CHILEAN JOURNAL
of AGRICULTURAL RESEARCH

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REVIEW

Content and antioxidant capacity of phenolic compounds in quinoa seed: A review

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Received: 25 May 2023; Accepted: 7 August 2023, doi:10.4067/S0718-58392023000600754

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 pseudocereal 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 these cases, 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 bioaccessibility 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 (TFC) 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 TFC, 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 ‘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 TFC 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 TFC 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.

Table 1. Extraction solvents to determine total phenolic content in quinoa seeds by the Folin-Ciocalteu assay. GAE: Gallic acid equivalents; GA: gallic acid; DM: dry matter; FM: fresh matter.

Ecotype/cultivar/variety	Extraction solvent(s)	Results	Unit	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)	250-792	mg GAE 100 g^{-1} DM	Abderrahim et al. (2015)
Quinoa (cultivated in Bolivia)	Methanol	71.7	mg GAE 100 g^{-1} DM	Àlvarez-Jubete et al. (2010)
Quinoa (USA)	Aqueous methanol acidified with acetic acid; 4 M sodium hydroxide; 6 M hydrochloric acid; 2 M hydrochloric acid	137	mg GAE 100 g^{-1} DM	Balakrishnan and Goodrich-Schneider (2020)
Greenhouse cultivated quinoa (Colombia)	Ethanol 96% (v/v) ultrasound assisted	1500 (freeze-dried) 700 (air-dried)	mg GAE 100 g^{-1} DM	Buitrago et al. (2019)
Quinoa variety Real (Argentina)	Ethanol (0, 40, 80% v/v)	67.50-102.86	mg GAE 100 g^{-1} DM	Carciochi et al. (2015)
28 varieties (Peru, USA, Bolivia, Denmark, Chile)	Methanol:water (4:1 v/v)	269-589	mg GAE 100 g^{-1} DM	Chen et al. (2019)
Quinoa (Peru)	Hydrochloric acid (0.1%) and methanol:water (80:20 v/v)	130	mg GAE 100 g^{-1} DM	Chirinos et al. (2013).
Quinoa pre-cooking (sweet from Ecuador, bitter from Peru)	Methanol:water 80:20 (v/v)	772 (sweet) 864 (bitter)	mg GAE 100 g^{-1} DM	Dini et al. (2010)
Quinoa (varieties Regalona, B080, AG2010 Chile)	Methanol 99% (v/v)	330 (field) 580-640 (greenhouse)	mg GAE 100 g^{-1} DM	Fischer et al. (2013)
Jinli-1 (China)	Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate	200.40	mg GAE 100 g^{-1} DM	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	167.15-308.32	mg GAE 100 g^{-1} DM	Han et al. (2019b)
Quinoa variety Real (Argentina)	Ethanol (30, 50 and 70% v/v)	179-229	mg GAE 100 g^{-1} DM	Luisetti et al. (2020)
Ecotypes Ancovinto, Cancosa, Cahuil, Faro, Regalona and Villarrica (Chile)	Methanol (90%) + acetic acid (0.5%)	14.22-65.53	mg GA 100 g^{-1} DM	Miranda et al. (2011)
Ecotypes Regalona and Villarrica (Chile)	Methanol (90%) + acetic acid (0.5%)	19.20 Regalona cultivated in the north (Vicuña) and 12.39 in the south (Temuco) 31.92 Villarrica cultivated in the north (Vicuña) and 22.87 in the south (Temuco)	mg GA 100 g^{-1} DM	Miranda et al. (2013)
Ecotypes Ancovinto, Cancosa, Cahuil, Faro, Regalona and Villarrica (Chile)	Ethanol 99% (v/v)	3.71-16.55	mg GA 100 g^{-1} DM	Miranda et al. (2014)

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 C 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-C 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 ortho-di-hydroxyls 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 (Table 2), 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).

Table 2. Extraction solvents to determine total flavonoid content in quinoa seeds using colorimetric methods. CE: Catechin equivalents; QE: quercetin equivalents; DM: dry matter; FM: fresh matter.

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

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 (Zulueta et al., 2009; Mishra et al., 2012). 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).

Table 3. Antioxidant capacity in quinoa seeds by DPPH, FRAP, ORAC, ABTS methods. TE: Trolox equivalents; DM: dry matter; FM: fresh matter.

Ecotype/cultivar/ variety/origin	Solvent(s) and extraction method	Method	Result	Unit	Reference
Quinoa (Bolivia)	Methanol	DPPH FRAP	57.7 92.1	mg TE 100 g^{-1} DM	Álvarez-Jubete et al. (2010)
28 varieties (Peru, USA, Bolivia, Denmark, Chile)	Methanol:water (4:1 v/v)	DPPH	106-248	mg TE 100 g^{-1} DM	Chen et al. (2019)
Quinoa (Peru)	Hydrochloric acid (0.1%) and methanol:water (80:20 v/v)	ABTS DPPH ORAC	830 530 1590	μmol TE 100 g^{-1} DM	Chirinos et al. (2013)
Quinoa (sweet Ecuador, bitter Peru)	Methanol:water (80:20 v/v)	DPPH FRAP	287 (sweet) 671 (bitter) 256 (sweet) 873 (bitter)	μmol TE 100 g^{-1} DM	Dini et al. (2010)
Quinoa (Regalona, B080, AG2010 Chile)	Methanol 99% (v/v)	DPPH CUPRAC	140-320 (field condition) 480-610 (greenhouse) 140-170 (field condition) 210-230 (greenhouse)	mg GAE 100 g^{-1} DM	Fischer et al. (2013)
Jinli-1 (China)	Acetone 80% (v/v); methanol, 2 M sodium hydroxide; 6 M hydrochloric acid; ethyl acetate	FRAP ORAC	196.43 1405	mg TE 100 g^{-1} DM	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 ORAC	110.26-216.71 37.61-70.48	mg TE 100 g^{-1} DM	Han et al. (2019b)
BRS-Piabiru (Brazil)	Methanol	FRAP DPPH	15.25 30.34	mg TE 100 g^{-1} DM	Nickel et al. (2016)
Quinoa	Ethanol 70% (v/v)	FRAP	13.13 (Korea) 8.42 (USA) 7.12 (Peru)	mM Fe^{2+} kg^{-1} DM	Park et al. (2017)
Quinoa (Bolivia)	Methanol, 0.16 M hydrochloric acid and water (8:1:1)	ABTS DPPH FRAP	2719 3884 497	mmol TE kg^{-1} DM mmol TE kg^{-1} DM mmol Fe^{2+} kg^{-1} DM	Paśko et al. (2009)
Commercial Red, white and black quinoa (Spain, Bolivia, Peru)	Methanol 80% and acetone 70% (v/v)	ABTS DPPH FRAP	388-776 194-501 237-457	mg TE 100 g^{-1} FM	Pellegrini et al. (2018)
24 Quinoa accessions (Peru)	Ethanol:water (1:1)	ABTS DPPH	878.44-1507.94 486.08-1195.74	μmol TE 100 g^{-1} FM	Valencia et al. (2017)
	Methanol:water (2:1)	ABTS DPPH	1036.55-1835.78 474.21-972.87	μmol TE 100 g^{-1} FM	
Ecotypes (Chile) Ancovinto Cancosa Cahuil, Faro, Regalona, Villarrica	Acetone:water (4:1)	DPPH ORAC	10.74-20.17 22.25-73.16	mmol TE 100 g^{-1} DM	Vega-Gálvez et al. (2018)

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.

Acknowledgements

Authors express their gratitude to the Agronomy Science postgraduate program, Universidad de Concepción, Chillán, Chile.

References

Abderrahim, F., Huanatico, E., Segura, R., Arribas, S., Gonzalez, M.C., Condezo-Hoyos, L. 2015. Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (*Chenopodium quinoa* Willd.) from Peruvian Altiplano. Food Chemistry 183:83-90. doi:10.1016/j.foodchem.2015.03.029.

Alencar, N.M.M., Oliveira, de C.L. 2018. Advances in pseudocereals: Crop cultivation, food application, and consumer perception. In Mérillon, J.M., Ramawat, K. (eds.) Bioactive molecules in food. Reference Series in Phytochemistry. Springer, Cham, Switzerland. doi:10.1007/978-3-319-54528-8_63-1.

Alseekh, S., Perez de Souza, L., Benina, M., Fernie, A. 2020. The style and substance of plant flavonoid decoration; towards defining both structure and function. Phytochemistry 174:112347. doi:10.1016/j.phytochem.2020.112347.

Alvárez-Jubete, L., Wijngaard, H., Arendt, E., Gallagher, E. 2010. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry 119:770-778. doi:10.1016/j.foodchem.2009.07.032.

Amarowicz, R., Pegg, RB. 2019. Natural antioxidants of plant origin. Advances in Food and Nutrition Research 90:1-81. doi:10.1016/bs.afnr.2019.02.011.

Balakrishnan, G., Goodrich-Schneider, R. 2020. Quinoa flavonoids and their bioaccessibility during in vitro gastrointestinal digestion. Journal of Cereal Science 95:103070. doi:10.1016/j.jcs.2020.103070.

Bermúdez-Soto, M-J., Tómas-Barberán, F-A., Gracía-Conesa, M-T. 2007. Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to in vitro gastric and pancreatic digestion. Food Chemistry 102(3):865-874. doi:10.1016/j.foodchem.2006.06.025.

Bommegowda, H., Singh, R. 2020. Phenolic acids from vegetables: A review on processing stability and health benefits. Food Research International 136:109298 doi:10.1016/j.foodres.2020.109298.

Bridi, R., Troncoso, M., Folch-Cano, C., Fuentes, J., Speisky, H., López-Alarcón, C. 2014. A polyvinylpolypyrrolidone (PVPP)-assisted Folin-Ciocalteu assay to assess total phenol content of commercial beverages. Food Analytical Methods 7:2075-2083. doi:10.1007/s12161-014-9856-0.

Buitrago, D., Buitrago-Villanueva, I., Barbosa-Cornelio, R., Coy-Barrera, E. 2019. Comparative examination of antioxidant capacity and fingerprinting of unfractionated extracts from different plant parts of quinoa (*Chenopodium quinoa*) grown under greenhouse conditions. Antioxidants 8(8):238. doi:10.3390/antiox808023.

Campos, D., Chirinos, R., Gálvez Ranilla, L., Pedreschi, R. 2018. Chapter Eight-Bioactive potential of Andean fruits, seeds, and tubers. In Toldrá, F. (ed.) Advances in Food and Nutrition Research 84:287-343. doi:10.1016/bs.afnr.2017.12.005.

Carciochi, R.A., Manrique, G.D. Dimitrov, K. 2015. Optimization of antioxidant phenolic compounds extraction from quinoa (*Chenopodium quinoa*) seeds. Journal of Food Science and Technology 52(7):4396-4404. doi:10.1007/s13197-014-1514-4.

Carocho, M., Morales, P., Ferreira, I.C.F.R. 2018. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends in Food Science & Technology 71:107-120. doi:10.1016/j.tifs.2017.11.008.

Çelik, S., Özyürek, M., Güclü, K., Apak, R. 2010. Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. Talanta 81(4-5):1300-1309. doi:10.1016/j.talanta.2010.02.025.

Chaudhary, N., Walia, S., Kumar, R. 2023. Functional composition, physiological effect and agronomy of future food quinoa (*Chenopodium quinoa* Willd.): A review. Journal of Food Composition and Analysis 118:105192. doi.org/10.1016/j.jfca.2023.105192.

Chen, Y.S., Aluwi, N.A., Saunders, S.R., Ganjyal, G.M., Medina-Meza, I.G. 2019. Metabolic fingerprinting unveils quinoa oil as a source of bioactive phytochemicals. Food Chemistry 286:592-599. doi:10.1016/j.foodchem.2019.02.016.
Chen, X., Zhang, Y., Cao, B., Wei, X., Shen, Z., Su, N. 2023. Assessment and comparison of nutritional qualities of thirty quinoa (Chenopodium quinoa Willd.) seed varieties. Food Chemistry 19:100808. doi.org/10.1016/j.fochx.2023.100808.
Chirinos, R., Pedreschi, R., Rogez, H., Larondelle, Y., Campos, D. 2013. Phenolic compound contents and antioxidant activity in plants with nutritional and/or medicinal properties from the Peruvian Andean region. Industrial Crops and Products 47:145-152. doi:10.1016/j.indcrop.2013.02.025.
Chito Trujillo, D., Ortega Bonilla, R., Ahumada Mamián, A., Rosero López, B. 2017. Quinoa (*Chenopodium quinoa* Willd.) versus soja (*Glycine max* [L.] Merr.) en la nutrición humana: revisión sobre las características agroecológicas, de composición y tecnológicas. Revista Española de Nutrición Humana y Dietética 21(2):184-198. doi:10.14306/renhyd.21.2.256.
Contreras-Jiménez, B., Torres-Vargas, O.L., Rodríguez-García, M.E. 2019. Physicochemical characterization of quinoa (*Chenopodium quinoa*) flour and isolated starch. Food Chemistry 298:124982. doi:10.1016/j.foodchem.2019.124982.
Dakhili, S., Abdolalizadeh, L., Hosseini, S.M., Shojaee-Aliabadi, S., Mirmoghtadaie, L. 2019. Quinoa protein: Composition, structure and functional properties. Food Chemistry 299:125161. doi:10.1016/j.foodchem.2019.125161.
de Araújo, F.F., de Paulo Farias, D., Neri-Numa, I.A., Pastore, G.M. 2021. Polyphenols and their applications: An approach in food chemistry and innovation potential. Food Chemistry 338:127535. doi:10.1016/j.foodchem.2020.127535.
de Menezes, B.B., Frescura, L.M., Duarte, R., Villetti, M.A., da Rosa, M.B. 2021. A critical examination of the DPPH method: Mistakes and inconsistencies in stoichiometry and IC_{50} determination by UV-Vis spectroscopy. Analytica Chimica Acta 1157:338398. doi:10.1016/j.aca.2021.338398.
Dini, I., Tenore, G.C., Dini, A. 2010. Antioxidant compound contents and antioxidant activity before and after cooking in sweet and bitter *Chenopodium quinoa* seeds. LWT - Food Science and Technology 43(3):447-451. doi:10.1016/j.lwt.2009.09.010.
Durazzo, A. 2017. Study approach of antioxidant properties in foods: Update and considerations. Foods 6(3):17. doi:10.3390/foods6030017.
Escuredo, O., González, M., Wells, G., Fischer, S., Hernández, J. 2014. Amino acid profile of the quinoa (*Chenopodium quinoa* Willd.) using near infrared spectroscopy and chemometric techniques. Journal of Cereal Science 60:67-74. doi:10.1016/j.jcs.2014.01.016.
Ferreira, I.C.F.R., Martins, N., Barros, L. 2017. Chapter One – Phenolic compounds and its bioavailability: in vitro bioactive compounds or health promoters? Advances in Food and Nutrition Research 82:1-44. doi:10.1016/bs.afnr.2016.12.004.
Filho, A.M., Pirozi, M.R., Borges, J.T., Pinheiro Sant'Ana, H.M., Chaves, J.B., Coimbra, J.S. 2017. Quinoa: Nutritional, functional, and antinutritional aspects. Critical Reviews in Food Science and Nutrition 57(8):1618-1630. doi:10.1080/10408398.2014.1001811.
Fiorito, S., Preziuso, F., Epifano, F., Scotti, L., Bucciarelli, T., Taddeo, V.A., et al. 2019. Novel biologically active principles from spinach, goji and quinoa. Food Chemistry 276:262-265. doi:10.1016/j.foodchem.2018.10.018.
Fischer, S., Wilckens, R., Jara, J., Aranda, M. 2013. Variation in antioxidant capacity of quinoa (*Chenopodium quinoa* Will) subjected to drought stress. Industrial Crops and Products 46:341-349. doi:10.1016/j.indcrop.2013.01.037.
Galili, S., Hovav, R. 2014. Determination of polyphenols, flavonoids, and antioxidant capacity in dry seeds. Polyphenols in Plants 1:305-323. doi:10.1016/B978-0-12-397934-6.00016-4.
Gordillo-Bastidas, E., Díaz-Rizzolo, D., Roura, E., Massanés, T., Gomis, R. 2016. Quinoa (*Chenopodium quinoa* Willd), from nutritional value to potential health benefits: An integrative review. Journal of Nutrition & Food Science 6:497. doi:10.4172/2155-9600.1000497.
Gülçin, I. 2012. Antioxidant activity of food constituents: an overview. Archives of Toxicology 86(3):345-391. doi:10.1007/s00204-011-0774-2.
Gülçin, I. 2020. Antioxidants and antioxidant methods: an updated overview. Archives of Toxicology 94(3):651-715. doi:10.1007/s00204-020-02689-3.
Gutteridge, J.M., Halliwell, B. 2010. Antioxidants: Molecules, medicines, and myths. Biochemical and Biophysical Research Communications 393(4):561-564. doi:10.1016/j.bbrc.2010.02.071.

Han, Y., Chi, J., Zhang, M., Zhang, R., Fan, S., Dong, L., et al. 2019a. Changes in saponins, phenolics and antioxidant activity of quinoa (*Chenopodium quinoa* Willd) during milling process. LWT-Food Science and Technology 114:10838. doi:10.1016/j.lwt.2019.108381.

Han, Y., Chi, J., Zhang, M., Zhang, R., Fan, S., Dong, L., et al. 2019b. Characterization of saponins and phenolic compounds: antioxidant activity and inhibitory effects on α-glucosidase in different varieties of colored quinoa (*Chenopodium quinoa* Willd). Bioscience, Biotechnology and Biochemistry 83(11):2128-2139. doi:10.1080/09168451.2019.1638756.

Hemalatha, P., Pedenla Bomzan, D., Sathyendra Rao, B.V., Sreerama, Y. 2016. Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chemistry 199:30-338. doi:10.1016/j.foodchem.2015.12.025.

Hoensch, H., Oertel, R. 2015. The value of flavonoids for the human nutrition: Short review and perspectives. Clinical Nutrition Experimental 3:8-14. doi:10.1016/j.yclnex.2015.09.001.

Iqbal, H., Yaning, C., Waqas, M., Shareef, M., Raza, S.T. 2018. Differential response of quinoa genotypes to drought and foliage-applied H_2O_2 in relation to oxidative damage, osmotic adjustment and antioxidant capacity. Ecotoxicology and Environmental Safety 164:344-354. doi:10.1016/j.ecoenv.2018.08.004.

Jamwal, K., Bhattacharya, S., Puri, S. 2018. Plant growth regulator mediated consequences of secondary metabolites in medicinal plants. Journal of Applied Research on Medicinal and Aromatic Plants 9:26-38. doi:10.1016/j.jarmap.2017.12.003.

Khalid, M., Saeed-ur-Rahman, Bilal, M., Huang, D-f. 2019. Role of flavonoids in plant interactions with the environment and against human pathogens — A review. Journal of Integrative Agriculture 18(1):211-230. doi:10.1016/S2095-3119(19)62555-4.

Kinyua Muthuri, L., Nagy, L., Nagy, G. 2021. Chronopotentiometric method for assessing antioxidant activity: A reagentless measuring technique. Electrochemistry Communications 122:106907. doi:10.1016/j.elecom.2020.106907.

Kumar, M., Dahuja, A., Tiwari, S., Punia, S., Tak, Y., Amarowicz, R., et al. 2021. Recent trends in extraction of plant bioactives using green technologies: A review. Food Chemistry 353:129431. doi:10.1016/j.foodchem.2021.129431.

Lan, Y., Zhang, W., Liu, F., Wang, L., Yang, X., Ma, S., et al. 2023. Recent advances in physiochemical changes, nutritional value, bioactivities, and food applications of germinated quinoa: A comprehensive review. Food Chemistry 426:136390. doi.org/10.1016/j.foodchem.2023.136390.

Lester, G., Lewers, K., Medina, M.B., Saftner, RA. 2012. Comparative analysis of strawberry total phenolics via Fast Blue BB vs. Folin-Ciocalteu: Assay interference by ascorbic acid. Journal of Food Composition and Analysis 27(1):102-107. doi:10.1016/j.jfca.2012.05.003.

Lin, M., Han, P., Li, Y., Wang, W., Lai, D., Zhou, L. 2019. Quinoa secondary metabolites and their biological activities or functions. Molecules 24(13):2512. doi:10.3390/molecules24132512.

Liu, K., Zha, X-Q., Li, Q-M., Pan, L-H., Luo, J-P. 2021. Hydrophobic interaction and hydrogen bonding driving the self-assembling of quinoa protein and flavonoids. Food Hydrocolloids 118:106807. doi:10.1016/j.foodhyd.2021.106807.

Luisetti, J., Lucero, H., Ciappini, M.C. 2020. Preliminary study to optimize the extraction of bioactive phenolic compounds from quinoa (*Chenopodium quinoa* Willd). Revista de Ciencia y Tecnología 33(1):94-99. doi:10.36995/j.recyt.2020.33.01.

Mark, R., Lyu, X., Lee, J.J.L., Parra-Saldívar, R., Chen, W. 2019. Sustainable production of natural phenolics for functional food applications. Journal of Functional Foods 57:233-254. doi:10.1016/j.jff.2019.04.008.

Martínez-Villaluenga, C., Peñas, E., Hernández-Ledesma, B. 2020. Pseudocereal grains: Nutritional value, health benefits and current applications for the development of gluten-free foods. Food and Chemical Toxicology 137:111178. doi:10.1016/j.fct.2020.111178.

Miranda, M., Delatorre-Herrera, J., Vega-Gálvez, A., Jorquera, E., Quispe-Fuentes, I., Martínez, E.A. 2014. Antimicrobial potential and phytochemical content of six diverse sources of quinoa seeds (*Chenopodium quinoa* Willd.) Agricultural Sciences 5(11):1015-1024. doi:10.4236/as.2014.511110.

Miranda, M., Vega-Gálvez, A., López, J., Parada, G., Sanders, M., Aranda, M., et al. 2010. Impact of air-drying temperature on nutritional properties, total phenolic content and antioxidant capacity of quinoa seeds (*Chenopodium quinoa* Willd.) Industrial Crops and Products 32(3):258-263. doi:10.1016/j.indcrop.2010.04.019.

Miranda, M., Vega-Gálvez, A., Martínez, E., López, J., Marín, R., Aranda, M., et al. 2013. Influence of contrasting environments on seed composition of two quinoa genotypes: nutritional and functional properties. Chilean Journal of Agricultural Research 73:108-116. doi:10.4067/S0718-58392013000200004.

Miranda, M., Vega-Gálvez, A., Quispe-Fuentes, I., Rodríguez, M., Maureira, H., Martínez, E. 2012. Nutritional aspects of six quinoa (*Chenopodium quinoa* Willd.) ecotypes from three geographical areas of Chile. Chilean Journal of Agricultural Research 72:175-181. doi:10.4067/S0718-58392012000200002.
Miranda, M., Vega-Gálvez, A., Uribe, E., López, J., Martínez, E., Rodríguez, M.J., et al. 2011. Physico-chemical analysis, antioxidant capacity and vitamins of six ecotypes of Chilean quinoa (*Chenopodium quinoa* Willd). Procedia Food Science 1:1439-1446. doi:10.1016/j.profoo.2011.09.213.
Mishra, K., Ojha, H., Chaudhury, N.K. 2012. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry 130(4):1036-1043. doi:10.1016/j.foodchem.2011.07.127.
Mufari, J., Miranda-Villa, P., Calandri, E. 2018. Quinoa germ and starch separation by wet milling, performance and characterization of the fractions. LWT-Food Science and Technology 96:527-534. doi:10.1016/j.lwt.2018.06.010.
Multari, S., Marsol-Vall, A., Keskitalo, M., Yang, B., Suomela, J-P. 2018. Effects of different drying temperatures on the content of phenolic compounds and carotenoids in quinoa seeds (*Chenopodium quinoa*) from Finland. Journal of Food Composition and Analysis 72:75-82. doi:10.1016/j.jfca.2018.06.008.
Navruz-Varli, S., Sanlier, N. 2016. Nutritional and health benefits of quinoa (*Chenopodium quinoa* Willd.) Journal of Cereal Science 69:371-376. doi:10.1016/j.jcs.2016.05.004 0733-5210.
Nickel, J., Spanier, L.P., Botelho, F.T., Gularte, M.A., Helbig, E. 2016. Effect of different types of processing on the total phenolic compound content, antioxidant capacity, and saponin content of *Chenopodium quinoa* Willd grains. Food Chemistry 209:139-143. doi:10.1016/j.foodchem.2016.04.031.
Nowak, V., Du, J., Charrondière, U.R. 2016. Assessment of the nutritional composition of quinoa (*Chenopodium quinoa* Willd.) Food Chemistry 193:47-54. doi:10.1016/j.foodchem.2015.02.111.
Ou, J., Wang, M., Zheng J., Ou, S. 2019. Positive and negative effects of polyphenol incorporation in baked foods. Food Chemistry 284:90-99. doi:10.1016/j.foodchem.2019.01.096.
Park, J.H., Lee, Y.J., Kim, Y.H., Yoon, K.S. 2017. Antioxidant and antimicrobial activities of quinoa (*Chenopodium quinoa* Willd.) seeds cultivated in Korea. Preventive Nutrition and Food Science 22(3):195-202. doi:10.3746/pnf.2017.22.3.195.
Paśko, P., Barton, H., Zagrodzki, P., Gorinstein, S., Folta, M., Zachwieja, Z. 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chemistry 115(3):994-998. doi:10.1016/j.foodchem.2009.01.037.
Pękal, A., Pyrzynska, K. 2014. Evaluation of aluminium complexation reaction for flavonoid content assay. Food Analytical Methods 7:1776-1782. doi:10.1007/s12161-014-9814-x.
Pellegrini, M., Lucas-González, R., Fernández-López, J., Ricci, A., Pérez-Alvares, J., Lo Sterzo, C., et al. 2017. Bioaccessibility of polyphenolic compounds of six quinoa seeds during in vitro gastrointestinal digestion. Journal of Functional Foods 38(Part A):77-88. doi:10.1016/j.jff.2017.08.042.
Pellegrini, M., Lucas-Gonzales, R., Ricci, A., Fontecha, J., Fernández-López, J., Pérez-Álvarez, J., et al. 2018. Chemical, fatty acid, polyphenolic profile, techno-functional and antioxidant properties of flours obtained from quinoa (*Chenopodium quinoa* Willd) seeds. Industrial Crops and Products 111:38-46. doi:10.1016/j.indcrop.2017.10.006.
Pereira, E., Cadavez, V., Barros, L., Encina-Zelada, Ch., Stojkovic, D., Sokovic, M., et al. 2020. *Chenopodium quinoa* Willd. (quinoa) grains: A good source of phenolic compounds. Food Research International 137:109574. doi:10.1016/j.foodres.2020.109574.
Prior, R. 2015. Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactives and health benefits. Journal of Functional Foods 18(Part B):797-810. doi:10.1016/j.jff.2014.12.018.
Prior, R.L., Wu, X., Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53(10):4290-4302. doi:10.1021/jf0502698.
Ramzani, P.M.A., Shan, L., Anjum, S., Khan, W.-u.-D., Ronggui, H., Iqbal, M., et al. 2017. Improved quinoa growth, physiological response, and seed nutritional quality in three soils having different stresses by the application of acidified biochar and compost. Plant Physiology and Biochemistry 116:127-138. doi:10.1016/j.plaphy.2017.05.003.
Reguera, M., Conesa, C.M., Gil-Gómez, A., Haros, C.M., Pérez-Casas, M.Á., Briones-Labarca, V., et al. 2018. The impact of different agroecological conditions on the nutritional composition of quinoa seeds. PeerJ 6:e4442. doi:10.7717/peerj.4442.
Romano, N., Ureta, M.M., Guerrero-Sánchez, M., Gómez-Zavaglia, A. 2020. Nutritional and technological properties of a quinoa (*Chenopodium quinoa* Willd.) spray-dried powdered extract. Food Research International 129:108884. doi:10.1016/j.foodres.2019.108884.

Schaich, K.M., Tian, X., Xie, J. 2015. Reprint of “Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays”. Journal of Functional Foods Part B 18:782-796. doi:10.1016/j.jff.2015.05.024.

Shahidi, F., Ambigaipalan, P. 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review, Journal of Functional Foods 18(Part B):820-897. doi:10.1016/j.jff.2015.06.018.

Siddeeg, A., AlKehayez, N.M., Abu-Hiamed, H.A., Al-Sanea, E.A., AL-Farga, A.M. 2021. Mode of action and determination of antioxidant activity in the dietary sources: An overview. Saudi Journal of Biological Sciences 28(3):1633-1644. doi:10.1016/j.sjbs.2020.11.064.

Świeca, M., Sęczyk, Ł., Gawlik-Dziki, U., Dziki, D. 2014. Bread enriched with quinoa leaves – The influence of protein–phenolics interactions on the nutritional and antioxidant quality. Food Chemistry 162:54-62. doi:10.1016/j.foodchem.2014.04.044.

Tang, Y., Tsao, R. 2017. Phytochemicals in quinoa and amaranth grains and their antioxidant, anti-inflammatory and potential health beneficial effects: a review. Molecular Nutrition & Food Research 61(7):1-16. doi:10.1002/mnfr.201600767.

Teixeira, J., Gaspar, A., Garrido, E.M., Garrido, J., Borges, F. 2013. Hydroxycinnamic acid antioxidants: an electrochemical overview. BioMed Research International 2013:251754. doi:10.1155/2013/251754.

Thakur, M., Bhattacharya, S., Khosla, P.K., Puri, S. 2019. Improving production of plant secondary metabolites through biotic and abiotic elicitation. Journal of Applied Research on Medicinal and Aromatic Plants 12:1-12. doi:10.1016/j.jarmap.2018.11.004.

Valcárcel-Yamani, B., Lannes, da S.S. 2012. Applications of quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* spp.) and their influence in the nutritional value of cereal-based foods. Food and Public Health 2(6):265-275. doi:10.5923/j.fph.20120206.12.

Valencia, Z., Cámara, F., Ccapa, K., Catacora, P., Quispe, F. 2017. Compuestos bioactivos y actividad antioxidante de semillas de quinua peruana (*Chenopodium quinoa* W.) Revista de la Sociedad Química del Perú 83(1):16-29. http://www.scielo.org.pe/pdf/rsqp/v83n1/a03v83n1.pdf.

Vázquez-Luna, A., Fuentes, F., Rivadeneyra, E., Hernández, C., Díaz-Sobac, R. 2019. Nutrimental content and functional properties of quinoa flour from Chile and Mexico. Ciencia e Investigación Agraria 46(2):144-153. doi:10.7764/rcia.v46i2.209.

Vega-Gálvez, A., Zura, L., Lutz, M., Jagus, R., Agüero, M.V., Pastén, A., et al. 2018. Assessment of dietary fiber, isoflavones and phenolic compounds with antioxidant and antimicrobial properties of quinoa (*Chenopodium quinoa* Willd.) Chilean Journal of Agricultural & Animal Science 34(1):57-67. doi:10.4067/S0719-38902018005000101.

Verma, N., Shukla, S. 2015. Impact of various factors responsible for fluctuation in plant secondary metabolites. Journal of Applied Research on Medicinal and Aromatic Plants 2(4):105-113. doi:10.1016/j.jarmap.2015.09.002 2214-7861.

Wianowska, D., Gil, M. 2019. Critical approach to PLE technique application in the analysis of secondary metabolites in plants. TrAC Trends in Analytical Chemistry 114:314-325. doi:10.1016/j.trac.2019.03.018 0165-9936.

Xiao, F., Xu, T., Lu, B., Liu, R. 2020. Guidelines for antioxidant assays for food components. Food Frontiers 1(1):60-69. doi:10.1002/fft2.10.

Zhang, B., Zhang, Y., Li, H., Deng, Z., Tsao, R. 2020. A review on insoluble-bound phenolics in plant-based food matrix and their contribution to human health with future perspectives. Trends in Food Science & Technology 105:347-362. doi:10.1016/j.tifs.2020.09.029.

Zulueta, A., Esteve, M.J., Frígola, A. 2009. Analytical methods ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. Food Chemistry 114(1):310-316. doi:10.1016/j.foodchem.2008.09.033.