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PHYSICO-CHEMICAL CHARACTERISTICS OF QUINOA SEEDS

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Abstract

Chenopodium quinoa Willd. (quinoa), is a stress-tolerant plant, cultivated in the Andean region, used as grain, due to its remarkable content in of nutritional elements given by minerals, vitamins, fatty acids, phenolic compounds and antioxidants.The present study aimed at highlighting physical (turbidity, liquid color and cream sample) and chemical parameters (polyphenols, saponins, proteins and total sugars) from the seeds of three quinoa varieties grown in Romania in 2017. The seeds of the three varieties were subjected to the same physico-chemical determinations in three repetitions each.The obtained results have highlighted the difference between varieties in terms of the statistically influenced physical parameters compared to the values of the chemical parameters where the obtained values are not statistically assured. Thus, color liquor had values between 129 and 430 determined at 430 nm; sample cream ranged between 299.1 and 5269.70 mg/L; protein content varied between 885.19 - 962.96 mg/L; saponins between 87.61 - 92.62 mg/L and the total sugar content between 43.47 - 49.60 %.

Key words*: Chenopodium quinoa, physical and chemical parameters.*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), is an Amaranthacean, stress-tolerant plant, cultivated in the Andean region, known about 7000 years ago, used as grain, most consumed for macronutrients (Ismail et al., 2015; Jacobsen, 2003; Vega-Galvez et al., 2010). As grain, quinoa is a well example of nutritional food given by minerals, vitamins, fatty acids, phenolic compounds and antioxidants (Filho et al., 2017). Quinoa grains may be used in the production of food processing and also be consumed in a similar manner to cooked rice, or soup, yoghurt and salad, or ground like flour (Jacobsen, 2003; Nickel et al., 2016; Tang et al., 2015).

It is known from the specialized literature that the pericarp of quinoa seeds is protected by a rich layer of saponins, which, for industrialization, it makes them impractical in their presence (Jacobsen, 2003). Therefore, for industrialization, certain chemical and physical parameters, which may have some advantages, must be known in the processing of quinoa seeds.

Despite the fact that quinoa seeds are beneficial to the body, preventing hypertension (Huang et al., 2014) and anti-inflammatory activities (Yao et al., 2014), quinoa saponins have a bitter taste, high turbidity levels, some cases of intoxication having been reported due to their activity. Saponins (aglycone unit linked to one / more glucide chains) are a group of chemical natural compounds found in in large quantities in seeds quinoa species (Khalil and El-Adawy, 1994).

In this respect, the present work aims at evaluating the physical and chemical parameters of three quinoa varieties newly introduced in the country, that have cultivation and processing potential for the food industry.

MATERIALS AND METHODS

1. The biological material used in research was represented by three varieties of quinoa (Puno, Titicaca and Vikinga), approved and registered in Denmark. Puno and Titicaca were recorded in Europe in 2009 and Viking in 2015, being therefore newly introduced. Currently, nine cultivations are approved at European level, five in the Netherlands, three in Denmark and one in France (Jacobsen, 2017). The initial seeds that were used to establish the quinoa culture were produced at Ku Farm in Denmark (Figure 1). In 2018 the three cultivars were sown in an experimental stationary in Cudalbi

locality, Galati county, a unit that uses a sustainable system of cultivation of agricultural species, a unit that does not over-fertilize the soil with fertilizers based on nitrogen, the total quantity used being under 170 kg N s.a./ha/year, similar to the organic farming systems (Stoleru, 2013).

Figure 1. Quinoa seeds from trails

In order to determine the physical indicators, 50 g of quinoa seeds from the three varieties were dried in the oven at 70°C after which a 1-liter solution of each sample was prepared, in three repetitions.

2. Physical analysis

2.1. Moisture: Moisture content was determined in vacuum at 70ºC temperature of oven. Moisture content (AOAC, 1999).

2.2. The turbidity of each sample was measured using a portable ISO Turbidimeter, HI 98713, (HANNA) at room temperature. (AOAC Method 970.14, Haze (Total) of Beer after Chilling, AOAC International, www.aoac.org.)

2.3. The color of the entire liquid was measured at 460 nm using the PG Instruments model spectrometer, UV-VIS spectrophotometer and UV WIN 5.05 software. The absorbance of each sample was recorded and the color value was calculated according to Lmabert Beer's law: $A=\varepsilon \times l \times c$. According to the law of equivalent proportions: $C_1V_1=C_2V_2$, where C_1, C_2, V_1, V_2 are the concentrations, respectively, the volumes of the solution in the vat, respectively in the Eppendorf tube, thus the concentration of the total solution was calculated. (AOAC Stakeholder Panel on Dietary Supplements (SPDS). https://www.aoac.org/AOAC_Prod_Imis/AOAC_Docs/SMPRs/SMPR%202016_003.pdf).

2.4. Sample cream. Each sample was transferred into four 15 mL Eppendorf tubes and kept cold. Then the samples are stored (4°C) to induce the formation of the cream in the absence of light. After incubation for 12 h, the mixtures from the Eppendorf tubes were immediately centrifuged at 3500 rpm for 30 min. The supernatant was removed for phytochemical analysis, while the cream sediment was carefully transferred to a pre-weighed sterilized vessel. The samples were washed twice with 5 mL of distilled water. They were then dried for 12 h at 85°C. The dry sample creams were weighed after being carefully removed from the oven and the amount of cream formed was determined by calculating the difference between total solids and dry mixture (according to AOAC Official Method 936.12 Oil (Tea Seed) in Olive Oil Qualitative Color Test. 2013-09-10, http://files.foodmate.com/2013/files2987.html)

3. Chemical analysis

3.1. Crude Fat: Soxhlet method was used for determination of crude fat content in samples (http://www.aafco.org/Portals/0/SiteContent/Laboratory/Fat_Best_Practices_Working_Group/Crude_Fat_Methods_Considerations.pdf).

3.2. Ash: Tahini halvah samples were set on fire at 550 ± 20ºC in ash oven.

$$\%\ \text{Ash (dry basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

(AOAC, Method Number 930.05, http://www.eoma.aoac.org/methods/info.asp?ID=31326).

3.3. Total and reducing sugars: Total and reducing sugars were determined by the AOAC International, 2002 method.

Soluble solids (%) were measured by using a Bausch - Lomb refractometer at 20°C, according Codex general standard for fruit juices and nectars (CODEX STAN 247-2005) (http://www.justice.gov.md/file/Centrul%20de%20armonizare%20a%20legislatiei/Baza%20de%20date/Materiale%202010/Legislatie/Codex%20Stan%20247-2005.pdf).

3.4. The total polyphenol substances (TP) (also known as total soluble polyphenol substances) were determined using the Folin-Ciocalteau Test (FC) and expressed in gallic acid equivalents (AOAC, SMPR 2015.XXX; Version 2; December 5, 2014 Method Name: Estimation of Total Phenolic Content Using the FC Assay, http://www.aoac.org/aoac_prod_imis/AOAC_Docs/NEWS/SMPR_Phenolicv2.pdf), (Butnariu, 2014).

3.5. The amount of soluble protein present in the tea was determined using the Biuret test. The diluted infusion samples were mixed with an equal volume of Biuret reagent compared to a standard curve and were left to react for 20 min for absorption. The reading is done at 540 nm. Well-diluted samples (1 mL / 4 mL) in triplicate tubes were transferred to other tubes, in which 4 mL of Biuret reagent solution was added (Eed and Burgoyne, 2015). The mix was agitated on the Vortex mixer for 20 minutes at room temperature. The absorbance values were measured using PG Instruments, UV-VIS spectrophotometer and UV WIN 5.05 software. The amount of protein was obtained by calculating the absorbance of the samples compared to the standard curve (concentration vs. absorption). For the standard curve, dilutions of 10, 8, 6, 4, 2 and 1 mg / mL (bovine serum albumin / water, weight / volume) and the control sample (0 mg / mL) were prepared. It was first applied for the spectrometer before measuring the absorbance of the samples (Hassan and Soleimani, 2016).

3.6. The amount of saponins

The qualitative analysis of saponin was done through identification reactions, foaming test (Standard Test Method for Foaming Characteristics, Active Standard ASTM D892), thin-layer chromatography. To determine the content of lipophilic substances (chlorophyll, waxes, vegetable oils, etc.) and their removal, the vegetable product was degreased using Soxhlet with non-polar solvents (Saidi et al., 2017). The difference between the final and initial values of the plant product cartridge shows the mass of the lipophilic substances, which is 5.8 %. After degreasing the plant product, the triterpene saponin are extracted with methanol twice with 400 and 200 mL of 70 % methanol, at reflux in a water bath for 3 h and 1.5 h, respectively. The combined extractive solutions are concentrated on the rotary evaporator (50°C) until the methanol is completely removed (Ayadi Hassan and Belbasi, 2017). The residue is dissolved in 50 mL of methanol and poured into a thin wire, continuously agitated, over 500 mL of acetone. It is kept cold for about 30 min, then it decants. The precipitate is washed with 125 mL of ether, filtered, redissolved in methanol and the precipitation is repeated in ether. Finally, it is filtered through the Buchner funnel in the vacuum. The precipitate is dried in a vacuum desiccator on calcium chloride. The crude saponin is white-yellow in ether, forming a flocculent precipitate, but after filtering and drying it becomes brownish-yellow. After drying, the powder is weighed and the result expressed as a percentage. Percentage of saponin = (WEP/WS) X 100 Where, WEP = Weight of oven dried end product. WS = Weight of powdered sample taken for test (Hariri Moghadam et al., 2018).

For the qualitative reactions, an infusion of 1:10 was prepared by heating the shredded plant product in the water bath for 10 min. After cooling, the infusion was filtered and used for the necessary reactions (Aramesh and Ajoudanifar, 2017). The thin layer chromatographic analysis of the saponin: chromatographic plate preparation (stationary phase): 13 x 18 cm silica gel and 0.25 mm thickness; solution to be analysed: 2 g of plant product are extracted with 10 mL of 70 % ethanol, for 10 min at reflux. The filtrate is concentrated to 5 mL; the ethanol solution: methanol solution of Merck saponin; mobile phase: chloroform: methanol: water (70: 44: 10); the applied quantity: 20 μL of the sample and 10 μL of the standard solution; Identification: Liebermann - Burchard reagent and then heating in the oven at 110ºC for 5 min (Mohammadhassan et al., 2018). The dosage of the saponin material from saponinquinoa samples was performed through the gravimetric method. Saponin have been identified through several methods, namely: color reactions (Sakowscki, Kobert, Liebermann-Burchard, Lafon reactions, sodium nitrite solution and concentrated sulfuric acid), precipitation (lead acetate, alcoholic solution of cholesterol).

4. Statistical analyses. The experimental data processing was carried out using specific mathematical and statistical method. All analyses were carried out in the three replications. Standard deviation (±SD) was calculated for each data series as an indicator of dataset scatter (n=3). The differences among the average values for each experimental variant were compared by using the Tukey test at $p<0.05$ probability level, computed by the IBM SPSS version 21.

RESULTS AND DISCUSSIONS

The data regarding the physical parameters of the solution obtained from the quinoa seeds are presented in Table 1. The moisture content of the seeds determined after drying in the oven at 7 0^oC varied between 1.61% for the Puno cultivar and 1.90 for the Vikinga cultivar. Thus, the differences between the three varieties in relation to bound water content are considered insignificant. Similar results have also been obtained by D'Amico et al., 2019 for the processing of quinoa seeds by milling.

The color of the liquid obtained for determining the cream sample and turbidity had shades ranging from 129 for the Viking cultivar where the lowest turbidity level was recorded, between 38.8-39.4 Day 0/1, NTU to 480 for Titicaca, where the highest turbidity level or opalescence was recorded, the values ranging between 65.4-78.2 Day 0/1, NTU.

The creation of the cream from the solution obtained from quinoa seeds is in correlation with the chemical parameters. Thus, the cream sample had values that ranged from 299.1 ± 12.59 mg / L for Puno and 5269.70 ± 171.54 mg / L for Vikinga, indicating that the three varieties can be used in a differentiated way in processing, depending on the destination of the final product.

Table 1. Physical analysis of quinoa seeds

Parameters/variety	Puno	Titicaca	Vikinga
Moisture %	1.61±0,13	1.72±0,08	1.90±0,06
Liquor Color	216,00±40,00	430,00±28,00	129,00±11,00
Samples Cream (mg/L)	299.10±12,59	303.60±12.16	5269.70±171,54
Turbidity (Day 0/1, NTU)	53.70±14,99	71.80±9,05	39.10±0,42

The results of the chemical analyses of the quinoa seeds from the three varieties are presented in Table 2.

Ash content varied between 1.44 ± 0.14 % for Titicaca and 1.72 ± 0.25 % for Vikinga, the differences between the three varieties being insignificant, similar results being obtained by other authors as well (Tang et al., 2015 D'Amico et al., 2019). Crude fat ranged between 33.00% for Puno and 37.40% for Viking, the differences between varieties being insignificant and not statistically ensured. D'Amico et al., 2019 obtained results ranging between 8.59% and 12.71% on quinoa processing by milling, depending on the size of the particles.

The Brix content of aqueous solution ranged from 49 % for Titica and 56% for Viking, values that are positively correlated with the total sugar values. The total sugar content varied between 43.47 ± 4.74% and 49.60 ± 10.28%, values similar to other data obtained in the scientific literature (Vega-Galvez et al., 2010; D'Amico et al., 2019).

The total protein content determined in aqueous solution ranged from 885.19 ± 12.77 mg / L for Vikinga and 962.96 ± 15.06 mg / L for Titicaca, the differences between the three varieties being insignificant. The values obtained in protein content are similar to grain legumes (Bozhanska, 2017). The data in Table 2 highlight the fact that there is an inversely proportional relationship between protein content and total sugar. Similar data are presented by other authors of the universal scientific literature as well (Filho et al., 2017; D'Amico et al., 2019).

Table 2. Chemical analysis of quinoa seeds

Samples	Ash (%)	Crude fat (%)	Brix (%)	Total sugar (%)	Protein (mg/L)	Total phenolics (g/L)
Titicaca	1.44±0.14	33.26±2.80	49.00±9.90	43.47±4.74	962.96±15.06	2.02±0.27
Vikinga	1.72±0.25	37.40±3.68	56.00±7.07	49.60±10.28	885.19±12.77	1.61±0.27
Puno	1.68±0.20	33.00±4.81	52.00±4.24	47.51±2.11	888.89±11.92	1.70±0.14

The total phenolic (TP) content in the aqueous solution had values ranging from 1.61 ± 0.27 g / L for Vikinga and 2.02 ± 0.27 g / L for Titicaca, the differences between the three varieties not being statistically significant. TP values correlate positively with total protein content, similar values being analysed by other authors as well (Ismail et al., 2015; Tang et al., 2015; Nickel et al., 2016; Ouis and Hariri, 2017).

Depending on the content of saponins in the seeds, quinoa varieties are classified as "sweet varieties" when they have less than 0.11% saponins or as "bitter varieties" when the saponin content is greater than 0.11% (Koziol).

Depending on the saponin values obtained from the seed tegument of the three samples, the quinoa varieties are classified in the sweet varieties class, where the values range within

limits from 87.61 ± 3.04 to 92.62 ± 5.36 mg /L (Fig. 2.).

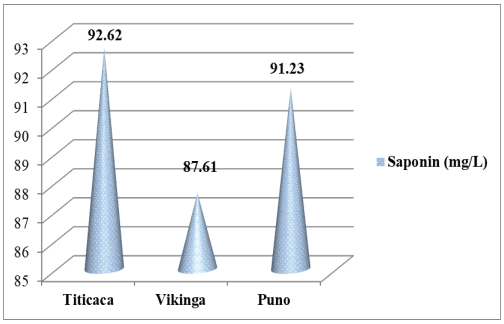

Figure 2. Saponin content in the three samples of quinoa seed

The results obtained in the case of the foaming analysis reveal that for the Titicaca variety, triterpenoid saponins are present and in the case of the Vikinga variety, there are or are predominant the steroidal saponins (Fig. 1.).
The chromatogram obtained under the experimental conditions mentioned above is shown in figure 3.

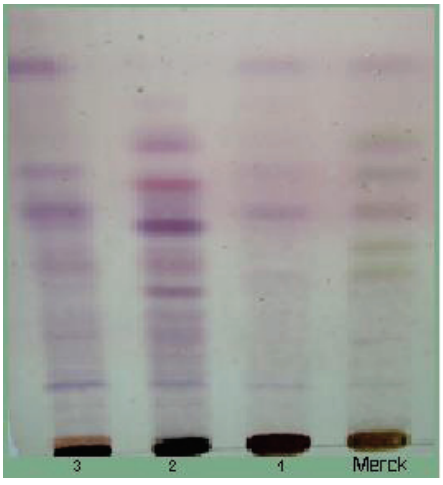

Figure 3. Thin-layer chromatography (TLC) – saponins in ultraviolet light
(1. Puno; 2. Titicaca; 3. Vikinga; Merck Saponin)

The chromatogram analysis shows that the color of the spots varies from violet-pink to brown-gray (Figure 3).
All analyzed saponins have the same color reaction as the Merck saponin. The Rf values and the color of the spots for saponin and extract are given in the table below.

Table 3. Color reaction of saponins present on quinoa seeds compared to the Merck saponin

Species	Rf	Color
Merck Saponin	0,2	Violet
	0,43	Violet
Puno Saponin sample	0,16	Gray
Titicaca Saponin sample	0,22	Brown-violet
Vikinga Saponin sample	0,34	Violet

Previous studies have shown that saponin content in plant tissue may change depending on environmental conditions or abiotic stress during growth (Fiallos-Jurado et al., 2016; Ojogu et al., 2017).

CONCLUSIONS

The physical parameters show different values between the three samples, sometimes the values are very significant, which denotes that physical parameters such as turbidity, cream sample or color liquor are genetically influenced.
The values of the chemical parameters are generally between the average values of the literature data, with the exception of crude fat, where the values obtained are even twice as high.
Quinoa plants grown under the same conditions and without stress have accumulated relatively similar and reduced amounts of saponins, which can be easier to process.
The high protein content, TP, total sugar correlated with a low saponin content, creates from the three varieties an increased opportunity for the industrial processing of quinoa seeds.

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