Vitamin C, total polyphenols and antioxidant activity in raw, domestically processed and industrially processed Indian *Chenopodium quinoa* seeds

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**ABSTRACT**

Total antioxidant activity, Vitamin C and total polyphenols were determined for the first time in Indian *Chenopodium quinoa* seeds. The raw seeds were subjected to domestic processing method by soaking and germination to see the effect on antioxidant activity, Vitamin C and total polyphenols as compared to the industrially processed seeds. Antioxidant activities were determined by DPPH and FRAP method. Total phenolic content and flavonoid was determined colorimetrically and vitamin C by N-bromosuccinimide (NBS) method. The results show that domestically processed seeds have higher vitamin C, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity as compared to the raw and industrially processed seeds. Antioxidant activity was found significantly correlated to the total phenolic content in raw, domestically processed and industrially processed seeds. The results suggest use of domestic processing of quinoa seeds to retain nutrient value and also infer dietary importance of Indian *Chenopodium quinoa*.

**INTRODUCTION**

*Chenopodium quinoa*, an underutilized crop belonging to family Chenopodiaceae is an ancient crop with modern perspectives. It originated and sprung initially in Andean region of South America with Peru and Bolivia being the main producers. It is known to have protein quality comparable to that of milk protein, casein, protein content (16%) higher than maize and wheat (Palombini et al., 2013), ash content (3 - 4%) higher than common cereals like wheat and rice (Miranda 2012) and fibre content (14-16% total dietary fibre; 2-3% crude fibre) more than that present in corn and wheat (Repo-Carrasco-Valencia 2010). It contains all essential amino acids with presence of both lysine (5.4%) and methionine (2.1%), which makes it a unique complete food (USDA 2005). Quinoa seeds are found rich in isoflavones, polyphenols and known to exhibit good antioxidant properties (Pasko et al., 2009). Isoflavones like genistein and daidzein have been found in quinoa. It is found to contain 11-17mg/100g bound phenolics like coumeric, benzoic, vanillic and ferulic acid and 96-164mg/100g free phenolics with quercetin, acacetin and kaempferol being majorly present (Gomez-Caravacca et al., 2014). Good antioxidant activity in quinoa may also be attributed to the presence of vitamin C and tocopherols (Tang et al., 2015) which also protects against oxidation of its fatty acids. High nutritional and phytochemical content make quinoa a wonder grain with many health benefits (Arneja et al., 2015). Successful propagation of quinoa in field trials at world’s hottest and driest place, Arabian peninsula, prove this crop’s versatility to adapt in adverse climatic and ecological conditions (Rao and Shahid 2012). Thus, astounding features of this crop such as, tolerance to stressful environmental conditions like soil salinity, drought, frost, high temperature etc., ability to grow at up to 4500m of altitude from sea level and impeccable nutritional benefits permit advancement of quinoa cultivation out from the boundaries of its Andean motherland to different parts of world.
Also the increasing demand of quinoa across the world has prompted many agriculturists and researchers worldwide for quinoa cultivation (The wire 2015). After being successfully promoted in western countries, quinoa has found its way to Asia with keen interest for the crop mainly in the Indian subcontinent (Bhargava et al., 2006). India being a land of diverse climatic regions (tropical wet, tropical dry, subtropical humid and mountains) and quinoa being a crop profoundly known to adapt well to unusual environmental conditions is found apt to grow in Indian boundaries (Bhargava et al., 2006). Initially being found grown in foots of Himlayan hills, the crop has been grown successfully for the first time in year 2013 under the project named “Anantha” in plains of drought prone area of Anantpur region in Andhra Pradesh (Deccan Chronicle 2013). The craze and demand for quinoa among Indians has grown far more over than their demand for the staple traditional crops and millets like sorghum, pearl millet, finger millet etc. (The wire, 2015).

Post harvesting grains undergo industrial processing, mainly the process of dehulling or decortications, to remove the outer layers of the grain. Dehulling is known to improve grain quality by lowering the content of anti nutrients and enhancing the sensory parameters, hence the acceptance and palatability of the grain. Despite these benefits of dehulling, it reported to cause loss of nutrients from grains. Thus, to minimize loss nutrients and increase bioavailability of nutrients, researchers recommend use of common traditional domestic processing methods for grains (Pawar and Machewar, 2006). Soaking and germination are the commonly used methods for domestic processing of seed. Antioxidant activity and phenolic content have been reported to be greatly affected by domestic processing (Dini et al., 2013)

It is known that the polyphenol concentration varies widely not only in different plant species, but even between cultivars, and it is influenced by numerous site-specific and environmental factors. In addition, polyphenol concentrations and health-promoting properties may be affected by harvesting and storage conditions (Kalt et al., 1999) and many important bioactive compounds may suffer during inappropriate processing. Thus, these factors are crucial determinants of the quality of food materials used in various functional-type food products and supplements. Keeping this in mind, this work involved the analysis of Indian Chenopodium quinoa grains for antioxidant activity, vitamin C content and total polyphenols and impact of processing viz. domestic (soaking and germination) and industrial processing on the said parameters.

MATERIALS AND METHODS

Seed procurement

Quinoa seeds, (Variety: Royal; Ecotype: White), raw and industrially processed were procured from Andhra Pradesh Institute of Rural Development (APARD), Hyderabad, India. Raw seeds were procured directly from the site of cultivation while industrially processed seeds were procured after the seeds had been dehulled by industrial method prior to packaging and marketing. Both raw and industrially processed seeds were initially rinsed with ethanol and soaked in 2% sodium hypochlorite solution for 10 minutes for seed sterilization. They were then washed with deionized water until pH 7 was obtained. The seeds were dried in vacuum drying oven at 40±5°C and stored at 4°C until further chemical analysis.

Preparation and processing of seeds for analysis:

Raw and industrially processed seeds: Dried raw and industrially processed seeds (500 g) obtained after the process of surface sterilization were ground to finely powdered flour with a laboratory grounder (Philips HL1606/03 500 W Mixer Grounder) and stored at 4°C for further analysis.

Domestic processing of raw seeds

Raw seeds were subjected to domestic processing by soaking and germination method.

Soaking

Raw Chenopodium quinoa (500g) seeds were soaked for 24 hours in deonized water (1:5 w/v) obtained through Millipore (Merck-Milli-Q® Direct 8 Water Purification System, USA). Water used for soaking was changed thrice at regular interval of 8 hours. After 24 hours, the water was discarded and seeds were washed once with de ionized water. Soaked seeds were further dried in vacuum drying oven at 40±5°C. Dried seeds were ground to finely powdered flour with laboratory grounder (Philips HL1606/03 500 W Mixer Grounder). Soaked quinoa seed flour was stored at 4°C for further analysis.

Germination

Raw Chenopodium quinoa (500g) seeds were thoroughly washed with and soaked in de ionized water (1:5 w/v) obtained through Millipore (Merck-Milli-Q® Direct 8 Water Purification System, USA) for 12 hours. Seeds were then spread on to petri dishes covered with autoclaved filter paper and incubated at 20°C in an incubator (Biotechnics, India) for 72 hours (Carciochi, 2014). Water was changed every 8 hours. Germinated seeds were then dried in vacuum drying oven at 40±5°C. Dried germinated seeds were ground to flour with laboratory grounder (Philips HL1606/03 500 W Mixer Grounder). Finely powdered germinated quinoa seed flour was stored at 4 °C for further analysis.

Extract Preparation

Finely ground seeds (in flour form) of Indian Chenopodium quinoa (5g) i.e. raw, industrially processed and domestically processed (soaked and germinated) were extracted at room temperature with 50 ml of 80% ethanol and 0.2M hydrochloric acid in ratio 9:1. The mixture was sonicated in an ultra sonic bath sonicator (Fisher scientific, UK) for about 10 minutes. It was then agitated for 1 hour at 120 rpm in an orbital shaker. Then it was transferred to centrifugation tubes and centrifuged for 15 minutes at 9000rpm. Subsequently, the supernatant was separated and the residue was re-extracted in the
same way. Both the supernatants were then combined and filtered through syringe filters (Agilent premium syringe filter, 0.45µm) to obtain clear extracts. Extracts prepared were stored at −80°C in a freezer for further analysis.

**Vitamin C analysis**

N-bromosuccinimide (NBS) method for determination of vitamin C as given by Miranda *et al.*, 2010 was used for determination of vitamin C in quinoa samples. Slight modifications were made in analysis accordingly. The method included preparation of standard ascorbic acid solution, standardization of NBS with ascorbic acid and estimation of ascorbic acid in sample extract.

a) Preparation of standard ascorbic acid: Standard ascorbic acid of concentration 0.4mg ml⁻¹ was prepared by dissolving 200mg ascorbic acid in 500 ml distilled water.

b) Standardization of NBS Solution: Standard ascorbic acid solution (20ml) was added to a flask containing 4 ml of 4% potassium iodide solution (KI), 1.6 ml of 10% acetic acid (CH₂COONa), 4 drops of 1% starch (used as an indicator) and 25 ml distilled water. It was then titrated with NBS (0.2mg ml⁻¹). Appearance of permanent blue color was considered as end point of titration.

c) Estimation of ascorbic acid in sample: Quinoa extracts, acidified with 0.4 g oxalic acid was added to a flask containing 10 ml of 4% potassium iodide solution (KI), 4 ml of 10% acetic acid (CH₂COONa), 4 drops of 1% starch (used as an indicator) and 40 ml distilled water. Final vitamin C content was expressed as mg 100g⁻¹ using following equation:

\[
\text{Vit C content} = \frac{\text{concentration of standard } \times \text{ volume of NBS corresponding to quinoa extract (ml)}}{\text{volume of NBS corresponding to standard ascorbic acid solution (ml)} \times \text{Sample mass (g)}}
\]

**Total phenolic content (TPC)** was assessed according to method described by Ainsworth and Gillespie, (2007) using Folin-ciocalteau reagent. Sample extract (0.5ml) was diluted and volume was made up to 1ml. After 2 minutes 2ml of 10% folin-ciocalteau reagent was added and vortexed thoroughly. At sixth minute, 8 ml of 700mM Na₂CO₃ was added and mixture was incubated for 2 hours. Mixture (2ml) was transferred to quartz cuvette and absorbance was read at 765nm using spectrophotometer.

**Determination of ferric reducing antioxidant power**

The ability of the extracts to reduce ferrous ions to ferrous ions was determined by the method as described by Jubete *et al.*, (2010) and Benzie and Strain, (1996) with some modification. FRAP reagent was prepared by mixing 2.5 ml of 0.01M 2,4,6-Tripyridyl-s-Triazine (TPTZ) in 0.04M hydrochloric acid, 2.5ml of 0.02M ferric chloride and 25 ml of 0.3M sodium acetate buffer (pH 3.6).

Quinoa extracts (100µl-300µl) and 2ml of FRAP reagent was taken in a 5ml volumetric flask. Distilled water was used to make up the volume. Solutions were kept in dark at 37°C for 60 minutes. Absorbance was read at 595nm using spectrophotometer (Shimadzu, Japan).

**Statistical analysis**

The experiments were performed in triplicates and the data was expressed as Mean ± SD. The data was analyzed on Microsoft Office Excel, 2007 and Graph pad prism 5 Software (La jolla, CA, USA). Means were compared using one way analysis of variance (ANOVA) followed by Tukeys multiple comparison test for comparison between means. The values were considered significant at p≤0.05.
RESULTS AND DISCUSSION

Vitamin C content of seeds

Vitamin C content in Indian Chenopodium quinoa is shown in Table 1. Vitamin C content of raw quinoa seeds was found to be 13 mg 100 g⁻¹ which is within the range of results reported by Miranda et al., (2010) (12.2-3 mg 100 g⁻¹) and corresponds closely to Cahuil variety (13.8 mg 100 g⁻¹) among six chilean quinoa ecotypes. The value reported in our study is less than Miranda et al., (2013) (22.31 mg 100 g⁻¹) in two quinoa genotypes from Temuco and Vacuna localities in Chile. This difference in vitamin C content may be due to different environmental and storage conditions, as factors like light intensity, amount of nitrogen fertilizers, frequency of irrigation and temperature of the region strongly affect the vitamin C content in crops (Lee and Kader, 2000).

Also there is significant difference in vitamin C content of raw and domestically processed Indian quinoa seeds (p<0.05). As depicted in our study, vitamin C content increased by 15% in germinated seeds of raw and industrially processed seeds was observed. This is because industrial processing methods like dehulling, pearling, shelling etc. lead to loss of nutritional content in seeds (Singh and Jambunathan 1990).

Table 1: Vitamin C content of Raw, domestically and industrially processed Indian Chenopodium quinoa seeds (mg 100 g⁻¹) X

| Indian Chenopodium quinoa | Vitamin C (mg/100g) |
|---------------------------|---------------------|
| Domestically Processed    |                     |
| Soaked                    | 15.09±0.17          |
| Germinated                | 19.38±0.28          |
| Industrially processed    | 9.45±0.35           |

X values are mean± SD, n=3. Values followed by same letter in same column are not significantly different (p>0.05).

Also the value of TPC content found in our study lies close to reported the values of TPC in raw quinoa seeds by Carciochi et al., (2014) (39 mg GAE 100 g⁻¹), Pasko et al., (2009) (38 mg GAE 100 g⁻¹), higher than as reported by Miranda et al., (2010) (28 mg GAE 100 g⁻¹). Higher reported values in our study can be explained as raw seeds (direct from the field) used were with the seed coat while the quinoa seeds procured by these authors were as available in the local market, which might be industrially processed for removal of seed coat which leads to decrease in phenolic content. Significant difference in phenolic contents reported by various other authors may be due to different environment conditions for growth, extraction solvents (Marmouzi et al., 2015), quinoa varieties with colored testa (Tang et al., 2015). Total phenolic content in soaked quinoa seeds was significantly less (28%, P≤0.05) as compared to raw seeds. The result corresponds to 26-56% loss in total phenolic content of black beans (Phaseolus vulgaris L.) reported by Xu and Chang (2008). Germinated seeds were found to exhibit 134% increase in total phenolic content as compared to raw quinoa seeds. This is because germination leads to increase in phenolic content of seeds as synthesis of phenolic acid is enhanced by seed growth during germination (Cevallos-Casals and Cisneros-Zevallos, 2010). Increase in total phenolic content in germinated quinoa has also been reported by Carciochi et al., (2014) (56 % after 48 hours and 101.2% after 72 hours of germination) and Jubete et al., 2010 (107% after 82 hours of germination). Industrial processed quinoa seeds exhibited 20% decrease in total phenolic content (34 mg GAE 100 g⁻¹). Similar decrease in phenolic compounds of pearled quinoa (abrasion degree of 30%) was reported by Gomez-Caravaca et al., (2014) with 21.5% and 35.2% decrease in free and bound phenolic compounds respectively.

Total phenolic content

Total phenolic content (TPC) of Indian Chenopodium quinoa is shown in Table 2. TPC of raw Indian quinoa seeds, in our study, was reported to be 43 mg PAE 100 g⁻¹ which corresponds well to TPC content reported by Repo-Carrasco-Valencia et al., (2010) (42 mg GAE 100 g⁻¹) and Vollamannova et al., (2013) (45 mg GAE 100 g⁻¹) in Carmen variety of quinoa.

Table 2: Total phenolic content (TPC) of Raw, domestically and industrially processed Indian Chenopodium quinoa seeds X

| Indian Chenopodium quinoa | Total Phenolic Content (mg GAE/100g) |
|---------------------------|------------------------------------|
| Raw                       | 43.2±0.25                          |
| Domestically Processed    |                                    |
| Soaked                    | 31.1±0.35                          |
| Germinated                | 101.2±0.29                         |
| Industrially processed    | 34.6±0.33                          |

X values are mean± SD, n=3. Values denoted by same letter within a column are not significantly different (p<0.05).

Also the value of TPC content found in our study lies close to reported the values of TPC in raw quinoa seeds by Marmouzi et al., (2015); Carciochi et al., (2014) and Chlopika et al., (2012).

Total flavonoids

Total flavonoid content (TFC) of quinoa seeds is shown in Table 3. Total flavonoid content of raw Indian quinoa was reported as 11.40 mg QE 100 g⁻¹. Results agree with the findings of Carciochi et al., (2014) (11.06 mg QE 100 g⁻¹) and Chirinos et al., 2013 (11 mg QE 100 g⁻¹). The total flavonoid content reported in our study is significantly different to the values reported by Marmouzi et al., (2015); Carciochi et al., (2014) and Chlopika et al., (2012).

Table 3: Total flavonoid content (TFC) of Raw, domestically and industrially processed Indian Chenopodium quinoa seeds.

| Indian Chenopodium quinoa | Total Flavonoids (mg QE/100g) |
|---------------------------|-------------------------------|
| Raw                       | 11.4±0.03                     |
| Domestically Processed    |                                |
| Soaked                    | 7.2±0.08                      |
| Germinated                | 18.02±0.21                    |
| Industrially processed    | 5.8±0.10                      |

X values are mean± SD, n=3. Values denoted by same letter within a column are not significantly different (p<0.05).

This might be due to different solvents used for extraction, difference in temperature during extraction process and different methods of flavonoid analysis used (HPLC or spectrophotometry). Total flavonoid content of soaked quinoa seeds decreased by 36% (7.2 mg QE 100 g⁻¹). Similar decrease in
flavonoid content after soaking has been found in white sorghum (Afiffy et al., 2012). Germination of quinoa seeds lead to significant increase (56%) in flavonoid content (18 mg QE 100g⁻¹). Similar increase in flavonoid content (59%) has been reported by Carciochi et al., 2014 in germinated quinoa seeds. The increase in flavonoid content on germination of seeds is due to synthesis of metabolites like flavonoids by phenylproponoid pathway, common to all plants, during process of seed germination (Wu et al., 2011). Industrial processing of quinoa seeds led to reduction in flavonoid content by 47%. The findings may be attributed to the fact that most of the flavonoids are contained in the seed coat and industrial processing involves removal of outer layer of seed thus causing decrease in the flavonoid content (Xu and Chang, 2008).

**Antioxidant activity (FRAP and DPPH)**

Antioxidant activity of Indian *Chenopodium quinoa* seeds is shown in Table 4. Raw quinoa seeds, were reported to have 59.6 mg TE 100g⁻¹ and 37.3 TEAC antioxidant activity as calculated by DPPH method and 84.4 mg TE 100g⁻¹ as calculated by FRAP method. The results were close to antioxidant activity reported by Jubete et al., (2010) (57.7 mg TE/100g and 34.8 TEAC by DPPH method and 84.1 mg TE 100g⁻¹ by FRAP method). Antioxidant activity of quinoa reported by Ranilla et al., (2009) (by DPPH) was much higher (86 mg TE 100g⁻¹) than as reported in our study (59.6 mgTE 100g⁻¹). This is because red quinoa were used by Ranilla et al., (2009) and difference in color of seeds strongly effects antioxidant activity with dark colored seed coats exhibiting highest antioxidants activities (Tang et al., 2015). Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. The result is supported by findings of Afiffy et al., (2012), who reported decrease in antioxidant activity in soaked white sorghum. Xu and Chang (2008) also reported decrease in antioxidant activity of soaked green pea (9%), yellow pea (8%) and lentil (7%). As phenols and flavonoids contribute significantly to antioxidant activity, the decrease may be due to leaching of phenols and flavonoids in water used for soaking the seeds (Afiffy et al., 2012). Antioxidant activity of germinated seeds (after 4 days or 48 hours) was found to increase by 90% (calculated by DPPH method). The result is supported by findings of Carciochi et al., (2014) which showed 100% increase in antioxidant activity of germinated quinoa seeds as evaluated by DPPH method. Similar increase in antioxidant activity of germinated quinoa seeds has also been reported by Pasko et al., (2009). FRAP values of germinated quinoa seeds increased by 89%. The result is supported by increase in FRAP values of quinoa sprouts (79%) as reported by Jubete et al., (2010). However industrial processing of the seeds lead to decline in antioxidant activity. Processed quinoa seeds showed decline of 14% and 19% antioxidant activity as evaluated by FRAP and DPPH, respectively. The result is supported by decrease in antioxidant activity of barley (Holtekkjolen et al., 2008) after undergoing industrial processing methods like decortications and pearling. Decline in antioxidant activity after processing can be due to removal of hulls which are majorly responsible for antioxidant activity (Zielinski and Kozlowska, 2000). We also observed strong linear correlation between total phenolic content and antioxidant activity (DPPH) of Indian *Chenopodium quinoa* seeds. As illustrated in Figure 2, a strong linear correlation was observed between total phenolic content (TPC) and DPPH of raw (R² = 0.9951, y=0.5395 x - 15.851), soaked (R² = 0.9854, y = 2.4204x - 53.323), germinated (R² = 0.9924, y=15.343x - 980.08) and industrially processed (R² = 0.9982, y = 14.373x - 407.1) Indian quinoa seeds. Observed correlation is in agreement with previously reported literature by Hirose et al., (2010). The significant correlation observed in our study is also supported by observations of Jubete et al., (2010), where similar correlation (r=0.99) was observed between TPC and DPPH antioxidant activity of quinoa seeds and sprouts.

![Graph](image)

**Fig. 1:** Vitamin C, TPC, FRAP and DPPH for raw and processed Indian quinoa seeds. Vitamin C in mg/100g, TPC in mg GAE/100g, FRAP and DPPH in mgTE/100g
CONCLUSION

Indian Chenopodium quinoa seeds grown in Anantapur district of Hyderabad, India were studied for the first time for its vitamin C content, total polyphenols and antioxidant activity. The study involved comparison of effect of domestic and industrial processing techniques on total polyphenols, vitamin C content and antioxidant activity. The results revealed that domestic processing of quinoa seeds mainly by the process of germination enriches its vitamin C, polyphenol content and antioxidant activity. Germinated quinoa being rich in antioxidants can be used in daily diets for cure of various degenerative diseases and hence, it can be inferred from the present study that domestically processed quinoa by germination could be preferred over industrially processed quinoa.

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