Journal of Chromatography B, 1011 (2016) 108–113

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Preparative separation of sesamin and sesamolin from defatted sesame meal *via* centrifugal partition chromatography with consecutive sample injection

Je-Seung Jeon [a], Chae Lee Park [a], Ahmed Shah Syed [a], Young-Mi Kim [a], Il Je Cho [b], Chul Young Kim [a,*]

[a] College of Pharmacy and Institute of Pharmaceutical Science and Technology, Hanyang University, Ansan, Gyeonggi-do 15588, Republic of Korea
[b] College of Korean Medicine, Daegu Haany University, 1, Hannydaero, Gyeongsan, Gyeongsangbukdo 38610, Republic of Korea

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form
28 December 2015
Accepted 30 December 2015
Available online 4 January 2016

Keywords:
Centrifugal partition chromatography
Consecutive sample injection
Sesamin
Sesamolin
Antioxidant response element

ABSTRACT

A preparative separation method using consecutive sample injection centrifugal partition chromatography (CPC) was developed to obtain sesamin and sesamolin from defatted sesame meal extracts. A two-phase solvent system consisting of *n*-hexane–ethyl acetate–methanol–water (8:2:8:2, v/v) was applied in reversed-phase mode (descending mode). Preliminary experiments with an SCPC-100 (column volume: 100 mL) were performed to select the appropriate two-phase solvent system and sample injection times; these parameters were then used with an SCPC-1000 (column volume: 1000 mL) in a 10-fold scale-up preparative run. A sample containing 3 g of crude extract was consecutively injected four times onto the SCPC-1000, which yielded 328 mg of sesamin and 168 mg of sesamolin. These compounds were analyzed by high-performance liquid chromatography and determined to have purities of 95.6% and 93.9%, respectively. Sesamin and sesamolin (30 μM) increased antioxidant response element (ARE) luciferase activity 2.6-fold and 1.9-fold, respectively.

1. Introduction

Sesamin and sesamolin (Fig. 1), two major components of sesame, exert many health-promoting effects, such as antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive and neuroprotective activities [1]. Sesamin has also been shown to prolong the mean life span of fruit flies by upregulating superoxide dismutase, catalase, *methuselah* and Rpn11 expression [2]. Recently, a mixture of *Schisandra* fruit extract and sesamin was also shown to improve human liver function [3] and sesamin exerted hepatoprotective properties against CCl_4 induced oxidative stress-mediated apoptosis in mice *via* JNK pathway [4].

Defatted sesame meal after oil extraction is mainly used as a feed ingredient for domestic animals or is composted. Antioxidant components have been reported to be present mainly in the hull of the sesame seed [5]. Moreover, sesame oil extracted from seeds with hulls has been shown to be more stable than that extracted from dehulled seeds, and the sesame coat also showed significant antioxidant activity in various *in vitro* systems [6], indicating that antioxidant components may exist in the sesame hull. In addition, sesame cake extract contained considerable amount of sesamin and sesamolin [7]. Thus, defatted sesame meal is an ideal source for the isolation and purification of sesamin and sesamolin. Furthermore, as sesame and its major lignans, sesamin and sesamolin, exerted antioxidant activities *in vitro* or *in vivo* [4,6], their ability to induce antioxidant response element (ARE) were evaluated.

Centrifugal partition chromatography (CPC), an efficient tool for the separation and purification of constituents from natural products, is a solid-free separation technique based on continuous liquid–liquid partitioning. Compared with high-performance liquid chromatography (HPLC), CPC offers many advantages such as a higher sample loading capacity, the absence of sample loss due to irreversible sample absorption to the solid column, a flexible operation mode and the choice of a wide range of solvent systems [7–11]. In previous studies, sesamin and sesamolin have usually been extracted from sesame seeds or oil, and then separated by conventional purification methods such as alumina, macroporous resins, RP-18, or a combination of saponification and fractional crystallization methods [12–14]. Although high-speed counter-current chromatography (HSCCC) has also been used to purify sesamin and sesamolin from sesame seeds, this process was time-consuming and only yielded product on the milligram scale [15]. Thus, this

* Corresponding author. Fax: +82 31 400 5958.
E-mail address: chulykim@hanyang.ac.kr (C.Y. Kim).

http://dx.doi.org/10.1016/j.jchromb.2015.12.062
1570-0232/

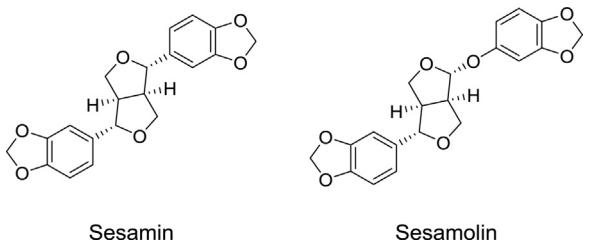

Fig. 1. Chemical structures of sesamin and sesamolin.

process requires consecutive sample injection and a scale-up process to obtain an adequate amount of target compound, making repeated sample injection in CPC less convenient than in HPLC. To date, repeated sample injections within a single run in CPC operation have been reported several times [16–21]. However, amount of injection sample was small [17] or interval for another injection time was too long [20,21] to purify large amount of target compounds. Multiple dual-mode CPC developed for only semi-purified extract or mixtures of standard compounds [18,19].

In this study, we report a consecutive sample injection CPC protocol developed for isolation and purification of sesamin and sesamolin from defatted sesame meal. The resultant method is simple and efficient for obtaining a large amount of pure target compounds. We also confirm the efficacy of the compounds isolated by antioxidant response element (ARE) activation in HepG2 cells.

2. Materials and methods

2.1. Apparatus

Centrifugal partition chromatography was performed on an Armen fully integrated SCPC-100 + 1000CPC spot instrument (Armen Instrument, St-Ave, France). This instrument is a fully automated system consisting of a CPC column compartment, a pump, an injector, a UV/vis detector, a fraction collector, a digital screen flat PC, and Armen Glider CPC software (Armen Instrument). The analytical Agilent 1260HPLC system (Agilent Technologies, Palo Alto, CA, USA) consisted of a G1312C binary pump, a G1329B autosampler, a G1315D DAD detector, a G1316A column oven and ChemStation software.

2.2. Reagents and materials

All HPLC-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA). The organic solvents used for extraction and CPC operation were obtained from Daejung (Gyeonggi-do, Korea). The NMR solvent ($CDCl_3$) was purchased from Cambridge Isotope Laboratories, Inc. (USA). Defatted sesame meal was obtained at a local market in Ansan, Gyeonggi-do, Korea.

Table 1
Partition coefficients (K) of sesamin and sesamolin in different solvent systems.

Solvent system (v/v)	Partition coefficient (K)		Separation factor (α)[a]
n-Hexane/ethyl acetate/methanol/water	Sesamin	Sesamolin	
9:1:9:1	0.20	0.26	0.30
8:2:8:2	0.44	0.62	1.41
7:3:7:3	0.77	1.12	1.45
7:3:6:4	1.53	2.19	1.43
6:4:6:4	1.92	2.85	1.48
7:3:5:5	7.47	13.0	1.74
6:4:5:5	7.85	12.94	1.65

[a] $\alpha = K_2/K_1$, $K_2 > K_1$.

2.3. Preparation of defatted sesame meal extracts

Defatted sesame meal (1.16 kg) was extracted three times with methanol (3 L) using an ultrasonic apparatus for 3 h at room temperature and then concentrated *in vacuo* to yield 68.2 g of crude extract and stored in a refrigerator (4 °C) for CPC separation.

2.4. HPLC analysis of crude extracts

All crude extracts and CPC peak fractions were analyzed by HPLC. HPLC analysis was performed using an Agilent 1260 HPLC system equipped with an Inno C18 column (250 mm × 4.6 mm, 5 μm, YoungJin Biochrom Co., Ltd., Seongnam, Korea). The flow rate was maintained at 1 mL/min for the mobile phase, and was controlled by binary pumps at 40 °C. The mobile phase was a linear gradient of acetonitrile with 0.1% formic acid (A) and water with 0.1% formic acid (B). The gradient extended from 0 to 20 min and consisted of 40–100% A. The resultant effluent was monitored at 280 nm; the UV spectrum range of the effluent was 200–400 nm.

2.5. Preparation of the two-phase solvent system

A two-phase solvent system was determined according to the appropriate partition coefficients ($0.5 < K < 2.0$) of the target compounds in a series of solvent systems of pre-equilibrated *n*-hexane–ethyl acetate–methanol–water. The K values were calculated by HPLC analysis. Briefly, approximately 2 mg of sample was added to each test tube, 2 mL of each phase of a pre-equilibrated two-phase solvent system was added, and the resultant mixture was thoroughly mixed. Each test tube was rigorously shaken for several minutes and then allowed to attain equilibrium. Then, 20 μL of the upper and lower phases were analyzed by HPLC at 280 nm. The K value was defined as the peak area of each compound in the upper phase divided by the peak area of the lower phase.

2.6. Scale-up and development of the consecutive sample injection procedure for CPC separation

Preliminary CPC operation was performed using the SCPC-100. According to the appropriate K values, CPC separation was performed with a two-phase solvent system consisting of *n*-hexane–ethyl acetate–methanol–water with a volume ratio of 7:3:7:3 (v/v) or 8:2:8:2 (v/v).

The CPC channel was first filled entirely with the upper organic layer as the stationary phase. Then, the rotor was set at 2500 rpm and the lower aqueous phase was pumped into the channel at a flow rate of 1 mL/min, with the instrument set in descending mode. After equilibrium was reached, defined as the elution of the mobile phase from the outlet (70 mL of the stationary upper phase was eluted, at a pressure of 80 bar), the sample solution (500 mg of sample in 4 mL of a mixture of the upper and lower phases) was injected. The resultant eluate was monitored at 254 and 280 nm with a UV detector (flow rate: 10 mL/min), and each fraction was collected with a fraction collector. Scale-up of SCPC-1000 separation was based on the SCPC-100 results. The SCPC-1000 conditions were as follows: flow rate, 10 mL/min; rotation speed, 1200 rpm. For scale-up, sample solution (3 g of sample in 16 mL of a mixture of the upper and lower phases) was injected into the instrument, and the UV detector was set to 254 and 280 nm. After optimizing conditions for the SCPC-1000, four repeated sample injections were performed according to the anticipated elution times of sesamin and sesamolin. Sasamin