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

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ABSTRACT

BACKGROUND: *Chenopodium quinoa,* an ancient crop sprung initially in Andean region of South America is well recognized for its outstanding nutritional composition and versatility. Quinoa from various geographical regions like Bolivia, Argentina, Kenya, Japan and Morocco has been studied, however there is no study on Indian *Chenopodium quinoa*. Thus, the present study aims to determine vitamin C, antioxidant activity and total polyphenols in Indian quinoa. Also, it compares effect of domestic and industrial processing on the grain.

RESULTS: The results show that domestically processed seeds have higher vitamin C, total phenolic content (TPC), total flavonoid content (TFC) and also 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.

CONCLUSION: Domestically processed quinoa, mainly by germination is reported to be rich in antioxidants, vitamin C and higher phenolic content. The results suggest use of domestic processing of quinoa seeds to retain nutrient value and also infer dietary importance of Indian *Chenopodium quinoa.* Further studies are required on antioxidant and polyphenol profiling of Indian quinoa.

INTRODUCTION

Chenopodium quinoa, an underutilized crop belonging to family chenopodiaceae is an ancient crop with modern perspectives. Originated and sprung initially in Andean region of South America with Peru and Bolivia being the main producers, the crop is gaining popularity worldwide due to its extraordinary nutritional composition and versatility to adapt extreme environmental conditions. Quinoa is an annual plant, 1 to 4 m tall, having erect cylindrical stem, green to pale yellow leaves, flowers with no petals and achene fruit. Quinoa grain is dicotyledonous and thus regarded as a pseudocereal. The grain is found with wide range of colour variety like red, black, pink, purple and white, depending on the presence of pigments and geographical area of cultivation. Talking about the nutritional aspects of quinoa, it is found to have protein content (16%) higher than maize and wheat. Protein quality of quinoa is found comparable to that of milk protein, casein. It contains all essential amino acids with

presence of both lysine (5.4%) and methionine (2.1%), which gives it a unique feature and makes it a complete food[51]. Quinoa is a gluten free grain appropriate for consumption by celiac patients. It has ash content (3 - 4%) higher than common cereals like wheat and rice and fibre content (14-16% total dietary fibre; 2-3% crude fibre) which is more than that present in corn and wheat. In addition quinoa seeds and sprouts are found rich in isoflavones, polyphenols and known to exhibit good anti oxidant properties[17]. 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. Good antioxidant activity in quinoa may also be attributed to the presence of vitamin C and tocopherols[50] which also protects against oxidation of its fatty acids. Ascorbic acid also known as Vitamin C has metabolic and therapeutic benefits when included in human diet. High nutritional and phyto chemical content make quinoa a wonder grain with many health benefits. 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[42]. 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. In addition to the extraordinary features, the increasing demand of quinoa across the world has prompted many agriculturists and researchers worldwide for quinoa cultivation[49]. After being successfully promoted in England (1970), Denmark, Europe (1993) and Kenya[13], quinoa has found its way to Asia with keen interest for the crop mainly in the Indian subcontinent[8]. Crop is more widespread in Pakistan, Nepal and India. India being a land of diverse climatic regions (tropical wet, tropical dry, subtropical humid and mountains) and quinoa being a crop profoundly known as well adapted to unusual environmental conditions is found apt to grow in Indian boundaries[8]. 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[18]. The project has highly promoted cultivation of quinoa among various private companies in South India. 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[49].

Post harvesting and pior to marketing, 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[36] and enhancing the sensory parameters, hence the acceptance and pallatabilty of the grain. Despite these benefits of dehulling, it reported to cause loss of nutrients from grains. Thus, to minimize loss nutrients and increase bioavailabilty of nutrients, researchers recommend use of common traditional domestic processing methods for grains[39, 32]. 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[23, 20].

After many studies revealing nutritional and phytochemical composition of american quinoa[17, 10, 26, 50], recently quinoa grown in Kenya, Japan and Morocco was studied for its seed quality, yield level, antioxidative properties, proximate, flavonoid, fatty acid and mineral compostion. The studies demonstrate significant influence of grain variety and climatic conditions of area of cultivation on nutritional and phytochemical composition of the seed. Till now no study has been done on Indian quinoa seeds. The aim of our study is to investigate antioxidant activity, content of vitamin c and total polyphenols in Indian *Chenopodium quinoa* grains. We also compared the effect of domestic and industrial processing of Indian quinoa grain on antioxidant activity, content of vitamin c and total polyphenols, which has never been discussed before.

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MATERIAL AND METHOD

Seed procurement

White *Chenopodium quinoa* seeds (*Chenopodium quinoa* Willd.), 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[45] for 10 minutes for seed sterilization. They were then washed with deionized water until pH 7 was obtained. The seeds were dried in vaccum 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 grinded to finely powdered flour with a laboratory grinder (Philips HL1606/03 500 W Mixer Grinder) 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.

a. Soaking

Raw *Chenopodium quinoa* (500g) seeds were soaked for 24 hours in deionized 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 grinded to finely powdered flour with laboratory grinder (Philips HL1606/03 500 W Mixer Grinder). Soaked quinoa seed flour was stored at 4°C for further analysis

b. Germination

Raw *Chenopdium 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[10]. Water was changed every 8 hours. Germinated seeds were then dried in vacuum drying oven at 40±5°C. Dried germinated seeds were grinded to flour with laboratory grinder (Philips HL1606/03 500 W Mixer Grinder). Finely powdered germinated quinoa seed flour was stored at 4°C for further analysis.

Extract preparation

Finely grounded 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 Barakat et al.1955 and Miranda et al. (2010) was used for determination of vitamin C in quinoa samples. Slight modifications were made in analysis accordingly. The method includes 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 concentration0.4mg ml^{-1} was prepared by dissolving 200mg ascorbic acid in 500 ml distilled water. b) Standardization of NBS Solu-

tion: 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 (CH_3COONa), 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 colour 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 (CH_3COONa), 4 drops of 1% starch (used as an indicator) and 40 ml distilled water. Final vitamin C content was expressed as mg 100^{-1} using following equation:

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

Determination of total phenolic content

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 upto 1ml. After 2 minutes 2ml 0f 10% folin-ciocalteau reagent and vortex thoroughly. At sixth minute, add 8 ml of 700mM Na_2CO_3 and incubate the mixture for 2 hours. Transfer 2 ml of mixture to quartz cuvette and read the absorbance at 765nm using spectrophotometer (Shimadzu, Japan). The readings were compared to gallic acid standard curve (linearity range 50-250 mg/ml and R^2=0.991). Final total phenolic content expressed as gallic acid equivalent (GAE) per 100 g in dry weight basis (dwb).

Determination of total flavonoid

Total flavonoid was determined according to procedure followed by Carciochi et al. (2014a). Quinoa extract (0.5ml) was taken in a test tube. To the test tube 4 ml of distilled water and 0.5 ml of 20% NaNO2 (Sodium nitrite) was added. Mixture was allowed to stand for about 5 minutes and then 0.3ml of 10% $AlCl_3$ (Aluminium Chloride) was added. After 1 minute 0.5 ml 0f 2M NaOH (Sodium hydroxide) was added to the reaction mixture. Absorbance was read at 510nm using spectrophotometer (Shimadzu, Japan). Quercitin was used as standard. Final results were expressed as mg of quercitin equivalent/100g i.e. mgQE/100g.

Determination of DPPH

DPPH i.e. 2,2,-diphenyl-2-picryl-hydrazyl assay was proceeded according to method followed by Jubete et al. (2010b) and Sun and Ho, (2005) with some modification. Aliquots of quinoa extract, in increasing trend, (i.e. 100, 200, 400, 800, 1000 µl) were taken for serial dilution. Diluted quinoa extracts (1 ml) from each serial dilution was added to cuvettes respectively. DPPH solution of concentration 200µM (absorbance 1.4) was freshly prepared and 1 ml of this solution was added to each cuvette containing quinoa extract. The mixture was vortexed and incubated in dark for 30 minutes. Absorbance was measured at 517 nm using spectrophotometer (Shimadzu, Japan). DPPH was expressed as mgTE/100g. Inhibitory concentration at 50% was also calculated (IC_{50} values) and denoted as TEAC (Trolox equivalent antioxidant capacity). TEAC was calculated according to method used by Jubete et al. (2010b), using formula $TEAC_{50} = IC_{50trolox}/IC_{50sample} \times 10^5$. Trolox (0.02M) was used as standard. The result was expressed as trolox equivalents (mgTE/g). Inhibitory concentration (IC_{50}) was estimated by interpolation to 50% inhibition and expressed as $TEAC_{50}$.

Determination of FRAP

FRAP i.e. Ferric reducing ability of plasma assay was proceeded according to procedure followed by Jubete et al. (2010b) and Benzie and Strain, (1996) with some modification. FRAP reagent was prepared by mixing 2.5 ml of 0.01M 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). Trolox stock solution of 0.02M was used as standard for the assay FRAP

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reagent (2ml), made up to 5 ml in a volumetric flask was used as blank.

Statistical analysis

The experiments were performed in triplicates and the data was expressed as mean±standard deviation. The data was analyzed on Microsoft office excel, (2007) and Graphpad 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 with values considered significant at $p \leq 0.05$.

RESULT 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, in our study, was found to be 13mg $100g^{-1}$ which is within the range of vitamin c content as reported by Miranda et al. (2010) (12-23mg 100^{-1}) and corresponds closely to Cahuil variety (13.8 mg $100g^{-1}$) among six chilean quinoa ecotypes studied in his study. The value reported in our study is greater than the values reported by Koziol (1992) (4mg $100g^{-1}$) and less than as reported by Ruales and Nair (1993) (16.4 mg $100g^{-1}$), Miranda et al. (2013) (22-31 mg $100g^{-1}$) 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[35].

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 soaked quinoa seeds and by 46% in germinated quinoa seeds. Higher increase in germinated seeds was observed might be due to synthesis of vitamin C in process of germination (Sattar et al. (1995) and Fernandez-Orozco *et al.* (2006). Yang et al. (2001) reported vitamin C content in sprouted mungbean where as nil vitamin C content was reported in raw seeds, which confirms vitamin C synthesis during germination process. Increased vitamin C content in germinated indian quinoa seeds is also supported by findings of Khattak et al. (2007) where linear relationship was observed between germination time and content of vitamin C in chickpea seeds. Also significant difference in Vitamin C content of raw and industrially processed seeds was observed. Industrial processing decreased the vitamin C content by 30%. De hulling, pearling, shelling etc are post harvest industrial treatments applied to cereal grains which lead to loss of their nutritional content [47].

TABLE 1 : Vitamin C content of raw, domestically and industrially processed Indian *Chenopodium quinoa* seeds (mg $100g^{-1}$) [A]

Indian *Chenopodium quinoa*		Vitamin C (mg /100g)
Raw		13.43±0.4[a]
Domestically Processed	Soaked	15.09±0.17[b]
	Germinated	19.38±0.28[c]
Industrially processed		9.45±0.35[d]

[A] values are mean± SD, n=3. Vales followed by same letter in same coloumn are not significantly different ($p< 0.05$)

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 mgGAE $100g^{-1}$ which corresponds well to TPC content reported by Repo-Carrasco-Valencia et al. (2010) (42 mg GAE 100^{-1}) and Vollamannova et al. (2013) (45mgGAE $100g^{-1}$) in Carmen variety of quinoa.

Also the value of TPC content found in our study

TABLE 2 : Total phenolic content (TPC) of raw, domestically and industrially processed Indian *Chenopodium quinoa* seeds [A]

Indian *Chenopodium quinoa*		Total Phenolic Content (mg GAE/100g)
Raw		43.2±0.28[a]
Domestically Processed	Soaked	31.1±0.35[b]
	Germinated	101.2±0.29[c]
Industrially processed		34.6±0.33[a]

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

lies close to reported the values of TPC in raw quinoa seeds by Carciochi et al. (2014a) (39 mgGAE $100g^{-1}$), Pasko et al. (2009) (38mgGAE $100g^{-1}$), higher than as reported by Gorenstein et al. (2007) (30 mgGAE $100g^{-1)}$ and Miranda et al. (2010) (28mg GAE $100g^{-1}$). 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[40]. The values obtained are lower than values reported by Repo-Carrasco-Valencia et al. (2011) (142-197mg GAE 100^{-1}), Palombini et al. (2013) (63 mgGAE $100g^{-1)}$, Carciochi et al. (2014b) (67 to 103 mgGAE $100g^{-1}$), Chlopika, (2012) (280mg GAE $100g^{-1)}$ and Tang et al. (2015) (518 mgGAE $100g^{-1}$). Significant difference in phenolic contents reported by various other authors may be due to different environment conditions for growth[43, 34], extraction solvents[11], quinoa varieties with coloured testa[9, 50]. Results of our study are also significantly different than as reported by Nsimba et al. (2008) due to different origin of quinoa varieties and different standard solutions used for analysis of total phenols. Total phenolic content of quinoa is higher than other wheat[2] and some Peruvian Andean seeds like kwicha, haba and tuber oca[16]. Total phenolic content in soaked quinoa seeds was reported as 31.1 mgGAE $100g^{-1}$, which is 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). Lower phenolic content was also reported in soaked faba beans by Siah et al. (2015). They explain hydrolysis and leaching of some condensed polyphenols during prolonged soaking period into water used for soaking as reason for reduction in TPC in soaked seeds. Germinated seeds were found to exhibit 134% increase in total phenolic content as compared to the raw quinoa seeds. Germination leads to increase in phenolic content of seeds[22] as synthesis of phenolic acid is enhanced by seed growth during germination[14]. Increase in total phenolic content in germinated quinoa has also been reported by Carciochi et al. 2014a (56 % after 48 hours and 101.2% after 72 hours of germination) and Jubete et al. 2010b (107% after 82 hours of germination). Difference in total phenolic increase can be explained on the basis of varying germination time and techniques. Industrial processed quinoa seeds exhibited 20% decrease in total phenolic content (34 mgGAE $100g^{-1}$). Common process involved in industrial seed processing is decortication, also known as pearling, which removes its saponins present mainly in outer layer of quinoa[15]. 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. Decrease in TPC after decortication (industrial abrasive dehulling) has also been reported in soyabean (11%), chickpeas (37%), and yellow peas (34%). By Xu et al. (2007). Han and Baik, (2008) (in wheat) and Madhujith et al. (2006) (in barley), Cardador-Martý´nez et al. (2002) (in common beans) and Price et al. (1998) (in green beans) have also reported decrease in TPC after decortication of seeds.

Total flavonoids

Total flavonoid content (TFC) of quinoa seeds is shown in TABLE 3. In our study, TFC of raw Indian quinoawas reported as 11.4 mg QE $100g^{-1}$. Results agree with the findings of Carciochi et al. 2014a (11.06 mg QE $100g^{-1}$) and Chirinos et al. 2013 (11 mg QE $100g^{-1}$). Similar value (11.6 mg QE $100g^{-1}$) was reported by Repo-carrasco-Valencia, (2010) in an ecotype of quinoa (Salcedo INIA).

The result reported in our study also lie within the range of values of total flavonoid reported by Miranda et al. 2014 (7.7- 14.37 mg QE $100g^{-1}$) stud-

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 ± 0.08^{a}
Domestically Processed	Soaked	7.2 ± 0.08^{b}
	Germinated	$18.02\pm0.^{2c}$
Industrially processed		5.8 ± 0.10^{d}

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

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ied in six different ecotypes of quinoa. The total flavonoid content reported in our study is significantly different to the values reported by Marmouzi et al. (2015); Carciochi et al. (2014a), Pasko et al. (2008) and Chlopika et al. (2012). 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). Intensity of seed coat colour majorly affects total flavonoid content in quinoa. Red and black quinoa have 50-100% more flavonoid content as compared to white quinoa seeds[50]. Higher total flavonoid content in quinoa than other Peruvian Andean grains (Haba, Kwicha), fruits(Tuna, Guinda, Granadilla, Tumbo) and tubers (Oca, Mashua) has been reported[16]. Total flavonoid content of soaked quinoa seeds decreased by 36% (7.2 mg QE $100g^{-1}$). Similarly decrease in flavonoid content after soaking has been found in pinto bean, chick pea[46] and white sorgum. Although quinoa is reported to be rich in quercitin derivatives but Hirose et al. 2010 could not detect any flavanols like quercetin and kaempferol upon quantitative analysis in hydrolysed quinoa seeds, which may be due to leaching of these flavanols on hydrolysis. Although the flavonoid content decreased after soaking of quinoa seeds but the difference is not significant ($p<0.05$) as significant decrease are found at higher soaking temperature and not at room temperature[46]. Germination of quinoa seeds lead to significant increase, by 56%, in flavoniod content (18 mg QE $100g^{-1}$). Similarly increase in flavonoid content by 59% has been reported by Carciochi et al. 2014a in germinated quinoa seeds. The result agrees with the findings of Guajardo-Flores et al. (2014) where two fold increase in flavonoid content of germinated black beans was reported. Uchegbu. (2015) also reported increase in flavonoid content of germinated African yam bean. HPLC analysis of flavonoids by Carciochi et al. (2014a) reported 4.4 times increase in total flavonoid content of germinated quinoa seeds with quercitin (increased by 56%) in abundant amount and kaempferol glycosides in moderate amounts[2]. The increase in flavonoid content on germination of seeds is due to synthesis of metabolites like flavonoids[41] by phenylproponoid pathway, common to all plants, during process of seed germination[52, 21]. Industrial processing of quinoa seeds lead to reduction in flavonoid content by 47%. Result is supported by reduction in flavonoid content of buckwheat on processing reported by Dietrych-Szostak, (1999). 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[53].

Antioxidant acivity (FRAP and DPPH)

Antioxidant activity of Indian *Chenopodium quinoa* seeds is shown in TABLE 4. Raw quinoa seeds, in our study, were reported to have 59.6 mgTE $100g^{-1}$ and 37.3 TEAC as calculated by DPPH method and 84.4 mg TE $100g^{-1}$ as calculated by FRAP method. The results were close to antioxidant activity according to Jubete et al. (2010b) (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

TABLE 4 : Antioxidant activity (FRAP and DPPH) of Raw, domestically and Industrially processed Indian *Chenopodium quinoa* seeds [A]

Indian *Chenopodium quinoa*		Antioxidant activity		
		FRAP mg TE/g	DPPH	
			TEAC ($IC50_{Trolox}$/IC50) x 10^5	mg TE/g
Raw		84.46±5.9[a]	37.3±0.45[a]	59.61±0.39[a]
Domestically Processed	Soaked	96.46±1.5[a]	34.99±1.42[b]	53.51±0.56[b]
	Germinated	159.23±0.[b]	70.48±0.3[c]	61.41±1.89[c]
Industrially processed		72.35±1.82[a]	30.73±1.9[d]	49.69±1.5[a]

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

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. Quinoa seeds exhibit higher antioxidant activity (evaluated by FRAP and DPPH) as compared to grain Amaranth[55,17] and some Peruvian Andean fruit like Tuna and grain Kwicha[16]. It is found to exhibit lower antioxidant activity as compared to buckwheat[2], oat and rice[29], higher than amaranth[2] and almost similar to wheat[2].

Antioxidant activity of quinoa also depends on intensity of colour of seed coat. Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. The results is supported by findings of Afiffy et al. (2012), who reported decrease in antioxidant activity in soaked white sorghum. Xu et al. (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[48], the decrease may be due to leaching of phenols and flavonoids in water used for soaking the seeds. Quinoa seeds germinated in day light for 7 days exhibit significantly high antioxidant activity than raw seeds[17]. In our study 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. (2014a) which showed 100% increase in antioxidant activity of germinated quinoa seeds as evaluated by DPPH method. Similarly, increase in antioxidant activity of germinated quinoa seeds has also been reported by Pasko et al. (2008). 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. (2010b). Quinoa (*Chenopodium quinoa*) sprouts have lower antioxidant activity (evaluated by FRAP) as compared to Amaranth (*Chenopodium album*) sprouts[17]. 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 rye Zielinski & Kozlowska (2004), wheat[33], barley[31] after undergoing industrial processing like decortications and pearling. Decline in antioxidant activity after processing can be due to removal of hulls which are majorly responsible for antioxidant activity[56] and Cardador-Martý´nez et al. (2002). As illustrated in Figure 2, a strong linear correlation was observed between total phenolic content (TPC) and DPPH of raw, soaked, germinated and industrially processed Indian quinoa. Observed correlation is in agreement with previously reported literature by Hirose et al. (2010). The significant correlation observed in our study is supported by observations of Jubete et al. (2010b), where similar correlation (r=0.99) was observed between TPC and DPPH antioxidant activity of quinoa seeds and sprouts. Palombini et al. (2013) observed good correlation (r=0.87) between total phenolic content and antioxidant activity in amaranth and quinoa seeds.

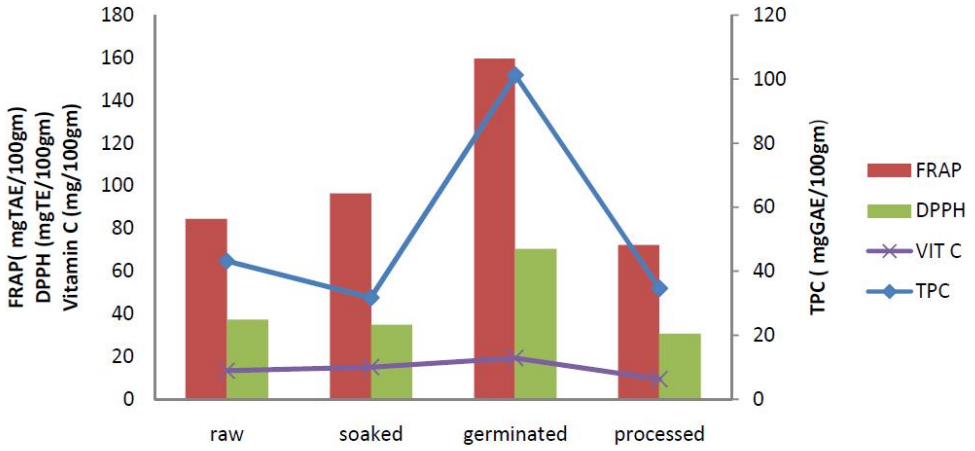

Figure 1 : Vitamin C, TPC, FRAP and DPPH for raw and processed Indian quinoa seeds. Vitamin C in mg/100g, TPC in mg GAE/100g, FRAP and DPPH in mgTE/100g

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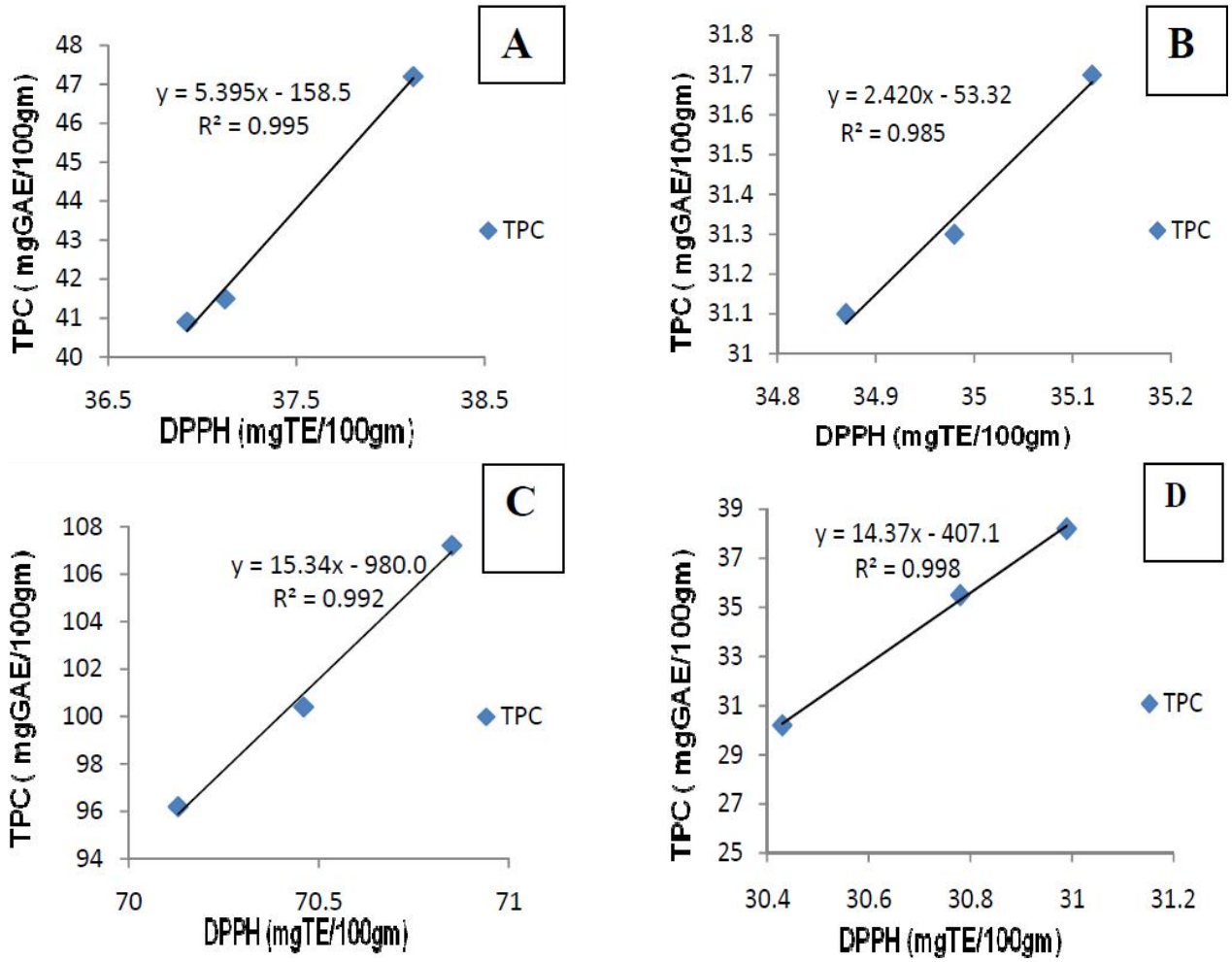

Figure 2 : Correlations between TPC and DPPH parameters of Indian quinoa seeds: (A) raw seeds (R^2 = 0.9951, y=0.5395 x - 15.851) (B) soaked seeds (R^2 = 0.9854, y = 2.4204x - 53.323) (C) germinated seeds (R^2 = 0.9924, y=15.343x - 980.08 (D) industrially processed seeds (R^2 = 0.9982, y = 14.373x - 407.1)

CONCLUSION

Indian *Chenopodium quinoa* seeds grown in Anantpur 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 reveal 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. Domestically processed quinoa by germination should be preferred over industrially processed quinoa.

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