LWT - Food Science and Technology 65 (2016) 464–470

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Improvements in the quality of sesame oil obtained by a green extraction method using enzymes

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ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form
11 August 2015
Accepted 17 August 2015
Available online 19 August 2015

Keywords:
Enzymes
Sesame
Phytosterols
Antioxidant capacity

Chemical compounds studied in this article:
γ-tocopherol (PubChem CID: 92729)
Stigmasterol (PubChem CID: 5280794)
Sitosterol (PubChem CID: 222284)
Campesterol (PubChem CID: 173183)
α-linolenic acid (PubChem CID: 5280934)
Linoleic acid (PubChem CID: 5280450)

ABSTRACT

The quality of vegetable oils is related to the presence of bioactive compounds, in which its contents may vary according to the extraction process. This study aimed to evaluate a clean technology for sesame oil extraction by enzymatic aqueous extraction, comparing to conventional extraction methods, such as pressing and solvent, in relation to the composition of bioactive lipophilic compounds. Two enzymes were used: Pectinex Ultra SPL and Alcalase 2.4L, and three factors were evaluated: concentration of enzymes (mL 100 mL^{-1}), sample/water ratio (g mL^{-1}) and extraction time (hours) through a 2^3 factorial design with center point in triplicate. The results showed variations in extraction yield and composition. The sesame oil extracted using enzymes showed the highest antioxidant capacity in the DPPH and L-ORAC (against peroxyl radical) assays, 128,54 and 349,98 μmol Trolox g^{-1} of oil, respectively, as well a higher content of total phytosterols (249 mg 100 g^{-1} of oil), total polyunsaturated and omega-6 fatty acids. No significant difference in γ-tocopherol content was observed, by Tukey test ($p < 0.05$), among the extraction methods. The enzymatic aqueous extraction improved the quality of sesame oil using a green methodology, free of toxic solvents.

1. Introduction

Sesame grains (*Sesamum indicum L.*) are grown worldwide. The Asian continent is the main producer, accounting for 51.3% of production in 2013, followed by Africa, 44.9%; America, 3.7%; and Europe, 0.1% (Faostat, 2015). Among the different application areas of this oilseed (use of grain and oil), could be highlighted the gastronomy (Beltrão et al., 2013), biofuel production (Sarve, Sonawane, & Varma, 2015), application in the pharmaceutical field (Jeevana & Sreelakshmi, 2011), cosmetics production (Rocha-Filho et al., 2014), and human nutrition (Finco, Garmus, Bezerra, & Córdova, 2011).

Many of the components found naturally in vegetable oils have properties that are beneficial to health, such as fatty acids, phytosterols, carotenoids, natural antioxidants and tocopherols (Huang, Ou, & Prior, 2005). Studies have shown that intake of dietary sesame oil could effectively ameliorate the cerebral ischemia (Ahmad et al., 2006) and has synergistic effect with anti-diabetic medication, providing an effective improvement of hyperglycemia (Sankar, Sambandam, Rao, & Ali, 2011). Moreover, the dietary substitution of sesame oil has an additive effect in the reduction of blood pressure and plays an important role in the modulation of electrolytes and in the reduction of lipid peroxidation and elevation of antioxidants (Sankar, Sambandam, Rao, & Pugalendi, 2004). The chemical composition of the sesame oil, characterized by a low level of saturated fatty acids and the presence of antioxidants have been attributed to reduction of proliferation of certain cancers (Kanu, Bahsoon, Kanu, & Kandeh, 2010; Miyahara, Hibasami, Katsuzaki, Imai, & Komiya, 2001).

In general, the extraction method using solvents is one of the

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http://dx.doi.org/10.1016/j.lwt.2015.08.053
0023-6438/

most widely used in industry due to high oil yield. However, many highly toxic and flammable organic solvents comes from non-renewable sources and after the process, it requires many steps of treatment of its residues. Extraction by pressing, a conventional method, does not use solvents, but this technique is combined with other extractions that associate solvents to improve the extraction yield.

Considering the global concern in relation to organic solvents and the damage that can be caused to the environment, the development of alternative methods of oil extraction and the quality of the products need to be evaluated. The enzymatic aqueous extraction is a clean technology which presents itself as a promising alternative to technique using organic solvents for extraction of vegetable oils, taking into account the principles of green chemistry.

The enzymatic aqueous extraction employs enzymes that hydrolyze the cell wall and membranes of oleosomes (Botaneco, 2015), releasing the oil into the aqueous medium. Despite the fact that the cost of the enzymes is still high, recently published studies found that controlling of the some parameters could make the extraction process feasible (Nascimento, Couri, Antoniassi, & Freitas, 2008; Soto, Chamy, & Zúñiga, 2007; Zhang et al., 2012).

A large number of enzymes used in industries for different applications have been reportedly produced in solid state fermentation (SSF) at large-scale. These include alpha amylase, glucoamylase, pectinase, protease, lipase, phytase and other enzymes. Many research studies have shown the enzymes production through SSF using different low-cost agro-industrial residues as the substrate, which is very attractive for bioprocessing, since it adds value by decreasing the cost of enzyme production, reducing the amount of solid waste and boosting the environmentally friendly management of agricultural and domestic wastes (Bansal, Tewari, Soni, & Soni, 2012; Delabona et al., 2013; Graminha et al., 2008; Hansen, Lübeck, Frisvada, Lübeck, & Andersen, 2015; Kaushik, Mishra, & Malik, 2014; Thomas, Larroche, & Pandey, 2013).

This study aimed to evaluate a clean technology for sesame oil extraction by enzymatic aqueous extraction, comparing to conventional extraction methods, such as pressing and solvent, in relation to the composition of bioactive lipophilic compounds.

2. Materials and methods

2.1. Sample preparation

The sesame grains were purchased in local market in Maringá-PR, Brazil. The grains were ground in a Wiley mill to obtain a fine flour that was then sieved, using the fraction that passed through a 16 mesh Tyler series sieve (WS Tyler, USA). Pectinex Ultra SPL (pectinase – active pectolytic enzyme preparation produced by a selected strain of *Aspergillus aculeatus* that contains mainly pectintranseliminase, polygalacturonase, and pectinesterase and small amounts of hemicellulases and cellulases. The pectinase hydrolyzes pectin, which is a component of the cell wall) and Alcalase 2.4L (endo-protease that hydrolyze most peptide bonds within a protein molecule) enzymes were obtained from Sigma (USA).

2.2. Enzymatic aqueous extraction

The enzymatic aqueous extraction of sesame oil was conducted from a 2^3 factorial design with center point in triplicate (Table 1) using the Design Expert software, version 7.1.3. The extraction yield response was evaluated by the influence of different concentrations of Pectinex Ultra SPL and Alcalase 2.4L enzymes, sample/water ratio and extraction time.

The extraction experiments were performed according to Santos and Ferrari (2005) with adaptations. The samples were subjected to heat treatment at 105 °C for 45 min. After, 5.0 g of sample was weighed and mixed with distilled water at a ratio of 1/6, 1/8 or 1/10 (g mL^{-1}). The pH of the mixture was adjusted to 4.5 with 1.0 mol L^{-1} aqueous HCl solution and then added Pectinex Ultra SPL enzyme in concentrations of 6, 8 and 10 mL 100 mL^{-1}. Afterwards, the mixture was maintained at 50 °C for 4, 6 and 8 h, with shaking at 100 rpm in an incubator shaker (CT 712). In the second step, the pH was adjusted to 7.0 through the addition of a 1.0 mol L^{-1} aqueous NaOH solution followed by the addition of the Alcalase 2.4 L enzyme in concentrations of 6, 8 and 10 mL 100 mL^{-1}. Then, the sample was incubated at 55 °C under the same conditions mentioned in the first step. After, the mixture was heated at 60 °C for 15 min and the extract was centrifuged for 15 min. The free oil was collected with a micropipette and weighed to determine the extraction yield.

Table 1
Factors and levels for the 2^3 factorial design with center point.

Factors	Symbol	Unit	Type	Levels		
				−1	0	+1
Enzymes	X_1	mL 100 mL^{-1}	Numeric	6	8	10
Sample/water	X_2	g mL^{-1}	Numeric	1/6	1/8	1/10
Time	X_3	h	Numeric	4	6	8

2.3. Solvent extraction

The sample was submitted to a lipid extraction process with a mixture chloroform-methanol-water (2:2:1.8 mL:mL:mL), respectively, according to Bligh and Dyer (1959).

2.4. Extraction by pressing

For lipid extraction by pressing, 100.0 g of sample, previously dried in a fan oven at 50 °C for about 14 h, was placed in a stainless steel cylinder (PEM – PHP 30 tons) under a pressure of 10 tons for 5 h.

2.5. Fatty acid composition

Fatty acid methyl esters (FAME) were prepared by the methylation of lipids (Hartman & Lago, 1973). The FAME were separated by gas chromatography (Trace Ultra 3300 – Thermo Scientific) equipped with a flame ionization detector (FID) and a cyanopropyl capillary column (100 m × 0.25 i.d., 0.25 μm film thickness, CP 7420 Varian). The injector, detector and gases conditions, and the main operational parameters were performed according to Sargi et al. (2013).

Quantification of fatty acids was performed using tricosanoic acid methyl ester (Sigma, USA) as an internal standard (23:0) (Joseph & Ackman, 1992). Theoretical FID (flame ionization detector) correction factor values (Visentainer, 2012) were used to obtain concentration values.

2.6. Phytosterols and tocopherols

Phytosterols and tocopherols were simultaneously evaluated by gas chromatography coupled to mass spectrometer (GC–MS) (Du & Ahn, 2002). The extracted oils were previously derivatized (Beveridge, Li, & Drover, 2002) and the analysis was performed in a gas chromatograph (Thermo–Finnigan, Thermo Focus GC) equipped with a capillary column DB-5 (5% phenyl, 95% methylpolysiloxane) fused silica, 30 m, 0.25 mm i.d and 0.25 μm thick film stationary phase (J & W Scientific, Folson, CA) coupled to a mass

Table 2
Factorial design 2^3 with center point and enzymatic aqueous extraction yield of sesame oil.

Coded variable				Actual variable			
Run	X_1	X_2	X_3	x_1	x_2	x_3	Y (%)
1	−1	−1	−1	6	1/6	4	26.74
2	+1	−1	−1	10	1/6	4	29.55
3	−1	+1	−1	6	1/10	4	20.33
4	+1	+1	−1	10	1/10	4	21.96
5	−1	−1	+1	6	1/6	8	35.42
6	+1	−1	+1	10	1/6	8	36.65
7	−1	+1	+1	6	1/10	8	27.34
8	+1	+1	+1	10	1/10	8	27.61
9	0	0	0	8	1/8	6	28.98
10	0	0	0	8	1/8	6	29.12
11	0	0	0	8	1/8	6	30.04

X_1, X_2, and X_3: enzymes concentration, sample/water ratio and extraction time, respectively. x_1, x_2, and x_3: enzymes concentration (mL 100 mL^{-1}), sample/water ratio (g mL^{-1}) and extraction time (h), respectively. Y represents the enzymatic aqueous extraction yield (%).

spectrometer (Thermo-Finnigan, DSQ II) equipped with an electron ionization (EI) source. The conditions for analysis by GC–MS were previously described by Zanqui et al. (2015). The system of data acquisition was performed by Xcalibur software accompanying database of spectra contained in the NIST MS Search spectral library version 2.0. Quantification was carried out in relation to the internal standard 5-α-cholestane (Sigma, Brazil) (Li, Beveridge, & Drover, 2007).

2.7. Antioxidant capacity

2.7.1. DPPH radical assay

The DPPH radical scavenging activity assay was performed as described by Masuda et al. (1999) and Brand-Williams, Cuvelier, and Berset (1995) including modifications according to Ma et al. (2011). The absorbance of the solutions was measured at 517 nm using a UV–Vis spectrophotometer (Thermo Scientific – Genesys 10S).

2.7.2. Lipophilic – oxygen radical absorbance capacity (L-ORAC)

The lipophilic antioxidant capacity was determined using the L-ORAC assay (Prior et al., 2003), with randomly methylated β-cyclodextrin (RMCD) as a solubility enhancer (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002). The decay of the fluorescence spectra was obtained with a spectrofluorimeter (Perkin Elmer Victor – X4) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

The results of both assays were expressed in Trolox equivalent antioxidant capacity per gram of oil.

2.8. Statistical and principal components analysis (PCA)

Data were expressed as mean values ± standard deviations of the analytical error propagation. The results were submitted to variance analysis (ANOVA) and mean values were compared by Tukey's test, using the Statistica software, version 8.0. The principal component analysis was performed with the Statgraphics software, version 16.1.03. Principal and interaction effects resulted of factorial design were calculated and the variance analysis was used to evaluate the effect of independent variables on the response using the mathematical model expressed by Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \varepsilon \quad (1)$$

where Y is the expected response; X_1 = enzymes concentration, X_2 = sample/water ratio, X_3 = extraction time; and the other terms refers to interactions effects.

3. Results and discussion

3.1. Total lipids (TL)

Table 2 shows the yields obtained for the enzymatic aqueous extraction of sesame oil from the 2^3 factorial design with center point in triplicate.

The highest yield obtained from the extraction of sesame oil from the factorial design was 36.65% under the conditions of 10 mL 100 mL^{-1} pectinase and protease, a sample/water ratio of 1/6 (g mL^{-1}) and 8 h of extraction.

3.2. Analysis of variance (ANOVA)

Table 3 shows the ANOVA for the extraction yield of sesame oil.

The study of the effects showed that only the terms enzyme concentration, sample/water ratio and extraction time were significant, in which the most significant factors in the extraction process were time and the sample/water ratio with a contribution of 43.36 and 51.92%, respectively. The ANOVA indicated that the model was significant and the effects can be noted in the response surface models (Fig. 1). The interaction effects were not significant, so these terms were removed from the model equation. The mathematical equation and the regression coefficient obtained for the enzymatic aqueous extraction of sesame oil are shown in Eq. (2).

$$Y = 28.20 + 0.74 \cdot X_1 - 3.89 \cdot X_2 + 3.56 \cdot X_3 \quad R^2 = 0.985 \quad (2)$$

where Y = extraction yield; X_1 = enzymes concentration;

Table 3
Analysis of variance for the response enzymatic aqueous extraction yield of sesame oil.

Source	Degrees of freedom	Sum of squares	Mean square	F value	*p*-value
X_1	1	4.41	4.41	7.48	0.0340
X_2	1	121.06	121.06	205.24	<0.0001
X_3	1	101.10	101.10	171.41	<0.0001
Curvature	1	3.04	3.04	5.15	0.0637
Residual	6	3.54	0.59	–	–
Lack of fit	4	2.88	0.72	2.17	0.3397
Pure error	2	0.66	0.33	–	–
Total	10	233.15	–	–	–

X_1 = enzymes concentration; X_2 = sample/water ratio; X_3 = extraction time.

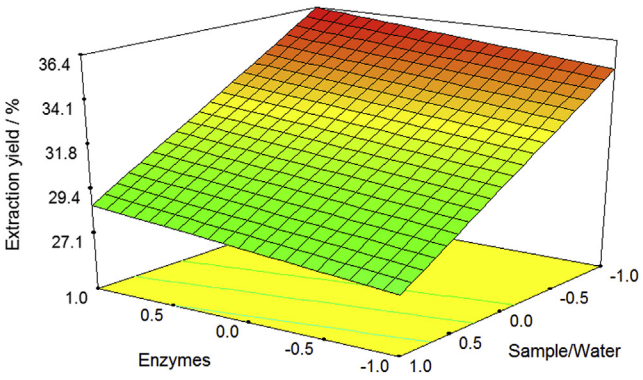

Fig. 1. Response surface for the enzymatic aqueous extraction of sesame oil.

Table 4
Extraction yield of sesame oil obtained by different methods.

Method	Yield (%)
Solvents	59.97[a] ± 0.81
Pressing	42.00[b] ± 0.21
Enzymatic*	36.65[c] ± 0.18

*The maximum extraction yield obtained from the factorial design. Mean ± standard deviation of triplicate analyses. Different letters in the same column indicate significant difference at 95% by the Tukey test ($p < 0.05$).

Table 5
Antioxidant capacity of sesame oil extracted by different methods.

Methods	Assays (μmol Trolox g^{-1} of oil)	
	DPPH	L-ORAC
Solvents	68.28[c] ± 1.79	204.04[c] ± 10.21
Pressing	88.85[b] ± 2.57	230.22[b] ± 2.97
Enzymatic	128.54[a] ± 4.60	349.98[a] ± 0.66

Mean ± standard deviation of triplicate analyses. Different letters in the same column indicate significant difference at 95% by the Tukey test ($p < 0.05$).

X_2 = sample/water ratio; X_3 = extraction time.

3.3. Extraction by conventional methodologies

Table 4 shows the yield for the extraction of sesame oil by conventional (solvent and pressing) and enzymatic extraction techniques.

The enzymatic aqueous extraction showed the lowest yield compared to conventional techniques, though its efficiency corresponds to 87% in relation to pressing method. However the response surface (Fig. 1) indicates that changes in the levels of some factors may still cause an increase in the extraction yield of sesame oil using enzymes.

3.4. Antioxidant capacity

To evaluate the antioxidant capacity of the sesame oil extracted either enzymatically or using conventional methods, two assays were performed: DPPH radical scavenging activity and antioxidant capacity by inhibiting oxidation induced by peroxyl radicals through the transfer of hydrogen atoms using the L-ORAC assay. Table 5 shows the results obtained from these tests.

The sesame oil obtained through enzymatic aqueous extraction demonstrated superior antioxidant capacity compared to the oils extracted using conventional methods in both assays, being 71.5% and 88.3% higher against DPPH and peroxyl radicals, respectively, compared to oil extracted by solvents. This indicates that the enzymatic method was probably able to extract a higher amount of bioactive compounds with antioxidant capacity.

3.5. Phytosterols and tocopherols

The γ-tocopherol and the three major phytosterols (campesterol, stigmasterol and sitosterol) were identified in sesame oil. Table 6 shows the quantification of these compounds present in the oil sample.

The enzymatic aqueous extraction exhibited the oil with the highest sitosterol and campesterol contents compared to conventional methods. The stigmasterol and γ-tocopherol contents showed no significant differences among the oil extraction methods.

According to the Codex Alimentarius (2013), the levels of campesterol, stigmasterol and sitosterol found in unrefined sesame oil are usually from 10.1 to 20.0; 3.4 to 12.0 and 57.7 to 61.9 g, respectively, relative to 100 g of total sterols. The contents obtained in this study are compatible with those reported levels, while the results obtained for sitosterol are above those reported, at 73.1 g, 74.1 g and 73.3 g for the methodologies using solvents, pressing and enzymes, respectively, to 100 g of phytosterols. Antoniassi et al. (2013) also obtained values above the levels from the Codex for sitosterol in oil of sesame grains of different genotypes that were grown in different locations. The sterol profile is used to set the standard identity of vegetable oils and countries could claim the amendment of the standard to Codex Alimentarius (2013), based on results of local varieties (Antoniassi et al., 2013).

Considering the data obtained for total phytosterols, is possible to observe that extraction using enzymes showed the highest sum of these bioactive compounds which brings benefits to human health (Awad, Chinnam, Fink, & Bradford, 2007; Awad, Fink, Williams, & Kim, 2001; Awad & Fink, 2000; Alappat, Valerio, & Awad, 2010; Cilla, Attanzio, Barberá, Tesoriere, & Livrea, 2015; Martins, Silva, Novaes, & Ito, 2004; Schröder & Vetter, 2012; Woyengo, Ramprasath, & Jones, 2009).

3.6. Fatty acids

Table 7 shows the results of the fatty acid quantification from the sesame oil obtained by solvent extraction, pressing and

Table 6
Quantification of tocopherol and phytosterols in sesame oil extracted by different methods.

Method	γ-Tocopherol (mg 100 g^{-1})	Phytosterols (mg 100 g^{-1})			Total phytosterols (mg 100 g^{-1})
		Campesterol	Stigmasterol	Sitosterol	
Solvents	46.94[a] ± 0.94	45.29[ab] ± 1.45	18.49[a] ± 1.30	173.78[ab] ± 7.20	237.56[ab] ± 7.45
Pressing	42.93[a] ± 5.51	37.93[b] ± 6.32	19.50[a] ± 0.77	164.61[b] ± 4.02	222.04[b] ± 7.16
Enzymatic	44.60[a] ± 1.27	46.92[a] ± 4.18	19.61[a] ± 4.24	182.43[a] ± 9.36	248.96[a] ± 11.10

Mean ± standard deviation of triplicate analyses. Different letters in the same column indicate significant difference at 95% by the Tukey test ($p < 0.05$).

Table 7
Fatty acid quantification of sesame oil.

Fatty acids	Method (mg g^{-1} total lipids)		
	Solvents	Pressing	Enzymatic
14:0	$0.171^{b} \pm 0.008$	$0.195^{a} \pm 0.008$	$0.169^{b} \pm 0.005$
16:0	$96.112^{a} \pm 1.430$	$94.131^{ab} \pm 0.289$	$93.786^{b} \pm 0.651$
16:1n-7	$1.534^{a} \pm 0.045$	$1.402^{b} \pm 0.017$	$1.510^{a} \pm 0.033$
17:0	$0.377^{a} \pm 0.019$	$0.439^{a} \pm 0.013$	$0.410^{a} \pm 0.017$
18:0	$53.191^{a} \pm 0.735$	$45.969^{b} \pm 0.457$	$51.972^{a} \pm 0.369$
18:1n-9	$366.047^{b} \pm 1.999$	$373.633^{a} \pm 2.218$	$362.202^{b} \pm 1.570$
18:1n-7	$5.491^{c} \pm 0.106$	$6.204^{a} \pm 0.086$	$5.844^{b} \pm 0.023$
18:2n-6	$428.127^{b} \pm 1.632$	$429.154^{b} \pm 1.203$	$433.397^{a} \pm 1.466$
18:3n-3	$2.725^{c} \pm 0.077$	$3.312^{a} \pm 0.035$	$2.884^{b} \pm 0.021$
20:0	$5.395^{a} \pm 0.127$	$4.740^{b} \pm 0.024$	$5.202^{a} \pm 0.182$
20:1n-9	$1.368^{b} \pm 0.007$	$1.430^{a} \pm 0.024$	$1.367^{b} \pm 0.013$
20:4n-6	$1.060^{a} \pm 0.004$	$0.859^{b} \pm 0.020$	$1.031^{a} \pm 0.041$
24:0	$0.645^{a} \pm 0.011$	$0.588^{b} \pm 0.041$	$0.504^{c} \pm 0.026$
Summations and ratio (mg g^{-1} total lipids)			
SFA	$155.927^{a} \pm 1.614$	$146.062^{c} \pm 0.542$	$152.044^{b} \pm 0.760$
MUFA	$373.914^{b} \pm 2.001$	$382.197^{a} \pm 2.219$	$370.422^{b} \pm 1.570$
PUFA	$431.911^{b} \pm 1.634$	$433.325^{b} \pm 1.203$	$437.312^{a} \pm 1.467$
n-6	$429.186^{b} \pm 1.632$	$430.013^{b} \pm 1.203$	$434.428^{a} \pm 1.467$
n-3	$2.725^{c} \pm 0.077$	$3.312^{a} \pm 0.035$	$2.884^{b} \pm 0.021$
PUFA/SFA	$2.770^{c} \pm 0.031$	$2.967^{a} \pm 0.014$	$2.876^{b} \pm 0.017$

Mean ± standard deviation of triplicate analyses. Different letters in the same line indicate significant difference at 95% by the Tukey test ($p < 0.05$). SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6: total omega-6 fatty acids; n-3: total of omega-3 fatty acids.

enzymatic aqueous extraction.

The fatty acids identified for the sesame oil, in which the major acids were 16:0, 18:0, 18:1n−9 and 18:2n−6, are in accordance with the profiles reported in previous studies (Aued-Pimentel, Takemoto, Antoniassi, & Badolato, 2006; Botelho et al., 2014; Corso et al., 2010). The enzymatic aqueous extraction showed the highest levels of polyunsaturated fatty acids (PUFA) and omega-6 (n-6), 437.312 mg g^{-1} and 434.428 mg g^{-1}, respectively, compared with conventional extraction methods. The main fatty acid responsible for these sums was linoleic acid (18:2n−6), considered as a strictly essential fatty acid. The fatty acid quantification of sesame oil extracted using solvents showed the highest sum of saturated fatty acids (SFA) and one of the smallest sums of polyunsaturated fatty acids (PUFA). Polyunsaturated fatty acids are considered healthier compared to saturated (Lawrence, 2010), therefore in evaluating these points and the PUFA/SFA ratio, the enzymatic aqueous extraction proved to be a methodology able to extract oil with a better quality compared to techniques using solvents.

3.7. Principal components analysis (PCA)

A PCA analysis was applied to confirm the effects of different extraction techniques. Loadings were referred to as SFA, TPS (total phytosterols), n-6, n-3 (omega-3), PUFA, MUFA (total monounsaturated fatty acids), DPPH and L-ORAC assays and the scores were the different extraction techniques (S: solvents, P: pressing and E: enzymatic aqueous extraction). PCA plots for the data obtained are shown in Fig. 2.

In Fig. 2A, the first principal component (PC1) had the highest eigenvalue, 5.15, and accounted for 64.47% of the variability in the data set. The second (PC2) had an eigenvalue of 2.84 and accounted for 35.52% of the variance in the data. Following the Kaiser's rule, an eigenvalue greater than 1.0 is considered a significant descriptor of data variance. The remaining six generated PCs (PC3–PC8) yielded progressively smaller eigenvalues ($P < 1$), so PC1 and PC2 better describe the data.

In the PCA plot with two components, the data shows that the techniques are significantly different, comparing the composition

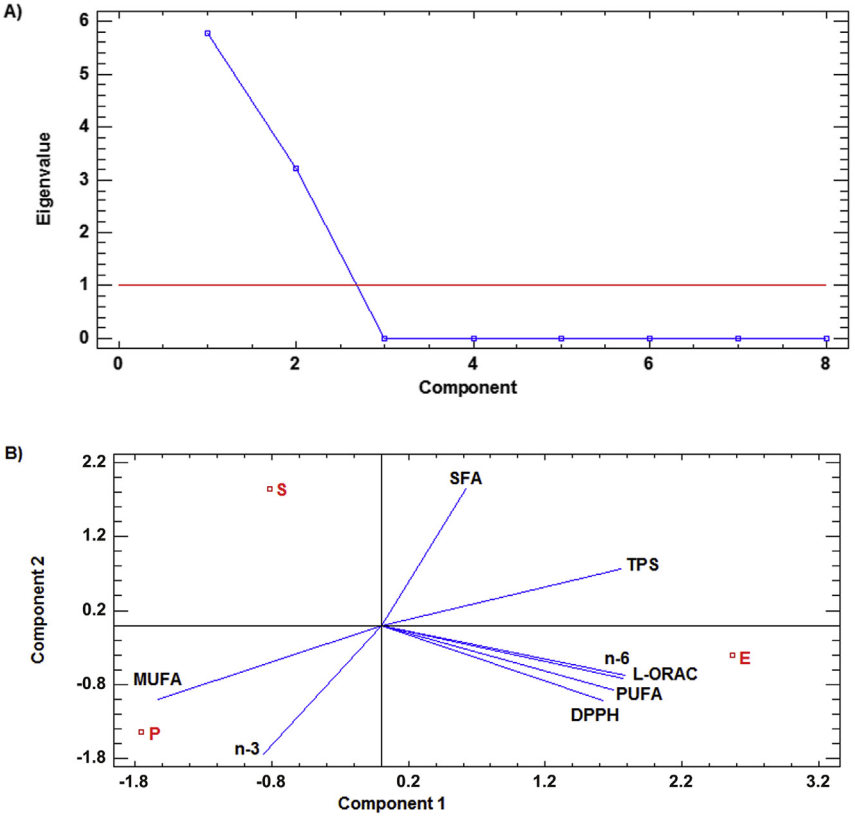

Fig. 2. Eigenvalue values (A) and principal component analysis biplot (B) of different techniques of sesame oil extraction.

of the sesame oils (Fig. 2B). Enzymatic aqueous extraction approximated the maximum values of n−6, L-ORAC, TPS, PUFA and DPPH, showing a good correlation with these analyses, compared to the other extraction techniques. Pressing extraction, on the other hand, approximated the maximum values of MUFA and n−3, and solvent extraction showed the highest composition of SFA. So, PCA confirmed that the quality of sesame oil can be improved by applying enzymatic aqueous extraction.

4. Conclusions

Aimed at obtaining vegetable oil with higher quality using methods without any toxic solvents during the process, the enzymatic aqueous extraction proved to be an alternative methodology for sesame oil. This extraction revealed superior quality oil, improving the antioxidant capacity, contents of total phytosterols, total polyunsaturated fatty acids and omega−6, compared to oil obtained using conventional methods.

The factorial design showed a significant mathematical model in terms of the studied factors: enzymes concentration, sample/water ratio and extraction time. The levels that showed the higher yield (36.65%) were 10 mL 100 mL^{-1} of pectinase and protease enzymes, a sample/water ratio 1/6 and 8 h of extraction for each step of the analysis.

The conventional methods showed higher extraction yield, however, considering that most of the methods for extraction of vegetable oils utilize solvents, it is interesting to develop and enhance new technologies that are able to extract oils that preserve the bioactive compounds naturally present, reducing contamination by toxic solvents and minimizing the damages to the environment. In light of these facts, the enzymatic aqueous extraction process could be optimized in order to obtain products with high quality and industrial application.

Acknowledgments

The authors thank Capes and CNPq for the financial support.

References

Ahmad, S., Yousuf, S., Ishrat, T., Khan, M. B., Bhatia, K., Fazli, I. S., et al. (2006). Effect of dietary sesame oil as antioxidant on brain hippocampus of rat in focal cerebral ischemia. *Life Sciences, 79*, 1921–1928.

Alappat, L., Valerio, M., & Awad, A. B. (2010). Effect of vitamin D and β-sitosterol on immune function of macrophages. *International Immunopharmacology, 10*, 1390–1396.

Alimentarius, C. (2013). *Codex standards for named vegetable oils. Codex Stan 210*. Italy: Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO).

Antoniassi, R., Arriel, N. H. C., Gonçalves, E. B., Freitas, S. C., Zanotto, D. L., & Bizzo, H. R. (2013). Effect of cultivation conditions on the composition of sesame seed and oil. *Ceres, 60*, 301–310.

Aued-Pimentel, S., Takemoto, E., Antoniassi, R., & Badolato, E. S. G. (2006). Composition of tocopherols in sesame seed oil: an indicative of adulteration. *Grasas y Aceites, 57*, 205–210.

Awad, A. B., Chinnam, M., Fink, C. S., & Bradford, P. G. (2007). β-sitosterol activates Fas signaling in human breast cancer cells. *Phytomedicine, 14*, 747–754.

Awad, A. B., & Fink, C. S. (2000). Phytosterols as anticancer dietary components: evidence and mechanism of action. *Journal of Nutrition, 130*, 2127–2130.

Awad, A. B., Fink, C. S., Williams, H., & Kim, U. (2001). In vitro and in vivo (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells. *European Journal of Cancer Prevention, 10*, 507–513.

Bansal, N., Tewari, R., Soni, R., & Soni, S. K. (2012). Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. *Waste Management, 32*, 1341–1346.

Beltrão, N. E. M., Ferreira, L. L., Queiroz, N. L., Tavares, M. S., Rocha, M. S., Alencar, R. D., et al. (2013). *O gergelim e seu cultivo no semiárido brasileiro*. Natal: IFRN.

Beveridge, T. H. J., Li, T. S. C., & Drover, J. C. G. (2002). Phytosterol content in American ginseng seed oil. *Journal of Agricultural and Food Chemistry, 50*, 744–750.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Botaneco. Oleosomes technology overview. http://www.botaneco.com/index-10.html. Accessed on 2.06.2015.

Botelho, J. R. S., Medeiros, N. G., Rodrigues, A. M. C., Araújo, M. E., Machado, N. T., Santos, A. G., et al. (2014). Black sesame (*Sesamum indicum L.*) seeds extracts by CO_2 supercritical fluid extraction: Isotherms of global yield, kinetics data, total fatty acids, phytosterols and neuroprotective effects. *The Journal of Supercritical Fluids, 93*, 49–55.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*, 25–30.

Cilla, A., Attanzio, A., Barberá, R., Tesoriere, L., & Livrea, M. A. (2015). Anti-proliferative effect of main dietary phytosterols and β-cryptoxanthin alone or combined in human colon cancer Caco-2 cells through cytosolic Ca^{+2} – and oxidative stress-induced apoptosis. *Journal of Functional Foods, 12*, 282–293.

Corso, M. P., Fagundes-Klen, M. R., Silva, E. A., Cardozo-Filho, L., Santos, J. N., Freitas, L. S., et al. (2010). Extraction of sesame seed (*Sesamum indicum L.*) oil using compressed propane and supercritical carbon dioxide. *The Journal of Supercritical Fluids, 52*, 56–61.

Delabona, P. S., Pirota, R. D. P. B., Codima, C. A., Tremacoldi, C. R., Rodrigues, A., & Farinas, C. S. (2013). Effect of initial moisture content on two Amazon rainforest *Aspergillus* strains cultivated on agro-industrial residues: biomass-degrading enzymes production and characterization. *Industrial Crops and Products, 42*, 236–242.

Du, M., & Ahn, D. U. (2002). Simultaneous analysis of tocopherols, cholesterol, and phytosterols using gas chromatography. *Journal of Food Science: Food Chemistry and Toxicology, 67*, 1696–1700.

Faostat. Food and agriculture organization of the United Nations. http://faostat3.fao.org/browse/Q/QC/E. Accessed on 10.06.2015.

Finco, A. M. O., Garmus, T. T., Bezerra, J. R. M. V., & Córdova, K. R. V. (2011). Yogurt-making with addition of sesame flour. *Ambiência, 7*, 217–227.

Graminha, E. B. N., Gonçalves, A. Z. L., Pirota, R. D. P. B., Balsalobre, M. A. A., Silva, R., & Gomes, E. (2008). Enzyme production by solid-state fermentation: application to animal nutrition. *Animal Feed Science and Technology, 144*, 1–22.

Hansen, G. H., Lübeck, M., Frisvada, J. C., Lübeck, P. S., & Andersen, B. (2015). Production of cellulolytic enzymes from ascomycetes: comparison of solid state and submerged fermentation. *Process Biochemistry, 50*, 1327–1341.

Hartman, L., & Lago, R. C. A. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice, 22*, 475–477.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry, 50*, 1815–1821.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

Jeevana, J. B., & Sreelakshmi, K. (2011). Design and evaluation of self-nanoemulsifying drug delivery system of flutamide. *Journal of Young Pharmacists, 3*, 4–8.

Joseph, J. D., & Ackman, R. G. (1992). Capillary column gas chromatographic method for analysis of encapsulated fish oils and fish oil ethyl esters: collaborative study. *Journal of AOAC International, 75*, 488–506.

Kanu, P. J., Bahsoon, J. Z., Kanu, J. B., & Kandeh, J. B. (2010). Nutraceutical importance of sesame seed and oil: a review of the contribution of their lignans. *Sierra Leone Journal of Biomedical Research, 2*, 4–16.

Kaushik, P., Mishra, A., & Malik, A. (2014). Dual application of agricultural residues for xylanase production and dye removal through solid state fermentation. *International Biodeterioration & Biodegradation, 96*, 1–8.

Lawrence, G. D. (2010). *The fats of life: Essential fatty acids in health and disease* (18th ed.). Canada: Rutgers University Press.

Li, T. S. C., Beveridge, T. H. J., & Drover, J. C. G. (2007). Phytosterol content of sea buckthorn (*Hippophae rhamnoides L.*) seed oil: extraction and identification. *Food Chemistry, 101*, 1633–1639.

Martins, S. L. C., Silva, H. F., Novaes, M. R. C. G., & Ito, M. K. (2004). Efeitos terapêuticos dos fitosteróis e fitostanóis na colesterolemia. *Archivos Latinoamericanos de Nutrición, 54*, 257–263.

Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T., & Andoh, T. (1999). Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. *Journal of Agricultural and Food Chemistry, 47*, 1749–1754.

Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S., Zhan, R., et al. (2011). Polyphenolic compounds and antioxidant properties in mango fruits. *Scientia Horticulturae, 129*, 102–107.

Miyahara, Y., Hibasami, H., Katsuzaki, H., Imai, K., & Komiya, T. (2001). Sesamolin from sesame seed inhibits proliferation by inducing apoptosis in human lymphoid leukemia Molt 4B cells. *International Journal of Molecular Medicine, 7*, 369–371.

Nascimento, R. J. S., Couri, S., Antoniassi, R., & Freitas, S. P. (2008). Fatty acids composition of açaí pulp oil obtained by enzymatic technology and hexane. *Revista Brasileira de Fruticultura, 30*, 498–502.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*, 3273–3279.

Rocha-Filho, P. A., Maruno, M., Oliveira, B., Bernardi, D. S., Gumiero, V. C., & Pereira, T. A. (2014). Nanoemulsions as a vehicle for drugs and cosmetics. *Nanoscience and Technology, 1*, 1–5.

Sankar, D., Sambandam, G., Rao, M. R., & Ali, A. (2011). Sesame oil exhibits synergistic effect with anti-diabetic medication in patients with type 2 diabetes mellitus. *Clinical Nutrition, 30*, 351–358.

Sankar, D., Sambandam, G., Rao, M. R., & Pugalendi, K. V. (2004). Sesame oil exhibits additive effect on nifedipine and modulates oxidative stress and electrolytes in hypertensive patients. *Journal of Nutraceuticals, Functional & Medical Foods, 4*, 133–145.

Santos, R. D., & Ferrari, R. A. (2005). Aqueous enzymatic of soybean oil. *Food Science and Technology, 25*, 132–138.

Sargi, S. C., Silva, B. C., Santos, H. M. C., Montanher, P. F., Boeing, J. S., Santos Júnior, O. O., et al. (2013). Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax, and perilla. *Food Science and Technology, 33*, 541–548.

Sarve, A., Sonawane, S. S., & Varma, M. N. (2015). Ultrasound assisted biodiesel production from sesame (*Sesamum indicum L.*) oil using barium hydroxide as a heterogeneous catalyst: comparative assessment of prediction abilities between response surface methodology (RSM) and artificial neural network (ANN). *Ultrasonics Sonochemistry, 26*, 218–228.

Schröder, M., & Vetter, W. (2012). Investigation of unsaponifiable matter of plant oils and isolation of eight phytosterols by means of high-speed counter-current chromatography. *Journal of Chromatography A, 1237*, 96–105.

Soto, C., Chamy, R., & Zúñiga, M. E. (2007). Enzymatic hydrolysis and pressing conditions effect on borage oil extraction by cold pressing. *Food Chemistry, 102*, 834–840.

Thomas, L., Larroche, C., & Pandey, A. (2013). Current developments in solid-state fermentation. *Biochemical Engineering Journal, 81*, 146–161.

Visentainer, J. V. (2012). Analytical aspects of the flame ionization detector response of fatty acid esters in biodiesels and foods. *Química Nova, 35*, 274–279.

Woyengo, T. A., Ramprasath, V. R., & Jones, P. J. H. (2009). Anticancer effects of phytosterols. *European Journal of Clinical Nutrition, 63*, 813–820.

Zanqui, A. B., Moraes, D. R., Silva, C. M., Santos, J. M., Gomes, S. T. M., Visentainer, J. V., et al. (2015). Subcritical extraction of flaxseed oil with n-propane: composition and purity. *Food Chemistry, 188*, 452–458.

Zhang, Y., Li, S., Yin, C., Jiang, D., Yan, F., & Xu, T. (2012). Response surface optimisation of aqueous enzymatic oil extraction from bayberry (*Myrica rubra*) kernels. *Food Chemistry, 135*, 304–308.