Indian Journal of Traditional Knowledge
Vol 21(04), October 2022, pp 876-882
DOI: 10.56042/ijtk.v21i4.33004

Effect of germination on selected phytochemicals and antioxidant activity of quinoa (*Chenopodium quinoa*)

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Received 25 March 2020; revised 23 July 2022; accepted 28 September 2022

Quinoa (*Chenopodium quinoa*) is a pseudo-cereal native of the Andean region of South America and has gained importance due to its potentially good nutrient content and natural antioxidants. In this research undertaking, quinoa seeds were subjected to germination and subsequent tray drying at 40ºC to study the changes in polyphenols, tannins, saponins and antioxidant activity during different germination stages. The total phenolic content (TPC) of germinated quinoa samples ranged from 150.8±2.26 mg GAE/100 g to 171.2±1.69 mg GAE/100 g with the highest value for the 48 h germinated sample. The total tannin content was highest (343±1.41 mg TA/100 g) in raw sample which decreased by 14% during 48 h of germination. Total saponin content decreased from 1.597 g SE/100 g to 0.938 g SE/100 g during 48 h germination. There was a significant two-fold increase in digestible starch during germination. The antioxidant activity was determined using 3 methods: DPPH scavenging activity, Ferric Reducing Antioxidant Power (FRAP) and ABTS reducing capacity. The DPPH scavenging activity of the germinated sample was found to have increased by 7.5% compared to the raw sample. Moreover, the antioxidant activity of the 48 h germinated sample was found to be higher than the standard ascorbic acid. The antioxidant activity of the 48 h germinated sample determined by FRAP (412.85 mg AAE/100 g) accounts for a 48.68% increase. The % ABTS reducing capacity of 48 h germinated sample was 86.65% higher than the raw quinoa seeds. Germination of quinoa improved the phenolic content and antioxidant activity of quinoa seeds and decreased the saponin content. Germinated quinoa can be ideally used in functional food formulations.

Keywords: Antioxidant activity, Germination, Quinoa, Saponins, Tannins, Total phenols

IPC Code: Int Cl.[22]: A23J 1/12, A23L 7/00, A61P 39/06

Quinoa seed has gained recognition as an extremely nutritious grain compared to traditional cereals providing relatively high quantity and quality of protein, essential fatty acids and dietary fibre[1]. National Academy of Sciences of the United States considered quinoa among "golden grains" because of its high nutritional value. NASA has incorporated quinoa in the diet of astronauts[2]. FAO in its thirty-seventh session of the General Conference declared the year 2013 as the International Year of Quinoa considering its potential to fight against hunger and malnutrition[3].

In India, the Himalayan region and North Indian plains have cultivated the crop with good yield. CSIR-NBRI, Lucknow initiated systematic trials of cultivation of quinoa in North East regions of India[4]. Quinoa was successfully grown under the project "Ananta" in Hyderabad and Anantapur region of Andhra Pradesh[5]. Though quinoa is considered a Super Food, its consumption is very limited in India and majority of the Indian population is still unaware of its potential health benefits[5].

Quinoa is a herbaceous annual plant of about 1-2 m height and usually grown for its seeds[6]. The disk-shaped seed have a flat equatorial band around its periphery[7]. Quinoa seed is actually a fruit and depending on its variety the colors differ - green, yellow, red or purple[8]. The embryo is dicotyledonous and surrounds the perisperm. It forms a part of the bran fraction of the seed. The perisperm holds the storage reserves for the developing embryo rather than the endosperm and this makes quinoa fall in to the category of pseudo cereal[9].

Starch content of quinoa varies from 51-61% and consists of uniform, small granules. Quinoa starch has industrial applications due to its high viscosity. The grains have an average of about 4.1% fiber with a range of 1.1-16.32%. Quinoa grains contain large

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amounts of minerals and the total mineral content of quinoa (3.4%) is comparatively higher than that of rice (0.5%). The calcium and iron contents are significantly higher than most of the commonly used cereals. The grain has been reported to contain an oil content of 5.0-7.2% on average[4]. Ruales and Nair[10] stated that quinoa oils are rich in essential fatty acids and natural antioxidants like α-tocopherol and γ-tocopherol.

Quinoa has appreciable amounts of thiamin, folic acid and vitamin C. The protein content in grain ranges from 7.47 to 22.08% and the protein quality of quinoa is superior with a balanced amino acid composition[10].

Jian *et al.*[11] reported that on germination a horde of biochemical processes leads to changes in the primary and secondary metabolites composition and impacts the intrinsic phenolic compounds profile and antioxidant activity. Germination seems to have the potential to enhance the nutrient and phyto nutrients profile of some seeds. The current study was thus undertaken to determine the effect of germination on the phytonutrients and antioxidant capacity of quinoa seeds (*Chenopodium quinoa* Willd.) and to analyze the impact of duration of germination on the same.

Materials and Methods

Quinoa seeds (*Chenopodium quinoa* Willd.) were procured from a super market in Chennai. It was stored at 4ºC in an air tight container for further use.

Germination and oven drying

As outlined by Carciochi *et al.*[1] the following method was adapted for the germination process with slight modification: Quinoa seeds (200 g) were soaked in 1% sodium hypochlorite solution for 5 min for surface sterilization. The seeds were washed with distilled water to neutral pH and soaked in distilled water overnight and drained. To facilitate hydration of the seeds by capillarity, the seeds were distributed into and covered by a wet muslin cloth and then incubated at 20°C (80–90% relative humidity). Each day the seeds were hydrated with 2.5 mL of sterile distilled water. A sample of quinoa seeds from 0 to 2 days at 24 h intervals was taken and identified henceforth as soaked (SQF), stages 1 and 2 of germination (G1QF and G2QF), respectively. Raw seeds and sprouts corresponding to each stage of germination were dried at 40°C in a tray drier till the time constant weight was recorded. The dried seeds were milled using a laboratory grinder, sieved (32 mesh) and stored in an air tight container. Codes assigned to quinoa samples are given in Table 1.

Preparation of extracts

The method adapted for extract preparation was outlined by Carciochi *et al.*[1]. Two gram of quinoa flour was homogenized with 20 mL of 80% methanol and kept in an orbital shaker for 30 min at 160 rpm. Then, the homogenate was centrifuged for 30 min at 3000 rpm and the supernatant was collected. The extraction was repeated once again for the residue at the same conditions. The supernatants were pooled and stored at -18°C for further assays.

Phytochemical and antioxidant activity assays

Qualitative phytochemical testing of crude extracts was done to study the presence of various phytochemical constituents- tannins, phenolic acids and flavonoids, using standard tests as outlined by Shah and Yadav[12].

Quantitative analysis was carried out for the following phytochemicals – total tannins, total poly phenols and total saponin. Total tannin content was determined using tannic acid as standard following Folin-Ciocaltaeu method outlined by Chandran *et al.*[13].

The total phenolic content was determined[14] by Folin-Ciocaltaeu assay. The TPC was expressed as milligrams of gallic acid equivalents (GAE)/ 100 g of dried sample from extrapolation of calibration curve which was made by preparing standard gallic acid solution. Vanillin-Sulphuric acid assay was used for determining total saponin content by the method outlined by Le *et al.*[15]. The TSC of the samples was then expressed in g of standard saponin (from quillaja bark, Sigma) equivalents (SE) /100 g of sample.

In a plant food matrix, the antioxidant compounds act through varied mechanisms and more than one method of evaluating the antioxidant activity is a

Table 1 — Codes assigned to Quinoa samples

RQF	Quinoa seeds washed with 1% sodium hypochlorite solution and distilled water; tray dried (40°C); milled.
SQF	Quinoa seeds soaked in 1% sodium hypochlorite for 5 min; washed and soaked in distilled water overnight (12 h- Day 0); tray dried (40°C) milled.
G1QF	Quinoa seeds after 24 h germination (Day 1); tray dried (40°C); milled.
G2QF	Quinoa seeds after 48 h germination (Day 2); tray dried (40°C); milled.

generally acceptable practice[1] and hence the antioxidant potential of the quinoa flour was determined by the following three methods:

DPPH (*1, 1-Diphenyl-2-Picrylhydrazyl*) Free Radical Scavenging Activity Assay was determined as illustrated by Carciochi *et al.*[1] 1 mL of 0.1 mM DPPH in methanol was mixed with 2 mL of sample extracts. The control was prepared by mixing 1 mL of DPPH solution with methanol. After incubation at room temperature in dark for 30 min, the absorbance was measured at 517 nm using spectrophotometer. Ascorbic acid was used as the standard. The percentage of scavenging activity of each extract on DPPH radical was calculated as %inhibition of DPPH (I%) using the following equation:

$$I\% = [(Ao-As)/Ao] \times 100,$$

Where, Ao is the absorption of control and As is the absorption of the tested extract solution.

ABTS [*2,2'-Azinobis* (*3-Ethylbenzothiazoline-6-Sulphonic Acid*)] free radical scavenging activity assay was carried out with slight modifications of the method given by Re *et al.*[16] The ABTS assay uses ABTS radicals pre-formed by oxidation of ABTS with potassium persulphate. The $ABTS^{\bullet+}$ stock (aqueous) solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate in equal quantities and the mixture was allowed to stand in the dark for 12-16 h at room temperature. The working solution of $ABTS^{\bullet+}$ was prepared by diluting the stock solution in methanol to give an absorbance of 0.70±0.02 at 734 nm. 2.0 mL of $ABTS^{\bullet+}$ working solution was mixed with 1 mL of sample extracts of different concentrations. The control was prepared by mixing 2.0 mL of $ABTS^{\bullet+}$ solution with 1 mL of methanol. Ascorbic acid was used as the standard. The mixture was incubated for 10 min keeping in the dark at room temperature and the absorbance was measured at 734 nm using spectrophotometer. The percentage of scavenging activity was calculated as % inhibition (I%) using the following equation: $I\% = [(Ao-As)/Ao] \times 100$, where Ao is the absorption of control and As is the absorption of the tested extract solution.

Following the procedure of Benzie and Strain[17], Ferric Reducing Antioxidant Power (FRAP) was determined based on the ability of the sample to reduce $Fe3^{+}$ to $Fe2^{+}$ ions. At low pH, in the presence of TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), ferric-tripyridyltriazine ($Fe3^{+}$ -TPTZ) complex is reduced to the ferrous ($Fe2^{+}$ -TPTZ) form with the formation of an intense blue colour having an absorption maximum at 593 nm. Ascorbic acid was used as the standard. 2.3 mL of the FRAP reagent was mixed with 0.7 mL of the sample extracts, incubated at 37ºC for 30 min in the dark and absorbance was measured at 593 nm against blank. Results were expressed in mg of ascorbic acid equivalents (AAE) /100 g.

α-Amylase inhibitor activity (AIA) was analyzed using the protocol stated by Deshpande *et al.*[18]. Briefly 1 g of sample was extracted with 10 mL of deionized water for 12 h at 4ºC and the supernatant was used for AIA testing. 0.25 mL supernatant was incubated with α-amylase enzyme (0.003% in 0.2 M sodium phosphate buffer, pH 7.0) for 15 min at 37ºC. Following, 0.5 mL of 1% starch solution pre-incubated at 37ºC was added. At the end of 3 min, the reaction was terminated by addition of DNS reagent and heating for 10 min in boiling water bath. The absorbance was measured at 540 nm. One unit of enzyme activity was defined as that which liberates, from soluble starch, one micromole of reducing group – calculated as maltose per min at 37ºC/ pH 7.0 under specified conditions. One unit of α-amylase inhibited was defined as one α-amylase inhibitory unit.

In vitro starch digestibility was analyzed following the method outlined by Englyst *et al.*[19] with slight modifications. The sample (50 mg, dwb) was incubated at 37ºC with pancreatin and amyloglucosidase. Digestible starch (DS) was quantified as the glucose released after 120 min incubation. After the enzyme incubation, the reaction mixture was hydrolyzed with potassium hydroxide (7 M) and further incubated with amyloglucosidase. This was indicative of the total starch (TS). Glucose released was determined by glucose oxidase/peroxidase kit. Digestible/ total starch was calculated as mg of glucose x 0.9.

Statistical analysis

Microsoft excel 2016 program was used for statistical analysis and calculations. Data were expressed as Mean±standard deviation for 3 sample replicates in each group. Student's t was used to compare means and study the significant difference between the RQF & SQF; RQF & G1QF; and RQF & G2QF respectively. The difference between compared groups was considered to be significant when $p<0.05$.

Results and Discussion

Qualitative analysis

Qualitative phytochemical testing of crude extracts was done to study the presence of various phytochemical constituents such as tannins, phenolic acids, flavonoids, glycosides and saponins using standard tests. The results of the same are provided in Table 2.

Phytochemical analysis of the extract of quinoa samples revealed the presence of most of the biochemicals tested for. Phenols, flavonoids and tannins were found to be present in raw and germinated samples. Glycosides and saponins were not detected in germinated quinoa extracts. The qualitative screening of raw and germinated samples of quinoa indicated that there was a positive effect of germination on the phytochemical properties of quinoa seeds. This directed the study to quantitative estimation of these biochemicals to understand the effect of germination in quinoa seeds.

Quantitative analysis

Phytochemicals quantified in raw and germinated quinoa samples are presented in Table 3.

Total phenolic content (TPC)

Dietary phenolic compounds are antioxidative, anti-allergic, anti-inflammatory, antiviral and anti-carcinogenic activities and possess cardiovascular protective properties. Gorinstein *et al.*[20] stated that quinoa seeds being a good source of bioactive-polyphenols might alter antioxidant status and fend off oxidative stress. In a study by Pasko *et al.*[21], quinoa seeds exhibited modest protective action by reducing lipid peroxidation against fructose-induced metabolic changes in rats.

Asao and Watanabe[22] reported that total polyphenolic content of quinoa was much higher than that of other grains such as amaranth, barley, wheat and rice. In a study, total phenolic content of 2.8±1.01 mg GAE/g was reported for cooked quinoa extracts[23]. In the current study, the total phenolic content of raw sample was found to be 154.8±2.26 mg GAE/100 g. The 48 h germinated sample had a 10.59 % significant ($p<0.05$) increase in TPC (171.2±1.69 mg GA/100 g). A similar research study,[1] reported that 72 h germination resulted in 101.2% increase in the total phenolics compared to raw seeds. Alvarez-Jubete *et al.*[24] reported that after 82 h of germination there was a two-fold increase in the total phenols in quinoa sprouts. Metabolic changes and surge in the activity of the endogenous hydrolytic enzymes in the seeds during the germination process could be possible explanations for the increase in total phenolic content. Kim *et al.*[25] have demonstrated that as sprouting progressed in germinated buckwheat (*Fagopyrum esculentum* Moench) there was a remarkable increase in the polyphenol content. Different cultivars of barley (*Hordeum vulgare*) sprouts showed a significant increase in total phenolic content and antioxidant activity compared with raw seeds.

Table 2 — Qualitative screening for the presence of phytochemicals

Parameter	Experiment name	RQF	SQF	G1QF	G2QF
Phenolic compounds	$FeCl_3$ test	–	–	+	+
	Lead Acetate test	+	+	+	+
Tannins	Gelatin test	+	+	+	+
Flavonoids	Alkaline reagent test	+	+	+	+
Glycosides	Libermann's test	–	–	–	–
	Salkowski's test	+	+	–	–
	Keller-Kilani test	–	–	–	–
Saponins	Shaking test	+	–	–	–

Table 3 — Phytochemicals in raw and germinated quinoa

Sample/Parameters	RQF	SQF	G1QF	G2QF
TPC (mg GA/100 g)	154.8±2.26	150.8±2.26 (-2.59%)	159.6±1.13 (+3.1%)	171.2±1.69 (+10.59%)
Tannin (mg TA/100 g)	343±1.41	347±4.24 (+1.16%)	313±7.07 (-8.74%)	294±2.82 (-14.28%)
Total Saponin content (g SE/100 g)	1.597±0.14	1.787±0.04 (+11.94%)	1.523±0.01 (-4.63%)	0.938±0.01 (-41.26%)

Values are Mean ± SD (triplicate samples)
Values in parenthesis indicates percent increase (+) or (-) decrease over RQF values

Total tannin content

Tannins are known to form complexes with dietary proteins and digestive enzymes[26]. The raw sample has the highest tannin content (343±1.41 mg TA/100 g) while 48 h germinated sample has the lowest (294±2.82 mg TA/100 g). Germination of quinoa had led to a decrease in the tannin content by 8.74% and 14.28% in 24 h and 48 h germination, respectively.

Tannins are present in outer coat of seed, which gets ruptured during germination and some loss could occur due to leaching of tannins in soaking water. This could be the possible explanation for the reduction in tannins due to germination. Padmashree *et al.*[5] in their research on quinoa observed a decrease in the tannin content during germination. In commonly consumed legumes, germination showed a significant reduction in tannin content in a study by Singh *et al.*[27]. A similar trend was obtained by Vijayakumari *et al.*[28] for *Vigna aconitifolia* and *Vigna sinensis*.

Total saponin content (TSC)

Saponins are composed of monosaccharide or oligosaccharide moieties linked to steroidal aglycones or triterpenoid. Saponins are bitter in nature and exhibit toxic anti-nutrient properties when consumed in large quantities. Quinoa seeds, in its seed coat or epicarp contain around 1%-1.2% saponins[29]. Polishing the seeds and washing with water[30] before processing or consumption can reduce the saponin content. Approximately, 34% of total saponins in quinoa is present in the hull. Hence, dehulling removes a major portion of the saponins. Washing the seeds is also found to decrease the saponins by approximately 3 times.[31] The total saponin content of raw sample was found to be 1.597±0.14 g SE /100 g. The saponin content decreased on germination to 0.938±0.01 g SE/100 g. This accounts for a 41.26% significant ($p<0.05$) decrease in the total saponin content on 48 h germination. There was a 11.94% increase in saponin content on soaking and 4.63% decrease in 24 h germination. The total saponin content of raw sample is comparable to studies by Gee *et al.*[32] and Chauhan *et al.*[33]. Ridout *et al.*[34] had stated that processing of quinoa led to a reduction in saponin levels in the final product. Beniwal *et al.*[35] in their research study found that saponin content was significantly decreased following different processing treatments and the highest reduction was noted in germinated quinoa and amaranth flours.

Alpha amylase inhibitor activity

No alpha amylase inhibitor activity was detected in any of the samples. Ranilla *et al.*[23] reported that α-amylase inhibitory activity was not detected in any of the Andean grains. This might be due to the high amylase activity in quinoa as reported by Lorenz and Nyanzi[36].

% Digestible starch

The total and digestible starch composition of quinoa samples are given in Table 4. The % total starch of raw sample was found to be 43.75% which increased significantly ($p<0.05$) to 76.25% during 48-h germination. 24 h and 48 h germination had recorded 45.7% and 74.28% increase. The % digestible starch was found to be 23.65% and 51.35% in raw and 48 h germinated sample, respectively. This accounts for a significant ($p<0.05$) 117% increase in the 48-hour germinated sample. Soaking and 24 h germination recorded a 75-76% increase in digestible starch.

Antioxidant activity

DPPH scavenging activity

The antioxidant activity of 100 mg/mL raw and germinated quinoa sample extracts was determined. The values of quinoa samples (Table 5) were similar to that of ascorbic acid at the same concentration (100 mg/mL). The antioxidant activity of raw sample was found to be 82.23%. The DPPH antioxidant activity of 24 h germinated sample and 48 h germination were 85.5% and 88.92% respectively. There is a significant ($p<0.05$) increase (7.5%) in the antioxidant activity of 48 h germinated sample compared to the raw sample. Also, this is higher than the antioxidant activity of

Table 4 — Total starch and digestible starch of quinoa flours

Sample/Parameters	RQF	SQF	G1QF	G2QF
% Total starch	43.75	60 (+37.14%)	63.75 (+45.71%)	76.25 (+74.28%)
% Digestible starch	23.65	41.5 (+75.47%)	41.85 (+76.95)	51.35 (+117.2%)

Values in parenthesis indicates percent increase (+) or (-) decrease over RQF values

Table 5 — % DPPH, FRAP and % ABTS scavenging activity of quinoa samples

Sample/Parameters	RQF	SQF	G1QF	G2QF
% DPPH Radical Scavenging Activity	82.23±2.08	82.305±0.40 (0.08%)	85.522±0.04 (4%)	88.923±0.04 (7.5%)
FRAP (mg AAE/100 g)	277.12±30.30	295±15.15 (6.45%)	348.57±30.30 (25.7%)	412.85±20.20 (48.68%)
% ABTS scavenging activity	29.06±1.13	49.33±0.37 (70%)	52.13±0.18 (79.65%)	54.13±0.37 (86.65%)

Values are Mean ± SD (triplicate samples)
Values in parenthesis indicates percent increase (+) or (-) decrease over RQF values

standard ascorbic acid (86.43%). A research study[23] also reported that quinoa exhibited the highest antioxidant activity among all Andean grains (86%).

Ferric reducing antioxidant power assay (*FRAP*)

The FRAP value as expressed in mg Ascorbic acid equivalents (AAE) /100 g was determined. The FRAP value for raw sample was found to be the lowest with 277.12±30.30 mg AAE/100 g. The FRAP value was highest for 48 h germinated quinoa sample with a value of 412.85±20.20 mg AAE/100 g. There was a 48.68% increase in the antioxidant activity of 48 h germinated sample compared to that of raw sample (Table 5). A significant difference ($p<0.05$) was found between raw sample and 48 h germinated sample and between 24 h germinated sample and 48 h germinated sample.

The FRAP value as determined by Jubete *et al.*[24] for raw quinoa was 92.1 mg TE/100 g and that of quinoa sprouts was found to be 164 mg TE/100. This difference in the FRAP values might be due to the difference in the standard used.

Azino-bis (*3-ethylbenzthiazoline-6-sulfonic acid*) *radical cation* (*ABTS*) *scavenging activity*

The % ABTS reducing activity of raw sample was found to be 29.06±1.13%. The % ABTS reducing activity of 48 h germinated sample was found to be 54.13±0.37 % which accounts for an 86.65% significant ($p<0.05$) increase in the antioxidant capacity of germinated sample compared to raw sample (Table 5).

All the methods of assessing antioxidant showed an increase in the antioxidant power during germination. Antioxidant activity of quinoa observed kindles interest to research further regarding its utilization as a natural antioxidant[4].

Conclusion

Germination process improved on the phenolic content in quinoa seeds and enhanced the antioxidant potential. Germination had brought a significant reduction in saponin content, which is a primary anti-quality factor. Reduction in tannin content and increase in digestible starch on germination had made it possible for Quinoa to be considered an ideal dietary ingredient. Therefore, the current study recommends the usage of dried germinated quinoa seeds in the formulation of functional foods.

Acknowledgement

The authors thank the Principal and Management of M.O.P. Vaishnav College for Women, Chennai for providing the necessary support for this work.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

Authors' Contributions

NS carried out the research work under the guidance of HA. NS prepared the draft; HA reviewed & edited the research paper.

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