Optimization of the saponin removal process to improve quinoa product quality and increase protein content

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Summary
Highest quality quinoa products require maximal extraction of saponins to improve taste and eliminate digestive inhibitors. However, current saponin removal methods have not been optimized to achieve this quality, nor tested to improve other important nutritional and operational factors. This study aimed to maximize the removal of saponins, improve protein content and effectively eliminate water usage by employing and optimizing the scarification process. The response surface method was applied to study the two key variables that affect saponin removal in the scarification process: rotational frequency and mass flow. The analysis of variance test was applied to optimize these two variables and the model was represented in both 2-D contour and 3-D response surface graphs. The final results showed over 99.0% saponin extraction as well as an 18.6% increase in protein content, both significant improvements over existing saponin removal methods. As scarification is a dry process, it eliminates the use, remediation and disposal of water required in standard saponin removal, and thus improves the overall sustainability of quinoa processing.

Keywords
quinoa; saponin; scarification; process optimization; sustainability; protein

Quinoa is a pseudo-cereal that has been cultivated for over five thousand years in the Andes Region of South America, and has received worldwide recognition for its exceptional agricultural and nutritional characteristics, as well as its environmental adaptability [1]. Currently it is produced for consumption predominantly in its native Peru and Bolivia, as well as in Chile and Ecuador [2].

Quinoa differs nutritionally from traditional grains by the biological value of its protein, which is similar to beef (740 $g \cdot kg^{-1}$) [3], as well as by the amino acid composition, which is similar to whole milk and close to the ideal recommended by the Food and Agriculture Organization [4]. In part, this is due to quinoa's high content of lysine [5, 6], an amino acid essential for human growth and development, which is generally found only in low quantities in traditional grain proteins [7]. Across all quinoa varieties, Navruz-Varli and Sanlier [7] found that the protein content was in a range of 138–165 $g \cdot kg^{-1}$, with an average of 150 $g \cdot kg^{-1}$. This protein content, accompanied with an absence of gluten, makes quinoa a good alternative to barley, wheat or rye carbohydrates, for those who suffer from complications due to gluten intolerance [8].

Another aspect of quinoa's high-value status is that it is a hardy and stress-resistant crop that flourishes in harsh environments, such as those found in its native Andes region of Peru and Bolivia. Additionally, it requires little pesticide application due to the presence of saponin, a highly effective anti-predator component [9] that increases in quantity during blooming [10], and resides in its seed coat. Low saponin quinoa cultivars have been developed but are difficult and expensive to manage because they require significant pesticide application to compensate for their lack of natural defences [11].

However, saponin is an anti-nutrient, a phytic acid and toxic alkaloid, which is traditionally removed to eliminate the bitter taste it imparts. It was found that saponin adversely affects quinoa's nutritional properties by decreasing the absorp-

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tion of proteins, starch digestibility [12, 13] and bioavailability of the minerals iron, magnesium and zinc [14].

While recent research suggests that saponins and their extracts might offer promising benefits in the cosmetic or pharmaceutical industries [15], current quinoa product quality standards require that they be removed as extensively as possible without regard to their preservation. Two dominant processes for industrial saponin removal are available, namely, a wet-process that entails extensive washing with water, and a dry-process of mechanical abrasion known as scarification [9]. At times, the removal process incorporates both of them.

The wet-process has been used for saponin extraction since antiquity. In the modern version that encompasses soaking, washing and rinsing, Mercado and Gladys [16] determined that 100 kg of quinoa would need 1 100 l of water for proper saponin removal. This coincides with the findings of Escalera Vásquez et al. [17] that 1 000 kg of quinoa requires between 5 000 l and 14 000 kl of water for adequate saponin removal, while generating considerable volumes of saponin-contaminated effluents that are often released into the environment without treatment.

Due to the high germinative power of quinoa, the wet-process also poses quality risks because the grains germinate during washing [12] and thus the mineral and protein contents are reduced. According to Chaparro Rojas et al. [18], quinoa only needs to be in contact with water for 6 h at 30 °C to start germinating, and the final product shows a 41 $g \cdot kg^{-1}$ decrease in protein content after the first day of soaking. Total cost of the product is also increased, as immediate drying is required to avoid not only germination, but also fermentation and proliferation of microorganisms [19].

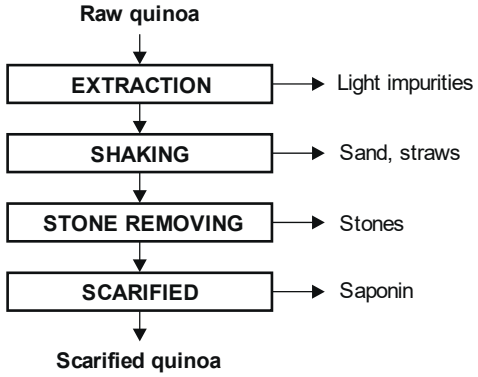

Fig. 1. Sequence of operations carried out to obtain scarified quinoa.

The dry process was introduced as a way to reduce the costs of the entirely wet process, originally using a rice peeling scarifier [20]. Quinoa-specific scarifiers were developed by removing the metallic net required in the rice peeling process, as Birbuet and Machicado [20] found that quinoa grains, due to inherent characteristics of their seed coats, create the abrasive friction necessary for effective epicarp and perisperm removal.

This research aimed to build on the previous research on saponin removal by optimizing the scarification process using industrial volumes and machinery, while avoiding completely the wet process with its higher costs, water waste and problems with disposal of contaminated water. Additionally, the study aimed to improve protein content not only by eliminating germination losses from the wet process, but also through the incidental effects of the scarification process on the unique quinoa seed structure.

MATERIALS AND METHODS

The present study was carried out at Ging Maquinas Peru, a grain machinery manufacturer located in the city of Lima, Peru, with their model ESC-2017 Scarifier with a 14.9 kW motor. The experimentation and data collection were carried out in January 2018.

Grains of white quinoa variety Salcedo INIA were used. Stones and impurities (organic and inorganic) were removed. Fig. 1 shows the entire sequence of operations carried out to obtain pre-scarified quinoa.

To evaluate extraction of saponin, the afrosimetric method was used. It is a physical method for measurement of the content of surfactants. When dissolved in water and stirred, saponins give a stable foam, the height of which is related to the saponin content of the grains [21]. Two samples were taken: one sample in the hopper before entering the scarifier, and the other sample after the scarification process. This was done for each treatment and repetition.

For the saponin extraction (SE) efficiency quantification, results were given by Eq. 1:

$$SE = \frac{(S_0 - S)}{S_0} \times 100 \quad (1)$$

where S_0 is initial saponin content and S is final saponin content. SE efficiency is expressed in percent.

Analyses of quinoa and scarified quinoa were performed according to the AOAC methods as follows: moisture (945.15), protein (979.09), lipids

(945.38), ash (923.03) and fibre (945.38) [22].

The response surface method (RSM) was applied to the two significant operational variables of the scarification process, mass flow and rotational frequency. Nine treatments were obtained, to which three repetitions were made, resulting in 27 experimental units. The tests were performed randomly. The values presented in Tab. 1 were considered and the combination of treatments and their repetitions was performed as indicated in Tab. 2, with all results entered into the Statgraphics Centurion XV.15.2.06 (Statgraphics Technologies, The Plains, Virginia, USA) for RSM calculations.

RESULTS AND DISCUSSION

With the levels of the factors and the experimental values ($\overline{Y}$) for each experimental point (Tab. 2), multiple regression analysis was carried out with the values obtained, generating the following polynomial equation of second degree (Eq. 2):

$$\overline{Y} = -256.485 + 0.70532X_1 + 0.26428X_2 + \\ + 0.000436X_1^2 - 0.000106X_1X_2 - \\ - 0.000118X_2^2 \quad (2)$$

where $\overline{Y}$ represents saponin extraction efficiency in percent, X_1 represents the estimated rotational frequency and X_2 represents the estimated mass flow.

The analysis of variance (Tab. 3) and statistical evaluation (Tab. 4) were carried out for the quadratic model of Eq. 2. The low p-value for the interactions and the quadratic terms suggested that there was a curvature in the response surface, as will be discussed later. The conditions recommended by the second order model that optimized the process were 11.67 Hz of rotational frequency and 797.49 kg·h^{-1} of mass flow. Due to the difficulties of exactly regulating mass flow (the input should be regulated micrometrically), mass flow was adjusted to 800 kg·h^{-1}. Although the lack of adjustment was significant, it did not invalidate the model's predictive purpose, because R^2 was 90.1 %. Thus, there was an adequate correlation between the values obtained and estimates of the response, and thus it was appropriate to represent the relationship between saponin extraction efficiency and the two variables studied.

Fig. 2 shows the contour graph of the response surface estimated for the saponin extraction efficiency, where both variables have a significant effect on increasing the response, reaching the best conditions when it is at a load between 680 kg·h^{-1} and 920 kg·h^{-1}, with a rotational frequency between 7.5 Hz and 11.67 Hz. Fig. 3 shows the estimated response surface for the saponin extraction efficiency in three dimensions, where there is a better visualization of the increased tendency of optimal values that occur between the intervals previously described.

After the optimization stage using RSM, the parameters that maximized the scarification process were determined. For the final optimization, the response corresponding to the highest

Tab. 1. Experimental factors and their levels for response surface methodology design.

Factor levels	Rotational frequency [Hz]	Mass flow [kg·h^{-1}]
Lower level (–1)	8.33	600
Intermediate level (0)	10.00	800
Higher level (+1)	11.67	1 000

Tab. 2. Optimization of saponin extraction with response surface methodology design.

Treatment	Rotational frequency [Hz]	Mass flow [kg·h^{-1}]	Extraction of saponin [%]
	$X_1(x_1)$	$X_2(x_2)$	$\overline{Y}_1$
1	10.00	800	92.0
2	8.33	600	73.1
3	8.33	1 000	77.9
4	11.67	1 000	94.3
5	10.00	1 000	91.0
6	8.33	800	77.8
7	10.00	600	82.6
8	11.67	600	94.3
9	11.67	800	100.0
10	10.00	800	95.4
11	8.33	600	70.6
12	8.33	800	87.7
13	11.67	600	92.4
14	10.00	1 000	88.7
15	8.33	1 000	82.6
16	10.00	1 000	89.3
17	11.67	800	92.4
18	11.67	800	100.0
19	10.00	800	92.4
20	8.33	600	66.9
21	8.33	1 000	73.1
22	11.67	1 000	97.5
23	10.00	1 000	91.5
24	8.33	800	80.4
25	10.00	600	93.5
26	11.67	600	95.1
27	11.67	800	98.1

Tab. 3. Analysis of variance of the quadratic model for the percentage of saponin extraction in the optimization stage.

Source	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	*P*-value
Rotational frequency (X_1)	1685.48	1	1685.48	139.55	0
Mass flow (X_2)	72.8424	1	72.8424	6.03	0.0239
X_1^2	114.058	1	114.058	9.44	0.0063
X_1X_2	54.656	1	54.656	4.53	0.0467
X_2^2	135.66	1	135.66	11.23	0.0034
Blocks	14.9564	2	7.47818	0.62	0.5489
Error total	229.475	19	12.0776		
Total corrected	2307.13	26			

Coefficient of determination (R^2): 90.05 %; coefficient of determination (R^2) adjusted for degrees of freedom: 87.69 %.
F-ratio – ratio of the mean square values, X_1^2 – quadratic effect of rotational frequency, X_1X_2 – effect of the interactions between rotational frequency and mass flow, X_2^2 – quadratic effect of mass flow, Blocks – experimental units in groups.

Tab. 4. Statistical evaluation for the quadratic model.

Coefficient	Estimate	Standard deviation
Intercept	–256.48500	58.91310
Rotational frequency (X_1)	0.70532	0.17190
Mass flow (X_2)	0.26428	0.06319
X_1^2	0.00043	0.00013
X_1X_2	–0.00010	0.00004
X_2^2	–0.00011	0.00003

Coefficient of determination (R^2): 90 %, adjusted coefficient of determination (R^2): 88 %, standard deviation of the model's residuals: 3.48, mean absolute error: 2.19.

saponin extraction efficiency was used and is presented where rotational frequency and mass flow were found to have the most significant effect on the process (Fig. 2).

Regarding previous saponin extraction efficiency reported in the literature, Candia Danz and Olaguivel Quisocala [23] found the saponin extraction efficiency of 48.5 % for white quinoa Serranita variety with the variables 12.75 Hz and 90 kg·h^{-1}. Armada et al. [24] indicated that at 25 Hz and 25 kg·h^{-1}, the extraction efficiency of 69.2 % was obtained for white quinoa Real variety. According to Candia Danz and Olaguivel Quisocala [23], scarifier machines with paddles usually reached the saponin removal efficiency of 95.0 %, but it depended on the quinoa variety used as the raw material. These values are similar to those of Quiroga et al. [25], who affirmed that the saponin extraction efficiency in a scarifier was between 90.0 % and 95.0 %.

Coinciding with the previously mentioned findings of Birbuet and Machicado [20], Quiroga et al. [25] determined that when friction occured between the grains, much more effective and homogeneous saponin extraction occured because the friction forces were equal to or lower than those produced when the grains are rubbed on an abrasive surface. Thus, a better control of the scarification process can be exercised to generate greater and more uniform removal of the

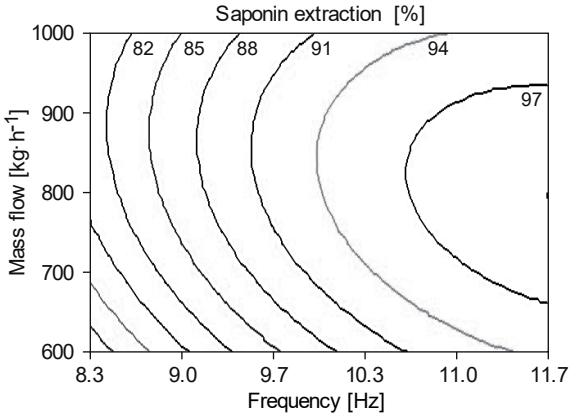

Fig. 2. Contour graph of the response surface estimated for the percentage of saponin extraction.

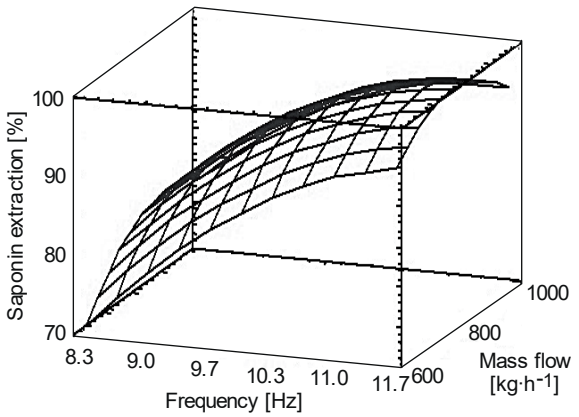

Fig. 3. Response surface estimated for the percentage of extraction of saponin.

Tab. 5. Comparison of nutritional content before and after scarification.

Component	Quinoa [g·kg^{-1}]	Scarified quinoa [g·kg^{-1}]	Difference [%]	Description
Moisture	122	115	–5.3	Decrease
Protein	161	186	15.1	Increase
Fat	44	47	7.5	Increase
Fibre	23	23	–1.7	Decrease
Ash	24	20	–17.7	Decrease
Carbohydrates	626	610	–2.6	Decrease
Saponin	2	0	–100.0	Decrease

episperm and pericarp, and hence also saponins, while preserving the integrity of the nutritionally important grain. This improved control could explain why the saponin extraction efficiency of (99.6 ± 0.83) % (n = 10) for the white quinoa variety Salcedo INIA found in this study exceeded the previous research reports. This could also be due to the fact that none of the previous studies aimed to determine the optimal operating variables that achieve the highest saponin extraction efficiency.

The greater control of the scarification process due to the inherent nature of quinoa's abrasive properties could also explain the 15.0% increase in protein content found (Tab. 5). Most previous studies suggested, at best, conservation of the protein content after saponin extraction (by either the wet method or a combination of wet and dry methods), although there are many reports that found reductions [25]. While in most cereals such as rice, the majority of proteins is located outside the grain and are therefore removed in the first stages of processing, the majority of quinoa proteins is located inside the grain and in the embryo [26]. Quiroga et al. [25] found that removing the outer layer, rich in saponins, fibre and flavonoids but low in protein, allows the consumable grain to gain approximately 6.0% relative weight of proteins. Thus, the difference between those results and the protein increase of 15.1 % found in this study, could be attributed to the optimized scarification for saponin extraction that incidentally removed more of episperm and pericarp that are poor in protein, without adversely affecting the protein-rich grain or embryo, and thus increased the relative weight of proteins.

By applying RSM, the reduction of saponin in white quinoa, Salcedo INIA variety, was maximized in a scarifier by optimizing the variables of mass flow and rotational frequency to the following parameters: mass flow of 800 kg·h^{-1} and rotational frequency of 11.67 Hz, for a saponin extraction efficiency of 99.6 %. Before saponin extraction, the raw white quinoa variety Salcedo INIA was composed of 122 g·kg^{-1} humidity; 161 g·kg^{-1} protein; 44 g·kg^{-1} fat; 23 g·kg^{-1} fibre; 24 g·kg^{-1} ash; 626 g·kg^{-1} carbohydrates; 2 g·kg^{-1} saponin (Tab. 5), and its average particle diameter was 1.4 mm with 55.3% retention in a 1.4 mm sieve, all values within the reported and desired ranges of a high-quality product.

After scarification under the study's optimized parameters, the final product was composed of 115 g·kg^{-1} moisture; 186 g·kg^{-1} protein; 47 g·kg^{-1} fat; 23 g·kg^{-1} fibre; 20 g·kg^{-1} in ash; 610 g·kg^{-1} carbohydrate; 0 g·kg^{-1} saponin (Tab. 5), and its average particle diameter was 1.4 mm with 67.6% retention in an 1.4 mm sieve. In terms of product quality, these values demonstrate that the optimized scarification process studied did not deteriorate the integrity of the grain or its physico-chemical properties, was highly effective in removing saponins, and increased the protein content as the most valued nutritional characteristic of quinoa. Additionally, this process does not require the use, remediation and disposal of water and thus drastically reduces the environmental impact and associated costs compared to the standard saponin extraction by the wet-process.

Further quinoa process research should validate these results and compare them directly with other saponin extraction processes across different quinoa varieties, including those with large (> 1.7 mm) and small (< 1.4 mm) grain sizes. Further product quality and nutritional research should focus on confirming the increase in protein content across different quinoa varieties, again including those with large and small grain sizes, while maintaining the desired high saponin removal efficiency.

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