Food Chemistry 136 (2013) 342–347

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Analytical Methods

Separation and purification of sulforaphene from radish seeds using macroporous resin and preparative high-performance liquid chromatography

Pengqun Kuang, Dan Song, Qipeng Yuan *, Rui Yi, Xinhua Lv, Hao Liang

State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, China

ARTICLE INFO

Article history:
Received 24 February 2011
Received in revised form 5 May 2012
Accepted 31 August 2012
Available online 13 September 2012

Keywords:
Radish seeds
Sulforaphene
Macroporous resin
Preparative HPLC
Separation
Purification

ABSTRACT

This present study described a rapid and cost-effective method for the separation and purification of natural sulforaphene from radish seeds by SP-700 macroporous resin and preparative high-performance liquid chromatography (HPLC). Sulforaphene with high purity and recovery was obtained by preparative HPLC with a C18 column and 30% methanol in ultra-pure water as the mobile phase. 12.5 kg of radish seeds, which contained 87.5 g of sulforaphene, produced 117.5 g of sulforaphene-rich extract of 65.8% sulforaphene after primary separation by SP-700 macroporous resin. 5.9 g of 96.5% sulforaphene was obtained from 9.5 g of the sulforaphene-rich extract after purification by preparative HPLC. The purified compound was assessed by analytical HPLC and characterised by ESI/MS, ^{1}H NMR and ^{13}C NMR. Standard curve was developed using the purified sulforaphene to allow quantification of sulforaphene in the extracts of radish seeds by analytical HPLC.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Epidemiological studies indicate that diet rich in fruit and vegetables, especially cruciferous vegetables such as broccoli (*Brassica oleracea* var. *italica*), cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var *acephala*), cauliflower (*Brassica oleracea* var. *botrytis*), and radish (*Raphanus sativus*), is associated with a decreasing risk of developing many cancers and cardiovascular diseases (Higdon, Delage, Williams, & Dashwood, 2007; Joseph et al., 2004; Lam et al., 2010; Lee et al., 2006; Truong, Baron-Dubourdieu, Rougier, & Guenel, 2010). This chemoprotective effect is related to isothiocyanates, a type of hydrolysis products of glucosinolates, which are relatively unique into cruciferous vegetables (Beevi, Mangamoori, Subathra, & Edula, 2010; Force, O'Hare, Wong, & Irving, 2007; O'Hare et al., 2009; Williams, Critchley, Pun, Chaliha, & O'Hare, 2010). When cruciferous vegetables are ground or chopped, glucosinolates are hydrolyzed by myrosinase enzyme (β-thioglucoside glucohydrolase, EC3.2.3.1) to a variety of biological products such as isothiocyanates, thiocyanates, nitriles, oxazolidine-thiones and epithionitriles (Bones & Rossiter, 2006; Vaughn & Berhow, 2005).

Radish sprouts have been shown to be capable of inducing the phase II enzyme, quinone reductase, *in vitro* in murine hepatoma cell line (Lee & Lee, 2006; O'Hare et al., 2009), and activating the antioxidant response element in a stably transfected hepatoma cell line (Hanlon & Barnes, 2011). These biological activities have been remarkably attributed to the radish sprouts' glucosinolate composition. Radish sprouts contain glucoraphenin, a glucosinolate that can be hydrolyzed to form sulforaphene. Sulforaphene, possessing a double bond in the alkyl chain (Fig. 4), has been identified as being particularly potent in its ability to induce the phase II enzymes (Posner, Cho, Green, Zhang, & Talalay, 1994), similar to sulforaphane hydrolyzed from glucoraphanin (Zhang, Talalay, Cho, & Posner, 1992). Sulforaphene has been shown to be capable of inhibiting the proliferative growth of human and murine erythroleukemic cells, human T-lymphoid cells, human cervix carcinoma cells and H3-T1-1 cells (Nastruzzi et al., 1996, 2000). In recent years, it has been reported that sulforaphene could reduce cell proliferation in a dose-dependent manner and induce apoptosis in LoVo, HCT-116, and HT-29 colon carcinoma cell lines (Barillari et al., 2008; Papi et al., 2008). And sulforaphene was found to be 1.3–1.5 times stronger than sulforaphane for the *in vitro* antimutagenicity activities in the TA100 strain in the presence of Aroclor 1254-induced rat liver S9 (Shishu & Kaur, 2009).

Although there are some publications reported on biological activities mentioned above, sulforaphene has yet not been studied actively on its potential in the field of preventing cancers and other diseases because of the high price of pure sulforaphene. As a result, in order to carry out studies evaluating on the biological activities of sulforaphene in animal or clinical trials, it is necessary to obtain considerably large amounts of highly purified sulforaphene for

* Corresponding author. Address: State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, No. 15 Beisanhuan East Road, Beijing 100029, China. Tel./fax: +86 10 6443 7610.
E-mail address: yuanqp@mail.buct.edu.cn (Q. Yuan).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.08.082

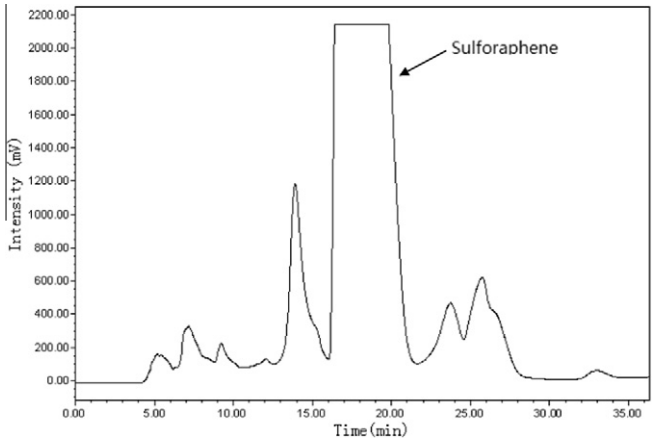

Fig. 1. The preparative HPLC chromatogram of the sulforaphene-rich extract. The preparative HPLC column was a reversed phase C18 column (19 × 300 mm, 7 μm, Symmetry Prep™). The mobile phase was 30% methanol in ultra-pure water. The flow rate was 10 ml/min. And the detection wavelength was 254 nm. Two hundred and fifty milligrams of the sulforaphene-rich extract separated by resin-based column chromatography was dissolved in 10 ml of the mobile phase, and then filtered with a 0.22 μm membrane filter in preparation for further purification by preparative HPLC.

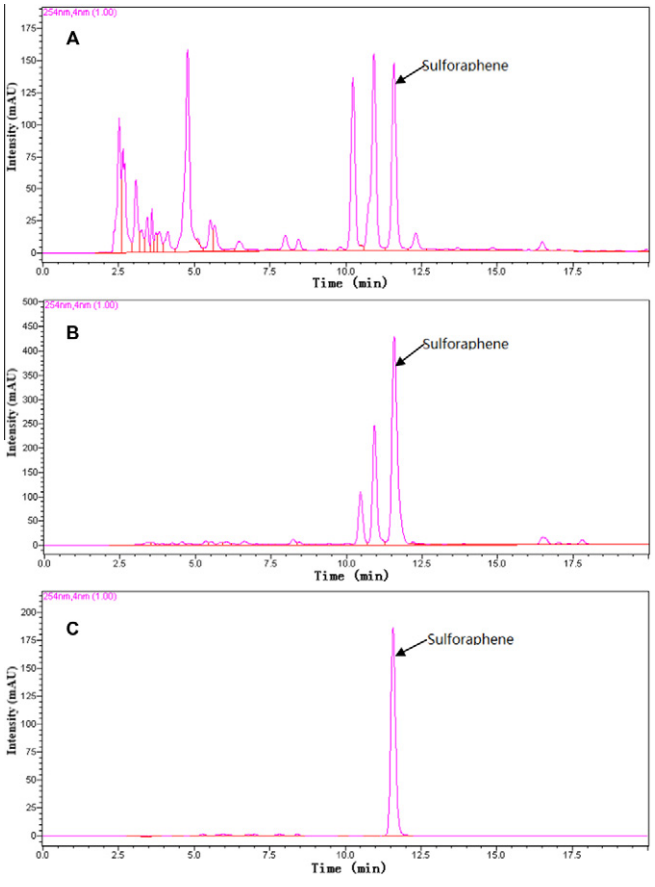

Fig. 2. The analytical HPLC chromatograms of sulforaphene product obtained by (A) crude extraction, (B) SP-700 macroporous resin-based column chromatography and (C) preparative HPLC. The analytical HPLC column was a reversed phase C18 column (4.6 × 250 mm, 5 μm, Diamodsil™). The mobile phase system consisted of acetonitrile as mobile phase A and 0.02% (v/v) TFA in ultra-pure water as mobile phase B. Firstly, the solvent system consisted of 20% A in B, then changed linearly over 20 min to 40% A, and then raised to 100% A immediately and maintained for 2 min to purge the column. The flow rate was 1.0 ml/min. The column oven temperature was 30 °C. And the detection wavelength was 254 nm.

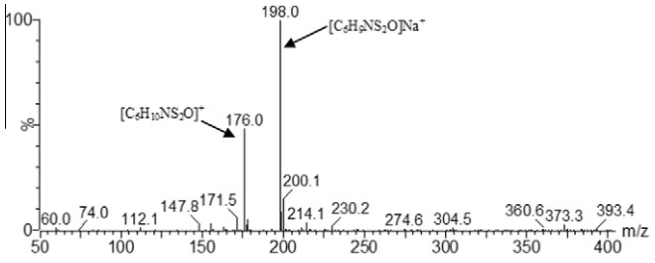

Fig. 3. The ESI/MS spectrum of the sulforaphene product (M+H: *m*/*z* 176.0, M+Na: *m*/*z* 198.0) purified by preparative HPLC.

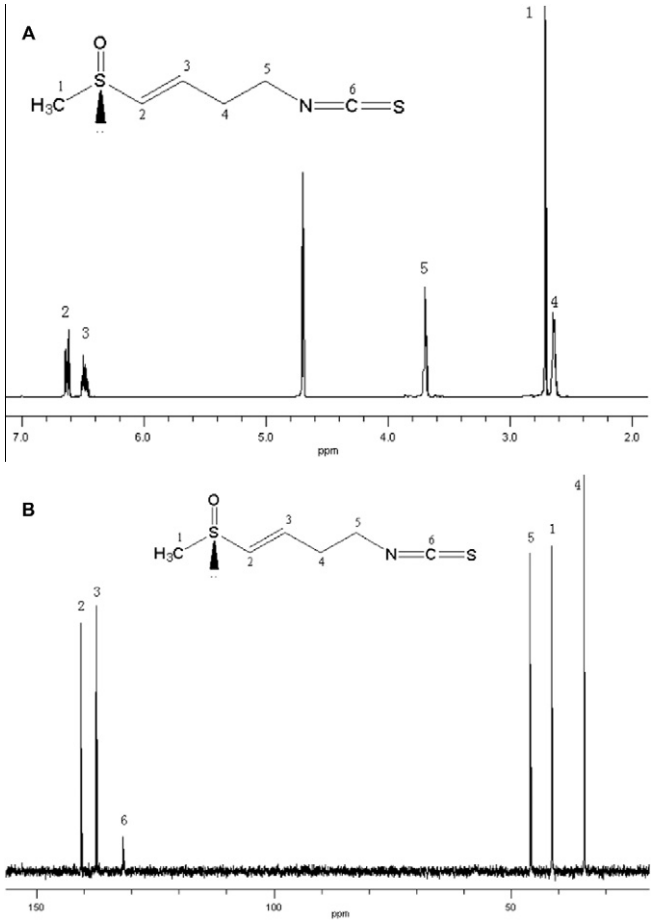

Fig. 4. The NMR spectra (D_2O, 600 MHz) of the sulforaphene product purified by preparative HPLC: (A) ^1H NMR spectrum and (B) ^{13}C NMR spectrum.

experimental purposes. But to the best of our knowledge, there are few publications focusing on the method for separation and purification of sulforaphene.

The goal of this research was to develop a new and economical method for the separation and purification of sulforaphene from radish seeds using macroporous resin-based column chromatography and preparative high-performance liquid chromatography, and to establish a fast and low-cost method for quantification of sulforaphene in the extracts of radish seeds using an analytical HPLC system. The chemical structure of the purified compound was verified by ESI/MS, ^1H NMR and ^{13}C NMR analysis.

2. Experimental

2.1. Chemicals and reagents

Radish seeds were purchased from Beijing TongRenTang Co. Ltd., (Beijing, China). Sinigrin standard with purity of more than

Download English Version:

https://daneshyari.com/en/article/10540514

Download Persian Version:

https://daneshyari.com/article/10540514

Daneshyari.com