EFFECT OF ULTRASONIC PRETREATMENT ON EXTRACTABILITY OF GLUCOSINOLATES FROM CABBAGE OUTER LEAVES

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ABSTRACT

White cabbage outer leaves have noted to contain various beneficial glucosinolates. Several techniques have been used to extract these compounds with various degrees of success. However, it might be possible to enhance the extraction efficiency of these (and other) compounds through structural modification of the cabbage matrix. In this study, the effect of structural modification via the use of ultrasonic pretreatment on the amount of total glucosinolates extractable from cabbage outer leaves was investigated. Use of ultrasonic pretreatment prior to extraction led to more structural damage and higher content of extractable glucosinolates than in the case where no pretreatment was applied.

INTRODUCTION

White cabbages are among the popular vegetables in Thailand and have been reported to contain various beneficial glucosinolates, which are sulfur-rich plant secondary metabolities possessing anticarcinogenic property [1, 2]. Since it is not possible to consume an excessive amount of cabbages to obtain a significant dose of glucosinolates, several techniques have been used to extract these compounds from the vegetables with various degrees of success. Omirou et al. [3], for example, studied the extraction of glucosinolates from Eruca sativa seeds and soil using microwave-assisted extraction (MAE) in comparison with selected other extraction methods. It was found that MAE and conventional extraction method exhibited higher yields of glucosinolates than ultrasound-assisted extraction.

Several pretreatment methods may be applied to plant materials prior to extraction to either help reduce the losses of an interested compound during extraction or to help enhance the extractability of such a compound. Tanongkankit et al. [4] investigated the effect of blanching on total glucosinolates contents of cabbage outer leaves. It was noted that use of steam blanching led to higher retention of glucosinolates compared with water blanching. This processing might also help enhance glucosinolates yield during extraction since steam blanching could help soften the cabbage structure, leading consequently to easier extraction. In fact, it is possible to enhance the extraction efficiency of bioactive compounds through structural modification of the material matrix. Uquiche et al. [5], for example, observed the effect of pretreatment with microwave irradiation on the extraction yield of oils from Chilean hazelnuts (Gevuina avellana Mol) and reported that microwave pretreatment helped modify the cellular walls, resulting in greater porosity and increase in mass transfer of the oils. Hiranvarachat et al. [6] studied the effects of different pretreatments prior to extraction on the extractability of β-carotene from carrots. It was observed that carrots blanched in water and in citric acid exhibited more damaged structures, leading to higher β-carotene contents during extraction when compared with carrots that undergone no pretreatment and carrots soaked in acid.

Ultrasonic pretreatment is a non-thermal technique that can help modify the material structure without posing any problem to the heat-sensitive bioactive compounds of interest [7]. Ultrasonic pretreatment relies on acoustic cavitation, which causes disruption of cell walls of plant materials, leading subsequently to better release of the internal cell compounds [8]. Rodrigues and Fernandes [9], for example, showed that the use of ultrasonic pretreatment led to more tissue damage of melons treated only osmotic dehydration. This resulted in better water diffusion from the melon matrix due to formation of microscopic channels. In terms of extraction, Pananun et al. [10] studied the effect of ultrasonic treatment on extraction yield of soybean isoflavones. Rupture of defatted soy flakes was noted when ultrasonic treatment was applied. The rupture in turn led to the reduction in the particle size, inducing an increase in the surface area available for isoflavones extraction and hence enhanced extraction.

The present study investigated the effect of structural modification via ultrasonic pretreatment prior to microwave-assisted extraction on the extraction yield of glucosinolates from cabbage outer leaves. The microstructural information was used to explain the observed changes of the extraction yield.

MATERIALS AND METHODS

Materials

Outer leaves of cabage (Brassica oleracea L. var. capitata) were obtained from a local market; 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. After that the leaves were chopped using an electric chopper (Moulinex, DPA141, Ecully, France) for 2 min.

Ultrasonic pretreatment

Five grams of chopped cabbage outer leaves dispersed in 50 mL of 99.9% (v/v) ethanol solution in a 100 mL beaker was placed into an ultrasonic bath (Elma, Elmasonic P, Singen, Germany) and pretreated either at a frequency of 37 or 80 kHz at a power of 160 or 320 W (or absorbed ultrasonic power of 3.60 or 7.19 W/g, respectively) for 5 min.

MAE set-up

A domestic microwave oven (Samsung, GE-872D, Port Klang, Malaysia), which is capable of operating at a maximum input of 850 W at a frequency of 2450 MHz was modified for MAE as described by Hiranvarachat et al. [6]. A 1000-mL round bottom flask containing a solvent and a sample was placed inside the microwave oven cavity. The flask was fitted with a condenser to condense the vaporized extract, which was then collected in a graduated cylinder. Cold water (~4 °C) was used as condensing medium.

MAE of cabbages

The whole content of a pretreated sample (chopped leaves and ethanol solution) was subjected to microwave irradiation at 300 W (or absorbed microwave power of 6.47 W/g) for 7 min to extract glucosinolates from the sample. After extraction the condensed extract was mixed with the remaining extract in the extraction flask. The combined extract was filtered through a filter paper (Ø110 mm, Cat. no. 1001, Schleicher and Schuell GmbH, Dassel, Germany) and the filtrate was concentrated using a rotary evaporator (Buchi, R-215, Flawil, Switzerland) at 50 °C for 10 min to produce a crude extract. The crude extract was dissolved in 5 mL of 99.9% (v/v) ethanol solvent and kept at -18 °C in a vial until further analysis.

Determination of glucosinolates content

The determination of the total glucosinolates content was performed following the method of Tanongkankit et al. [4]:

Preparation of Sephadex pyridine-acetate column

Total glucosinolates were separated using an ion-exchange column. The column was prepared by adding 25 mg of dry DEAE-Sephadex A-25 (Sigma-Aldrich, Steinheim, Germany) into a syringe (0.8×4 cm), which was filled subsequently with deionized water. The syringe was then left overnight at room temperature and kept in refrigerator until use.

Before loading an extract into the column, the pH of the eluate was adjusted into neutral by passing 0.5 N of NaOH (5 mL) and water (10 mL) through the column. The column was converted into an acetate form by adding 5 mL of 0.5 M pyridine acetate solution and 10 mL of water. Three mL of an extract was then added into the prepared column. The column was washed twice with 2 mL of water, 2 mL of 30% (v/v) formic acid and 2 mL of water in order to discard the eluate each time. Finally, the column was eluted twice with 4.75 mL of 0.3 M potassium sulfate; the content was adjusted by adding 0.3 M potassium sulfate to 10 mL. The extract eluate from the column was kept in refrigerator at -18 °C prior to analysis.

Quantification of glucosinolates

One mL of an extract eluate was transferred to a tube. After that 7 mL of 80% (v/v) sulphuric acid and 1 mL of 1% (w/v) thymole solution were added. The solution was mixed by a vortex mixer (Vortex-Genie 2, G560E, Bohemia, NY) for 30 s and placed in a water bath (Heto, AT 110, Allerod, Denmark) at 100 °C for 60 min. The tube was cooled with tap water and mixed again by the vortex mixer for 30 s. Spectrophotometer (Shimadzu, UV 21101 PC, Kyoto, Japan) was used to measure the absorbance of glucosinolates at 505 nm; 0.3 M of potassium sulphate was used as a blank. Sinigrin standard (Sigma, St. Louis, MO) solution (3 μmol/mL) was applied to calculate the glucosinolates concentration by the following equation:

$$C = \frac{\mathrm{Abs_x}}{K} \times \frac{D}{W} \quad (1)$$

where C = total glucosinolates concentration (μmol/mL), $\mathrm{Abs_x}$ = absorbance of the extract, K = absorbance factor (absorbance/concentration of standard) (mL/μmol), D = dilution factor (50), W = mass of the extract taken (g).

Determination of microstructural changes of cabbage outer leaves

Chopped leaves sample were dehydrated in liquid CO2 using a critical point dryer (Tousimis, Samdri 780-A, Rockville, MD). The dehydrated sample was then coated with gold in a sputter coater (Balzers Union, SCD-040, Balzers, Liechtenstein). The microstructural changes of the sample was observed by a scanning electron microscope (JEOL, JSM-5410LV, Tokyo, Japan) at 350× magnification.

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) and are presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests; Differences were considered at a confidence level of 95%. All statistical analyses were performed using SPSS® software (version 17) (SPSS Inc., Chicago, IL). All experiments were performed in duplicate unless specified otherwise.

RESULTS AND DISCUSSION

Effects of ultrasonic pretreatment on extractable glucosinolates

Table 1 lists the contents of total glucosinolates extractable from the non-pretreated and ultrasonically pretreated cabbages at different conditions. The extractable glucosinolates content from cabbage outer leaves without ultrasonic pretreatment was 455.06±10.94 μmol/100 g dry mass, which was lower than the contents extractable from the other pretreated cabbages.

Ultrasonic pretreatment at a frequency of 37 kHz and a power of either 160 or 320 W led to higher extractable glucosinolates contents than that at a frequency of 80 kHz at the same power levels. This is probably because lower ultrasonic frequency (37 kHz) led to increased cavitation forces, resulting in more damage of the cabbage structure [11] and hence higher extent of glucosinolates release from the cabbage matrix. At the same frequency, however, ultrasonic pretreatment at a higher power of 320 W led to higher contents of extractable glucosinolates. This might be

because higher ultrasonic power presented higher power intensity to cause mechanical vibrations, promoting more structural disruption [12] and inducing more glucosinolates release during extraction.

Cabbages pretreated with ultrasonic at a frequency of 80 kHz and a power of 160 W exhibited no significantly different amount of extractable glucosinolates compared with the non-pretreatment sample. This might be due to the combined effect of higher frequency and lower power of ultrasound mentioned earlier.

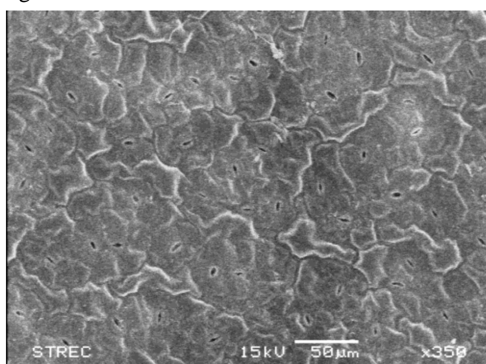
Table 1 Total glucosinolates contents of the extracts

Method	Total glucosinolates content (μmol/100 g dry mass)
Non-pretreatment + MAE	455.06 ± 10.94^{a}
Ultrasonic pretreatment (37 kHz, 160 W) + MAE	602.88 ± 6.64^{c}
Ultrasonic pretreatment (37 kHz, 320 W) + MAE	623.17 ± 10.04^{d}
Ultrasonic pretreatment (80 kHz, 160 W) + MAE	465.21 ± 7.53^{a}
Ultrasonic pretreatment (80 kHz, 320 W) + MAE	568.10 ± 2.51^{b}

Same letters in the same column indicate that values are not significantly different (p>0.05)

Microstructural changes of cabbage outer leaves

Fig. 1a and 1b show the microstructure of the non-pretreated cabbages and cabbages after ultrasonic pretreatment (37 kHz, 320 W) for 5 min. For non-pretreated cabbages clear cell periphery, cell integrity and extended smooth surface areas were observed in Fig. 1a. Fig. 1b, on the other hand, shows much more significant intercellular and collapse of cabbage structure. This is probably because of the microstreaming of the solvent due to collapsing bubbles as well as the mechanical vibrations during acoustic cavitation. This led to an increase in glucosinolates release during extraction. Ultrasonic pretreatment at other conditions led to lower glucosinolates yields because the combined effect of frequency and power of ultrasound at those other conditions might result in less damage of the cabbage structure.



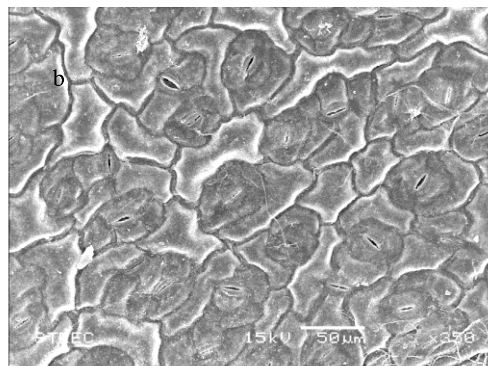


Fig. 1 SEM images (350×) of cabbage outer leaves: (a) fresh cabbages; (b) cabbages after ultrasonic pretreatment (37 kHz, 320 W)

CONCLUSIONS

Ultrasonic pretreatment prior to extraction helped damage the structure of cabbage outer leaves, thus resulting in a higher extraction yield of glucosinolates. A study on the release of other bioactive compounds (i.e., sulforaphane, vitamin C, α-tocopherol and phenolics) from cabbage upon ultrasonic pretreatment prior to MAE is recommended as a future work.

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