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MECHANICAL SCARIFICATION OF QUINOA SEEDS (*CHENOPODIUM QUINOA* WILLD.) AND OBTAINING OIL FROM THE SEED COAT BY SUPERCRITICAL CO_2 EXTRACTION

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ABSTRACT

The paper presents development of a high-performance process for extracting oil from quinoa seeds (*Chenopodium quinoa* Willd.) by supercritical CO_2 extraction. The pre-treatment of seeds involving mechanical scarification was applied. The abrasive material gradation and time of abrasion were optimized. Optimum scarification conditions were obtained using the abrasive gradation P40 at scarification time of 50-100 min. Under these conditions, a seed coat was obtained in an amount of 10% of the seed weight and it contained 20 g oil/100 g seed. The oil was separated from the seed coats by supercritical fluid extraction. Approximately 61% of oil recovery was obtained under extraction conditions: pressure 25 MPa, temperature 40° C and extraction time 120 min. From seeds containing 5.6 g oil/100 g seed, after scarification and extraction with supercritical carbon dioxide, approx. 1.2 g oil/100 g seed was obtained from the seed coat.

1.Introduction

Oils from seeds, fruits and other raw materials are obtained by various methods. The conventional method of oil extracting from seeds with high content of oil, such as rapeseed and sunflower, is expression without heating or expression with heated press (Bogaert *et al.*, 2018; Savoireet *et al.*, 2012). However, in many cases, complex methods are used including pressing, heating, solvent extraction, ultrasounds and microwaves pretreatments or enzymatic extraction (Koubaa *et al.*, 2016, Kumar *et al.*, 2017). Oil content in quinoa is at a low level ranging from 2 to 9.5 g oil/100 g seed, with an average of 5.0–7.2 g oil/100 g seed, therefore, in order to achieve high extraction efficiency, complex extraction methods are required.

Quinoa (*Chenopodium quinoa* Willd.) is a food plant of the family *Amaranthaceae*, subfamily *Chenopodiaceae* and genus *Chenopodium*. The edible seeds of quinoa are small, round and flat, and they may measure from about 1.5 mm in diameter to 2.5 mm (Koziol, 1993; Maradini-Filho, 2017; Novak et al., 2016; Vega-Gálvez *et al.*, 2010).

Quinoa is a pseudocereal of Andean origin and is used principally in the same manner as wheat and rice (Maradini-Filho, 2017). Quinoa has been considered as an alternative oilseed crop, due to the quality and quantity of its lipid fraction being rich in essential fatty acids such as linoleic and α-linolenic and contains high concentrations of natural antioxidants such as α- and γ-tocopherol (Bhargava, 2006; Koziol, 1993; Maradini-Filho, 2017; Vega-Gálvez *et al.*, 2010). Quinoa is also an excellent example of "functional food" which may help to reduce the risk of various diseases. Its functional properties

may be related to the presence of fibres, minerals, vitamins, fatty acids, antioxidants and phytonutrients, which favourably contribute to human nutrition (Hager *et al.*, 2014; Maradini-Filho, 2017; Miranda *et al.*, 2010; Vega-Gálvez *et al.*, 2010).

Thus, quinoa is an alternative ingredient in the gluten-free diet and can be used by persons who suffer from celiac disease (Cordeiro *et al.*, 2012). Furthermore, due to the relatively high protein content with very favourable amino acid composition, it is a potential raw material for obtaining protein preparations (isolates, concentrates), which in turn can be used to enrich food (Bastidas *et al.*, 2016; Hager *et al.*, 2014). Quinoa can be eaten as a wheat replacement, as a hot breakfast cereal or can be boiled in water to make infant cereal food. The seeds can even be popped like popcorn. Seeds can be ground and used as flour (bakery products, noodles, flakes etc.).

Important methods of seed scarification include heat, freeze-thaw, mechanical and acid scarification (Bastidas *et al.*, 2016; Kimura and Islam, 2012). Heat scarification is the method that uses high temperatures to break seed coat. Freeze-thaw scarification is a method that breaks the seed coat by exposing seeds to temperature alternations between low and high. Acid scarification is chemical method to melt seed coat and soften hard seed (Kimura and Islam, 2012). However, due to ecological reasons, mechanical processes are used. The mechanical processing concerns abrasion of the outer layer of the seed coat and can be carried out using a thresher or scarifier with sandpaper. In the case of quinoa, this process is not easy to perform due to the small size of seeds. The scarification of quinoa seed is often applied before sowing. Seed scarification reduces the thickness and weakens the strength of seed coat, which improves germination (Alderete-Chávez *et al.*, 2010; Kimura and Islam, 2012; Martin *et al.*, 2013; Rostami and Shasavar, 2009). The residue after the scarification of seeds is used as an additive to animal feed.

Due to concentration of oil in the outer layer of seeds (pericarp) (Prego *et al.*, 1998), studies were carried out on the possibility of extracting oil from the coats by using supercritical fluid extraction (SFE). SFE is widely used as an alternative to traditional techniques like mechanical pressing, organic solvent extraction. The supercritical carbon dioxide (SC-CO_2) is the most common solvent because of its unique properties, namely, it is non-flammable, non-toxic, inexpensive, cost efficient and high selectivity to non-polar molecules such as oils (Rai *et al.*, 2016). Its solvent properties can be changed dramatically with small changes in pressure and temperature. SC-CO_2 also separates easily from the extract, once the pressure is released, leaving no traces on the extract (Benito-Román *et al.*, 2018). One possibility under consideration is to use supercritical fluids technology which provides extraction yields very similar to those obtained by conventional liquid solvents extraction processes in which solvent-free extracts are obtained in relatively mild conditions which avoid thermal degradation, thus making it the ideal solvent for natural products (Follegatti-Romero *et al.*, 2009; Gracia *et al.*, 2011). In previous papers, application of SFE for extraction of oil and oil with increased amount of tocopherols was presented (Przygoda and Wejnerowska, 2015; Wejnerowska and Ciaciuch, 2018). Under optimum conditions, high oil recovery was obtained (~ 89%) and content of tocopherols in oil was increased from ~ 73 to 336 mg tocopherol/100 g oil. Regardless of the method of extraction (extraction with solvent or SFE) and type of oil extracted, no significant differences were found in profile of fatty acids (Benito-Román *et al.*, 2018; Follegatti-Romero *et al.*, 2009; Wejnerowska and Ciaciuch, 2018). Additionally, quinoa oil extracted using CO_2 presented higher antioxidant capacity and tocopherol content than quinoa oil extracted with solvent (hexane), regardless the quinoa variety used (Benito-Román *et al.*, 2018).

The paper presents result of the studies on the method of oil extraction from seed coat after seed pre-treatment by mechanical scarification. In order to recover oil, the seed coats obtained

as a result of mechanical scarification was subjected to extraction with supercritical carbon dioxide.

2. Materials and methods

2.1. Materials

Quinoa seeds (*Chenopodium Quinoa* Willd.) were bought from a local shop (country of origin Bolivia). Total humidity of seeds was determined by moisture analyzer MA30 (Sartorius, Germany) and was equal to 8.2%. Size of seeds before grinding was 1.6-2.0 mm (91%). Size of seeds and ground seeds was determined by performing sieve analysis Fritsch analysette 3 (RoTH, Germany). For sieve analysis, sieves with mesh sizes from 0.5 to 2.24 mm were used.

2.2. Reagents and standards

Carbon dioxide (99.5%) was obtained from Linde Gas (Poland). HPLC grade *n*-hexane and ethanol were purchased from Merck (Darmstadt, Germany).

The fine-grain sand for Soxhlet and SFE was sieved on sieves and fractions of 0.2–0.3 mm were collected. Then, the sand was purified by successive elution with warm distilled water, methanol and hexane. The sand was dried after each stage of elution.

2.3. Soxhlet extraction

Samples of 10 g o

f ground quinoa seeds were weighted with accuracy of 0.0001 g and then were mixed with 10 g of sand to determine the oil content by Soxhlet extraction using *n*-hexane at 60 °C for 16 h. After extraction, *n*-hexane was evaporated under vacuum at 40 °C and subsequently the solvent was totally removed by nitrogen steam. After evaporation of solvent, the oil content was determined gravimetrically. The mass of extracted oil was assumed to be 100% of the extractable matter (Przygoda and Wejnerowska, 2015; Wejnerowska and Ciaciuch, 2018).

2.4. Scarification of seeds

The working chamber of the scarifier (designed in our laboratory) was made of stainless steel and inside covered with replaceable, fine-grained abrasive material. Moreover, a flexible clamp supporting the abrasion of seeds was mounted. After starting the device, the chamber with a diameter of 40 cm rotated at a speed of 20 rpm. 100.0 g of seeds were poured into the chamber and after the cover was applied, the abrasion process was started. The principle of the scarifier operation consists in rubbing the seeds by rotating abrasive material at the moment of passing under the pressing down element. Scarification was carried out at constant drum revolutions, changing the time (10, 50 and 100 min) and using three types of abrasive gradations P40 (425 μm), P60 (269 μm) and P80 (201 μm). Scarification was performed three times for each time and abrasive gradation. Seeds that were not subjected to scarification were accepted as a control sample. After scarification, the seeds along with the seed coats were removed from the chamber by means of a manual vacuum cleaner and sieve analysis of the obtained material was performed. Based on the results of sieve analysis of the tested samples, the equivalent diameter (d) and content of fractions in the set were calculated. The particle size distribution of the tested material was approximated by Rosin, Rammler, Sperling and Bennet (RRSB) distribution function:

Where:

$$\sum R = e^{\left(-\frac{d}{d^*}\right)^n} \quad (1)$$

ΣR – total residue on the sieve,

d – diameter of sieve fraction of seed weight, defined as a geometric mean of mesh sizes of two adjacent sieves [mm],
d* – mean of linear dimensions of all seeds in a set [mm],
n – uniformity coefficient of graining.

Efficiency of the seed coat abrasion was investigated depending on time of scarification and abrasive gradation. The efficiency was expressed in amount of subscreen fraction obtained (diameter < 500 μm) (Table 1). The results were statistically analysed using one-way and multivariate analysis of variance (ANOVA) and Tukey's test to determine the significance of differences between variables.

In order to assess the effects of mechanical scarification, microscopic observation, analysis of oil content in the obtained seed coats and quantity of coat were used. A polarizing light microscope Eclipse E400 POL (Nikon, Japan) equipped with camera adapter for enlarged photos (20 x) was used for microscopic observations.

2.5. Supercritical fluid extraction procedures

A laboratory-scale SFE system Lizard 2001 SEKO-K s.r.o (Brno, Czech Republic) was used in this study. The seed coats (fraction < 0.5 mm) was loaded into the extractor cell of 1.2 mL capacity (0.5 cm internal diameter (I.D.) and 6.1 cm of effective height). 1 g of sample containing sand and seed coats at different ratios i.e. 1:1 - 1:3 (coat:sand w/w) were located into the cell and the content of cell was stirred for 5 min by use of rotary stirrer. The modifier (ethanol) was spiked directly into the sample in the extraction vessel before the extraction cell was attached to the SFE system. The extract was collected into 12 mL vials (previously weighted). The experiments were carried out in temperature 40 °C, at pressure 25 MPa and time 60, 90 and 120 min.

The adjustment of CO_2 flow is not possible in this SFE system. Measurements of CO_2 flow rate were performed at the end of capillary (restrictor) with diameter of 45 μm and length of 7 cm. The SC-CO_2 flows were dependent on extraction conditions and they were within the range from 15 to 22.0 L/h.

The extraction yield was determined by comparing the weight of oil obtained by SFE with the weight of oil obtained by Soxhlet extraction. All experimental results reported are average values from three repeated independent experimental runs.

3.Results and discussions

3.1. Scarification of quinoa seeds

The aim of studies was to carry out mechanical scarification in conditions allowing to remove as much of the seed coat as possible while maintaining the proper seed structure. Seeds after scarification can be used for food purposes and for sawing.

The scarification optimization was performed taking into account variable work parameters, i.e. time of scarification (10, 50 and 100 min) and the thickness of abrasive used (P40, P60 and P80). After each scarification process, a sieve analysis was performed, making allowance for the obtained amount of subscreen fraction. In the next stage of testing, oil was extracted from the subscreen fraction. Due to the small amount of obtained subscreen fraction after 10 min of scarification, regardless of the abrasive used, these results were not taken into consideration in statistical analysis of results.

Table 1. Coefficients of the RRSB equation

	Abrasive gradation									
	Control	**P80**			**P60**			**P40**		
Time (min)	0	10	50	100	10	50	100	10	50	100
d* (mm)	1.87	1.84	1.88	1.83	1.84	1.82	1.80	1.78	1.72	1.72
n	11.06	9.47	7.49	7.16	9.47	7.29	6.66	6.27	5.35	4.45
R^2	0.93	0.99	0.91	0.92	0.99	0.94	0.90	0.89	0.86	0.76

The seed coat layer obtained as a result of the scarification is a subscreen fraction of < 0.5 mm, the rest of the fraction are the different size seeds devoid of a hard shell (coat). The results of screening of control and scarified materials were subjected to linear regression analysis by determining the RRSB equation coefficients (Table 1). The calculated parameters indicate a good fitting of the RRSB model. The average grain diameter in the set (d*) decreases gradually with decreasing abrasive gradation and increasing time of scarification. The n parameter informing about the uniformity of the grain set is the smallest for the longest scarification time and the lowest abrasive gradation. It can be noticed that the equation well describes the granulometric distribution studied (high values of R^2).

Statistical analysis of the effect of the scarification time and the abrasive type on the amount of subscreen fraction obtained (Table 2) was carried out. Both the abrasive gradation and time of scarification have a statistically significant high impact on the amount of subscreen fraction obtained. Similarly, the analysis showed a highly statistically significant interaction between abrasive gradation and scarification time.

Table 2. Multifactorial analysis of variances with interactions (anova $\alpha = 0.01$) of the effect of abrasive gradation and time of scarification on the amount of subscreen fraction obtained

Factor	**Statistical value**	
	F	***p***
Abrasive gradation	2153.370	< 0.0001
Time of scarification	331.464	< 0.0001
Abrasive gradation × Time of scarification	152.992	< 0.0001

The mean results of the amount of subscreen fraction obtained depending on the scarification parameters used were presented in Table 3. The statistical analysis showed no significant differences between the control sample (no scarification) and the samples subjected to scarification with abrasive gradation of P80. Similarly, no differences were found for abrasives gradation of 60 in time of 50 min. Abrasive gradation of P40 resulted in a larger amount of subscreen fraction compared to the other gradations in each applied scarification time. The highest share of subscreen fraction was obtained by scarifying quinoa seeds with abrasive gradation of 40 for 100 min (9.32%).

The amount of subscreen fraction increases on the average two- or four-fold with the longer scarification time for 50 and 100 min, respectively. The results presented in Table 3 show that due to the amount of subscreen fraction obtained, the most favourable is application of abrasive with gradation of P40 and scarification time of 100 min. According to our observations, it is the most favourable to remove 10% by weight of the outer layer of seeds (seed coat). The optimum conditions of scarification are indicated by a high oil content in the separated seed coat (Table 4) and the results of microscopic observations. This is clearly illustrated in Fig. 1 where the whole seeds (1a), seeds after scarification (1b), seeds after scarification and separation of seed coats (1c) and separated seed coats (1d) are presented. It can be distinctly seen in Fig. 1(c) that the seeds were not damaged in scarification process and the oil-rich layer of endosperm was separated from the seeds.

The selected fractions were subjected to analysis of oil content by Soxhlet method (Table 4). Satisfactory results were obtained which indicated that seed coats obtained by scarification (fraction < 0.5 mm) contained 20.0 g oil/100 g seed coats and in the other combined

fractions (0.5-2.4 mm) the oil content decreased from 5.6 (whole seeds) to 3.3 g oil/100 g seed.

Table 3. The amount of subscreen fraction obtained depending on the abrasive gradation and time of scarification

Abrasive gradation	Time of scarification [min]	Subscreen fraction [%]
control	-	$0.60^{a} \pm 0.06$
P80	50	$0.63^{a} \pm 0.05$
P80	100	$0.84^{a} \pm 0.07$
P60	50	$0.70^{a} \pm 0.09$
P60	100	$1.61^{b} \pm 0.24$
P40	50	$5.39^{c} \pm 0.27$
P40	100	$9.32^{d} \pm 0.29$

a, b, c, d – values denoted with the same letter indicate belonging to a homogeneous group that does not differ statistically at $\alpha = 0.05$.

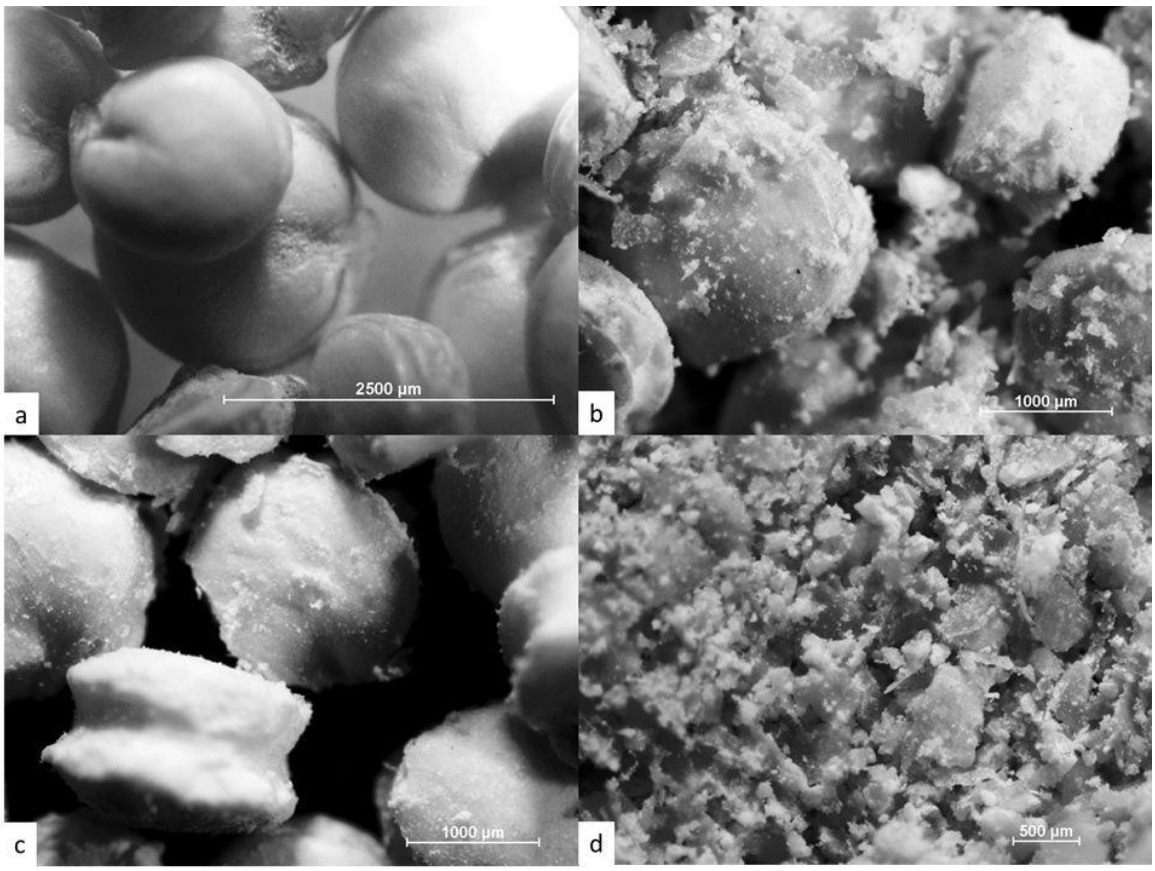

Figure 1. Quinoa seeds, a – whole seeds (control), b – seeds after abrasion (P40 grad., 100 min.), c – 0.5–2.25 mm, d – subscreen fraction (seed coat) < 0.5 mm

Table 4. Oil content (soxhlet method) in seeds and in the selected fractions after scarification of quinoa seeds for 100 min

Operating conditions	Particle size (mm)	Oil content (Soxhlet) (%)
Control (no scarification)	> 2.24-0.5	5.6 ± 0.2
Seed coat scarified. P60	> 2.24-0.5	5.5 ± 0.2
Seed coat scarified. P40	> 2.24-0.5	3.3 ± 0.1
Seed coat scarified. P40	< 0.5	20.0 ± 0.9

3.2. Supercritical fluid extraction

As a result of scarification of quinoa seeds, the material containing over three times (20.0 g oil/100 g seed coats) more oil was obtained compared to whole seeds (5.6 g oil/100 g seed).

The oil contained in the seed coats obtained by scarification was subjected to extraction with supercritical carbon dioxide. Optimization studies on oil separation from quinoa seeds have been presented in our previous paper (Wejnerowska and Ciaciuch, 2018). The most favourable parameters for extraction with supercritical CO_2 were determined during our previous studies. These parameters were also applied for extraction of oil from the seed covers, i.e. temperature of 40 °C and pressure of 25 MPa. In order to increase the extraction efficiency, ethanol was used as cosolvent in the amount of 10% w/w. The tests were carried out at a variable extraction time of 60, 90 and 120 min. Our previous studies showed that it was preferable to add an inert filling (sand) to a material such as ground seeds (Przygoda and Wejnerowska, 2015; Wejnerowska and Ciaciuch, 2018; Wejnerowska *et al.*, 2013). Extraction of oil from seed coats, obtained as a result of scarification, were carried out with addition of various amounts of filling i.e. 1:1-1:3 (coat:sand, w/w). The amount of oil obtained and the efficiency of SFE process are shown in Table 5.

Table 5. Amount of extract obtained from seed coats and degree of recovery depending on time of extraction and amount of inert filling added (25 MPa, 40 °C and 10% w/w ethanol as cosolvent)

Seed coat:inert filling [w/w]	Amount of oil obtained [g/100 g of coat]			Yield [% w/w]		
	60 min	90 min	120 min	60 min	90 min	120 min
0	5.2 ± 0.2	6.4 ± 0.3	8.4 ± 0.4	24.5 ± 1.1	28.6 ± 1.2	40.1 ± 1.8
1:1	7.0 ± 0.3	8.2 ± 0.4	11.7 ± 0.5	35.1 ± 1.6	40.9 ± 1.8	58.5 ± 2.9
1:2	11.4 ± 0.5	11.8 ± 0.6	12.2 ± 0.7	57.2 ± 2.7	59.0 ± 3.0	60.9 ± 3.2
1:3	10.1± 0.5	10.1 ± 0.4	10.4 ± 0.5	50.5 ± 2.3	50.6 ± 2.4	52.2 ± 2.5

A positive effect of an inert filling addition was observed in the case of the seed coats. Addition of filling to the seed coat in a ratio of 1:2 results in an approx. 100% increase in extraction efficiency compared to extraction carried out without addition of filling. This is related to looseness of the batch structure and facilitated penetration of supercritical carbon dioxide between its particles. On the other hand, too much (1:3) of filling added results in a slight decrease in the yield.

A longer time of extraction caused that more oil from extracted material was obtained. Extending the extraction time from 60 min to 120 min has a significant effect on extraction efficiency if extraction is carried out without addition of filling or if it is in amount of 1:1. It can be concluded that adding the optimum amount (1:2) of filling to the seed coat results in maximum process efficiency in a shorter time. Extraction of oil from seed coat of quinoa with supercritical CO_2 allowed us to obtain about 12

g of oil from 100 g of seed coats. In the case of oil extraction from quinoa seeds, we received a maximum of 6.7 g of oil from 100 g of seeds (Wejnerowska and Ciaciuch, 2018). Taking into account that the coasts of supercritical extraction conducted on industrial scale are high, it is much more advantageous to extract from the material (seed coats) containing a larger amount of oil.

4. Conclusions

The paper presents a high-performance method of using seed coats of quinoa seeds to obtain an oil. Mechanical pretreatment with scarification prior to extraction was used. As a result of our tests, the optimum amount of seed coat which can be removed by any mechanical scarification method was determined and it was 9-10% of the seed weight. Seeds subjected to scarification can be used for seeding or after removal of hard shell and bitter saponins can be used for food purposes. A valuable product that is obtained as a result of scarification is a seed coat containing large amounts of oil (20 g oil/100 g seed coats). For this purpose, supercritical fluid extraction with supercritical carbon dioxide was applied. The recovery of oil from the seed coats was approx. 60% and the amount of oil obtained from 100 g of the coats was approx. 12 g.

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