Goat Manure Fertilization Effect on Saponin and Protein Content in Quinoa (Chenopodium quinoa Willd) Grain of Different Origin

Article · July 2020

DOI: 10.36632/mejar/2020.9.2.34

Juan Antonio González
Fundación Miguel Lillo

Middle East Journal of Agriculture Research Volume : 09 | Issue : 02| April-June | 2020
EISSN: 2706-7955 ISSN: 2077-4605 Pages:434-443
DOI: 10.36632/mejar/2020.9.2.34

Goat Manure Fertilization Effect on Saponin and Protein Content in Quinoa (*Chenopodium quinoa* Willd) Grain of Different Origin

Juan Antonio González[1], Emilio Fernando Lizarraga[1,2], Luis Ernesto Erazzú[3], Luciana Martinez-Calsina[3], Sebastián Edgardo Buedo[1] and Daniela Alejandra González[4]

[1]Instituto de Ecología, Fundación Miguel Lillo. Miguel Lillo 251. Tucumán (Argentina). jagonzalez@lillo.org.ar
[1]Instituto de Fisiología Animal. Fundación Miguel Lillo. Miguel Lillo 251. Tucumán (Argentina). sebuedo@lillo.org.ar.
[2]Facultad de Ciencias Naturales e Instituto Miguel Lillo. Universidad Nacional de Tucumán. Miguel Lillo 205. Tucumán (Argentina). eflizarraga@lillo.org.ar
[3]Instituto Nacional de Tecnología Agropecuaria (INTA). Famaillá, Tucumán (Argentina) – Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán. erazzu.luis@inta.gob.ar
[4]Instituto de Bioprospección y Fisiología Vegetal (INBIOFIV). CONICET-UNT; Facultad de Ciencias Naturales e Instituto Miguel Lillo. danigonz37@gmail.com

Received: 07 April 2020 / Accepted 10 June 2020 / Publication date: 20 June 2020

ABSTRACT

Background and objectives: A field experiment using two quinoa varieties (Regalona Baer and CICA) was performed to determine the effects of goat manure fertilization on saponin and protein grain content was performed. A trial was conducted in a valley at 2,000 m a.s.l (Tucumán, Argentina) and at different levels of goat manure addition equivalent to 0, 32, 64, 128, 192 kg N/ha.

Findings: Saponin and protein content increased almost linearly as goat manure fertilization increased. However, there was a varietal difference, R. Baer synthesized more saponin than CICA at the same nitrogen level. ***Conclusions:*** The study indicated that the quinoa saponin content, in absence of water stress and salinity, but under the same agronomical managements, increased linearly with goat manure addition. Probably the differences in response to fertilization of both varieties were related to the genotypes origin, one from high mountain (CICA) and the other from lowland (R. Baer).

Significance and novelty: Goat manure fertilization on quinoa produced more proteins but the saponin synthesis increased in the same way. Additionally, quinoa varieties showed variability for the saponin content. Thus, it is necessary to found an equilibrium point to produce a grain for human consumption with high protein and low saponin content or a grain with high protein and high saponin content for a later industrial separation of both compounds.

***Keywords*:** Quinoa, fertilization, nitrogen, saponin, protein.

Introduction

Quinoa (*Chenopodium quinoa* Willd.), that is originated from the Andes, is today considered one of the world´s healthiest and has a relatively new introduction to our daily diet. At least 110 countries are evaluating quinoa as a complementary crop (Bazile and Baudron 2015). Quinoa has been selected due to its high nutritional values, which are essential amino acids, minerals, vitamins and other compounds like polyphenols, phytosterols and phenols (González, Roldán, Gallardo, Escudero & Prado, 1989) González, Eisa, Hussin & Prado, 2015). These all means that quinoa should be considered as a nutraceutical or functional food (Graf *et al*., 2015). Furthermore, quinoa can grow at high and low elevation, within marginal soils and also extremely stressful weather conditions (González *et al.,* 2015). However, beyond the aforementioned advantages quinoa contains saponins with bitter taste (Kuljanabhagavad and Wink, 2009). In plants, saponins acts as a chemical barrier or shield in the plant defense system to counter pathogens and herbivores (Augustin, Kuzina, Anderson & Bak, 2011). However, if the quinoa grain is used for human food saponins should be removed before consumption. This bitter water-soluble compound is accumulated in the external layers of the grain (pericarp) (Prado, Gallardo & González, 1996). Due to saponin is located in the pericarp and for its water solubility this compound can be easily removed by using a rubbing method or water

Corresponding Author: González, Juan Antonio, Instituto de Ecología, Fundación Miguel Lillo. Miguel Lillo 251. Tucumán (Argentina). E-mail: jagonzalez@lillo.org.ar

dissolution. Saponins, from a chemical point of view, are a non-volatile secondary metabolite distributed mainly in plants. They are glycosidic triterpenoids, steroids, and to a lesser extent alkaloid. Triterpenoid saponins are mainly abundant in dicotyledons while steroidal saponins are abundant in monocotyledons (Netala, Ghosh, Bobbu, Anitha & Tartte, 2014). The aglycone portion of saponins is known as sapogenin and the glucidic part are generally oligosaccharides. The sugar associates are glucose, arabinose and galactose. The number or position of sugar units attached to the triterpene, or steroidal skeleton, originate several structures present in more than 100 plant families (Güçlü-Ustündağ and Mazza, 2007). Saponins in quinoa are basically triterpenoids with glucose constitution about 80% of the weight and they are a complex mixture of glycosides triterpenic and has been report for this complex: oleanolic acid, hederagine, phytoacagenic acid, serjanic acid, 3β-Hydroxi-23-oxo-olean-12-en-28-oic acid, 3β-Hydroxi-27-oxo-olean-12-en-28-oic acid and 3β,23α,30β-Trihydroxi-olean-12-en-28-oic acid.

The variation of the quinoa saponin content depends on the genotype's origin. In general, some Ecuatorian, Peruvian and Bolivian quinoa cultivars (between 3,000 to 4,200 m asl) had low levels of grain saponin (Reichter, Tatarynovich &Tyler, 1986). However, those from lowland site (near sea level) in Chile presented much higher saponin content (Miranda *et al.,* 2012). In general, saponin content depends on the growth, biotic inducers such as pathogens (fungi or bacteria), herbivores (Szakiel, Paczkowski & Henry, 2010), abiotic factors such as light, temperature, humidity (Szakiel, Paczkowski & Henry, 2011), soil salinity (Gómez-Caravaca, Segura-Carretero, Fernández-Gutierrez & Caboni, 2012) and agronomic conditions and post-harvest treatments (Fenwick, Price, Tsukamoto & Okubo, 1991). For example, water stress during quinoa development, reduced saponins content nearly 45 % (Gómez-Caravaca, Segura-Carretero, Fernández-Gutiérrez & Caboni, 2011), while salinity conditions increased it (Pulvento *et al.,* 2012). As a crop management, irrigation and fertilizers addition are regular agronomic practices used to increase grain production and to obtain higher protein content. Unfortunately, these practices produce high saponin contents (Miranda *et al.,* 2012; Bilalis *et al.,* 2012). However, little information is available related to the response varieties and agronomic practices. Therefore, the aim of this work was to study the relationship between goat manure fertilization, protein content and saponin accumulation on quinoa grains of two contrasting quinoa varieties (one from sea level and other from mountain place) without water stress and similar crop managements.

Materials and Methods

Plant material and experiment

Two quinoa varieties, CICA from the Perú high valleys and Regalona Baer from the Chile lowland, were used. CICA is cultivated in Argentinean Northwest since the FAO Project American and European Quinoa Test (1996-1998) (Mujica, Jacobsen, Izquierdo & Marathee, 2001) and since then it is the most commonly used variety. CICA has been used at Encalilla (Amaicha, Tucumán, Argentina) since 2000. In the last 5 five years, R. Baer variety was incorporated in experimental fields.

During the growing seasons of 2014- 2015 and 2015-2016 a field experiment was performed in an arid valley of the Argentinean Northwest (Encalilla, Amaicha del Valle, 22º 31´S, 65º 59´W, 1,995 m a.s.l., Tucumán, Argentina). The soil of Encalilla is classified as Xeric Torriorthent type (USDA, 2010) with sandy clay loam texture (0–50 cm soil depth), pH 8.4, electrical conductivity (EC) 2.0 dS m^{-1}, exchangeable sodium (ES) 38.6% and cation exchange capacity (CEC) 12.3 cmol kg^{-1} (equal to meq/100 g), organic matter 0.6 % and total nitrogen content 0.055 % (González, Konishi, Bruno, Valoy & Prado, 2011). A randomized complete block design experiment with three replications for each variety was performed. The treatments were: CC: CICA (C) Control (without N), C2, C4, C8 and C12 (CICA with 2, 4, 8 and 12 t manure/ha respectively) and RC: Regalona Baer (R) Control (without N), R2, R4, R8 and R12 (Regalona Baer with 2, 4, 8 and 12 tn manure/ha respectively). In all the case 2, 4, 8 and 12 tn manure/ha were equivalent to 32, 64, 128 and 192 kgN/ha respectively. The goat manure was applied twice: one just before sowing and other at the beginning of reproductive phase. Quinoa was sown by hand at a depth of 2-3 cm. five rows (5-m long) per plot, 10 cm interplant spacing and 50 cm between rows was the spatial arrange. When the first two leaves emerged, a handthinning was carried out to give a seedling rate of 100,000 plants ha^{-1}. Drip irrigation was applied twice a week during the first month and then weekly during the following months. At 150 days after sowing, 10 plants from the three central rows were hand-harvested and then the grains were analyzed for their saponin and protein content.

Goat manure fertilization

Fertilization was based on organic goat manure obtained from local farmers located of the same location were quinoa grown. Manure analysis was performed and results were summarized in Table 1. The analysis of the goat manure revealed a high EC, and a low content of P, K Ca and Mg (Table 1).

Table 1: Goat manure analysis

pH	7.8
Humidity (%)	44.5
Electrical conductivity (dS m^{-1})	10.4
Organic matter (%)	28.8
Total nitrogen (%)	1.6
K (%)	0.1
Ca (%)	1.01
K (%)	0.1
Mg (%)	0.23
P (%)	0.31

Saponin and protein contents determinations

The total saponin content was determined according to slightly modified method proposed by Helaly, Soliman, Soheir & Ahmed (2001). The foundation of this spectrophotometric method is the reaction of oxidized triterpene saponins with vanillin (Li *et al.,* 2010). Sulfuric acid was employed as oxidant and the purple colour is a general characteristic of this reaction. Saponin was extracted from 20 g of quinoa grains (control and treatments) by exhaustive mechanical maceration with 80 % and 50% ethanol during 24 h respectively. Both extracts were gathered and concentrated at low pressure obtaining a gummy residue. All extracts were stored at -4 ºC until used. The extracts of each sample were solubilized with 50% aqueous ethanol at 5 mg/mL and an extract aliquot (0.125 mL) was added at the same volume of 8% vainillin in absolute ethanol (prepared freshly). Then the tubes were set in a bath at 5 ºC and 1.25 mL of 72% sulfuric acid aqueous was added and mixed for 20 seconds. For the blank tube, 0.125 mL of absolute ethanol was mixed with the same volumes of 8% vainillin and 72% sulfuric acid cited above. The tubes were then set in a thermostat at 60 ºC for 20 min and them at 0 ºC for 5 min. Measurements were performed at 538 nm in a Specord S600 spectrophotometer. Total saponin was expressed as mg oleanolic acid equivalents (mg OAE) per grams of extract and grains. All total saponin values are presented as means of triplicate analyses. Calibration curves, using oleanolic acid, were made by diluting stock standards in absolute ethanol to yield 0.005- 0.01 mg/mL. Five calibration points were used and analyses were replicated three times for each calibration point (n = 3). Linear regression of data followed the correlation coefficient was R^2= 0.9944. The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25. All methodologies followed the recommendations of AOAC (1990). All measurements were done for triplicate.

Statistical analyses

Data were expressed as the mean values ± standard deviation. The data were also analyzed by one-way analysis of variance using one-way ANOVA using InfoStat® (Infostat, 2008) statistical software. Duncan's multiple range test was employed to compare the significant differences among mean of the treatments at 95 % level of confidence.

Results

Grain nitrogen and protein content

As we increased goat manure fertilization, percentage of nitrogen in the grain also increased, reaching the maximum at 12 tn manure/ha dose (equal to 192 kgN/ha). The maximum grain nitrogen content for the two quinoa varieties in comparison with control was 43 %. A linear pattern was found in both varieties (Fig. 1). Concerning protein content, CICA increased from 15.5 % in CC to 22.1 % in C192 treatment, while in Regalona Baer it raised from 15.0 % to 21.4 % for CC and C12 treatments, respectively.

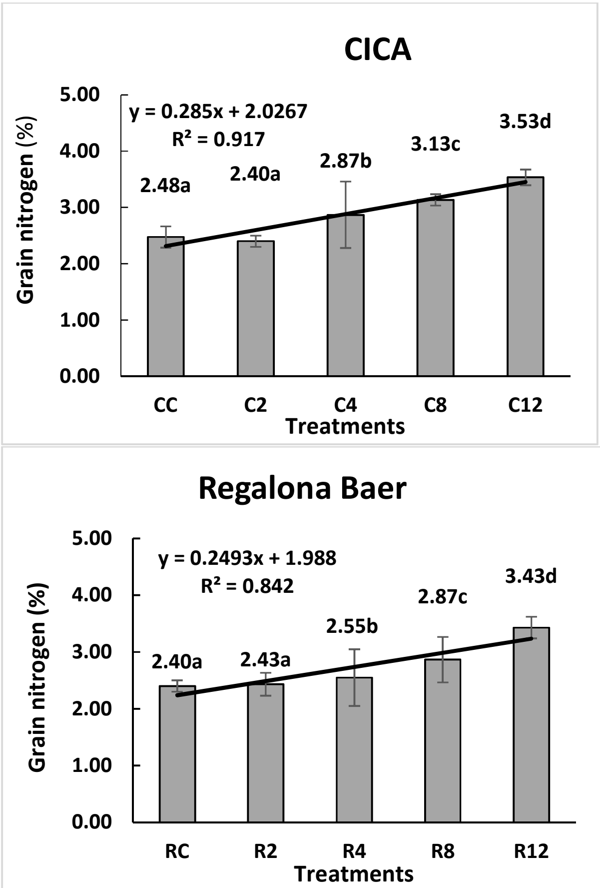

Fig. 1: Grain nitrogen content of two quinoa varieties growing under different nitrogen fertilization. Bars show mean values ±SE (n=4). Different letters represent significant difference among treatments at $P \leq 0.05$.

Grain saponin content

Differences in saponin content were detected between quinoa varieties under different goat manure fertilizations. At any treatment, R. Baer showed the highest saponin content in relation to CICA (Fig. 2). Maximum saponin content recorded at 12 tn manure/ha (equal to 192 kgN/ha) was 8.61 % and 6.93 % for Regalona Baer and CICA respectively. Thus, comparing maximum dose to control treatments, saponin content was 2.0 and 1.7 times higher for CICA and Regalona Baer, respectively. Both varieties showed a linear increase in saponins synthesis as fertilization increased (Fig. 2).

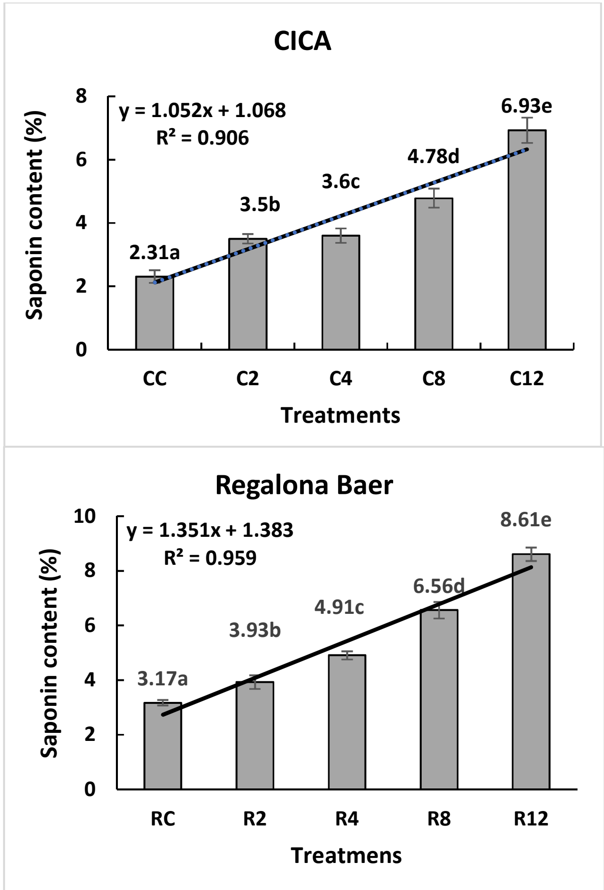

Fig. 2: Grain saponin content of two quinoa varieties growing under different nitrogen fertilization. Bars show mean values ±SE (n=4). Different letters represent significant difference among treatments at $P \leq 0.05$.

Discussion

Among others elements goat manure has higher nitrogen that is an essential mineral nutrient required by plants for its development. In general, the effect of nitrogen fertilization on plant morphology and physiology is well documented (Gastal and Lemaire 2002). Particularly in quinoa as it is known that yield and metabolism respond strongly to nitrogen fertilization (Almadini, Badran and Algosaibi, 2019; Bascuñan-Godoy *et al.,* 2018; Kakabouki *et al.,* 2014; Bilalis *et al.,* 2012; Schulte auf'm Erley, Kaul, Kruse & Aufhammer, 2005; Basra, Iqbal and Afzal, 2014; Berti *et al.,* 2000). However, little attention has been placed on the relationship between quinoa nitrogen fertilization and the production of saponins. Bilalis *et al.* (2012) found a positive correlation between both parameters and our study showed similar result. However, it is necessary to take into account that saponin content may be affected by tillage system and fertilization (Bilalis *et al.,* 2012) and other environmental and agronomic factors associated with plant growth (Fenwick *et al.,* 1991). Considering the Bilalis *et al.* (2012) study we performed our experiment maintaining the same tillage and watering control. The last was controlled because water stress has an effect on saponin content (Pulvento *et al.,* 2012; Martinez, Veas, Jorquera, San Martín & Jara, 2009). Thus, the probable effect of water stress on saponin synthesis in our experiment would have been negligible due to drip irrigation during the entire plant cycle. In the same way, there was no influence of salinity on saponin synthesis due to the low salinity content (electrical conductivity of 2.0 dS m^{-1}) in the soil where the quinoa crop was grown (Prado, Fernández-Turiel, Tsarouchi, Psaras & González, 2014). Other factors that can influence the synthesis of saponins are attacks by fungi and bacteria, herbivory; or even the effect of light, temperature and humidity (Faizal and Geelen, 2013; Szakiel, Paczkowski & Henry, 2011). In our study we did not detected fungi or bacteria and the two varieties used were under the same light, temperature and humidity regime.

It is worth noting that the saponin increments under different nitrogen fertilization levels obtained in Encalilla site were independent of the geographical quinoa origin because the same linear increment pattern was obtained for CICA, originated in the high mountain of Perú, and for Regalona Baer from Chile lowland. Thus, the increase in the content of saponins under different nitrogen treatments should be taken into account by farmers in general and in particular by those who are cropping CICA and Regalona Baer in the Argentinean northwest.

According to some references the expected quinoa grain saponin content in different quinoa varieties ranges from 2 to 5 % (Medina-Meza, Aluwi, Saunders & Ganjyal, 2016; Vega-Gálvez *et al.,* 2010). We obtained values from 2.3 to 6.9 % in CICA and 3.2 to 8.6 % in R. Baer. Quinoa was classified in a semi-quantitative scale as "sweet", "semi-sweet" or "bitter" according to its saponin content (Fenwick, Price, Tsukamoto & Okubo, 1991). As reported by Koziol (1991) a quantitative scale is possible considering a "sweet" grain if the saponin content is less than 0.11 % in a fresh weight basis. According to Mastebroek, Limburg, Gilles & Marvin (2000) the saponin content in grain of sweet genotypes varied from 0.2 – 0.4 g/kg dry weight (0.02 % to 0.04 %) and "bitter" if the saponin content is > 4.7 g/kg dry weight (> 0.47 %). Some sensory tests determined that the maximum tolerance for saponin content in the cooked grain was below 0.1 % (Nieto and Soria, 1991). According to these results the maximum acceptable level of saponin in quinoa for human consumption varies from 0.06% to 0.12% (Bacigalupo and Tapia, 1990; Vega-Gálvez *et al.,* 2010). Our results showed that CICA and R. Baer should be classified as "bitter" under any fertilization treatments used. According to bibliography, there is little previous information about saponin content in these varieties. The only references are related to Regalona, grown in south Chile, with saponin content from 2.2 to 3.2 % (Miranda *et al.,* 2012). According to Medina-Meza *et al.* (2016) the bitterness was due to the presence of phytolaccagenic acid. The bitterness is undesirable for quinoa consumption but interesting from the pharmaceutical point of view due to clinical applications or agricultural one like antifungal or commercial applications (Güçlü-Ustündağ and Mazza, 2007). For example, there are also precedents that saponins have beneficial effects for health, acting as strengthening of the immune system (Verza *et al.* 2012), anti-carcinogenic, hypocholesterolemic (Shi *et al*. 2004) and even anti-inflammatory properties (Yao, Xiushi, Zhenxing & Guixing, 2014; Norato *et al*. 2014; Norato, Murphy & Chew 2019). Some agronomic applications, as antifungal, would also be possible (Martin, Ndjoko & Hostettmann, 2008; Stuardo and San Martin 2008). From the human nutrition point of view, the ideal situation would be to obtain a "sweet quinoa" with a low saponin and high protein content. These can be obtained through the aid of biotechnology and undoubtedly requires knowledge of the genes involved in the synthesis of saponins. Data from Fiallos-Jurado *et al.* (2016) could be the beginning, although is necessary to point out that saponins are necessary for the plant defense against pathogens. Thus, the complex interrelation among genotypes origin, gentoypes x environment interaction, agricultural practices, cost/benefit calculus and saponin and protein synthesis will be necessary to be evaluated in a near future. Undoubtedly, the screening of varieties or quinoa populations from different origins will be the starting point for these future investigations.

Conclusions

The study indicated that the quinoa saponin content, in absence of water stress and salinity, but under the same agronomical conditions, increased linearly with goat manure fertilization. This result is independent of the variety origin (lowland or high mountain) but more saponin is synthetized in a lowland genotype. Although quinoa is a multipurpose species, at present all crops are aimed to produce grains for human consumption. However, until a quinoa with a high protein value, low in saponin content is obtained, without implying a lower resistance to pests, saponins should be considered as a byproduct of high biological value.

Acknowledgements

This work was supported by Miguel Lillo Foundation ("Ecophysiology of selected *Chenopodium quinoa* varieties and other species of nutritional value in field and lab". (Project B-0004-1) and National Institute of Agricultural Technology (INTA). Our thanks to Ilce Gabriela Medina-Meza (Michigan State University – USA) for her valuable comments.

Conflict of interest: the authors declare do not have any conflict of interest

References

Al Madini, A.M., Badran, and A.M. Algosaibi, 2019. Evaluation of Efficiency and Response of Quinoa Plant to Nitrogen Fertilization levels. Middle East J. Appl. Sci., 09: 839-849. DOI:10.36632/mejas/2019.9.4.1

AOAC. 1990. Official method of analysis. 15th ed. Association of Official Analytical Chemists (AOAC), Washington, DC, USA.

Augustin, J.M., V. Kuzina, S.B. Anderson, and S. Bak, 2011. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry, 72, 435-457.
https://doi: 10.1016/j.phytochem.2011.01.015.

Bacigalupo, A. and M. Tapia, 1990. Potencial agroindustrial de los cultivos andinos subexplotados. In: Tapia, M. (Eds.), Cultivos andinos subexplotados y su aporte a la alimentación, 136-163. Santiago, Chile: FAO, Ediciones Gegra S.A.

Bascuñan-Godoy, L., C. Sanhueza, K. Pinto, F. Cifuentes, M. Reguera, V. Briones, A. Zurita-Silva, R. Álvarez, A. Morales, and H. Silva, 2018. Nitrogen physiology of contrasting genotypes of *Chenopodium quinoa* Willd. (Amaranthaceae). Scientific Reports, 8: 17524.
https://doi:10.1038/s41598-018-34656-5

Basra, S.M.A., S. Iqbal, and I. Afzal, 2014. Evaluating the response of nitrogen application on growth, development and yield of quinoa genotypes. Int. J. Agric. Biol. 16, 886- 892.
https://doi: 13–1486/2014/16–5–886–892.

Bazile, D. and F. Baudron, 2015. The dynamics of the global expansion of quinoa growing in view of its high biodiversity. Chapter 1.4. In FAO and CIRAD. State of the art report of quinoa in the world in 2013, 42-45. Rome.

Berti, M., R. Wilckens, F. Hevia, H. Serri, I. Vidal, C. Mendez, 2000. Fertilizacion nitrogenada en quinoa (*Chenopodium quinoa* Willd.). Ciencia Investigación Agraria 27:81-90. DOI:10.7764/rcia.v27i2.999.

Bilalis, D., I. Kakabouki, A. Karkanis, I. Travlos, V. Triantafyllidis, and D. Hela, 2012. Quinoa seed and saponin production of organic quinoa (*Chenopodium quinoa* Willd.) for different tillage and fertilization. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40, 42-46. https://doi: http://dx.doi.org/10.15835/nbha4017400.

Faizal A, and D. Geelen 2013. Saponins and their role in biological processes in plants. Phytochem. Rev. 12(4): 877-893.

Fenwick, G. R., K. R. Price, C. Tsukamoto, and K. Okubo, 1991. Saponins. In: J.P.F. D'Mello, C.M. Duffus, and J.H. Duffus (Eds.), Toxic Substances in Crop Plants. The Royal Society of Chemistry, Cambridge, 285–327.

Fiallos-Jurado, J., J. Pollier, T. Moses, P. Arendt, N. Barriga-Medina, E. Morillo, V. Arahana, M.L. Torres, A. Goossens, and A. Leon-Reyes, 2016. Saponin determination, expression analysis and functional characterization of saponin biosynthetic genes in *Chenopodium quinoa* leaves. Plant Science 250, 188–197.
https://doi: 10.1016/j.plantsci.2016.05.015

Gastal, F. and G. Lemaire, 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. J. Exp. Bot. 53, 789–799.
https://doi.org/10.1093/jexbot /53.370.789

Güçlü-Ustündağ, O. and G. Mazza, 2007. Saponins: Properties, Applications and Processing. Critical Reviews in Food Science and Nutrition, 47:231–258.
https://doi.10.1080/1040839 0600698197

Gomez-Caravaca, A.M., G. Iafelice, A. Lavini, C. Pulvento, M.F. Caboni, and E. Marconi, 2012. Phenolic compounds and saponins in Quinoa samples (*Chenopodium quinoa* Willd.) Grown under different saline and non-saline irrigation regimens. J.Agr. Food Chem. 60, 4620−4627. https://doi.org/.1021/jf3002125

Gómez-Caravaca, A.M., A. Segura-Carretero, M. Fernández-Gutiérrez, and F. Caboni, 2011. Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd) by a liquid chromatography diode array detection electrospray ionization time-of-flight mass spectrometry methodology. J. Agric. Food Chem., 59, 10815–10825. https://doi.10.1021/jf202224j

González, J.A., A. Roldán, M. Gallardo, T. Escudero, and F.E. Prado, 1989. Quantitative determinations of chemical compounds with nutritional value from Inca Crops: *Chenopodium quinoa* ("quinoa"). Plant Foods for Human Nutrition, 39, 331-337. https://doi.10.1007/BF01092070

González, J.A., Y. Konishi, M. Bruno, M. Valoy and F.E. Prado. 2011. Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (*Chenopodium quinoa*) cultivars from two different agroecological regions. J. Sci. Food Agric. 2012; 92: 1222-1229.
DOI: 10.1002/jsfa.4686

González, J.A., S. Eisa, S. Hussin, and F.E. Prado, 2015. Quinoa: an Incan crop to face global changes in agriculture. In: Murphy, K.S., Matanguihan, J. (Eds.). Quinoa: Improvement and sustainable production (pp. 1-18). Wiley-Blackwell, Hoboken, NJ, USA.

Graf, B.L., P. Rojas-Silva, L.E. Rojo, J. Delatorre-Herrera, M.E. Baldeón, and I. Raskin, 2015. Innovations in health value and functional food development of quinoa (*Chenopodium quinoa* Willd.). Comprehensive Reviews in Food Science and Food Safety, 14, 431-445.
https://doi. 0.1111/1541-4337.12135

Grandón S. A., B.M. Espinosa, L.D. Ríos, O.M. Sánchez, C.K. Sáez, S.V. Hernández, and A. Becerra, 2013. Variation of saponin contents and physiological status in Quillaja saponaria under different environmental conditions. Natural Product Communications, 8: 1697 – 1700. https://doi.org/10.1177/1934578X1300801209

Helaly, F.M., H.S.M. Soliman, A.D. Soheir, and A.A. Ahmed, 2001. Controlled release of migration of molluscicidal saponin from different types of polymers containing Calendula officinalis. Advances in Polymer Technology, 20: 305–311. https://doi.org/10.1002/adv.10005

Infostat. 2008. InfoStat, Versión 2008. Grupo InfoStat, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba, Córdoba,Argentina.

Kakabouki, I., D. Bilalis, A. Karkanis, G. Zervas, E. Tsiplakou, and D. Hela, 2014. Effects of fertilization and tillage system on growth and crude protein content of quinoa (*Chenopodium quinoa* Willd.): an alternative forage crop. Emirates Journal of Food and Agriculture, 26: 18-24. https://doi.10.9755/ejfa.v26i1.16831

Koziol, M.J., 1991. Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (*Chenopodium quinoa* Willd). Journal of the Science of Food and Agriculture, 54, 211-219. https://doi.org/10.1002/jsfa.2740540206

Kuljanabhagavad. T. and M. Wink, 2009. Biological activities and chemistry of saponins from Chenopodim quinoa Willd. Phytochemistry Reviews, 8, 473-490.
https://doi.org /10.1007/s11101 -009-9121-0

Li, J., Y.G. Zu, Y.J. Fu, Y.C. Yang, S.M. Li, Z.N. Li, and M. Wink, 2010. Optimization of microwave-assisted extraction of triterpene saponins from defatted residue of yellow horn (Xanthoceras sorbifolia Bunge.) kernel and evaluation of its antioxidant activity. Innovative Food Science and Emerging Technologies, 11, 637-643.
https://doi.org/10.1016 /j.ifset.2010.06.004

Martin, R.S., K. Ndjoko, and K. Hostettmann, 2008. Novel molluscicide against Pomacea canaliculate based on quinoa (*Chenopodium quinoa*) saponins. Crop Protection, 27: 310-319. https://doi.org/10.1016/j.cropro.2007.03.015

Martínez, E.A., E. Veas, C. Jorquera, R. San Martín, P. Jara, 2009. Re-Introduction of quinoa into Arid Chile: Cultivation of two lowland races under extremely low irrigation. J. Agron. Crop. Sci. 195:1-10. https://doi.org/10.1111/j.1439-037X.2008.00332.x

Mastebroek, J.D., H. Limburg, T. Gilles, and H.J.P. Marvin, 2000. Occurrence of sapogenins in leaves and seeds of quinoa (*Chenopodium quinoa* Willd). Journal of the Science of Food and Agriculture, 80, 152-156.
https://doi.org/10.1002/(SICI)1097-0010(20000101)80:1<152: AID-JSFA503>3.0.CO;2-P

Medina-Meza, I.G., N.A. Aluwi, S.R. Saunders, and G.M. Ganjyal, 2016. Profiling of triterpenoid saponins from 28 quinoa varieties (*Chenopodium quinoa* Willd) grown in Washington State by GC-MS. Journal of Agricultural and Food Chemistry, 64: 8583-8591. https://doi.10.1021/acs.jafc.6b02156

Miranda, M., A. Vega-Gálvez, I. Quispe-Fuentes, M.J. Rodriguez, H. Maureira, and E.A. Martinez, 2012. Nutritional aspects of six quinoa (*Chenopodium quinoa* Willd.) ecotypes from three geographical areas of Chile. Chilean Journal of Agricultural Research, 72: 175-181. https://doi.org/10.4067/S0718-58392012000200002.

Mujica, A., S. E. Jacobsen, J. Izquierdo, J. P. Marathee, 2001. Resultados de la Prueba Americana y Europea de la Quinua. Puno: FAO; UNA; CIP.

Netala, V.R., S.B. Ghosh, P. Bobbu, D. Anitha, and Tartte, V. 2014. Triterpenoid saponins: A review on biosynthesis, applications and mechanism of their action. International Journal of Pharmacy and Pharmaceutical Sciences, 7: 24–28.
https://doi.innovareacademics.in/journals/index.php/ijpps/article/view/2807.

Nieto, C., and M. Soria, 1991. Procesamiento de quinua en Ecuador. 94 p. Proyecto 3P-85-0213. Informe final de labores. INIAP-UTA-CIID. Quito, Ecuador.

Noratto, G., R. Carrión-Rabanal, G. Medina, A. Mencia, I. Mohanty, D. A. González and K. Murphy, 2014. Quinoa protective effects against obesity-induced intestinal inflammation. Experimental Biology. EB Call 4 Abstracts. USA.

Noratto, G.D., K. Murphy, and B.P. Chew, 2019. Quinoa intake reduces plasma and liver cholesterol, lessens obesity-associated inflammation, and helps to prevent hepatic steatosis in obese db/db mouse. Food Chemistry 287: 107-114. DOI: 10.1016/j.foodchem.2019.02.061

Prado, F.E., M. Gallardo, and J.A. González, (1996). Presence of saponin-bodies in pericarp cell of *Chenopodium quinoa*. Willd ("quinoa"). Biocell, 20: 261 266.

Prado, F.E., J.L. Fernández-Turiel, M. Tsarouchi, G.K. Psaras, and J.A. González, 2014. Variation of seed mineral concentrations in seven quinoa cultivars grown in two agroecological sites. *Cereal Chemistry,* 91: 453-459. https://doi.org/10.1094 / CCHEM-08-13-0157-R

Pulvento, C., M. Riccardi, A. Lavini, G. Iafelice, E. Marconi, and R. d'Andria, 2012. Yield and quality characteristics of quinoa grown in open field under different saline and non-saline irrigation regimes. Journal of Agronomy and Crop Science, 198: 254-263. https://doi.org/10.1111/j.1439-037X.2012.00509.x

Reichter, R.D., J.T. Tatarynovich, and R.T. Tyler, 1986. Abrasive dehulling of quinoa (*Chenopodium quinoa*): Effect on saponin content as determined by an adapted hemolytic assay. Cereal Chemistry, 63: 471-475

Schulte auf˝m Erley, G., H.P. Kaul, M. Kruse, and W. Aufhammer, 2005. Yield and nitrogen utilization efficiency of the pseudocereals amaranth, quinoa and buckwheat under differing nitrogen fertilization. European Journal of Agronomy, 22: 95-100. https://doi.10.1016/j.eja.2003.11.002

Shi, J., K. Arunasalam, D. Yeung, Y. Kakuda, G. Mittal, and Y. Jiang, 2004. Saponins from edible legumes: Chemistry, processing, and health benefits. Journal of Medicinal Food, 7: 67–78. https://doi.10.1089/109662004322984734.

Stuardo, M. and R. San Martin, 2008. Antifungal properties of quinoa (*Chenopodium quinoa* Will d) alkali treated saponins against Botrytis cinereal. Industrial Crops and Products, 27, 296-302. https://doi.org/10.1016/j.indcrop.2007.11.003

Szakiel, A., C. Paczkowski, and M. Henry, 2010. Influence of environmental biotic factors on the content of saponins in plants. Phytochemistry Reviews. 10: 493–502.
https://doi.org/10.1007/s11101-010-9164-2

Szakiel, A., C. Paczkowski, and M. Henry, 2011. Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews, 10: 471-491.
https://doi.org/ 10.1007/s11101-010-9177-x

USDA, Keys to Soil Taxonomy (11th edn). 2010. Natural Resources Conservation Service, Washington, DC.

Vega-Gálvez, A., M. Miranda, J. Vergara, E. Uribe, L. Puente. and E.A. Martinez, 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* Willd.) and ancient Andean grain : a review. Journal of the Science of Food and Agriculture, 90: 2541-2547. https://doi.10.1002/jsfa.4158

Verza, S.G., F. Silveira, S. Cibulski, S. Kaiser, F. Ferreira, G. Gosmann, P.M. Roehe, and G.G. Ortega, 2012. Immunoadjuvant activity, toxicity assays, and determination by UPLC/Q-TOF-MS of triterpenic saponins from *Chenopodium quinoa* seeds. Journal of Agricultural and Food Chemistry, 60, 3113-3118. https://doi. 10.1021/jf205010c

Yao, Y., Y. Xiushi, S. Zhenxing, and R. Guixing, 2014. Anti-inflammatory activity of saponins from quinoa (*Chenopodium quinoa* Willd.) Seeds in lipopolysaccharide-stimulated RAW 264.7 macrophages cells. Journal of Food Science, 79: 1018-1023. https://doi. 10.1111/1750-3841.12425.