

## Effect of Extraction Time on Degradation of Bioactive Compounds (*Zingiber Officinale* Roscoe)

Nor Ilia Anisa Aris<sup>a,b</sup>, Noor Azian Morad<sup>a,b\*</sup>

<sup>a</sup>Centre of Lipid Engineering and Applied Research (CLEAR), Universiti Teknologi Malaysia, Kuala Lumpur Campus, 54100 Kuala Lumpur, Malaysia

<sup>b</sup>Malaysia-Japan International Institute of Technology (MIIT), Universiti Teknologi Malaysia, 54100 UTM Kuala Lumpur Campus, Kuala Lumpur, Malaysia

\*Corresponding author: azian@ic.utm.my

### Article history

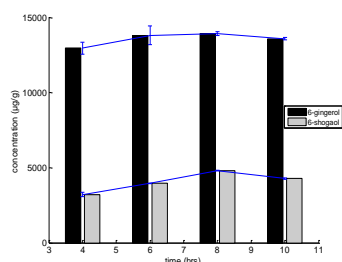
Received :14 October 2013

Received in revised form :

30 December 2013

Accepted :24 February 2014

### Graphical abstract



### Abstract

Ginger, the rhizome of *Zingiber officinale* contains the bioactive compounds that have a long history of medicinal usage. The two most abundant ginger bioactives identified are 6-gingerol and 6-shogaol. It is important that the availability of these bioactives are first identified. This is done through ethanol extraction. Thus, this paper identified the effect of extraction time using ethanol on the overall yield and concentration of 6-gingerol and 6-shogaol. Kinetic studies on degradation of the bioactive compounds also have been studied. Fourier transform infrared (FTIR) spectroscopy was applied as an additional tool for the characterization of the compounds and in identifying the degradation circumstances. Results showed that the highest overall yield was obtained after 8 hours of extraction. In consistent with the overall yield of extraction, the concentration of 6-gingerol and 6-shogaol reached equilibrium within 8 hours. The concentration of 6-gingerol and 6-shogaol are 13923.26 and 4816.84 µg/g, respectively. Conclusively, the saturated concentration of zingiber bioactive compounds could be determined through ethanol extraction within 8 hours.

**Keywords:** Ginger; bioactive compounds; saturated concentration; ethanol extraction

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### 1.0 INTRODUCTION

Ginger, the rhizome of *Zingiber officinale* is one of the widely used species of the zingiber family (zingiberace) and is commonly used for various foods and beverages. Recently, ginger has gained much attention for anticancer [1], anti-inflammatory [2], antioxidatives [3] due to the presence of bioactive constituents. Ginger contains different number of bioactive constituents which primarily depends on the origin and freshness of the ginger. [4-5]

The pungency of fresh ginger is due to the gingerols, which are homogenous series of phenols and the most abundant bioactive constituents in ginger is 6-gingerol [5]. However, there are minor constituent of gingerols such as 8-, and 10-gingerols. Meanwhile, shogaol which is the dehydration product of gingerols constituent (6-gingerol to 6-shogaol) caused the pungency of dried ginger (Figure 1). Other gingerol and shogaol related compounds such as 6-paradol, 6- and 10-dehydrogingerdiolones, 6- and 10-gingerdiolones, 4-, 6-, 8-, and 10-gingerdiols, 6-methylgingerdiol, zingerone, 6-hydroxyshogaol, and 6-, 8-, 10-dehydroshogaols and diarylheptanoids have also been reported. However, these minor constituents only contributes from 1 to 10% of the overall gingerols and shogaols [3]. Soxhlet extraction is a standard method of extraction and applied as a benchmark technique in evaluating the performance of other extraction methods, in particular for solid-liquid extraction [7]. Bogdanov and Svinyarov [8] applied this

technique in establishing the bioactive alkaloids present within *Glaucium flavum* Crantz raw material. Indeed, the saturation concentration has also been determined through this technique. The saturation concentration is essentially identified for modelling and extraction effectiveness purposes.

The objective of this paper is to study the effect of extraction time on determining the saturation concentration of 6-gingerol and 6-shogaol. The extraction time is varied in obtaining the optimum time and identifying the degradation effect.

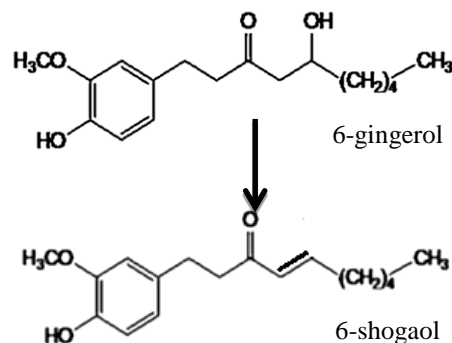


Figure 1 Schematic diagram of degradation 6-gingerol to 6-shogaol [6]

## 2.0 MATERIAL AND METHODS

### 2.1 Sample Preparation and Chemicals

Dried ginger (*Zingiber officinale* Roscoe) was supplied from local supplier (Sabah, Malaysia). All samples were stored in a refrigerator at 4°C for maintaining the freshness before being used in experiments thought.

HPLC grade methanol (99.9%, ACS, Houston, USA), acetonitrile (99.99%, Fisher, Loughborough, UK) and ultrapure water with resistivity > 18.2 MΩ cm (Barnstead, USA) were used as mobile phase in HPLC analysis. 6-gingerol and 6-shogaol standards were purchased from ChromaDex (Irvine, CA).

### 2.2 Soxhlet Extraction

20 g of dried and ground ginger with 2.0 mm of size particles was weighed and extracted with 200mL of ethanol for varying time (4, 6, 8 and 10 hrs) using soxhlet extraction apparatus. The extraction temperature was constant at the boiling point of ethanol (78.1°C) and monitored using infrared laser thermometer (AR300, China). The sample was gradually filled with condensed fresh ethanol from a distillation flask (Figure 2). Once the liquid reaches overflow level, a siphon aspirates the extracted sample from sample matrix and unloads it back into the distillation flask [9]. The process is repeated for each prescribed time. Then, the ethanol evaporated overnight in the oven at 40°C before further analysis. The experiments were conducted in triplicate and standard deviations were calculated (<5%).

### 2.3 Determination of Overall Yield

The overall yield (wt % of dry basis) was calculated as following equation,

$$\text{yield}(\text{wt}\%) = \frac{W_a - W_b}{W_r} \times 100 \quad (1)$$

where  $W_a$ ,  $W_b$  and  $W_r$  are the weight of extracted sample before evaporated overnight (g), weight of extracted sample after evaporated (g) and weight of raw material (20.0 g).

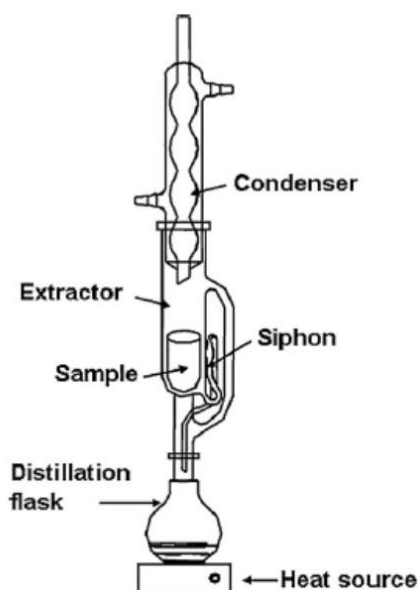


Figure 2 Schematic diagram of soxhlet extractor [7]

### 2.4 HPLC Analysis

The extracted samples obtained through soxhlet extraction process were homogeneously dissolved in 10mL of methanol. The mixtures were then filtered through 0.45 μm membrane filter (Nylon, Waters Corporation). The analysis was performed by HPLC (e2695, Waters, USA) with a photo diode array detector (PDA). The compounds were separated on C18 column (symmetry®) 5.0 μm particle size, (150 mm × 4.6 mm) and detected at 282 nm wavelength. The gradient mobile phase consisted of (A) water, 50%: methanol, 50% and (B) acetonitrile at a flow rate of 0.8 ml/min. The elution of binary solvent was conducted in gradient fashion, using the following profile: 0-8 min, 45% B; 8-10 min, 65% B; 10-11 min, 55% B; 11-20 min, 55% B and column temperature was set at 30°C ± 5°C.

### 2.5 FTIR Analysis

An FTIR Spectrometer, PerkinElmer Spectrum 100 Series (Perkin Elmer, USA), was used for infrared spectra analysis. Each spectrum was obtained at 4 cm<sup>-1</sup> resolution in the 650 cm<sup>-1</sup>-4000 cm<sup>-1</sup> region, scanned in transmittance vs. wavenumber mode at room temperature.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Effects of Time on the Overall Yield

The time of extraction is one of the parameter that plays an important role in the extraction of bioactive compounds. In optimizing the extraction process, the time of extraction is crucial for thermal labile bioactives and cost saving considerations. The bioactive compounds are thermal labile and will degrade in prolonged extraction time [10]. The effect on overall yield at different extraction times is shown in Figure 3. The figure shows that the optimum time was 8 hrs with the overall yield at 7.0 ± 0.36 wt%. The percentage of yield which represents the percentage of oleoresin was approximately (7-11%) to the findings by Kadam *et al.* [11].

The percentage of overall yield increasing until reaching the optimum time (8 hrs) and began to decrease at 10 hrs of extraction. An increasing in extraction time is reported to improve the overall extraction because of the enhancement of mass transfer and diffusivity of ethanol into the ginger matrix. The time of extraction increases, the solubility of bioactive compounds in ethanol also increases. The ethanol will diffuse into the ginger matrix and the bioactive compounds from the active site within the matrix will dissolve into the solvent. As postulated by Veličković *et al.* [12], the solvent plays a role on the classical process of extraction of the bioactive compounds from plant material via diffusion of bioactive compounds or 'targeted compounds' through the cell wall.

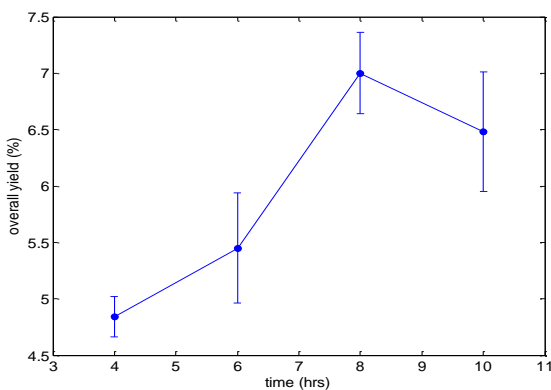


Figure 3 The effect of time on overall yield

The circumstances of the time in extraction process can be explained by Fick's second law of diffusion which defines that the diffusivity of 'targeted compounds' can reach the equilibrium and saturation after a certain time at prescribed conditions between the compounds in the matrix and solvent [13]. Thus, once the extraction reaching saturation condition, the yield decreased at 10hrs due to the degradation of bioactives.

### 3.2 Effect of Prolonged Extraction Time on Compound Structural Changes

In understanding the effect of time during extraction on the structure of ginger bioactive compounds, the spectrum analysis of bioactive compounds studies were carried out by using FTIR. The spectrum of ginger extracts displayed the phenol group as cited by Saptarini *et al.* [14]. As shown in Figure 4, the trend of spectrums for 4, 6 and 8 hrs almost similar which indicated the presence of aliphatic O-H groups in the range of 3000 to 3600  $\text{cm}^{-1}$ . However, at 10hrs of extraction the aliphatic O-H absorption was missing completely. This was due to the dehydration of gingerol to shogaol since shogaol has no aliphatic O-H [15].

The bands at 2800 to 2950  $\text{cm}^{-1}$  displayed two stretching peaks at lower extraction times of 4 and 6 hrs, respectively. The  $\text{CH}_2$  and  $\text{CH}_3$  stretching were well separated (2944 and 2832  $\text{cm}^{-1}$ ). As time increases, the bands became a broad peak due to the changes structure of compounds. While C-O group was well presented at 1030  $\text{cm}^{-1}$  for all the extraction times.

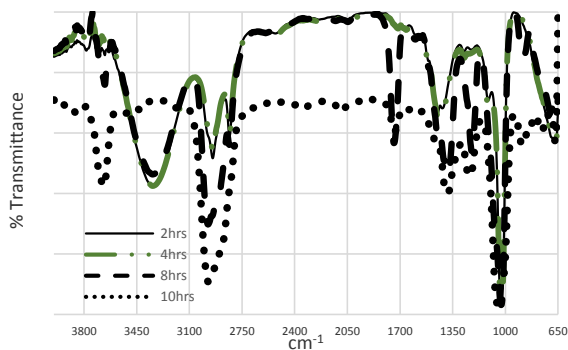


Figure 4 FTIR spectrum for ginger extract in soxhlet extraction

### 3.3 Effects of Time Varied on the Recovery of Bioactive Compounds

In investigating the concentration of ginger bioactive compounds (6-gingerol and 6-shogaol) present in the oleoresin extracted, the bioactive concentrations are plotted against time as shown in Figure 5. The concentration of each bioactive compounds are consistent to the overall yield as discussed previously. The highest amount of bioactives obtained at 8 hrs, thus it was concluded that 8 hrs was the optimum time to extract bioactive compounds using ethanol. In addition, the concentration at 8 hrs also could be classified as saturated concentration of ginger bioactive compounds. The ethanol extraction introduced a fresh solvent for each cycle during the extraction process. The freshness of ethanol was introduced until reaching the equilibrium state. At the equilibrium, there was probably all the compounds that present in ginger matrix were diffused out to the ethanol. As all the compounds had been diffused out, the interaction of bioactive compounds would be changed from diffuse out to ethanol to the internal interaction (within bioactive compounds). The unstable bioactive compounds will easily degrade to the other substances or derivatives. Thus, as increasing time, the concentration of each compound will be decreased due to the degradation and reduction of driving force.

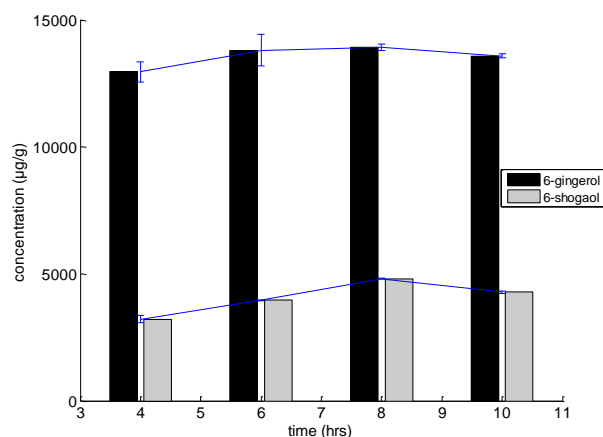


Figure 5 Effect of extraction time on amount of 6-gingerol and 6-shogaol presented

Besides, 6-shogaol also presented the lowest quantity of bioactive compounds in zingiber using ethanol extraction. As claimed by [16], the optimum temperature in obtaining 6-shogaol at 170°C which is 1.5 times higher than 6-shogaol operated in soxhlet extraction (73.5°C). Thus, temperature plays a role in presenting the 6-shogaol.

### 4.0 CONCLUSION

The experimental results showed that the extraction time played an important role on the extraction of bioactive compounds. The degradation effect should be considered while extract the bioactive compounds at the prolonged time. The optimum or saturation concentration of 6-gingerol and 6-shogaol were presented at 8 hrs in soxhlet extraction. The FTIR studies also showed that there was dehydration of bioactive compounds above than 8 hrs of extraction time.

### Acknowledgement

The financial support provided for this project by Malaysia-Japan International Institute of Technology is gratefully acknowledged. Authors also would like to acknowledge the staff in Universiti Putra Malaysia for technical support.

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