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Original Article

Afrosymetric method for quantifying saponins in *Chenopodium Quinoa* Willd. from Colombia

Método afrosimétrico para quantificação de saponinas em *Chenopodium Quinoa* Willd. da Colômbia

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal that, in addition to presenting good nutritional characteristics in the grain, has secondary metabolites in the episperm of the seeds and, within them, saponins. Saponins are characterized by being emulsifying, foaming and generating a bitter taste. This metabolite has not been completely studied in quinoa materials from Colombia. For this reason, the objective of this research was to quantify the content of saponins present in quinoa materials from the department of Boyacá using three afrosimetric methods. For this, a completely randomized design (CRD) was implemented with a factorial arrangement of 3 (afrosimetric methods) x 5 (quinoa materials). From the quantification of saponins, it was determined that the Amarilla de maranganí genotype, was the one that presented the highest content in all the evaluated methods; the standard afrosimetric method being the most efficient. Finally, the conglomerate analysis allowed to discriminate the materials in sweet quinoas such as Tunkahuan and Blanca de Jericó with saponin contents lower than 0.06%, and in bitter quinoas (Negra de la Colorada, Dorada and Amarilla de Maranganí) with contents higher than 0.11%. The biochemical characterization of the germplasm will allow a selection of genotypes suitable for consumption and for the industry, given the potential use that saponins currently have.

Keywords: Andean crop, biochemical characterization, secondary metabolite, seeds, nutritional compounds.

Resumo

A quinoa (*Chenopodium quinoa* Willd.) é um pseudocereal que, além de apresentar boas características nutricionais no grão, possui metabólitos secundários no episperma das sementes e, dentro deles, saponinas. As saponinas são caracterizadas por serem emulsificantes, espumantes e gerarem um sabor amargo. Este metabólito não foi completamente estudado em materiais de quinoa da Colômbia. Por esta razão, o objetivo desta pesquisa foi quantificar o teor de saponinas presentes em materiais de quinoa do departamento de Boyacá usando três métodos afrosimétricos. Para isso, foi implementado um delineamento inteiramente casualizado (CRD) com arranjo fatorial 3 (métodos afrosimétricos) x 5 (materiais de quinoa). A partir da quantificação das saponinas, determinou-se que o genótipo Amarilla de maranganí foi o que apresentou o maior teor em todos os métodos avaliados; sendo o método afrosimétrico padrão o mais eficiente. Por fim, a análise de conglomerados permitiu discriminar os materiais em quinoas doces como Tunkahuan e Blanca de Jericó com teores de saponina inferiores a 0.06%, e em quinoas amargas (Negra de la Colorada, Dorada e Amarilla de Maranganí) com teores superiores a 0.11%. A caracterização bioquímica do germoplasma permitirá a seleção de genótipos adequados ao consumo e à indústria, dado o potencial de uso que as saponinas têm atualmente.

Palavras-chave: cultura andina, caracterização bioquímica, metabólito secundário, sementes, compostos nutricionais.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal from the Amarantaceae family, grown mainly in the Andes region in countries such as Peru, Ecuador, Chile, Bolivia and Colombia (Hernández-Ledesma, 2019). Currently, demand has increased both domestically and internationally because of its nutritional properties. Quinoa seeds have a high content of protein, essential amino acids, vitamins, minerals, antioxidants and fiber (Granado-Rodríguez et al., 2021). However, seeds also have compounds in the episperm that reduce the nutritional quality of the grain, including saponins, which are anti-nutritional, imparting a bitter taste, and are highly toxic when consumed in large quantities (El-Hazzam et al., 2020; Pathan and Siddiqui, 2022).

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Saponins are secondary metabolites with a hydrophobic part, called aglycone, that can have a triterpene or steroidal nucleus, and a hydrophilic part, which is sugar, an amphiphilic characteristic that reduces surface tension and forms foam when in contact with water and stirred (El-Hazzam et al., 2020). The saponins found in quinoa seeds are important because of biological properties that can be used in pharmaceutical and cosmetic industries. These saponins have biological activities that include hemolytic, anti-inflammatory, antioxidant, anticancer, antifungal, and antibacterial activities, among others (Lin et al., 2019; Abdelaleem and Elbassiony, 2021). In addition, they can be used to produce various agroindustrial products, such as soaps, shampoos, detergents, beer and biopesticides (Han et al., 2019; Velásquez-Flórez and Vélez-Salazar, 2020).

On the other hand, the surfactant property of saponins means they can be quantified in quinoa grains with the Afrosymetric method because the formation of a stable and persistent foam at the liquid/air interface indicates the presence of saponins (Amankeldi et al., 2018; Yousif et al., 2021). The standard afrosymetric method was initially developed by Koziol (1991), in the search for a simple technique to extract and determine the presence of saponins in quinoa grains and was used to evaluate more than 5000 quinoa genotypes from Latinreco (Ecuador), with significant results. However, over the years, this methodology has been modified, adapting it to environmental conditions and the needs of farmers (Guzmán et al., 2013; León-Roque et al., 2019).

Several studies have used this method to quantify saponins in different species, such as *Ocinum gratissimum*, *Vernonia amygdalina*, *Moringa oleifera*, *Nilotic Acacia*, *Parkia biglobosa* and *Chenopodium pallicudae* (Guzmán et al., 2013; Ajayi et al., 2018; Olumide et al., 2019;). Advantages include the ease of use and good correlation between the height of the foam with the content of saponins in the grains. In addition to extracting and quantifying the content, this method classifies quinoa materials as sweet (<0.11%) or bitter (>0.11%) (Gómez-Caravaca et al., 2014). Although these compounds are of great importance in the medicinal industry because of their nutraceutical properties, in Colombia, studies on saponins in quinoa are limited, especially in the Department of Boyacá, which has a germplasm collection that has not been characterized in terms of saponin contents. As such, these compounds are underused and limit consumption and marketing. This study aimed to determine the saponin content of five quinoa materials from the Department of Boyacá by evaluating three Afrosymetric methods, which could be a new sustainable source for these compounds in high quantities given the increase in production and global expansion of this crop.

2. Material and Methods

2.1. Plant material

Five quinoa (*C. quinoa*) materials were evaluated from the seed collection of the Laboratorio de Biotecnología Vegetal de la Secretaria de Fomento Agropecuario de la Gobernación de Boyacá, in the city of Tunja, located at an altitude of 2,820 m.a.s.l, with an average temperature of 13°C, constant photoperiod 12:12, relative humidity 78% and with an average annual rainfall of 1142 mm. The characteristics and common names of these quinoa materials are described below:

Blanca de Jericó (BJ): erect habit, height between 1.20 and 2.10 m. Green panicle, branched, white grain, sweet taste (Montes-Rojas et al., 2018).

Tunkahuan (TK): height between 1.60 and 2.30 m, branched, slightly compact, purple to orange purple at the beginning of maturity, white and large grains, diameter greater than 2 mm (Montes-Rojas et al., 2018).

Negra de la Colorada (NC): branched up to the lower-third; height between 1.08-1.67 m. Vegetative cycle of 70 days. Green panicle at flowering and green-yellow at maturity, branched. Black seeds, 1.66 – 1.79 mm in diameter (Montes-Rojas et al., 2018).

Amarilla de Maranganí (AM): slightly branched, erect, 1.80 m. high, large grain with orange color (2.5 mm.), vegetative cycle of 180 to 210 days (late), high content of saponins (Montes-Rojas et al., 2018).

Quinua Dorada (DO): large grain with yellowish color, high content of saponins (Guerrero et al., 2017).

2.2. Quantification of saponins

The saponin quantification was carried out in the Laboratorio de Biología Molecular y Celular Vegetal at the Universidad Pedagógica y Tecnológica de Colombia, Tunja. A fully randomized experiment design (CRD) was used, with a 3x5 factorial arrangement, where the first factor was the three methodologies (standard, fast and modified), and the second factor was the five quinoa materials described above, for a total of 45 experiment units.

2.3. Standard afrosymetric method (MAE)

For this methodology, 0.5 g of seeds were weighed, which were added to a test tube, 15 cm length and 15 mm diameter, along with 5 ml of distilled water. The tube was covered and stirred vigorously for 30 seconds, leaving at rest for 30 minutes. Then, it was stirred again for 20 seconds and left to rest for 30 more minutes, stirred again for 30 seconds with strong agitation, and left at rest for 5 minutes. The height of the foam was measured using a ruler with an accuracy of 0.1 cm (Koziol, 1991) The values were placed in Equation 1.

$$\%saponin = \frac{0.646 \times (h) - 0.104}{w \times 10} \quad (1)$$

h: height of the foam column (cm); w: sample weight (g).

2.4. Modified afrosymetric method (MAM)

0.5 g of seeds were weighed, macerated, added to a test tube along with 10 ml of distilled water, and placed in an incubation for 30 minutes to 95°C to dryness. After cooling, the material was covered and stirred vigorously for 30 seconds. Following 30 minutes at rest, the height of the foam was measured to the nearest 0.1 cm (Navarro del Hierro et al., 2018). The values were placed in equation 1.

2.5. Fast afrosymetric method (MAR)

0.5 g of each evaluated quinoa material were weighed, and 5 ml of distilled water were added, with vigorous stirring for 30 seconds. The tubes were left to stand for 10 seconds, and the height of the foam was measured with a ruler with an accuracy of 0.1 cm (Bergesse et al., 2019). The values were placed in Equation 2.

$$\%saponin = \frac{0.441 \times (h) - 0.001}{w \times 10} \quad (2)$$

h: height of the foam column after 30 seconds (cm weight (g).

2.6. Statistical analysis

The data from the saponin quantification using the three Afrosymetric methods were subjected to a descriptive analysis, Levene homogeneity of variances test, and Shapiro-Wilk normality test. After checking the assumptions of homogeneity, an analysis of variance (ANOVA) was performed, and the Fisher Least significant difference (LSD) Test was applied to find significant differences ($p<0.05$) using the Statistical Program Infostat (Di Rienzo et al., 2020). The error bar chart was used with the R program ggplot2 package. For the cluster analysis, the algorithms included in the factoextra package in the R program were used (Kassambara and Mundt, 2020). The dendrogram was constructed using Euclidean distance and Ward's minimal variance hierarchical grouping method. These analyses were performed using the FactoMineR package in the R program (R Core Team, 2020).

3. Results

In the evaluation of the afrosymetric method for the quantification of saponin in quinoa germplasm from the department of Boyacá, it was possible to observe as shown in the Figure 1, the foam height was variable and depended on the material evaluated. However, the afrosymetric method was capable of extracting some proteins that can influence foam stability, where the foam height changed rapidly in both directions, which could lead to an overestimation of saponin contents (León-Roque et al., 2019; El-Hazzam et al., 2020; Yousif et al., 2021).

The literature has reports that quinoa accessions with foam heights less than 0.6 cm can be classified as sweet, and those exceeding 6.6 cm can be classified as bitter (Medina-Meza et al., 2016). Here, Blanca de Jericó and Tunkahuan presented foam heights less than 0.5 cm and were considered sweet materials. Amarilla de Maranganí, Negra de la Colorada and Dorada were bitter because they had heights between 6.9 and 8.5 cm according to the standard afrosymetric (MAE) and fast methods (MAR). On the other hand, the modified afrosymetric method (MAM) for all materials presented a foam height of less than 3 cm.

The analysis of variance for the variable saponin content detected significant differences ($p<0.05$) between the materials and the afrosymetric methods evaluated. In addition, the interaction of these two factors (material and method) affected the quantification of the saponin content, with highly significant differences between the treatments ($p<0.05$) (Table 1).

According to the Fisher Mean Comparison Test (LSD), Blanca de Jericó and Tunkahuan had saponin contents equal to or less than 0.06%. Amarilla de Maranganí, Negra de la Colorada and Dorada had a high percentage of saponins, but the content varied according to the Afrosymetric method evaluated (Figure 2).

Amarilla de Maranganí, Negra de la Colorada and Dorada were classified as bitter with saponin contents greater than 0.11% (Table 2).

The dendrogram obtained from the cluster analysis (Figure 3) showed that the quinoa materials were grouped into two clusters according to their low or high saponin content, respectively. The first group contained Tunkahuan and Blanca de Jericó, which presented low saponin contents of less than 0.6 mg/g of seeds, and the second group had Amarilla de Maranganí, Negra de la Colorada and Dorada, with high saponin contents in a range between 1.5 and 19 mg/g of seeds. The cophenetic correlation coefficient was 0.98, which indicated a high level of reliability in the clusters.

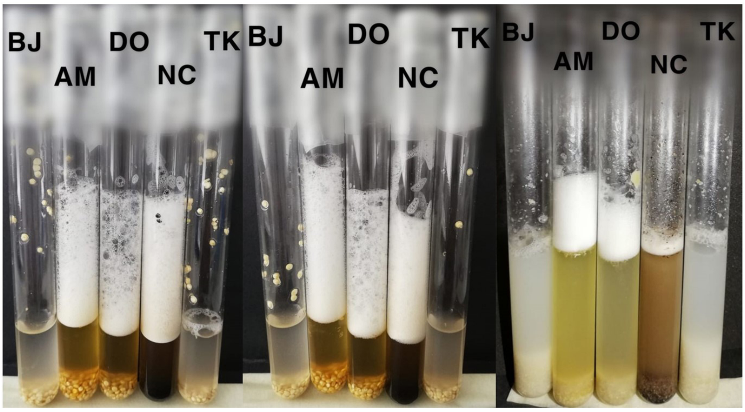

Figure 1. Foam column in quinoa materials for each Afrosymetric method. (A) Standard Afrosymetric Method (MAE); (B) modified afrosymetric method (MAM); (C) Rapid Afrosymetric Method (MAR).

Table 1. Analysis of variance for the saponin content (mg/g seed).

Source of Variation	Sum of squares	DF	Middle square	F	p-valor
Modele	600.32	14	42.88	554.95	<0,0001
Material	316.5	4	79.12	1,024.03	<0,0001
Method	165.84	2	82.92	1,073.12	<0,0001
Material*Method	117.99	8	14.75	190.87	<0,0001
Error	2.32	30	0.08		
Total	602.64	44			

DF: Degree of freedom. *Significance test after 1000 permutations. F: Critical value of rejection of the hypothesis.

Table 2. Saponin Percentages in the quinoa grains of the five materials.

Material	% Saponins			Classification
	MAE	MAR	MAM	
Amarilla de Maranganí	0.93 ± 0.03	0.71 ± 0.02	0.13 ± 0.01	Bitter
Blanca de Jericó	0	0.0002	0.0033	Sweet
Dorada	0.91 ± 0.02	0.68 ± 0.01	0.09 ± 0.01	Bitter
Negra de la Colorada	0.81 ± 0.03	0.57 ± 0.02	0.12 ± 0.02	Bitter
Tunkahuan	0.02	0.0045	0.03	Sweet

MAE (standard afrosymetric method); MAR (fast afrosymetric method); MAM (modified afrosymetric method).

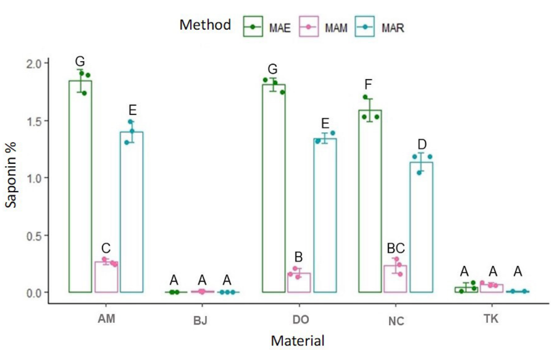

Figure 2. Percentage of saponin from quinoa materials in each Afrosymetric method. Different letters indicate significant differences according to the Fisher's mean comparison test (LSD) (p≤0.05). Vertical bars indicate standard error (n=3).

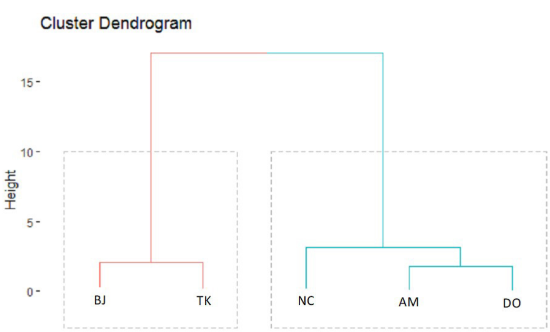

Figure 3. Dendrogram of five quinoa materials, grouped by the saponin content in the seeds.

4. Discussion

Saponins are the secondary metabolites, found on the layer of grains, which provide bitter taste (Pathan and Siddiqui, 2022). Lim et al. (2020) reported saponin contents in different parts of quinoa plants, such as leaves (0.97), grains (1.26), sprouts (1.29), stems (3.67), and bran (8.34%). Different factors such as locations, abiotic stresses, and varieties influence saponin contents. Different factors, such as drought and salinity, decrease the quantify of saponins, and the sweet quinoa variety has lower saponin contents than the bitter variety (Pathan and Siddiqui, 2022).

The production of quinoa without those bitter saponins has been a breeding target, and some naturally non-bitter quinoa accessions have been identified (Jarvis et al., 2017). For breeding and phenotyping purposes, testing for saponins is desirable. Over 90 different saponins have been found in quinoa (Mad et al., 2006). Saponins are composed of an aglycone backbone with sugar moieties, whose combination generates the great variety of saponins that we can find in nature (Mad et al., 2006).

For phenotyping purposes in plant breeding, a simple detection test may be used that utilizes the foaming characteristics of saponins. When shaken in water, saponins foam, a property that is used for the afrosimetric method (Koziol, 1991). Colorimetric methods such as the use of spectrophotometry can also be used for saponin detection (Hirich et al., 2021). However, these methods also detect the phytosterols in plants. Jarvis et al. (2017) validated the results of an afrosimetric test using a more specific detection method, i.e., gas chromatography–mass spectrometry (GC–MS), on a mapping population, which segregated for saponins.

The afrosimetric test and GC–MS method corroborated the absence or presence of saponins.

Studies on the extraction and quantification of saponins in quinoa have shown variability in this characteristic in the evaluated germplasm (Otterbach et al., 2021). The quantification of this molecule has been carried out with different methods, such as afrosymetric, hemolytic, spectrophotometric, and chromatographic methods, among others (Koziol, 1991; El-Aziz et al., 2019). The Afrosymetric method presented optimal results despite the fact that the high polarity and structural complexity of saponins make their isolation, identification, and quantification a complex process (Yousif et al., 2021). This method is based on the property of saponins that decreases the surface tension of water and forms a relatively stable foam, the height of which correlates with the saponin content in quinoa seeds (Gargiulo et al., 2019; Wisetkomolmat et al., 2019).

The literature has reports that quinoa accessions with foam heights less than 0.6 cm can be classified as sweet, and those exceeding 6.6 cm can be classified as bitter (Medina-Meza et al., 2016). In this sense, Blanca de Jericó and Tunkahuan were considered sweet materials (Heights less than 0.5 cm Table 2) and Amarilla de Maranganí, Negra de la Colorada and Dorada were bitter because they had heights between 6.9 and 8.5 cm according to the standard afrosymetric (MAE) and fast methods (MAR) (Figure 1). Similar results were obtained by other researchers (Bonilla et al., 2019), who evaluated Hualhuas and Recuay varieties with the afrosymetric method and classified them as sweet and bitter genotypes with foam heights of 0.5 cm and 7.5 cm, respectively.

Several studies have reported on the quantification of saponins in quinoa seeds with the Afrosymetric method (Gargiulo et al., 2019; León-Roque et al., 2019) however, the reported methodologies are variable.

According to the Fisher Mean Comparison Test (LSD), Blanca de Jericó and Tunkahuan had saponin contents equal to or less than 0.06%. Amarilla de Maranganí, Negra de la Colorada and Dorada had a high percentage of saponins (Table 2), but the content varied according to the Afrosymetric method evaluated (Figure 2). The results showed that these variations depended on the specific type of saponin contained in each genotype (Escribano et al., 2017).

Other authors (Montes-Rojas et al., 2018) have reported similar results when using the standard Afrosymetric method to obtain saponin contents from Blanca de Jericó and Tunkahuan, reporting 0.0012% and 0.0050%, respectively. In addition, these genotypes have a low content of saponin in the seeds and may be suitable for human consumption after a light washing or scarification.

Amarilla de Maranganí, Negra de la Colorada and Dorada were classified as bitter with saponin contents greater than 0.11% (Table 2). This is consistent with other research on saponins in quinoa (Guerrero et al., 2017; Pathan and Siddiqui, 2022), since materials with contents greater than 0.11% are considered bitter. The results were similar to those found by other authors (Bergesse et al., 2019) who evaluated four quinoa materials, finding saponin percentages greater than 0.5% (Figure 2).

The variability in the saponin content observed in the quinoa materials from the Department of Boyacá may have been due to the effect of the genotype and the environmental conditions. The content of saponins is determined by abiotic factors such as precipitation, temperature, photoperiod, and soils with high salinity, along with the response of plants to different types of stress (Lim et al., 2020). Processing of grains can play an important role in enhancing the nutritional components especially bioactive compounds without alarmingly increasing the antinutritional factors in the grains which reduce the bioavailability of the nutrients. With respect to quinoa, few studies have been carried out on the processing effects on saponins (Han et al., 2019; Mhada et al., 2020), however Sharma et al. (2022) reported that moist heating reduced saponin contents by 14-64% whereas dry heating increased them by 9-25% antinutritional components in raw and processed grains were found to be within safe limits. Therefore, thermal processing can be used to improve the potentiality of quinoa as functional food ingredient for providing technological and health benefits.

Recent research has shown that saponins have a potential use in industrial and pharmaceutical processes (Singh and Chaudhuri, 2018; Han et al., 2019; Wisetkomolmat et al., 2019), which generates added value in quinoa since it also plays an important role in responses to biotic and abiotic factors. The results identified an economical and easy-to-implement methodology that can be used to quantify saponins in different quinoa germplasm to achieve sustainable use of this plant's genetic resource and respond to the current needs of farmers, producers, and consumers.

The quinoa materials from the Department of Boyacá varied in terms of the saponin content (Figure 3). Notably, Amarilla de Maranganí could be a promising source of saponins and could be used in genetic improvement programs that seek the sustainable use of this plant's genetic resource.

Of the methods evaluated for the quantification of saponin contents, the most efficient was the standard afrosymetric method, followed by the fast afrosymetric method, which, given its shorter analysis time, can be used in field trials to identify *in situ* materials with high or low saponin contents.

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