

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)

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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

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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; Polasa and Nirmala, 2003; 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

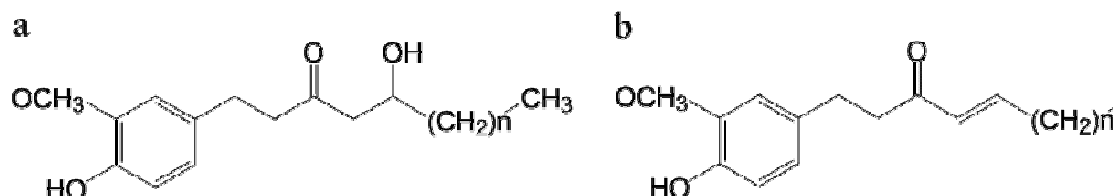


Figure 1. Basic chemical structures of selected: (a) Gingerols ($n = 2, 4, 6, 8$, and 10 ; 4–, 6–, 8–, 10–, and 12–gingerols) and (b) Shogaols ($n = 4, 6$, and 8 ; 6–, 8–, and 10–shogaols).

Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH[•]), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ammonium salt (ABTS^{•+}), 2,4,6-tripyridyl-*s*-triazine (TPTZ), FeCl₃·6H₂O, 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

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 (0–1500 µM), standard solutions (0.2 mg mL⁻¹) or samples were mixed with 1 mL of DPPH[•] solution and 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 515 nm. A methanol blank was also submitted to the same procedure and measured in parallel to the standards and samples.

The ABTS^{•+} assay was based on the method of Thaipong *et al.* (2006). When combined with an oxidant (2.45 mM potassium persulfate), ABTS^{•+} (5 mM in 20 mM sodium acetate buffer, pH 4.5) reacted to create a stable, dark blue–green radical solution following 12–16 hours of incubation in the dark (4°C). The solution was then diluted to an absorbance of 0.6±0.01 at 731 nm to form the test reagent. Reaction mixtures containing 20 µL of Trolox

(50–4,000 µM), standard solutions or samples and 1 mL of ABTS^{•+} 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 731 nm. A 20 mM sodium acetate buffer (pH 4.5) blank was also submitted to the same procedure and measured in parallel to the standards and samples.

The ferric reducing/antioxidant power (FRAP) assay was done according to Wootton–Beard *et al.* (2011). The stock solutions included 300 mM acetate buffer (3.1 g CH₃COONa·3H₂O and 16 mL CH₃COOH), 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 including 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, 250×4.6 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 1. Comparison of the antioxidant abilities of [6]–, [8]–, and [10]–gingerols, as well as [6]–, [8]–, and [10]–shogaols using DPPH[•], ABTS^{•+}, and FRAP assays.^a

Methods	DPPH [•]	ABTS ^{•+}	FRAP
Standard curves	$A = -0.0004 \times C + 0.7516$	$A = -0.0001 \times C + 0.541$	$A = 0.0009 \times C - 0.0077$
R ²	0.9985	0.9989	1
Linearity range (μM TE)	0–1500	50–4000	0–1000
Antioxidant abilities (μmol TE g ⁻¹)	[6]–G	4712 ± 166 ^b	6060 ± 96 ^b
	[8]–G	3774 ± 272 ^c	4479 ± 168 ^c
	[10]–G	3791 ± 156 ^c	3330 ± 170 ^c
	[6]–S	7308 ± 131 ^a	12690 ± 160 ^a
	[8]–S	4370 ± 45 ^b	4473 ± 55 ^c
	[10]–S	4616 ± 394 ^b	4108 ± 157 ^d

^a "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 ≈ [8]–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 also indicates that [6]-shogaol > [6]-gingerol ($P < 0.05$) using all the three tests; [8]-shogaol > [8]-gingerol ($P < 0.05$) using DPPH $^{\bullet}$ test; [8]-shogaol \approx [8]-gingerol ($P > 0.05$) using ABTS $^{++}$ and FRAP tests; [10]-shogaol > [10]-gingerol ($P < 0.05$) using DPPH $^{\bullet}$ 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 > [6]-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 $^{\bullet}$, ABTS $^{++}$, and FRAP had a good reproducibility and the maximum absorbance of DPPH $^{\bullet}$, 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.

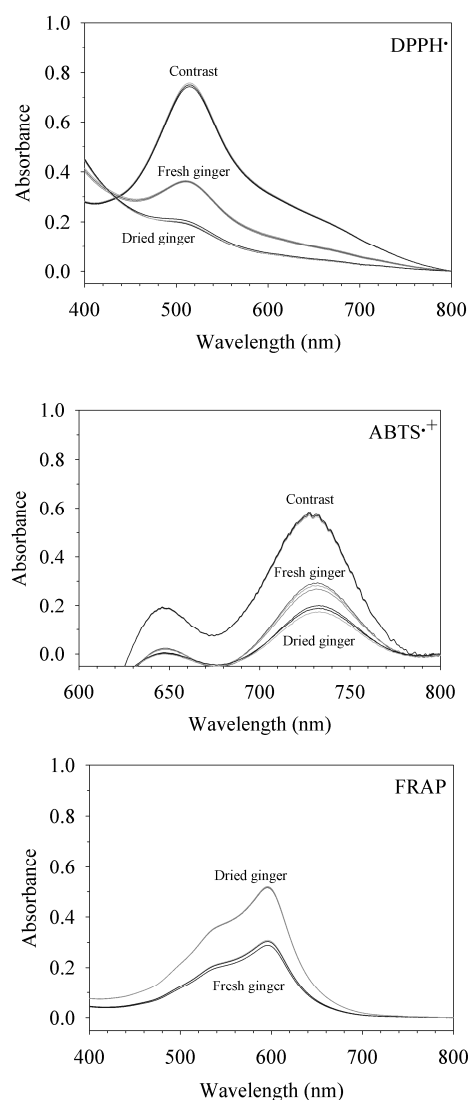


Figure 2. Absorption spectra of DPPH $^{\bullet}$, ABTS $^{++}$ and FRAP tests for contrast, fresh ginger and dried ginger.

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]-,

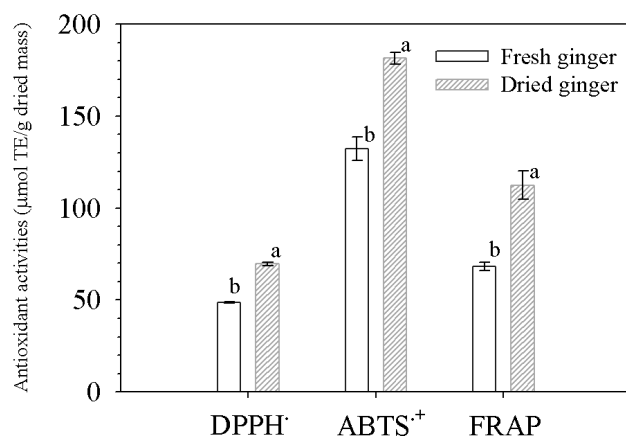


Figure 3. Comparison of the antioxidants abilities of fresh and dried ginger using DPPH[•], ABTS^{•+} and FRAP assays. The letter a and b indicate significant difference in all the three columns ($P < 0.05$); data are the mean \pm standard deviation of a single sample with triplicate measurements.

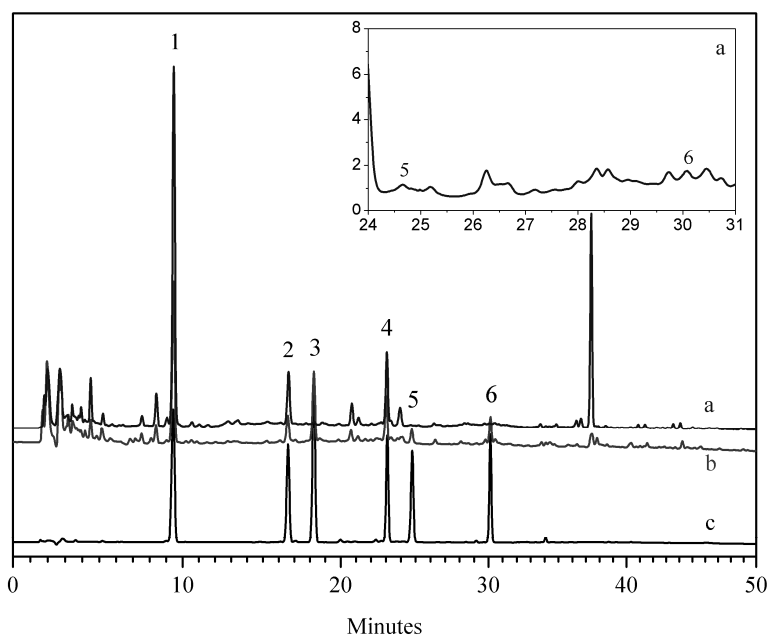


Figure 4. HPLC separation of the pungent principles (280 nm) from: (a) Fresh ginger; (b) Dried ginger, and (c) A standard mixture of (1) [6]-gingerol (0.08 mg mL^{-1}), (2) [8]-gingerol (0.08 mg mL^{-1}), (3) [6]-shogaol (0.08 mg mL^{-1}), (4) [10]-gingerol (0.08 mg mL^{-1}), (5) [8]-shogaol (0.06 mg 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.^a

Standards	Standard curves	R ²	Linearity range (μg mL ⁻¹)	Found (μg mL ⁻¹)		Contents (mg g ⁻¹ dried mass)	
				FG	DG	FG	DG
[6]–G	$A = 7608650 \times C - 1532$	1	1–200	165.70±1.67	58.79±0.91	8.29 ± 0.08 ^a	2.94 ± 0.05 ^b
[8]–G	$A = 5350397 \times C - 1545$	1	1–200	38.64±0.60	7.72±0.09	1.93 ± 0.03 ^a	0.39 ± 0.00 ^b
[6]–S	$A = 8858646 \times C - 3797$	0.9998	0.1–200	0.73±0.01	11.93±0.24	0.12 ± 0.04 ^b	1.19 ± 0.02 ^a
[10]–G	$A = 17717292 \times C - 3797$	0.9996	1–200	47.75±1.01	23.86±0.42	2.39 ± 0.05 ^a	1.19 ± 0.02 ^b
[8]–S	$A = 6553879 \times C - 1200$	0.9999	0.1–200	0.47±0.01	6.76±0.14	0.02 ± 0.00 ^b	0.34 ± 0.01 ^a
[10]–S	$A = 6667492 \times C - 2026$	0.9997	0.1–200	0.76±0.02	11.51±0.23	0.04 ± 0.00 ^b	0.58 ± 0.01 ^a

^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]–gingerols, as well as [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 ketones 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).

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مقایسه خواص آنتی اکسیدانی برخی جینجرها و شوگول ها و رابطه مقدار آنها با توان آنتی اکسیدانی زنجبیل تازه و خشک (*Zingiber officinale* Roscoe)

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چکیده

زنجبیل (*Zingiber officinale* Roscoe) دارای خواص بیولوژیکی مواد غیر تصعیدی استاندارد با طعم تند است که به نام جینجرولهای [۶]، [۸] و [۱۰] و شوگول های [۶]، [۸] و [۱۰] شناخته می شوند. تاکنون توان آنتی اکسیدانی شوگول ها و جینجرول ها به جزئیات مورد مقایسه قرار نگرفته و گزارش نشده است. بنا براین در این پژوهش توان آنتی اکسیدانی این شش ماده اصلی بررسی و مقایسه شد. بر اساس نتایج، جینجرولهای [۶]، [۸] و [۱۰] و نیز شوگول های [۶]، [۸] و [۱۰] فعالیت آنتی اکسیدانی چشمگیری در آزمایشگاه نشان دادند. نتایج سنجش با مواد $ABTS^{++}$ و $DPPH^*$ ، و FRAP نشان داد که در بین شش ماده مطالعه شده، توان آنتی اکسیدانی شوگول [6] بیشترین بود ($P < 0.05$) و اینکه توان شوگول های [۶]، [۸] و [۱۰] از جینجرول های نظیرشان بیشتر بود. این امر ممکن است به خاطر حضور نیمه کتون های غیر اشباع آلفا و بتا (α, β -unsaturated ketones moieties) باشد. به علاوه، این مشاهده که توان آنتی اکسیدانی جینجرول [۶] از جینجرولهای [۸] و [۱۰] بیشتر بود ($P < 0.05$) چنین اشاره داشت که زنجیره کوتاه تر کربن در جینجرول [۶] و شوگول [6] نقش عمده ای در افزایش توان آنتی اکسیدانی آنها در مقایسه با چهار ماده دیگر که زنجیره کربنی طولانی تری دارند داشته است. این یافته را می توان به این نسبت داد که جینجرول ها به علت از دست دادن آب در طی خشک شدن در اجاق به شوگول تبدیل می شوند. نتایج ما اطلاعات تازه ای در باره توان آنتی اکسیدانی جینجرول ها و شوگول ها فراهم آورده است.