Food Chemistry 120 (2010) 1041–1046

Contents lists available at ScienceDirect

Food Chemistry

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

Extraction, separation and characterisation of sesame oil lignan for nutraceutical applications

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ARTICLE INFO

Article history:
Received 27 March 2009
Received in revised form 17 October 2009
Accepted 23 November 2009

Keywords:
Sesamum indicum
Sesamin
Sesamolin
USM
Nutraceuticals

ABSTRACT

Nutraceutical aspects of sesame oil (SO) are well reported. However, an efficient process for commercial production has not yet been reported. In this study we have aimed at separating lignans from SO aiming at use as nutraceuticals. SO was subjected to sequential extraction with methanol under selected conditions of temperature (70 °C), time (100 min) and solvent:oil ratio (1:1). Under the optimised conditions, the yields of pooled methanolic extract concentrate and residual oil were 10.09 ± 1.0 g and 89.2 ± 1.0 g, respectively. On HPLC analysis, the methanol concentrate showed a total lignan content of 9.32 ± 0.19% (6.54 ± 0.12% sesamin and 2.78 ± 0.31% sesamolin). The concentrate was subjected to low temperature crystallization (4 °C) for the separation of lignan crystals and 51% of the lignans in the oil with 94.4% purity. The crystal-removed methanolic concentrate was saponified and purified; the total lignan content (sesamin and sesamolin) in the unsaponifiable matter (USM) was 64%.The distribution of sesamin and sesamolin in the purified USM was in the proportion 46:54, unlike that in the pure crystals (84:16). Lipid classes (triglycerides, TG; free fatty acids, FFA; diglycerides, DG; monoglycerides, MG; polar lipid, PL) in SO, methanolic extract concentrate and residual oil were separated using thin-layer chromatography (TLC). The amounts of lipid classes were determined by relating the total area of the fatty acid peaks to the area of the peak for internal standard (methyl heptadecanoate), using gas chromatography (GC). The process reported here describes a simple and less cumbersome procedure to produce lignans with high yield and purity for nutraceutical applications.

1. Introduction

Oil from sesame (*Sesamum indicum Linn.*), is markedly different from all other vegetable oil due to its high nutritional and therapeutic values and it is widely utilised in tropical and sub tropical regions. India and China are the major producers of sesame seed, contributing about 70% of the total world production of about 1.2 million metric tones (MMT). Sesame seed yields about 45–50% by weight of highly stable oil with a distinct flavour, and is widely used in Indian traditional medicine; "Ayurveda" and their medicinal applications are referred to in the traditional medical texts of India and China. Sesame oil (SO) is also used for culinary purposes and deoiled meal is mainly utilized as cattle feed. Several epidemiological and clinical studies have indicated a strong positive association of intake of dietary and non-dietary phytochemicals and health. In this context, several plant-based nutraceuticals are developed with health-protective roles. Plant materials that have been consumed as part of the diet or traditional medicines would require less rigorous screening protocols and can be used for nutraceutical development. Sesame seed/SO, from this perspective, is a rich source of lignans, known for a variety of biological activities and has been used as food or medicine for ages by a large section of the population. Recent studies, using modern methods, have revealed potential health benefits of sesame, such as antioxidative (Shahidi, Liyana-Pathirana, & Wall, 2006) antihypertensive (Sankar, Sambandam, Rao, & Pugalendi, 2005) hypocholesteremic (Hirose et al., 1991), anticancer (Miyahara, Hibasami, Katsuzaki, Imai, & Komiya, 2001) and immunoregulation (Nonaka, Yamashita, Izuka, & Namiki, 1997). Beneficial health effects of sesame are primarily attributed to lignans and their glycosides. Lignans are a class of secondary plant metabolites produced by oxidative dimerization of two phenyl propanoid units. The main lignans in SO are sesamin and sesamolin and are reported to be in the concentration range 1.0–2.0%. They account for about 10% of the unsaponifiable matter (USM) in SO (Hemalatha & Ghafoorunissa, 2004).

Perusal of the earlier reports related to recovery of lignans from sesame seed/SO would reveal that none of them were industrially

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doi:10.1016/j.foodchem.2009.11.047

feasible and economical. Shinmen et al. (1993) used an immiscible organic solvent for extracting the lignans from sesame and obtained a lignan content of only 32.23% in the extract, based on total lignans in oil. They also tried supercritical extraction of oil and also different chromatographic techniques for isolating desired lignans from the extract. However, the yields and purities by these techniques have not been reported. Namiki, Kobayashi, and Hara (2001) subjected SO to supercritical extraction and the reported % yield of extracts ranged from 21% to 24%; the lignan content in the extract was only 1.5–3.5%. Akimoto, Shinmen, Yamada, Shimizu and Sugano (1994) extracted SO using organic solvent and administered it to animals and reported that the composition showed inhibitory effects and could be used in treatment of inflammation, thrombosis or hypertension. However, the yield and purity were not reported. Similarly, other techniques have also been tried, wherein the oil was subjected to liquid extraction, followed by saponification and precipitation at 4 °C; again the yield of extraction was very poor (Dachtler, VandePut, Stijn, Beindorff, & Fritsche, 2003). The objective of the present work was to develop a commercially feasible and economical process to produce a range of products containing lignans of 60–95% purity for nutraceutical applications with emphasis on maximising recovery of the oil for reuse.

2. Methods

2.1. Raw material and chemicals

White SO was supplied by M/s. Arjuna Natural Extracts Limited (Aluva, Kerala, India). Standards of fatty acids methyl esters (FAME), methyl heptadecanoate, sesamin and sterols (Stigmasterol, campesterol and β-sitosterol) were purchased from Sigma (St Louis, MO, USA). γ-Tocopherol was purchased from Merck (Darmstadt, Germany). HPLC-grade solvents from Merck (Darmstadt, Germany) were used for HPLC analysis. All other chemicals were of laboratory grade from Ranbaxy (New Delhi, India).

2.2. Experimental apparatus and extraction procedure

Hundred grams of SO were mixed with methanol in different ratios (1:1, 1:2 and 1:0.5 w/v), and was then placed in an extraction vessel (1 l) consisting of a three necked flask equipped with a motor-driven stirrer, reflux condenser, thermometer assemblage and heating mantle. The mixture of SO and methanol, in different ratios, as described, was then subjected to continuous stirring at different extraction temperatures (50, 60 and 70 °C) for 10 min, after which the mixture temperature was lowered to 50 °C. The mixture was then transferred into a separating funnel and, after 15 min of settling time, the methanol extract and residual oil were separated. The separated residual oil from the first extraction was stripped of solvent and subjected to a second extraction with a fresh batch of methanol, as has been described and, likewise, 10 sequential extractions were performed. The methanolic extracts separated from the 10 sequential extractions were pooled and stripped of solvent using a flash evaporator to obtain the methanolic extract concentrate. The resultant residual oil from the 10 sequential extractions was also concentrated and yields were determined by gravimetry. The sequential extraction process was repeated with three batches of fresh SO at an oil to solvent ratio of 1:1(w/v) and temperature of 70 °C.

2.3. Crystallization of lignans from methanolic extract concentrate

The methanolic extract concentrate, as obtained above, was dispersed in petroleum ether (1:0.5 w/v) and the mixture was subjected to cryoscopic temperature conditons of 4 to 10 °C for a time duration of 24–48 h in order to facilitate crystallization of the lignans. The lignan crystals were separated from the mixture by vacuum-filtration, washed with chilled petroleum ether until oil-free, dried in a vacuum oven at a temperature below 60 °C for 1 h and weighed.

2.4. Separation of lignans from methanolic extract concentrate by saponification

Saponification of the crystal-removed methanolic extract was carried out by adding (1:1 w/v) of potassium hydroxide (KOH) in water (60:40 w/v) and ethanol (1:6 v/v) to the extract and then refluxing it in a boiling water bath for 1 h. After completion of saponification, water (1:4 v/v) was added to the mixture which was then extracted with petroleum ether (1:10 v/v) six times, each time separating the petroleum ether phase. The separated petroleum ether fractions from the six extractions were pooled and then washed with 10% ethanol until alkali-free. The combined alkali-free petroleum ether extract was flash-evaporated under reduced pressure and then dried in a vacuum oven at a temperature below 60 °C for 30 min to 1 h to get the USM.

2.5. Purification of USM

Purification of USM was carried out by washing it with petroleum ether (1:0.7 w/v) 10 times, followed by vacuum-filtration to remove the impurities and it was then dried in a vacuum oven below 60 °C for 1 h to obtain the purified USM.

2.6. Analytical methods

2.6.1. Lignans, tocols and sterols

Lignans (sesamin and sesamolin), tocol and sterols analyses were performed with a Shimadzu HPLC (Kyoto, Japan) with LC-10 AD model pump, a 7125 model Rheodyne injector (Cotati, CA) fitted with a 20 μl sample loop, and a SPD-10 A ultraviolet (UV)–visible detector. The peaks were recorded using a C-R7Ae plus integrator. Reverse-phase HPLC equipped with a Luna 5 μm C 18 (2) column (250 × 4.6 mm), was used for the analysis of lignans in samples (oil, methanol extract concentrate, residual oil, crystal and USM). Lignans were separated using methanol/water (70:30 v/v) at a flow rate of 1 ml/min. The UV detector was set at 290 nm (Hemalatha & Ghafoorunissa, 2004). Quantitation of separated peaks was done by calibration with standard sesamin. The peak identified at a retention time of 12.9 min was confirmed as sesamin in comparison with standard sesamin, and that at a retention time of 17.3 min was confirmed as sesamolin, based on earlier reports, and was quantitated using the response factor of sesamin. For the analysis of tocols, a Phenomenex–NH_2 (M) column (25 cm × 4.6 mm id × 5 μm, Kyoto, Japan) was used in the normal phase with the solvent system *n*-hexane/isopropanol (92:8 by vol) at a flow rate of 1 ml/min. The UV detector was set at 297 nm (Renuka Devi, Suja, Jayalekshmy, & Arumughan, 2000) and the peaks were identified and quantitated using tocol standards. For the analysis of sterols, a reverse-phase Zorbax ODS column (25 cm × 4.6 mm id × 5 μm) was used with solvent system methanol/water (96.5:3.5 by vol) at a flow rate of 1.2 ml/min. The UV detector was set at 206 nm (Holen, 1985) and the separated peaks were identified and quantitated using sterol standards.

2.6.2. Separation of lipid classes

About 20 mg of SO, methanolic extract concentrate and residual oil were spotted and separated into triacylglycerols (TG), free fatty acid (FFA), diacylglycerol (DAG), monoacylglycerol (MG) and polar lipid (PL) classes by thin-layer chromatography (TLC) on a 1 mm