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Enhancement of microwave-assisted extraction of bioactive compounds from cabbage outer leaves via the application of ultrasonic pretreatment

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

[a] Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, 126 Pracha u-tid Road, Tungkru, Bangkok 10140, Thailand
[b] PTT Group Frontier Research Center, PTT Public Company Limited, 555 Vibhavadi Rangsit Road, Chatuchak, Bangkok 10900, Thailand
[c] 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

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ABSTRACT

Although microwave-assisted extraction (MAE) has proved to be a rapid alternative to extract bioactive compounds from plant materials, it might be possible to enhance MAE further through structural modification of the plant matrix. In this study, the effect of plant structure modification via ultrasonic pretreatment prior to MAE was investigated. The evolutions of selected bioactive compounds, namely, glucosinolates, sulforaphane, vitamin C and phenolics, and antioxidant activity of the extract from cabbage outer leaves during sonication, which is indeed equivalent to ultrasonic-assisted extraction (UAE), were first monitored to identify a suitable time for the pretreatment prior to subsequent MAE. For comparison MAE and Soxhlet extraction were conducted and their results compared with those belonging to UAE and UAE + MAE. Microstructural changes of cabbages, as observed via confocal laser scanning microscopy (CLSM) and quantified via the use of the fractal dimension, undergone different extraction methods were observed and used to explain the extraction results. Energy consumption of different extraction methods was also evaluated. UAE + MAE led to higher contents of extractable bioactive compounds due to the effects of acoustic cavitation and subsequent internal heating within the plant cells by microwave irradiation, which resulted in more structural damage and hence better release of the compounds. The contents of the extractable bioactive compounds from UAE, MAE and UAE + MAE were significantly lower than those from Soxhlet extraction in almost all cases; Soxhlet extraction time was much longer, however. MAE exhibited the highest energy efficiency compared with UAE, UAE + MAE and Soxhlet extraction.

1. Introduction

Microwave-assisted extraction (MAE) has recently received much attention as an alternative means to extract bioactive compounds from plant materials [3]. Although MAE has noted to possess a number of advantages over a more traditional solvent extraction, including rapid extraction and less consumption of an extraction solvent, the extraction yield achievable through MAE is still generally low [13]. Any means that can help enhance MAE and hence to obtain a higher yield is desirable.

It is possible to enhance the extractability of any extraction methods, including MAE, by modifying the structure of a material to be extracted. Several pretreatment methods have been applied to plant materials prior to extraction to help enhance the extractability of bioactive compounds through plant structure modification. Tanongkankit et al. [24], for example, investigated the effect of blanching pretreatment on the extraction yield of glucosinolates from cabbage outer leaves. Use of steam blanching helped enhance glucosinolates extraction since blanching led to softening of the cabbage structure, leading to easier extraction. Hiranvarachat et al. [7] investigated the effects of selected pretreatment methods on the bioaccessibility, which is closely related to the extractability, of β-carotene in carrots. Blanching led to carrot cell wall disruption, leading to more extensive release of β-carotene from the carrot matrix, resulting in higher bioaccessibility.

Despite its advantage in terms of structural modification capability, thermal pretreatment may lead to thermal degradation of bioactive compounds. Non-thermal pretreatments are therefore of interest. Among many alternatives, ultrasonic pretreatment has widely been applied to help modify the structure of many

* Corresponding author at: Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, 126 Pracha u-tid Road, Tungkru, Bangkok 10140, Thailand. Tel.: +66 2 470 9244; fax: +66 2 470 9240.
E-mail address: sakamon.dev@kmutt.ac.th (S. Devahastin).

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materials without posing a significant problem to heat-sensitive bioactive compounds [15]. Ultrasonic pretreatment relies on acoustic cavitation, which causes disruption of cell walls of plant materials, leading to a more extensive release of internal cell compounds [14]. Bagherian et al. [1], among others, investigated the effect of ultrasonic pretreatment prior to MAE on the yield of pectin from grapefruit. Ultrasonic pretreatment led to higher extraction yield of pectin when comparing with the values obtained from both conventional solvent extraction and MAE without pretreatment. Pananun et al. [19] and Izadifar [9] also observed a similar effect of ultrasonic pretreatment on the extraction yields of isoflavones from soybean and phenolics from wheat dried distiller's grain (DDG), respectively. More recently, Pongmalai et al. [20] studied the effect of ultrasonic pretreatment prior to MAE on the extraction yield of glucosinolates from cabbage outer leaves and found that ultrasonic pretreatment at a frequency of 37 kHz and a power of 320 W prior to MAE led to more extensive breakdown of cabbage structure than at other tested conditions. This in turn resulted in a higher extraction yield of glucosinolates. Nevertheless, the evolution of glucosinolates (and other important compounds in cabbages) during sonication was not reported although monitoring the changes of compositional profiles is of importance if an effective ultrasonic pretreatment protocol is to be designed.

Despite some prior studies on the use of ultrasonic pretreatment to enhance the extractability of bioactive compounds during subsequent extraction, including MAE, limited information is available on how a suitable condition, especially in terms of the treatment time, for ultrasonic pretreatment could be determined. The first aim of this study was therefore to determine a suitable ultrasonic pretreatment time prior to MAE; cabbage outer leaves were used as the test material. The evolutions of selected bioactive compounds, namely, glucosinolates, sulforaphane, vitamin C and phenolics, as well as antioxidant activity of the extract from cabbages were monitored along with the change in the temperature during ultrasonic pretreatment, which is indeed equivalent to ultrasonic-assisted extraction (UAE). The yields of all the interested bioactive compounds achievable upon subsequent MAE (or in fact UAE + MAE) were then determined. For comparison MAE and Soxhlet extraction were conducted and their results compared with those belonging to UAE and UAE + MAE. Microstructrural changes of cabbages, as observed via confocal laser scanning microscopy (CLSM) and quantified via the use of the fractal dimension, undergone different extraction methods were observed and used to explain the extraction results. Energy consumption of UAE, MAE, UAE + MAE and Soxhlet extraction was also evaluated and compared.

2. Materials and methods

2.1. Materials and chemicals

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, Ecully, France) for 2 min to obtain an average size of cabbages of 1.7–2.5 mm.

Ascorbic acid standard, gallic acid as well as 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were obtained from Sigma–Aldrich (Steinheim, Germany), Folin–Ciocalteu reagent was purchased from Carlo Erba (Milan, Italy), while sinigrin and sulforaphane standards were obtained from Sigma–Aldrich (St. Louis, MO). 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonuim salt (ABTS) was purchased from Sigma–Aldrich (Oakville, Canada) and 6-hydoxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma–Aldrich (Buchs, Switzerland).

2.2. Methods

2.2.1. Ultrasonic pretreatment or UAE

Experiments were first performed to determine a suitable time for ultrasonic pretreatment by monitoring the evolutions of selected bioactive compounds of the extract from cabbages. The results are indeed representative of the UAE of the compounds.

Five g of chopped cabbages was dispersed in 50 mL of 99.9% (v/v) ethanol, which was used as an extraction solvent, in a 250-mL beaker, which was placed in an ultrasonic bath (Elma, Elmasonic P, Singen, Germany) containing 1 L of distilled water and sonicated 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 chopped cabbages and ethanol). This condition was selected based on the results of Pongmalai et al. [20] who reported that sonication at a frequency of 37 kHz and a power of 320 W led to a higher extractable glucosinolates content than at other conditions. The sonication time was, on the other hand, varied from 0 to 40 min.

After sonication an ethanolic extract was filtered through a filter paper. The filtrate was concentrated using a rotary evaporator (Buchi, R-215, Flawil, Switzerland) at 50 °C for 7 min before being diluted with another solvent; the type and amount of the added solvent depended on the required subsequent chemical analysis. Five mL of 99.9% (v/v) ethanol was added if glucosinolates content, total phenolics content (TPC) and antioxidant activity, both by the DPPH and ABTS assays, were to be determined. For sulforaphane analysis, 2 mL of acetonitrile was added, while for vitamin C analysis 2 mL of 3% (w/v) metaphosphoric acid was added. A diluted sample was kept at −18 °C in a vial until further analysis.

2.2.2. MAE

A domestic microwave oven (Samsung, GE-872D, Port Klang, Malaysia), which is capable of operating at a maximum input power of 850 W at a frequency of 2450 MHz, was modified for MAE as described by Hiranvarachat et al. [8]. The whole content of a sample (chopped leaves and 99.9% (v/v) ethanol) was subject to microwave irradiation at 100 W (or absorbed microwave power of 0.63 W/g) for 2 min; the reasons for the selected conditions will be given in Section 3.2. The extract was filtered through a filter paper and the filtrate was concentrated using the rotary evaporator at 50 °C for 7 min to produce a crude extract. The content was diluted with different extraction solvent, which was again added depending on the type of the required chemical analysis. A diluted sample was kept at −18 °C in a vial until further analysis.

In the case of UAE + MAE, a sample was ultrasonically pretreated as described in Section 2.2.1. The sonication time for the pretreatment was 30 min; this time selection decision will be explained in Section 3.1. After sonication the whole content of the pretreated sample was subject to microwave irradiation at 100 W (or absorbed microwave power of 0.63 W/g) for 2 min as mentioned earlier.

2.2.3. Soxhlet extraction

A Soxtec System HT (Soxtec Extraction Unit 1043 and Service Unit 1046, Tecator, Höganäs, Sweden) was used for Soxhlet extraction. Five gram of chopped cabbages was placed in a thimble, which was placed in an extraction chamber and dipped into a cup containing 50 mL of 99.9% (v/v) ethanol. The cup was heated to 50 ± 2 °C for 3 h. An extract was filtered through a filter paper and the filtrate was concentrated using the rotary evaporator at