μg $g^{-1}$ dry weight) and other samples, this was the trend for the chlorophyll a, chlorophyll b and carotenoids contents. In all samples the concentration of carotenoids in less than the sum of chlorophylls. Furthermore, it is observed that, the content of total pigments in the quinoa samples are higher than commercial teas. The large variation of total pigments content is in agreement with other reports, which suggest that the quality of commercial teas is clearly influenced by processing and generally the commercial drying leads to losses and chemical transformations of some bioactive components[26].

**Determination of polyphenols and flavonoids:** Total polyphenols content and total flavonoid content of two quinoa cultivars Salcedo and Altiplano leaves and other samples are presented in Table 3. The study reports a higher content of total phenolic and flavonoid compounds in the green tea and cats claw. The contents of 10.55 mg GAE $g^{-1}$ and 8.69 mg rutin $g^{-1}$, respectively were in the Salcedo leaves and whereas, that in Altiplano the contents 10.72 mg GAE $g^{-1}$ and 9.14 mg rutin $g^{-1}$. According to this study, the contributions of flavonoids to the content of total phenolic compounds of Salcedo and Altiplano leaves were significantly lower than that in other samples. On the other hand, a study in the *Chenopodium quinoa* leaves extracts allowed for the identification of the aglycones of ten four major phenolic acids and flavonoids in the polyphenolic fraction. The main phenolic acids were ferulic, sinapinic and gallic acids, whereas, kaempferol and isorhamnetin were the most abundant flavonoids[13]. The polyphenol

Table 3: Total phenolic and flavonoid contents (mg $g^{-1}$ dry weight) in two quinoa cultivars Salcedo and Altiplano leaves and other samples

| Resources | Total phenolics | Total flavonoids | References |
|---|---|---|---|
| Salcedo | 10.55±0.35[d] | 8.69±0.49[e] | |
| Altiplano | 10.72±0.27[d] | 9.14±0.42[e] | |
| Coca | 33.02±1.38[c] | 13.64±0.26[d] | |
| Lemon verbena | 33.67±0.55[c] | 24.55±0.36[c] | |
| Cats claw | 65.51±2.88[b] | 35.09±0.32[a] | |
| Green tea | 89.38±1.86[a] | 26.69±0.41[b] | |
| *Mentha longifolia* | 85.10 | 70.60 | Stanisavljevic *et al.*[28] |
| *Zingiber officinale* | 33.10 | 5.54 | Ghasemzadeh *et al.*[29] |
| *Ginkgo biloba* | 62.81 | 24.20 | Zilic *et al.*[30] |
| *Salvia officinalis* | 69.99 | 35.94 | Zilic *et al.*[30] |
| *Melissa officinalis* | 113.15 | 64.20 | Zilic *et al.*[30] |
| Sweet potato leaf | 35.40 | 3.40 | Fu *et al.*[31] |

All analyses are the mean of triplicate measurements±standard deviation, [a]mg rutin $g^{-1}$ of dry plant material, [b]mg gallic acid $g^{-1}$ of dry plant material. Means followed by different letters in the column indicate statistically significant differences by Duncan test at least $p<0.05$

Table 4: Antioxidant activities (μmol TE $g^{-1}$ dry weight) of Salcedo and Altiplano leaves and other samples

| | Antioxidant activity | | Antioxidant index score* | | |
|---|---|---|---|---|---|
| Resources | DPPH | ABTS | DPPH index | ABTS index | Antioxidant potency composite index |
| Coca | 225.18±3.05[a] | 780.42±8.62[c] | 100.00 | 59.27 | 79.64 |
| Green tea | 139.36±1.46[b] | 1316.63±21.04[a] | 62.09 | 100.00 | 81.04 |
| Cats claw | 126.60±3.89[c] | 1050.44±15.19[b] | 55.22 | 79.78 | 67.50 |
| Lemon verbena | 116.46±0.46[d] | 422.58±3.75[d] | 51.09 | 32.09 | 41.59 |
| Altiplano | 62.65±3.06[e] | 77.05±0.33[e] | 26.76 | 5.85 | 16.31 |
| Salcedo | 46.00±2.11[f] | 54.58±0.82[f] | 19.74 | 4.15 | 11.95 |

*Antioxidant index score = [(sample score/best score)×100], averaged for all seven tests for each beverage for the antioxidant potency composite index. Means followed by different letters in the column indicate statistically significant differences by Duncan test at least $p<0.05$

content analyzed by HPLC reports around 1362.96 μg $g^{-1}$, whereas, the content of flavonoids 118.60 μg $g^{-1}$ (mainly kaempferol, quercetin, rutin and isorhamnetin). The plants with high total polyphenol content in leaves has been previously observed[27] and related to the accelerated polyphenol synthesis by light exposure as a filtration mechanism against UV-β-radiation.

**Antioxidant activity:** The DPPH and ABTS scavenging activity and the antioxidative potency of Salcedo and Altiplano leaves and other samples extracts is shown in the Table 4. The antioxidant potency composite index determined for the samples based on ranking of two antioxidant assays. Generally, the total polyphenol contents are highly correlated with antioxidant activity and the bioavailability of polyphenols has been reported[32].

The order of antioxidant potency in the sample leaves as follows: Green tea>coca>cats claw>lemon verbena> Altiplano>Salcedo. However, the most widely used methods of determining antioxidant capacity is the DPPH and ABTS method and these methods gave a rank order different, especially in the following samples: DPPH method: Coca>green tea>cats claw and ABTS method: Green tea>cats claw>coca. These values were similar or superior to those reported by Bordoloi *et al.*[33] for different edible herbs used in Jorhat, Assam, India measured with the DPPH assay by Wojdyło *et al.*[34] for different spices extracts measured with the ABTS assay and by Chirinos *et al.*[35] using different assays (DPPH, ABTS and ORAC) in fruits, grains, leaves, seeds, roots and tubers from 27 different Peruvian Andean plants used in folk medicine or/and as food.

**Correlation between mean antioxidant index scores:** The correlations between mean antioxidant index scores, obtained by combination of the two antioxidant methods used (DPPH and ABTS) and total phenolic and flavonoid contents are displayed in Fig. 1. In the study, antioxidant potency showed the positive correlation coefficients with phenolics ($r^2$ = 0.6575), flavonoids ($r^2$ = 0.3896) and the correlation between phenolics and flavonoids ($r^2$ = 0.6744).

These results are similar to those reported by Fu *et al.*[31] indicating a high correlation between antioxidant activity and the polyphenol content, whereas the correlation between the flavonoid content was low. Chirinos *et al.*[35] reported high correlations between total phenolic contents and the ABTS and DPPH assays ($r^2$ = 0.8063 and 0.8527, respectively).

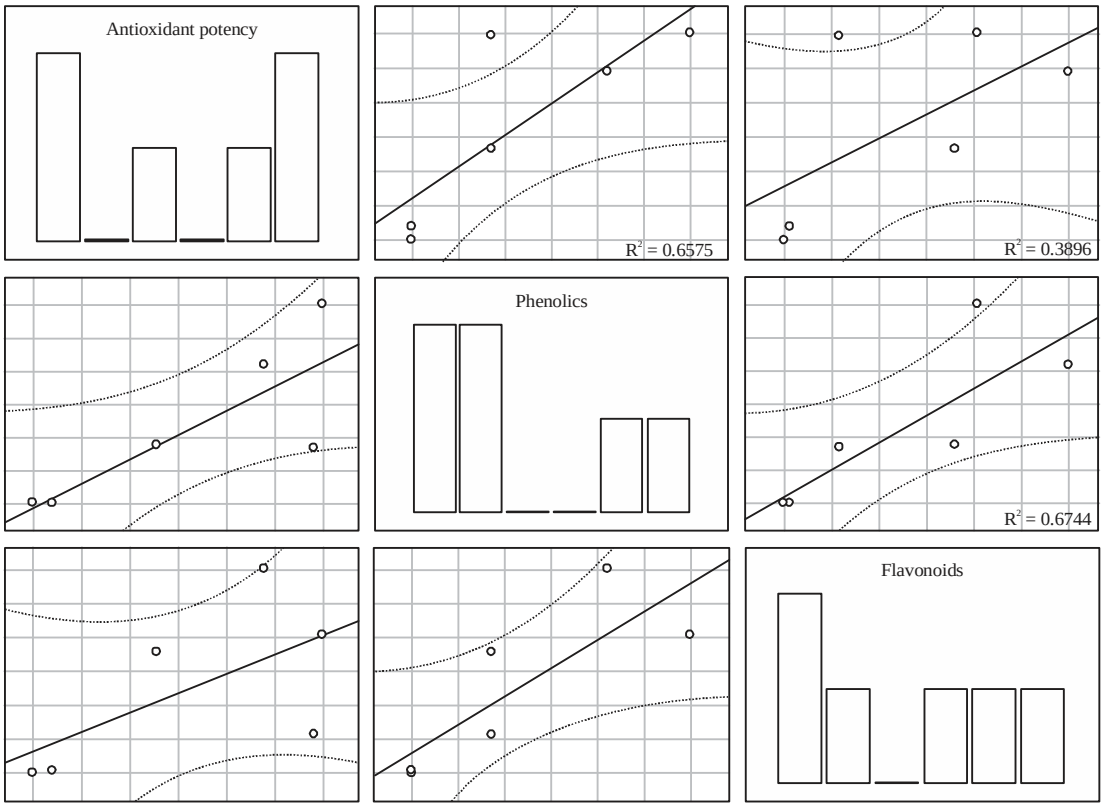


Fig. 1: Correlation between mean antioxidant index scores, obtained by combination of the two antioxidant methods used (DPPH and ABTS) and total phenolic and flavonoid contents

## CONCLUSION

This study can be concluded that the leaves of two quinoa cultivars (Salcedo and Altiplano) are excellent resources of bioactive components and can be used in the food industry as infusions. Studies are needed on the profile of polyphenols to know which are the phenols responsible for antioxidant activity.

## ACKNOWLEDGMENTS

The authors are grateful to the Ministry of Science, Technology and Innovative for financing this research and the Instituto de InnovaciónAgraria (INIA) to provide the samples for this study and to the Universidad Peruana de Ciencias Aplicadas for partially funding the research.

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