

Antioxidant and Anti Nutritional Composition of Germinated Quinoa (*Chenopodium quinoa Willd*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MNSS has performed the research analysis, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BAK has designed the study, guided in writing the protocol and first draft of the manuscript. Authors KUM and KBSD have monitored the proof reading of the article. Author WJS has managed literature search. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To analyse the antioxidant and antinutritional components of germinated quinoa.

Sample: Whole (HGQ) and dehulled (DGQ) quinoa grain (*Chenopodium quinoa Willd*) was procured from Department of Agronomy, College of Agriculture, PJTS Agricultural University, Rajendranagar, Hyderabad and commercially processed quinoa seed (CGQ) purchased from local market was germinated at 20°C temperature for 4hrs and analyzed.

Study Design: Analysing antioxidants and antinutritional components.

Place and Duration of Study: Analysis was conducted in PGRC Laboratory, PJTSAU, Rajendranagar, Hyderabad.

Results: The total phenol content and flavonoid content increased upon germination and was high for HGQ and DGQ was high compared to CGQ. IC₅₀ value for antioxidant value of CGQ in methanol and water extract was low compared to HGQ and DGQ. The oxalate content of germinated quinoa ranged from 6.17 ±0.01 to 9.45±0.02mg/100g. The saponin content of germinated quinoa was high for HGQ and DGQ compared to CGQ. When compared to the raw there was 104.16% reduction was seen in HGQ, whereas it reduced to 404.85% in DGQ. But when compared to the commercial variety the saponin contents were 23.07% (DGQ) and 69.35% (HGQ) more. This may be because of processing conditions of commercial variety.

Keywords: Germinated quinoa; antioxidants; phenols; flavonoids; saponins and oxalates.

1. INTRODUCTION

Quinoa (*Chenopodium quinoa Wild*) belongs to the Goosefoot family "Chenopodiaceae", is a pseudo cereal traditionally consumed by Andean cultures that is attracting attention worldwide as a functional food. [1]. Quinoa is a stress-tolerant plant cultivated along the Andes for the last 7000 years, challenging highly different environmental conditions [2].

India being a land of diverse climatic regions and quinoa being a crop profoundly known to adapt well to unusual environmental conditions is found appropriate to grow in India [3].

In view of global need to identify crops with potential to produce quality food, quinoa has a high potential both for its nutritional benefits and its agricultural versatility to contribute to food security in various regions of the planet, especially in countries which are limited in food production or where the population has no access to protein sources [4].

Quinoa has dietary fiber content that acts as a functional and bioactive ingredients and natural antioxidants like phenolic compounds. Phenols are defined to carry many potential beneficial health effects, such as reduction of risks of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, osteoporosis, scavenging free radicals and providing metal chelating activities. In food, polyphenols may contribute to bitterness, astringency, colour, flavour, oxidative stability of products [5].

Saponins and phytic acid are the main antinutritional factors in quinoa. Other inhibitors like trypsin inhibitor and tannins are present in low levels. The pericarp of the seed contains as much as 6% saponins. Saponins may interfere

with digestion by directly interacting with the digestive enzymes, preventing absorption of nutrients. They are removed either by the wet method, i.e. washing and rubbing in cold water or by dry method, i.e. toasting and subsequent rubbing of the grains to remove the outer layers. On commercial scale, saponins are removed by abrasive dehulling but in this method; some saponins remain attached to the perisperm phenylpropanoid pathway [6].

Quinoa is not genetically modified and is rarely allergenic due to absence of gluten [7]. Quinoa has been recommended as part of a gluten-free diet for celiac patients and also to people having wheat allergy [8].

There are different processing technologies available for processing of quinoa in order to improve the nutrient composition and for development of different products. Washing caused a reduction in saponins content and an abrasion of 30% degree was sufficient to obtain sweet quinoa [9].

Process of dehulling is known to improve grain quality by lowering the content of anti nutrients and enhancing the sensory parameters. Even though there are benefits of dehulling, it causes loss of nutrients from grains. Thus, to minimize loss and increase bioavailability of nutrients, use of common traditional domestic processing methods for grains like soaking and germination are the most common [10].

The quinoa composition allows for greater storage potential than that of other oil seeds due to the greater chemical stability of the starches and lipids. But, quinoa loses viability more rapidly than cereals because of the porosity in the outer covering, allows seed to gain or lose moisture easily and may initiate germination in the panicle [11]. Germination of edible seeds modifies both

the palatability and the nutritional profile of grains and is a potential means of reducing off flavours [12].

Processing methods like soaking, germination and fermentation are shown to increase solubility of iron in quinoa. Malting under moderate thermal treatment resulted in increase in antioxidant activity of quinoa [13].

Germination activates the natural enzymes, improves vitamin status and softens the grain. Germination increases different nutritive factors such as vitamin concentrations and bioavailability of trace elements and minerals. Quinoa has short germination period of 4-5 hours in comparison to other grains that require at least 12-14 hours. Germination of quinoa reduces the antinutritional factors like saponins [14].

Research on quinoa has mainly focused on composition of the whole seed, protein quality, starch functionality and incorporation of quinoa into food products. Considering the expansion of area under quinoa cultivation, the growing popularity of the grain in domestic and foreign markets, and the deficit of scientific information about the effect of germination on nutritional composition, the current study aimed to evaluate the influence of different time and temperature conditions on quinoa seed germination, nutritional value of germinated seeds and antioxidant activity of germinated quinoa seeds.

2. MATERIALS AND METHODS

2.1 Procurement of Raw Materials

Quinoa seeds were obtained from Department of Agronomy, College of Agriculture, PJTS Agricultural University, Rajendranagar, Hyderabad. The other ingredients were procured from local market of Hyderabad. The glassware and equipment were available at Post Graduate & Research Centre, PJTSAU, Rajendranagar, Hyderabad, were used throughout the study.

2.2 Preparation of Aqueous / Methanolic Sample Extracts

In order to compare the total phenolic, flavonoids, antioxidant activity soluble in different polarity solvents and to determine the total phenolic content, flavonoids, antioxidant activities of the extracts, the germinated quinoa samples were extracted in 2 types of solvents: distilled water and 95% methanol.

Firstly 2.0 g of germinated quinoa samples were subjected to extraction by cold maceration in 100 ml of methanol for 24 hrs followed by centrifugation at 3000 rpm for 10 min and filtered through Whatman No. 41 filter paper to obtain clear extracts. The clear filtrate was collected and preserved at 4°C until further use. All the analysis was carried out in triplicates.

2.3 Preliminary Phytochemical Screening of Germinated Quinoa Sample

The preliminary tests for carbohydrate, alkaloids, proteins, amino acids, flavonoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatins, phenols and quinines were carried out as per the procedure given by [15].

The total flavonoid content (TFC) of germinated quinoa samples was estimated by using the method of [16]. The total phenol content (TPC) in extracts was determined by using the Folin - Ciocalteu reagent following the method described by [17]. The antioxidant activity was estimated by using 1-diphenyl-2-picrylhydrazil (DPPH) method as described by [18]. The estimation of oxalate content of germinated samples was determined by using the titrimetric method described by [19] method. The saponins content of germinated quinoa was estimated by the Spectrophotometric method of [20]. The saponin and oxalate content was estimated in methanol extracts.

2.4 Statistical Analysis

The results were statistically analysed [21] the results were presented as mean \pm standard deviation. Difference between the variables was tested for significance by ANOVA using SAS version 9.1.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity of Germinated Quinoa Extracts

3.1.1 Phytochemical screening of germinated quinoa

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants. These substances have been found to be beneficial to human health as well as possessing antioxidant activity [22]. Plant derived compounds are well known for their therapeutic values since ancient times.

Phytochemicals could act as an antioxidant and anti-inflammatory substances. They play a vital role in detoxification of harmful and deleterious chemicals of the body [23]. The phytochemical tests were carried out using standard methods of analysis of carbohydrates, alkaloids, proteins, amino acids, flavonoids, fixed oils and fats, terpenoids, cardiac glycosides, steroids, saponins, tannins, phlobatanins, phenols and quinines. The results of phytochemical screening are given in Table 1.

Results of present study show that carbohydrates, proteins, flavonoids, fixed oils and fats were detected in all the samples. Amino acids, glycosides, phenols and saponins were strongly detected in experimental samples (HGQ and DGQ) than commercial quinoa (CGQ). Steroids, alkaloids, phlobatanins and quinines were not detected (Table 1).

3.1.2 Total phenol content (TPC) of germinated quinoa

Mean values of TPC of the methanol and water extracts of germinated quinoa samples were statistically analysed and presented in Table 2(a). The total phenol content of methanol extract of HGQ was (48.3±0.48 mg RE/ 100 g), DGQ was (42.2±0.32 mg RE/ 100 g) and CGQ was (30.8 ±0.42 mg RE/ 100 g). The total phenol content of water extract of germinated quinoa HGQ was (42.4±0.28 mg RE/ 100 g), DGQ was (38.4 ±0.28 mg RE/ 100 g) and CGQ was (27.4 ±0.28 mg RE/ 100g). The IC₅₀ values were calculated and given in Table 2(b). IC₅₀ value of CGQ in methanol and water extracts was 6.84±0.40, 7.47±0.10; HGQ in methanol and water extracts was 4.98±0.01, 5.33±0.18 and DGQ in methanol and water extracts was 6.06±0.20, 6.10±0.09 as given in Table 2(b) indicating high phenol content in HGQ which is significantly higher ($p \leq 0.05$) than DGQ and CGQ.

Affect of germination on the total phenolic compound of the amaranth seed was reported by [24]. The results show that TPC content was 4.50±0.02 mg GAE/g in raw amaranth flour to 7.3±0.03 mg GAE/g in 72h germinated amaranth seed. The higher TPC of germinated amaranth flours compared to non-germinated amaranth flours could be due to the biosynthesis of phenol compounds caused by enzyme hydrolysis during germination. This increase could also be attributed to the production of β -glucosidase during germination.

3.1.3 Total flavonoid content (TFC) of germinated quinoa

The quantitative analysis of flavonoid content was done in germinated quinoa and the results are given in Table 3(a). The total flavonoid content of germinated quinoa in methanol extract of HGQ was 10.04± 0.28 mg RE/ 100 g, DGQ was 7.80± 0.48 mg RE/ 100 g and CGQ was 4.28± 0.39 mg RE/ 100 g. The TFC of germinated quinoa in water extract was 8.34± 0.78 mg RE/ 100 g in HGQ, DGQ was 6.2± 0.98 mg RE/ 100 g and CGQ was 3.84 ± 0.28 mg RE/ 100 g as given in Table 3 (a). Different inhibitions were calculated at different concentrations and plotted in Fig. 1. IC₅₀ values CGQ in methanol and water extract was 62.5±0.37, 78.5±0.39; HGQ 36.75±0.28, 44.6±0.32 and DGQ was 41.6±0.32, 48.6±0.28. The IC₅₀ value of CGQ was more compared to DGQ and HGQ (Table 3b).

Germination of quinoa seeds leads to significant increase (56%) of flavonoid content in quinoa (18 mg QE 100 g⁻¹). The increase is due to synthesis of metabolites like flavonoids by phenylpropanoid pathway, during the process of seed germination [25].

A significant increase of TPC and TFC values throughout the germination period was reported by [26]. After 72 hrs of germination a considerable increase of 101. 2% was reported.

3.1.4 Free radical scavenging assay by DPPH method

The intensity of the radical scavenging effect is measured by calculating half-inhibition concentration using IC₅₀ which was the efficient concentration required for decreasing initial free radical concentration by 50 percent [27]. IC₅₀ was obtained by representing data at various concentrations. In the present study, the antioxidant activity of extracts was carried out by *in-vitro* antioxidant models in relation to ascorbic acid.

DPPH radical scavenging activity Antioxidants react with DPPH, which is a stable free radical and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicated the radical scavenging potential of the antioxidant components [28].

As the concentration increased, the antioxidant activity increased in the germinated quinoa

samples. Lower IC₅₀ value indicates the more antioxidant activity. IC₅₀ value for CGQ in methanol and water extract was 4.09±0.04, 3.98±0.01, HGQ in methanol and water extract was 2.07±0.24, 2.07±0.06 and DGQ in methanol and water extract was 3.32±0.02, 3.87±0.08 as given in Table 4.

Table 1. Phytochemical screening of germinated quinoa in methanol and water extracts

S. No	Phytochemicals	Test	CGQ		HGQ		DGQ	
			M	W	M	W	M	W
1.	Carbohydrates	Molisch test	+	+	+	+	+	+
2.		Mayer's test	-	-	-	-	-	-
	Alkaloids	Wagner's test	-	-	+	-	-	-
		Hager's test	-	-	+	-	+	-
3.	Proteins	Kjeldahl method	+	+	+	+	+	+
4.	Amino acids	Ninhydrin test	+	+	++	+	++	+
5.	Flavonoids	With ammonia solution	+	+	+	+	+	+
6.	Fixed oils and fats	Foam test	+	+	+	+	+	+
7.	Terpenoids	-	+	-	+	-	+	-
8.	Cardiac glycosides	-	+	+	++	++	++	+
9.	Steroids	Liebermann Bucharid test	-	-	-	-	-	-
10.	Saponins	Foam test	+	+	++	++	++	++
11.	Tannins	FeCl ₃ test	+	+	+	-	+	-
12.	Phlobatanins	With HCl	-	-	-	-	-	-
13.	Phenols	Ferric chloride test	+	+	++	+	++	+
		Liebermann's test	+	+	++	+	+	+
14.	Quinones	With conc. HCl	-	-	-	-	-	-

*CGQ – commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ – dehulled germinated quinoa, M- methanol extracts, W- water extracts, + = detected, ++ = strongly detected, - = not detected
All screening tests were carried out in triplicates

Table 2(a). Total phenol content of germinated quinoa in methanol and water extracts

Sample	TPC (M) (mg/ 100 g RE)	TPC (W) (mg/ 100 g RE)
CGQ	30.8 ^a ±0.42	27.4 ^a ±0.28
HGQ	48.3 ±0.48	42.4±0.28
DGQ	42.2 ^b ±0.32	38.4 ^b ±0.28
Mean	40.4±0.40	36.06±0.28
CD	0.56	0.62
SE of mean	0.57	0.59
CV (%)	1.25	1.59

*values are expressed as mean ± standard deviation for all the three determinants. means within the same column followed by common letter do not significantly differ at p≤ 0.05. CGQ – commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ – dehulled germinated quinoa, M – methanol extract, W- water extract, TPC – total phenol content in rutin equivalents (mg RE/ 100 g)

Table 2 (b). Inhibitory activity of phenols of germinated quinoa in methanol and water extracts

S. No	Parameter	CGQ(M)	HGQ (M)	DGQ (M)	CGQ (W)	HGQ (W)	DGQ (W)
1.	IC ₅₀	6.84 ± 0.4	4.98 ^a ± 0.01	6.06 ^b ± 0.2	7.47±0.1	5.33 ^a ±0.18	6.10 ^b ±0.09
2.	Mean	6.17			6.09		
3.	CD	0.89			0.51		
4.	SE of mean	0.88			0.22		
5.	CV (%)	6.37			3.73		

*Values are expressed as mean ± standard deviation for all the three determinants. Means within the same column followed by common letter do not significantly differ at p≤ 0.05. CGQ – Commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ – Dehulled germinated quinoa, M – Methanol extract, W- Water extract

Table 3(a). Total flavonoid content (TFC) of germinated quinoa in methanol and water extracts

Sample	TFC (M) (mg/ 100g RE)	TFC (W) (mg/ 100g RE)
CGQ	4.28 ^a ± 0.39	3.84 ^a ± 0.28
HGQ	10.04± 0.28	8.34± 0.78
DGQ	7.80 ^b ± 0.48	6.2 ^b ± 0.98
Mean	7.37 ±0.38	6.12±0.68
CD	0.78	0.85
SE of mean	0.04	0.05
CV (%)	6.25	4.26

*values are expressed as mean ± standard deviation for all the three determinants. means within the same column followed by common letter do not significantly differ at $p \leq 0.05$. CGQ – commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ– dehulled germinated quinoa, M – methanol extract, W- water extract, TFC – total flavonoid content (mg RE/ 100 g)

Table 3(b). Inhibitory activity of flavonoids of germinated quinoa in methanol and water extracts

S. No	Parameter	CGQ (M)	HGQ (M)	DGQ (M)	CGQ (W)	HGQ (W)	DGQ (W)
1.	IC ₅₀	62.5± 0.37	36.75 ^a ±0.28	41.67 ^b ± 0.32	78.5 ±0.39	44.6 ^a ±0.32	48.6 ^b ±0.28
2.	Mean	54.69			69.93		
3.	CD	0.98			0.94		
4.	SE of mean	0.96			0.99		
5.	CV (%)	2.51			12.5		

* values are expressed as mean ± standard deviation for all the three determinants. means within the same column followed by common letter do not significantly differ at $p \leq 0.05$. CGQ – commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ – dehulled germinated quinoa, M – methanol extract, W- water extract

Table 4. Antioxidant activity of extracts of germinated quinoa in methanol and water

S. No	Parameters	CGQ	HGQ	DGQ	CGQ	HGQ	DGQ
		M	M	M	W	W	W
1.	IC ₅₀	4.09±0.04	2.07 ^a ±0.24	3.32 ^b ±0.02	3.98 ^b ±0.01	2.07 ^a ±0.06	3.87 ^b ±0.08
2.	Mean	3.16			3.30		
3.	CD	0.53			0.19		
4.	SE of mean	0.30			0.31		
5.	CV (%)	7.51			2.56		

*values are expressed as mean ± standard deviation for all the three determinants. CGQ – commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ – dehulled germinated quinoa, M – methanol extracts, W- water extracts

The germination of quinoa extract to DPPH solution caused a rapid decrease in IC₅₀ value as indication to its good scavenging capacity. Phytochemical analysis showed high total flavonoid contents in the germinated quinoa extracts suggesting that the flavonoid compounds present in the extract could be responsible for the observed DPPH radical scavenging activity.

The IC₅₀ value of germinated *Amaranth viridis* flour was 1.57 mg/ml in a study conducted by [24] which is significantly lower than IC₅₀ of HGQ 2.07±0.24.

3.2 Antinutritional Components of Germinated Quinoa

3.2.1 Total oxalate content

The oxalate content of germinated quinoa ranged from 6.17 ±0.01 to 9.45±0.02 mg/100 g. The decreasing order of oxalate content of germinated quinoa was HGQ (9.45± 0.02) > DGQ (8.16±0.02) > CGQ (6.17± 0.01 mg/100 g). The values of the study are significantly higher when compared to the study reported by [24] which was 5.10± 1.05 in germinated amaranth flour.

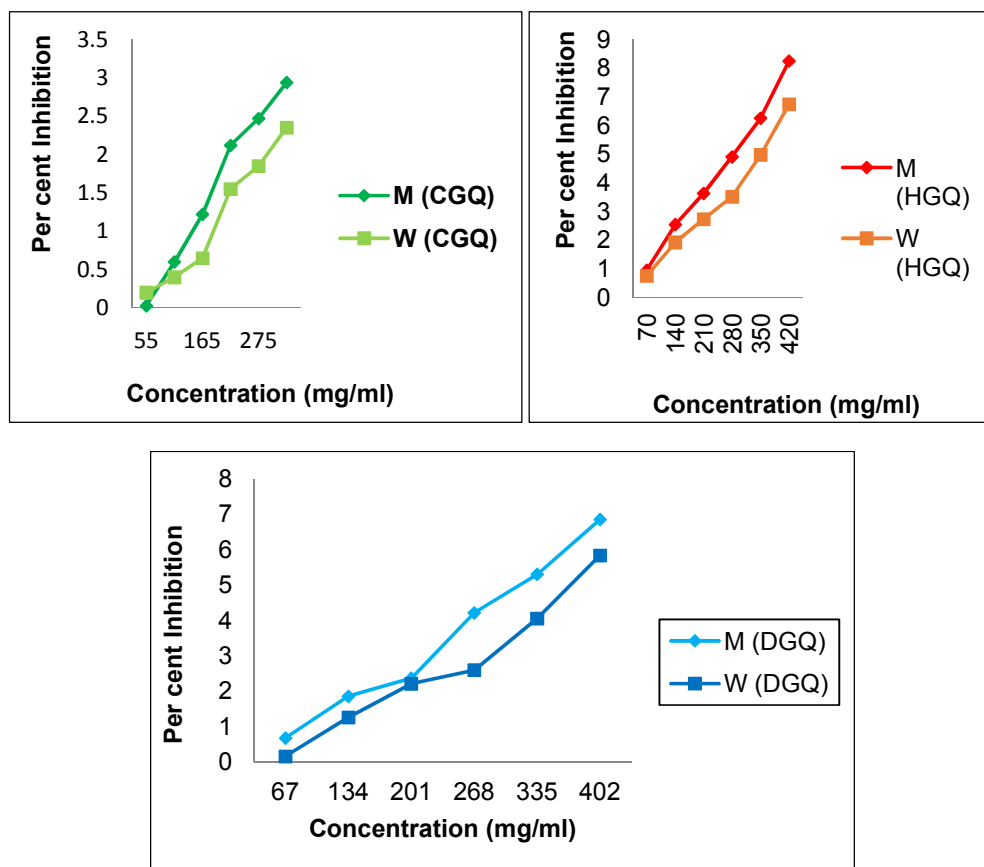


Fig 1. Percentage inhibition of TFC in methanol and water extracts

Table 5. Antinutritional components of germinated quinoa Oxalates by titremic and Saponins by Spectrophotometric method

Sample	Oxalates (mg/100g)	Saponins (mg/ 100 g)
CGQ	6.17±0.01	1.90±0.04
HGQ	9.45±0.02	6.24±0.01
DGQ	8.16±0.02	2.47±0.01
RQ	-	12.75±0.08
Mean	7.92	2.54
CD	0.04	0.10
SE of mean	0.47	0.19
CV (%)	0.27	1.80

*values are expressed as mean ± standard deviation for all the three determinants. CGQ – commercial germinated quinoa, HGQ – with husk germinated quinoa, DGQ – dehulled germinated quinoa, RQ – raw quinoa

3.2.2 Total saponins content

Saponins are naturally occurring glycosides in many plants, such as solanum and Alliums pp, oats, soya, clover and variety of herbs and seeds. Saponins are also considered as natural antimicrobial compounds which make part of plant's defence system [29]. Quinoa can be classified on the basis of its saponins content

which is dependent on the quinoa variety: 'sweet' (free from or containing < 0.11% of free saponins) or 'bitter' (containing > 0.11% of free saponins) as reported by [30].

The saponin content of germinated quinoa ranged from 1.90±0.04 mg/100 g to 6.24±0.01 mg/100 g. The increasing order of saponins content of quinoa was CGQ

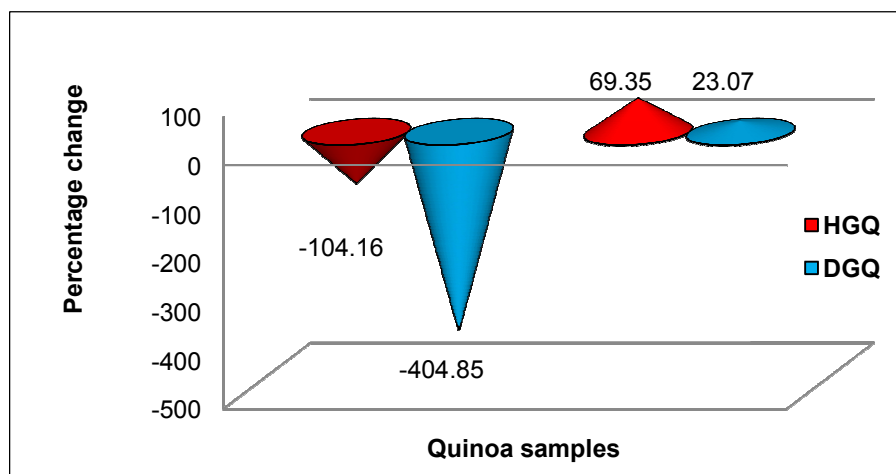


Fig. 2. Percent change in saponins content in methanol extracts of germinated quinoa

(1.90 ± 0.04 mg/100g) < DGQ (2.47 ± 0.01 mg/100 g) and HGQ (6.24 ± 0.01 mg/ 100 g). The values of the saponin content reported was significantly similar to the values reported by [24]. The saponin content in raw quinoa grain was 12.47 ± 0.08 mg/100 g (Table 5). Compared to the raw quinoa there was 104.16% reduction seen in HGQ, reduced to 404.85% in DGQ. But when compared to the commercial variety the saponin contents were with 23.07% (DGQ) and 69.35% (HGQ) more increased. This may be because of processing conditions of commercial variety (Fig. 2).

4. CONCLUSION

The total phenol and flavonoid contents of methanol and water extracts of HGQ were high compared to DGQ and CGQ. The oxalate content of germinated quinoa ranged from 6.17 ± 0.01 to 9.45 ± 0.02 mg/100 g. The saponin content of germinated quinoa ranged from 1.90 ± 0.04 to 6.24 ± 0.01 mg/100 g. When compared to the raw quinoa, there was a 104.16% reduction was seen in HGQ, whereas a 404.85% diminution was noticed in DGQ. But when compared to the commercial variety the saponin contents were 23.07% (DGQ) and 69.35% (HGQ) more. This may be because of processing conditions of commercial variety.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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