

# EFFECT OF SOAKING AND GERMINATION ON ANTINUTRITIONAL FACTORS OF QUINOA (*Chenopodium quinoa*)

## Abstract

Quinoa is a nutritional powerhouse, packed with protein, fiber, vitamins and minerals. However, it also contains certain compounds that impact nutrient availability. To address this, processing methods like soaking and germination have emerged as effective traditional treatments. They not only enhance the nutritional and bioactive potential but also diminish the anti-nutritional components in these grains, elevating their overall quality. This study examines the effect of soaking and germination treatments on anti-nutritional characteristics of quinoa. The result shows that an increase in germination percentages increases with longer germination times. Notably, seeds soaked in lukewarm water containing 2% salt for 24 hours showed the highest germination percentage (82.89%), while the number of non-germinated (16.27%) and abnormally germinated (0.32%) seeds decreased and greatest radical length (10.25 mm) when compared to seeds soaked in normal water. However, as the germination period extended, quinoa exhibited increased levels of vitamin C, notably more pronounced in lukewarm water with a 2% salt concentration. Additionally, germinated quinoa presents reduced levels of phytic acid (68.22 mg/100 g), tannin (0.36 mg/100 g), saponin (75.13 mg/100 g), and oxalates (42.55 mg/100 g) in comparison to raw quinoa. Hence, soaking and germination emerge as effective methods for reducing antinutritional components and enhancing the nutritional and bioactive potential of quinoa.

**Key words** - Quinoa, Germination properties, Antinutritional content, Vitamin C

## Introduction

Pseudocereals, being dicotyledonous and gluten-free, are frequently regarded as alternatives to true cereals. Quinoa (*Chenopodium quinoa*; family *Chenopodiaceae*) is a well-known pseudocereal that is widely utilized around the world. It grows in a wide range of climates and altitudes, from sea level to the top of Bolivia Altiplano plateau. Due to its wide genetic diversity, quinoa can adapt to a variety of harsh conditions, including highland and frost

(Jacobsen, 2003). It is one of the few crops that can grow in Southern Bolivia and Northern Chile on soil with a high saline level (Jacobsen, 1999). It is consumed as a staple food, similar to maize and potato. Quinoa was once a prominent staple food in the Andean region, but it has been mostly replaced by foods such as rice and pasta (Repo, 2003). Pseudocereals can be utilized like regular cereals to make value-added food products because of their high starch content (Thakur *et al.*, 2021). In recent times, pseudocereals have surged in popularity among consumers due to their exceptional nutritional quality.

Nowadays, quinoa is distinguished by its high protein content and quality, with a varied range of amino acids that are well-balanced and particularly high in lysine and methionine. It contains significant amounts of fiber, along with essential minerals like calcium and iron. Additionally, it is rich in antioxidants like polyphenols. Moreover, these grains are abundant in phenolic components, contributing to their extensive health benefits (Hirose *et al.*, 2010, Repo, 2011). In recent times, it has become a popular raw material in the diet of vegetarians. This pseudocereal has piqued the curiosity of researchers as a possible component in product formulations.

Despite being extremely nutritious, these grains have low bioavailability due to the presence of anti-nutritional components such as tannins and phytic acid, which bind with nutrients and render them inaccessible to our bodies. A variety of conventional processing procedures can be used to boost the bioavailability of many nutrients in these grains (Hotz and Gibson, 2007). Soaking and germination are the most regularly used processes and may be considered the simplest, most cost-effective, and most widely utilized strategies for improving nutritional quality and reducing anti-nutritional components in food grains.

Soaking is a domestic treatment used for the hydration of grains in water for a few hours. Thus, it is quite useful in decreasing and eliminating the anti-nutritional components present in the food grains (Kajihaua *et al.*, 2014). Numerous studies have described that soaking for 12–18 hrs is the most effective treatment to reduce the levels of anti-nutritional components such as proteolytic enzyme inhibitors as well as phytic acid, which are partly or wholly solubilized in soaked water (Embaby, 2010; Kajihaua *et al.*, 2014). Germination follows subsequently the soaking where the soaked seeds are kept in a moist environment until they get sprouted. It results in enhancing the bio-active potential, as well as the sensory characteristics (Kuo and Faber Taylor, 2004) of germinated grains, and reduces the anti-nutritional components at the same time. Various researchers have described that 24–48 hrs of germination can successfully result in the augmentation of nutritional properties and reducing the anti-nutritional factors of grains (Urbano *et al.*, 2005). Considering the beneficial effects of soaking and germination on

enhancing the nutritional and bioactive properties of processed grains, our study focuses on standardizing processing techniques encompassing diverse soaking treatments and germination methods for quinoa grains. Its primary goal is to evaluate their collective impact on mitigating anti-nutrient levels in the resultant quinoa flour. Implementing these quinoa processing procedures holds potential for increasing the usage of these grains, which are being underutilized.

## **MATERIAL AND METHODS**

**Location of the study** - University of Agricultural Sciences, GKVK, Bangalore.

### **Procurement of raw materials**

Quinoa was procured from Kilaru Naturals Private Limited Hyderabad, Telangana. Prior to analysis, the grains underwent a meticulous cleaning process was employed to remove any extraneous materials, followed by storage in a deep freezer for subsequent analysis. Additionally, all other necessary ingredients for the study were procured from the local market in Bangalore.

### **Standardization of time and temperature for germination of quinoa**

To conduct the germination tests, petri plates were thoroughly cleaned and rinsed with distilled water and then sterilized. Autoclaving the filter paper at 120 °C was undertaken to minimize the risk of contamination. After a thorough grading process, 100 seeds were manually counted to exclude any damaged or deformed seeds. These selected seeds were then soaked in 15 ml of distilled water for 6 hrs. Following the soaking period, the seeds were transferred to sterilized petri plates, each covered with filter paper, and placed in a dark environment at room temperature.

### **Experimental Design**

**The experiment was laid out in a Completely Randomised Design (CRD) with four treatment being as Main Factors: T1- Soaking in normal water, T2- Soaking in normal water with 2 % salt, T3- Soaking in normal lukewarm water and T4- Soaking in normal lukewarm water with 2 % salt.** The experiment involved three replications, each consisting of 100 seeds. At 4 hrs intervals, the quinoa seeds were examined for both physical characteristics and vitamin C content throughout the germination process.

### **Assessment of physical characteristics during germination**

Following a 4 hr interval, the counts of germinated **seeds** (GRS), abnormally germinated **seeds** (AGS) (characterized by cotyledon emergence before the radical), and non-germinated seeds (NGS) were recorded and expressed as a percentage relative to the

total seed count, following the methodology by Hager (2014). Furthermore, the length of the protruding radical was measured for 10 seeds from each Petri dish at each time point, utilizing a digital caliper.

### **Estimation of vitamin C**

Vitamin C (ascorbic acid) estimation was done by a titrimetric method using Iodate Solution (Ranganna, 1996).

### **Analysis of anti-nutritional factors of processed quinoa**

Assessing antinutritional factors in fermented quinoa flour is crucial for ensuring nutritional quality and safety. This evaluation informs the optimization of processing methods to minimize antinutritional factors, enhancing the overall nutritional profile of the final product and promoting consumer acceptance. Anti-nutrients viz., tannins, phytic acid, oxalates and saponins were estimated for the different processed methods, unprocessed grains and processed grains. The following method was adopted for estimation:

### **Estimation of Tannins**

One ml of extract was taken in 100 ml volumetric flask to which 3ml of Folin Denis Reagent (FDR) and 10 ml of Sodium carbonate were added. The contents were mixed and diluted to 100 ml using distilled water and allowed to stand for 30 mins and absorbance was measured at 760 nm. The tannins content of the samples was calculated as tannic acid equivalent from standard graph (Ranganna, 2003).

The tannin content of the samples was calculated as tannic acid equivalents from standard graph.

$$\text{Tannic acid (\%)} = \frac{\text{mg of tannic acid} \times \text{dilution} \times 100}{\text{Aliquot taken for the estimation} \times \text{weight of the sample}} \times 10$$

### **Estimation of phytic acid**

The phytic acid estimation was based on the principle that phytate is extracted with TCA (trichloroacetic acid) and precipitated as ferric salt. The iron content of the precipitate was determined calorimetrically and phytate phosphorous content calculated from this value assuming a constant 4Fe: 6P molecular ratio in the precipitate (Raghuramulu *et al.* (2003)).

$$\text{Phytic acid} = \frac{\% \text{Fe(NO}_3)_3 \times 15}{\text{Weight of the sample}}$$

### Estimation of oxalates

Oxalic acid was estimated according to the method of Raghuramulu *et al.* (2003)

$$\text{Oxalic acid} \frac{\text{mg}}{100\text{g}} = \text{Titre value} \times \text{N of KMnO}_4 \times \frac{0.45}{0.01} \times \text{dilution factor} \times \frac{100}{\text{wt of sample}}$$

### Estimation of saponin

The absorbance of the mixture was measured by spectrophotometer at wavelength of 544nm. Diosgenin was used as a reference standard (Hai *et al.*, 2012).

Saponin concentration was obtained from the standard graph.

$$\text{Saponin content} \frac{\text{mg}}{100} = \frac{\text{Absorbance of sample} \times \text{dilution factor} \times \text{gradient of graph}}{\text{weight of the sample}} \times 100$$

**Statistical Analysis** - Mean and standard deviation was calculated using the SPSS 16.0 software. One way ANOVA was used to test the significance of the data.

## Result and Discussion

### Germination properties of quinoa

The germination of quinoa is crucial for optimizing processing operations. A comprehensive understanding of quinoa's germination properties is essential for advancing novel food products and processes within the field of food science. Table 1 provides a comprehensive overview of the germination characteristics exhibited by quinoa grains. The results highlight a discernible correlation between the germination percentage of quinoa seeds and the duration of germination. As the germination time increases, there is a clear upward trend in germination percentages from 29.14 to 68.07 per cent for T1, 41.80 to 74.24 per cent for T2, 47.16 to 79.98 per cent for T3 and 52.33 to 82.89 per cent for T4, respectively. Notably, seeds soaked in normal lukewarm water with 2 per cent salt for 24 hrs demonstrated the highest germination percentage (82.89 %), while seeds soaked in normal water for the same duration exhibited the lowest germination percentage (68.07 %). Conversely, with the extension of germination time in all variations, there is a noticeable decrease in the count of non-germinated seeds. Particularly T4, which statistically displayed a lower percentage of non-germinated seeds at 16.27 per cent compared to T1 (31.84 %). However, the prolonged germination time led to an increase in the

occurrence of abnormally germinated seeds. In contrast, the frequency of abnormally germinated seeds was notably higher in seeds soaked in normal water for 24 hrs (0.43 %), whereas it was comparatively lower in seeds soaked in lukewarm water with 2 per cent salt for the same duration (0.32 %). This observation suggests that the latter condition may create a more conducive environment for achieving the highest germination capacity, emphasizing the potential influence of time and salt concentration on the germination process.

The observed improvement in germination may be attributed to soaking quinoa in salt water during germination and it enhances the germination process by eliminating bitter compounds such as saponins. This, in turn, improves osmotic adjustment, facilitating better water uptake. Additionally, the salt water soaking helps control microbial growth, neutralize inhibitory substances and stimulate enzyme activity, all of which contribute to more efficient nutrient breakdown and ultimately result in higher germination percentages. In a parallel study conducted by Panuccio *et al.* (2014) reported that soaking quinoa seeds in salt water can lead to an increased germination rate. This implies that a greater number of seeds are likely to sprout under such conditions.

Furthermore, a study indicated that quinoa seeds display increased tolerance to specific salts, including NaCl and KCl, throughout the germination process and initial seedling growth (Barbieri *et al.*, 2019). Contrastingly, findings from Gomez-Ramirez *et al.* (2017) reported that soaking quinoa seeds in salt water can modify the surface chemistry of the seeds, enhancing the germination process. They suggested that plasma activation could be a contributing factor to the improved germination of quinoa seeds under these conditions. As a result, salts during germination may trigger specific physiological and molecular responses in quinoa, ultimately resulting in an augmented germination rate.

**Table 1: Effect of germination properties of quinoa grains**

| Time (hr) | T1                              |                                 |                              | T2                              |                                 |                                | T3                              |                                 |                   | T4                              |                                 |                |
|-----------|---------------------------------|---------------------------------|------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------------|---------------------------------|-------------------|---------------------------------|---------------------------------|----------------|
|           | GRS                             | NGS                             | AGS                          | GRS                             | NGS                             | AGS                            | GRS                             | NGS                             | AGS               | GRS                             | NGS                             | AGS            |
| 4         | 29.14<br>±<br>0.17 <sup>f</sup> | 70.96<br>±<br>0.88 <sup>a</sup> | 0.28 ±<br>0.06 <sup>ab</sup> | 41.80<br>±<br>0.42 <sup>f</sup> | 57.92<br>±<br>0.46 <sup>a</sup> | 0.01<br>±<br>0.01 <sup>c</sup> | 47.16<br>±<br>0.17 <sup>f</sup> | 53.18<br>±<br>1.03 <sup>a</sup> | 0.00<br>±<br>0.00 | 52.33<br>±<br>0.67 <sup>f</sup> | 48.39<br>±<br>1.20 <sup>a</sup> | 0.00 ±<br>0.00 |
| 8         | 34.66<br>±<br>0.34 <sup>e</sup> | 65.81<br>±<br>0.42 <sup>b</sup> | 0.22 ±<br>0.09 <sup>b</sup>  | 49.33<br>±<br>0.57 <sup>e</sup> | 50.77<br>±<br>1.04 <sup>b</sup> | 0.31<br>±<br>0.01 <sup>b</sup> | 51.73<br>±0.54 <sup>e</sup>     | 28.96<br>±<br>0.78 <sup>d</sup> | 0.00<br>±<br>0.00 | 58.00<br>±<br>0.33 <sup>e</sup> | 41.67<br>±<br>0.87 <sup>b</sup> | 0.00 ±<br>0.00 |
| 12        | 43.55<br>±<br>0.51 <sup>d</sup> | 56.45<br>±<br>1.38 <sup>c</sup> | 0.33 ±<br>0.01 <sup>ab</sup> | 55.31<br>±<br>0.61 <sup>d</sup> | 43.79<br>±<br>0.55 <sup>c</sup> | 0.30<br>±<br>0.01 <sup>b</sup> | 58.84<br>±<br>0.65 <sup>d</sup> | 40.78<br>±<br>0.38 <sup>b</sup> | 0.00<br>±<br>0.00 | 64.05<br>±<br>1.00 <sup>d</sup> | 35.27<br>±<br>0.50 <sup>c</sup> | 0.00 ±<br>0.00 |
| 16        | 52.85<br>±<br>0.17 <sup>c</sup> | 47.10<br>±<br>0.77 <sup>d</sup> | 0.61 ±<br>0.05 <sup>a</sup>  | 62.09<br>±<br>0.88 <sup>c</sup> | 38.04<br>±<br>0.23 <sup>d</sup> | 0.61<br>±<br>0.05 <sup>a</sup> | 65.47<br>±<br>0.58 <sup>c</sup> | 33.84<br>±<br>0.59 <sup>c</sup> | 0.32<br>±<br>0.01 | 75.19<br>±<br>0.41 <sup>c</sup> | 26.25<br>±<br>0.79 <sup>d</sup> | 0.00 ±<br>0.00 |

|             |                                 |                                 |                              |                                 |                                 |                                |                                 |                                 |                                 |                                 |                                 |                              |
|-------------|---------------------------------|---------------------------------|------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|------------------------------|
|             |                                 |                                 |                              |                                 |                                 |                                |                                 |                                 | ab                              |                                 |                                 |                              |
| 20          | 58.59<br>±<br>0.57 <sup>b</sup> | 41.84<br>±<br>0.60 <sup>e</sup> | 0.51 ±<br>0.34 <sup>ab</sup> | 69.00<br>±<br>0.43 <sup>b</sup> | 31.75<br>±<br>0.88 <sup>e</sup> | 0.60<br>±<br>0.01 <sub>b</sub> | 72.98<br>±<br>0.12 <sup>b</sup> | 25.12<br>±<br>1.16 <sup>e</sup> | 0.33<br>±<br>0.05 <sub>ab</sub> | 80.90<br>±<br>0.93 <sup>b</sup> | 18.97<br>±<br>0.65 <sup>e</sup> | 0.34 ±<br>0.01 <sup>ab</sup> |
| 24          | 68.07<br>±<br>0.74 <sup>a</sup> | 31.86<br>±<br>0.18 <sup>f</sup> | 0.43 ±<br>0.29 <sup>ab</sup> | 74.24<br>±<br>0.40 <sup>a</sup> | 25.11<br>±<br>0.94 <sup>f</sup> | 0.31<br>±<br>0.01 <sub>b</sub> | 79.23<br>±<br>0.84 <sup>a</sup> | 18.13<br>±<br>1.04 <sup>f</sup> | 0.33<br>±<br>0.05 <sub>ab</sub> | 82.89<br>±<br>0.85 <sup>a</sup> | 16.27<br>±<br>0.48 <sup>f</sup> | 0.32 ±<br>0.01 <sup>ab</sup> |
| Mean        | 47.81                           | 52.34                           | 0.40                         | 58.63                           | 41.23                           | 0.36                           | 62.57                           | 33.33                           | 0.16                            | 68.89                           | 31.14                           | 0.11                         |
| F-<br>Value | *                               | *                               | *                            | *                               | *                               | *                              | *                               | *                               | *                               | *                               | *                               | *                            |
| SEm±        | 0.27                            | 0.46                            | 0.11                         | 0.34                            | 0.43                            | 0.01                           | 0.32                            | 0.50                            | 0.00                            | 0.43                            | 0.46                            | 0.01                         |
| CD @<br>5 % | 0.85                            | 1.44                            | 1.01                         | 1.05                            | 1.35                            | 0.02                           | 0.99                            | 1.57                            | 0.01                            | 1.35                            | 1.43                            | 0.02                         |

Note: T1- Soaking in normal water, T2- Soaking in normal water with 2 % salt, T3- Soaking in normal lukewarm water and T4- Soaking in normal lukewarm water with 2 % salt. GRS – Germinated seeds, NGS – Non germinated seeds, AGS – Abnormally germinated seeds Mean within the same column followed by common letter do not significantly differ at  $p \leq 0.05$ . NS- Non significant, \*Significant at 5 % level

### Length of germinated quinoa

Table 2 illustrates the progression in length of germinated quinoa grains. It demonstrates a steady increase in length as the germination period extends. Among the various soaking conditions, the samples immersed in lukewarm water with 2 per cent salt exhibited the greatest length (10.25 mm), followed by those in lukewarm water (9.22 mm), normal water with 2 per cent salt (8.20 mm) and the shortest length observed in samples soaked in normal water (7.09 mm). Additionally, the length of germinated quinoa grains consistently increases over time, displaying a noticeable positive correlation between longer germination periods and specific soaking conditions with grain length at each time interval (Fig.1).

This may be attributed to osmotic effect caused by saltwater solutions create, influencing seed water uptake by seeds. Simultaneously, soaking seeds in lukewarm water increases sprout length by creating an environment favorable for enzymatic activity. Germination related enzymes thrive within specific temperature ranges and lukewarm water often aligns closely with their optimal functioning temperature. Conversely, soaking seeds in lukewarm water increases sprout length due to the gentle warmth promoting enzymatic activity. Enzymes crucial for germination thrive within specific temperature ranges, and lukewarm water often aligns well with these ranges, boosting their activity. This enhanced enzymatic activity aids in breaking down stored nutrients and initiating faster growth.

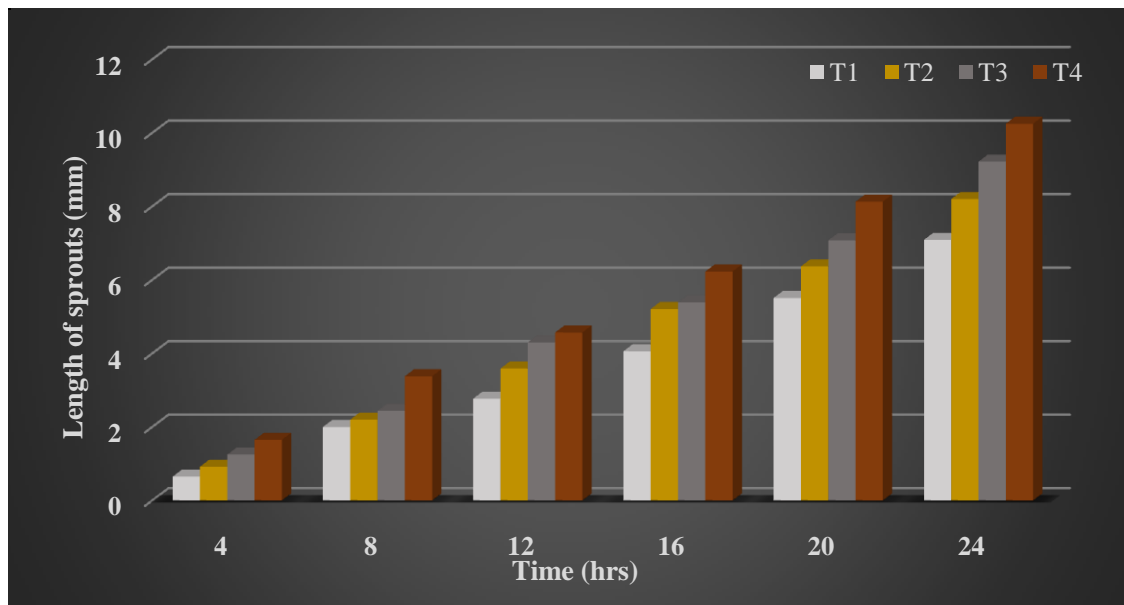
Additionally, lukewarm water assists in softening the seed coat, enhancing water penetration and activating germination related enzymes. This process facilitates hydration and

speeds up water uptake, particularly compared to soaking seeds in colder water. Furthermore, some seeds have innate dormancy mechanisms that prevent immediate germination even in ideal conditions. Soaking in lukewarm water can help overcome certain types of dormancy by signalling to the seed that conditions are suitable for germination, thus prompting the seed to start the germination process more readily. The results were on par with the study conducted by Adolf *et al.* (2013) found that soaking in salts water, increased the germination rate but not the germination percentages, compared to the control. The study also demonstrated that quinoa's tolerance to high salinity at the primary stages of seed germination is based on the presence of specific salts.

**Table 2: Length of germinated quinoa**

| Time (hr) | T1                       | T2                       | T3                       | T4                        |
|-----------|--------------------------|--------------------------|--------------------------|---------------------------|
|           | LS (mm)                  | LS (mm)                  | LS (mm)                  | LS (mm)                   |
| 4         | 0.65 ± 0.09 <sup>e</sup> | 0.92 ± 0.04 <sup>f</sup> | 1.25 ± 0.23 <sup>f</sup> | 1.65 ± 0.39 <sup>f</sup>  |
| 8         | 2.00 ± 0.20 <sup>d</sup> | 2.20 ± 0.31 <sup>e</sup> | 2.44 ± 0.49 <sup>e</sup> | 3.38 ± 0.45 <sup>e</sup>  |
| 12        | 2.77 ± 0.40 <sup>d</sup> | 3.59 ± 0.54 <sup>d</sup> | 4.33 ± 0.43 <sup>d</sup> | 4.37 ± 0.56 <sup>d</sup>  |
| 16        | 4.06 ± 0.92 <sup>c</sup> | 5.29 ± 0.34 <sup>c</sup> | 5.21 ± 0.42 <sup>c</sup> | 6.23 ± 0.15 <sup>c</sup>  |
| 20        | 5.51 ± 0.47 <sup>b</sup> | 6.37 ± 0.42 <sup>b</sup> | 7.08 ± 0.62 <sup>b</sup> | 8.13 ± 0.16 <sup>b</sup>  |
| 24        | 7.09 ± 0.12 <sup>a</sup> | 8.20 ± 0.33 <sup>a</sup> | 9.22 ± 0.29 <sup>a</sup> | 10.25 ± 0.30 <sup>a</sup> |
| Mean      | 3.68                     | 4.43                     | 4.92                     | 5.67                      |
| F-Value   | *                        | *                        | *                        | *                         |
| SEm±      | 0.27                     | 0.21                     | 0.25                     | 0.21                      |
| CD @ 5 %  | 0.83                     | 0.65                     | 0.78                     | 0.66                      |

Note: T1- Soaking in normal water, T2- Soaking in normal water with 2 % salt, T3- Soaking in normal lukewarm water and T4- Soaking in normal lukewarm water with 2 % salt. LS- Length of sprouts in milli meter. Mean within the same column followed by common letter do not significantly differ at  $p \leq 0.05$ . NS-Non significant, \*Significant at 5 % level.



**Fig. 1: Length of germinated quinoa**

Note: T1- Soaking in normal water, T2- Soaking in normal water with 2 % salt, T3- Soaking in normal lukewarm water and T4- Soaking in normal lukewarm water with 2 % salt. LS - Length of sprouts in milli meter.

### Vitamin C content of germinated quinoa

Table 3 illustrates the vitamin C content of germinated quinoa grain. The results showed a progressive increase in vitamin C levels as the germination period increases. Particularly, samples soaked in normal lukewarm water with a 2 per cent salt concentration exhibited an increased vitamin C content (22.20 mg) after a 24 hrs period (Fig.2). Conversely, samples soaked in normal water (15.38 mg) for the same duration exhibited relatively lower vitamin C levels. This may be due to observations that vitamin C synthesis occurred during germination, a result of the vitamin C biosynthesis within the seeds. This may be the reason for the continuous increase in vitamin C level at all the time periods. The findings were consistent with the study conducted by Srujana *et al.* (2017). The vitamin C content in unprocessed (raw) seeds was 0.38 mg/100 g. Throughout the germination process, there was a steady increase over several days, ultimately reaching a level nearly 16 times the initial content after 72 hrs.

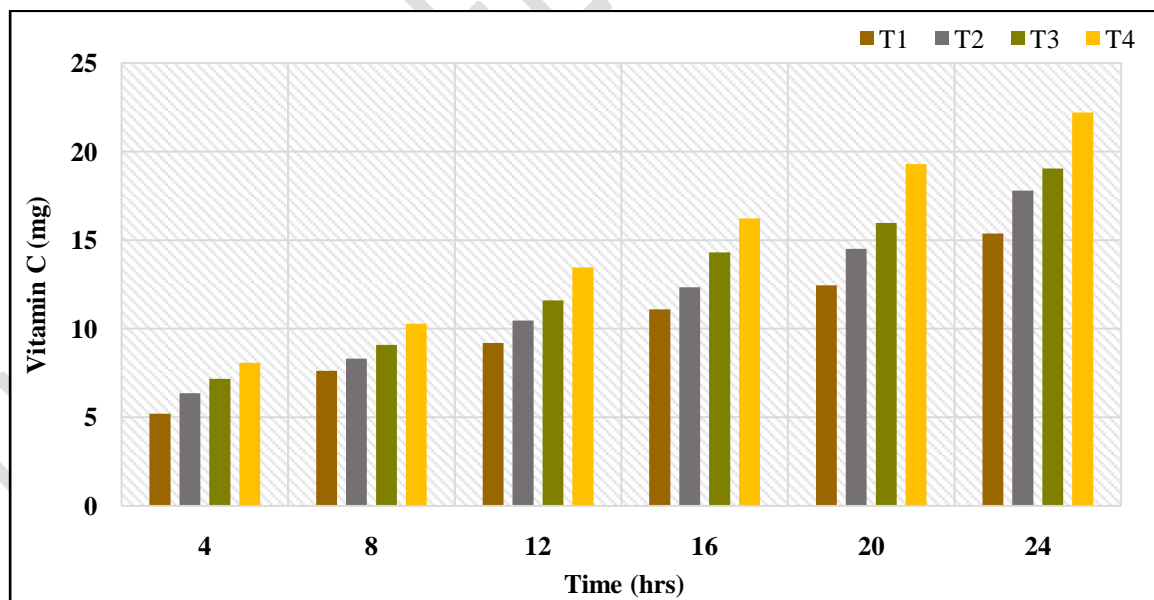
In accordance with the research conducted by Kaur and Tanwar., (2016), their findings are consistent with a study examining the impact of germination on the vitamin C content of quinoa. Result revealed that domestic processing of quinoa seeds, including the germination process, led to an increase in its vitamin C content. On the other hand, the results obtained diverged significantly from those reported by Hager *et al.* (2014). Their study indicated radical protrusion at distinct time intervals, specifically 5 hrs at 25 °C and 9 hrs at 5 °C, underscoring a

pronounced temperature-dependent effect. In summary, soaking quinoa in salt water can be potential to boost the germination rate, trigger antioxidant mechanisms, elongate protrusion and improve the nutritional profile of germinated quinoa, including an enhancement in vitamin C content.

**Table 3: Effect of germination on vitamin C content of quinoa**

| Time (hr) | T1                        | T2                        | T3                        | T4                        |
|-----------|---------------------------|---------------------------|---------------------------|---------------------------|
|           | VC (mg)                   | VC (mg)                   | VC (mg)                   | VC (mg)                   |
| 4         | 5.21 ± 0.23 <sup>d</sup>  | 6.35 ± 0.44 <sup>e</sup>  | 7.17 ± 0.30 <sup>f</sup>  | 8.08 ± 0.14 <sup>f</sup>  |
| 8         | 7.62 ± 0.55 <sup>c</sup>  | 8.31 ± 0.42 <sup>f</sup>  | 9.08 ± 0.13 <sup>e</sup>  | 10.28 ± 0.44 <sup>e</sup> |
| 12        | 9.02 ± 0.85 <sup>c</sup>  | 10.46 ± 0.77 <sup>d</sup> | 11.60 ± 0.55 <sup>d</sup> | 13.45 ± 0.67 <sup>d</sup> |
| 16        | 11.10 ± 0.42 <sup>b</sup> | 12.34 ± 1.00 <sup>c</sup> | 14.30 ± 0.67 <sup>c</sup> | 16.22 ± 0.36 <sup>c</sup> |
| 20        | 12.45 ± 1.28 <sup>b</sup> | 14.51 ± 0.49 <sup>b</sup> | 15.97 ± 0.93 <sup>b</sup> | 19.29 ± 0.47 <sup>b</sup> |
| 24        | 15.38 ± 1.12 <sup>a</sup> | 17.80 ± 0.35 <sup>a</sup> | 19.03 ± 0.72 <sup>a</sup> | 22.20 ± 0.39 <sup>a</sup> |
| Mean      | 10.13                     | 11.63                     | 12.86                     | 14.92                     |
| F-Value   | *                         | *                         | *                         | *                         |
| SEm±      | 0.48                      | 0.36                      | 0.35                      | 0.26                      |
| CD @ 5 %  | 1.49                      | 1.12                      | 1.10                      | 0.79                      |

Note: T1- Soaking in normal water, T2- Soaking in normal water with 2 % salt, T3- Soaking in normal lukewarm water and T4- Soaking in normal lukewarm water with 2 % salt. VC- Vitamin C in milligrams.



**Fig. 2: Effect of germination on vitamin C content of quinoa**

Note: T1- Soaking in normal water, T2- Soaking in normal water with 2 % salt, T3- Soaking in normal lukewarm water and T4- Soaking in normal lukewarm water with 2 % salt. VC-Vitamin C in milligrams.

**Anti nutrients content in germinated quinoa**

The levels of antinutrients (phytic acid, tannins, saponins, and oxalates) in raw and germinated quinoa flour are presented (Table 4). Statistically significant differences were observed between the processing variations. The findings indicated that raw sample exhibited the highest phytic acid content (142.47 mg/100 g) compared to the germinated sample (68.22 mg/ 100g) respectively. This is attributed to the activation of endogenous enzymes, such as phytase activity, which degrade antinutrients, including phytic acid. Similarly, Darwish *et al.* (2021) reported that during germination, the activity of phytase increases. Phytase catalyzes the enzymatic breakdown of phytic acid into inositol and phosphates, leading to a reduction in the overall concentration of phytic acid during the germination of quinoa compared to its raw quinoa.

The extent of phytate degradation ranged from 30 to 73 per cent of the initial content in raw quinoa, as observed after the fermentation of dry-roasted and milled quinoa grains (Castro-Alba *et al.*, 2019). In alignment with this, Maldonado-Alvarado *et al.* (2023) conducted a study reporting a significant increase in phytase activity across all varieties during germination. Concurrently, there was a significant reduction in phytic acid content, with levels decreasing by 32 to 74 per cent during the germination period.

Quinoa seeds exhibited notably lower levels of tannins in comparison to other cereals. The study results underscored this observation, indicating that the tannin content ranged from 0.77 mg/100 g in raw quinoa to a mere 0.36 mg/100 g in germinated quinoa. This finding is significant as it suggests that quinoa, even in its raw state, possesses relatively low tannin content and germination further contributes to a substantial reduction in these compounds. This occurs due to processes such as germination and saltwater soaking, where endogenous enzymes like tannase become activated, breaking down tannins. Moreover, the soaking water may promote ion exchange processes, causing the displacement of tannins from the quinoa.

Additionally, saltwater soaking assists in leaching tannins from the seeds. The synergy of these processes results in a significant reduction of tannin levels, thereby improving both the flavor and nutritional profile of quinoa. The findings align with the study conducted by Pritham *et al.* (2019) revealed that popping led to a decrease in tannin contents (0.026 mg/100 g). This reduction may be attributed to the salt pre-treatment, distinguishing it from germination and soaking processes, which resulted in higher tannin levels (0.423 and 0.846 mg/ 100 g, respectively). Likewise, the decline in tannin levels can be attributed to enzymatic transformations occurring during the germination phase of the seed (Megat Rusydi and Azrina, 2012).

It's noteworthy that, processing methods have a significant impact on reducing saponins in quinoa. According to the table, the highest reduction in tannins was observed in germinated quinoa flour (75.13 mg/100 g) compared to the raw quinoa flour (348.67 mg/100 g). This indicates that the process of germination is particularly effective in decreasing the saponin content in quinoa. The reduction in saponin levels is linked to the enzymatic breakdown of compounds and the leaching effect of saltwater, disrupting interactions and facilitating the removal of saponins that causes bitter taste. Specifically, saltwater soaking, especially when used as a pre-treatment, helps leach out water-soluble components, including saponins.

Saponins are amphipathic molecules, meaning they have both hydrophilic (water-attracting) and hydrophobic (water-repelling) parts. The salt in the water may disrupt the interactions that keep saponins in the quinoa, facilitating their removal into the soaking water. In a parallel study, Suarez-Estrella *et al.* (2020) similarly reported that mass spectrometer analysis revealed a reduction in saponin content during quinoa germination. Specifically, the content decreased from about 0.4 per cent after 12 hrs of sprouting to 0.05 per cent in the seeds.

Oxalates are compounds present in various plant-based foods, capable of forming crystals when combined with calcium. Recognized as a toxic substance, oxalate poses a significant health risk. The germination process was observed to contribute to the reduction of oxalates. The results indicate that oxalate content was at its lowest, 42.55 mg/100 g, during germination, while it was highest 128.16 mg/100 g, in raw quinoa flour. The observed reduction in oxalate content during the germination process of quinoa may be attributed to the use of saline water. This practice could potentially impact the germination and early seedling growth of quinoa, causing changes in the levels of primary metabolites and enzyme activity, which may contribute to the reduction of oxalates during the germination process (Panuccio *et al.*, 2014).

Likewise, a study conducted by Estrella *et al.* (2021) reported that the processes of soaking and boiling lead to a reduction in oxalates in quinoa, with the observed decrease ranging from 19 to 87 per cent. Therefore, the observed significant reduction in antinutrient levels during the germination process, in comparison to the antinutrient content in raw quinoa flour, indicates that the transformative effects of germination play a pivotal role in diminishing the concentration of these compounds. This, in turn, suggests a potential enhancement in the overall nutritional quality and bioavailability of quinoa, emphasizing the positive impact of germination on its nutritional profile.

**Table 4: Effect of processing on anti-nutrients in germinated quinoa (mg/100 g)**

| Treatments | Antinutrients |         |          |          |
|------------|---------------|---------|----------|----------|
|            | Phytic acid   | Tannins | Saponins | Oxalates |

|          |                            |                          |                            |                            |
|----------|----------------------------|--------------------------|----------------------------|----------------------------|
| RQF      | 142.47 ± 0.76 <sup>a</sup> | 0.77 ± 0.18 <sup>a</sup> | 348.67 ± 2.08 <sup>a</sup> | 128.16 ± 0.95 <sup>a</sup> |
| GQF      | 68.22 ± 0.42 <sup>b</sup>  | 0.36 ± 0.01 <sup>b</sup> | 75.13 ± 1.00 <sup>b</sup>  | 42.55 ± 0.59 <sup>b</sup>  |
| Mean     | 105.34                     | 0.36                     | 211.9                      | 85.35                      |
| F-Value  | *                          | *                        | *                          | *                          |
| SEm±     | 0.35                       | 0.07                     | 0.94                       | 0.46                       |
| CD @ 5 % | 1.42                       | 0.30                     | 3.80                       | 1.84                       |

Note: RQF- Raw Quinoa flour, GQF- Germinated quinoa flour, mean within the same column followed by common letter do not significantly differ at  $p \leq 0.05$ . NS-Non significant, \*-Significant at 5 % level

## CONCLUSION

Quinoa is a nutrient dense grain that contains fiber, protein, vitamins and minerals. Nevertheless, it includes substances that impact the availability of nutrients. Simple methods to enhance the nutritional value and sensory qualities of quinoa include soaking and germination, which don't require complicated equipment. In present study results showed that soaking in salt water with 2 per cent salt and germinating could increase in germination percentage, length of germination radical and vitamin C content, as well as a decrease in antinutritional content when compared to raw quinoa. Hence, employing processing techniques can significantly enhance the nutritional value and functional properties of these underutilized grains, making them an ideal dietary choice.

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