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Extraction of bioactive compounds from sesame (*Sesamum indicum* L.) defatted seeds using water and ethanol under sub-critical conditions

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ABSTRACT

Sesame seeds contain a vast array of lignans and phenolic compounds having important biological properties. An optimized method to obtain these seed components was designed by using water and ethanol at high pressure and temperature conditions. The maximum concentrations of lignans, total phenolics, flavonoids and flavonols compounds were achieved at 220 °C extraction temperature and 8 MPa pressure, using 63.5% ethanol as co-solvent. Under these conditions, the obtained sesame extracts gave the best radical scavenging capacity. Kinetic studies showed a high extraction rate of phenolic compounds until the first 50 min of extraction, and it was in parallel with the highest scavenging capacity. The comparison of our results with those obtained under conventional extraction conditions (normal pressure, ambient temperature) suggests that recovery of sesame bioactive compounds may be markedly enhanced using water/ethanol mixtures at sub-critical conditions.

1. Introduction

Sesame (*Sesamum indicum* L.), a member of the Pedaliaceae family, is an important oilseed crop; sesame oil production ranks eighth in the world's oil market. Sesame seeds are also consumed directly or incorporated as ingredients in several edible products.

Presently, there is good concern about health benefits derived from both sesame oil and sesame seed consumption, including hypocholesterolemic, anti-inflammatory, and antimutagenic effects (Chen et al., 2005; Lazarou, Grougnet, & Papadopoulos, 2007). Moreover, sesame consumption has been associated to risk reduction of cardiovascular, atherosclerosis, and oxidative stress-related diseases (Gouveia, Cardoso, de Oliveira, Rosa, & Moreira, 2016). Increasing evidences suggest that these biological effects are due in part to a specific type of lignans - namely sesamin, sesamolin, sesamol and sesaminol – which are abundantly present in sesame seeds (Kochhar, 2002; Rangkadilok et al., 2010). In addition to lignans, sesame seeds contain several phenolic compounds (ferulic, vanillic, cinnamic and *p*-coumaric acids, among others) (Ben Othman, Katsuno, Kanamaru, & Yabe, 2015; Mohdaly, Ramadan-Hassanien, Mahmoud, Sarhan, & Smetanska, 2013) which have been also reported to display important biological properties.

Several *in vitro* studies confirm that lignans and phenolics present in sesame seeds display strong antioxidant properties (Ben Othman et al., 2015; Kim et al., 2014; Kumar & Singh, 2014; Sarkis, Michel, Tessaro, & Marczak, 2014; Shahidi, Liyana-Pathirana, & Wall, 2006; Suja, Abraham, Thamizh, Jayalekshmy, & Arumughan, 2004; Suja, Jayalekshmy, & Arumughan, 2005). For instance, Suja et al. (2004, 2005) demonstrated that purified sesame extracts, composed mainly by free and glycosylated lignans, have higher antioxidant activity than that of the BTH, a well-known synthetic antioxidant. Kumar and Singh (2014) reported strong free radical scavenging capacity from sesame lignans; moreover, these compounds showed synergistic effect with γ-tocopherol. On the other hand, Ben Othman et al. (2015) found that lignan glycosides were the main antioxidants in butanol-soluble fractions extracted from defatted sesame seeds, whereas purified water-soluble fractions contained ferulic and vanillic acids as the main bioactive components. Regarding applications in food

Abbreviations: BBD, Box-Behnken design; BHT, butylhydroxytoluene; DB, dry basis; DSS, defatted sesame seed; GAE, gallic acid equivalent; OE, optimal extract; QE, quercetin equivalent; RSC, radical scavenging capacity; RSM, response surface methodology; SSC, sesame seed cake; SWE, sub-critical water extraction; TFC, total flavonoid content; TFlC, total flavonol content; TPC, total phenol content; TLC, total lignan content.

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systems, it has been found that sesame ethanolic extracts can stabilize soybean, sunflower and safflower oils (Mohdaly, Smetanska, Ramadan, Sarhan, & Mahmoud, 2011; Suja et al., 2004), and olein butter (Nadeem et al., & Khan, 2014) better than BHT. Other studies also reveal the potential of sesame lignans to extend the shelf-life of natural foods – for example as inhibitors of browning reactions in fruit pulps (Kumar & Singh, 2014) – or processed foods obtained by means of high temperature treatments (Yeo, Park, & Lee, 2011).

Extraction of sesame antioxidant compounds has been mostly carried out by using conventional methods (maceration, soxhlet) with different organic solvents, such as methanol, ethanol, acetone, diethyl ether, and ethyl acetate, as they provide high antioxidant yields due to their hydrogen-bonding ability, which is crucial for the extraction of phenolics or lignans, as those present in sesame seeds. These methods, however, have some drawbacks including long processing times, large amounts of solvents, and elimination of solvent residues that are often prohibited by food regulations.

One avenue that has not been thoroughly explored is the extraction of the solid material (for example, defatted milled seeds) with water at temperatures above the boiling point, and pressure sufficiently high to maintain the liquid state. Under these conditions, viscosity and surface tension of water are reduced and, at the same time, diffusivity characteristics are increased thus favouring the extraction process. It should be bear in mind that extraction from natural solid materials is a mass transfer process involving transport of the solvent into the matrix (inner transport), dissolution of the solutes (solubility), and release of solutes from the solid matrix to the global solvent phase (external transport). Thus, an increase in water diffusivity could reduce mass transfer limitation and increase extraction yields. It has been also observed that at high pressure and temperature extraction conditions, water behaves like certain organic solvents with the capacity of dissolve a wide range of medium and low polarity compounds (Teo Tan, Yong, Hew, & Ong, 2010). Dielectric constant of water is function of temperature, and affects solubility of the solutes in water; hence, changes in temperature influence extraction selectivity. In addition, the dielectric constant of water - and its polarity - can be modified by addition of organic co-solvents, such as ethanol. In summary, several factors including temperature, pressure, composition of the solvent system, and solvent-to-solid ratio, may be modified to enhance the extraction capacity of pure water or water mixed with organic solvents.

The extraction process mentioned above is known as sub-critical or superheated water extraction (SWE) (also named as pressurized hot water extraction), and it has been largely applied to the extraction of different kinds of bioactive compounds (Aliakbarian, Fathi, Perego, & Dehghani, 2012; Ko, Cheigh, & Chung, 2014; Xu, Wang, Liu, Yuan, & Gao, 2015; Zeković et al., 2014).

Sesame seed cake (SSC) is the by-product obtained after oil removal, usually by cold pressing. The production of SSC is estimated to be high, considering that approximately 70% of sesame seed production is destined to the oil industry (Ben Othman et al., 2015). Sesame seed cake is an undervalued by-product; it is commonly used as poultry or cattle feed in several producing countries. Previous works have reported the presence of both lignan and phenolic compounds in defatted sesame seeds (Ben Othman et al., 2015; Mohdaly et al., 2013; Sarkis et al., 2014; Suja et al., 2004). The present study was aimed to optimize the extraction conditions of these bioactive components using water and ethanol at high pressure and temperature. The extracts obtained were compared in terms of total lignan and phenolic contents, and antioxidant activity.

2. Materials and methods

2.1. Plant material

Sesame seeds (*Sesamum indicum* L.) were obtained from commercial plantations located at Salta province, Argentina. Whole seeds were partially defatted using a pilot plant scale screw-press (Komet, Model CA 59 G, Mönchengladbach, Germany) following procedures described elsewhere (Martínez, Penci, Marin, Ribotta, & Maestri, 2013). The resulting material was milled, fully defatted by Soxhlet extraction (*n*-hexane), and washed three times with distilled water (100 g/L) in order to remove soluble sugars and proteins (Suja et al., 2004). Finally, the material obtained (henceforth defatted sesame seed, DSS) was subjected to oven drying with forced air convection at 35° C, and stored until use in amber glass containers at −20 °C under nitrogen atmosphere.

2.2. Sub-critical water extraction

Sub-critical water extraction (SWE) was carried out in an in-house developed apparatus according to the experimental setup reported in Barrera Vázquez et al. (2015). It consists of a stainless steel high-pressure extractor cell (18.5 mL internal volume), a HPLC pump (ELDEX OPTOS 2SM, California, USA) having a maximum flow rate of 10 mL/min, a coiled preheated, and a downstream back pressure regulator (BPR). The extraction cell is equipped with aluminum heating jackets with two electrical resistances and connected to a temperature regulator. To maintain the set temperature, the cell is mounted within a thermally insulated box. The pressure in the extractor is measured with a pressure gauge (Dynisco Dynipack 16, Franklin, Massachusetts, USA). The experimental apparatus is completed with stainless steel 1/8″ connecting lines and accessories. In all experimental runs, stainless steel membrane cartridges were filled with 0.5 g of DSS and located into the extraction cell. The temperature was set at the desired extraction conditions, and pressure was achieved by controlling the pumped solvent with the BPR. The solvents used were distilled water and absolute ethanol. The volumetric flow was regulated by the HPLC pump to obtain solvent mass flow of 5 g/min. For each run, a final extract volume equivalent to 150 g was collected, centrifuged at 10,000 rpm for 20 min, and stored in amber glass bottles under nitrogen at −20 °C until analysis.

2.3. Extract analyses

Total phenol content (TPC) was determined using the Folin-Ciocalteau reagent according to Singleton, Orthofer, & Lamuela-Raventós (1999). The TPC from sample extracts obtained at the different SWE conditions was quantified by comparison of the absorbance value (725 nm) with those from a standard curve using gallic acid (GA). TPC was expressed as mg GAE/g DSS (DB).

Total flavonoid content (TFC) was determined according to Harborne (1989). A 1 mL-aliquot of ethanolic 2% aluminum chloride was added to 1 mL of each sample extract. After 10 min incubation at room temperature, the absorbance at 420 nm was measured. TFC was determined by comparison of the absorbance value with those from a standard curve using quercetin, and expressed as quercetin equivalent (mg QE/g DSS, DB).

Total flavonol content (TFlC) was determined by the method of Kumaran and Karunakaran (2007). Aliquots of 2 mL ethanolic 2% aluminum chloride, and 3 mL (50 g/L) sodium acetate solutions were added to 2 mL of each sample extract. After 2.5 h incubation at 20 °C, the absorbance at 440 nm was measured. TFlC was

calculated using the equation based on the calibration curve using quercetin as standard.

Total Lignan content (TLC) was determined according to Kwan-Su, Lee, and Lee (2006). Aliquots of 0.2 mL of each sample extract were appropriately diluted using methanol (HPLC grade) and, after shaking in a vortex, the absorbance at 290 nm was measured. TLC was quantified by comparison of the absorbance values with those from a standard curve using sesamin, and expressed as sesamin equivalent (mg SE/g DSS, DB).

2.4. Antioxidant activity

2.4.1. Radical scavenging capacity (RSC)

Scavenging capacity against the free stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (DPPH·) was assessed according to Apak et al. (2013). Three concentrations of each sample extract were added separately to 2 mL DPPH· solution (10^{-4} M) and the absorbance of each mixture was determined after 30 min of mixing using a UV–visible spectrophotometer at 515 nm. RSC toward DPPH· was estimated by means of the following equation:

$$\mathrm{DPPH}^{\cdot}_{r} = 1 - \left[\frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100\right] \quad (1)$$

where $\mathrm{DPPH}^{\cdot}_{r}$ expresses the amount of the radical that remains in the medium after antioxidants present in the extract are depleted. The RSC was expressed as IC_{50} (extract concentration, which causes 50% decrease of the initial DPPH· concentration). A lower IC_{50} value indicates higher antiradical activity. Also, the IC_{50} values of the sample extracts were compared with those from synthetic standards (BHT and Trolox).

Radical scavenging capacity was also determined by using the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay as described by Sarkis et al. (2014), with some modifications. ABTS radical was produced by mixing an ABTS solution (7 mM) with potassium persulfate (2.45 mM), and keeping the mixture in the dark at room temperature for 12 h. Afterwards, the ABTS radical solution was diluted in ethanol 95% until absorbance at 734 nm reached 0.7 (± 0.02). Aliquots from 10 to 80 μL of sesame extracts were mixed with 3 mL of the ABTS solution, and absorption was determined after 6 min. The percentage of ABTS radical inhibition was calculated using the following equation:

$$\mathrm{RSC_{ABTS}}(\%) = \left[1 - \frac{A}{A_0}\right] \times 100 \quad (2)$$

2.4.2. β-Carotene-linoleic acid bleaching test

This method is based on spectrophotometric measurements of β-carotene bleaching induced by the oxidative degradation products of linoleic acid. In brief, 0.2 mg β-carotene in 1 mL chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were transferred into a round-bottom flask. Once the chloroform had been removed in a rotary evaporator at 35 °C, 50 mL distilled water was added and the resulting mixture was stirred vigorously. Four mL of this emulsion were transferred to tubes containing the sesame extract or standard (BHT or Trolox) in the same concentration (200 μg/mL). After mixing, the absorbance (470 nm) at zero (A^0) time was recorded. The remaining sample was placed in a water bath at 50 °C for a period of 2 h and the absorbance (A^{120}) was measured at 15 min intervals. A mixture, prepared as described previously, without β-carotene, was used as a blank. All determinations were carried out in duplicate. The data were expressed as antioxidant activity (AA %) calculated according to the following equation:

$$\mathrm{AA}\ \% = \left[1 - \frac{A^0_{\mathrm{Sample}} - A^{120}_{\mathrm{Sample}}}{A^0_{\mathrm{Control}} - A^{120}_{\mathrm{Control}}}\right] \times 100 \quad (3)$$

2.5. Experimental design and response surface analysis

The experimental design for bioactive compound extraction from DSS was carried out using Response Surface Methodology (RSM). A Box-Behnken design with three factors was selected to identify relationships between the response variables and the process parameters, as well as those conditions that optimized the extraction process. The response variables selected were: IC_{50} (mg DSS/mL DPPH) (Y_1), TPC (mg GAE/g DSS) (Y_2), TFC (mg QE/g DSS) (Y_3), TFlC (mg QE/g DSS) (Y_4), TLC (mg SE/g DSS) (Y_5). The independent variables were temperature (X_1: 140–220 °C), pressure (X_2: 8–14 MPa), and concentration of ethanol as extraction co-solvent (X_3: 0–95%). The values of the ranges for each of these three factors were based on preliminary experimental studies (data not shown). The design consisted of fifteen randomized runs including three replicates at the central point (Table 1).

Quadratic polynomials were fitted to express the responses (Y_n) as a function of factors; where Y is the response, β_0 is the constant term, β_i represents the coefficients of the linear parameters, X_i represents the factors, β_{ii} represents the coefficients of the quadratic parameter, and β_{ij} represents the coefficients of the interaction parameters.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum\sum_{i<j}^{3}\beta_{ij} X_i X_j \quad (4)$$

The experimental results were analyzed to obtain the regression models. The model fitness quality was evaluated by ANOVA. The fit of the model to the experimental data was given by the determination coefficient (R^2) which explains the extent of the variance in a modeled variable that can be explained with the model. Only models with high determination coefficient were included in the study. Multiple regression equations included only significant coefficients ($p < 0.05$). All determinations were performed in triplicate, randomly, and replicas of the central point were done to allow estimation of pure error as square sums. Statistical analyses were performed using Statgraphic Plus software (v5.1, USA). Finally, for the model validation, the response variables were measured after extraction under optimal conditions, in triplicate. The experimental and predicted values were compared in order to validate the model.

2.6. Variability of antioxidant capacity through the extraction process

Recovery of phenolic compounds under the optimum extraction condition was analyzed as a function of time. Extractions were performed during a total time of 75 min. For all fractions obtained, TPC and antioxidant activity (RSC, DPPH method) were determined. For this latter, 200-mL aliquots were used.

3. Results and discussion

3.1. Fitting the response surface models

Experimental results from the investigated response variables (dependent variables) are reported in Table 1. Statistical analyses indicated that all of them fitted well to second order polynomial models (the polynomial equation for IC_{50} is shown below for illustration). The fit quality was established based on the determination coefficients (R^2), which were found to vary between 0.7598 and 0.9851. The "fitness" of the models was studied through the lack-of-fit test which indicates the suitability of the models to accu-

Table 1
Box–Behnken experimental design with coded SWE conditions and experimentally obtained values of antioxidant activity (IC_{50}), total phenol content (TPC), total flavonoid content (TFC), total flavonol content (TFlC), and total lignan content (TLC).

Run order	Independent variable			Investigated response				
	X_1 Temperature (°C)	X_2 Pressure (MPa)	X_3 Ethanol (%)	Y_1 IC_{50} (mg DSS/mL DPPH)	Y_2 TPC (mg GAE/g DSS)	Y_3 TFC (mg QE/g DSS)	Y_4 TFlC (mg QE/g DSS)	Y_5 TLC (mg SE/g DSS)
1	180	8	0	1.94 ± 0.06	5.12 ± 1.01	0.44 ± 0.01	ND	2.37 ± 0.05
2	220	11	0	0.88 ± 0.25	13.4 ± 0.73	0.58 ± 0.01	0.30 ± 0.01	3.85 ± 0.17
3[a]	180	11	48	0.95 ± 0.10	8.08 ± 0.49	0.69 ± 0.02	0.48 ± 0.04	5.59 ± 0.05
4	140	11	0	3.91 ± 0.73	6.16 ± 0.40	0.41 ± 0.01	ND	1.29 ± 0.17
5	140	11	95	3.55 ± 0.40	7.75 ± 0.51	0.39 ± 0.01	0.50 ± 0.09	0.68 ± 0.10
6[a]	180	11	48	0.94 ± 0.10	5.89 ± 0.58	0.55 ± 0.01	0.50 ± 0.08	3.13 ± 0.17
7	220	11	95	0.71 ± 0.16	16.0 ± 2.82	0.90 ± 0.07	1.29 ± 0.02	5.27 ± 0.17
8	180	14	95	2.57 ± 0.06	4.96 ± 0.25	0.37 ± 0.01	0.83 ± 0.02	1.07 ± 0.07
9	140	14	48	2.94 ± 0.50	2.80 ± 0.08	0.39 ± 0.01	0.20 ± 0.01	2.99 ± 0.03
10	220	8	48	0.27 ± 0.03	37.1 ± 1.31	2.64 ± 0.04	2.96 ± 0.02	19.9 ± 0.20
11	180	14	0	2.16 ± 0.48	4.84 ± 0.08	0.36 ± 0.01	ND	1.35 ± 0.03
12	180	8	95	2.12 ± 0.10	6.00 ± 1.05	0.53 ± 0.05	0.30 ± 0.01	1.43 ± 0.10
13	220	14	48	0.51 ± 0.08	17.5 ± 0.81	1.12 ± 0.01	0.95 ± 0.11	7.46 ± 0.10
14[a]	180	11	48	1.18 ± 0.16	7.19 ± 0.04	0.55 ± 0.01	0.52 ± 0.01	4.56 ± 0.07
15	140	8	48	3.31 ± 0.49	3.15 ± 0.16	0.41 ± 0.01	ND	1.28 ± 0.05

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalent; QE, quercetin equivalent; DSS, defatted sesame seed.
[a] Central point. ND: Not detected. Data from the investigated responses are expressed as arithmetic mean ± standard deviation (n = 2).

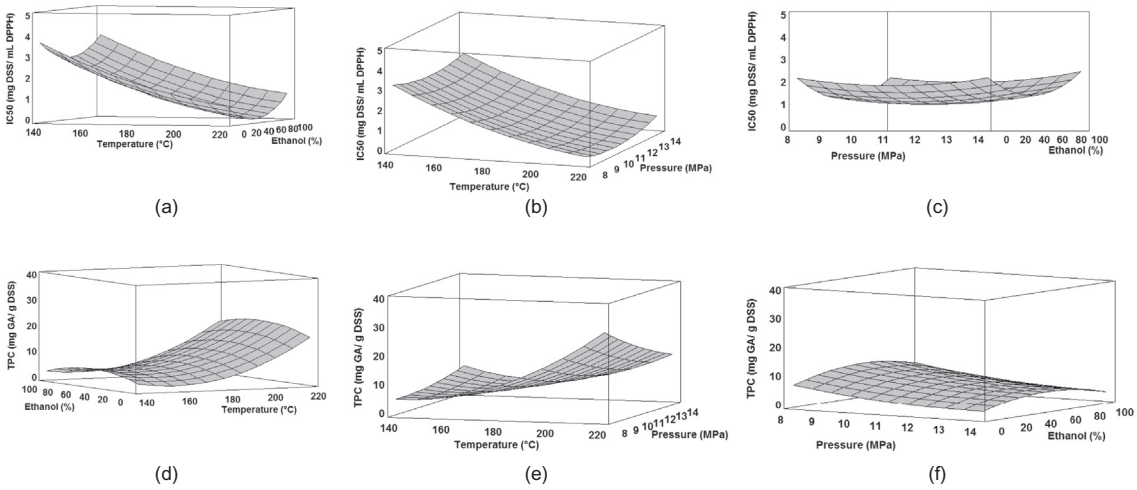

Fig. 1. Response surface plots showing combined effects of temperature (°C), pressure (MPa) and ethanol concentration (%) on IC_{50} value (a,b,c), total phenol content (TPC) (d,e,f), total flavonoid content (TFC) (g,h,i), total flavonol content (TFlC) (j,k,l), and total lignan content (TLC) (m, n, o).

rately predict the variation. For all response variables considered the lack of fit was not significant ($p > 0.05$) which suggests that the models are adequate for the observed data at a confidence level of 95%. So, all the investigated response variables were included in SWE optimization. The response surface graphs (Fig. 1) for dependent variables were plotted in function of two factors (independent variables) while the third factor was kept constant at middle level.

3.2. Influence of extraction parameters on the investigated response variables

For antioxidant activity (IC_{50}, Y_1), the 2nd order polynomial equation was as follow:

$$Y_1 = 23.5173 - 0.14051X_1 - 1.04329X_2 - 0.0436719X_3 + 0.00024974X_1^2 + 0.00127083X_1X_2 + 0.0000247396X_1X_3 + 0.0371759X_2^2 + 0.000399306X_2X_3 + 0.000364402X_3^2 \quad (5)$$

The effect of the extraction temperature was significant ($p < 0.05$) for both first-order linear effect (X_1) and second-order quadratic effect (X_1^2). The effects of pressure and ethanol concentration were significant only in second-order quadratic effect (X_2^2 and X_3^2). None interactive effect was observed. The experimentally measured values varied from 0.27 to 3.91 (mg DSS/mL DPPH) (Table 1). The lowest IC_{50} value (the highest antioxidant activity) was obtained at the highest temperature tested, and medium ethanol percentage (Fig. 1 a, c). At constant pressure (8 MPa) and ethanol concentration (48%) an increase in temperature had a strong effect on antioxidant activity. The RSC, measured by the $DPPH^{\cdot}$ inhibition percentage, was raised from 8.5% at 140 °C to 79.3% at 220 °C.

Regarding TPC (Y_2), only the effect of the extraction temperature (first-order linear effect) was significant. The experimental values from TPC determinations varied from 2.8 to 37.1 (mg GAE/g DSS) (Table 1); the maximum one was obtained at 220 °C and 8 MPa, using 48% ethanol concentration. The extraction temperature had a dramatic effect on phenolic compound recovery. At constant pressure (8 MPa) and ethanol concentration (48%), TPC increased almost thrice when temperature was raised from

Table 2
Predicted and experimental values at the optimal extraction condition (220 °C extraction temperature, 8 MPa pressure, 63.5% ethanol as co-solvent).

	IC_{50} (mg DSS/mL DPPH)	TPC (mg GAE/g DSS)	TFC (mg QE/g DSS)	TFlC (mg QE/g DSS)	TLC (mg SE/g DSS)
Predicted	0.21	30.7	2.21	2.36	17.5
Experimental	0.24 ± 0.025	32.3 ± 2.96	2.56 ± 0.28	2.12 ± 0.19	21.6 ± 0.12

Abbreviations: Antioxidant activity is expressed as IC_{50} values; TPC, total phenol content; TFlC, total flavonol content; TLC, total lignan content; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalent; QE, quercetin equivalent; SE, sesamin equivalent; DSS, defatted sesame seed.

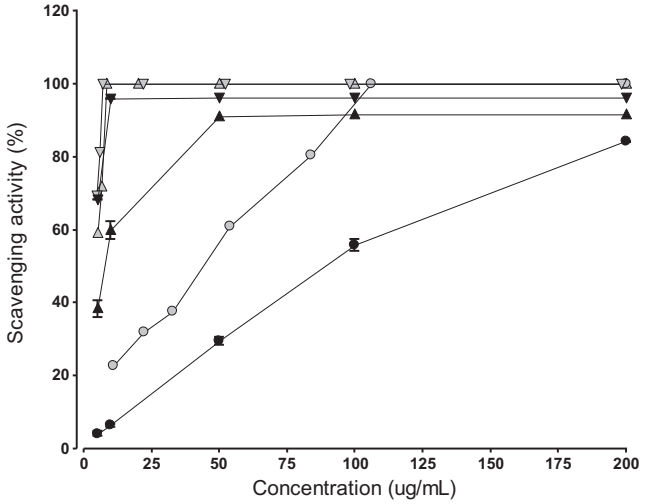

Fig. 2. Radical scavenging activity from sesame extract (DPPH method ●, ABTS method ●) obtained using the combination of process variables that maximized phenolics and lignans extraction. The synthetic antioxidants BHT (DPPH ▲, ABTS ▲) and Trolox (DPPH▼, ABTS ▼) were used as references. Each point represents the arithmetic mean ± standard deviation (n = 3).

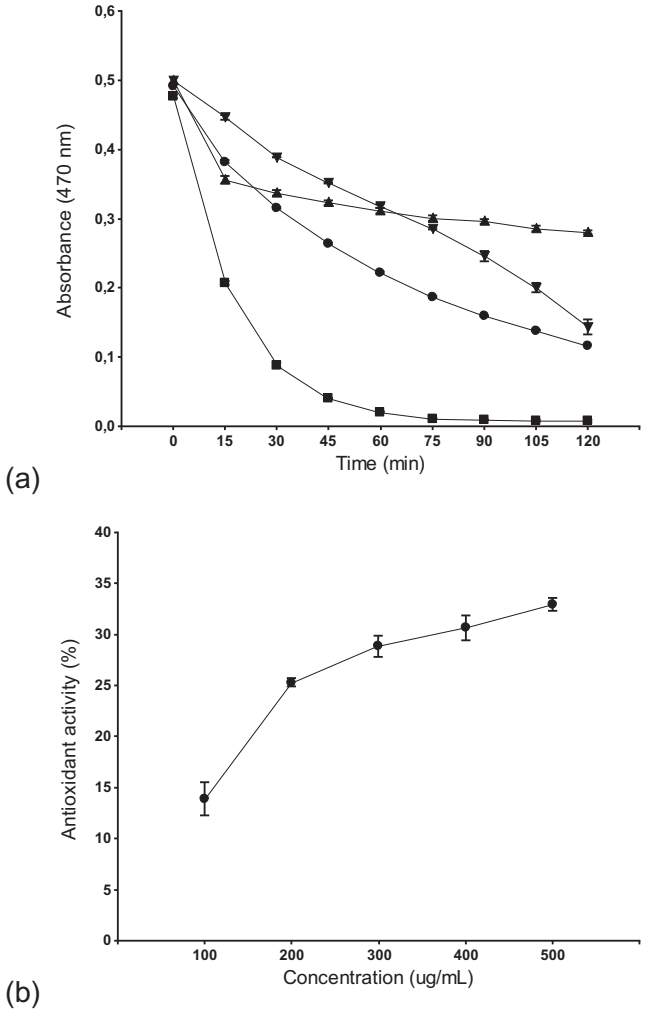

Fig. 3. a) Antioxidant activity (β-carotene-linoleic acid bleaching test) from sesame extract (●) obtained by using the combination of process variables that maximized phenolics and lignans extraction. The synthetic antioxidants BHT (▲) and Trolox (■), and a control sample (■) without any antioxidant substance were used as references. b) Antioxidant activity from sesame extract at different concentrations. Each point represents the arithmetic mean ± standard deviation (n = 3).

180 to 220 °C. At this latter temperature, an increase in pressure caused a significant reduction in TPC. The effect of ethanol concentration was negligible; nevertheless, extraction was slightly improved by using 1:1 (v/v) ethanol:water ratio (Fig. 1 d–f). The maximum TPC we reached (37.1 mg GAE/g DSS) was approximately ten times higher than the highest extracted amount (3.55 mg GAE/g) reported by Sarkis et al. (2014) using ethanol:water (48:52, v/v) at 58 °C, and similar sample (defatted seeds) preparation method. Similarly, Reshma, Namitha, Sundaresan, and Kiran (2012) reported an average TPC of 4.1 mg GAE/g defatted seeds sequentially extracted with ethyl acetate, methanol, methanol–water 70:30 (v/v), and pure water.

For both TFC (Y_3) and TFlC (Y_4) only the effect of extraction temperature (X_1) was significant. Coincidently with results from TPC, the highest TFC (2.64 mg QE/g DSS) and TFlC (2.96 mg QE/g DSS) values were achieved at the highest temperature tested (220 °C), at 8 MPa pressure, using 48% ethanol as co-solvent (Fig. 1 g–l). The highest value we reached for TFC was something higher than that reported by Reshma et al. (2012) (2.2 mg GAE/g defatted seeds) under the extraction conditions mentioned earlier.

The extracts obtained at the various SWE conditions showed TLC ranging between 0.68 and 19.9 mg SE/g DSS (Table 1). The similarity in the response surface plots indicates that conditions that enhanced phenolics extraction (Fig. 1 d –f) also favored lignans recovery (Fig. 1 m–o). As it can be seen, temperature was the most important factor affecting extraction; pressure and ethanol concentration had minor effects. Comparisons of the highest TLC reached here with those obtained with organic solvents and conventional extraction methods (Kim et al., 2014; Suja et al., 2005) suggest that sesame lignans may be better extracted using water/ethanol mixtures at sub-critical conditions.

About the solvent effect, it seems clear that pure water is not very effective for extraction of the mentioned compounds with the SWE conditions used here. Although viscosity of ethanol (1.074 cP, at 25 °C) is higher than that of water (0.89 cP) – this fact could reduce solvent diffusivity when they are mixed –, its polarity is markedly lower (polarity index 5.2 *vs* 9.0, dielectric constant 24 *vs* 78.5, for ethanol and water, respectively). So, addition of ethanol to water may reduce the dielectric constant of this latter thus enhancing the capacity to dissolve a wider range of compounds, including those of medium polarity such as phenolics and lignans present in sesame seeds.

Table 3
Kinetic of sesame phenolic extraction at the optimal extraction condition (220 °C extraction temperature, 8 MPa pressure, 63.5% ethanol as co-solvent).

Extraction time (min)	TPC (mg GAE/g DSS)	Accumulated %	RSC (DPPH)%
0–10	2.50 ± 0.37	5.63	28.9 ± 2.54
10–20	12.6 ± 3.82	33.9	67.7 ± 8.04
20–30	17.3 ± 1.18	72.8	79.7 ± 7.53
30–50	8.32 ± 1.61	91.5	30.8 ± 8.13
50–75	3.76 ± 0.39	100	17.6 ± 1.35

Abbreviations: TPC, total phenol content; GAE, gallic acid equivalent; DSS, defatted sesame seed; RSC, radical scavenging capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical. Data are expressed as arithmetic mean ± standard deviation (n = 3).

3.3. Optimization and verification of mathematical models

Multiple graphical and numerical optimizations were run in order to determine the optimum levels of independent variables with desirable response goals. Predicted and experimental values for the investigated response variables at the optimal extraction condition are given in Table 2. According to the desirability function, the combination of variables for an optimal process that maximized extraction of both phenolics and lignans, and gave the highest antioxidant activity, was 220 °C, 8 MPa, and 63.5% ethanol concentration. Under these conditions, extractions were run in triplicate and averaged experimental values for IC_{50}, TPC, TFC, TFlC and TLC were found to be 0.24 (mg DSS/mL DPPH), 32.3 (mg GAE/g DSS), 2.56 (mg QE/g DSS), 2.12 (mg QE/g DSS) and 21.6 (mg SE/g DSS), respectively. No significant differences were found between each of these values and the corresponding predicted values ($p \leq 0.05$), thus suggesting good fit of the model to experimental data.

3.4. Antioxidant activity under optimal extraction conditions

Further antioxidant activity assays were done to sesame extracts obtained under optimal extraction conditions. Fig. 2 shows data of RSC compared with those from BHT and Trolox, at different concentrations. Antiradical activity of extracts against both DPPH and ABTS radicals increased linearly with extract concentration. At concentration of 200 μg/mL, sesame extracts reached DPPH inhibition percentages (approximately 84%) something lower to those from BHT (91.7%) or Trolox (96.2%), but markedly higher than those reported by Abdelazim, Mahmoud, and Ramadan-Hassanien (2013) (60% at 200 μg/mL), and Ben Othman et al. (2015) (30% at 2500 μg/mL), who used methanol and ethanol (70%), respectively, both at normal pressure conditions, as extracting solvents. On the other hand, scavenging activity against ABTS radical reached 100% inhibition percentage at extract concentration near 100 μg/mL.

Antioxidant activity tested by means of the β-carotene-linoleic acid bleaching test (Fig. 3), calculated using the equation stated previously (Eq. (3)), showed sesame extract having lesser AA% than those from both BHT and Trolox. The value obtained (25 AA%) for the former suggests low antioxidant activity in oil in water emulsion systems. These results do not agree with those from by Mohdaly et al. (2011) who reported sesame cake extracts having better AA% than BHT.

3.5. Extraction kinetics

Table 3 shows TPC and RSC values as a function of the extraction time. Findings indicate that most phenolics (more than 90%) can be recovered before 50 min extraction; negligible amounts are obtained later. Extracts achieved between 10 – 20 min and between 20 and 30 min had the highest scavenging capacity. All these facts suggest that extraction times shorter than 50 min may be sufficient to get concentrated extracts with good antioxidant activity; longer times may cause dilution of phenolic compounds in the final extract.

4. Conclusions

A process to obtain bioactive compounds from defatted sesame seeds (DSS) was designed by using water and ethanol at high pressure and temperature conditions. Theoretical models were scanned against experimental data in order to optimize extraction conditions and antioxidant activity of targeted compounds. Hydroalcoholic mixtures showed to be effective as extracting solvents since they may scan a wide range of polarities regarding the compounds to be extracted. The maximum concentrations of lignans, and total phenolics, flavonoids and flavonols compounds were achieved at 220 °C extraction temperature and 8 MPa pressure, using 63% ethanol as co-solvent. Under these conditions, the obtained DSS extracts gave the best radical scavenging capacities, as measured by both DPPH and ABTS methods, similar to those reached by using synthetic antioxidants. Kinetic studies showed a high extraction rate of phenolic compounds until the first 50 min of extraction, and it was in parallel with the highest scavenging capacity. This represents an extraction time markedly shorter than the usually used in conventional process at normal pressure and ambient temperature. The comparison of our results with those obtained under conventional extraction conditions (normal pressure, ambient temperature) suggests that recovery of both lignan and phenolic compounds may be markedly enhanced using water/ethanol mixtures at sub-critical conditions. The process, based on the use of solvents accepted in the food industry, provides a method to extract both lignan and phenolic compounds simultaneously. It could be adapted by changing process conditions to selectively extract different kinds of bioactive compounds.

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