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Optimisation of Oil Extraction from *Quinoa* Seeds with Supercritical Carbon Dioxide with Co-solvents

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Abstract

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In the present work supercritical fluid extraction with carbon dioxide was performed to obtain oil from quinoa seeds. The effects of extraction variables – namely pressure, temperature, time, particle size, and co-solvent, on supercritical carbon dioxide extraction are investigated. Total extraction yields and compositions using pure CO_2 and CO_2 + selected co-solvents are compared. The maximum recovery for quinoa oil is found to be about 89%, and is obtained when extractions are carried out at 25 MPa, 40°C for 80 minutes. A significant effect on the oil recovery is exerted by size reduction of seeds to a particle size ≤ 0.50 mm and addition of co-solvent to seed in an amount of 20% – methanol/ethanol (1 : 1, w/w). Irrespective of the extraction method and conditions, the fatty acid composition is not substantially changed.

Keywords: quinoa seed oil; seed oil extraction; supercritical fluid extraction

Quinoa (*Chenopodium quinoa* Willd.) is a food plant of the family *Amaranthaceae*, subfamily *Chenopodiaceae* and genus *Chenopodium*. Quinoa (*Chenopodium quinoa* Willd.) is an Andean pseudo-cereal grain about 1 mm thick which ranges in diameter from 1 mm to 2.5 mm. Several studies have revealed that the oil content in quinoa ranges from 1.8% to 9.5%, with an average of 5.0–7.2% (Koziol 1993; Bhargava *et al*. 2006; Nowak *et al*. 2016; Maradini-Filho 2017). Quinoa offers a high quality oil rich in natural antioxidants such as tocopherols and unsaturated fatty acids like linoleic acid (~ 50%) and oleic acid (~ 25%) (Koziol 1993). In addition, it has a wide range of vitamins and microelements. The gluten-free nature also makes quinoa a valuable dietary source of digestible protein for people with gluten sensitivity and celiac disease. Its antioxidant activity is associated with its content of tocopherol (vitamin E) and phenolic compounds (Bhargava *et al*. 2006; Miranda *et al*. 2010). Although high in unsaturated fatty acids, quinoa oil is stable due to its high amounts of vitamin E, which acts as a natural antioxidant to prevent rapid lipid oxidation (Ng *et al*. 2007). The antioxidant activity of quinoa might be of particular interest to the medical researchers and needs more attention towards its utilization as a potent antioxidant. Due to the fact that quinoa contains high quality oil and that some varieties show fat concentrations up to 9.5%, it has been considered as a potentially valuable new oil crop (Koziol 1993; Bhargava *et al*. 2006).

Supercritical fluid extraction (SFE) is an alternative method used to recover and separate extracts from plants, which generally provides extract of higher quality when compared to conventional methods (Dogenski *et al*. 2016). SFE is widely used as an alternative to traditional techniques like mechanical pressing, organic solvent extraction. 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,

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non-polar, easy to remove and also offers a higher extraction rate (Rai *et al*. 2016). Due to the advantages mentioned above, there is interest in developing the new extraction methods to obtain extracts that contain a high amount of valuable compounds and no residual solvent. One possibility under consideration is to use supercritical fluid technology which provides extraction yields very similar to those obtained by conventional liquid solvent 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 (Gracia *et al*. 2011).

The main drawback of SC-CO_2 is its low polarity, a problem that can be overcome by employing a polar co-solvent (modifier, entrainer) to change the polarity of the supercritical fluid and to increase its solvating power towards the analyte of interest. For example, the addition of relatively small percentages (1–10%) of polar co-solvent (e.g. methanol, ethanol, isopropanol, acetone) to carbon dioxide expands its extraction range to include more polar analytes (Herrero *et al*. 2010). Ethanol is commonly used as co-solvent for the extraction of natural products because the toxicity of ethanol to the human body is low. Furthermore, it can be easily removed from the food matrix although there is limited information regarding the effect of ethanol and other co-solvents on the SC-CO_2 extraction (Solana *et al*. 2014).

To the best of our knowledge, there is no report on the extraction of oil from quinoa seeds by supercritical fluid techniques. In our previous publication, we presented the results of studies on the isolation of extract from quinoa seeds with increased content of tocopherols (Przygoda & Wejnerowska 2015). Thus, the objective of this study was to investigate an effect of the type and concentration of polar co-solvent (methanol, ethanol), particle size distribution of ground seeds as well as parameters such as: temperature, time and pressure on the extraction efficiency of oil from quinoa seeds by using SC-CO_2.

MATERIAL AND METHODS

Material. Quinoa (*Chenopodium quinoa* Willd.) seeds were bought from a local shop (country of origin Bolivia). Total humidity of seeds was determined with a Sartorius MA30 moisture analyser and was equal to 8.2%. Size of seeds before grinding was 2.1 mm (79.2%). Quinoa seeds were ground with Ultra Centrifugal Mill ZM 200 (Retsch GmbH, Germany) and kitchen mill Bomann CB 425 (Germany). In the Ultra Centrifugal Mill ZM 200 the size reduction takes place by impact and shearing effects between the (12-tooth) rotor and the fixed ring sieve (trapezoid holes 1.00 mm). The feed material passes through the hopper (with splash-back protection) onto the rotor. Centrifugal acceleration throws it outward with great energy and it is precrushed on impact with the wedge-shaped rotor teeth moving at a high speed. It is then finely ground between the rotor and the ring sieve. The size of ground seeds was determined by performing sieve analysis with Fritsch Analysette 3 PRO and their fractional composition is presented in Table 4.

Reagents and standards. Carbon dioxide (99.5%) was obtained from Linde Gas (Poland). HPLC grade *n*-hexane, methanol, and ethanol were purchased from Merck (Germany). Standard mixture of fatty acids – Mix RM-2 – was purchased from Supelco (USA).

The fine-grain sand for Soxhlet extraction 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.

Soxhlet extraction. Samples of 10 g of ground quinoa seeds were weighed to the nearest 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 hours. After extraction, *n*-hexane was evaporated under vacuum at 40°C and subsequently the solvent was totally removed by nitrogen steam. After solvent evaporation, the oil content was determined gravimetrically. The mass of extracted oil was assumed to be 100% of the extractable matter.

Supercritical fluid extraction procedures. A laboratory-scale SFE system Lizard 2001 SEKO-K s.r.o (Czech Republic) was used in this study. Ground seeds of quinoa were loaded into the extractor cell of 1.2 ml capacity; 0.5 cm internal diameter (i.d.) and 6.1 cm of effective height. About 0.4 g of sand and 0.6 g of ground seeds (weighed to the nearest 0.0001 g) were located into the cell and the content of the cell was stirred for 5 min using a rotary stirrer. For all the modifier (co-solvent) studies, the modifier was spiked directly onto 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 weighed). The experiments were carried out at a temperature of 40–80°C, at a pressure from 18 to 30 MPa and time for 40–140 minutes.

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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 the 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 10.5 to 27.0 l/hour.

The extraction yield was determined by comparing the weight of oil obtained by SFE with the weight of oil obtained by Soxhlet extraction.

FAME analysis. The fatty acid methyl esters (FAMEs) were prepared by transesterification of oil with 2M KOH in methanol and isooctane. Gas chromatographic analyses were performed on an HP 6890 (Hewlett-Packard, USA) model equipped with an on-column injector and flame ionization detector (FID). The fused silica capillary column coated with a 1.0 μm film of HP-FFAP (polyethylene glycol), 30 m and 0.53 mm I.D. was used. Helium was used as a carrier gas at a constant flow rate of 1.2 ml/minute. Temperatures of FID detector and the on-column injector were 250 and 103°C, respectively. The oven temperature was 100°C (0 min), and then programmed to 230°C at 10°C/min and held there for 2 minutes. One microlitre of sample was injected into the column, and the fatty acids were identified by a comparison of the retention times with those of the methyl ester standards.

RESULTS AND DISCUSSION

Effect of SFE parameters on quinoa oil recovery. Optimization studies of oil extraction from quinoa seeds with supercritical carbon dioxide (SC-CO_2) were performed using the seeds with oil content of 7.0%. The seeds were ground with an ultra-centrifugal mill to the particle size from ≤ 0.05 to 1.4 mm (the composition is presented in Table 2).

Effect of co-solvent. Effect of the co-solvent on the efficiency of oil extraction from seeds was studied by using methanol, ethanol and methanol/ethanol mixture (1 : 1, w/w) in an amount of 1.3–20% (weight of the co-solvent with respect to weight of seeds).

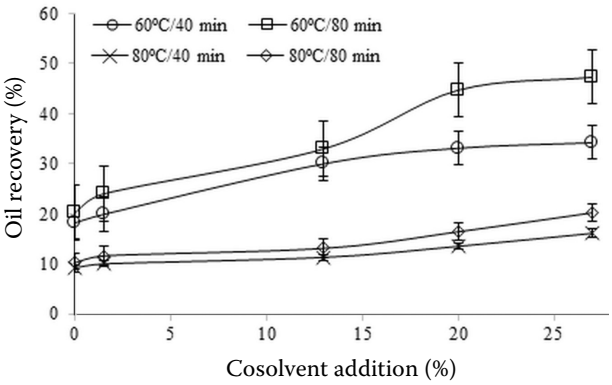

Figure 1. Effect of co-solvent addition (methanol/ethanol 1 : 1, w/w), extraction time and temperature on the oil recovery (extraction of quinoa ground seeds with supercritical CO_2 at 18 MPa.). Co-solvent addition is expressed in wt% relative to the total amount of ground seeds

Studies on extraction efficiency were performed under the constant conditions (18 MPa, 80°C, 60 min) by changing the type and amount of the co-solvent added. Results of studies are presented in Table 1.

It was observed that the addition of a polar co-solvent to quinoa seeds affected the amount of oil extracted with supercritical fluid. The extraction yield increased as the concentration of polar co-solvents was increased. Our research has shown a slight difference in the extraction efficiency in the case of using methanol or ethanol. However, the use of their mixture resulted in a noticeable increase in yield, therefore the methanol/ethanol mixture (1 : 1, w/w) was used in our further studies.

Effect of process parameters on quinoa oil recovery. Optimization studies of the supercritical extraction conditions were performed by adding different amounts of methanol/ethanol mixture (0 to 27%), at two temperatures (60 and 80°C), at two times of extraction (40 and 80 min) and at constant pressure (18 MPa).

The experimental results show that the lower temperature and longer time of extraction are more favourable for quinoa oil extraction (Figure 1). These data are consistent with the results presented in our previous paper (Przygoda & Wejnerowska 2015). In the case of oil extraction from amaranth

Table 1. Effect of co-solvent addition on the oil recovery (%)

Control	Methanol			Ethanol			Methanol/ethanol 1 : 1 (w/w)		
	1.3	13.2	20.0	1.3	13.2	20.0	1.3	13.2	20.0
8.5	8.5	8.7	9.9	8.5	8.8	9.1	8.8	9.3	10.9

Extraction of quinoa ground seeds with supercritical CO_2 (18 MPa, 80°C, 60 min); co-solvent addition is expressed in %wt relative to the total amount of ground seeds

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Table 2. Extraction of separated fractions of quinoa ground seeds with supercritical CO_2 compared to a conventional hexane extraction method (Soxhlet)

Size	Particle size (mm)	Composition (%)	Oil yield (g of oil/100 g of seed)		Recovery (% total oil)
			soxhlet	SFE	
1	0.80 ± ≥ 1.40	28.0	2.6	1.0	38.5
2	0.50 ± 0.80	44.2	7.7	3.1	40.3
3	≤ 0.05 ± 0.50	27.8	10.9	6.7	61.5
4*	0.12 ± 0.50	95.2	7.1	6.3	88.7

Extraction with supercritical CO_2 at 25 MPa, 40°C, for 80 min, co-solvent addition 20%wt (methanol/ethanol 1 : 1, w/w) relative to the total amount of ground seeds); SEF – supercritical fluid extraction; *seeds ground with a kitchen mill

(Wejnerowska *et al.* 2013), which contains a similar amount of oil in the seeds like quinoa, the same dependences were obtained.

Addition of the co-solvent to the seeds has a significant impact on the amount of extracted oil. It was observed that in extraction performed at a higher temperature (80°C), an increase in the amount of co-solvent – ethanol and methanol (1 : 1, w/w) from 20% to 27% resulted in the yield increase by ~ 20%, however, at a temperature of 60°C it was only by ~ 5%. It was decided to perform further studies on the effect of pressure on the extraction yield at lower temperatures (40 and 60°C) and with addition of the co-solvent in an amount of 20%. The effect of pressure on the extraction of oil from quinoa seeds was investigated with SC-CO_2 at pressures of 18, 25, and 30 MPa. The results are presented in Figure 2.

As expected, it was observed that the extraction yield increased with pressure, according to other studies on the oil extraction from seeds (Wejnerowska *et al.* 2013; Tai & Kim 2014; Li *et al.* 2016; Özkal & Yener 2016; Rai *et al.* 2016). The results presented

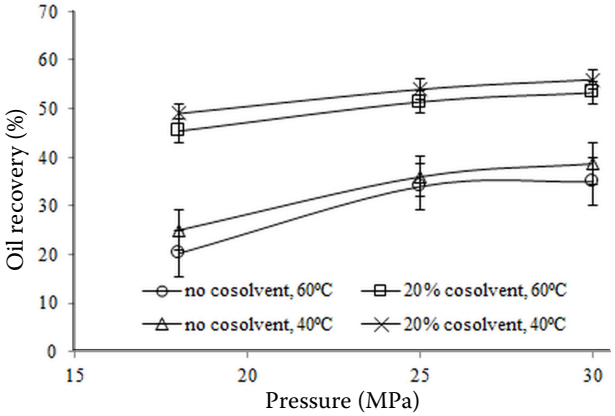

Figure 2. Effect of pressure on the oil recovery (extraction of quinoa ground seeds with supercritical CO_2 for 80 min; co-solvent addition is expressed in wt% relative to the total amount of ground seeds)

in Figure 2 show that over the range of pressures 18–30 MPa and for temperatures of 40–60°C, addition of the co-solvent has a higher effect on oil recovery. Addition of the co-solvent in an amount of 20% to seeds results in an increase in the yield of extraction (performed under the same conditions) even by 120%. However, an increase in pressure from 25 to 30 MPa results only in a slight increase in oil recovery from 54% to 56% (20% of co-solvent, 40°C) and from 51% to 53% (20% of co-solvent, 60°C).

Time of extraction, it is a very important parameter affecting the quantity of extracted oil. It is known that a longer time of extraction increases the amount of extracted oil. The dependence between time of extraction and oil recovery from seeds for two temperatures of 40 and 60°C is presented in Figure 3. In the initial time of extraction, its yield rapidly increases, whereas between 80 and 140 min only a slight increase in extraction yield is observed (2–3%).

Summarising the results of studies on optimization of the oil extraction from quinoa seeds, it was found that the highest recoveries of 55–56% were obtained under the following conditions: pressure from 25 MPa to 30 MPa, temperature 40°C, and co-solvent of 20%, the extraction time 80–120 minutes. Extraction with SC-CO_2 performed under conditions given above allowed us to obtain the yield of oil extraction from quinoa seeds by 10% higher than in our previous work (Przygoda & Wejnerowska 2015). Surely, the addition of co-solvent to seeds has the highest effect on this result.

Effect of particle size. The results of studies show that 45% of unextracted oil remains in quinoa seeds. Therefore, studies were undertaken in order to increase the extraction efficiency, taking into consideration the method of size reduction and particle size distribution of ground seeds. The seeds for tests were ground by using two mills – kitchen mill and ZM 200 ultra-centrifugal mill. An operating principle of

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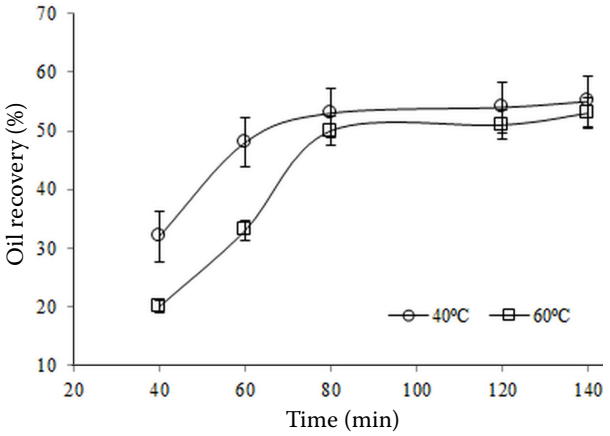

Figure 3. Effect of extraction time on the oil recovery (extraction of quinoa ground seeds with supercritical CO_2 at 25 MPa and co-solvent addition 20% (methanol/ethanol 1 : 1, w/w). Co-solvent addition is expressed in wt% relative to the total amount of ground seeds

mills and the obtained particle size distribution of ground seeds are different (Table 2). Quinoa seeds ground with the ZM 200 mill were applied in SFE optimization studies. Due to a wide range of particle sizes (≤ 0.05 to ≥ 1.40 mm), for further studies they were separated to three fractions (Table 2, size 1–3).

The oil content was determined by a reference method (Soxhlet) in seeds ground with both mills and in each fraction. Moreover, the yield and recovery of oil were determined by the SFE method. The results show extraction efficiency at the constant conditions: pressure of 25 MPa, temperature of 40°C, extraction time of 80 min and use of co-solvent in an amount of 20% – ethanol and methanol (1 : 1, w/w). The results are presented in Table 2.

The results show that the attention should be paid to two aspects. The one is significantly different oil content in fractions obtained after grinding with an ultra-centrifugal mill, i.e. from 2.6 to 10.9 g oil/100 g of seeds. The other consists in obtaining considerable differences in oil recovery from the tested fractions, depending on the particle size and method of grinding (recovery 38.5–88.7%).

Differences in oil content in the individual fractions can result from the structure of seeds. Oil in quinoa seeds occurs in the surface layer of seeds (endosperm) (Prego *et al*. 1998) and the individual parts of seeds are ground with an ultra-centrifugal mill to a different extent. The kitchen mill provides higher grinding efficiency and the obtained fine fraction of seeds has the mean oil content. Differences in the efficiency of grinding in both mills are clearly visible in Figure 4B–F. It can be observed in presented photographs that different parts of seeds occur in different fractions (Figure 4B–E). The highest oil content was obtained in ground seeds of small particle size (≤ 0.50 mm).

It can be observed that the extraction yield decreased when the particle size increased due to the interparticle diffusion resistance; the smaller the particle size, the shorter the diffusion path. The grinding process increases the surface area and may disrupt the cell walls, reducing mass transfer resistance and leaving the extracted oil more accessible to the solvent, and consequently, increasing the extraction rate. The total extraction yields obtained indicated that a reduction in particle size from size 1 to size 2 slightly increased the extraction recovery (from 38.5% to 40.3%), whereas a reduction in particle size from size 1 to size 3 increased the recovery from 38.5% to 61.5%.

Fatty acid composition. The profile of fatty acids (FAs) in quinoa oil obtained by the Soxhlet method with *n*-hexane and by SFE at optimized conditions is presented in Table 3. The results refer to the ground seeds of size ≤ 0.05 to ≥ 1.4 mm.

No significant differences were found in FA analysis between oils extracted with *n*-hexane in a Soxhlet apparatus or by SFE (with/without co-solvent) as shown in Table 3. There is no change in terms of FA distribution in all the extracted quinoa seed oils under

Table 3. Fatty acid profiles determined in the samples of quinoa oil obtained with conventional hexane extraction (Soxhlet) and with supercritical CO_2 extraction

Extraction methods	Fatty acid (% of total fatty acids)							
	C14:0	C16:0	C18:1	C18:2	C18:3	C20:0	other	UFA
Soxhlet	0.2	11.3	28.7	50.6	5.5	0.7	3.0	84.8
SFE 1	0.2	11.0	29.7	50.0	5.2	0.7	3.2	84.9
SFE 2	0.2	11.2	28.2	50.9	5.7	0.7	3.1	84.8

25 MPa, 40°C, 120 min; UFA – unsaturated fatty acids; SFE – supercritical fluid extraction; SFE 1 – co-solvent addition 20%wt (methanol/ethanol, 1 : 1, w/w) relative to the total amount of ground seeds; SFE 2 – no co-solvent

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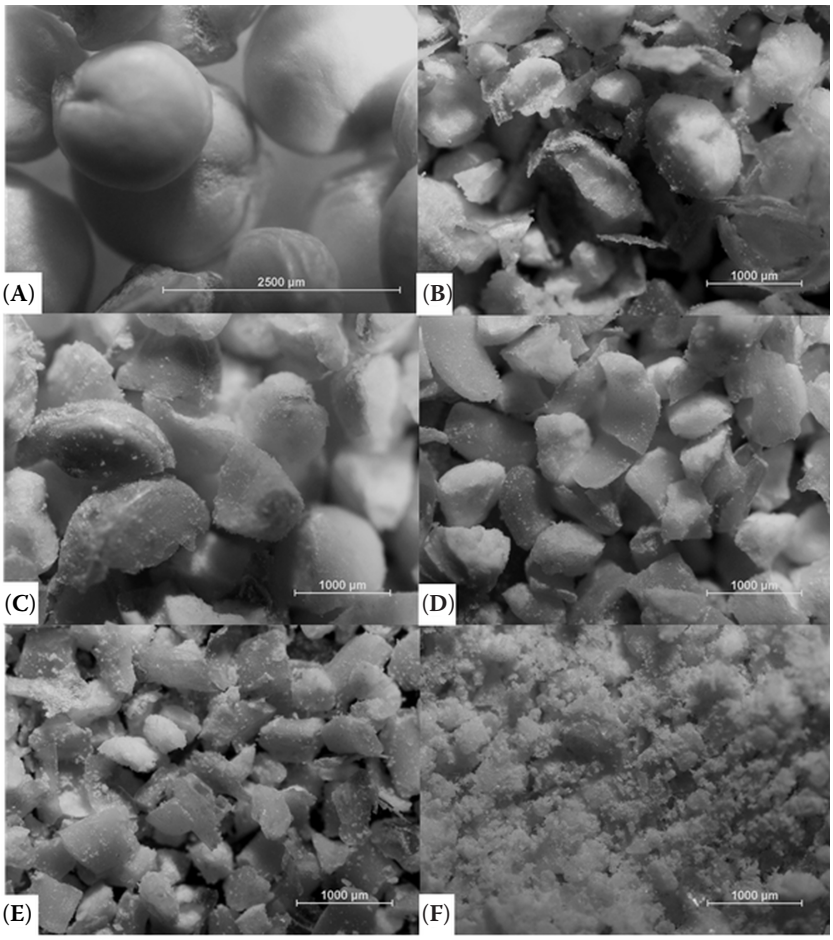

Figure 4. Microscope images of quinoa seeds: (**A**) whole seeds, (**B**) seeds ground with a ZM 200 mill, particle size from ≤ 0.05 to ≥ 1.40 mm, (**C**) particle size 0.80–1.00 mm, (**D**) particle size 0.50–0.71 mm, (**E**) particle size 0.05–0.35 mm, (**F**) seeds ground with a kitchen mill, particle size 0.12–0.50 mm

conditions of this study. Neither significant nor slight differences were observed by other researchers in oil extracted from different seeds by SFE. They also compared SFE results with other extraction techniques such as Soxhlet, cold pressed and ultrasound-assisted extractions (Sánchez-Vicente *et al.* 2009; Özcan *et al.* 2013; Ara *et al.* 2014). The FA composition in quinoa oil is similar to that of maize and soybean oil. Unsaturated fatty acids in quinoa oil reach up to 85% of the total FA. The main component in oils was linoleic acid (~ 50%), followed by oleic acid (~ 29%) and palmitic acid (~ 11%). These results agree with the FA amounts in quinoa oil found by other authors (Koziol 1993; Peiretti *et al.* 2013; Tang *et al.* 2015). The total acid composition includes about 56% of polyunsaturated fatty acids (PUFA). Although high in PUFAs, quinoa oil is stable due to its high amounts of vitamin E, which acts as a natural antioxidant to prevent rapid lipid oxidation (Ng *et al.* 2007). PUFAs were mainly from two essential FAs, linoleic acid (18:2ω6) and α-linolenic acid (18:2ω3).

Quinoa can be considered an alternative oilseed crop, due to the quality and quantity of its lipid fraction. A comparison of the fatty acid profile of quinoa oil with that of maize and soybean showed similar levels of oleic, linoleic and linolenic acids. Quinoa is also an excellent example of 'functional food' which may help 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. Quinoa contains a number of nutrients including oil with higher levels of essential fatty acids that have a beneficial effect on human health (Maradini-Filho 2017).

CONCLUSIONS

In the present study, the supercritical fluid extraction operating conditions were optimised to achieve an efficient oil extraction from quinoa seeds. The maximum oil extraction recovery was 88.7%, which was obtained at 25 MPa, 40°C, 80 min, and 20% of co-solvent, ethanol and methanol (1 : 1, w/w). The total extraction yield increased with an increase in

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pressure and time; the temperature rise reduced the process efficiency. The addition of co-solvent and suitable size reduction of seeds have a crucial impact on this result. A decrease in particle size increased the total oil yield. The presented result was obtained for the particle size ≤ 0.35 mm and co-solvent addition to seeds in an amount of 20% (co-solvent weight relative to the weight of seeds). The results indicated that the values for the oil content and the fatty acid compositions of supercritical and hexane extracts are similar.

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