Comparative Antioxidant Properties of Some Gingerols and Shogaols, and the Relationship of Their Contents with the Antioxidant Potencies of Fresh and Dried Ginger (Zingiber officinale Roscoe)

J. Guo¹², H. Wu¹, L. Du¹*, W. Zhang², and J. Yang²

ABSTRACT

Ginger (Zingiber officinale Roscoe) contains the biological properties of the major standard non-volatile pungent compounds of ginger, namely, [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols. So far, the comparative antioxidant potencies among shogaols and gingerols have not been studied in detail and reported. Accordingly, this study aimed to examine and compare the antioxidant abilities of the six main compounds. Results showed that [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols exhibited substantial in vitro antioxidant activities. The DPPH•, ABTS•+, and FRAP assays results showed that the antioxidant abilities of [6]–shogaol were greatest among the six gingerols and shogaols studied (P< 0.05), and those of [6]–, [8]–, and [10]–shogaols were greater than those of [6]–, [8]–, and [10]–gingerols, respectively, which can be attributed to the presence of α, β–unsaturated ketones moieties. Moreover, the observation that the antioxidant abilities of [6]–gingerol were greater than those of [8]– and [10]–gingerols (P< 0.05) indicated that the short carbon chains of [6]–gingerol and [6]–shogaol played a significant role in making them more potent antioxidants than the other four longer carbon chain compounds. This finding can be attributed to gingerols undergoing dehydration transformations into shogaols during oven drying. Our results provided some new information on the antioxidant abilities of gingerols and shogaols.

Keywords: α, β–unsaturated ketones moieties, Antioxidant potency, Gingerol, Shogaol, HPLC–UVD

INTRODUCTION

Ginger, the dried rhizome of the plant Zingiber officinale Roscoe, is one of the most widely used spices around the world and is a common condiment for a variety of compounded foods and beverages (Chrubasik et al., 2005; Gupta, 2008). As a medicinal plant, ginger has been widely used in traditional Chinese, Ayurvedic, and Tibb–Unani herbal medicines globally (Ali et al., 2008; Malekizadeh, et al., 2012). Recently, ginger has received increasing attention because of its remarkable antioxidant (El–Ghorab et al., 2010; Mesomo et al., 2012; Oboh et al., 2012), anti–inflammatory (Minghetti et al., 2007), antidiabetic (Afshari et al., 2007), and anticancer activities (Shukla and Singh, 2007; Cheng et al., 2011).

Ginger mainly contains essential oils and oleoresin. Oleoresin is a non–volatile pungent component, and its major constituents have been identified as [4]–, [6]–, [8]–, [10]–, and [12]–gingerols, as well as [6]–, [8]–, and [10]–shogaols, using high–performance liquid chromatography–mass spectrometry (He et al., 1998; Schweiggert et al., 2008; Hu et al., 2011). Shogaols are the corresponding dehydration product of

¹Analytical and Testing Center, Shanxi Normal University, Linfen, Shanxi 041004, People Republic of China.
²College of Engineering, Shanxi Normal University, Linfen, Shanxi 041004, People Republic of China.
*Corresponding author; email: lmd@dns.sxnu.edu.cn
gingerols (Bhattarai et al., 2001; Bhattarai et al., 2007; Wang et al., 2009; Schwertner and Rios, 2007). The chemical structures of [4]–, [6]–, [8]–, [10]–, and [12]–gingerol, as well as [6]–, [8]–, and [10]–shogaols are presented in Figure 1. Recent studies have focused on the relative potencies (Cheng et al., 2011; Dugasani et al., 2010; Pawar, et al., 2011) and quantification (Bhattarai et al., 2007; Balachandran et al., 2006; Qiao and Du, 2011; Salmon et al., 2012; Zhan et al., 2011) of the major standard non-volatile pungent compounds of ginger i.e. [6]–, [8]–, and [10]–gingerols, as well as [6]–shogaol. The extract of fresh ginger has a better flavor and is more pungent, and its major active ingredients are gingerols (Balachandran et al., 2006; Balachandran et al., 2006; Tiwari et al., 2006). However, many researchers have found that [6]–shogaol exhibits the most potent antioxidant properties among the three gingerols and [6]–shogaols (Cheng et al., 2011; Dugasani et al., 2010; Pawar et al., 2011). So far, the comparative antioxidant potencies among shogaols have not been reported, and the comparative antioxidant potencies among [6]–, [8]–, and [10]–gingerols and [6]–, [8]–, and [10]–shogaol, have not been reported yet.

In this study, we examined the antioxidant properties of standard [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols using DPPH, ABTS, and FRAP assays (Moon and Shibamoto, 2009). We also compared the relationships of their contents with the antioxidant potencies of fresh and dried ginger extracts using an HPLC system with an ultraviolet detector (UVD).

**MATERIALS AND METHODS**

1.1–Diphenyl–2–picrylhydrazyl (DPPH), 2’–azinobis–3–ethylbenzothiazoline–6–sulfonic acid ammonium salt (ABTS”), 2,4,6–tripryidyl–s–triazine (TPTZ), FeCl$_3$·6H$_2$O, and 6–hydroxyl–2,5,7,8–tetramethylchroman–2–carboxylic acid (Trolox) were purchased from Acros (New Jersey, USA). The standard [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols were obtained from Chromadex Inc. (Irvine, CA, USA). Standard solutions of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols were prepared with methanol at a concentration of 0.4 mg mL$^{-1}$, and diluted to 0.2 mg mL$^{-1}$ using methanol during antioxidant assays. A series of standards, namely, [6]–gingerol (0.08 mg mL$^{-1}$), [8]–gingerol (0.08 mg mL$^{-1}$), [10]–gingerol (0.08 mg mL$^{-1}$), [6]–shogaol (0.08 mg mL$^{-1}$), [8]–shogaol (0.06 mg mL$^{-1}$), and [10]–shogaol (0.06 mg mL$^{-1}$), was prepared by combining 0.4, 0.4, 0.4, 0.4, 0.3, and 0.3 mL of standard solutions (0.4 mg mL$^{-1}$), respectively. These solutions were diluted to different concentrations using methanol and filtered through a 0.22 µm micro–poly(ether sulfone) (PES) before HPLC analysis. All standards were capped and stored at –20°C until analysis. All chemicals used were analytical grade or HPLC grade. Distilled deionized water or ultra–pure water was used throughout the study.

**Plant Materials and Sample Preparation**

Fresh ginger purchased from Linfen, Shanxi province, China, was washed clean and cut into slices. Twenty grams of fresh ginger was

![Figure 1](image-url)
milled. Dried ginger was prepared from 20 g of fresh ginger slices through oven drying at 55°C for 72 hours until constant weight. The dried samples were obtained in powder form after oven drying at 55°C and grinding. Extractions of fresh and dried ginger were carried out at 40°C for 48 hours using methanol according to the method of ISO 13685: 1997 (E) with some modifications. Then, the samples were filtered, transferred to a 100 mL volumetric flask, diluted to mark with methanol, and thoroughly mixed for antioxidant assays. The samples were filtered through a 0.22 µm micro–PES flat membrane and stored until HPLC analysis.

Measurements of Antioxidant Assays Expressed as Trolox Equivalents (TE)

Antioxidant assays were performed using a Cary 300 UV–visible spectrophotometer (Varian, USA) with 2 or 10 mm quartz cells (Yixing jingke Optical Instrument Co., Ltd., China). The DPPH assay was done according to the method of Floegel et al. (2011) and Schwarz et al. (2001). The stock solution (1 mM) was prepared by dissolving 19.7 mg DPPH with 50 mL methanol and then stored at −20°C. The working solution was adjusted to 0.8±0.01 at 515 nm by methanol using the spectrophotometer. Then, 20 µL of Trolox (0–1,000 µM), standard solutions or samples and 4 mL of reagent were incubated in a water bath at 37°C for 30 minutes in the dark. After incubation, the spectra were scanned and the absorbance was measured at 596 nm. A stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O solution. All three solutions were mixed together in the ratio 10:1:1. Reaction mixtures containing 20 µL of Trolox (0–1,000 µM), standard solutions or samples and 4 mL of reagent were incubated in a water bath at 37°C for 30 minutes in the dark. After incubation, the spectra were scanned and the absorbance was measured at 596 nm. A stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O solution blank was also submitted to the same procedure and measured in parallel to the standards and samples. For all assays, the results were expressed in µmol TE g⁻¹ or kg gingerol, shogaol, or ginger mass.

HPLC Analysis

The standard solution was prepared as described in the section “Plant Materials and Sample Preparation”. The standard solutions and ginger extracts were analyzed on an HPLC system comprising a Waters 1525 binary HPLC pumps fitted with a 20 µL Hamilton syringe, a Waters 2489 dual wavelength UV–visible detector set at 280 nm, and a Waters Symmetry Shield RP–C18 column (5 µm, 250x4.6 mm²; Waters, Milford, MA, USA). The HPLC operating parameters were according to Hu et al. (2011), with some modifications: injection volume, 10 µL; flow rate, 1.0 mL min⁻¹; chromatographic run time, 62 minutes; and eluents, acetonitrile (A) and 1% glacial acetic acid (B). The gradient elution had the
following profile: 0–10 minutes, 45–50% A; 10–20 minutes, 65% A; 20–40 minutes, 95% A; 40–50 minutes, 100% A; 50–52 minutes, 45% A; and 52–62 minutes, 45% A. The column temperature was 48 °C. The peaks of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols in the ginger extracts were identified based on the comparison of their retention times with that of the corresponding standards. The concentrations in each sample were calculated by comparing their response with the corresponding standard curves.

**Statistical Analysis**

Each standard and sample was measured in triplicate. The mean and standard deviation (n= 3) were calculated. The data were statistically analyzed at the significant level of P< 0.05 using Levene’s test for homogeneity and Duncan’s multiple range test with SAS version 9.2 English (Rafiee et al., 2012).

**RESULTS AND DISCUSSION**

**Antioxidant Abilities of Gingerols and Shogaols.**

<table>
<thead>
<tr>
<th>Methods</th>
<th>DPPH’</th>
<th>ABTS**</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard curves</td>
<td>$A = -0.0004C + 0.7516$</td>
<td>$A = -0.0001C + 0.541$</td>
<td>$A = 0.0009C - 0.0077$</td>
</tr>
<tr>
<td>R²</td>
<td>0.9985</td>
<td>0.9989</td>
<td>1</td>
</tr>
<tr>
<td>Linearity range (µM TE)</td>
<td>0–1500</td>
<td>50–4000</td>
<td>0–1,000</td>
</tr>
<tr>
<td>[6]–G</td>
<td>4712 ± 166b</td>
<td>8040 ± 937b</td>
<td>6060 ± 96b</td>
</tr>
<tr>
<td>[8]–G</td>
<td>3774 ± 272c</td>
<td>5623 ± 475c</td>
<td>4479 ± 168c</td>
</tr>
<tr>
<td>[10]–G</td>
<td>3791 ± 156c</td>
<td>4673 ± 247c</td>
<td>3330 ± 170c</td>
</tr>
<tr>
<td>[6]–S</td>
<td>7308 ± 131a</td>
<td>14657 ± 1227a</td>
<td>12690 ± 160a</td>
</tr>
<tr>
<td>[8]–S</td>
<td>4370 ± 45b</td>
<td>5390 ± 265c</td>
<td>4473 ± 55c</td>
</tr>
<tr>
<td>[10]–S</td>
<td>4616 ± 394b</td>
<td>5607 ± 462c</td>
<td>4108 ± 157d</td>
</tr>
</tbody>
</table>

“A” is absorbance. The difference in the activities of different compounds was evaluated by Duncan’s multiple range test. Different letters in the same column indicate significant difference. Data are the mean±standard deviation of a single sample with triplicate measurements.

The antioxidant activities of the standards and ginger extracts by DPPH’, ABTS**, and FRAP assays were measured three times to test the reproducibility of the assays. The DPPH’, ABTS**, and FRAP standard curves were $A = -0.0004C + 0.7516$, $A = -0.0001C + 0.541$, and $A = 0.0009C - 0.0077$ respectively (C: µM TE). The linear ranges were 0–1,500 µM TE, 50–4,000 µM TE, and 0–1,000 µM TE, respectively. Table 1 shows the antioxidant activities of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols for scavenging DPPH’, ABTS**, and reducing ferric. The results indicated that [6]–shogaol > [6]–gingerol ≈ [10]–shogaol = [8]– shogaol > [10]–gingerol (P< 0.05) using DPPH’ test. Also, [6]–shogaol > [6]–gingerol > [8]–gingerol = [10]–shogaol = [8]– shogaol > [10]–gingerol (P< 0.05) using ABTS** test; while [6]–shogaol > [6]–gingerol > [8]–gingerol = [8]– shogaol > [10]–shogaol > [10]–gingerol (P< 0.05), using FRAP test. In summary, [6]–gingerol exhibited the highest antioxidant ability among the gingerols, and [6]–shogaol exhibited the highest antioxidant ability among the shogaols.

Table 1. Comparison of the antioxidant activities of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols using DPPH’, ABTS**, and FRAP assays.a
Table 1 also indicates that [6]–shogaol> [6]–gingerol (P< 0.05) using all the three tests; [8]–shogaol> [8]–gingerol (P< 0.05) using DPPH* test; [8]–shogaol = [8]–gingerol (P> 0.05) using ABTS** and FRAP tests; [10]–shogaol> [10]–gingerol (P< 0.05) using DPPH* test; [10]–shogaol> [10]–gingerol (P> 0.05) using ABTS** test; and [10]–shogaol> [10]–gingerol (P< 0.05) using FRAP test. In summary, the antioxidant abilities of [6]–, [8]–, and [10]–shogaols were greater than those of [6]–, [8]–, and [10]–gingerols, respectively.

In this study, [6]–shogaol exhibited the most potent antioxidant properties among the six compounds, which can be attributed to the presence of α, β–unsaturated ketone moieties. A previous study has found that the antioxidant abilities of [6]–shogaol> [10]–gingerol> [8]–gingerol (Dugasani et al., 2010). However, the present work proved that the antioxidant properties of [6]–gingerol were second only to [6]–shogaol. We concluded that the short carbon chains of [6]–gingerol and [6]–shogaol played a significant role in making them more potent among the six compounds. Our statistics differs from the viewpoint of the carbon chain length playing a significant role in making [10]–gingerol as the most potent among all the gingerols (Dugasani et al., 2010).

Comparison of the Antioxidant Abilities of Fresh and Dried Ginger

Figure 2 shows that the UV–visible spectra of DPPH*, ABTS**, and FRAP had a good reproducibility and the maximum absorbance of DPPH*, ABTS**, and FRAP were at 515 nm, 731 nm and 596 nm. Figure 3 shows that the antioxidant abilities of dried ginger were obviously higher than those of fresh ginger. The antioxidant abilities of dried ginger were approximately 1.4–, 1.4–, and 1.6–fold higher than those of fresh ginger, respectively. This finding justified the use of dried ginger in traditional medicine.

Contents of Three Gingerols and Three Shogaols of Fresh and Dried Ginger

Figure 4 shows that the HPLC chromatograms of the methanolic extracts of fresh and dried ginger are similar, with dried ginger extracts showing some constituents not present in fresh ginger. It was found that dried ginger showed considerably higher peak areas of [6]–, [8]–,
Figure 3. Comparison of the antioxidants abilities of fresh and dried ginger using DPPH•, ABTS•⁺ and FRAP assays. The letter a and b indicate significant difference in all the three columns (P < 0.05); data are the mean±standard deviation of a single sample with triplicate measurements.

Figure 4. HPLC separation of the pungent principles (280 nm) from: (a) Fresh ginger; (b) Dried ginger, and (c) A standard mixture of (1) [6]–gingerol (0.08 mg mL⁻¹), (2) [8]–gingerol (0.08 mg mL⁻¹), (3) [6]–shogaol (0.08 mg mL⁻¹), (4) [10]–gingerol (0.08 mg mL⁻¹), (5) [8]–shogaol (0.06 mg mL⁻¹) and (6) [10]–shogaol (0.06 mg mL⁻¹).
Table 2. Quantification of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols in fresh (FG) and dried (DG) ginger.

<table>
<thead>
<tr>
<th>Standards</th>
<th>Standard curves</th>
<th>Linearity range (µg mL⁻¹)</th>
<th>Found (µg mL⁻¹)</th>
<th>Contents (mg g⁻¹ dried mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6]–G</td>
<td>( A = 7608650 \times C – 1532 )</td>
<td>1 1–200</td>
<td>165.70±1.67 58.79±0.91</td>
<td>8.29 ± 0.08(^a) 2.94 ± 0.05(^b)</td>
</tr>
<tr>
<td>[8]–G</td>
<td>( A = 5350397 \times C – 1545 )</td>
<td>1 1–200</td>
<td>38.64±0.60 7.72±0.09</td>
<td>1.93 ± 0.03(^a) 0.39 ± 0.00(^b)</td>
</tr>
<tr>
<td>[6]–S</td>
<td>( A = 8858646 \times C – 3797 )</td>
<td>0.9998 0.1–200</td>
<td>0.73±0.01 11.93±0.24</td>
<td>0.12 ± 0.04(^a) 1.19 ± 0.02(^a)</td>
</tr>
<tr>
<td>[10]–G</td>
<td>( A = 17717292 \times C – 3797 )</td>
<td>0.9996 1–200</td>
<td>47.75±1.01 23.86±0.42</td>
<td>2.39 ± 0.05(^a) 1.19 ± 0.02(^b)</td>
</tr>
<tr>
<td>[8]–S</td>
<td>( A = 6553879 \times C – 1200 )</td>
<td>0.9999 0.1–200</td>
<td>0.47±0.01 6.76±0.14</td>
<td>0.02 ± 0.00(^b) 0.34 ± 0.01(^b)</td>
</tr>
<tr>
<td>[10]–S</td>
<td>( A = 6667492 \times C – 2026 )</td>
<td>0.9997 0.1–200</td>
<td>0.76±0.02 11.51±0.23</td>
<td>0.04 ± 0.00(^b) 0.58 ± 0.01(^b)</td>
</tr>
</tbody>
</table>

\(^a\) “A” is peak area; the letter a and b indicate significant difference in the same row (P< 0.05); data are the mean±standard deviation of a single sample with triplicate measurements.

and [10]–shogaols than fresh ginger, whereas fresh ginger showed considerably higher peak areas of [6]–, [8]–, and [10]–gingerols than dried ginger. This finding can be attributed to gingerols undergoing dehydration transformations into shogaols during oven drying. Table 2 shows that the contents of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols significantly differed (P< 0.05) between fresh and dried ginger. The levels of 6–, 8–, and 10–shogaols in dried ginger were approximately 9.9–, 17.0–, and 14.5–fold higher than those in fresh ginger, respectively. The relationship of the contents of [6]–, [8]–, and [10]–shogaols with the antioxidant potencies of fresh and dried ginger showed that compounds [6]–, [8]–, and [10]–shogaols played a more important role in dried ginger than in fresh ginger.

A previous study has shown that [6]–gingerol is degraded to form [6]–shogaol, and vice versa in aqueous solutions (Bhattarai et al., 2001). In the present study, we found that all three gingerols were partly degraded to form shogaols in dried ginger during oven drying coupled with a significant increase in antioxidant abilities. Because gingerols have acidic methylene protons, they tended to undergo dehydration to form shogaols (Fukuda et al., 1996), which caused the dehydration transformation of all of [6]–, [8]–, and [10]–gingerols to, respectively, [6]–, [8]–, and [10]–shogaols by thermal treatment. The results provided some different information on the antioxidant potencies of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols, which contributed to the extensive study on ginger. Our further research is being designed to increase the contents of [6]–, [8]– and [10]–shogaols through ginger process.

CONCLUSIONS

The DPPH•, ABTS•⁺, and FRAP assays results showed that the antioxidant abilities of [6]–shogaol were greatest among the six gingerols and shogaols we studied (P< 0.05), and those of [6]–, [8]–, and [10]–shogaols were greater
than those of [6]–, [8]–, and [10]–gingerols, respectively. This was attributed to the presence of α, β-unsaturated ketone moieties of [6]–shogaol, and the presence of short carbon chains of [6]–gingerol and [6]–shogaol, which made their antioxidants more potent than the other four long carbon chain compounds. Also, based on the results, there existed correlation between the antioxidant properties and the contents of [6]–, [8]–, and [10]–shogaols in ginger.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Funds of China (21171110) and Research Funds for the Doctoral Program of Higher Education of China (20091404110001).

REFERENCES

مقایسه خواص آنتی اکسیدانی برخی چنگل‌ها وشوگول‌ها و رابطه مقدار آنها با
توان آنتی اکسیدانی چنگل تازه و خشک (Zingiber officinale Roscoe)


cj. گو. ه. وو. ل. دو. و. زانگ. و. ج. یانگ

چکیده

α، β-unsaturated ketones (moieties) ممکن است به خاطر حضور لیمیت کتون های غیر اشباع آلفا و با (P < 0.05) آن مشاهده کننده نمایند که توان آنتی اکسیدانی جنگلولهایی [6] و شوگول [6] بیشتر بود (0.05 > P) چون اشکار داشت که زنجبیر خون‌های تر کریم در جنگلولهایی [6] و شوگول [6] نسبت به آن‌ها در مقایسه با چهار ماده دیگر که زنجبیر کریمی طولانی‌تر دارای داشته است. این یافته را می‌توان به این نسبت داده که جنگلول ها به علت از دست دادن آب در طی خشک شدن در اجاق به شوگول تبدیل می‌شوند. نتایج ما اطلاعات تازه ای درباره توان آنتی اکسیدانی چنگل‌ها وشوگول‌ها فراهم آورده است.