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Microwave pretreatment enhances the formation of cabbage sulforaphane and its bioaccessibility as shown by a novel dynamic soft rat stomach model

Patsaporn Pongmalai[a,b], Nan Fu[a,*], Somchart Soponronnarit[c], Naphaporn Chiewchan[b], Sakamon Devahastin[b,d,*], Xiao Dong Chen[a]

[a] Suzhou Key Laboratory of Green Chemical Engineering, School of Chemical and Environmental Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, Jiangsu 215123, China
[b] 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
[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
[d] The Academy of Science, The Royal Society of Thailand, Dusit, Bangkok 10300, Thailand

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ABSTRACT

Microwave (MW) pretreatment was used to increase the sulforaphane content in cabbages. *In vitro* digestion in rat stomach and shaking flask was then investigated to monitor the bioaccessible sulforaphane content in the pretreated cabbages. Microstructural changes of cabbages were observed and used to support the pretreatment and digestion results. MW treatment at 26.40 W/g for 15 s increased the sulforaphane content by 6.23 times compared with that in the fresh samples. Bioaccessible sulforaphane content after rat-stomach digestion increased 3.48–4.19 times compared with that in the pretreated samples before digestion. This increase was similar to that after shaking-flask digestion, probably because cabbage tissues are soft and can be easily digested even in a simplified batch *in vitro* digestion system. Nevertheless, sulforaphane concentration in the digested mixture during rat-stomach digestion became constant after 15 min, while that in the shaking flask reached the same concentration only after 75 min.

1. Introduction

Sulforaphane [1-isothiocyanato-4-(methylsulfinyl) butane] is well recognized to possess high anticarcinogenic activities (Guo, Yang, Wang, Guo, & Gu, 2014). Unfortunately, the compound is very heat-sensitive and easily degradable, especially in the presence of light and oxygen (Pongmalai, Devahastin, Chiewchan, & Soponronnarit, 2015). Therefore, any vegetables that can serve as the sources of sulforaphane should be prepared via the use of a suitable preparation method prior to consumption, or sulforaphane may suffer undesirable degradation. It is important to note that disruption of the plant microstructure by any pretreatment method is expected to result in the formation and release of sulforaphane from the plant cellular structure. This should in turn affect the bioaccessibility and bioavailability of the compound during subsequent consumption or digestion.

To understand the bioaccessibility of sulforaphane upon consumption, monitoring the changes in the sulforaphane content during digestion is crucial. Such an understanding can commonly be obtained via *in vitro* digestion experiments, where food is digested in synthetic digestive fluids in a shaker or a continuously stirring bioreactor (Corrêa et al., 2017; Gullon, Pintado, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2015; Kamiloglu et al., 2017; Sancho, Souza, Aliaga de Lima, & Pastore, 2017). Note, however, that most standardized *in vitro* digestion models, including SHIME, RIVM and Infogest model are static models, where food and digestive fluids are mixed and react under a controlled environment. As a result, these digestion models may not adequately simulate the real digestion system, which exhibits constantly changing biochemical reactions. Gastric mobility is also expected to result in significant structural modification and compound release from the food matrix (Thuenemann, 2015; Wu et al., 2017). For this reason, dynamic *in vitro* gastrointestinal digestion systems that offer continuous digestion reactions and closely resemble the *in vivo* digestion process have attracted increasing attention during the past decade (Krul et al., 2002). Among several dynamic gastrointestinal models, *in vitro* soft rat

* Corresponding authors at: Suzhou Key Laboratory of Green Chemical Engineering, School of Chemical and Environmental Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, 199 Ren-Ai Rd., Suzhou Industrial Park, Suzhou, Jiangsu 215123 , China (N. Fu); 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 (S. Devahastin).
E-mail addresses: nan.fu@suda.edu.cn (N. Fu), sakamon.dev@kmutt.ac.th (S. Devahastin).

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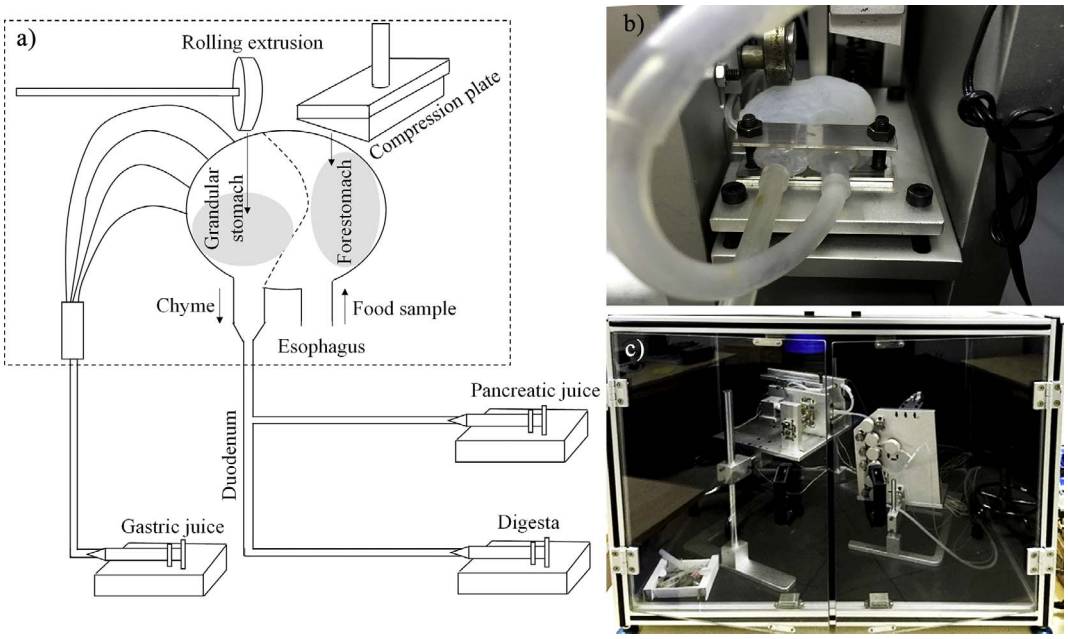

Fig. 1. Mechanized soft rat stomach model. (a) A schematic illustration of the system; (b) the soft-elastic rat stomach equipped on the apparatus; (c) the apparatus.

stomach model is an interesting option since this animal model exhibits physiological parameters very close to the *in vivo* digestion of a rat (Chen, Jayemanne, & Chen, 2013). Nevertheless, limited information is so far available both on how a suitable preparation method prior to digestion could improve the content of sulforaphane and its bioaccessibility as well as on the evolution of this bioactive compound upon digestion.

The present work aimed at studying the feasible use of microwave (MW) cooking, which is a widely used domestic food preparation method, to enhance the formation of sulforaphane. Outer leaves of white cabbage (*Brassica oleracea* L. var. *capitata*) were used as the test material. Since sulforaphane is a hydrolysis product of glucoraphanin, the evolutions of these two compounds, along with that of the activity of myrosinase, which is the enzyme responsible for the hydrolysis reaction, should first be monitored. Monitoring of such evolutions would allow the identification of a suitable MW pretreatment condition. A newly constructed mechanized soft rat stomach model was then utilized to perform dynamic *in vitro* digestion experiments of the pretreated cabbages in comparison with batch *in vitro* digestion experiments. Only gastric digestion was studied as it is the major mechanism for food disintegration and digestion and hence plays an important role on food structural modification, release and bioaccessibility of the bioactive compounds of interest. In order to understand the release and formation mechanisms of sulforaphane during dynamic *in vitro* digestion, the results were compared to that obtained with a simplified batch *in vitro* digestion system. Microstructural changes of cabbages, as observed via scanning electron microscopy (SEM) and quantified via the use of fractal dimension, were investigated and used to explain the MW pretreatment and digestion results.

2. Materials and methods

2.1. Materials and chemicals

Outer leaves of cabbages (*Brassica oleracea* L. var. *capitata*) were obtained from a local market in Suzhou; the leaves were kept at 4 °C until the time of an experiment but for no longer than 3 h. Prior to 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 (Braun, K600, Kronberg, Germany) for 1 min and immediately introduced to a treatment process.

α-amylase from *Bacillus subtilis*, pepsin from porcine gastric mucosa and mucin from porcine stomach were obtained from Sigma-Aldrich (St. Louis, MO). Glucoraphanin potassium salt was obtained from Chromadex (Irvine, CA), while sinigrin and sulforaphane standards were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. MW treatment

Five grams of chopped cabbages were introduced into a domestic MW oven (Galanz, HC-83510FR, Guangdong, China) at an input power of either 160, 320, 480, 640 or 800 W (or specific absorbed powers of 22.46, 24.47, 24.81, 26.40 and 26.94 W/g, respectively) for a maximum of 45 s. After that the pretreated cabbages were immediately placed into a 100-mL Erlenmeyer flask containing an extraction solvent to extract the bioactive compounds and to determine the myrosinase activity. Note that 50 mL of pure methanol and 50 mL of dichloromethane were used as the extraction solvents for glucoraphanin and sulforaphane extraction, respectively. On the other hand, 50 mL of 30-mM citrate/phosphate buffer (pH 7) was prepared for myrosinase activity determination.

The cabbage temperature during MW treatment was monitored using a fiber-optic thermometer (Luxtron, m600, Santa Clara, CA); the thermometer was placed in the center of the cabbages subjecting to microwave irradiation.

2.3. Dynamic in vitro rat stomach digestion

In vitro digestion was first performed within the mechanized soft rat stomach model (Bionic Rat Model II, Nantong Dong-Concept New Material Technology Ltd., Jiangsu Province, China) following the methods of Wu et al. (2017) with some modification. Prior to the start of the digestion process, one g of the pretreated cabbages was added to the stomach through the esophagus (see Fig. 1); this was followed by adding one g of simulated rat saliva. The mixture was mixed in the stomach for 30 s before loading one g of artificial gastric juice through the esophagus. Note that the saliva solution was prepared by dissolving 0.1128 ± 0.0005 g of α-amylase in 1.01 mL of deionized water with pH 7.80 ± 0.44 adjusted by using 1-M NaOH. Artificial gastric juice was prepared by dissolving pepsin (0.27 g/L or 250 U/mL) and mucin