The Journal of Supercritical Fluids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

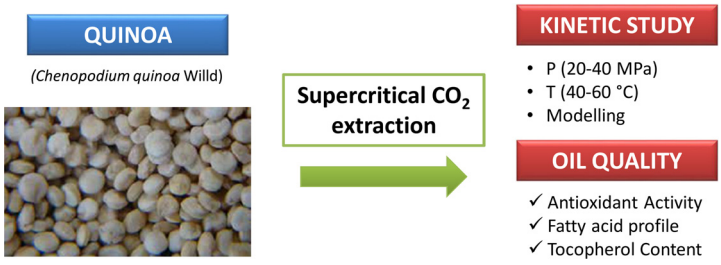
journal homepage: www.elsevier.com/locate/supflu

Supercritical carbon dioxide extraction of quinoa oil: Study of the influence of process parameters on the extraction yield and oil quality

Oscar Benito-Román*, María Rodríguez-Perrino, María Teresa Sanz, Rodrigo Melgosa, Sagrario Beltrán

Department of Biotechnology and Food Science (Chemical Engineering Section), Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos s/n, 09001, Burgos, Spain

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Supercritical fluid extraction
CO_2
Quinoa
Oil
Tocopherol

ABSTRACT

The supercritical CO_2 extraction of oil from four different quinoa varieties has been studied in this work. For this purpose, the influence of extraction temperature (40–60 °C), pressure (20–40 MPa) and raw material size (250–1000 μm) on the extraction rate has been considered. The extraction rate resulted to be faster the higher the pressure whereas the temperature had less influence on the extraction kinetics. The experimental data were modelled using the Sovova's kinetic model.

The quality of the oil extracted has been evaluated in terms of antioxidant activity (AA), fatty acid profile and tocopherol content. The highest AA was obtained for quinoa oil extracted at 40 MPa and 40 °C; this oil presented high content of polyunsaturated fatty acids (63% of the total) and significant amount of tocopherols (2.5 mg/g oil).

Quinoa oil extracted using CO_2 presented higher antioxidant capacity and tocopherol content than quinoa oil extracted with hexane, regardless the quinoa variety used.

1. Introduction

In recent years there has been a renewed interest in quinoa (*Chenopodium quinoa* Willd) due to its outstanding nutritional properties: it is rich in high quality proteins, vitamins and minerals [1]. Quinoa has been grown in South America for centuries [2], primarily in Bolivia and Peru [3]. This pseudo-cereal has the ability to grow in a wide diversity of environments due to its resistance to weather and soil conditions, unlike other cereals, which reinforces its attractiveness [4].

This renewed interest in quinoa has been fostered by The Food and Agricultural Organization of the United Nations (FAO) after having considered it as *"high nutritive"* due to its high protein content (in the range from 10 to 18%) and high quality oil (in the range from 4.5 to 8.75%) [5]. Moreover, quinoa shows a fatty acid profile rich in unsaturated acids (mainly oleic and linoleic) and a balanced content in aminoacids [6] such as lysine and methionine [1]. The increased

* Corresponding author.
E-mail address: obenito@ubu.es (O. Benito-Román).

https://doi.org/10.1016/j.supflu.2018.05.009
Received 28 March 2018; Received in revised form 8 May 2018; Accepted 8 May 2018

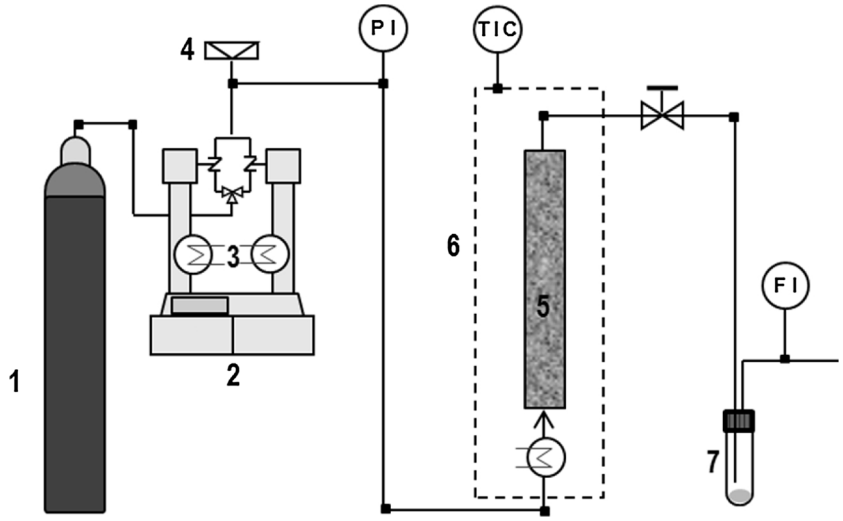


Fig. 1. Supercritical fluid extraction plant. 1: CO_2 reservoir; 2: syringe pump; 3: cryostat; 4: bursting disk; 5: high pressure extractor; 6: oven; 7: separator.

concern that consumers have about food safety and food functionality, willing to try functional and healthier products, is pushing the studying and growing of quinoa in European countries and Japan [7], where there is a great potential for this cultivar. In this sense the presence of essential fatty acids and tocopherols in the oil extracted from quinoa has attracted the attention of the cosmetic, pharmaceutical and food industries [3].

Despite all the excellent properties attributed to quinoa, there is a limited amount of research works about it [2]. The conventional methods to extract oil from seeds are based on the use of organic solvents at relative high temperatures followed by laborious purification procedures. To the best of our knowledge there is a limited number of works dealing with the extraction and characterization of the oil extracted from quinoa [8–10]. Alternatively to the organic solvents, the supercritical fluid extraction is a well-known technology used to extract high added value compounds from many different sources. Supercritical CO_2 (SC-CO_2) is one of the most common supercritical solvents due to its properties: mild critical point (critical temperature 31.7 °C and critical pressure around 7 MPa), non-toxicity, non-explosivity, low price and high selectivity to non-polar molecules such as oils. Its solvent properties can be changed dramatically with small changes in pressure and temperature [11]. SC-CO_2 also separates easily from the extract, once the pressure is released, leaving no traces in the extract. All these properties make SC-CO_2 a potentially successful solvent for the extraction of oil from quinoa [12]. Although it is possible to find in the literature numerous works that successfully deal with the extraction of oil from cereals, such as wheat bran [13], corn [14] or amaranth [15], there is only one work that uses supercritical CO_2 to extract oil from quinoa [3]. Przygoda and Wejnerowska, did not study the extraction of oil from a kinetic point of view: an experimental design was used instead of studying the extraction curves. From that work it is not possible to conclude what mechanism controls the extraction process, whether the internal diffusion, external transport or equilibrium. In that work temperature was varied from 35 to 120 °C, pressure from 8.5 to 28.5 MPa for experiments lasting up to 100 min. The oil yield and the tocopherol concentration were quantified and the effect of the process parameters was studied.

The purpose of this work is to study the influence of several extraction parameters (pressure, temperature and particle size) on the extraction rate of quinoa oil (cv. Titikaka, since it is the most extensively grown in Europe due to its adaptation to the climatic conditions). The Sovovás mathematical model [16] is used to describe the experimental extraction curves. In a second step of the work, the quality and stability of the quinoa oil obtained by SC-CO_2 is analysed and compared to the oil obtained using hexane, in terms of fatty acid profile, tocopherol content and antioxidant activity.

Finally the oil extracted from four quinoa varieties (Pasankalla, Collana and Altiplano and Titikaka) using either hexane or supercritical CO_2 is analysed and compared.

2. Materials and methods

2.1. Raw material

Four different varieties of quinoa (kindly provided by Quinoa Spain Productos Ecológicos S.L. and harvested in Álava (Spain)) were used in this work. The oil content of those varieties, named Titikaka, Altiplano, Collana and Pasankalla, was determined by Soxhlet extraction (Buchi B-8111) using hexane as solvent.

Quinoa seeds were ground in a ball mill (Fritsch) to get different particle sizes in the range from 250 to 1000 μm and study the influence of the particle size on the extractability of the oil by SC-CO_2.

2.2. Supercritical fluid extraction equipment and procedure

The extraction experiments were carried out in a lab-scale plant, whose diagram is shown in Fig. 1.

The maximum specifications of this experimental set-up are 150 °C and 50 MPa. The extractor has a volume of 26.5 mL with ½" internal diameter. In a typical experiment, around 12 g of quinoa were placed in the extractor, which was pressurized with CO_2 (Air Liquide S.A.) up to the extraction pressure. Then, the solvent flowed at the desired pressure and temperature (at a mass rate of 0.14 ± 0.02 kg/h) for the desired time (maximum 3 h). Different combinations of pressure and temperature were tried in order to study their influence on the extraction performance.

2.3. Analytical methods

2.3.1. Determination and quantification of the fatty acids profile

The fatty acids profile was determined by the AOAC official method [17]. According to this method the fatty acid methyl esters were firstly prepared and then analyzed by gas chromatography using a Hewlett Packard (6890N Network GC System) gas chromatograph equipped with an auto-sampler (model 7683B) and a flame ionization detector (FID). Helium was used as a carrier gas at a flow rate equal to 1.8 mL/min. A fused silica capillary column (OmegawaxTM-320, 30 m × 0.32 mm) was used.

Table 1
Oil content of four quinoa varieties obtained by Soxhlet extraction using hexane.

Quinoa Variety	g oil/g of insoluble solid
Titikaka	4.3 ± 0.1
Pasankalla	7.9 ± 0.1
Altiplano	6.0 ± 0.4
Collana	4.9 ± 0.3

Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Aldrich). Their quantification was made by relating the peaks area to the area of an internal standard (methyl tricosanoate) as indicated by the AOAC method [17]. Calibration curves were made for several pairs formed by internal standard + several representative chromatographic standards in order to find the corresponding response factors.

2.3.2. Determination and quantification of the tocopherol profile

Tocopherols determination was done by HPLC-DAD. 0.03 g of the sample were weighted and dissolved in 2 mL of hexane, shaken for a minute in a vortex mixer and filtered (pore size 0.45 μm). Tocopherols were determined following a modification of the IUPAC method [18] using a HPLC (Agilent series 1100) equipped with the software ChemStation, a degasser (G1322A), a quaternary pump (G1311A), an autosampler (G1329A), a column oven (G1316A) and a diode array detector (G1315A). The column used was ACE 5 Silica (250 × 4,6 mm). The mobile phase was 99% hexane (A) and 1% 2-propanol (B) at a flow rate of 1 mL/min. An isocratic gradient was used, the total run time was 15 min and the injection volume was 50 μL. All the tocopherols were monitored at 296 nm. The column was kept at 25 °C.

The individual compounds of α-, γ- and δ-tocopherols were identified and quantified using a calibration curve of the corresponding standard compounds.

2.3.3. Determination of antioxidant capacity: DPPH assay

The antioxidant capacity of the quinoa oil was evaluated using the DPPH assay. The free radical scavenging capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·). Briefly, 2 mL of the DPPH solution (10 μM) prepared in isooctane were mixed with 0.5 mL of isooctane quinoa oil solution (0.02 g/mL). The absorbance was measured (Jasco V-750 spectrophotometer) at 517 nm and 25 °C against a blank of pure isooctane for 120 min. The antioxidant capacity was presented in the form of percentage of inhibition of the DPPH radical versus time.

2.3.4. Total phenolics (TP) quantification

The Folin-Ciocalteau method was used to quantify the total phenolics content in the oil extracted from quinoa, following the method described by Ricciutelli et al. [19]. Briefly this analytical procedure took 2.5 mL of oil sample that were dissolved in 2.5 mL of hexane and then extracted for 5 min in a vortex stirrer using 2.5 mL of a methanol-water (80/20, v/v) mixture. The mixture was centrifuged at 5000 rpm and the supernatant was collected and extracted twice, using the methanol:water mixture. Finally 7.5 mL of methanolic extract was collectd. Afterwards the collected solution was washed with 2 × 2.5 mL of hexane and collected in a 50 mL volumetric flask where 2.5 mL of Folin-Ciocalteau reagent and 2.5 mL of 7.5% sodium carbonate solution were added. Finally, the solution was brought up to the flask volume with distilled water. After 240 min in the dark the absorbance was measured at 765 nm (Jasco V-750 spectrophotometer). The TP content was expressed in mg equivalents of gallic acid per kg of oil.

2.4. Statistical analysis

The experimental results were analysed using Statgraphics Centurion. The LSD (Least Significant Difference) test was run to determine which means were significantly different from which others at a 95% confidence level, in order to test the significance of the experimental conditions on the studied response.

3. Experimental results

3.1. Conventional solvent extraction

The oil content of the four quinoa varieties was determined by Soxhlet extraction using hexane as solvent. Results are presented in Table 1.

As can be seen in Table 1, significant differences in the oil content were observed among the varieties of quinoa used in this work, being oil content of Pasankalla (7.9 ± 0.1 g of oil/g of insoluble solid) almost two times higher than Titikaka (4.3 ± 0.1 g of oil/g of insoluble solid).

3.2. Supercritical CO_2 extraction

3.2.1. Influence of the process parameters

The extraction of a solute from a solid raw material involves three different stages: internal mass transfer, phase equilibrium and external mass transfer. In this section the extraction isotherms (40, 50, 60 °C) at three different pressures (20, 30, 40 MPa) were obtained in order to find out the stage that controls the extraction of oil from quinoa using supercritical CO_2.

Quinoa was milled to obtain three different particle sizes: 500-1000 μm; 250-500 μm and 125-250 μm. In these experiments the temperature was kept at 50 °C and pressure at 30 MPa. The best results were obtained for the intermediate particle size (range from 250 to 500 μm), as can be seen in Fig. 2. The largest particle size (500-1000 μm) presented a higher resistance to the internal mass transfer, and therefore the extraction was very slow since only part of the oil was accessible to the SC-CO_2. However, very small particle sizes did not help the extraction process: although the initial part of the extraction curve was very fast, the final extraction yield was significantly lower than the one for the intermediate particle size. Lower particle sizes involve more broken cells and therefore the oil is released and easily accessible to the SC-CO_2. In turn, small particles can induce the channeling of the CO_2, which would leave zones of the packed bed without contact with the solvent.

The effect of temperature on the extraction rate was studied at 40, 50 and 60 °C, at three different pressures (20, 30 and 40 MPa). The CO_2 mass rate was kept constant at 0.14 ± 0.02 kg/h. Results are shown in Fig. 3(a–c), where the extraction curves are presented. All the extraction curves share a common trend: an initial straight line (the extraction process is controlled by solubility) followed by a curved line, which indicates that the extraction is being controlled by the internal diffusion. The second part of the curve indicates a much lower extraction rate, therefore most of the oil is extracted in the first part of the extraction process.

In general, and regardless the extraction temperature considered, an increase of the oil extraction rate was observed when pressure was increased. This was in agreement with the results presented by Westerman et al. [15] for oil extracted from amaranth seeds, a cereal that shares similarities with quinoa. The faster extraction rates observed with increasing pressure at constant temperature can be attributed to an increase of the CO_2 solvent power, induced by the increase of the density.

Fig. 3 also shows that the first part of the extraction was not significantly affected by temperature: at a constant pressure, the initial slopes of the extraction curves obtained at different temperatures were similar. However, it was in the second part of the extraction curve when

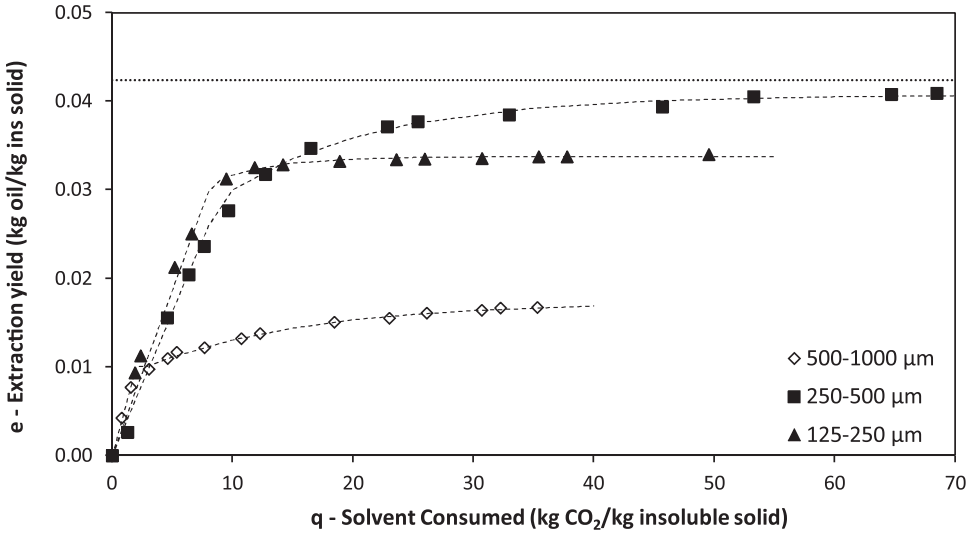


Fig. 2. Influence of the particle size on the extraction of oil from Titikaka quinoa at 50 °C and 30 MPa (◊ 500–1000 µm; ■250-500 µm; ▲ 125–250 µm). Dashed lines represent the extraction curve obtained with the Sovova's model.

the influence of the temperature was important: an increase in the amount of oil extracted was observed the higher the temperature. This phenomenon was observed for the three levels of pressure studied. At a given pressure, when temperature is increased the CO_2 density decreases, decreasing its solvent power, but the transport properties of the CO_2 are enhanced: internal mass transfer limitations are overcome as a consequence of the CO_2 lower viscosity which enhances its diffusion allowing to extract more oil. This was specially observed in the experiments done at 40 MPa. This trend was again similar to that presented by Westerman et al. [15] in the extraction of amaranth seeds.

3.2.2. Modelling of the supercritical extraction

In this work, the model proposed by Sovová [16] was used to describe the experimental extraction curves. As described by Rebolleda et al. [14] this model uses the simplification that assumes that the solute is a single pseudo-component. This model expresses the extraction yield, e, as:

$$e = \frac{E}{N_m} \tag{1}$$

Where E is the amount of extract (kg) and N_m the amount of insoluble solid (kg) loaded in the reactor. The amount of CO_2 consumed, q, is calculated according to the following expression:

$$q = \frac{Q \cdot t}{N_m} \tag{2}$$

Where Q is the solvent rate (kg/h) and t is the extraction time expressed in hours.

The model of Sovová considers that the extraction curve consists of two parts. The first one is linear, since the solute is easily accessible for the solvent being transferred directly; in the second part of the curve the solute diffuses first out of the intact cells and then to the fluid phase. The slope of the linear part of the curve has a value close to the solubility of the oil in the CO_2. The model proposed by Sovová is described in Eqs. (3) and (4), used to describe the first and the second part of the extraction curve, respectively:

$$e = q \cdot y_s, \text{for} 0 \le q \le q_c \tag{3}$$

$$e = x_u[1 - C_1\exp(-C_2 q)], \text{for} q > q_c \tag{4}$$

In the previous equations y_s is the experimental weight solubility datum, C_1 and C_2 are two adjustable parameters, q_c is the crossing point (separates the solubility controlled extraction from the internal diffusion controlled extraction) and x_u is the solute concentration in the untreated solid (kg solute/kg insoluble solid). These adjustable parameters were calculated using the software Statgraphics Centurion XVI using the Marquardt's algorithm. Two more parameters were calculated using the Sovová's model: the grinding efficiency, r, which is described as the volumetric fraction of broken cells in the particles; and the solid-mass transfer coefficient, $k_s a_s$. Both r and $k_s a_s$ were calculated according to the Eqs. (5) and (6), respectively:

$$r = 1 - C_1\exp\left(\frac{C_2 q_c}{2}\right) \tag{5}$$

$$k_s a_s = \frac{(1 - r)(1 - \varepsilon)QC_2}{N_m} \tag{6}$$

In Eq. (6) the flow rate, Q, is expressed in kg·s^{-1}, and ε refers to the porosity of the bed.

In Fig. 3(a–c) the experimental extraction data are represented together with the calculated curves using the Sovová's model (dashed lines). It can be seen that there is a good fitting between the experimental data and the curves described by the model. The mean relative deviations (MRD) between experimental and calculated yields were calculated (using the Eq. (7)) for each extraction curve:

$$MRD(\%) = \frac{1}{n}\sum_{i=1}^{n} abs\left(\frac{e_{exp} - e_{calc}}{e_{exp}}\right) \cdot 100 \tag{7}$$

The fitting parameters and the calculated error between the experimental and the calculated data using the model are presented in Table 2.

From the results presented in Table 2 the grinding efficiency (r) for the quinoa oil SC-CO_2 extraction is higher than that obtained for other seeds: whereas for quinoa r was in the range 0.40–0.82, for wheat bran was in the range 0.39–0.59 [13] or for corn germ oil it was reported to be in the range 0.32–0.48 [14]. An increase of the grinding efficiency was observed when pressure was increased at constant temperature at all temperatures studied in this work; however, temperature only produced slight changes at the same pressure. The particle size had a significant effect on this coefficient: higher values were observed the lower the particle size of the quinoa used for the extraction. The q_c value was in the range 4.4-22.5 kg CO_2/kg insoluble solid. This parameter showed a marked decrease with the CO_2 density used in the extraction process that changed from 723.7 kg/m^3 at 60 °C and 20 MPa to 956.1 kg/m^3 at 40 °C and 40 MPa.

The initial slope of the extraction curve (y_s, expressed in kg of oil/kg CO_2) increased with temperature and dramatically with pressure (see Table 2), proving that the solvent power of the CO_2 increased with density. The value of the slope can be related to the solubility of the oil in the SC-CO_2. Del Valle et al. [20] proposed a general equation to

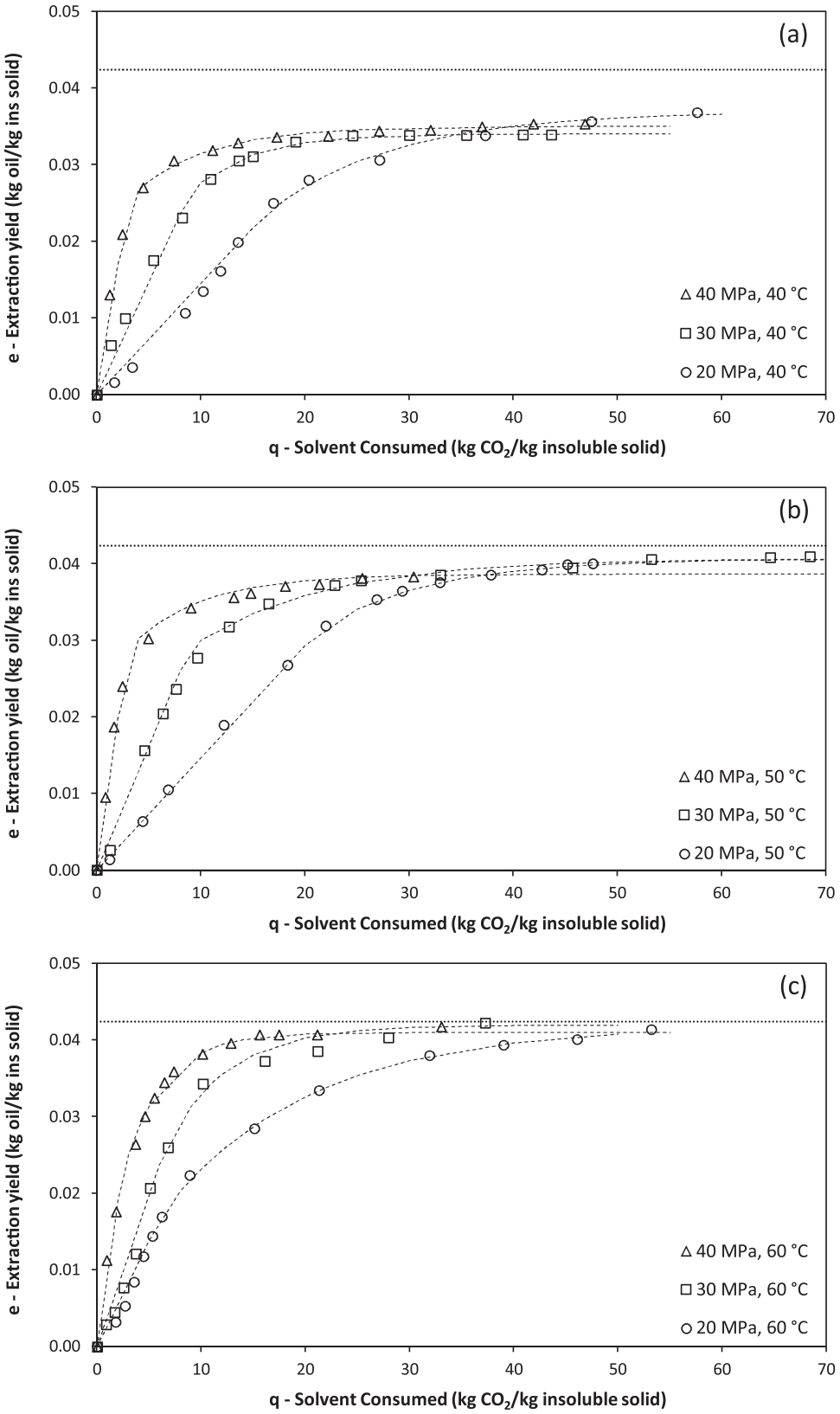


Fig. 3. Effect of pressure and temperature on the extraction of oil from quinoa. In figures a), b) and c) experiments were done at 40, 50 and 60 °C, respectively. ○ refers to experiments done at 20 MPa, □ to 30 MPa and △ to 40 MPa. Dashed line represents the extraction curve obtained with the Sovova's model, and dotted line the amount of oil extracted using hexane in a Soxhlet apparatus.

predict the solubility of vegetable oils in SC-CO_2 (with a ± 40% error). This equation is a function of both solvent density and temperature. In order to compare data obtained at temperatures different from 40 °C, a temperature correction term (TCT) was calculated and data were divided by this TCT term. The general equation proposed by Del Valle et al. and the TCT are the following:

Table 2
Fitting coefficients for the quinoa extraction with CO_2, modelled using the Sovova's model.

T (°C)	P (MPa)	y_s	x_u	C_1	C_2	$K_s \cdot a_s$	r	MRD (%)
40	**20**	0.0015	0.037	1.345	0.081	8.59E-05	0.45	8.8
40	**30**	0.0027	0.034	1.047	0.173	9.61E-05	0.60	6.9
40	**40**	0.0085	0.035	0.411	0.140	5.38E-05	0.70	2.2
50	**20**	0.0015	0.041	1.660	0.093	7.69E-05	0.40	3.7
50	**30**	0.0033	0.041	0.582	0.080	4.54E-05	0.60	6.0
50	**40**	0.0099	0.039	0.385	0.143	3.61E-05	0.73	4.0
60	**20**	0.0028	0.042	0.902	0.070	3.75E-05	0.47	9.9
60	**30**	0.0040	0.042	1.170	0.170	6.32E-05	0.61	12.9
60	**40**	0.0096	0.041	0.840	0.256	7.53E-05	0.72	2.5
500–1000 μm[a]		0.0050	0.017	0.525	0.077	3.41E-05	0.49	1.6
250–500 μm[a]		0.0033	0.041	0.582	0.080	4.54E-05	0.60	6.0
125–250 μm[a]		0.0037	0.034	0.490	0.207	5.11E-05	0.82	4.4

[a] Particle size effect experiments were carried out at 50 °C and 30 MPa.

$$c_{sat}(g \cdot kg^{-1}) = 8.07\left(\frac{\rho}{910}\right)^{[9.59-8.45((\rho/910)-1)-23.0((\rho/910)-1)^2]} * TCT \quad (8)$$

Where TCT is described in Eq. (9):

$$TCT = exp\left\{-4182\left[1 - 259\left(\frac{1}{T} - \frac{1}{313}\right)\right]\left(\frac{1}{T} - \frac{1}{313}\right)\right\} \quad (9)$$

In Fig. 4, the solubility data calculated using Del Valle's equation are presented together with the error limits allowed for the general equation. The experimental data obtained for the quinoa oil extraction are also presented in Fig. 4. It can be seen that, in general, our experimental data are within the limits proposed by the general equation. This fact indicates that the extraction process is being controlled by the solubility of the oil in the supercritical CO_2. However there are three values that are below the lower limit of solubility values predicted by Del Vallés equation; this can be due to the mass transfer resistance and effect of deviations from plug flow.

The solubility in SC-CO_2 of other oils extracted from cereals was in a similar order of magnitude to quinoa. For instance amaranth solubility was in the range from 0.0004 to 0.0074 kg oil/kg CO_2 at 60 °C and from 10 to 30 MPa [15]. He et al. [21] reported a solubility of oil from amaranth around 0.0080 kg oil/kg CO_2 at 40 °C and 25 MPa, obtained from the slope of the extraction curve. Both researchers using amaranth as raw material concluded that the solubility of its oil in CO_2 increased with pressure at constant temperature; however contradictory results were reported regarding the temperature effect: Westerman et al. [15] reported that solubility slightly increased with temperature at constant pressure whereas He et al. [21] reported a decrease. In any case the most important effect on solubility was attributed to pressure in both cases.

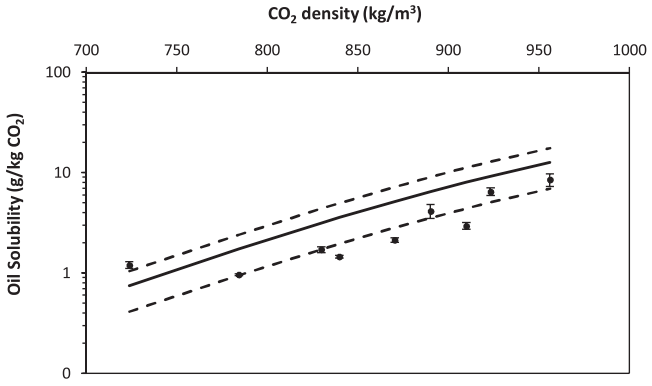


Fig. 4. Corrected (at 40 °C) experimental solubility values of quinoa oil as a function of the CO_2 density (●). Solid line represents the prediction of del Valle et al. [20] general equation and dashed lines represent the error limit of the general equation.

3.3. Characterization of the quinoa extracts: quality and stability

3.3.1. Characterization of Titikaka quinoa oil extracts

3.3.1.1. Antioxidant activity. The quality of the oils extracted from Titikaka quinoa variety was evaluated in terms of antioxidant activity using the DPPH method. With this method, it was seen that the oils extracted at 60 °C had similar antioxidant activity at all the pressures studied in this work: around 80% DPPH radical inhibition after one hour; after the 60 min and up to 120 min the DPPH radical inhibition remained constant. Between the extraction experiments carried out at 40 or 50 °C it was observed that the lower the temperature the higher the antioxidant activity. Pressure in turn had a significant influence on the antioxidant activity of the extracted oil, being higher the higher the pressure. In Table 3 the inhibition of the DPPH radical activity is presented for oils extracted under different SC-CO_2 conditions throughout the time, up to 60 min.

Oil extracted at 40 MPa and 40 °C exhibited the highest antioxidant activity, higher than 90% DPPH radical inhibition for the oil extracted from Titikaka variety. At 40 °C, a slight decrease in the antioxidant activity (80% inhibition of the DPPH radical) was observed when pressure was decreased down to 20 MPa.

Marmouzi et al. [8] reported a 47% inhibition of the radical DPPH for a quinoa oil extracted using hexane, from quinoa seeds that contained 4.9 ± 0.3% of fat. This value is significantly lower than ours, probably due to the extraction method and the differences in the quinoa varieties used in each study. The DDPH assay used by Marmouzi et al. consisted of the addition of 2.7 mL of 0.2 mM DPPH solution (in methanol) to 0.3 mL of the quinoa oil. The antioxidant activity of quinoa oil has been studied by several researchers; all of them conclude that quinoa is one of the cereals that shows a higher antioxidant activity. Paśko et al. [22] determined the polyphenols content in quinoa (no further information about the proximate composition), concluding that this cereal has the highest content compared to amaranth, which presents a higher antioxidant activity evaluated in terms of the FRAP and ABTS assays.

The literature on total polyphenols in quinoa oil is scarce. Most of the papers report the total polyphenols extracted from quinoa seeds (which is around 0.7 mg GAE/g for quinoa [23]). In our case the oil extracted from the Titikaka variety using SC-CO_2 resulted in a total polyphenols content of 112.9 ± 4.2 mg gallic acid/kg of oil. The total polyphenol content in quinoa oil is slightly lower than in other oils, such as olive oil: Owen et al. [24] reported that total phenolics content was around 196 ± 19 mg/kg oil, on average for different olive oils.

3.3.1.2. Determination and quantification of the tocopherol profile. The importance of tocopherols, some of the major lipid soluble antioxidants [25], is related to their antioxidant properties and to their several physiological functions [10]. The tocopherol profile extracted from Titikaka quinoa varieties using SC-CO_2 was quite similar at all the experimental extraction conditions used in this work: α-tocopherol resulted to be the most abundant (1.1-1.5 mg/g oil) followed by γ-tocopherol (0.9–1.2 mg/g oil) with very small amounts of δ-tocopherol (0.1–0.3 mg/g oil). It was observed that temperature enhanced the extraction of tocopherols, whereas high pressures contributed to improve the extraction of tocopherols at 40–50 °C. The highest amount of tocopherols was extracted at 50 °C and 40 MPa (3.3 mg/g oil extracted), decreasing at higher temperatures as can be seen in Fig. 5. The statistical analysis of the experimental data using the LSD test allowed identifying five homogenous groups (a homogenous group does not show significant differences among the members of the group). According to this test it was clearly seen that pressure had a clear effect at 50 °C, where a pressure increase led to a significant improvement in the extraction of tocopherols. At 60 °C a slight decrease in the tocopherol content was observed when pressure was increased; an

Table 3
DPPH radical activity inhibition (expressed in%) obtained using quinoa oil extracted under different experimental conditions (from 20 to 40 MPa, and from 40 to 60 °C).

Time (min)	20 MPa			30 MPa			40 MPa		
	40 °C	50 °C	60 °C	40 °C	50 °C	60 °C	40 °C	50 °C	60 °C
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	47.5 ± 4.8	47.3 ± 3.2	43.4 ± 5.2	42.8 ± 1.6	50.0 ± 1.2	41.9 ± 5.5	44.9 ± 3.0	43.5 ± 2.7	29.5 ± 2.0
5	63.6 ± 3.4	62.5 ± 2.8	61.5 ± 4.3	61.0 ± 1.2	67.1 ± 2.8	61.2 ± 5.1	65.4 ± 3.1	63.9 ± 1.4	53.2 ± 2.8
10	69.7 ± 4.7	66.8 ± 3.4	66.7 ± 3.6	66.7 ± 2.1	72.7 ± 3.3	66.6 ± 5.3	72.3 ± 3.2	70.4 ± 1.0	59.1 ± 2.3
15	73.2 ± 5.2	69.7 ± 4.3	69.8 ± 5.8	70.3 ± 1.1	76.4 ± 3.8	69.8 ± 5.0	76.4 ± 2.8	74.3 ± 1.2	61.3 ± 3.3
20	75.7 ± 5.2	71.9 ± 1.6	72.3 ± 5.4	72.6 ± 1.5	78.7 ± 3.9	72.0 ± 4.9	79.5 ± 2.7	77.3 ± 2.0	64.8 ± 2.5
30	78.8 ± 5.0	74.9 ± 2.6	75.5 ± 5.3	76.0 ± 1.2	81.8 ± 2.8	75.2 ± 4.7	84.1 ± 2.3	81.9 ± 1.5	68.8 ± 2.5
40	81.0 ± 4.9	77.1 ± 1.2	77.7 ± 5.3	78.3 ± 1.8	83.8 ± 4.3	77.5 ± 4.3	88.0 ± 2.2	85.7 ± 1.1	72.0 ± 2.3
50	82.5 ± 4.6	78.8 ± 2.4	79.2 ± 5.0	80.1 ± 2.2	85.1 ± 3.2	79.3 ± 4.1	91.6 ± 2.2	89.2 ± 0.9	75.0 ± 2.0
60	83.8 ± 4.2	80.4 ± 3.8	81.0 ± 4.5	81.5 ± 1.8	86.3 ± 4.1	80.7 ± 3.7	95.1 ± 1.9	92.8 ± 2.1	78.7 ± 2.0

opposite trend observed at 40 °C.

Przygoda and Wejnerowska [3] found that a pressure increase reduced the amount of tocopherols present in quinoa oil extracted with SC-CO_2, and for that reason they used low pressures (18.5 MPa), high temperatures (130 °C) and long extraction times (180 min) to maximize the extraction of tocopherols and obtain around 3.4 mg tocopherol/g oil extracted. This content is similar to that presented in our work, but ours was obtained at a significantly lower temperature. This probes that extremely high temperatures are not necessary to obtain high tocopherol recoveries in oils extracted from quinoa using SC-CO_2. The high stability of tocopherols from quinoa with high temperatures has been reported by [25]. These authors concluded that, although tocopherols are resistant to high temperatures, a high extraction temperature might affect the antioxidant capacity of the oil, and therefore should be avoided.

The characterization of quinoa oil in terms of tocopherol content has been carried out by other researchers. For instance Tang et al. [10] extracted oil from different quinoa varieties (white, red and black) using a mixture of MTBE and THF. The oil content was in the range 6.6-7.2%, whereas the highest tocopherol content was found in black quinoa (0.9 mg/g oil). No significant differences were observed between quinoa varieties regarding tocopherol form content, being γ-tocopherol the most abundant (around 80%). Álvarez-Jubete et al. [26] extracted quinoa oil using hexane. The oil content of quinoa resulted to be 7.2%, with tocopherol content close to 1 mg/g oil extracted, being γ-tocopherol the most frequent. When amaranth was used as raw material, lower tocopherol content was detected, and form β was the major one. The results presented by other researchers exhibited a significantly lower tocopherol content compared to the results obtained using SC-CO_2. Besides the higher efficiency of the supercritical extraction, aspects such as quinoa variety or particle size used for the extraction might explain the differences reported.

3.3.1.3. Fatty acid profile. The fatty acid profile of the oil extracted from Titikaka quinoa variety using SC-CO_2 at different pressures and temperatures was determined. No significant differences in the profiles were observed (data not shown since the fatty acid profile was basically similar to the fatty acid profile for Titikaka presented in Table 4). Only a slight PUFA decrease was observed at the highest temperature. The highest amount of total fatty acids was observed when the extraction was performed at 40 °C and 20 MPa, the mildest conditions, and resulted to be 911.7 ± 14.7 mg/g oil.

The variety used to perform the supercritical CO_2-extraction kinetic study, Titikaka, resulted to be the variety that contained the highest amount of PUFA (accounting for up to the 63% of the total fatty acids). Within the unsaturated fatty acids, the major resulted to be linoleic acid (56.1 ± 1.6%) followed by oleic acid (6.4 ± 0.2%). Palmitic acid was the major saturated fatty acid found in the oil extracted from Titikaka quinoa (approximately 9.5 ± 0.2%). This fatty acid profile was quite similar to that provided by other authors such as Rebolleda et al. [13] when extracting oil from wheat bran using SC-CO_2. Since the extraction method did not affect significantly the fatty acid profile, it is possible to compare our results with those obtained by other authors using organic solvents to extract oil from quinoa. Marmouzi et al. [8] reported an oil content in quinoa around 4.9%. The analysis of the fatty acid profile (oil was extracted with hexane) reported similar values to ours: a 66% of the total FA were PUFA, being linoleic and α-linolenic the most abundant (60.3% of the total fatty acids). Peiretti et al. [6] reported that the most abundant fatty acid in quinoa was linoleic (48.8% of the total fatty acid extracted) followed by oleic (23.9% of the total) and palmitic

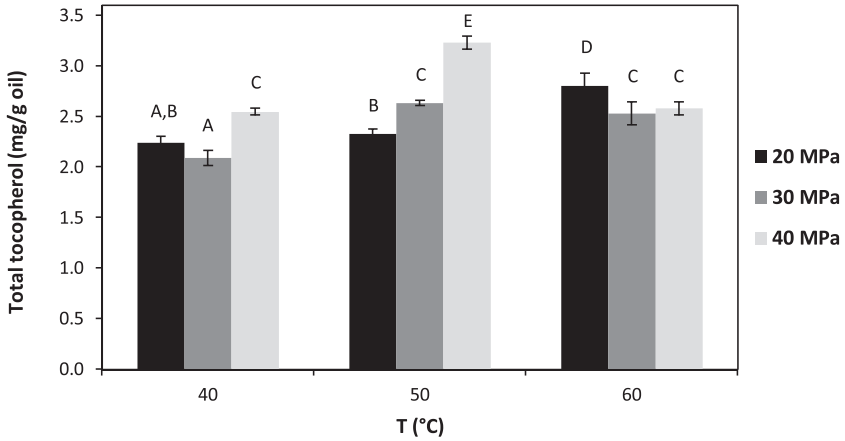


Fig. 5. Total tocopherols present in oils extracted from Titikaka quinoa using supercritical CO_2 as a function of the extraction pressure and temperature. Capital letters indicate the homogeneity among the groups using the LSD (Least Significant Difference) procedure.

Table 4
Fatty acid profile (expressed in% of fatty acid) of quinoa oil obtained from different quinoa varieties extracted with hexane (Soxhlet) and SC- CO_2 (40 °C, 40 MPa). Some minor fatty acids have not been considered.

Fatty acids (%)		Titikaka		Altiplano		Collana		Pasankalla	
		Hexane	SC-CO_2	Hexane	SC-CO_2	Hexane	SC-CO_2	Hexane	SC-CO_2
Palmitic	C16:0	9.0 ± 0.2	9.5 ± 0.2	8.4 ± 0.4	9 ± 1	9.1 ± 0.4	9.4 ± 0.5	11.9 ± 0.1	12.7 ± 0.9
Stearic	C18:0	0.6 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Oleic	C18:1n-9	21.4 ± 0.5	18.8 ± 0.5	31 ± 2	27.2 ± 0.5	27 ± 1	26 ± 1	26.4 ± 0.1	26 ± 2
Vaccenic	C18:1n-7	1.3 ± 0.1	1.4 ± 0.0	0.8 ± 0.0	1.1 ± 0.1	0.9 ± 0.0	1.1 ± 0.1	1.6 ± 0.1	1.4 ± 0.2
Linoleic cis&trans	C18:2n-6	56 ± 1	56 ± 2	46 ± 2	47 ± 1	52 ± 2	52 ± 3	43.2 ± 0.3	45 ± 3
α-linolenic	C18:3n-3	6.2 ± 0.2	6.4 ± 0.2	7.9 ± 0.5	8.2 ± 0.3	3.8 ± 0.2	3.6 ± 0.2	8.3 ± 0.3	9.1 ± 0.7
Arachidic	C20:0	0.5 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
Gondoic	C20:1n-9	1.4 ± 0.1	1.4 ± 0.0	1.6 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.9 ± 0.0	1.6 ± 0.1
SFA		11.5 ± 0.2	11.5 ± 0.3	11.5 ± 0.6	11.4 ± 0.2	12.1 ± 0.5	11.7 ± 0.6	14.5 ± 0.1	12.7 ± 0.9
MUFA		26.0 ± 0.7	25.9 ± 0.9	35 ± 2	33.7 ± 0.9	32 ± 1	32 ± 2	33.7 ± 0.3	33 ± 3
PUFA		63 ± 2	63 ± 2	55 ± 3	55 ± 2	56 ± 3	56 ± 3	51.8 ± 0.3	54 ± 4
Total FA (mg/g oil)		**821 ± 11**	**809 ± 12**	**870 ± 22**	**873 ± 12**	**844 ± 18**	**775 ± 20**	**765 ± 3**	**847 ± 31**

(9.6% of the total). Similar compositions were reported by Przybylski et al. [27], who performed one of the first quinoa lipid characterizations that can be found in the literature.

3.3.2. Characterization of the oil obtained at the optimal SFE conditions for the 4 quinoa varieties

According to the results presented in Section 3.3.1.1 the highest antioxidant activity of the oils extracted from Titikaka quinoa was obtained at 40 °C and 40 MPa. Under these extraction conditions, considered as optimal, the extraction of oil from the other three different quinoa varieties (Altiplano, Collana and Pasankalla) was carried out and the extracted oil was characterized and compared to those results obtained for Titiakaka variety. The oil from the all the quinoa varieties was also extracted using hexane in order to evaluate the effect of the extraction method on the quality of the oil extracted.

In Fig. 6 the antioxidant activity of oils extracted from several quinoa varieties using either SC-CO_2 or hexane is also shown. The antioxidant activity of the oil extracted using supercritical CO_2 was substantially higher than that obtained using hexane, as can be seen in Fig. 6. Whereas oil extracted from Titikaka quinoa using SC-CO_2 presented an inhibition of the DPPH radical higher than 90% after 60 min, oil extracted from the same quinoa using hexane only provided 60% of inhibition under the same conditions. Similar results were observed for the other three quinoa varieties studied: SC-CO_2 significantly improved the quality of the oil regardless the quinoa variety used, exception made to the Altiplano variety, which was not strongly affected by the extraction method. This behavior might be attributed to structural changes compared to the other quinoa varieties.

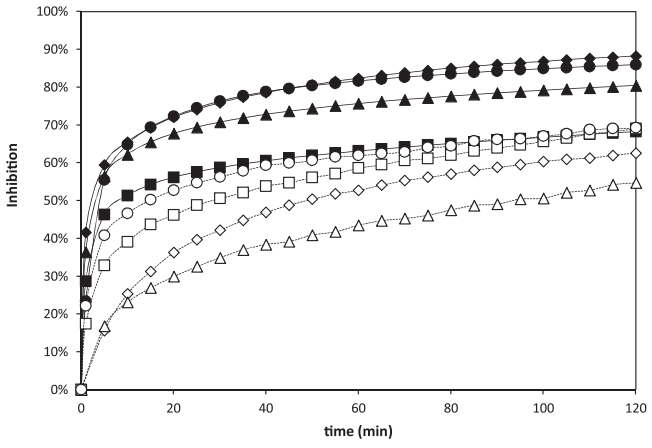


Fig. 6. Inhibition of the activity of the radical DPPH of oil extracts obtained from different quinoa varieties using CO_2-SC at 40 MPa and 40 °C (full symbols) and hexane (empty symbols). Key: ♦◊ Titikaka; ■□Altiplano; ▲△ Collana; ●○ Pasankalla.

No significant differences were observed in the fatty acid profile of the different varieties of quinoa studied when the SC-CO_2 extraction was carried out. As shown in Table 1, the variety Titikaka presented the lowest amount of oil in its composition. Despite this fact, Titikaka quinoa oil was mainly composed by PUFA (around 63%) whereas, for the rest of the analysed varieties, the percentage of PUFA decreased down to 54–56%. All the varieties had similar content in saturated fatty acids. The extraction method (either SC-CO_2 or hexane) did not influence the fatty acid profile, as can be observed in Table 3, since the content in SFA, MUFA and PUFA was quite similar. Tang et al. [10] did not report significant differences in the fatty acid profile obtained from different quinoa varieties, being SFA around 11% of the total, MUFA 32% and PUFA up to 57%. These researchers extracted oil from quinoa using hexane as solvent.

Larger differences were observed in the total tocopherol content, as presented in Fig. 7. In this figure, the total tocopherol content in oils extracted from different varieties of quinoa using either hexane or supercritical CO_2 (40 MPa, 40 °C) as solvents is presented. It can be clearly seen that supercritical CO_2 significantly improves the extractability of tocopherols compared to the results obtained when hexane was used. In the case of Titikaka quinoa, supercritical extraction doubles the amount of tocopherols extracted. The statistical analysis of the experimental results (LSD test, 95% confidence level) revealed that both, the extraction method and the quinoa variety, significantly affected the amount of tocopherols present in the oil. Przygoda and Wejnerowska [3] also probed that, compared to the conventional hexane extraction, supercritical CO_2-improved dramatically the extraction of tocopherols, leading to oils up to four times more concentrated in tocopherols. No other works have been found in the literature dealing with the SC-CO_2 extraction and characterization of quinoa oil.

All in all, the oil extracted from Titikaka variety with SC-CO_2 resulted to be probably the best of the oils extracted from the different varieties studied in this work, since it presented the highest PUFA content and antioxidant activity and the second highest tocopherols content.

4. Conclusions

In this work, supercritical CO_2 was used to extract oil from different varieties of quinoa. The extracted oil was compared to the oil extracted using hexane, probing the higher quality of the SFE oil in terms of antioxidant activity and tocopherols profile when extracted at 40 MPa and 40 °C. SC-CO_2 provided oils with a higher tocopherol content, regardless the quinoa variety used. Nevertheless, the extraction method did not influence the fatty acid profile. The highest PUFA content was

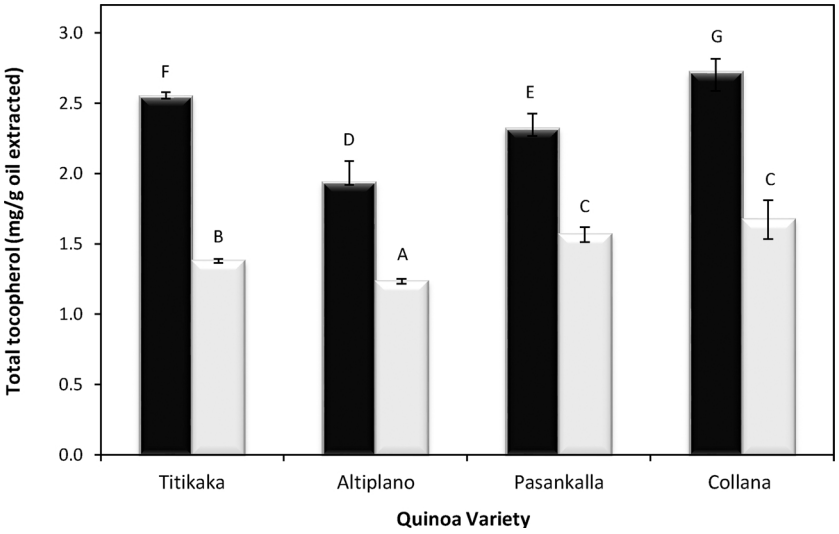


Fig. 7. Total tocopherols present in oils extracted from different quinoa varieties using supercritical CO_2 at 40 MPa and 40 °C (black bars) and hexane (grey bars).

detected for the oils extracted from the variety Titikaka.

The influence of the main process parameters was evaluated: the optimal quinoa particle size was found to be in the range 250-500 μm in order to avoid internal diffusion limitations (particle size larger than 500 μm) or the formation of preferential channels in case very small particle sizes are used. Regarding the effect of pressure, it was observed an increase of the extraction rate with pressure, favored by the higher density of the CO_2. The effect of temperature on the extraction of oil from quinoa did not provide a clear trend: in the initial period of the extraction, temperature is not playing a significant role on the extraction rate, just a slight increase in the solubility of the oil; however, once the maximum extraction rate is passed, it seems that temperature does affect the extraction rate, with higher extraction rates the higher the temperature. This phenomenon can be attributed to the better mass transfer properties of the CO_2 the higher the temperature, that decreases viscosity and improves its diffusivity through the quinoa grains.

All in all, it has been observed that supercritical fluid extraction using CO_2 is a suitable technology to extract high quality oil from different varieties of quinoa seeds.

Acknowledgments

To Junta de Castilla y León and the European Regional Development Fund (ERDF - FEDER) for the financial support of project BU055U16 and O. Benito-Román's Post-doctoral contract. R. Melgosa thanks Ministry of Economy and Competitiveness of Spain (MINECO) for his predoctoral contract (BES-2013-063937)

References

[1] A. Bhargava, S. Shukla, D. Ohri, Chenopodium quinoa – an indian perspective, Ind. Crops Prod. 23 (2006) 73–87, http://dx.doi.org/10.1016/j.indcrop.2005.04.002.

[2] C. Mestanza, R. Riegel, H. Silva, S.C. Vásquez, Characterization of the acetohydroxyacid synthase multigene family in the tetraploide plant Chenopodium quinoa, Electron. J. Biotechnol. 18 (2015) 393–398, http://dx.doi.org/10.1016/j.ejbt.2015.07.003.

[3] K. Przygoda, G. Wejnerowska, Extraction of tocopherol-enriched oils from Quinoa seeds by supercritical fluid extraction, Ind. Crops Prod. 63 (2015) 41–47, http://dx.doi.org/10.1016/j.indcrop.2014.09.038.

[4] S. Navruz-Varli, N. Sanlier, Nutritional and health benefits of quinoa (Chenopodium quinoa Willd.), J. Cereal Sci. 69 (2016) 371–376, http://dx.doi.org/10.1016/j.jcs.2016.05.004.

[5] C. Vilche, M. Gely, E. Santalla, Physical properties of quinoa seeds, Biosyst. Eng. 86 (2003) 59–65, http://dx.doi.org/10.1016/S1537-5110(03)00114-4.

[6] P.G. Peiretti, F. Gai, S. Tassone, Fatty acid profile and nutritive value of quinoa (Chenopodium quinoa Willd.) seeds and plants at different growth stages, Anim. Feed Sci. Technol. 183 (2013) 56–61, http://dx.doi.org/10.1016/j.anifeedsci.2013.04.012.

[7] Y. Hirose, T. Fujita, T. Ishii, N. Ueno, Antioxidative properties and flavonoid composition of Chenopodium quinoa seeds cultivated in Japan, Food Chem. 119 (2010) 1300–1306, http://dx.doi.org/10.1016/j.foodchem.2009.09.008.

[8] I. Marmouzi, N. El Madani, Z. Charrouf, Y. Cherrah, M.Y. El Abbes Faouzi, Proximate analysis, fatty acids and mineral composition of processed Moroccan Chenopodium quinoa Willd. and antioxidant properties according to the polarity, Phytotherapie 13 (2015) 110–117, http://dx.doi.org/10.1007/s10298-015-0931-5.

[9] S.C. Ng, A. Anderson, J. Coker, M. Ondrus, Characterization of lipid oxidation products in quinoa (Chenopodium quinoa), Food Chem. 101 (2006) 185–192, http://dx.doi.org/10.1016/j.foodchem.2006.01.016.

[10] Y. Tang, X. Li, P.X. Chen, B. Zhang, M. Hernandez, H. Zhang, M.F. Marcone, R. Liu, R. Tsao, Characterisation of fatty acid, carotenoid, tocopherol/tocotrienol compositions and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes, Food Chem. 174 (2015) 502–508, http://dx.doi.org/10.1016/j.foodchem.2014.11.040.

[11] M. Mukhopadhyay, Natural Extracts Using Supercritical Carbon Dioxide, (2000), http://dx.doi.org/10.1201/9781420041699.

[12] M.G. Bernardo-Gil, R. Roque, L.B. Roseiro, L.C. Duarte, F. Gírio, P. Esteves, Supercritical extraction of carob kibbles (Ceratonia siliqua L.), J. Supercrit. Fluids 59 (2011) 36–42, http://dx.doi.org/10.1016/j.supflu.2011.08.007.

[13] S. Rebolleda, S. Beltrán, M.T. Sanz, M.L. González-Sanjosé, Á.G. Solaesa, Extraction of alkylresorcinols from wheat bran with supercritical CO 2, J. Food Eng. 119 (2013) 814–821, http://dx.doi.org/10.1016/j.jfoodeng.2013.07.008.

[14] S. Rebolleda, N. Rubio, S. Beltrán, M.T. Sanz, M.L. González-Sanjosé, Supercritical fluid extraction of corn germ oil: study of the influence of process parameters on the extraction yield and oil quality, J. Supercrit. Fluids 72 (2012) 270–277, http://dx.doi.org/10.1016/j.supflu.2012.10.001.

[15] D. Westerman, R.C.D. Santos, J.A. Bosley, J.S. Rogers, B. Al-Duri, Extraction of Amaranth seed oil by supercritical carbon dioxide, J. Supercrit. Fluids 37 (2006) 38–52, http://dx.doi.org/10.1016/j.supflu.2005.06.012.

[16] H. Sovová, Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation, J. Supercrit. Fluids 33 (2005) 35–52, http://dx.doi.org/10.1016/j.supflu.2004.03.005.

[17] AOAC, Fatty acids in encapsulated fish oils and fish oil methyl and ethyl esters. gas chromatographic MEthods, Assoc. Anal. Chem. (1995) 41.1.30.

[18] IUPAC, Determination of Tocopherols and Tocotrienols in Vegetable Oils and Fats by High Performance Liquid Chromatography, IUPAC, 1998.

[19] M. Ricciutelli, S. Marconi, M.C. Boarelli, G. Caprioli, G. Sagratini, R. Ballini, D. Fiorini, Olive oil polyphenols: a quantitative method by high-performance liquid-chromatography-diode-array detection for their determination and the assessment of the related health claim, J. Chromatogr. A 1481 (2017) 53–63, http://dx.doi.org/10.1016/j.chroma.2016.12.020.

[20] J.M. Del Valle, J.C. De La Fuente, E. Uquiche, A refined equation for predicting the solubility of vegetable oils in high-pressure CO 2, J. Supercrit. Fluids 67 (2012) 60–70, http://dx.doi.org/10.1016/j.supflu.2012.02.004.

[21] H.P. He, H. Corke, J.G. Cai, Supercritical carbon dioxide extraction of oil and squalene from amaranthus grain, J. Agric. Food Chem. 51 (2003) 7921–7925, http://dx.doi.org/10.1021/jf030488y.

[22] P. Paśko, H. Bartoń, P. Zagrodzki, S. Gorinstein, M. Fołta, Z. Zachwieja, Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth, Food Chem. 115 (2009) 994–998, http://dx.doi.org/10.1016/j.foodchem.2009.01.037.

[23] L. Alvarez-Jubete, H. Wijngaard, E.K. Arendt, E. Gallagher, Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking, Food Chem. 119 (2010) 770–778, http://dx.doi.org/10.1016/j.foodchem.2009.07.032.

[24] R.W. Owen, W. Mier, A. Giacosa, W.E. Hull, B. Spiegelhalder, H. Bartsch, Phenolic

compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignansand squalene, Food Chem. Toxicol. 38 (2000) 647–659, http://dx.doi.org/10.1016/S0278-6915(00)00061-2.

[25] M. Miranda, A. Vega-Gálvez, J. López, G. Parada, M. Sanders, M. Aranda, E. Uribe, K. Di Scala, Impact of air-drying temperature on nutritional properties, total phenolic content and antioxidant capacity of quinoa seeds (Chenopodium quinoa Willd.), Ind. Crops Prod. 32 (2010) 258–263, http://dx.doi.org/10.1016/j.indcrop.2010.04.019.

[26] L. Alvarez-Jubete, M. Holse, Å. Hansen, E.K. Arendt, E. Gallagher, Impact of baking on vitamin E content of pseudocereals amaranth, quinoa, and buckwheat, Cereal Chem. 86 (2009) 511–515, http://dx.doi.org/10.1094/CCHEM-86-5-0511.

[27] R. Przybylski, G.S. Chauhan, N.A.M. Eskin, Characterization of quinoa (Chenopodium quinoa) lipids, Food Chem. 51 (1994) 187–192, http://dx.doi.org/10.1016/0308-8146(94)90255-0.