Heat treatments of ginger root modify but not diminish its antioxidant activity as measured with multiple free radical scavenging (MULTIS) method

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Ginger (Zingiber officinale Rosc.) root (or rhizome) has been reported to have antioxidant properties such as reactive oxygen species scavenging activities. Using multiple free-radical scavenging method, we have newly determined the scavenging abilities of ginger roots against five reactive oxygen species, i.e., HO•, O2•−, RO•, tert-BuOO•, and O2. After heating grated ginger roots at 80°C for 2 h, nearly 50% decrease in scavenging ability was recorded against O2•− and tert-BuOO•. Conversely, the O2•− scavenging ability increased by about 56% after heat treatment. Based on the antioxidant activity measurement of the ginger’s components, i.e., 6-gingerol, 6-shogaol, and zingerone, active species acting as antioxidant capacity of ginger was shown. Additionally, ginger’s antioxidant capacity was quantitatively compared with that of rosemary extract, indicating that rosemary is peroxyl specific scavenger while ginger has higher scavenging ability against HO• and O2•−.

Key Words: ginger, antioxidant capacity, multiple free-radical scavenging method, MULTIS, ESR spin trapping

The rhizome (root) of ginger (Zingiber officinale Rosc.) is widely used as a spice and herbal medicine. The main pungent component gingerol has been shown to have a variety of biological properties, including anticancer, antioxidant, anti-inflammatory, and antifungal. Nevertheless, the effective component that is responsible to such activities is not well understood. 6-Gingerol, a ginger’s major pharmacologically active component is considered an important natural antioxidant of food origin and has been shown to decrease peroxidation of phospholipid liposomes in vitro. The other component 6-shogaol has many biological effects such as antibacterial and antioxidant activities. Zingerone and shogaol, the dehydrated form of gingerol, are formed during thermal processing and long-term storage of ginger roots. Based on the scavenging ability against a synthetic free radical compound, Dugasani et al. have demonstrated that 6-shogaol may have potent free radical scavenging properties. However, the direct scavenging abilities against various biologically-relevant active species have not been evaluated yet.

In previous studies, the scavenging activities of antioxidants are determined by using stable synthetic free radical such as DPPH (diphenyl picryl hydrazly) or galvinoxyl. However, it is reasonable to speculate that the reactivity of antioxidants with stable synthetic radical may be drastically different from those with various biologically active species. Recently, ESR (electron spin resonance) spin-trapping based multiple free-radical scavenging (MULTIS) method has been developed. In that method, various reactive oxygen species were photochemically generated, against which antioxidant’s scavenging ability was quantified. Thus, direct scavenging abilities of hydrophilic antioxidants were determined in cattle and human sera against reactive oxygen species, such as hydroxyl, superoxide, and singlet oxygen. Also, the MULTIS method has been shown to be a useful method in the determination of comparative antioxidant capacity in plant specimens.

In this study, both lipophilic and hydrophilic antioxidants in ginger were solubilized and diluted in a mixture of acetonitrile and water. Acetonitrile itself has negligible active species scavenging ability and was expected to act as a pure medium. After such modification, we have determined the direct scavenging abilities of gingers (Zingiber officinale) produced in Japan against five reactive oxygen species (hydroxyl radical, superoxide, alkoxyl radical, peroxyl radical, and singlet oxygen). This paper is the first to show that the MULTIS method is useful in the determination of the antioxidant activity in food. We found that the heat treatment of the ginger specimen either diminished or enhanced antioxidant activities, depending on the reactive oxygen species scavenged. Also, we elucidated the cause of heat-mediated alteration of the antioxidant capacity.

Materials and Methods

Materials. Spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was purchased from Tokyo Chemical Ind. (Tokyo, Japan) and was used for the detection of hydroxyl (HO•) and alkoxyl (RO•) radicals. For superoxide (O2•−) and alkoxyl peroxyl radical (tert-BuOO•), 5-(2,2-dimethyl-1,3-propoxycyclophosphoranyl)-5-methyl-1-pyrroline N-oxide (CYPMO) was used because CYPMO has better trapping ability for O2•− and tert-BuOO• than DMPO. CYPMO was obtained from Shidai Systems (Saitama, Japan). For the detection of singlet oxygen (1O2), 4-hydroxy-2,2,6,6-tetramethylpiperidine (4-CHO-TEMP) was obtained from Tokyo Chemical Ind. The precursors and sensitizers for the formation of active species were reported elsewhere. Ginger-related antioxidant compounds (6-gingerol and zingerone (purity >98%) were obtained from Tokyo Chemical Ind. and used as received. 6-Shogaol was prepared according to the method reported by Okamoto et al. Identification was made by 1H NMR (nuclear resonance) spin-trapping based multiple free-radical scavenging (MULTIS) method has been developed. In that method, various reactive oxygen species were photochemically generated, against which antioxidant’s scavenging ability was quantified. Thus, direct scavenging abilities of hydrophilic antioxidants were determined in cattle and human sera against reactive oxygen species, such as hydroxyl, superoxide, and singlet oxygen. Also, the MULTIS method has been shown to be a useful method in the determination of comparative antioxidant capacity in plant specimens.

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magnetic resonance) measurements (purity >97%). Acetonitrile (Wako Pure Chemical Industries Ltd., Osaka, Japan) and water purified by distillation were used as a mixture solvent.

**Methods.** The essence of the MULTIS method is to photolitically generate a finite amount of free radicals (reactive oxygen species) in the absence or presence of the known amount of antioxidant-containing specimen. The decrease of active species concentration due to the sample’s antioxidant activity is quantified with ESR spin trapping method.

**Sampling procedures of ginger and ESR measurements.** Yellow gingers (Zingiber officinale) harvested in two prefectures were used (sample A and B): the ginger harvested in October was stored at ambient temperature and 90% humidity. Liquid ginger samples (n = 3) were obtained through grating ginger roots followed by squeezing with a piece of cloth. The grated ginger samples (contains 3–10 mM $\cdot$BuOO •) were stored at ambient temperature and 90% humidity. Liquid samples such as plant extracts has been hampered by the lack of standard. In the case of pure antioxidant, trolox has been used as a standard antioxidant and antioxidant capacity is often expressed in trolox equivalent unit. In this study, we used scavenging rate (ST) undergo competitive scavenging reactions against free radical (R') as follows:

$$\text{ST} + \text{R'} \rightarrow k_{\text{ST}} \rightarrow \text{Spin adduct}$$

$$\text{AOx}(1) + \text{R'} \rightarrow k_{1} \rightarrow \text{Product-1}$$

$$\text{AOx}(2) + \text{R'} \rightarrow k_{2} \rightarrow \text{Product-2}$$

$$\vdots$$

$$\text{AOx}(n) + \text{R'} \rightarrow k_{n} \rightarrow \text{Product-n}$$

Therefore, ginger’s total scavenging ability can be expressed as the sum of each component’s scavenging rate:(9)

$$\frac{v_{\text{ginger}}}{v_{\text{ST}}} = \frac{I_{0} - I}{I} = \frac{\sum_{i=1}^{n} k_{i} \alpha_{i} [\text{AOx}]_{0}}{k_{\text{ST}} [\text{ST}] [\text{R'}]} = \frac{\sum_{i=1}^{n} k_{i} \alpha_{i} [\text{AOx}]_{0}}{k_{\text{ST}} [\text{ST}] [\text{R'}]}$$

where the $[\text{AOx}]_{0}$ symbol denotes the initial concentration (M) of spin traps and the ginger antioxidant components. [AOx] and [ST (mol)] express the concentration in volume (%) and ST amount by mole, respectively. $\alpha_{i} = [\text{AOx}]_{i}/[\text{ST}]_{i}$ are constants. Plotting $(I_{0} - I)/I$ against $[\text{AOx}]_{0}/[\text{ST}]_{0}$ enables us to calculate relative scavenging rate of grated ginger (100%) in 1 mM ST solution ($v_{\text{ginger}}/v_{\text{ST}}$).

**Results**

Antioxidant activities of grated ginger and components. Using the ESR peak heights $I_{0}$ and $I$ in the absence and presence of grated ginger, the relative scavenging rates ($v_{\text{ginger}}/v_{\text{ST}}$) against 1 mM ST were calculated using Eq. (2). The results for the ginger samples at 25°C and heated at 80°C for 2 h are listed in Table 1, together with the active species scavenging rates of rosemary extracts. The ESR measurements of antioxidant abilities were repeated 5 times for the same ginger and rosemary samples, and the error was shown as probable error. Trolox was selected as the standard scavenger and relative scavenging rates of grated ginger and rosemary extract against 100 mM trolox solution were expressed in trolox equivalent units (TEU/100) (Table 1).

The relative scavenging rates for ginger samples (1 and 2) against reactive oxygen species can be expressed by using Eq. (2) as follows:

$$\frac{v_{\text{ginger}}}{v_{\text{ST}}} = \frac{I_{0}/I_{\text{ginger}} - 1}{I_{0}/I_{\text{ST}} - 1}$$

Fig. 1a and b show the radar chart for the scavenging rates in heat-treated ginger samples (A and B). Furthermore, Fig. 1c illustrates the radar chart for the antioxidant activities (scavenging rates) of grated ginger and rosemary extract as relative numbers with respect to the standard antioxidant trolox.
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**Table 1.** Relative scavenging abilities of grated ginger against five reactive oxygen species in acetonitrile and water (1:1, v/v) mixture at room temperature

| Ginger          | HO• | O₂• | RO• | tert-BuOO• | ¹O₂ |
|-----------------|-----|-----|-----|------------|-----|
| Sample A        | 3,360 ± 180 | 1,400 ± 102 | 345 ± 18 | 655 ± 31 | 233,000 ± 9,000 |
| Sample A (heated) | 3,810 ± 160 | 2,180 ± 78 | 344 ± 16 | 324 ± 10 | 98,000 ± 3,900 |
| Sample B        | 4,720 ± 110 | 1,930 ± 98 | 645 ± 32 | 662 ± 29 | 299,000 ± 12,000 |
| Sample B (heated) | 4,800 ± 120 | 2,980 ± 120 | 535 ± 10 | 440 ± 9 | 190,000 ± 11,000 |
| Rosemary (Jan. 2017) | 1,780 ± 170 | 7,570 ± 830 | 3,010 ± 50 | 5,000 ± 250 | 251,500 ± 18,400 |
| Trolox (100 mM) | 3,190 ± 330 | 8,390 ± 230 | 11,500 ± 300 | 2,830 ± 130 | 236,000 ± 7,000 |

ST = DMPO for HO• and RO•, CYP-MPO for O₂• and tert-BuOO•, and 4-HO-TEMP for ¹O₂. The HO• scavenging ability in an acetonitrile-water mixture (3:7, v/v). Alkoxyl radical derived from AAPH. Peroxyl radical derived from tert-butylhydroperoxide. Rosemary harvested in January 2017.

![Fig. 1. Radar chart illustration of relative scavenging rates for ginger sample A (a) and sample B (b). Radar charts show the MULTIS values in liquid ginger samples at 25°C (black line) and ones heated at 80°C (gray line). The MULTIS values at 25°C are set to 1.0, thus its radar chart is a normal pentagon (black line). (c) The relative scavenging rates of grated ginger sample B and rosemary extract harvested in Okayama region (January 2017). TEU100 (equivalent to trolox using 100 mM trolox solution as standard) was employed as a standard MULTIS number.](image)

Discussion

Using the ESR spin-trapping based MULTIS method, we have determined the scavenging abilities of grated ginger against five reactive oxygen species. By heat treatment, we showed that grated ginger increased O₂• scavenging ability and decreased the ¹O₂ and tert-BuOO• scavenging abilities. Furthermore, scavenging activities of ginger-related antioxidant compounds (6-gingerol, 6-shogaol, and zingerone) were evaluated against five reactive oxygen species.
processing and long-term storage. Shown to be chemically produced from gingerol during thermal compounds in ginger, such as shogaol and zingerone, have been for singlet oxygen and peroxyl radical decreased. The pungent samples (Fig. 1a and b). Conversely, the antioxidant activities was about 1.5-fold increase for superoxide scavenging in all ginger (Fig. 1c). This chart clearly indicates that ginger and rosemary each has specific scavenging pattern. Rosemary is good at scavenging singlet oxygen and peroxyl radical, while ginger is good at scavenging hydroxyl radical and singlet oxygen.

The existing data for antioxidants have made it possible to speculate the scavenging mechanism by ginger’s antioxidant components. 6-Gingerol showed relatively high scavenging ability against hydroxyl and singlet oxygen (Table 2). Previously, HO• scavenging by 6-gingerol was shown to be taken place predominantly via radical adduct formation (RAF) mechanism.17 Also, previous studies showed that •O2 quenching reaction by flavonoid antioxidants proceeds via a charge-transfer intermediate.18 Because antioxidants having smaller oxidation potential Eox tends to show higher •O2 scavenging rates, the relatively small Eox of 6-gingerol (0.39 V)19 suggests that 6-gingerol is an effective quencher for •O2 and that 6-gingerol’s •O2 quenching reaction proceeds via charge-transfer mechanism.

Finally, Fig. 3 shows a radar chart illustration for the antioxidant abilities of the present ginger sample A, together with the MULTIS values estimated from the component quantity of 6-gingerol (29.4% (w/w)) and 6-shogaol (4.3% (w/w)). The HO• scavenging ability of 6-gingerol + 6-shogaol is adjusted to 1.0, because it has been shown that scavenging abilities of antioxidant compounds against HO• radical was unselective.20 The scavenging

| Antioxidant       | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} (heated) | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} | k_{Ox}/k_{Ox,gingerol} |
|-------------------|------------------------|-------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| 6-Gingerol        | 4.50 ± 2.3             | 22.8 ± 1.2                    | 4.24 ± 0.14            | 8.23 ± 0.24            | 4.230 ± 130            |
| 6-Gingerol (heated)| 52.4 ± 1.9             | 30.6 ± 1.5                    | 3.79 ± 0.18            | 2.84 ± 0.13            | 1.840 ± 90             |
| 6-Shogaol         | 41.6 ± 2.9             | 37.2 ± 2.0                    | 4.34 ± 0.13            | 1.96 ± 0.09            | 2.380 ± 180            |
| Zingerone         | 67.1 ± 2.3             | 34.9 ± 1.8                    | 5.73 ± 0.22            | 3.75 ± 0.19            | 9.350 ± 410            |
| Trolox            | 31.9 ± 3.3             | 83.9 ± 2.3                    | 115 ± 3                | 28.3 ± 1 3             | 2,360 ± 70             |

*The HO• scavenging ability in an acetonitrile-water mixture (3:7, v/v).
ability of 6-gingerol is nearly the same as that of the grated ginger sample, suggesting that 6-gingerol plays the major role in the overall scavenging activity.

Conclusion

The present study demonstrated that the heat treatments of ginger specimens either increase or decrease the scavenging activity, depending on the kind of reactive oxygen species subject to scavenging. This means that unlike other antioxidant-containing vegetables, ginger’s antioxidant activity is not necessarily lost by cooking at elevated temperatures. Active species acting as anti-oxidant capacity of ginger was revealed. In addition, we show that cooking at elevated temperatures. This means that unlike other antioxidant-containing species acting as anti-oxidant capacity of ginger was revealed. In addition, we show that the illustration of radar charts of different plant extracts such as ginger and rosemary provides insightful information on specific scavenging capacity.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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