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# Efecto de la disponibilidad de agua y de azufre sobre las características agronómicas, actividad antioxidante y aminoácidos azufrados de quinoa (*Chenopodium quinoa* Willd.)

Tesis para optar al grado de Doctora en Ciencias de la Agronomía

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# RESUMEN

Una forma de afrontar los retos del cambio climático es conocer la capacidad de adaptación de los cultivos a diferentes ambientes edafoclimáticos. El objetivo de este estudio fue evaluar el efecto de la disponibilidad de agua y de azufre en las características agronómicas, actividad antioxidante y aminoácidos azufrados de la quinoa. El ensayo se realizó en macetas con sustrato de la serie Santa Bárbara, al aire libre durante 2019/20 y 2020/21. El diseño fue de bloques divididos de dos factores, disponibilidad de agua (WA) de 25%, 50% y 100% en la etapa de llenado de grano y disponibilidad de azufre (SA) en concentraciones de 8, 12 y 20 ppm en el cultivo.

En las características agronómicas y productivas no existió interacción significativa ($p>0,05$) entre los factores (WA*SA). En el análisis individual, la menor WA (25%) disminuyó significativamente ($p\leq0,05$) el rendimiento y la altura, mientras que, la mayor fertilización de azufre aumentó significativamente ($p\leq0,05$) la altura, la longitud de la panoja, peso mil semillas y el rendimiento. En antioxidantes, el contenido de fenoles totales (TPC) y flavonoides (TFC) presentaron diferencias significativas ($p\leq0,05$) entre los tratamientos de 100% y 25% WA. En tanto, SA ejerció diferencias significativas ($p\leq0,05$) entre 8 y 20 ppm en los ensayos de TPC y el poder antioxidante reductor del ión férrico (FRAP). La interacción WA*SA generó diferencias significativas ($p\leq0,05$) en los TFC y la capacidad antioxidante del radical oxígeno (ORAC) en la segunda temporada. Al correlacionar los resultados de cantidad y capacidad antioxidante, sólo en 2019/2020 existió una correlación válida de los resultados entre TPC y FRAP. En ambas temporadas, la interacción WA*SA no generó diferencias significativas ($p>0,05$) en las proteínas y en los aminoácidos azufrados. En proteínas, individualmente WA y SA, presentaron diferencias significativas ($p\leq0,05$) entre 25 y 100%, así como, entre 8 y 20 ppm, respectivamente. En los aminoácidos azufrados, metionina evidenció diferencias significativas ($p\leq0,05$) con 12 ppm de SA en la primera temporada.

# ABSTRACT

One way to face the challenges of climate change is to know the adaptation capacity of crops to different edaphoclimatic environments. The objective of this study was to evaluate the effect of water and sulfur availability on the agronomic characteristics, antioxidant activity and sulfur amino acids of quinoa. The test was carried out in pots with Santa Barbara series substrate, outdoors during 2019/20 and 2020/21. The design was a two-factor split block design, water availability (WA) of 25%, 50% and 100% in the grain filling stage and sulfur availability (SA) at concentrations of 8, 12 and 20 ppm in the crop.

For agronomic and productive characteristics, there was no significant interaction ($p>0.05$) between the factors (WA*SA). In the individual analysis, the lowest WA (25%) significantly decreased ($p\leq0.05$) the yield and height of the plant, while the highest sulfur fertilization significantly increased ($p\leq0.05$) the height, panicle length, thousand seed weight and yield. In antioxidants, the content of total phenols (TPC) and flavonoids (TFC) presented significant differences ($p\leq0.05$) between the 100% and 25% WA treatments. Meanwhile, SA exerted significant differences ($p\leq0.05$) between 8 and 20 ppm in the TPC and ferric ion reducing antioxidant power (FRAP) tests. The WA*SA interaction generated significant differences ($p\leq0.05$) in TFC and oxygen radical antioxidant capacity (ORAC) in the second season. When correlating the results of quantity and antioxidant capacity, only in 2019/2020 was there a valid correlation of the results between TPC and FRAP. In both seasons, the WA*SA interaction did not generate significant differences ($p>0.05$) in proteins and sulfur amino acids. In proteins, individually WA and SA, presented significant differences ($p\leq0.05$) between 25 and 100%, as well as between 8 and 20 ppm, respectively. In the sulfur amino acids, methionine showed significant differences ($p\leq0.05$) with 12 ppm of SA in the first season.

## CAPITULO 1:

### 1.1 Introducción

La producción agrícola enfrenta el reto de abastecer de alimentos a la población mundial. Sin embargo, la escasez hídrica y la falta de suelos productivos que imperan actualmente, generan un ambiente adverso, dificultando el cumplir con la creciente demanda. La falta de agua reduce el crecimiento, la calidad y la productividad de los cultivos, pudiendo llegar a reducir hasta en un 50% el rendimiento del grano (Moraga et al., 2022).

Entre los nutrientes vegetales, el azufre ha sido vinculado a la tolerancia de las condiciones desfavorables que enfrentan las plantas. Se reconoce como el cuarto nutriente más importante para el desarrollo y crecimiento, después del nitrógeno, fósforo y potasio (Prasad & Singh, 2018). Mundialmente los suelos agrícolas han evidenciado un déficit de azufre (Anjum et al., 2015), lo cual, se ha asociado al cambio de formulación de los fertilizantes, dado el impacto negativo en las emisiones atmosféricas descrito en la literatura.

La quinoa gracias a su variabilidad genética, puede crecer en suelos marginales y con condiciones ambientales adversas (Pereira et al., 2019) como son el frío (Hussain et al., 2018; Iqbal et al., 2018), la salinidad y la sequía (Bascuñán-Godoy et al., 2018; Manaa et al., 2019). La aclimatación de las plantas al déficit de agua se ha descrito como un proceso complejo y que involucra numerosos cambios, tanto a nivel fisiológico, como morfológico y bioquímico.

Disminuye el crecimiento de la planta, ocurren cambios en la expresión de los genes, se incrementan los niveles de ácido abscísico, se acumulan solutos y proteínas protectoras, además de aumentar el contenido de antioxidantes (García-Morales et al., 2013).

El atractivo nutricional de la quinoa radica en la alta calidad de sus proteínas además de contar con vitaminas, minerales y compuestos fenólicos, cuyos efectos promueven la salud (Dumshott et al., 2022).

La respuesta de la planta de quinoa ante la escasez de agua, así como, las consecuencias del déficit de azufre se encuentran descritas en la literatura. Sin embargo, no existen antecedentes de la interacción de estos factores y su efecto en conjunto, tanto a nivel productivo como su incidencia en los compuestos considerados relevantes nutricionalmente en la semilla como son las proteínas, aminoácidos y antioxidantes. Debido a lo anterior, el objetivo de este estudio fue evaluar el efecto de la disponibilidad de agua y de azufre sobre las características agronómicas, la actividad antioxidante y el contenido de aminoácidos azufrados en quinoa (*Chenopodium quinoa* Willd.) genotipo Regalona.

## 1.2 Hipótesis

La restricción en la disponibilidad de agua y de azufre en el cultivo de la quinoa, no afecta en forma negativa las características agronómicas de la planta, la actividad antioxidante y la cantidad de aminoácidos azufrados en las semillas producidas.

## 1.3 Objetivos

### 1.3.1 Objetivo general

Evaluar el efecto de la disponibilidad de agua y de azufre en el cultivo sobre las características agronómicas de la planta, actividad antioxidante y el contenido de aminoácidos azufrados en la semilla de quinoa (*Chenopodium quínoa* Willd.) genotipo Regalona.

### 1.3.2 Objetivos específicos

- Determinar las características agronómicas de la planta de quinoa al ser cultivada en condiciones de disponibilidad de agua y de azufre diferente.

- Analizar el contenido de compuestos fenólicos y capacidad antioxidante de las semillas de quinoa a las distintas condiciones de disponibilidad de agua y de azufre.

- Correlacionar la capacidad antioxidante con el contenido de compuestos fenólicos expresados en la semilla de quinoa expuesta a diferente disponibilidad de agua y de azufre.

- Cuantificar el contenido de proteínas y contenido de aminoácidos azufrados en las semillas de quinoa producidas con diferente disponibilidad de agua y de azufre en el cultivo.

## 1.4 Conclusión general

El aumento en la fertilización con azufre ejerce un efecto positivo sobre las características agronómicas, el rendimiento y la capacidad antioxidante según el poder de reducción antioxidante del ión férrico. Por su parte, el déficit hídrico disminuye la productividad de la planta, pero se incrementa el contenido fenólico en la semilla, tanto en fenoles totales como en flavonoides.

En ambas temporadas, al incrementar la disponibilidad de agua aumentó el contenido de proteínas en la semilla, similar efecto, se generó con la disponibilidad de azufre. En tanto, en los aminoácidos la concentración media (12 ppm) de azufre, ejerció un efecto negativo en el contenido de metionina y su precursor serina en la primera temporada. En la segunda temporada, este efecto lo ejerció sobre serina y prolina, más no sobre los aminoácidos azufrados.

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# **CAPÍTULO 2:** Content and antioxidant capacity of phenolic compounds in quinoa seed: A review

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## ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is an ancestral pseudocereal native to the Andean region of South America. Due to its wide genotypic diversity, it has been described as a species capable of adapting to different agroclimatic environments, which influence the composition of the seed. Quinoa is known for its high protein and essential amino acid content. It is also rich in vitamins, minerals, and phenolic compounds. This review summarizes the scientific information on the content of phenolic compounds and the in vitro antioxidant capacity of quinoa seeds determined by spectrophotometric methods. Discrepancies in the available data, resulting from the use of different experimental variables, have also been addressed. The data analysis identified a clear need for standardized methodology to produce comparable results. Regarding antioxidant potential, in vitro studies provided basic information, which is complemented by in vivo bioavailability studies. According to the information collected, future research is required to evaluate the effect of environmental stress, geographic aspects, and quinoa growing conditions on the antioxidant potential of the seed.

**Key words:** Antioxidant capacity, phenolic compounds, quinoa seeds, spectrophotometric methods.

# INTRODUCTION

Secondary metabolites (SM) are recognized as essential compounds, playing important roles in plant defense and adaptation (Jamwal et al., 2018). The production of SM depends on the development and physiological stage of the plant, constituting less than 1% of dry mass (DM) content (Thakur et al., 2019). Based on the biosynthetic pathways, SM can be grouped into terpenes, N-containing compounds, and phenolic compounds. The main synthesis routes of phenolic compounds are the malonic acid and shikimic acid pathways (Verma and Shukla, 2015; Kumar et al., 2021). Phenolic compounds in plants have a role in the uptake of reactive oxygen species (ROS), aiding the plant in recovering from oxidative stress (Iqbal et al., 2018). This type of stress occurs when there is an imbalance between ROS production and the antioxidant defense, resulting in damage and changes in the tissue environment (Tang and Tsao, 2017; Siddeeg et al., 2021).

Phenolic compounds have been associated with the prevention of various degenerative diseases caused by oxidative stress due to their antioxidant and/or modulating action on numerous enzymatic functions (Carciochi et al., 2015; Hemalatha et al., 2016). Their potential biological activity has been widely examined through in vitro assays and post-ingestion bioavailability studies (Pereira et al., 2020). However, various technical and conceptual limitations have been reported when evaluating biological activity through this type of assay (Schaich et al., 2015). In this sense, a study conducted by Pellegrini et al. (2017)

demonstrated that chemical identification or in vitro evaluations do not predict the potential effect on human health. Furthermore, Ferreira et al. (2017) mention that bioavailability is a complex process that involves the release of compounds from the food matrix, absorption, distribution, metabolism, and excretion.

Quinoa (*Chenopodium quinoa* Willd.) is a pseudoceral native to South America. In recent decades, around 193 SM have been identified in its seed, among these are phenolic acids, flavonoids, terpenoids, steroids and nitrogenous compounds (Lin et al., 2019). The protein content in quinoa seed is particularly high, varying between 13.8% and 16.5% on a dry basis (Navruz-Varli and Sanlier, 2016; Multari et al., 2018; Lan et al.,2023). It also contains 16 types of amino acids, nine of which are considered essential for humans (Escuredo et al., 2014; Filho et al., 2017), especially for the development and maintenance of various metabolic needs (Chito Trujillo et al., 2017). Quinoa seed is rich in B complex vitamins, vitamins E and C (Valcárcel-Yamani and Lannes, 2012; Tang and Tsao, 2017), and minerals such as Ca, Fe, Mg, Mn, P, K, Cu and Zn (Miranda et al., 2012; Filho et al., 2017; Ramzani et al., 2017; Contreras-Jiménez et al., 2019; Dakhili et al., 2019; Han et al., 2019a; Chaudhary et al., 2023). In addition, the lipid content of quinoa ranges between 4.0 and 7.6 g 100 $g^{-1}$ (Nowak et al., 2016), with linoleic acid (39.68–58.15%) being the most predominant compound, followed by oleic acid (13.57–25.98%), palmitic acid (6.47–13.73%) and α-linolenic acid (6.57–12.95%) (Chen et al., 2023). Palmitic acid is the predominant saturated fatty acid in quinoa, representing

10% of the total content (Lan et al., 2023).

This review summarizes the scientific information on the content of phenolic compounds and antioxidant capacity of quinoa seeds. It focuses on the available data obtained by in vitro analytical methods, also analyzing the factors that have led to discrepancies in the levels of compounds reported in the literature.

## Phenolic compounds

Phenolic compounds are a heterogeneous group of SM biosynthesized through the pathways of pentose phosphate, shikimate and phenylpropanoid (Mark et al., 2019; de Araújo et al., 2021). They are hydrophilic in nature, being mainly found in the quinoa seed coat (Tang and Tsao, 2017; Mufari et al., 2018). These compounds can be found in free, soluble conjugated (with sugars or other components of low molecular mass), and insoluble-bound forms. Most phenolic compounds in quinoa are present in the free form, ranging from 167.2 to 308.3 mg gallic acid equivalents 100 g$^{-1}$ dw (Tang and Tsao, 2017; Martínez-Villaluenga et al., 2020). Free phenolic acids are found in the vacuole of the plant cell (Zhang et al., 2020), while bound phenolic acids are attached to components such as cellulose, hemicellulose and proteins that constitute the cell wall (Multari et al., 2018), being linked by covalent bonds, hydrogen bonds and/or hydrophobic interactions (Zhang et al., 2020), i.e., they remain in the residue after the aqueous-organic extract. Some of these compounds are hydrolysable tannins like phenolic and hydroxycinnamic acids. In this case there are bound to carbohydrates and proteins, as well as macromolecules, such as condensed tannins (proanthocyanidins).

The antioxidant function of phenolic compounds lies in their ability to generate a defensive barrier against oxidative stress by inhibiting reactive oxygen species (ROS). This function that is attributed to the presence of hydroxyl groups attached to aromatic rings in their molecular structure (Buitrago et al., 2019). Phenolic compounds of natural sources have been classified as primary antioxidants because they directly inhibit oxidation reactions, unlike secondary antioxidants, which act indirectly (Amarowicz and Pegg, 2019). According to the biological potential, phenolic compounds have demonstrated activity both in vitro and in vivo against various diseases and metabolic conditions (Pellegrini et al., 2018).

Phenolic compounds are ingested as complex mixtures present in a food matrix (Bermúdez-Soto et al., 2007), and their bioactivity in humans depend on the processes of digestion, absorption, and metabolism (Pellegrini et al., 2017; Balakrishnan and Goodrich-Schneider, 2020; de Araújo et al., 2021). Bioavailability depends on factors such as compound concentration, chemical structure, conjugation with other compounds, molecular size, polymerization degree, and solubility (de Araújo et al., 2021). In vivo, soluble phenolic compounds in free and conjugated forms can be released into the digestive fluids of the stomach, with approximately 5%-10% absorbed in the small intestine. The remaining 90%-95% of phenolic compounds that are not absorbed, including soluble and insoluble compounds bound to the food matrix, continue their passage to the colon where they are subjected to digestive enzymes or interactions with the gut microbiota (Bommegowda and Singh, 2020; Zhang et al., 2020). The benefits of insoluble phenolic compounds depend on the type of food matrix and colonic fermentation, as well as the amount and type of compounds released, and their potential synergistic effects. In fact, phenols can act by exerting bioactive functions in the intestinal epithelium, endothelial cells, and systemic circulation, modulating inflammatory processes and mediating cell signaling pathways (Zhang et al., 2020).

Balakrishnan and Goodrich-Schneider (2020) examined the impact of in vitro gastrointestinal digestion on the bio accessibility of flavonoid compounds present in quinoa, reporting that seven of the eleven flavonoid compounds identified were found intact after digestion, with a significant increase in flavonoid concentration, which led to an approximately two-fold increase in antioxidant capacity. The authors suggested that efficient extraction by digestive enzymes could release compounds that are associated with health benefits and bound to other nutrients. Additionally, Han et al. (2019a) have indicated that binding phenolic compounds, when ingested, could survive the gastrointestinal digestion process, exerting protective effects within the colon.

One method commonly used to determine the total phenolic content (TPC) in foods is the spectrophotometric method developed in 1927, which uses the Folin-Ciocalteu (FC) reagent (Lester et al., 2012; Ou et al., 2019). This method has wide application in evaluating antioxidant extracts from various sources, including extracts from herbs, spices and fruits, cereals, and legumes, and plants (Gülçin, 2020). The principle of the method is based on the ability of phenolic compounds to reduce a mixture of phosphomolybdic acid/phosphotungstic acid, in an alkaline medium, to blue oxides of tungsten and molybdenum, respectively (Bridi et al., 2014). The resulting blue compounds exhibit maximum absorbance at 760 nm (Galili and Hovav, 2014). Under reaction conditions, the FC reagent can quantify oxidation of non-phenolic compounds as well as some inorganic substances, providing a high apparent result (Bridi et al., 2014; Pękal and Pyrzynska, 2014). Sugars (fructose and sucrose), ascorbic acid, aromatic amines, Fe (II), organic acids and sulfur dioxide can interfere with the measurement of TPC, therefore, it is crucial to remove these interfering substances for accurate determination (Lester et al., 2012). The TPC is commonly expressed as gallic acid equivalents per 100 g dry matter (mg GAE 100 g$^{-1}$ DM) or other phenolic compound equivalents, such as caffeic acid, catechin, chlorogenic acid, or ferulic acid equivalents.

Han et al. (2019a) studied the Chinese quinoa cv. Jinli-1, and reported levels of free phenolic compounds of 162.90 mg GAE 100 g$^{-1}$ DM, and lower levels of bound compounds of 37.50 mg GAE 100 g$^{-1}$ DM. Similar results were reported in red, white and black quinoa of Chinese and Peruvian origin, ranging from 124.73 to 173.23 mg GAE 100 g$^{-1}$ DM and from 42.42 to 143.47 mg GAE 100 g$^{-1}$ DM for free and bound forms, respectively (Han et al., 2019b). This agrees with Vega-Gálvez et al. (2018), who evaluated six Chilean quinoa cultivars and reported values of free fractions two to seven times higher than the bound fractions. Additionally, a study on colored quinoa from the Peruvian highlands indicated a TPC range of 250 to 792 mg GAE 100 $g^{-1}$ DM, with values that fluctuated between 128 and 452 mg GAE 100 $g^{-1}$ DM, and between 123 and 341 mg GAE 100 $g^{-1}$ DM for bound and free fractions, respectively (Abderrahim et al., 2015). Such differences cannot be attributed to a specific factor since the studies differ in type and origin of the quinoa cultivar, edaphoclimatic and crop conditions, and extraction methodologies (Table 1).

According to Abderrahim et al. (2015) and Valencia et al. (2017), ecotype is one of the factors that influences the concentration of TPC in quinoa. An ecotype is a group of cultivars delimited by morphological, distribution, agronomic and ecological criteria. However, other authors have suggested that environmental conditions, agricultural or cultivation management techniques, and genetic factors are also responsible for the differences observed in TPC in quinoa (Filho et al., 2017; Han et al., 2019b). Pellegrini et al. (2018) have noted that such differences could be associated with the extraction procedures used in the laboratory and the reactivity of the Folin reagent with the other non-phenolic compounds present in the sample, such as vitamins, amino acids, and proteins. In addition, Wianowska and Gil (2019) have attributed variations in TPC to the location of the metabolites in the sample, the type of interactions between metabolites and other compounds, as well as the type and concentration of interfering substances.

Kumar et al. (2021) have highlighted the importance of the extraction solvent, which depends on several factors such as the chemical nature of the compounds to be extracted, quantity and position of their hydroxyl groups, molecular size, solvent concentration, temperature, contact time, particle size, and mass-solvent ratio, among others. In quinoa, Luisetti et al. (2020) recommend using 39% ethanol, with a liquid/solid ratio of 31:1 and a temperature of 54 °C, identifying the percentage of alcohol as the main factor influencing extraction. Similarly, Carciochi et al. (2015) and Fiorito et al. (2019) agree on the use of ethanol as extraction solvent but differ in terms of concentration and temperature. They reported a phenolic contents of 102.86 mg GAE 100 $g^{-1}$ DM in quinoa seeds extracted with 80% (v/v) ethanol at 60 °C. In contrast, Hemalatha et al. (2016) reported that the highest extraction of phenolic compounds in quinoa was achieved using methanol (80%) acidified with 1% hydrochloric acid, with stirring in a thermoregulated bath at 50 °C for 3 h.

Abderrahim et al. (2015) have reported that using a mixture of sulfuric acid and methanol is efficient for extracting bound phenolic compounds in quinoa, yielding better results than methanol and hydrochloric acid. In addition, Han et al. (2019b) have highlighted the importance of estimating both bound and free phenolic compounds, otherwise TPC would be underestimated. The authors have also addressed the potential health benefits of phenolic compounds in food, as they are slowly released and continue to exert effects through the microflora in the colon. When studying bioavailability in humans, it has been found that the absorption of phenolic compounds from the diet is generally incredibly low, with plasma concentrations ranging from micromolar to nanomolar levels. The time required to reach maximum concentration varies between 30 min and several hours, depending on the site of absorption (Bermúdez-Soto et al., 2007).

**Phenolic acids.** Phenolic acids are represented by derivatives of benzoic and cinnamic acid, which consist of seven and nine carbon atoms, respectively (Teixeira et al., 2013). The benzoic acid derivatives are called phenolic acids and cinnamic acid derivatives are referred to as phenylpropanoids (Shahidi and Ambigaipalan, 2015). Benzoic acid derivatives include *p*-hydroxybenzoic, 3,4-dihydroxybenzoic, gallic, protocatechuic, vanillic, and syringic acids, while cinnamic acid derivatives include caffeic, *p*-coumaric, rosmarinic, ferulic, cinnamic, and chlorogenic acids (Gülçin, 2012). In quinoa, around 29 analogues of phenolic acid have been identified and, according to their structural characteristics, classified as benzoic acid analogues, which include benzoic, gallic, protocatechuic, syringic, vanillic acids, as well as cinnamic acid derivatives such as caffeic, chlorogenic, cinnamic, coumaric, ferulic, rosmarinic, sinapinic acids (Lin et al., 2019).

**Flavonoids.** Flavonoids are cyclized diphenylpropanes found in plant foods and plants (Shahidi and Ambigaipalan, 2015; Gülçin, 2020). These compounds accumulate in the apical meristem and the hypodermis of stems and leaves, while they are also found in pollen (Khalid et al., 2019). Flavonoids are derived from malonyl CoA and phenylalanine, synthesized in the cytosol, and stored in the vacuole or transported to other parts of the plant, where they function as bioactive molecules (Alseekh et al., 2020). The general structure of flavonoids is a fifteen-carbon skeleton with two benzene rings connected by a heterocyclic pyrene ring (Lin et al., 2019). The presence of functional groups in the rings and the presence of conjugated double bonds in the molecule structure enhances their antioxidant properties. Flavonoids exhibit a wide range of activity due to their functional versatility and structural diversity. In fact, flavonoids module ROS in plant tissues, regulate auxin transport and contribute to the coloration of flowers (Khalid et al., 2019). In quinoa seeds, flavonoids are the second most abundant phenolic compound (Martínez-Villaluenga et al., 2020). The main compounds reported are flavanols, quercetin (Gordillo-Bastidas et al., 2016; Multari et al., 2018), kaempferol and its glycosides (Alencar and Oliveira, 2018; Campos et al., 2018; Buitrago et al., 2019; Lin et al., 2019; Balakrishnan and Goodrich-Schneider, 2020; Pereira et al., 2020; Chaudhary et al., 2023), as well as myricetin (Pellegrini et al., 2018).

One way to determine the total flavonoid content (TFC) is by spectrophotometry using the aluminum chloride ($AlCl_3$) reagent. In fact, $AlCl_3$ forms stable acidic complexes with the C4 keto group, either the C3 or C5 hydroxyl groups, and the orthodihydroxyl groups on ring B (Chirinos et al., 2013). The addition of $AlCl_3$ solution is selective for flavones and flavonols (Pękal and Pyrzynska, 2014). In the case of quinoa, Han et al. (2019a) reported a TFC of 224.56 mg EC 100 $g^{-1}$ DM, corresponding to 147.95 and 76.61 free and bound flavonoids, respectively. A similar trend was observed in red, white, and black quinoa from China and Peru, with free flavonoids ranging from 96.09 to 174.44 mg EC 100 $g^{-1}$ DM, and bound flavonoids ranging from 43.26 to 151.27 mg EC 100 $g^{-1}$ DM (Han et al., 2019b).

Flavonoid content is strongly influenced by genotype, soil, environmental conditions, plant maturity, harvest, and postharvest conditions (Pellegrini et al., 2018). The determination of TFC depends on the extraction method, solvent composition, temperature, and nature of the plant matrix (Carciochi et al., 2015). The polarity of flavonoids varies depending on glycosides, isoprenoids, and substitution (aliphatic or aromatic), and allowing for the use of different solvents ranging from water to ethyl ether, for their extraction (Khalid et al., 2019).

In human metabolism, flavonoids are captured and absorbed mainly by the mucosa of the small intestine and later in the colon, where they are metabolized by the microbial flora. They function as prebiotics properties by promoting the growth of beneficial bacteria such as bifidobacteria and lactobacilli, thus influencing the composition of the intestinal flora (Hoensch and Oertel, 2015). Balakrishnan and Goodrich-Schneider (2020) reported a greater release of these compounds from the matrix under physiological conditions compared to those obtained in the chemical extraction process conducted with methanol in the laboratory. Seven of the eleven compounds identified were found intact after the in vitro digestion process. The availability of flavonoid compounds in the human intestinal tract can be negatively affected by gastric acids, enzymes, and bile salts (Liu et al., 2021). Additionally, flavonoids can exhibit both antioxidants and pro-oxidants activities in the gastrointestinal tract and cell culture systems (Gutteridge and Halliwell, 2010).

### Antioxidant activity

The antioxidant activity of foods depends on numerous factors such as concentration, temperature, oxygen tension as well as the presence of other antioxidants and nutrients (Miranda et al., 2010). Antioxidants exert their activities through one of seven mechanisms: Sequestering free radicals from the environment, chelating metallic ions, inhibiting enzymes that produce free radical, activating endogenous antioxidant enzymes, preventing lipid peroxidation; avoiding DNA damage, and inhibiting protein modification and sugar destruction. Phenolic compounds function through radical scavenging mechanisms, in which antioxidants donate an electron or a hydrogen atom from their hydroxyl, thus stabilizing the radical (Carocho et al., 2018).

**Evaluation of in vitro antioxidant activity:** An antioxidant is a substance that can significantly delay or prevent substrate oxidation, even at low concentrations compared to the oxidizable substrate (Carocho et al., 2018; Gülçin, 2020). Real samples often contain a mixture of antioxidant species; therefore, antioxidant character is considered as a collective property. The analysis results are often compared to the action of a species used for calibration (Kinyua Muthuri et al., 2021). The chemical diversity of natural antioxidants, along with the presence of glycosides and isomers in plant-based matrices, poses challenges in their separation and individual quantification (Çelik et al., 2010; Xiao, et al., 2020). In fact, these are influenced by reaction mechanisms, interactions between antioxidants and their biological role (Durazzo, 2017).

The terms “antioxidant activity” and “antioxidant capacity” are often used interchangeably. However, some authors prefer to introduce a distinction between antioxidant activity, the constant of a reaction occurring between an antioxidant and an oxidant (Gülçin, 2020), and antioxidant capacity, the concentration of free radicals scavenged (Siddeeg et al., 2021). Antioxidants are classified into primary and secondary antioxidants depending on their mechanism of action. Primary antioxidants can inhibit oxidation reactions, while secondary antioxidants act indirectly, i.e., they can react with pro-oxidants or be capable of eliminating oxygen. As primary antioxidants, phenolic compounds, act according to two mechanisms: hydrogen atom transfer (HAT) or single-electron transfer (SET) (Amarowicz and Pegg, 2019).

The HAT-based assays include various methods such as the total radical-trapping antioxidant parameter (TRAP) assay, oxygen radical absorbance capacity (ORAC) assay, cellular antioxidant activity (CAA) assay, β-carotene bleaching assay and thiobarbituric acid (TBA) assay (Xiao et al., 2020). While assays that involve SET-based reactions include Trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), inhibition of diphenyl-1-picrylhydrazyl radical (DPPH), cupric (or copper (II) ion) reducing antioxidant capacity (CUPRAC), and TPC using the Folin-Ciocalteu reagent (Prior, 2015). The main factors that determine the type of mechanism and efficacy of antioxidants are binding dissociation energy and ionization potential. Depending on the characteristics of the samples, in vitro assays use different extraction solutions such as ethanol, ethanol/water, acetone/water, methanol/water, acid methanol/water followed by acetone/water (Çelik et al., 2010).

In HAT-based assays using temperature, the antioxidant and the substrate compete for peroxyl radicals through the decomposition of azo compounds. On the other hand, SET-based assays measure the ability of an antioxidant to reduce an oxidant, resulting in a color change (Mishra et al., 2012; Zulueta et al., 2009). HAT-based reactions are described as rapid reactions, occurring within seconds or minutes. The high reactivity could be attributed to the presence of reducing agents (Prior et al., 2005). In contrast, SET-based reactions are slow and may require more time to reach completion (Siddeeg et al., 2021), so calculations are based on the decomposition rate of the product rather than kinetics. Interference from trace components, particularly metals, can lead to high variability, low reproducibility, and consistent results (Prior et al., 2005). Changes in absorbance in SET-based assays are compared to the concentration of a standard antioxidant used to produce a linear curve called the calibration curve. Therefore, the change in absorbance correlates with the concentration and capacity of the antioxidant (Siddeeg et al., 2021).

At the molecular level, the antioxidant potential of phenolic compounds depends on the number and arrangement of the hydroxyl groups present, binding site, and presence of other functional groups, such as double bonds and their conjugation with hydroxyl or ketone groups (Mishra et al., 2012; Schaich et al., 2015; Gülçin, 2020). When estimating antioxidant activity, various variables should be considered, including concentration, temperature, light level, substrate type, physical state of the system, and microcomponents that function as pro-oxidants or synergists (Gülçin, 2020). In quinoa seeds, antioxidant capacity has been determined using both HAT- and SET-based assays (Table 3).

It is important to point out that the comparability of results in the literature is limited due to variations in experimental conditions among different laboratories (Álvarez-Jubete et al., 2010). However, reference ranges can be established. By using extraction solvents with different polarities, Çelik et al. (2010) determined that it is possible to obtain significant differences in the results depending on the predominant assay mechanism. Zhang et al. (2020) addressed the need for an efficient extraction method that does not cause degradation to dissociate insoluble phenolic compounds covalently bound or trapped in the macromolecules of the food matrix through ester, ether, or C-C bonds or by hydrophobic interactions and hydrogen bonds. Various techniques, including acid, alkaline or enzymatic hydrolysis assisted by ultrasounds or microwaves, as well as solvent/liquid pressure extractions, can be used.

In the absence of an official standardized method to determine antioxidant capacity, Zulueta et al. (2009) have recommended the use of different oxidation conditions and quantification methodologies. Schaich et al. (2015) have also reported on the lack of standardization of the results, indicating that a time of 20 and 30 min may not allow for a stable reaction state, which generates an underestimation of radical scavenging activity for slow-reacting molecules (Mishra et al., 2012). When expressing results, de Menezes et al. (2021) suggest that, although the 50% inhibitory concentration ($IC_{50}$) can be easily determined, care must be taken to avoid errors in data interpretation and improve measurement reliability.

In quinoa, differences in antioxidant capacity have been related to genetic and agrotechnical factors, as well as environmental conditions (Miranda et al., 2011). When studying different varieties of quinoa, Fischer et al. (2013) attributed such differences to the interaction between genotype and environment. Ecotype and geographic location of the crop also play a role in antioxidant capacity (Reguera et al., 2018; Vega-Gálvez et al., 2018). However, it is important to note that in vitro antioxidant activity does not necessarily reflect the bioavailability and activity of antioxidants in biological systems, considering the high concentrations used in in vitro tests and the varied results from clinical trials (Prior, 2015; Amarowicz and Pegg, 2019). It should be noted that a bioactive compound is available for absorption once the gastrointestinal digestion process has elapsed (Pellegrini et al., 2017). In this sense, understanding the contribution mechanisms of phytochemicals to human nutrition and health, both individually and collectively, is crucial (Tang and Tsao, 2017).

Depending on the nature and combination of antioxidants, the mechanisms used by antioxidants in a food matrix vary from no interaction between bioactive compounds to concerted and synergistic actions or antagonistic interactions (Durazzo, 2017). In this context, the pH of the food environment, the structure and stability of antioxidants have been identified as key variables (Siddeeg et al., 2021). At the level of interactions, Świeca et al. (2014) reported that the joint action of proteins and phenolic compounds could affect both the antioxidant capacity and bioavailability of these compounds. Despite the beneficial effects of phenolic compounds on human health, they can function as pro-oxidants when ingested in high concentrations, resulting in toxicological effects (Shahidi and Ambigaipalan, 2015; de Araújo et al., 2021). Additionally, Gutteridge and Halliwell (2010) point out that excessive consumption of antioxidants may also affect endogenous production and the immune system.

Currently, consumers awareness regarding food quality and functionality, has led to the recognition of quinoa as a promising ingredient. Its nutritional composition and bioactive ingredients make it an attractive option to meet population requirements. In a study by Romano et al. (2020), it was demonstrated that appropriate technique like pray drying, can preserve the nutritional value, and improve the technological parameters of quinoa extracts, which could be used as functional ingredients to diversify the supply of products based on this pseudocereal.

## CONCLUSIONS

The scientific information summarized in this review provides evidence of the composition of quinoa seeds in terms of phenolic compounds and antioxidant capacity. The data correspond to the results obtained by spectrophotometric methods, with values expressed in the same unit of measurement. However, due to differences in geographic and genetic diversity as well as in analytical conditions, the results could not be analyzed comparatively. However, the analyzed information allowed establishing reference ranges and identifying the factors involved in the different methods used for compound determination. The reproducibility and reliability of the results could be improved by further limiting the experimental variables.

### Author contribution

Writing-original draft & editing: M.O. Review & Supervision: S.F. Methodology: C.F. Conceptualization: I.F., A.P. All co-authors reviewed the last version and approved the manuscript before submission.

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Table 1. Extraction solvents to determine total phenolic content in quinoa seeds by the Folin-Ciocalteu assay.

| Ecotype/cultivar/variety | Extraction solvent(s) | Unit | Results | Reference |
|---|---|---|---|---|
| Colored quinoa from the Peruvian highlands (13 types) | Methanol acidified with hydrochloric acid/water (50:50 v/v), acetone/water (70:30 v/v) and methanol/sulfuric acid (90:10 v/v) | mg GAE 100 $g^{-1}$ DM | 250-792 | Abderrahim et al. (2015) |
| Quinoa (cultivated in Bolivia) | Methanol | mg GAE 100 $g^{-1}$ DM | 71.7 | Álvarez-Jubete et al. (2010) |
| Quinoa (USA) | Aqueous methanol acidified with acetic acid; 4 M sodium hidroxide; 6 M hydrochloric acid; 2 M hydrochloric acid | mg GAE 100 $g^{-1}$ DM | 137 | Balakrishnan and Goodrich-Schneider (2020) |
| Greenhouse cultivated quinoa (Colombia) | Ethanol 96% (v/v) ultrasound assisted | mg GAE 100 $g^{-1}$ DM | 1500 (freeze-dried)<br>700 (air-dried) | Buitrago et al. (2019) |
| Quinoa variety Real (Argentina) | Ethanol (0, 40, 80% v/v) | mg GAE 100 $g^{-1}$ DM | 67.50-102.86 | Carciochi et al. (2015) |
| 28 varieties (Peru, USA, Bolivia, Denmark, Chile) | Methanol:water (4:1 v/v) | mg GAE 100 $g^{-1}$ DM | 269-589 | Chen et al. (2019) |
| Quinoa (Peru) | Hydrochloric acid (0.1%) and methanol:water (80:20 v/v) | mg GAE 100 $g^{-1}$ DM | 130 | Chirinos et al. (2013). |
| Quinoa pre-cooking (sweet from Ecuador, bitter from Peru) | Methanol:water 80:20 (v/v) | mg GAE 100 $g^{-1}$ DM | 772 (sweet)<br>864 (bitter) | Dini et al. (2010) |
| Quinoa (varieties Regalona, B080, AG2010 Chile) | Methanol 99% (v/v) | mg GAE 100 $g^{-1}$ DM | 330 (field)<br>580-640 (greenhouse) | Fischer et al. (2013) |
| Jinli-1 (China) | Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate | mg GAE 100 $g^{-1}$ DM | 200.40 | Han et al. (2019a) |
| Red, white and black quinoa (China and Peru) | Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate | mg GAE 100 $g^{-1}$ DM | 167.15-308.32 | Han et al. (2019b) |
| Quinoa variety Real (Argentina) | Ethanol (30, 50 and 70% v/v) | mg GAE 100 $g^{-1}$ DM | 179-229 | Luisetti et al. (2020) |

| | | | | |
|---|---|---|---|---|
| Ecotypes Ancovinto, Cancosa, Cahuil, Faro, Regalona and Villarrica (Chile) | Methanol (90%) + acetic acid (0.5%) | mg GA 100 g$^{-1}$ DM | 14.22-65.53 | Miranda et al. (2011) |
| Ecotypes Regalona and Villarrica (Chile) | Methanol (90%) + acetic acid (0.5%) | mg GA 100 g$^{-1}$ DM | 19.20 Regalona cultived in the north (Vicuña) and 12.39 in the south (Temuco)<br>31.92 Villarrica cultived in the north (Vicuña) and 22.87 in the south (Temuco) | Miranda et al. (2013) |
| Ecotypes Ancovinto, Cancosa, Cahuil, Faro, Regalona and Villarrica (Chile) | Ethanol 99% (v/v) | mg GA 100 g$^{-1}$ DM | 3.71-16.55 | Miranda et al. (2014) |
| BRS-Piabiru (Brazil) | Methanol | mg GAE 100 g$^{-1}$ DM | 97.60 | Nickel et al. (2016) |
| Quinoa | Ethanol 70% (v/v) | mg GAE 100 g$^{-1}$ DM | 14.50 (Korea)<br>15.33 (Peru)<br>16.28 (USA) | Park et al. (2017) |
| Quinoa (Bolivia) | Methanol, 0.16 M hydrochloric acid and water (8:1:1) | mg GAE 100 g$^{-1}$ DM | 375 | Paśko et al. (2009) |
| 24 quinoa accessions (Peru) | Ethanol:water (1:1) | mg GAE 100 g$^{-1}$ FM | 78.3-343.7 | Valencia et al. (2017) |
| Quinoa (Chile and M**e**xico) | Methanol (10%) | mg GAE 100 g$^{-1}$ FM | 319.1 (Chile)<br>180.4 (Mexico) | Vázquez-Luna et al. (2019) |
| Ecotypes (Chile) Ancovinto Cancosa Cahuil, Faro, Regalona, Villarrica | Acetone:water (4:1) | mg GAE 100 g$^{-1}$ DM | 97-164 | Vega-Gálvez et al. (2018) |

GAE: Gallic acid equivalents; GA: gallic acid; DM: dry matter; FM: fresh matter.

Table 2**.** Extraction solvents to determine total flavonoid content in quinoa seeds using colorimetric methods.

| Ecotype/cultivar/variety | Extraction solvent(s) | Unit | Results | Reference |
|---|---|---|---|---|
| Greenhouse cultivated quinoa (Colombia) | Ethanol 96% (v/v) ultrasound assisted | mg QE 100 $g^{-1}$ DM | 504 (freeze-dried)<br>154 (air-dried) | Buitrago et al. (2019) |
| Quinoa variety Real (Argentina) | Ethanol (0, 40, 80% v/v) | mg QE 100 $g^{-1}$ DM | 1.65-26.93 | Carciochi et al. (2015) |
| Quinoa (Peru) | Hydrochloric acid (0.1%) and methanol:water (80:20 v/v) | mg QE 100 $g^{-1}$ DM | 110 | Chirinos et al. (2013) |
| Quinoa pre-cooking (sweet from Ecuador, bitter from Peru) | Methanol:water (80:20 v/v) | mg CE 100 $g^{-1}$ DM | 139 (sweet)<br>81 (bitter) | Dini et al. (2010) |
| Jinli-1 (China) | Acetone 80% (v/v); 2 M methanol, 6 M sodium hydroxide; hydrochloric acid; ethyl acetate | mg CE 100 $g^{-1}$ DM | 224.56 | Han et al. (2019a) |
| Red, white and black quinoa (China and Peru) | Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate | mg CE 100 $g^{-1}$ DM | 139.35-307.11 | Han et al. (2019b) |
| Quinoa (India) | Methanol 80% (v/v) and HCl 1% | mg CE 100 $g^{-1}$ DM | 109 | Hemalatha et al. (2016) |
| Ecotypes Ancovinto, Cancosa, Cahuil, Faro, Regalona and Villarrica (Chile) | Ethanol 99% (v/v) | mg QE 100 $g^{-1}$ DM | 7.77-14.37 | Miranda et al. (2014) |
| Quinoa | Ethanol 70% (v/v) | mg QE 100 $g^{-1}$ DM | 20.91 (Korea);<br>13.24 (USA);<br>11.51 (Peru). | Park et al. (2017) |
| 24 quinoa accessions (Peru) | Ethanol:water (1:1) | mg CE 100 $g^{-1}$ FM | 19.9-102.9 | Valencia et al. (2017) |
| Quinoa (Chile and Mexico) | Methanol (10%) | mg QE 100 $g^{-1}$ FM | 70.1 (Chile)<br>25.4 (Mexico) | Vázquez-Luna et al (2019) |
| Ecotypes (Chile) Ancovinto Cancosa Cahuil, Faro, Regalona, Villarrica | Acetone:water (4:1) | mg CE 100 $g^{-1}$ DM | 109.4-211.1 | Vega-Gálvez et al. (2018) |

CE: Catechin equivalents; QE: quercetin equivalents; DM: dry matter; FM: fresh matter.

Table 3. Antioxidant capacity in quinoa seeds by DPPH, FRAP, ORAC, ABTS methods.

| Ecotype/cultivar/ variety/origin | Solvent(s) and extraction method | Method | Unit | Result | Reference |
|---|---|---|---|---|---|
| Quinoa (Bolivia) | Methanol | DPPH<br>FRAP | mg TE 100 g$^{-1}$ DM | 57.7<br>92.1 | Álvarez-Jubete et al. (2010) |
| 28 varieties (Peru, USA, Bolivia, Denmark, Chile) | Methanol:water (4:1 v/v) | DPPH | mg TE 100 g$^{-1}$ DM | 106-248 | Chen et al. (2019) |
| Quinoa (Peru) | Hydrochloric acid (0.1%) and methanol:water (80:20 v/v) | ABTS<br>DPPH<br>ORAC | µmol TE 100 g$^{-1}$ DM | 830<br>530<br>1590 | Chirinos et al. (2013) |
| Quinoa (sweet Ecuador, bitter Peru) | Methanol:water (80:20 v/v) | DPPH<br>FRAP | µmol TE 100 g$^{-1}$ DM | 287 (sweet)<br>671 (bitter)<br>256 (sweet)<br>873 (bitter) | Dini et al. (2010) |
| Quinoa (Regalona, B080, AG2010 Chile) | Methanol 99% (v/v) | DPPH<br>CUPRAC | mg GAE 100 g$^{-1}$ DM | 140-320 (field conditions)<br>480-610 (greenhouse)<br>140-170 (field conditions)<br>210-230 (greenhouse) | Fischer et al. (2013) |
| Jinli-1 (China) | Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate | FRAP<br>ORAC | mg TE 100 g$^{-1}$ DM | 196.43<br>1405 | Han et al. (2019a) |
| Red, white and black quinoa (China and Peru) | Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate | FRAP<br>ORAC | mg TE 100 g$^{-1}$ DM | 110.26-216.71<br>37.61-70.48 | Han et al. (2019b) |
| BRS-Piabiru (Brazil) | Methanol | FRAP<br>DPPH | mg TE 100 g$^{-1}$ DM | 15.25<br>30.34 | Nickel et al. (2016) |

| | | | | | |
|---|---|---|---|---|---|
| Quinoa | Ethanol 70% (v/v) | FRAP | mM Fe$^{2+}$ kg$^{-1}$ DM | 13.13 (Korea)<br>8.42 (USA)<br>7.12 (Peru) | Park et al. (2017) |
| Quinoa (Bolivia) | Methanol, 0.16 M hydrochloric acid and water (8:1:1) | ABTS<br>DPPH<br>FRAP | mmol TE kg$^{-1}$ DM<br>mmol TE kg$^{-1}$ DM<br>mmol Fe$^{2+}$ kg$^{-1}$ DM | 2719<br>3884<br>497 | Paśko et al. (2009) |
| Commercial Red, white and black quinoa (Spain, Bolivia, Peru) | Methanol 80% and acetone 70% (v/v) | ABTS<br>DPPH<br>FRAP | mg TE 100 g$^{-1}$ FM | 388-776<br>194-501<br>237-457 | Pellegrini et al. (2018) |
| 24 Quinoa accessions (Peru) | Ethanol:water (1:1) | ABTS<br>DPPH | µmol TE 100 g$^{-1}$ FM | 878.44-1507.94<br>486.08-1195.74 | Valencia et al. (2017) |
| | Methanol:water (2:1) | ABTS<br>DPPH | µmol TE 100 g$^{-1}$ FM | 1036.55-1835.78<br>474.21-972.87 | |
| Ecotypes (Chile) Ancovinto Cancosa Cahuil, Faro, Regalona, Villarrica | Acetone:water (4:1) | DPPH<br>ORAC | mmol TE 100 g$^{-1}$ DM | 10.74-20.17<br>22.25-73.16 | Vega-Gálvez et al. (2018) |

TE: Trolox equivalents; DM: dry matter; FM: fresh matter.

# **CAPÍTULO 3:** Effect of sulfur and water availability on the agronomic characteristics, yield, content and antioxidant capacity of quinoa

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## ABSTRACT

The challenge of feeding the world's population implies producing a greater quantity and better quality of food. Therefore, a deeper knowledge of the adaptation capacity of crops to different edaphoclimatic environments is required to face the challenges of climate change. The objective was to evaluate the effect of water and sulfur availability on agronomic characteristics, yield, total phenol content and antioxidant capacity in quinoa cv. Regalona.

The test was carried out in outdoor pots in the 2019/20 and 2020/21 seasons. The substrate was soil from the Santa Bárbara series. The design was a two-factor split block design, sulfur availability (SA) with 8, 12 and 20 ppm sulfur, and water availability (WA) of 25%, 50% and 100% in the grain filling stage.

Likewise, there was no significant interaction ($p>0.05$) between the factors (WA*SA) for the agronomic and productive characteristics. However, in the individual analysis, the lowest WA (25%) significantly decreased ($p\leq0.05$) the yield and height of the plant, while the highest sulfur fertilization increased significantly ($p\leq0.05$) plant height, panicle length, thousand seed weight and yield.

In antioxidants, treatments with 100% and 25% WA generated significant differences ($\alpha\leq0.05$) in total phenols (TPC) and flavonoids (TFC) content. Meanwhile, SA exerted significant differences ($p\leq0.05$) between 8 and 20 ppm in the TPC and ferric ion reducing antioxidant power (FRAP) tests. The interaction of the factors (WA*SA) generated significant differences ($\alpha\leq0.05$) in the flavonoid content and the oxygen radical absorbance capacity (ORAC) in the 2020/2021 season. The highest flavonoid content was with 25% AW and 12 ppm SA and the ORAC antioxidant capacity was with 100% WA and 12 ppm SA. At lower SA, the antioxidant capacity increased significantly ($\alpha\leq0.05$) according to the FRAP test. When correlating the data from the quantity and antioxidant capacity tests, only in the 2019/2020 season was there a valid correlation of the results between TPC and FRAP.

Keywords: sulfur deficiency, water availability, antioxidant capacity, quinoa.

# INTRODUCTION

Plants are exposed to environmental factors that affect bioavailability of essential nutrients as well as plant growth, development, and yield (Bouain et al., 2019; Sanaeifar et al., 2023). Drought, which is a period of below-normal water availability, can trigger physiological, biochemical, and molecular responses by plants (Iqbal et al., 2018; Sharma et al., 2022), with secondary metabolites being particularly important as they can act as osmoprotectants, osmolytes, antioxidants and/or exert protective functions in response to stress conditions. Additionally, water restriction negatively impacts the absorption of macronutrients such as nitrate, phosphate, and sulfate by plants, which produce metabolites to cope with this type of stress (Chan et al., 2013). Sulfur is an essential macronutrient for plants since it is part of a variety of chemical structures such as amino acids, membrane sulfolipids, cell walls, vitamins, pigments, phosphonucleotides, metabolites, and cofactors (Zhang et al., 2020). The storage of sulfur is limited, being mainly absorbed from the soil, plant or animal residues, or external nutrient supply (Prasad & Shivay, 2018; Zhang et al., 2020).

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal that belongs to the dicotyledonous class, Amaranthaceae family, Chenopodiaceae subfamily, and Chenopodium genus (Contreras et al., 2019). The species can adapt to adverse conditions due to its genetic variability (Pereira et al., 2019), including abiotic stress factors such as drought (Maestro-Gaitán et al., 2022). From a nutritional point of view, quinoa stands out for its high contents of essential amino acids, vitamins, minerals, and phenolic compounds (Ramzani et al., 2017). Phenols are bioactive compounds that act as antioxidants, which have health-promoting effects (Ren et al., 2022) and are associated with the prevention or reduction of the risk of diseases such as cancer, cardiovascular and inflammatory diseases (Gómez-Caravaca et al., 2014), diabetes, obesity, and neurodegenerative diseases (Pereira et al., 2020).

The effect of drought on quinoa has been described in the literature, but the response of quinoa plants to induced water restriction in sulfur-deficient soils has not been studied yet. The objective of this study was to evaluate the effect of sulfur and water availability on the agronomic characteristics, yield, content, and antioxidant capacity of quinoa seed (*Chenopodium quinoa* Willd.) cv. Regalona.

## MATERIALS AND METHODS

### Experimental conditions

The experiment was carried out outdoors in pots during the 2019/20 and 2020/21 seasons at the El Nogal Experimental Station (36°35'56.5"S, 72°05'02.8"W), Faculty of Agronomy, University of Concepción, Chile. The site is characterized by a temperate Mediterranean climate, with an average temperature of 13.1ºC (Fischer et al., 2013). The meteorological parameters between sowing and harvest of quinoa were obtained from the

Agrometeorological Station of the University of Concepción (36° 35.715'S, 72° 4.794'W) (Table 1).

## Experimental design

The experiment was carried out in a split-block design with two factors (sulfur and water availability) and three levels per factor. Sulfur availability (SA) of 8,12 and 20 ppm were applied in the crop, adjusting the content in two different plant development stages. Water availability (WA) was adjusted when 50% of the seeds were in the grain filling stage, with levels of 25%, 50% and 100% of available water at field capacity (Fischer et al., 2017). The experimental unit consisted of four pots of 30 cm in diameter and 50 cm in depth. The substrate used in each pot corresponded a soil the Santa Bárbara series with 30.8% sand and 54.8% silt and y 15.2% clay, classified as medial, amorphic, Typic Haploxerands (Stolpe, 2005), with a porosity of 72.4 %.

## Agronomic conditions

Five quinoa seeds genotype cv. Regalona were sown per pot. Seedling were thinned when they had four true leaves, leaving one plant per pot. Fertilization applied before and during cultivation was estimated according to the initial soil analysis (Table 2). At sowing, 1.56 g of triple superphosphate pot$^{-1}$ (80 kg of $P_2O_5$ ha$^{-1}$) and 0.78 g of urea pot$^{-1}$ (40 kg of N ha$^{-1}$) were added. Subsequently, a second dose of 1.17 g urea pot$^{-1}$ (60 kg of N ha$^{-1}$) was added to all the treatments at the beginning of flowering. When the plant developed two true leaves, two different fertilizers were applied, Ecosul ® and Sulpomag ® as a source of potassium and sulfur (S); the latter also contains magnesium. Doses of 1.60 g pot$^{-1}$ of Sulpomag and 0.60 g pot$^{-1}$ of Ecosul were applied to the 8 and 12 ppm S treatments, whereas respective doses of 2.65 g pot$^{-1}$ and 0.20 g pot$^{-1}$ were applied of the treatment of 20 ppm S.

To prevent the incidence of mildew (*Peronospora variabilis*), a common fungus in quinoa crops, the fungicide Mancozeb 80 WP (1.4 g L$^{-1}$) was applied in two different plant development stages: when the plant had three true leaves and a week after the first application.

Weed control and irrigation were carried out manually. From sowing to grain filling, water availability was maintained at 100% field capacity, with water restriction beginning 85 days from sowing in the 2019/20 season and 93 days from sowing in the 2020/21 season, which corresponds to the period when the grain presents a milky-pasty consistency. Water doses to be added were determined using three analog capacitive sensors per block, placed 8 cm deep from the surface of the pot and, vertically, 5 cm from the plant stem.

### Evaluations

Agronomic and yield parameters: the following determinations were made in the two evaluated seasons (2019/20 and 2020/21):

- Days to flowering: the period from emergence to 50% flowering of quinoa plants.
- Days to grain filling: the period from emergence to physiological maturity, in which the grain presents resistance to penetration when pressed by the nails.

- Days to maturity: period between emergence and physiological maturity, when 50% of the panicles have a brown color.
- Plant height (cm) and panicle length (cm): plant height was measured from the base of the stem to the apex of the inflorescence on the main stem; and length of the main panicle of each plant was determined at harvest using a tape measure.
- Seed yield (g): quinoa plants were cut and placed in a paper bag and dried in the open air for ten days to homogenize humidity. Relative humidity reached 43%, while average maximum and minimum temperatures were 30.3ºC and 11.5ºC, respectively. Subsequently, the panicles were threshed in a stationary thresher (Baldor 1425 rpm), and the seeds obtained were dry cleaned at a speed of 21 m $s^{-1}$. Yield was obtained by determining the mass (g) of the seeds per plant in each of the treatments.
- One thousand seed weight (g): the average mass in a sample of 500 seeds per experimental unit was obtained and multiplied by two.

In vitro antioxidant activity of the extracts: total phenolic content (TPC) and total flavonoids content (TFC) were determined. Antioxidant activity was assessed by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays. A Synergy HTX multi-mode microplate reader and Gen5 software were used in all the assays.

Preparation of the extracts: 5 g of ground seeds (Laboratory Mill 3100 Perten) were sieved through a 1 mm sieve (U.S. standard sieve N°18) and mixed with

50 mL of the extraction solution (70% v/v acetone acidified to 0.5% v/v glacial acetic acid) in a vortex shaker (Boeco, Germany) at 1600 rpm for 3 minutes at room temperature. The mixture was placed in a thermoregulated bath (Lauda, Germany) at 20°C for one hour, and then centrifuged (Boeco M-24 A, Germany) at 3000 rpm for 10 minutes at 22°C. The supernatant was collected, and the residue was subjected to extraction again. Both supernatants were combined in an amber bottle and refrigerated until analysis.

- Total phenolic compounds were determined according to the methodology of Singleton et al. (1999), with some modifications. The results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of dry matter (DM).
- Total flavonoid (flavones and flavonols) content (TFC) was calculated using the aluminum chloride colorimetric method according to Lin & Tang (2007), with some modifications. The results were expressed as milligrams of quercetin equivalents (mg QE) per 100 g of dry matter (DM).
- The ferric reducing antioxidant power (FRAP) assay was applied according to the method of Ou et al. (2002), with some modifications. FRAP values were calculated using a $FeSO_4$ calibration curve and the results were expressed as micromoles of iron (µmol $Fe^{2+}$) per 100 g of dry matter (DM).
- The oxygen radical absorbance capacity (ORAC) assay was performed according to the methodology of Wu et al. (2004), with some modifications. The results were expressed as micromoles of Trolox equivalents (µmol TE) per 100 g of dry matter (DM).

### Statistical analysis

Data were analyzed normality and homoscedasticity using the Shapiro-Wilk and Bartlett tests, respectively. Subsequently, the results of each trial were subjected to an analysis of variance and means were compared by the Least Significant Difference test (α=0.05). All statistical analyses were performed using SAS University Edition software (2016), including the correlation analysis between the assays of TPC and antioxidant capacity.

## RESULTS AND DISCUSSION

### Agronomic and yield parameters

The phenology of quinoa plants genotype cv. Regalona varied between the evaluated seasons (Table 3), recording 43 and 48 days from emergence to flowering, 85 and 93 days from emergence to grain filling, and 110 and 121 days from emergence to maturity for the 2019/20 and 2020/21 seasons, respectively. These differences could be explained by temperature variation between the seasons, with maximum and minimum temperatures in the range of 17.2 - 34.1ºC and 3.0 - 14.8ºC in the 2019/20 season, respectively; and 16.5 - 31.2ºC and 2.6 - 14.4ºC in the 2020/21 season, respectively. The life cycle span observed in the present study is shorter than that observed by Voronov et al. (2023), who reported 135 - 140 days when studying ten commercial accessions of quinoa grown in the United States, Peru, Denmark, and Chile. Furthermore, De Santis et al. (2016) reported several 61 days from emergence and 50%

flowering in a study on quinoa cv. Regalona grown in a clay loamy soil, while Gámez et al. (2019) reported 86 and 111 days from sowing and grain filling for quinoa plants Rainbow (coastal zone of Chile) and Illpa (highlands of Chile), respectively. Difference between the results obtained in the present work and those of the previously mentioned studies could be attributed to edaphoclimatic conditions as well as the effects of nutrient deficiency and water restriction on quinoa plants. In this regard, Maestro-Gaitán et al. (2022) have described that the phenology of quinoa is affected by water stress, resulting in advanced flowering and a shorter grain filling stage.

Plant height reached higher values in the 2019/20 season, ranging from 97.1 to 114.9 cm in the SA treatments (Table 4) and from 100.4 and 110.7 cm in the WA treatments (Table 5), whereas values in both treatments fluctuated between 70.8 and 82.3 cm and between 70.2 and 80.5 cm in the 2019/20 and 2020/21 seasons, respectively. This situation could be explained by differences in rainfall levels between the seasons, reaching respective levels of 25.5 and 6.9 mm. It is important to note that the plants were covered to prevent exposure to the rainfall event (89.1 mm) in January 2021.

Regarding panicle length, values ranging between 12.1 and 16.0 cm and between 12.6 and 14.8 cm were observed in the 2019/20 season in the SA and WA experiments, respectively. However, higher values ranging from 14.4 to 18.1 cm and from 15.9 to 16.3 cm were observed in the following season for both factors, respectively (Table 4 and 5). A study of Reguera et al. (2018) reported a value of 17 cm for cv. Regalona, which agrees with the results obtained herein for the treatment of 100% WA and 20 ppm SA.

With respect one thousand seed weight, values varied between 2.5 and 2.7 g in both the SA and WA trials in the 2019/20 season. However, differences were observed between the two factors in the 2020/21 season, with values ranging from 2.5 to 2.9 g and from 2.5 to 2.8 g for the two factors, respectively (Table 4 and 5). These results are close to those obtained by Voronov et al. (2023), who reported a range of 1.6 – 3.0 g for this pseudocereal. Similarly, Bhargaba et al. (2007) studied 29 lines of *Chenopodium* from different geographical areas (Peru, Mexico, Bolivia, Bolivia, Argentina, and Chile) and reported values ranging from 0.78 to 4.09 g for this parameter.

Yield per plant varied between 21.2 and 29.1 g and between 19.9 and 28.9 g in the 2019/20 season for the SA and WA trials, respectively, while values ranged from 9.8 to 14.6 g and from 10.4 to 13.6 g per plant in the 2020/21 season. These results are lower than those reported by Reguera et al. (2018), who obtained yields between 26.5 and 54.1 g per plant. This can be attributed to the restriction levels established in this study.

In both seasons, the sulfur dose of 8 ppm showed a negative and significant effect ($p \leq 0.05$) on plant height, panicle length, yield, and one thousand seed weight compared to treatment of 20 ppm (Table 4). The negative effect observed on plant growth and yield agrees with the findings of Cao et al. (2023) and Prasad & Shivay (2018), who reported that a sulfur level of 10 mg kg$^{-1}$ is considered as the critical limit to avoid affecting these parameters, as well as the quality of the crop and the defense mechanisms against the abiotic stress.

WA had a significant effect ($p \leq 0.05$) on crop yield and plant height at 25% and 100% WA in both seasons (Table 5). In this sense, Aziz et al. (2018) found that biomass decreased as water deficit increased. In the 2020/21 season, there was a significant effect ($p \leq 0.05$) of WA on one thousand seed weight at 25% WA compared to the 50% and 100% WA treatments. In this sense, a study conducted by Ruiz et al. (2013) revealed that up to 50% water deficit due to drought has no effect on quinoa yield, which coincides with the findings of the present study. Furthermore, Geerts et al. (2008) recommends planned deficit irrigation, arguing that the use of half of the water required by quinoa plants stabilizes yield and reduces the incidence of fungal attacks, generating a beneficial effect when fertilization is limited. In contrast, Gámez et al. (2019) evaluated plants Rainbow and Illpa with irrigation levels of 100%, 50% and 20% of total field capacity in the grain filling stage and found no differences in yield.

The interaction between the factors did not show an effect on the agronomic and productive characteristics.

## Total phenolic content, flavonoid content and in vitro antioxidant capacity

When analyzing the sulfur content factor, TPC values per 100 g DM were in the range of 129.0 - 147.2 mg GAE and 158.3 - 163.3 mg GAE for the 2019/20 and 2020/21 seasons, respectively (Table 6). In addition, significant differences ($p \leq 0.05$) were found between the sulfur levels of 8 and 20 ppm S. Regarding the water availability factor, TPC per 100 g DM fluctuated between 135.5 and 145.5 mg GAE and between 153.4 and 170.4 mg GAE for the 2019/20 and 2020/21 seasons, respectively. Furthermore, there were significant differences ($p \leq 0.05$) between the 25% and 100% WA treatments in both seasons. These results coincide with Aziz et al. (2018), who reported a significant increase in the TPC of quinoa plants under drought conditions, but differences were not consistent for all the treatments. Similarly, Fischer et al. (2013) conducted a study on quinoa plants subjected to water stress, reporting TPC per 100 g DM values ranging between 330 and 380 mg GAE using a similar methodology for polyphenol quantification and 99% methanol as the extraction solvent. This could explain the lower TPC values obtained herein, using 70% acetone for the extraction. In this sense, Celik et al. (2010) found that TPC is influenced by the type of solvent, polarity, reaction mechanism, solubility parameters and electron transfer capacity. Furthermore, by using a similar extraction solvent and methodology in the determination of TPC, Vega-Gálvez et al. (2018) reported per 100 g DM a range of 97 - 164 mg GAE for six ecotypes of quinoa, being close to the values determined in this study. In this case, the differences could be related to genotype and cultivation conditions as described by Chaudhary et al. (2023).

Filho et al. (2017) have described that the method based on the Folin Ciocalteau reagent has the limitation of including other compounds such as ascorbic acid, phytic acid, tocopherols, sterols, carotenoids and ecdysteroids, which, without being phenolic compounds, have antioxidant activity.

In the SA experiment, the total flavonoid content (TFC), specifically flavones and total flavonols, per 100 g DM was in the range of 12.4 - 12.8 mg QE and 10.9 - 12.8 mg QE in the 2019/20 and 2020/21 seasons, respectively. Meanwhile, in the WA trial, per 100 g DM ranges of 12.3 - 13.0 mg QE and 11.8 - 12.4 mg QE were observed in the 2019/20 and 2020/21 seasons, respectively (Table 6). The number of flavonoids represents a small portion of the TFC, which agrees with Tang et al. (2015). Additionally, the values obtained in the present study agree with those of Carciochi et al. (2015), who reported values per 100 g DM of 1.65 - 26.93 mg QE. It should be noted that the 25% and 100% WA treatments showed a significant effect ($p \leq 0.05$) on the flavonoid content in both seasons (Table 7). Likewise, lower water availability tended to result in a greater flavonoid content.

No differences were found ($p > 0.05$) in terms of TFC when water availability at the three levels interacted with the sulfur level of 20 ppm. The interaction between the factors only generated significant differences between in flavonoids content 2020/2021 season. The highest flavonoid content was obtained with the treatment of 25% WA and 12 ppm SA (Table 8).

The results of the FRAP assay, SA resulted in values per 100 g DM of 952.3 - 1089.4 µmol $Fe^{2+}$ and 911.50 - 1010.83 µmol $Fe^{2+}$ in the 2019/20 and 2020/21 seasons, respectively. In both seasons, this factor decreased the FRAP values, with differences ($p \leq 0.05$) between the sulfur treatments of 8 and 20 ppm. Regarding the WA factor, per 100 g DM values ranged from 975.5 to 1040.7

µmol $Fe^{2+}$ and from 927.1 to 1015.3 µmol $Fe^{2+}$ for the 2019/20 and 2020/21 seasons, respectively (Tables 6 and 7). The FRAP values obtained in the present study are higher than the value per 100 g DM of 750 µmol $Fe^{2+}$ reported by Reguera et al. (2018) for quinoa. In this sense, it should be noted that these values represent only an indicator of the total antioxidant power, with no preventive effect in terms of antioxidant capacity as described by Ou et al. (2002).

Regarding the ORAC assay, the sulfur content treatments per 100 g recorded values of 58731.6 – 63220.0 µmol TE and 64036.2 - 68956.6 µmol TE for the first and second seasons, respectively. In both seasons, the treatment of 12 ppm SA presented significant differences ($p \leq 0.05$) with respect to the level of 20 ppm SA (Table 6), indicating that sulfur content affects the quality of antioxidants in quinoa seeds. Schaich, Tian & Xie (2015) indicated that the values obtained through the ORAC assay do not allow classifying food nor determining alternatives for food consumption because both the molecules detected through this technique and their physiology need to be considered.

In WA, the ORAC values per 100 g DM were in the range of 58731.6 – 63220.0 µmol TE and 64036.2 - 68956.6 µmol TE for the 2019/20 and 2020/21 seasons, respectively (Table 7). These values are higher compared to the results obtained in a study conducted by Rocchetti et al. (2017) using a similar analysis methodology for commercial quinoa, per 100 g DM the value was 34171 µmol TE, which can be explained by the different edaphoclimatic conditions and extraction solvent used, 70% methanol for the study already mentioned and 70% acetone in this study.

In the 2019/2020 season, the 50% WA treatment allowed obtaining the highest ORAC value, showing a significant effect with respect to the other two WA treatments evaluated (Table 7). However, in the 2020/21 season, the effect was exerted by the 100% WA treatment. Meanwhile, when water availability interacted with the sulfur content factor, the treatment 50% WA and 20 ppm SA presented differences ($p \leq 0.05$) with respect to the other treatments. The treatment 100% WA and 12 ppm SA recorded the highest antioxidant capacity value according to the ORAC assay (Table 9).

When analyzing the degree of correlation between TPC and antioxidant activity by the FRAP method, coefficients of 0.76 and 0.66 were found for the 2019/20 and 2020/21 seasons, respectively. In both cases, the null hypothesis was rejected. However, the correlation coefficient between the variables was greater than 0.75 in the 2019/20 season, being valid according to Castillo (2001). Álvarez-Jubete et al. (2010) studied quinoa of Bolivian origin and reported a high correlation (0.99) between these parameters. The authors explained that discrepancies with other studies were attributed to the interpretation of the results, presence of interfering substances, oxidant solubility, oxidation state, pH of the medium and type of substrate. In contrast, Park et al. (2017) found a negative relationship between TPC and FRAP values when comparing quinoa of Peruvian, American and Korean origin. The low correlation (-0.744) was attributed to the fact that the main antioxidant compounds of the extracts could be non-phenolic, e.g., ascorbic acid, phytic acid, α-tocopherol, β-carotene and saponins.

The correlation coefficients between TPC and the ORAC values were 0.39 and 0.42 for the 2019/20 and 2020/21 seasons, respectively. Tang et al. (2015) reported a higher correlation coefficient of 0.99 for commercial quinoa extracts, including both free and conjugated phenolic compounds and using a 70% methanol as an extraction solvent.

## CONCLUSION

In both seasons, the phenological stages of quinoa were shortened, time from sowing to flowering, as well as from sowing to grain filling and from sowing to seed maturity.

The interaction of sulfur and water availability factors did not show an effect on agronomic and productive characteristics. Fertilization with sulfur had a positive effect on plant height, panicle length, yield and thousand seed weight in both seasons. Meanwhile, the lower the availability of water, the height of the plants and the yield of the seeds decrease.

In antioxidants, the interaction of the study factors showed that the average dose of sulfur (12 ppm) with the lowest availability of water generates the greatest number of flavonoids content, while, with the highest availability of water (100%) obtains greater oxygen radical antioxidant capacity (ORAC). Meanwhile, individually, water reduction increases the content of both total phenols and flavonoids. However, the lower the availability of sulfur, the greater the antioxidant capacity in vitro according to the ferric ion reducing antioxidant power (FRAP) test.

In the first season of the study, the content of total phenols and the antioxidant capacity according to the FRAP assay were validly correlated. While no correlation was found between the content of total phenols and the antioxidant capacity by the assay (ORAC).

This study contributes to a better understanding of the impact of drought conditions and soil nutrient deficiency, specifically sulfur content, on agricultural production, which allows for a quantitative approach to the negative effects of those conditions on quinoa.

## Prospects

The lipid content of quinoa ranges between 5.3% and 14.5%, with 19 - 12.3% corresponding to saturated fatty acids (mainly palmitic acid), and 70 - 89% to unsaturated fatty acids such as linoleic, α-linolenic, and oleic acids (Chaudhary et al. 2023). Future research is required to evaluate the effect of different sulfur concentrations on the quantity and proportion of fatty acids, both saturated and unsaturated, in quinoa. Zhang et al. (2020) found that limiting the sulfur content in the cultivation of *Scenedesmus acuminatus* increased the amount of saturated (C16:0) and monounsaturated (C18:1) fatty acids, which might indicate that a similar effect on quinoa would increase the nutritional value of this pseudocereal.

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Table 1. Average temperature, rainfall, hours of sun, and solar radiation from sowing to harvesting of quinoa in Chillan, Ñuble región, Chile.

| Season | Month | Max. T° (°C) | Min. T° (°C) | Rainfall (mm month$^{-1}$) | Hours of sun (h) | Solar radiation (MJ m$^{-2}$ day$^{-1}$) |
|---|---|---|---|---|---|---|
| 2019/20 | October | 19.5 ± 2.3 | 6.1 ± 3.1 | 9.9 | 8.5 ± 1.4 | 18.4 ± 4.5 |
| | November | 24.3 ± 4.1 | 9.0 ± 2.5 | 6.2 | 9.9 ± 1.6 | 22.4 ± 4.4 |
| | December | 27.1 ± 2.8 | 11.0 ± 2.0 | 4.2 | 10.5 ± 1.2 | 24.8 ± 4.6 |
| | January | 29.2 ± 4.2 | 12.5 ± 2.3 | 3.9 | 10.6 ± 1.4 | 25.5 ± 3.6 |
| | February | 30.0 ± 4.1 | 11.6 ± 3.2 | 1.3 | 10.6 ± 0.4 | 25.5 ± 1.3 |
| 2020/21 | October | 21.9 ± 5.4 | 6.0 ± 3.4 | 4.7 | 9.0 ± 2.3 | 20.5 ± 5.0 |
| | November | 24.2 ± 2.3 | 8.3 ± 1.7 | 0 | 10.1 ± 1.3 | 23.1 ± 3.6 |
| | December | 26.6 ± 4.1 | 9.3 ± 2.3 | 2.2 | 10.5 ± 1.7 | 25.4 ± 5.0 |
| | January | 28.3 ± 4.3 | 11.5 ± 2.9 | 89.1 | 10.5 ± 1.5 | 24.9 ± 5.6 |
| | February | 26.9 ± 4.3 | 10.9 ± 2.1 | 0 | 9.2 ± 1.4 | 20.2 ± 5.3 |

Table 2. Soil analysis of the silt loamy soil used (Santa Bárbara series) in the experiments.

| Determination | Natural grassland | Normal category or level of sufficiency |
|---|---|---|
| pH | 6.03 | 6.0 - 7.0 |
| Organic matter (%) | 8.41 | 2.0 - 8.0 |
| Electrical cond (dS m$^{-1}$) (1:2,5) | 0.1 | <1.0 |
| Ammonium N-NH$_4$ (mg kg$^{-1}$) | 7.5 | >10 |
| Nitrate N-NO$_3$ (mg kg$^{-1}$) | 10.3 | >10 |
| Available N (mg kg$^{-1}$) | 17.8 | 20 - 60 |
| Available Phosphorus (mg kg$^{-1}$) | 3.6 | 20 |
| Exchangeable Potassium (cmol kg$^{-1}$) | 0.35 | 0.30 - 0.45 |
| Available Potassium (mg kg$^{-1}$) | 135.8 | 115 - 175 |
| Exchangeable Calcium (cmol kg$^{-1}$) | 6.66 | 4.0 - 8.0 |
| Exchangeable Magnesium (cmol kg$^{-1}$) | 1.32 | 0.6 - 1.5 |
| Exchangeable Sodium (cmol kg$^{-1}$) | 0.17 | <1.0 |
| Sum of bases (cmol kg$^{-1}$) | 8.5 | 5.0 - 10.0 |
| Available Sulfur (mg kg$^{-1}$) | 7.8 | 16 - 30 |
| Exchangeable Aluminum (cmol kg$^{-1}$) | 0.01 | <0.15 |
| CICE (cmol kg$^{-1}$) | 8.5 | >5.0 |

| | | |
|---|---|---|
| Al saturation (%) | 0.17 | <2.0 |
| K saturation (%) | 9.7 | 5.0 - 10.0 |
| Ca saturation (%) | 78.4 | 65 - 75 |
| Mg saturation (%) | 15.7 | 5.0 - 10 |
| Calcium/Magnesium | 5.0 | 4.0 - 6.0 |
| Potassium/Magnesium | 0.6 | 0.3 - 0.6 |
| Iron (mg kg$^{-1}$) | 8.6 | >2.5 |
| Manganese (mg kg$^{-1}$) | 3 | >3.0 |
| Zinc (mg kg$^{-1}$) | 1 | >1.0 |
| Copper (mg kg$^{-1}$) | 0.6 | >0.5 |
| Boron (mg kg$^{-1}$) | 0.7 | 0.6 - 1.5 |

Table 3. Length of the plant phenological stages of quinoa in the two consecutive seasons.

| Number of days | 2019/20 | 2020/21 |
|---|---|---|
| From emergence to flowering | 43 | 48 |
| From emergence to the initial phase of grain filling | 85 | 93 |
| From emergence to seed maturity | 110 | 121 |

Table 4. Mean values of agronomic parameters of quinoa under three levels of sulfur availability in two consecutive seasons in Chillán, Chile.

| Available sulfur (ppm) | Plant height (cm) | Panicle length (cm) | One thousand seed weight (g) | Yield (g/plant) |
|---|---|---|---|---|
| 2019/2020 | | | | |
| 20 | 114.9 a | 16.0 a | 2.7 a | 29.1 a |
| 12 | 108.3 a | 13.1 b | 2.6 b | 25.9 b |
| 8 | 97.1 b | 12.1 c | 2.5 b | 21.2 c |
| 2020/2021 | | | | |
| 20 | 82.3 a | 18.1 a | 2.9 a | 14.6 a |
| 12 | 75.3 b | 15.8 b | 2.6 b | 13.2 b |
| 8 | 70.8 b | 14.4 c | 2.5 b | 9.8 c |

* Different letters in a column indicate significant differences ($p \leq 0.05$) for the treatments in the season.

Table 5. Mean values of agronomic parameters of quinoa under three levels of water availability in two consecutive seasons in Chillán, Chile.

| Available water (%) | Plant height (cm) | Panicle length (cm) | One thousand seed weight (g) | Yield (g/plant) |
|---|---|---|---|---|
| **2019/2020** | | | | |
| 100 | 110.7 a | 14.8 a | 2.7 a | 28.9 a |
| 50 | 105.8 ab | 13.7 b | 2.7 a | 27.3 a |
| 25 | 100.4 b | 12.6 c | 2.5 a | 19.9 b |
| **2020/2021** | | | | |
| 100 | 80.5 a | 16.3 a | 2.8 a | 13.6 a |
| 50 | 78.1 a | 15.9 a | 2.7 a | 12.7 a |
| 25 | 70.2 b | 16.1 a | 2.5 b | 10.4 b |

* Different letters in a column indicate significant differences ($p \leq 0.05$) for the treatments in the season.

Table 6. Mean values of TPC, TFC, FRAP, ORAC per 100 g of dry matter (DM) of quinoa under three levels of water availability in two consecutive seasons in Chillán, Chile.

| Available sulfur (ppm) | TPC (mg GAE) | TFC (mg QE) | FRAP (µmol $Fe^{2+}$) | ORAC (µmol TE) |
|---|---|---|---|---|
| 2019/2020 | | | | |
| 20 | 129.0 b | 12.8 a | 952.4 b | 63220.0 a |
| 12 | 143.2 a | 12.6 a | 990.6 b | 59064.9 b |
| 8 | 147.2 a | 12.4 a | 1089.4 a | 58731.6 b |
| 2020/2021 | | | | |
| 20 | 158.3 b | 12.7 a | 911.5 b | 64770.9 b |
| 12 | 161.3 ab | 12.8 a | 969.7 a | 68956.6 a |
| 8 | 163.3 a | 10.9 b | 1010.8 a | 64036.2 b |

* Different letters in a column indicate significant differences ($p \leq 0.05$) for the treatments in the season. n =12 samples per treatment.

Table 7. Mean values of TPC, TFC, FRAP, ORAC per 100 g of dry matter (DM) of quinoa under three levels of water availability in two consecutive seasons in Chillán, Chile.

| Available water (%) | TPC (mg GAE) | TFC (mg QE) | FRAP (µmol $Fe^{2+}$) | ORAC (µmol TE) |
|---|---|---|---|---|
| 2019/2020 | | | | |
| 100 | 135.5 b | 12.3 b | 1040.7 a | 58839.1 b |
| 50 | 138.4 ab | 12.5 ab | 975.5 b | 62903.1 a |
| 25 | 145.5 a | 13.0 a | 1016.2 ab | 59273.8 b |
| 2020/2021 | | | | |
| 100 | 153.4 c | 11.8 b | 927.1 b | 68931.1 a |
| 50 | 159.1 b | 12.2 a | 949.7 b | 62689.4 c |
| 25 | 170.4 a | 12.4 a | 1015.3 a | 65165.6 b |

* Different letters in a column indicate significant differences ($p \leq 0.05$) for the treatments in the season. n =12 samples per treatment.

Table 8. Mean values of total TFC (mg QE per 100 g DM) for the interaction between a water and sulfur availability in the 2020/2021 season in Chillán, Chile.

| Available sulfur (ppm) | Available water (%) | | |
|---|---|---|---|
| | 25 | 50 | 100 |
| 8 | 11.4 cA | 11.3 bA | 10.1 bB |
| 12 | 13.4 aA | 12.6 aB | 12.4 aB |
| 20 | 12.4 bA | 12.7 aA | 12.9 aA |

* Different lowercase letters indicate significant differences ($p \leq 0.05$) within the column. Different capital letters indicate significant differences ($p \leq 0.05$) within the row.

Table 9. Mean values of the ORAC antioxidant capacity (µmol TE per 100 g DM) for the interaction between water and sulfur availability in the 2020/2021 season in Chillán, Chile.

| Available sulfur (ppm) | Available water (%) | | |
|---|---|---|---|
| | 25 | 50 | 100 |
| 8 | 64966.8 aA | 63408.4 aA | 63733.5 aA |
| 12 | 67330.5 aA | 66607.5 aA | 72931.8 aA |
| 20 | 63199.6 aAB | 60985.2 bB | 70127.9 aA |

* Different lowercase letters indicate significant differences ($p \leq 0.05$) within the column. Different capital letters indicate significant differences ($p \leq 0.05$) within the row.

# CAPÍTULO 4:

## 4.1 Introducción

La escasez hídrica producto del cambio climático ha alterado la producción de alimentos a nivel mundial. Se ha descrito un desplazamiento geográfico de las zonas de producción y una disminución de la superficie cultivable. Es complejo estimar con precisión la magnitud e impacto que esta crisis ambiental tendrá sobre la nutrición humana, considerando, la imperante necesidad de aumentar la cantidad y la calidad de los alimentos para abastecer a una población en constante crecimiento. Ante tal escenario, es indispensable contar con cultivos que aporten con los nutrientes necesarios para mantener una buena alimentación. En este contexto, una de las plantas que ha demostrado tener la habilidad de crecer en condiciones ambientales y nutricionales adversas es la quinoa (Pereira et al., 2019). Además, este pseudocereal posee un importante contenido de proteínas y aminoácidos esenciales, algunos de estos aminoácidos limitantes en cultivos altamente demandados como el trigo y el maíz (Vilcacundo & Hernández-Ledesma, 2017).

Actualmente, la proteína vegetal es una alternativa para la alimentación humana, debido a su versatilidad y menor costo frente a la proteína de origen animal. Por tanto, estudiar el efecto e interacción de la escasez de agua y la falta de azufre en el cultivo de la quinoa, aporta al conocimiento de este pseudocereal. Al respecto la literatura es escasa, a pesar de ser condiciones que pueden afectar la composición nutricional de esta semilla.

El objetivo de este estudio fue cuantificar el contenido de proteínas y de aminoácidos azufrados en las semillas de quinoa producidas con diferente disponibilidad de agua y de azufre en el cultivo.

## 4.2 Revisión Bibliográfica

### 4.2.1 Efecto de la disponibilidad de agua y de azufre en las proteínas y los aminoácidos

Las plantas viven expuestas a condiciones bióticas y/o abióticas desfavorables para su crecimiento y desarrollo, como son la infección por patógenos, el ataque de herbívoros, así como, condiciones de sequía, temperaturas extremas, deficiencia de nutrientes y exceso de sales o metales tóxicos en el suelo (Zhu, 2016). Las plantas contrarrestan el daño celular causado por las condiciones adversas, utilizando mecanismos para percibir señales externas y expresar respuestas adaptativas a nivel morfológico, fisiológico, bioquímico y molecular (García-Morales et al, 2013; Ali et al., 2018). Un ejemplo es la quinoa que, ante la falta de agua, las plantas equilibran la absorción y la pérdida de esta (Zurita-Silva et al., 2015). A su vez, aumentan el contenido de prolina y de azúcares solubles como la glucosa y la trehalosa para protegerse de las reacciones oxidativas (Dumshott et al., 2022).

La prolina es reconocida como un marcador de tolerancia, un mayor contenido de este aminoácido se asocia a una mayor capacidad de ajuste osmótico (Miranda-Apodaca et al., 2018). Entre las funciones de la prolina se encuentra que puede actuar como molécula chaperona, protegiendo la integridad y plegamiento de diferentes enzima, así como actuar como antioxidante, disminuyendo las especies reactivas de oxígeno e inhibiendo la peroxidación de los lípidos (Pereyra & Quiriban, 2014).

Las plantas pueden resistir de mejor forma la fluctuaciones de su entorno si disponen de los nutrientes necesarios para su crecimiento y desarrollo (Anjum et al, 2015). Específicamente se ha asociado al azufre a la tolerancia de las plantas ante las condiciones adversas (Kulczycki, 2021). El azufre es el cuarto nutriente vegetal más importante después del nitrógeno, fósforo y potasio (Prasad & Singh, 2018). El requerimiento de este nutriente depende de cada especie, sin embargo, se estima cercano a la demanda de fósforo (Kulczycki, 2021).

El déficit de azufre afecta la fijación de nitrógeno, por tanto, en el largo plazo causa disminución en el contenido de proteínas totales, clorofila, ARN y biomasa, además de generar clorosis en las hojas jóvenes (Samborska et al., 2019).

El azufre se puede absorber a la forma de óxido de azufre (IV) desde la atmósfera a través de los estomas de las hojas (Kulczycki, 2021), sin embargo, la principal vía se realiza a través del suelo, mediante las raíces a la forma de sulfato. Posteriormente, el sulfato es transportado a los cloroplastos de las hojas a través de un sistema de transporte activo, para luego, ser reducido a sulfuro (Anjum et al, 2015; Samborska et al., 2019). Una vez como sulfuro, es incorporado a la primera forma orgánica que es la cisteína, aminoácido precursor de distintas moléculas como el glutatión, los glucosinolatos y la metionina. Esta última a través de la S-adenosil metionina controla en la planta la síntesis del etileno (Anjum et al, 2015; Samborska et al., 2019).

Debido a la alta reactividad del grupo tiol que posee la cisteína (Cys), el cual se oxida fácilmente formando especies reactivas que inhiben las actividades enzimáticas y provocan un importante daño oxidativo, es fundamental mantener su homeostasis, para lo cual debe existir una acción coordinada entre la enzima de síntesis (o-acetil serina tiol sintasa) y la enzima de degradación (L-cisteína desulfhidrasa) (Gotor et al., 2015).

A través de las plantas el azufre llega a los animales y a los humanos. Después del calcio y el fósforo, el azufre es el elemento mineral más abundante en el organismo humano. Entre sus funciones se encuentra el mantener y promover el proceso de digestión y absorción del estómago, además de proteger al organismo contra sustancias tóxicas y participar en la síntesis de colágeno, incidiendo en el envejecimiento celular (Gupta & Gupta, 2014).

### 4.2.2 Síntesis de proteínas y aminoácidos en la planta

En las plantas el contenido de proteínas, por ende, el perfil aminoacídico puede variar de acuerdo con las condiciones climáticas, del suelo, la altitud y la latitud geográfica, los niveles de precipitación, las prácticas agrícolas y las variedades/cultivares utilizadas (Almeida et al.,2020).

En la célula vegetal los aminoácidos (aa) se sintetizan, utilizan y degradan en distintos compartimentos como, por ejemplo, los cloroplastos, el citosol, los peroxisomas, las mitocondrias y las vacuolas. Desde las hojas de origen, los aa son exportados e importados a las hojas nuevas y a las semillas en desarrollo. Son componentes esenciales del metabolismo, no sólo por constituir proteínas, sino también, por ser precursores de metabolitos secundarios y además portadores de nitrógeno orgánico entre los órganos de la planta (Dinkeloo, Boyd & Piloto, 2018).

### 4.2.3 Proteínas de la quinoa

El contenido de proteínas de la quinoa se encuentra en un rango entre 7,5% y 22,1%, con un promedio de 14,6% (Covarrubias et al., 2020). Esta cantidad es mayor a lo que poseen productos altamente consumidos como la cebada (11% b.s), el arroz (7,5% b.s) y el maíz (13,4% b.s) (Chaudhary et al., 2023).

En los alimentos, el valor nutricional de las proteínas depende de la presencia de los aminoácidos esenciales, así como, de la utilización biológica de estos (Maradini et al., 2017) y de los productos generados a través de su hidrólisis en el tracto gastrointestinal humano (Martínez-Villaluenga et al., 2020).

La ingesta diaria de proteínas en adultos por kg de peso corporal debiese estar entre 0,8 - 1,0 gramos, considerándose como límite para evitar la deficiencia 0,66 g. El valor aumenta a 1,1 g en mujeres embarazadas, así como a 1,3 g en mujeres en lactancia materna y entre 1,2 - 1,52 g en los lactantes (Mota et al., 2016; Almeida et al., 2020).

### 4.2.4 Aminoácidos

En la quinoa se han encontrado 16 tipos de aa, nueve de ellos considerados indispensables para el metabolismo, su importancia radica en que el organismo humano no tiene la capacidad de sintetizarlos, por tanto, deben ser incorporados a través de la dieta (Escuredo et al., 2014; Chito et al., 2017; Maradini et al., 2017). Dentro de los aminoácidos esenciales, por cada 100 g de proteína de quinoa, se destaca la lisina (2,4 a 7,8 g), metionina (0,3 a 9,1 g) y treonina (2,1 a 8,9 g) (Nowak et al., 2016; Iqbal et al., 2018).

Los cereales contienen bajos niveles de lisina, mientras que las legumbres son deficientes en los aa azufrados metionina y cisteína (Almeida et al., 2020). En el metabolismo humano, los aa azufrados poseen un rol destacado tanto en el funcionamiento como en la mantención del sistema inmunitario (Mota et al., 2016). Según la OMS/FAO, un adulto, mayor de 18 años requiere 2,2 g de aa azufrados por cada 100 g de proteína (Almeida et al., 2020).

Actualmente, los consumidores demandan por productos vegetales innovadores, altos en nutrientes como las proteínas y que aporten a mantener un mejor estado de salud. Por tanto, el desarrollo de nuevas matrices alimentarias ha centrado el interés en los pseudocereales, considerándolos una alternativa frente a los cereales para la formulación de alimentos sin gluten (Ahmad et al., 2018).

### 4.3 Reactivos y estándares

Los reactivos y disolventes utilizados fueron adquiridos en Merck (Darmstadt, Alemania). El estándar de aminoácidos se adquirió en Sigma-Aldrich (St. Louis, MO, EE. UU.).

## 4.4 Metodología

### 4.4.1 Análisis químicos

Las semillas limpias y tamizadas (tamiz de 1 mm U.S. Standard Sieve N°18) se almacenaron en un lugar fresco y seco a temperatura ambiente hasta su análisis.

**4.4.2 Determinación de proteínas:** según el método n°992.23 de la A.O.A.C. (Asociación Oficial de Análisis Químico, 1996). Se consideró el factor de conversión de nitrógeno total a proteínas de 6,25 como recomienda el método Oficial del Codex Alimentario. Todos los análisis se realizaron por triplicado.

### 4.4.3 Determinación de aminoácidos

- Tratamiento de las muestras: el acondicionamiento de las muestras se realizó según Wang et al. (2020).

- Extracción de aminoácidos libres: se realizó según Reguera et al. (2018) con algunas modificaciones. Se tomaron 150 mg de la muestra seca y molida, sin saponinas y desengrasada con 100% de hexano, la cual, fue homogeneizada en 400 µL de agua:cloroformo:metanol (3:5:12 v/v). Luego, se centrifugó (14.000 rpm a 2 min) y se recolectó el sobrenadante. Este proceso se realizó dos veces. Ambos sobrenadantes se combinaron y mezclaron con 200 µL de cloroformo y 300 µL de agua. La mezcla resultante se agitó (80 min a 600 rpm) y centrifugó (14.000 rpm a 2 min). El sobrenadante fue llevado a sequedad utilizando un baño termorregulado a 70 ºC en una campana de extracción de aire. Las muestras fueron congeladas a -80 ºC hasta su análisis.

Posteriormente, cada muestra fue suspendida en 500 µL de 0,1% de ácido trifluoracético (v/v), centrifugada (14000 rpm por 4 minutos a 4 ºC) y el sobrenadante fue filtrado (celulosa 0,22 µm) previo al análisis por UHPLC-MS.

- Identificación de aminoácidos: se realizó mediante UHPLC-DAD-MS/MS (Shimadzu UHPLC LCMS-8030, Kyoto, Japón). El equipo cuenta con bomba cuaternaria LC-30AD, unidad desgasificadora DGU-20A5R, horno CTO-20AC, automuestreador SIL-30AC, sistema controlador CBM-20A y un detector de arreglo de diodos (DAD, modelo SPD-M20A) acoplados con un espectrómetro de masas (MS). El control y el procesamiento de datos se realizaron con el software LabSolutions (versión 5.86).

Para la separación cromatográfica se utilizó una columna Shim-pack XR-ODS III (tamaño de partícula de 2,2 µm, 2,0 mm de d.i. y 200 mm de longitud, Shimadzu) con una precolumna GVP-ODS (2,0 mm de d.i. x 5 mm de longitud, Shimadzu). La columna fue mantenida a 50 °C con un flujo de 0,20 mL min$^{-1}$ con un gradiente de elución. El eluyente “A” estaba compuesto por agua con ácido fórmico al 0,1% (v v$^{-1}$) y eluyente "B" por acetonitrilo (ACN) con ácido fórmico al 0,1% (v v$^{-1}$). El programa del gradiente fue el siguiente: 0 - 20 min, 0% - 20% B; 21 - 26 min, 80% B; 27-30 min, 0% B. El espectrograma se registró entre 190 a 700 nm.

Para la cuantificación por HPLC-MS/MS se utilizó el modo de monitoreo de reacciones múltiples (MRM). Se fijó la fuente de ionización de electro-spray (ESI) y la temperatura de desolvatación a 400 °C y 250 °C, respectivamente.

Los flujos de gas de secado y nebulización se fijaron en 15 y 3 L min$^{-1}$, respectivamente. Para cada aminoácido analizado se optimizaron las transiciones MRM y se definieron las transiciones cuantitativas y cualitativas (Tabla A1 Anexo). La cuantificación se realizó con una curva de calibración externa de concentraciones 2,5; 5; 10; 20; 40; 80; 100 y 250 µM preparada a partir de una solución estándar de aminoácidos Sigma-Aldrich de 2,5 mM (Cód. 382499). Los aminoácidos en la muestra se identificaron por comparación de los tiempos de retención y transiciones MRM. La cuantificación se realizó obteniendo el área del peak de la transición MRM cuantitativa. Los resultados se expresan en g/100 g de proteína.

### 4.4.4 Análisis Estadístico

Se analizó la normalidad y la homogeneidad de los datos de mediante las pruebas de Shapiro-Wilk y Bartlett, respectivamente. Posteriormente, los resultados de cada ensayo fueron sometidos a un análisis de varianza (ANDEVA) y a la prueba de comparación de medias DMS (α=0,05), utilizando el software SAS University Edition (2016).

## 4.5 Resultados y Discusión

### 4.5.1 Proteínas en semillas de quinoa genotipo Regalona

El contenido de proteínas por cada 100 g de masa seca se encontró entre 13,91 a 16,16 g en la temporada 2019/2020 y entre 15,44 a 18,86 g en la temporada 2020/2021. Covarrubias et al. (2020) reportaron por cada 100 g de masa seca un rango entre 15,60 g a 17,40 g para igual genotipo, metodología de determinación de nitrógeno total y factor de conversión (6,25).

Cabe destacar que, en ambas temporadas, no existió una interacción significativa (p>0,05) entre los factores de disponibilidad de agua (WA) y de azufre (SA). En el análisis individual, al incrementar la fertilización con azufre, también lo hizo la cantidad de proteínas, cuyo efecto fue significativo (p≤0,05) entre los tratamientos de 8 y 20 ppm (Figura 1) en ambas temporadas de estudio. Lo anterior, concuerda con Prasad & Singh (2018) y Wilson et al. (2020) quienes destacan que un déficit de azufre perjudica la absorción de nitrógeno, lo cual, reduce la síntesis de proteínas, al ser un cofactor fundamental para algunas enzimas como por ejemplo la nitrato reductasa.

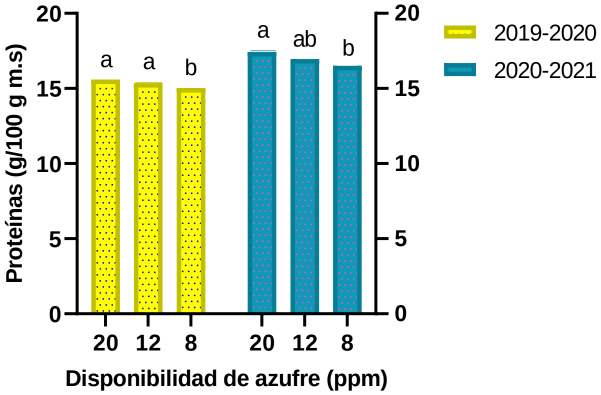

Figura 1 Contenido de proteínas (g/100 g m.s) en semillas de quinoa según disponibilidad de azufre (SA). Letras iguales significa que no existen diferencias significativas (p>0,05) para una misma temporada. **Fuente: Elaboración propia.**

En tanto, a mayor WA aumenta el contenido de proteínas en la semilla. Existiendo diferencias significativas (p≤0,05) entre los tratamientos del 25% y 100%, en ambas temporadas (Figura 2).

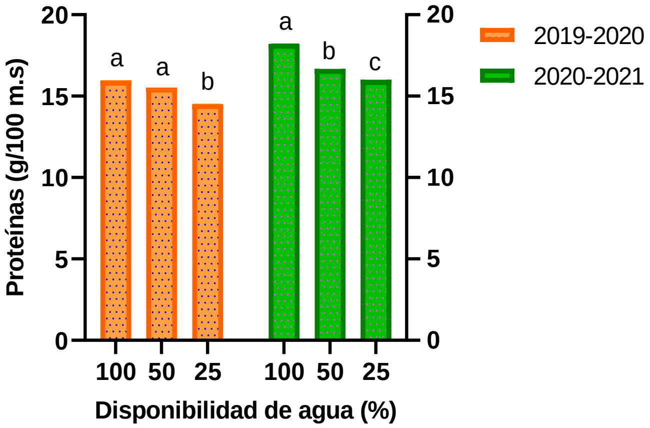

Figura 2 Contenido de proteínas (g/100 g m.s) en semillas de quinoa según disponibilidad de agua (WA). Letras iguales significa que no existen diferencias significativas (p>0,05) para una misma temporada. **Fuente: Elaboración propia.**

### 4.5.2 Aminoácidos azufrados

En ambas temporadas, por cada 100 g de proteína según disponibilidad de agua (%) y de azufre (ppm), el contenido de cisteína (Cys) fluctuó entre 0,01 - 0,03 g y entre 0,02 - 0,03 g respectivamente, en tanto, para metionina (Met) el rango fue de 0,69 - 2.22 g y entre 0,66 - 2,17 g respectivamente. Los valores encontrados son inferiores al rango de 0,05 - 0,27 g por cada100 g proteína para Cys y entre 0,22 - 4,48 g por cada 100 g proteína para Met reportados por Escuredo et al. (2014) para similar cultivar.

En ambas temporadas, no existió una interacción significativa (p>0,05) entre los factores WA y SA. De igual forma, los factores individualmente no ejercieron un efecto significativo (p>0,05) en el contenido de Cys (Tabla 1 y 2).

Tabla 1 Contenido de cisteína (Cys), metionina (Met), serina (Ser) y prolina (Pro) por cada 100 g de proteína de quinoa en temporadas 2019/2020 y 2020/2021 con tratamientos de disponibilidad de agua (%).

| Agua<br>(%) | Cys<br>(g/100 g proteína) | Met<br>(g/100 g proteína) | Ser<br>(g/100 g proteína) | Pro<br>(g/100 g proteína) |
|---|---|---|---|---|
| | | 2019/2020 | | |
| 100 | 0,03 a | 0,69 a | 0,11 b | 2,29 a |
| 50 | 0,03 a | 0,92 a | 0,14 a | 2,52 a |
| 25 | 0,03 a | 0,80 a | 0,14 a | 2,96 a |
| | | 2020/2021 | | |
| 100 | 0,01 a | 2,22 a | 0,12 b | 4,64 a |
| 50 | 0,02 a | 1,79 a | 0,18 a | 4,83 a |
| 25 | 0,02 a | 2,06 a | 0,14 ab | 4,51 a |

*Letras distintas indican diferencias significativas ($p \leq 0,05$) entre los tratamientos. n = 12
**Fuente: Elaboración propia.**

En Met sólo en la temporada 2019/2020 existieron diferencias significativas ($p \leq 0,05$) entre 12 ppm con respecto a 8 ppm y 20 ppm de SA (Tabla 2).

Tabla 2 Contenido de cisteína (Cys), metionina (Met), serina (Ser) y prolina (Pro) por cada 100 g de proteína de quinoa en temporadas 2019/2020 y 2020/2021 con tratamientos de disponibilidad de azufre (ppm).

| Azufre<br>(ppm) | Cys<br>(g/100 g proteína) | Met<br>(g/100 g proteína) | Ser<br>(g/100 g proteína) | Pro<br>(g/100 g proteína) |
|---|---|---|---|---|
| | | 2019/2020 | | |
| 20 | 0,03 a | 0,93 a | 0,14 a | 2,85 a |
| 12 | 0,03 a | 0,66 b | 0,12 b | 2,32 a |
| 8 | 0,03 a | 0,82 a | 0,14 a | 2,61 a |
| | | 2020/2021 | | |
| 20 | 0,02 a | 2,17 a | 0,12 b | 4,54 b |
| 12 | 0,02 a | 2,01 a | 0,13 b | 4,23 c |
| 8 | 0,02 a | 1,89 a | 0,19 a | 5,22 a |

*Letras distintas indican diferencias significativas ($p \leq 0,05$) entre los tratamientos. n = 12
**Fuente: Elaboración propia.**

La baja cantidad de Cys encontrada en este estudio se podría atribuir al contenido de azufre de los tratamientos. Al respecto, Azcón-Bieto & Talón (2008) mencionan que cuando la planta no dispone de suficiente sulfato, se activa el proceso de absorción y de asimilación, mediante la regulación de las enzimas ATP sulfurilasa y la adenosina 5'-fosfosulfato reductasa, lo que disminuye el suministro de las formas orgánicas de azufre reducido, como es la cisteína.

Por otra parte, al actuar la Cys como precursor de otras estructuras, como biotina, tiamina, coenzima A, ácido lipoico, grupos hierro-azufre y metionina pudiese estar conformando estas (Gotor et al., 2015).

Una tercera posibilidad del bajo contenido de Cys puede atribuirse al análisis de cuantificación, el cual determina los aminoácidos libres presentes en la muestra. Cabe destacar que para determinar el contenido de Cys y Met que conforman las proteínas, se necesitan diversas etapas de hidrólisis, además de la hidrólisis típica de HCl por 24 h, lo cual degrada los aa azufrados, independientemente de la duración de las etapas (Templeton & Laurence. 2015; Silverman et al. 2022).

La síntesis de los aminoácidos azufrados se relaciona estrechamente con la síntesis de serina (Ser), por tanto, el contenido de Ser en ambas temporadas por cada 100 g de proteína fluctuó entre 0,11 - 0,18 g según WA (%) y entre 0,12 - 0,19 g según SA. Al respecto, Escuredo et al. (2014) reportaron para Ser por cada 100 g de proteína un rango de 1,06 - 2,83 g para el mismo cultivar. El bajo contenido del aminoácido Ser encontrado en este estudio concuerda con la reducida cantidad de los aminoácidos Cys y Met. Cabe destacar que a partir de Ser se sintetizan los aa azufrados, en particular, la Cys se obtiene mediante la reacción secuencial que involucra a las enzimas serina acetil transferasa y la o-acetil serina sintasa según lo reportado por Gotor et al. (2015). En tanto, a partir del grupo metilo que se encuentra en el carbono β de la Ser, es posible sintetizar Met (Palíková et al. 2014). En las temporadas 2019/2020 y 2020/2021, no existió interacción de los factores WA y SA ensayados. Sin embargo, en ambas temporadas el contenido de Ser presentó diferencias significativas ($p \leq 0,05$) entre los tratamientos de 100% de WA con respecto a lo que consideraban el 25% y 50% (Tabla 1). En tanto, en la temporada 2019/2020, la dosis de 12 ppm de SA presentó diferencias significativas ($p \leq 0,05$) con respecto a 8 ppm y 20 ppm. En tanto en la siguiente temporada fue el tratamiento de 8 ppm, el cual presentó el mayor contenido, generando diferencias ($p \leq 0,05$) con respecto a 12 ppm y 20 ppm (Tabla 2).

A su vez, se determinó el contenido de prolina (Pro), aa sindicado por Miranda-Apodaca et al. (2018) y Trovato et al (2021) como un marcador de tolerancia la sequía. El mayor contenido de este aa se relaciona con una mayor capacidad de ajuste osmótico a las condiciones del medio. El rango obtenido en ambas temporadas por cada 100 g de proteína fue entre 2,29 - 4,83 g según WA (%) y entre 2,32 - 5,22 g según SA. Estos valores son superiores al rango de 1,22 - 2,52 g por 100 g proteína reportado por Escuredo et al. (2014) para el genotipo Regalona. La diferencia se podría atribuir, a la SA de este estudio, ya que, en ambas temporadas, la menor WA no ejerció un efecto significativo (p>0,05) en el contenido de Pro. A su vez, no existió interacción entre los factores WA y SA estudiados. En tanto, individualmente la SA en la temporadas 2020/2021 presentó diferencias significativas (p≤0,05) entre todos los tratamientos ensayados, siendo la dosis más baja (8 ppm) la que obtuvo el valor más alto de Pro (Tabla 2). En este contexto, Reguera et al. (2018) utilizando similar metodología que este estudio, en la extracción de aminoácidos libres, y genotipo Regalona, no encontraron diferencias en el contenido de los aminoácidos Met, Ser y Pro, en quinoa cultivada en Río Hurtado (30.3◦S, 70,6◦O, 1.462 m s.n.m.), Chile.

### 4.6 Conclusión

En ambas temporadas, al incrementar la disponibilidad de agua y de azufre se evidenció un aumento en el contenido de proteínas en la semilla. Sin embargo, en los aminoácidos azufrados cisteína y metionina, la disponibilidad de agua no ejerció efecto en la cantidad de estos. En tanto, en la primera temporada de estudio la concentración media (12 ppm) de azufre afectó negativamente la cantidad de metionina, así como su precursor serina. En tanto, en la segunda temporada, este efecto lo ejerció sobre serina y prolina, más no sobre los aminoácidos azufrados.

## 4.7 Referencias

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## 4.8 Glosario

aa = aminoácido.

ABTS = 2,2-azinobis-[3 etilbenzotiazolin-6-sulfónico].

Cys = cisteína.

CUPRAC = capacidad antioxidante.

DM = masa seca.

DPPH = 1,1-difenil 2-picrilhidracil.

FC = Folin Ciocalteau.

FM = materia fresca.

FRAP = poder antioxidante reductor del ión férrico.

GA = ácido gálico.

GAE = equivalentes de ácido gálico.

Met = metionina.

ORAC = capacidad de absorbancia del radical oxígeno.

ppm = partes por millón.

Pro = prolina.

QE = equivalentes de quercetina.

TE = equivalentes de trolox.

TPC = contenido de fenoles total.

TFC = contenido de flavonoides totales.

SA = disponibilidad de azufre.

Ser = serina.

SM = metabolitos secundarios.

WA = disponibilidad de agua.

## 4.9 Anexo

Tabla A1 Datos de tiempo de retención, transiciones, ión precursor predominante y voltajes para cada aminoácido según método establecido en el HPLC-MS/MS.

| Analito | Tiempo de retención (min) | Transición | Ión precursor (m/z) | Q1 (V) | Q3 (V) |
|---|---|---|---|---|---|
| Cisteína (Cys) | 2,19 | 122,1 - 76,05 | 122,10 | -20 | -15<br>-22<br>-19 |
| Metionina (Met) | 3,67 | 150,1 - 56,1 | 150,10 | -10 | -22<br>-21<br>-23 |
| Prolina (Pro) | 2,47 | 116 - 70,1 | 116,00 | -12 | -14<br>-11<br>-16 |
| Serina (Ser) | 2,09 | 106,1 - 60,15 | 106,10 | -17 | -23<br>-17<br>-18 |

**Fuente: Elaboración propia.**