Separation and Purification Technology 174 (2017) 338–344

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Enhancing the recovery of cabbage glucoraphanin through the monitoring of sulforaphane content and myrosinase activity during extraction by different methods

Patsaporn Pongmalai [a], Sakamon Devahastin [a,*], Naphaporn Chiewchan [a], Somchart Soponronnarit [b]

[a] Advanced Food Processing Research Laboratory, Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, 126 Pracha u-tid Road, Tungkru, Bangkok 10140, Thailand

[b] Division of Energy Technology, School of Energy, Environment and Materials, King Mongkut's University of Technology Thonburi, 126 Pracha u-tid Road, Tungkru, Bangkok 10140, Thailand

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 26 October 2016
Accepted 4 November 2016
Available online 5 November 2016

Keywords:
Enzymatic hydrolysis
Glucosinolates
Microwave
Steaming
Ultrasound
Temperature evolution

ABSTRACT

Since endogenous hydrolysis of glucoraphanin into sulforaphane is inefficient and difficult to control, a means to effectively extract glucoraphanin from its source, so that exogenous conversion can be later performed, is desired. In this study, selected extraction methods, i.e., combined ultrasound-assisted extraction and microwave-assisted extraction (UAE + MAE), vacuum microwave-assisted extraction (VMAE), UAE + VMAE and Soxhlet extraction, with water as an extraction solvent, were employed to extract glucoraphanin from cabbage outer leaves, with the aim to enhance the extraction yield and at the same time minimize the loss of the compound. Use of fresh versus steamed leaves for the extraction was also compared. Evolutions of the cabbage-water temperature as well as glucoraphanin and sulforaphane contents of the extracts along with the myrosinase activity were monitored. UAE + VMAE led to a significantly higher content of glucoraphanin than UAE + MAE and VMAE; Soxhlet extraction resulted in a lower glucoraphanin content and required a much longer time. Nevertheless, UAE + VMAE could not rapidly inactivate myrosinase in the fresh leaves, resulting in some conversion of glucoraphanin into sulforaphane, which suffered degradation during the extraction. UAE + VMAE of steamed cabbages proved to yield the highest maximum content of glucoraphanin, with the value of 650.09 μmol/100 g dry weight, 87% higher than that obtained via the use of the fresh cabbages in combination with UAE + VMAE. Simple kinetic modeling of the extraction processes was also attempted.

1. Introduction

Glucosinolates are a group of sulfur-containing plant secondary metabolites that occur in *Brassica* vegetables [1]. These compounds are naturally stable but biologically inactive. Glucosinolates can be divided into three classes based on the structure of different amino acid precursors, including aliphatic, aromatic and indole glucosinolates [2]. Glucoraphanin is well recognized as an aliphatic glucosinolate found in *Brassica* vegetables. When plant tissues are damaged, glucoraphanin is hydrolyzed by endogenous myrosinase, which is located in plant myrosin cells, to form sulforaphane [3].

Sulforaphane is an isothiocyanate derivative formed from glucoraphanin and is well known to possess high anticarcinogenic activity [4,5]. Many studies have therefore been devoted to the production and extraction of this compound from its source [6–8]. It is reported, however, that endogenous myrosinase is typically inefficient in converting glucoraphanin into sulforaphane. Jeffery and Stewart [9], for example, reported that endogenous myrosinase in homogenized broccoli could convert only 25% (or lower) of glucoraphanin into sulforaphane. This implies that direct intake of *Brassica* vegetables may not be sufficient to deliver the intended health benefits as glucoraphanin is still in its native form. Extraction of glucoraphanin from its source and addition of exogenous myrosinase to hydrolyze glucoraphanin into sulforaphane in a more effective and controllable manner should therefore be conducted.

To achieve the aforementioned goal, evolutions of the glucoraphanin and sulforaphane contents as well as myrosinase activity during extraction need to be monitored. This information can be used to design an effective means to extract glucoraphanin without causing much degradation to the compound, either by thermal degradation or unintentionally hydrolyzing it into sulforaphane, which is very heat-sensitive and hence easily degradable and not

* Corresponding author.
E-mail address: sakamon.dev@kmutt.ac.th (S. Devahastin).

http://dx.doi.org/10.1016/j.seppur.2016.11.003
1383-5866/

recoverable. Studies indeed exist on this kind of information. Ghawi et al. [10], for example, investigated the effect of thermal treatment (at 30–100 °C) on the changes of glucoraphanin and sulforaphane contents of broccoli; myrosinase activity was also determined. Thermal treatment at 80 °C for 4, 8 and 12 min led to an increase in the glucoraphanin content in broccoli. However, glucoraphanin degraded at 100 °C due to thermal degradation. Myrosinase activity remained rather constant when mild-heat treatment at 50 °C for 4 min was applied. A sudden decrease in the activity nevertheless occurred at higher temperatures (50–100 °C), leading to a lower sulforaphane content in boiled broccoli. Pérez et al. [7] later reported that myrosinase activity remained even after higher-temperature blanching (70–74 °C). Although the hydrolysis of glucoraphanin at a higher temperature (70–74 °C) took place, leading to sulforaphane formation, the content of this latter compound was very low due to its high thermal sensitivity. No information is so far available, however, on the evolutions of glucoraphanin and sulforaphane contents as well as myrosinase activity during extraction.

In terms of extraction, a number of advanced techniques have been applied to extract plant bioactive compounds from their sources. Ultrasound-assisted extraction (UAE) has recently received much attention due to its ability to modify plant structure via acoustic cavitation; disruption of plant cell walls results in enhanced release of internal cell compounds into an extraction solvent [11,12]. Microwave-assisted extraction (MAE) is also of interest as it helps induce rapid heating within plant cells, leading to disruption of cellular structure and hence increased extraction yield [8,13]. Pongmalai et al. [12] indeed combined UAE and MAE to enhance the extraction of bioactive compounds from cabbages. UAE + MAE was noted to result in higher contents of extractable bioactive compounds than either UAE or MAE alone; the enhancement is due to the combined effect of acoustic cavitation and rapid heating within the plant cells by microwave irradiation. On the other hand, Hiranvarachat et al. [14], who monitored the evolutions of extractable triterpene saponins and phenolics contents from *Centella asiatica* leaves during MAE and vacuum microwave-assisted extraction (VMAE), confirmed the benefit of VMAE over MAE, if the former is operated at an optimum condition. VMAE outperformed MAE due to the ability of the former to perform extraction at a lower temperature as a result of the reduced boiling point of an extraction solvent at a lower pressure.

In addition to their ability to enhance extraction, ultrasound and microwave have noted to help inactivate some enzymes during processing. Benlloch-Tinoco et al. [15], for example, studied the effect of microwave on the activity of enzyme in kiwifruit puree and found that microwave heating at a higher power with a shorter treatment time was more effective in decreasing the enzyme activity than heating in a water bath. Lopes et al. [16] confirmed that microwave treatment led to a more extensive inactivation of horseradish peroxidase than heating in a thermostatic bath. This is because the functional groups of the enzymes directly absorb microwave irradiation, leading to rapid heating and hence the destruction of the enzyme structure. In terms of the ability of ultrasound to inactivate enzymes, Cheng et al. [17] observed the ability of ultrasound to help inactivate polyphenol oxidase (PPO) in mushroom (*Agaricus bisporus*) during thermal processing. More recently, Sulaiman et al. [18] also observed the effect of ultrasound on PPO inactivation in fruit purees; ultrasonication is effective in reducing the PPO activity due to the formation and collapse of microbubbles that affect the enzyme structure.

Based on the above-mentioned arguments, it is interesting to determine if UAE + MAE, VMAE and UAE + VMAE can be used to extract glucoraphanin and at the same time inactivate myrosinase, so negligible hydrolysis of this compound into sulforaphane, which is very easily degradable and thus difficult to retain, would take place. Such one-step procedure, if successfully validated, could help reduce the complexity, time and energy consumption of the whole process as no additional step to inactivate the native enzyme would be required prior to the extraction. Cabbage outer leaves, which are low-value residues of the vegetable processing industry but contain a significant amount of glucoraphanin, were used as the test raw material. For comparison, steamed cabbage leaves, which no longer contained myrosinase of any significant activity, were allowed to undergo the extraction. The results obtained via Soxhlet extraction were also collected and compared.

2. Materials and methods

2.1. Materials

Outer leaves of cabbage (*Brassica oleracea* L. var. capitata) were obtained from Pakklong Talad market in Bangkok; the leaves were kept at 4 °C until the time of an experiment. Before starting of each experiment, the leaves were washed with tap water and drained on a screen to get rid of excess water. The leaves were then chopped with an electric chopper (Moulinex, DPA141, Écully, France) for 2 min to obtain an average size of cabbages of 1.7–2.5 mm. The chopped cabbages were immediately introduced to an extraction process.

2.2. Methods

2.2.1. UAE, MAE and VMAE

Five g of chopped cabbages was dispersed in 50 mL of deionized water (DI water), which was used as an extraction solvent, in a 250-mL beaker; the whole content was then placed in an ultrasonic bath (Elma, Elmasonic P, Singen, Germany) containing 1 L of distilled water. UAE was performed by sonicating the mixture of cabbages and DI water at a frequency of 37 kHz and a set power of 320 W (or absorbed ultrasonic power of 0.03 W/g of the mixture of cabbages and DI water) for 40 min; this condition was selected from the preliminary experiments as the value that led to the highest extractable glucoraphanin content (data not shown).

For combined extraction methods, i.e., UAE + MAE and UAE + VMAE, an ultrasonically treated mixture was placed in a round-bottom flask and then subject to microwave irradiation at 180 W. MAE and VMAE were performed in the same domestic microwave oven (Samsung, GE-872D, Port Klang, Malaysia), which was modified as detailed in Hiranvarachat et al. [19]. The microwave power of 180 W was selected to prevent excessive boiling of the extraction solvent at the tested vacuum pressure [9]. VMAE was conducted at an absolute pressure of 70 kPa as this pressure led to the highest recoverable glucoraphanin content in comparison with those at the other tested pressures, i.e., 30 and 50 kPa (data not shown). The duration of both MAE and VMAE was maintained at 10 min; this time was selected to deliberately extend the extraction to the point where myrosinase was completely inactivated as will be later discussed. VMAE without the prior use of UAE was also conducted; a sample was prepared in the same manner and subject directly to VMAE.

The temperature evolution of the cabbage-water mixture during UAE was measured using a type-T thermocouple, which was inserted into a beaker containing the mixture and connected to a data logger (Yokogawa, DX112, Tokyo, Japan). A fiber-optic thermometer (Luxtron, m600, Santa Clara, CA) was used to monitor the mixture temperature during MAE and VMAE; the thermometer was placed in the center of the flask containing the mixture. The specific absorbed microwave powers in the cases of UAE + MAE, UAE + VMAE and VMAE were 1.3, 1.3 and 1.19 W/g, respectively. Specific absorbed microwave powers, which are the powers