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

Suellen Andressa Oenning Ribeiro [a], Antonio Eduardo Nicacio [a], Ana Beatriz Zanqui [a], Polyana Batoqui França Biondo [a], Benício Alves de Abreu-Filho [b], Jesui Vergilio Visentainer [a], Sandra Terezinha Marques Gomes [a], Makoto Matsushita [a, *]

[a] State University of Maringa, Department of Chemistry, Colombo Avenue, 5790, 87020-900 Maringa, Parana State, Brazil
[b] State University of Maringa, Department of Biology, Colombo Avenue, 5790, 87020-900 Maringa, Parana State, Brazil

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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

* Corresponding author.
E-mail address: mmakoto@uem.br (M. Matsushita).

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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