

Research Article

## **[6]-gingerol content and bioactive properties of ginger (*Zingiber officinale* Roscoe) extracts from supercritical CO<sub>2</sub> extraction**

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Paper originally presented at Food Innovation Asia 2007

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**Abstract:** Ginger (*Zingiber officinale* Roscoe) is one of the most widely used herbs that contains several interesting bioactive constituents and possesses health promoting properties. [6]-gingerol, a major pungent ingredient of ginger, also has potent antioxidant activity. Monitoring of [6]-gingerol content during the drying process, ginger extraction with supercritical CO<sub>2</sub> and bioactive properties analysis of extracts were performed. Fresh mature ginger rhizomes with  $94.17 \pm 0.16\%$  moisture content were dried using a rotary air dryer at  $55 \pm 2^\circ\text{C}$  for 11 hours to achieve a moisture content of  $11.54 \pm 0.29\%$ . After the drying process, [6]-gingerol content of the ginger rhizomes were reduced from  $21.15 \pm 0.13$  to  $18.81 \pm 0.15$  mg/g dry weight basis. Dried ginger was then pulverized to coarse powder, approximately 0.5 mm in diameter, prior to extraction. The supercritical CO<sub>2</sub> extraction of ginger was undertaken with two conditions of 200 bar at 35°C and 230 bar at 40°C. The results showed that the extracts from both conditions had [6]-gingerol contents of  $238.94 \pm 0.79$  and  $170.50 \pm 0.45$  mg/g extract, total phenolic contents of  $183.96 \pm 1.25$  and  $126.04 \pm 0.72$  mg gallic acid/g extract, respectively. In addition, the ginger extracts showed antioxidant activities using DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay, compared with BHT standard, expressed as EC<sub>50</sub>, that were  $13.09 \pm 1.77$  and  $26.68 \pm 1.76$  µg/ml, respectively. The antioxidant activities using ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]) radical cation scavenging assay were  $813.33 \pm 6.67$  and  $724.44 \pm 7.70$  µmol Trolox/g extract, respectively.

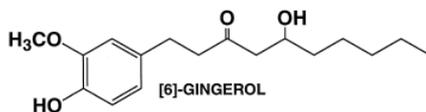
**Keywords:** [6]-gingerol, ginger extract, supercritical CO<sub>2</sub> extraction, bioactive properties, compounds, biochemistry

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## Introduction

Ginger (*Zingiber officinale* Roscoe) is a widely used herb and food-flavouring agent. Its nutraceutical properties have long been of interest to the food processing and pharmaceutical industries. The volatile essential oils contributing to the characteristic flavour of ginger, varies from 1.0-3.0%. While the oleoresin, responsible for the pungent flavour of ginger, varies from 4.0-7.5% and also possesses substantial antioxidant activity (Balachandran *et al.*, 2006). [6]-gingerol (structure shown in Figure 1) is the most abundant constituent of fresh ginger but it decreases during postharvest storage and processing, especially thermal processing (He *et al.*, 1998; Zhang *et al.*, 1994). The typical extraction methods for ginger, such as steam distillation or solvent extraction have several drawbacks in that they are time consuming and employ large amounts of chemical solvent. Recently, the supercritical fluid extraction process has been playing an increasingly important role in extracts from natural products, mainly because it can produce extracts that are free from residues. In addition, it can be conducted at moderate temperatures, which is necessary to retain the thermal-sensitive components intact. Carbon dioxide ( $T_C = 31.05\text{ }^\circ\text{C}$ ,  $P_C = 73.8\text{ bar}$ ) is the most frequently used solvent for this extraction because of its practical advantages, such as nontoxic, moderate critical temperature and easy separation after depressurization. Furthermore, extracts from the supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction can be regarded as all natural with the GRAS status for food applications (Diaz-Reinoso *et al.*, 2006).

The objectives of this study were to monitor [6]-gingerol content during the drying process of fresh ginger, to extract [6]-gingerol from dried ginger by using the SC-CO<sub>2</sub> extraction and to determine the bioactive properties of the ginger extracts.



**Figure 1.** Chemical structure of [6]-gingerol (5-hydroxy-1-(4'-hydroxy-3'-methoxyphenyl)-3-decanone).

## Materials and Methods

Fresh mature ginger rhizomes that were harvested at the age between 10-12 months and weighed in the range of 100-200 grams per rhizome, were purchased from Chumpon Province.

### 1. Material preparation

Fresh mature ginger rhizomes were graded, washed through with tap water, peeled and cut into cross-sections of  $2 \pm 1$  mm thickness.

### 2. Drying of fresh mature ginger

Cut ginger samples were dried in an air dryer at  $55 \pm 2^\circ\text{C}$  to achieve 10-12% moisture content. During drying for 0, 3, 6, 9 and 11 hours, samples were taken for the analyses of [6]-gingerol content and bioactive properties.

### 3. SC-CO<sub>2</sub> extraction of ginger

Dried ginger samples were pulverized and sieved through 35/40 mesh (approximately 0.5 mm diameter) prior to extraction with SC-CO<sub>2</sub> lab scale (5L/42MPa Equipment, Masson new separation technology, China). Two extraction conditions, 200 bar at 35°C and 230 bar at 40°C, were performed. 1,000g of ground ginger was used for each experiment and was packed into a stainless steel column (column capacity 2kg dry weight). CO<sub>2</sub> (>99.5% purity) was used as extraction solvent. The oleoresin extracts were collected, and at proximately 10 min extraction time, the extracts were taken for analyses of [6]-gingerol content and bioactive properties.

### 4. [6]-gingerol and bioactive property analyses

#### 4.1 [6]-gingerol content

The contents of [6]-gingerol were analyzed by high performance liquid chromatography (HPLC 1100, Agilent, Germany) equipped with a reversed phase column C18 (Hypersil<sup>®</sup>ODS 250mm x 4.0mm i.d., 5 µm), Elution was isocratic using a mixture of HPLC grade acetonitrile and water (55:45 v/v) flow rate 1.0ml/min, temperature 30°C. A Variable Wavelength Detector (VWD) set at 282nm was used. The compounds were identified and quantified based on retention time using [6]-gingerol as HPLC external standard. 10g of cut ginger were blended with 50ml methanol (HPLC grade) by electrical blender for 1min and centrifuged at 5,000rpm for 5min. The supernatant was subsequently filtered through a 0.20 µm Nylon membrane filter (Whatman, England). A 20 µl ginger extract was then subjected to HPLC for the [6]-gingerol analysis.

#### 4.2 Antioxidant activities

The antioxidant activities were determined with two radical scavenging assays: DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay and ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]) radical cation scavenging assay. DPPH assay was performed according to the method of Yamasaki *et al.* (1994). Results were expressed as EC<sub>50</sub> (Efficient Concentration, the amount of sample (µg) needed for 50% decrease in the initial DPPH concentration per 1.0ml of initial solution) and BHT was used as a standard (EC<sub>50</sub> = 13.82 ± 0.38 µg/ml). ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]) radical cation scavenging assay was conducted according to the method of Re *et al.* (1999), and compared with Trolox standards (final concentration 0-15 µM) in ethanol. The higher the value of µmol Trolox, the stronger the antioxidant activity. Results were expressed as µmol Trolox/g extract.

#### 4.3 Total phenolic content

Total phenolic content was determined by the Folin–Ciocalteu method (Miliauskas *et al.*, 2004) and gallic acid (final concentration 0-8 µg/ml) was used as the standard. Results were expressed as mg gallic acid/g extract.

## 5. Statistical analyses

The experimental design was a Complete Randomized Design (CRD). Data were analyzed and expressed as Means  $\pm$  standard deviation using paired T-test to determine statistically significant differences at the  $P \leq 0.05$ . All analyses were conducted using SPSS for Window Version 12.0.

## Results and Discussion

### 1. Determination of [6]-gingerol content and bioactive properties of fresh and dried ginger

The amounts of [6]-gingerol extracted from fresh and dried ginger are shown in Table 1. [6]-gingerol was the major ginger oleoresin. Molecular structure of gingerol consisted of  $\beta$ -hydroxyl keto functional group which was thermally labile. The thermal degradation products of [6]-gingerols including shogaols and aliphatic aldehydes possibly occurred during the drying process (Zhang *et al.*, 1994; Bhattarai *et al.*, 2001). As a result, the dried ginger had a smaller amount of [6]-gingerol compared to the fresh product.

The moisture content, [6]-gingerol and the bioactive properties of fresh and dried ginger including the total phenolic content (mg gallic acid/g extract), antioxidant activities as assayed by DPPH ( $EC_{50}$ ,  $\mu\text{g/ml}$ ) and ABTS ( $\mu\text{mol Trolox/g extract}$ ) are shown in Table 1. Dried ginger showed antioxidant activity significantly ( $P \leq 0.05$ ) higher than that of fresh ginger. The antioxidant activities of fresh and dried ginger, determined by DPPH assay, expressed as  $EC_{50}$ , were  $64.60 \pm 0.18$  and  $32.95 \pm 1.32$   $\mu\text{g/ml}$ , respectively. While ABTS assay of fresh and dried ginger were  $169.06 \pm 3.96$  and  $403.71 \pm 7.24$   $\mu\text{mol Trolox/g extract}$  and total phenolic contents were  $24.63 \pm 0.43$  and  $59.80 \pm 0.14$  mg gallic acid/g extract, respectively. The results indicated that the drying process of ginger caused a significant increase in the total phenolic content, contributing to stronger antioxidant activity. The decrease in the amount of [6]-gingerol during the drying process was observed, in contrast to the amount of total phenolic content and antioxidant activity. In addition, Kikuzaki and Nakatani (1993) reported that thermal degradation products of gingerols such as shogaols and zingerones also exhibited antioxidant activity.

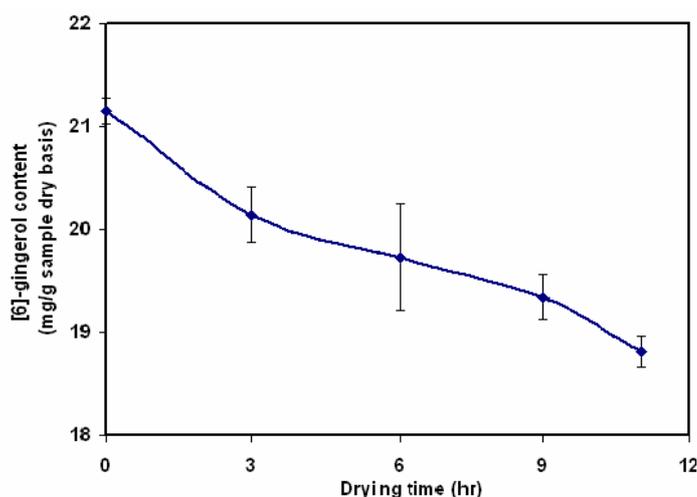
**Table 1.** Chemical and bioactive properties of fresh and dried ginger.

Chemical and bioactive properties	Materials	
	Fresh ginger	Dried ginger
Moisture content (%)	$94.17 \pm 0.16^a$	$11.54 \pm 0.29^b$
[6]-gingerol content (mg/g dry weight basis)	$21.15 \pm 0.13^a$	$18.81 \pm 0.15^b$
Total phenolic content (mg gallic acid/g extract)	$24.63 \pm 0.43^b$	$59.80 \pm 0.14^a$
$EC_{50}$ ( $\mu\text{g/ml}$ ) <sup>1</sup>	$64.60 \pm 0.18^a$	$32.95 \pm 1.32^b$
ABTS assay ( $\mu\text{mol Trolox/g extract}$ )	$169.06 \pm 3.96^b$	$403.71 \pm 7.24^a$

<sup>a,b</sup> means  $\pm$  standard deviation in the same row with different letters is significantly different ( $P \leq 0.05$ )<sup>1</sup> Efficient Concentration; The amount sample ( $\mu\text{g}$ ) needed for 50% decreasing in the initial DPPH concentration per 1.0 ml of initial solution.

## 2. Changes of [6]-gingerol content during drying process

Fresh mature ginger rhizomes containing an initial moisture content of  $94.17 \pm 0.16\%$  were dried in an air dryer at  $55 \pm 2^\circ\text{C}$  for 11 hours to achieve a moisture content of  $11.54 \pm 0.29\%$ . Changes in [6]-gingerol content during this drying process are shown in Figure 2. The results showed that [6]-gingerols decreased gradually as the drying time increased. The amount of [6]-gingerol was decreased from  $21.15 \pm 0.13$  to  $18.81 \pm 0.15$  mg/g dry weight basis. The results also implied that the drying process contributed to the reduction in [6]-gingerol content. Dried ginger contained lower [6]-gingerol content than that of fresh ginger (based on dry weight basis).



**Figure 2.** Changes in [6]-gingerol content during drying process at  $55 \pm 2^\circ\text{C}$ .

## 3. Determination of [6]-gingerol content and bioactive properties of ginger extract from SC-CO<sub>2</sub> extraction

Chemical and bioactive properties of ginger extracts from SC-CO<sub>2</sub> extraction are shown in Table 2. It was found that the ginger extracts using SC-CO<sub>2</sub> contained greater amounts of [6]-gingerols.

The extracts from the conditions at 200 bar and  $35^\circ\text{C}$  had significantly ( $P \leq 0.05$ ) greater amounts of [6]-gingerol than those at 230 bar and  $40^\circ\text{C}$ . The extraction conditions at 230 bar and  $40^\circ\text{C}$  also contributed to a decrease in the amounts of [6]-gingerol and total phenolic content. The extracts obtained from 200 bar at  $35^\circ\text{C}$  and 230 bar at  $40^\circ\text{C}$ , had antioxidant activity (with DPPH method, expressed as EC<sub>50</sub>) of  $13.09 \pm 1.77$  and  $26.68 \pm 1.76$   $\mu\text{g/ml}$ , respectively, compared to BHT standard  $13.82 \pm 0.38$   $\mu\text{g/ml}$ . The antioxidant activity with ABTS method, expressed as  $\mu\text{mol Trolox}$  were  $813.33 \pm 6.67$  and  $724.44 \pm 7.70$   $\mu\text{mol Trolox/g extract}$ , respectively. This study found that the extracts retained high phenolics and high antioxidant activities. The ginger extracts produced from SC-CO<sub>2</sub> extraction were also free from chemical solvents, providing a significant advantage in their potential application as functional ingredients for the food industry.

**Table 2.** Chemical and bioactive properties of ginger extracts from SC-CO<sub>2</sub> extraction.

Chemical and bioactive properties	Ginger extracts from SC-CO <sub>2</sub> extraction	
	200 bar, 35°C	230 bar, 40°C
[6]-gingerol content (mg/g extract)	238.94 ± 0.79 <sup>a</sup>	170.50 ± 0.45 <sup>b</sup>
Total phenolic content (mg gallic acid/g extract)	183.96 ± 1.25 <sup>a</sup>	126.04 ± 0.72 <sup>b</sup>
EC <sub>50</sub> (µg/ml)	13.09 ± 1.77 <sup>b</sup>	26.68 ± 1.76 <sup>a</sup>
ABTS assay (µmol Trolox/g extract)	813.33 ± 6.67 <sup>a</sup>	724.44 ± 7.70 <sup>b</sup>

<sup>a,b</sup> means ± standard deviation in the same row with different letters is significantly different ( $P \leq 0.05$ )

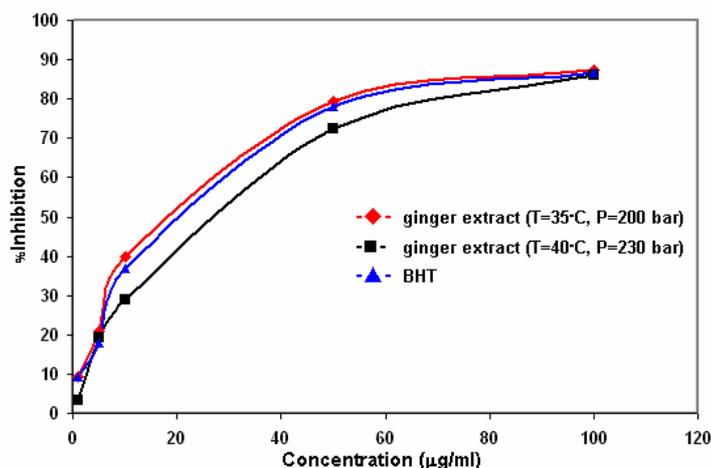
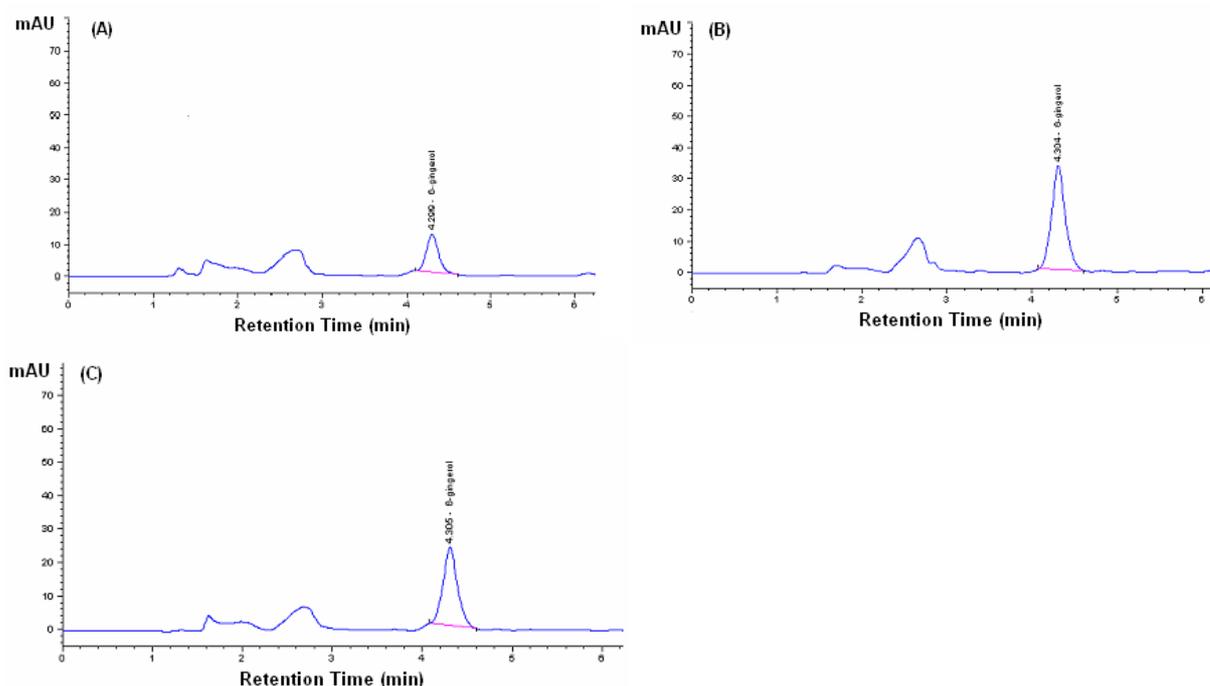
**Figure 3.** % inhibition of ginger extracts from SC-CO<sub>2</sub> extraction, based on DPPH scavenging method (compared with BHT standard).

Figure 3 shows changes in % inhibition (the percentage inhibition of DPPH radicals) of ginger extracts from SC-CO<sub>2</sub> extraction, based on DPPH scavenging method using BHT as a standard. Ginger extracts from SC-CO<sub>2</sub> extraction at 200 bar and 35°C had a higher % inhibition than that at 230 bar and 40°C. At the concentration of 100 µg/ml, ginger extract from 200 bar at 35°C showed 87.29% inhibition of DPPH radicals, while ginger extract from 230 bar at 40°C had 86.85% inhibition and was comparable to the 85.99% inhibition of BHT standard.

The HPLC chromatograms of [6]-gingerol from fresh ginger and extracts obtained from SC-CO<sub>2</sub> extraction are shown in Figure 4. The retention time of [6]-gingerol as shown in the chromatograms varied from 4.2 - 4.3 minutes.



**Figure 4.** The HPLC chromatogram of [6]-gingerol (A) fresh ginger, (B) ginger extract from SC-CO<sub>2</sub> extraction (T = 35 °C, P = 200 bar) and (C) ginger extract from SC-CO<sub>2</sub> extraction (T = 40 °C, P = 230 bar).

## Conclusion

The drying process affected [6]-gingerol content. As the drying time increased the amount of [6]-gingerol decreased. The ginger extract from SC-CO<sub>2</sub> extraction at 200 bar and 35°C had [6]-gingerol content and bioactive activities greater than those at 230 bar and 40°C. The SC-CO<sub>2</sub> extraction method is an ideal alternative, providing extracts with high bioactive properties.

## Acknowledgments

Financial support under the Excellence in Agro-Industry Scholarship for this research from the Faculty of Agro-Industry, Prince of Songkla University is gratefully acknowledged. The authors would also like to thank Ms. Songsuda Promthong, Scientific Equipment Centre, PSU and Dr. Pattama Pittayakhajonwut, Bioresources Research Unit, BIOTEC, for their technical support.

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