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# OPTIMIZATION OF THE ULTRASOUD-ASSISTED EXTRACTION OF SAPONINS FROM QUINOA (*CHENOPODIUM QUINOA* WILD) USING RESPONSE SURFACE METHODOLOGY

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## ABSTRACT

**Background.** Quinoa grain has a bitter tasting layer in the pericarp called saponin, a triterpenoid glycoside with industrial potential. Traditionally, quinoa saponins are extracted with a large amount of water, which is why ultrasound technology constitutes an emerging technological alternative which is considered efficient and profitable compared to traditional extraction methods. The objective of this research was to determine the amplitude, time, and concentration of ethanol that guarantee a higher content of saponin through extraction assisted by ultrasound.

**Materials and methods.** To find the optimal extraction conditions, the response surface methodology was used using the Box Behnken design with 5 central points, taking as a response the content of saponins (expressed in oleanolic acid as it is the most abundant sapogenin).

**Results.** According to the results obtained, the $R2$ values were in agreement with the adjusted $R2$, showing that the data fit the model well. The results showed that ethanol concentration has a significant effect ($p < 0.05$) on the saponin content in the extract. Optimization showed that the optimal extraction conditions were 70% ethanol, 59% amplitude and an exposure time of 12 min. These values were obtained experimentally to compare theoretical values and found residual error percentages less than 3%. The emulsifying activity was evaluated, reporting a value of 52,495 units of emulsion activity per milliliter (UAE/mL), and the foaming stability indicated that 87.54% of the initial foam was maintained after 5 min, indicating high stability.

**Conclusion.** The parameters of ethanol concentration, amplitude and time were optimized in the extraction of saponins, assisted by ultrasound. Furthermore, the extract obtained had good foaming and emulsifying characteristics, suggesting its suitability for use in industry.

**Keywords:** sapogenin, quinoa, ultrasound extraction, second order polynomial, Box Behnken design

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## INTRODUCTION

*Chenopodium quinoa* Wild is an annual dicotyledonous plant, it belongs to the Chenopodiaceae family (Wang and Zhao, 2014). Peru and Bolivia are the main quinoa producing countries (Gianna et al., 2012). Quinoa is not only rich in proteins and carbohydrates, but it is also rich in bioactive substances, polyphenols, flavonoids, and saponins (Graf et al., 2015). Saponins are distributed mainly in the pericarp of quinoa seeds (Woldemichael and Wink, 2001). Quinoa has relatively high levels of saponins depending on the variety and thickness of the pericarp (Jarvis et al., 2017). They are a large group of complex structures and have a biological activity of an organic nature (Osbour et al., 2011). The pericarp of quinoa is a by-product that is generally discarded because it is responsible for the astringent or bitter taste, so removal is sought prior to its consumption (Osbourn et al., 2011). Saponins also have foaming, emulsifying properties (Cheok et al., 2014), as well as an antioxidant, hepatoprotective, anti-inflammatory (Jesus et al., 2015), antibacterial (Sun et al., 2019), and molluscicidal properties (Jiang et al., 2018). The traditional extraction method uses large amounts of water and energy expenditure in concentration, whereas ultrasound-assisted extraction offers an alternative to minimize the use of water and energy (Panda and Manickam, 2019). Ultrasound extraction reduces extraction time compared to traditional methods (Picó, 2013). Ultrasound produces a cavitation effect, which can cause physical and mechanical changes in raw materials, facilitating the extraction of compounds (Chemat et al., 2017; Morales et al., 2020). The Box-Behnken Design Tool (BBD) is used for the optimization of biological compound extraction procedures and is an important surface methodology (RSM) (Box and Wilson, 1951). BBD is a second-order rotary design based on a three-level incomplete factorial design. This design is widely used as an inexpensive method to extract a large amount of information with a small number of experiments (Aslan and Cebeci, 2007). Also, the response surface methodology allows the monitoring of the interaction of the independent variables with the response variables by using a collection of statistical and mathematical methods (Heydari-Majd et al., 2014). The objective of this study was to evaluate the effect of the conditions of extraction of saponins assisted by ultrasound from the pericarp of quinoa, (a by-product of quinoa industrialization). The surface response methodology was used to optimize the parameters (amplitude, time, and concentration of ethanol) in the extraction of saponins, assisted by ultrasound, as well as to characterize their emulsifying and foaming function.

## MATERIAL AND METHODS

### Materials

The pericarp of the quinoa grains was of the Hualhuas variety, collected from the quinoa processing companies in the city of Huancayo, Junín region, Peru. Ethanol was purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). For the extraction of saponins from quinoa scarification, a compact ultrasonic laboratory device UP 100H (Hielscher Ultrasound Technology, Teltow, Germany) of 100 W and 30 kHz of power was used. This equipment can adjust the power output by adjusting the amplitude percentage (20–100%). A sonotrode MS 7 was used with a pulse control set to 1 cycle of continuous operation. The Thermo Fisher Genesis 10S UV spectrophotometer was used for spectrophotometric readings, and the Agilent 6890 N gas chromatograph was used for the identification of sapogenins.

### Sample preparation

The quinoa pericarp was ground to an approximate size of 0.6 mm, recommended by Laqui-Vilca et al. (2017), and then stored in plastic bags until use.

### Hydrolysis of saponin

To facilitate the quantification of total saponins, the method proposed by Medina-Meza et al. (2016) was used. An aliquot of about 0.8 mL of the crude concentrate was hydrolyzed with an equal proportion of 6 N HCl at 110°C for 2 hours. The hydrolyzate produced was cooled for 5 min with ice water. Later, it was neutralized with an ammonia solution. Then the neutralized solution was centrifuged at 3000 g for 5 min. The supernatant was extracted and combined with ethyl acetate (3 mL of ethyl acetate for every 5 ml of the sample). The fractions were then combined and filtered on a bed of anhydrous sodium sulfate. The final extract was stored at –20°C in an Eppendorf tube until later analysis. Oleanolic acid is a compound that

is found as a precursor for triterpenic saponins (Ahumada et al., 2016). This acid was used as a standard to elaborate the standard curve if it was also possible to quantify the saponins when used as the predominant acid (Medina-Meza et al., 2016).

### Oleanolic acid quantification

To determine the total saponins expressed in oleanolic acid, the proposed method by Medina-Meza et al. (2016) was used. In this method, 250 µL of the final extract with 1000 µL of the reagent mixture (glacial acetic acid / sulfuric acid 1:1 v/v) was placed in an Eppendorf tube to develop the color. It was shaken vigorously in a vortex for 30 s and then heated in a water bath at 60°C for 30 min, during which a faint lilac color developed. The mixture was then cooled in ice water for 5 min. The absorbance at 527 nm was measured. Glacial acetic acid was used as a blank.

### Determination of emulsifying activity

The emulsifying activity of saponins was carried out using vegetable oil in an aqueous medium and by adding 3 ml of the crude extract of saponins and 0.5 ml of vegetable oil. It was stirred vigorously for 2 min and incubated at room temperature for 1 hr without disturbance to separate the aqueous phase and the oil phase. The aqueous phase was carefully removed using a 1 mL micropipette and the absorbance was measured. The solution without any oil was taken as a blank (Ghagi et al., 2011). The absorbance of the emulsion was measured using a spectrophotometer at a wavelength established by complete scanning of the light spectrum. The wavelength that obtained the highest absorbance for the sample was taken as the optimal wavelength for all samples (this being 700 nm). The emulsification activity per ml (AE/ml) was calculated using the formula: emulsification unit = absorbance obtained × dilution factor

### Determination of foaming stability

The method used for measuring foaming power and foam stability was that used by Chen et al. (2010). A portion of the test solution was placed in a jacketed cylinder. Foam developed when a stream of the second portion of 200 mL test solution was added to the first portion of the test solution through a standard orifice from a 90 cm height. This resulted in turbulence and foam. The height of the foam generated was measured immediately and again after 5 min. The foam height at the initial stage indicates the foaming power of the surfactant solution. The parameter *R*5, defined as the ratio of the height of the foam at 5 min to that at the initial stage, is proposed as an evaluation of foam stability.

### Identification of sapogenins (GC)

It was performed using gas chromatography (Medina-Meza et al., 2016). It consists of taking an aliquot of the final dry extract. The extracted saponins were then derivatized, using 100 µL of anhydrous pyridine, 100 µL of bis (trimethylsilyl) trifluoroacetamide (BSTFA), and 10 µL of cholesterol decanoate (internal standard, IS). They are added to the dry extract and heated at 70°C for one hour. Derivatized extracts (2 µL) are injected into an Agilent 6890N gas chromatograph. A RESTEK Rxi-5HT capillary column (60 m × 0.25 mm × 0.25 mm) was used. The injector and detector temperatures were adjusted to 350°C, while the oven temperature was programmed from 160 to 220°C at 15°C/min, from 220 to 290°C at 10°C/min, for 7 min, from 290 to 330°C at 8°C/min maintained for 15 min.

### Experimental design

The ultrasound-assisted extraction of saponins was carried out in a 1:50 ratio (solvent ratio) (Wang et al., 2018). The ultrasound-assisted extractions of saponin from quinoa scarification were optimized by response surface methodology (RSM) using a Box Behnken

**Table 1.** Levels and coded values of the independent variables of the response surface model

| Independent variables | Levels | Coded values |
|---|---|---|
| Amplitude, % (*A*) | 55 | –1 |
| | 60 | 0 |
| | 65 | +1 |
| Ethanol concentration (*B*) | 70 | –1 |
| | 75 | 0 |
| | 80 | +1 |
| Time (*C*) | 10 | –1 |
| | 12.5 | 0 |
| | 15 | +1 |

design with 5 central points. The amplitude ($A$), exposure time ($B$), and ethanol: water ratio ($C$), were taken as independent variables and the saponin content ($Z$) as the dependent variable. The independent variables were evaluated to optimize the extraction of saponins from the scarified quinoa.

A second-order polynomial was used for the mathematical modeling of the relationship between the dependent and independent variables.

The total content of saponins at optimal conditions was estimated using second-order polynomials and was validated experimentally. Surface response methodology experiments were designed and analyzed using Design Expert 7 software.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where:

$Y$ – the response or dependent variable,
$X_1$, $X_2$, $X_3$ – independent variables,
$\beta_0$, $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ – the linear intersection, quadratic and interaction coefficients, respectively,
$\varepsilon$ – the residual.

## RESULTS AND DISCUSSION

### Fit to model

The content of saponins expressed as oleanolic acid obtained experimentally was used to calculate the coefficients of the second-order polynomial equation, the regression coefficients, and the $p$ values. The regression model was established between the oleanolic acid content ($Z$), the amplitude ($A$), the time ($B$), and the ethanol-water ratio ($C$). The equation for the multivariate quadratic adaptation of the regression model is:

$$Z = -3381.77714 + 90.84486 \times A + 4.63171 \times B + 18.12157 \times C - 0.03 \times A \times B + 0.213 \times A \times C - 0.018 \times B \times C - 0.86986 \times A^2 - 0.095429 \times C^2 - 0.19386 \times C^2$$

The value of $R^2$ and adjusted $R^2$ was 0.93 and 0.86 respectively. This also shows a significant value for the equation ($p < 0.01$) whilst the model's $F$ value of 13.37 implies that the model is significant. Values of Prob > $F$ less than 0.05 indicate that the model terms are significant.

### Model analysis

Figure 1 shows the response surface graphs obtained for the factors studied.

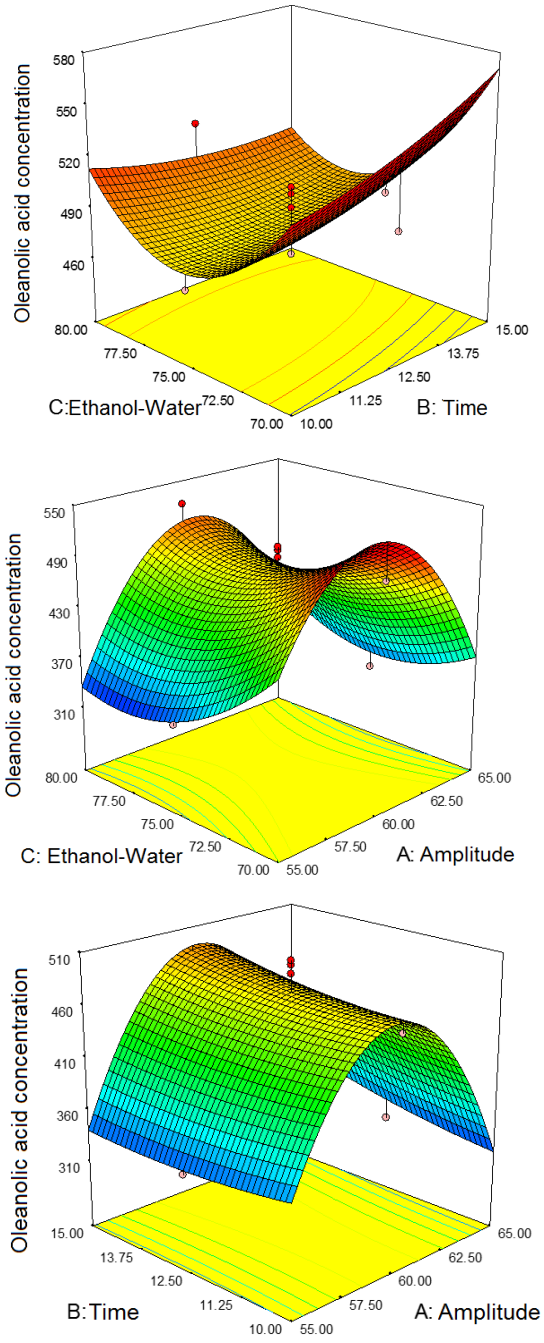


**Fig. 1.** Graphs of response surfaces of oleanolic acid extracted from quinoa pericarp affected by the amplitude, time, and concentration of ethanol

Medina-Meza et al. (2016) mention that the mixture of ethanol in water is used to extract the polar and nonpolar components of the saponin. In this work a dilution of ethanol in water was used, varying the polarity for the extraction of saponin. This was important in this work since the polar and apolar behavior is a structural characteristic of this glycoside, which is made up of a sugar and an aglycone (Wink, 2016). Regarding the ultrasonic extraction time for saponins, it is lower compared to other extraction methods. The extraction of saponins with water, for tea leaves, was reported by Yu (2018) as taking one hour. This was substantially higher in the time of ultrasonic extraction carried out, as well as the extraction time of three hours by Soxhlet reflux as reported by (Medina-Meza et al., 2016). Short times were taken based on preliminary results gathered and finding no significant difference in the time interval considered. Long extraction times tend to deteriorate metabolites and increase the temperature of the medium. The optimal extraction time found in this work is lower than that found by Wang et al. (2018), but the ethanol-water ratio is higher. These variations are caused by the type of reactor used. A rod-type reactor was used for this research and not an ultrasound bath, as supported by Panda and Manickam (2019) who state that the type of reactor intervenes in the extraction due to the manner in which the transfer of the ultrasonic waves is carried out.

It was observed that as the amplitude increases, the oleanolic acid content in the extracts also increased, but that it was not statistically significant for the solvent content and time studied. Chemat et al. (2017) advises a high amplitude to obtain the necessary mechanical vibrations that will lead to cavitation. Similar ultrasound equipment was used by Zardo and Espindola (2019), which tells us that the effects of ultrasound on extraction performance depend on the nature of the plant material. The main effects related to improved releases of plant material content can be attributed to cavitation (which disrupts the cell wall), reduction in particle size, intensification of mass transfer, and, consequently, an increase in extraction.

## Validation of optimal extraction conditions

The estimated levels of optimal extraction conditions, for the maximum response of oleanolic acid with desirability value, were: amplitude – 59%, time – 12 min, and ethanol concentration – 70%, and thus, obtaining an oleanolic acid concentration of 532.35 mg of oleanolic acid/ml of extract.

In order to verify the optimized results produced by the program, experimentation was carried out which obtained results very close to those expected. Therefore, we can say that the results are optimal for extraction.

Wang et al. (2018) obtained very similar values when optimizing the extraction of saponins from oat bran, but with longer times. This was probably due to the extraction material, which allows for less cavitation.

## Quantification of sapogenin by gas chromatography

Four sapogenins present in the extracts obtained by ultrasound were identified (oleanolic acid, hederagenin, and sejanic acid).

**Table 2.** Sapogenins obtained in gas chromatography

| Purified sapogenin | Holding time min | STD AO µg | STD AO area | AO µg |
|---|---|---|---|---|
| Oleanolic acid | 13.37 | 500 | 30 018 634 | 10.92 |
| Hedagerin | 14.39 | 500 | 30 018 634 | 8.55 |
| Sejánic acid | 16.65 | 500 | 30 018 634 | 5.62 |
| Others | 17.03 | 500 | 30 018 634 | 2.90 |

STD AO µg – standard oleonolic acid, µg; STD AO area – standard oleonolic acid, area; AO µg – oleonolic acid, µg.

Madl et al. (2006), as well as Jeong et al. Yoon (2019), also found these saponins in analyses of quinoa samples. The composition of sapogenins in saponins determine their emulsifying and foaming properties. Elias and Diaz (1988) reported similar contents for other varieties of quinoa planted in Perú.

## Emulsifying activity and foaming stability of saponins from quinoa pericarp

The sample had 52.49 units of emulsifier activity per milliliter (UAE/ml) and 84.5% foam stability (*R*5). They are two very important attributes in the industry and biological action (Ghagi et al., 2011).

The emulsifying activity and foaming stability of saponins are mainly due to the presence of a lipid--soluble aglycone and a water-soluble sugar chain, which show an amphiphilic nature. In this way, foaming is achieved (with liquid-gas phases), as is an emulsifying effect (with liquid-liquid phases), and dispersibility (with liquid-solid phases). Saponins with a sugar chain have the best foaming characteristics (Oleszek and Hamed, 2010). However, the emulsifying activity and foaming stability vary depending on the type of saponin and the aglycones present in its structure, for example, the saponins of *Sapindus mukorossi* studied by Ghagi et al. (2011), lower than 235 UAE/ml in its emulsifying activity.

The foam, as spherical bubbles surrounded by liquid or wet foam, presented small bubbles. As time passed, certain bubbles formed polyhedra. The reduced size of the bubbles indicated a decrease in surface tension, whilst the subsequent increase in surface, but with a low variation in the size of polyhedral bubbles, indicated foam stability (Stevenson, 2012). The change in foam height presented a value of *R*5 higher than 50% for what is considered a good stability. 87.54% was obtained for the *R*5 factor which is slightly higher than the 86% reported by (Chen et al., 2010). The result is quite similar to the 85% reported by (Böttcher and Drusch, 2016).

## CONCLUSIONS

Optimal conditions for the extraction of saponins from quinoa pericarp were optimized. They are: 60% amplitude, 12 minutes, and 70% ethanol. The saponin extracts extracted under these conditions had an emulsifying activity of 52.49 units of activity of emulsion per milliliter (UAE $ml^{-1}$) and foaming stability of 87.54% for factor *R*5, obtaining good stability. Saponins constitute an important biocomponent in the pharmaceutical and agro-industrial industries, suggesting the ultrasound-assisted extraction method as a good alternative.

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