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Analysis of the 6-gingerol Content in *Zingiber* spp. and their Commercial Foods using HPLC

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Abstract The content analysis of 6-gingerol, which is an active compound, in *Zingiber* spp. (*Z. officinale* and *Z. mioga*) and their commercial foods (ginger teas and powders) was conducted using high-performance liquid chromatography. A reverse phase system was used, with a gradient solvent system of water and acetonitrile. The 6-gingerol content was highest in the methanol extract of *Z. officinale* root (17.09 mg/g extract) and ginger powder B (15.92 mg/g extract). The results demonstrated that this method was simple and reliable for the quality control of *Zingiber* commercial foods.

Keywords 6-gingerol · high-performance liquid chromatography · LOD · LOQ · *Zingiber* spp.

Introduction

Zingiber spp. is an important natural material that provides useful products for food, spices, medicines, powders, perfumes, and dietary supplements (Bhunia and Mondal, 2012). It is well known as an ethnomedicine (Tushar et al., 2010), and some varieties, such as *Z. cassumunar*, *Z. officinale*, *Z. zerumbet*, and *Z. mioga*, are used in traditional medicine (Devi et al., 2014).

Ginger is the dried rhizome of *Z. officinale* (Mowrey and Clayson, 1982), and has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). It is cultivated in many tropical and subtropical countries including Australia, Nigeria, and Haiti; however, China and India are world's leading producers (Pawar et al., 2011). In Western herbal medicine, ginger is used as a treatment for colds, rheumatism, sore throats, and for digestive disorders, which include dyspepsia, vomiting, gastritis, nausea, and diarrhea (Zick et al., 2010). Moreover, ginger has been investigated *in vitro* and in animal models for its cancer prevention (Mowrey and Clayson, 1982), anti-inflammatory (Ghayur et al., 2005), and anti-diabetic activities (Al-Amin et al., 2006). Ginger has recently been increasingly used because of its low toxicity and the broad spectrum of its biological and pharmacological applications, such as its anti-tumor, anti-apoptotic, anti-oxidant, anti-proliferative, cytotoxic, and anti-platelet activities (Sekiwa et al., 2000; Young et al., 2005; Wei et al., 2005; Shukla and Singh, 2007).

All of the pungent compounds in ginger contain 4-hydroxy-3-methoxyphenyl moieties and ketone functional groups in their structures (Zick et al., 2010). The main classes of pungent or phenolic compounds in ginger are the gingerols, shogaols, paradols, and zingerones (Chrubasik et al., 2005). Gingerols are the major compounds and are biologically active constituents in the fresh roots. Shogaols are only found in small quantities in the fresh root, but are typically found in dried or thermally treated roots as 6-shogaol (Jolad et al., 2004). Among these compounds, 6-gingerol was found to possess anti-oxidative activity through the inhibition of phospholipid peroxidation (Aeschbach et al., 1994). Furthermore, 6-gingerol inhibits phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity, and skin tumor promotion in mice (Park et al., 1998), and has an inhibitory effect on xanthine oxidase, which is responsible for the generation of reactive oxygen species such as superoxide anions (Chang et al., 1994).

Many analytical methods, which include gas chromatography coupled with mass spectrometry, liquid chromatography coupled

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with mass spectrometry, nuclear magnetic resonance, thin layer chromatography, and capillary electrophoresis have been used for the analysis of gingerol-related compounds in ginger (Connell and McLachlan, 1972; Huang et al., 1997; He et al., 1998; Catchpole et al., 2003; Jolad et al., 2004).

The objective of this study was to analyze the 6-gingerol content of *Zingiber* spp. (*Z. officinale* and *Z. mioga*) and their commercial products (ginger teas and powders) using high-performance liquid chromatography (HPLC).

Materials and Methods

Plant materials and commercial foods. *Z. officinale* root was obtained from Omniherb Co., Ltd. (Korea). The methanol (MeOH) extracts of aerial and root parts of *Z. officinale*, and the whole plant and root parts of *Z. mioga*, were purchased from the Korea Plant Extract Bank, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea. Five ginger teas and two powders were purchased as commercial foods from Local Mart, Korea.

Apparatus and chemicals. Evaporation was conducted with an evaporator system (Eyela rotary vacuum evaporator N1100, Tokyo Rikakikai Co. Ltd., Japan) under reflux *in vacuo*. The HPLC analysis was performed using a Waters Breeze system (Waters Co., USA) equipped with a Waters 1525 binary HPLC pump and a 2489 system UV/VIS detector. The water, MeOH, and acetonitrile (ACN) used were of HPLC grade, and all other reagents were of analytical grade. 6-Gingerol (Fig. 1) was purchased from Sigma-Aldrich (USA).

Preparation of standards and samples. 6-Gingerol was weighed and dissolved in 80% MeOH to obtain a stock standard solution (1.0 mg/mL). Aqueous 6-gingerol solutions were prepared at concentrations of 0.016, 0.031, 0.062, 0.125, 0.25, 0.5, and 1 mg/mL for the construction of a calibration curve. For the analysis of 6-gingerol in the ginger and commercial foods, 5 g of each were extracted using MeOH (3×250 mL) under reflux and then evaporated *in vacuo*. Each extract was dissolved in 1 mL of 80% MeOH and filtered through a 0.45-μm filter before the HPLC analysis.

HPLC conditions. The HPLC separation of 6-gingerol for its qualitative and quantitative analysis was performed using a reverse phase system. A Waters Spherisorb® INNO C18 (4.6×250 mm, 5 μm) column was used with a mobile phase, which consisted of a gradient solvent system of water (containing 0.2% acetic acid) and ACN (from 50:50 to 100:0 over 20 min). UV detection was conducted at 230 nm, the injection volume was 10 μL, and the flow rate was 1 mL/min. All injections were performed in triplicate.

Limits of detection and quantification (LOD and LOQ). Validation of the HPLC method for 6-gingerol as a standard compound was performed using the LOD and LOQ. The method linearity was established using triplicate injections in the range of 0.016–1 mg/mL. Seven calibration solutions were injected in triplicate and their analyses were performed. Calibration curves

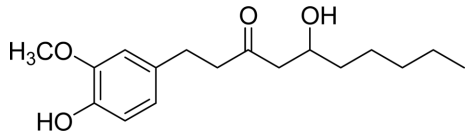

Fig. 1 Structure of 6-gingerol.

were constructed using linear regression analysis of the peak area ratios (Y) corresponding to 6-gingerol versus its concentration (X) in mg/mL. The relative standard deviation was used as a measure of repeatability. The percent recoveries were evaluated by calculating the ratio of amount of 6-gingerol detected versus the amount added. The LOD and LOQ values were separately determined at signal to noise ratios (S/N) of 3 and 10, respectively.

Calibration curve. Stock solutions (0.016–1 mg/mL) of 6-gingerol were prepared in 80% MeOH. The 6-gingerol contents of the samples were determined using the corresponding calibration curves. The calibration curve for 6-gingerol was calculated using the peak area (Y), concentration (X, mg/mL), and mean values (n =3) ± standard deviation (SD).

Results and Discussion

We determined the 6-gingerol contents of *Zingiber* spp. and their commercial foods using HPLC. The HPLC separations and analyses were performed using a reverse phase system with a mobile phase consisting of water and ACN gradient (50:50 to 100:0 over 20 min) and 6-gingerol was detected using a UV-Vis detector at 230 nm. The HPLC chromatograms of 6-gingerol, *Z. officinale* root, *Z. mioga* root (KRIBB), ginger tea C, and ginger powder B are shown in Fig. 2. The 6-gingerol concentration of the *Z. officinale* root extract from Omniherb was determined to be 13.24 mg/g extract. The 6-gingerol contents of the aerial part of *Z. officinale*, root of *Z. officinale*, whole plant of *Z. mioga*, and root of *Z. mioga* from KRIBB were determined to be 0.83, 17.09, 0.28, and 0.16 mg/g of extract, respectively (Table 1). The 6-gingerol content of *Z. officinale* was higher than that of *Z. mioga*. In addition, the 6-gingerol content in aerial part of the ginger was higher than that in the roots. 6-Gingerol is the major compound isolated from *Z. officinale* (Jolad et al., 2004). However, the major pungent compounds of *Z. mioga* were miogadial, mioganal, galanal A, and galanal B (Kim et al. 2005). In previous papers, HPTLC method was reported and validated for estimation of 6-gingerol in dried rhizome extracts of *Z. officinale* with high precision (Kumar et al. 2012). HPLC method was allowed for the detection of all ginger's pungent constituents simultaneously in run time of 25 minutes (Zick et al., 2010).

Additionally, the 6-gingerol concentrations of ginger teas and powders were determined (Tables 2 and 3). With regard to these commercial foods, the 6-gingerol content was highest in ginger powder B, with a concentration of 15.92 mg/g extract. Originally, the products of ginger powder A and B contain 36.8% and 100% ginger powders, respectively. In general, the 6-gingerol contents

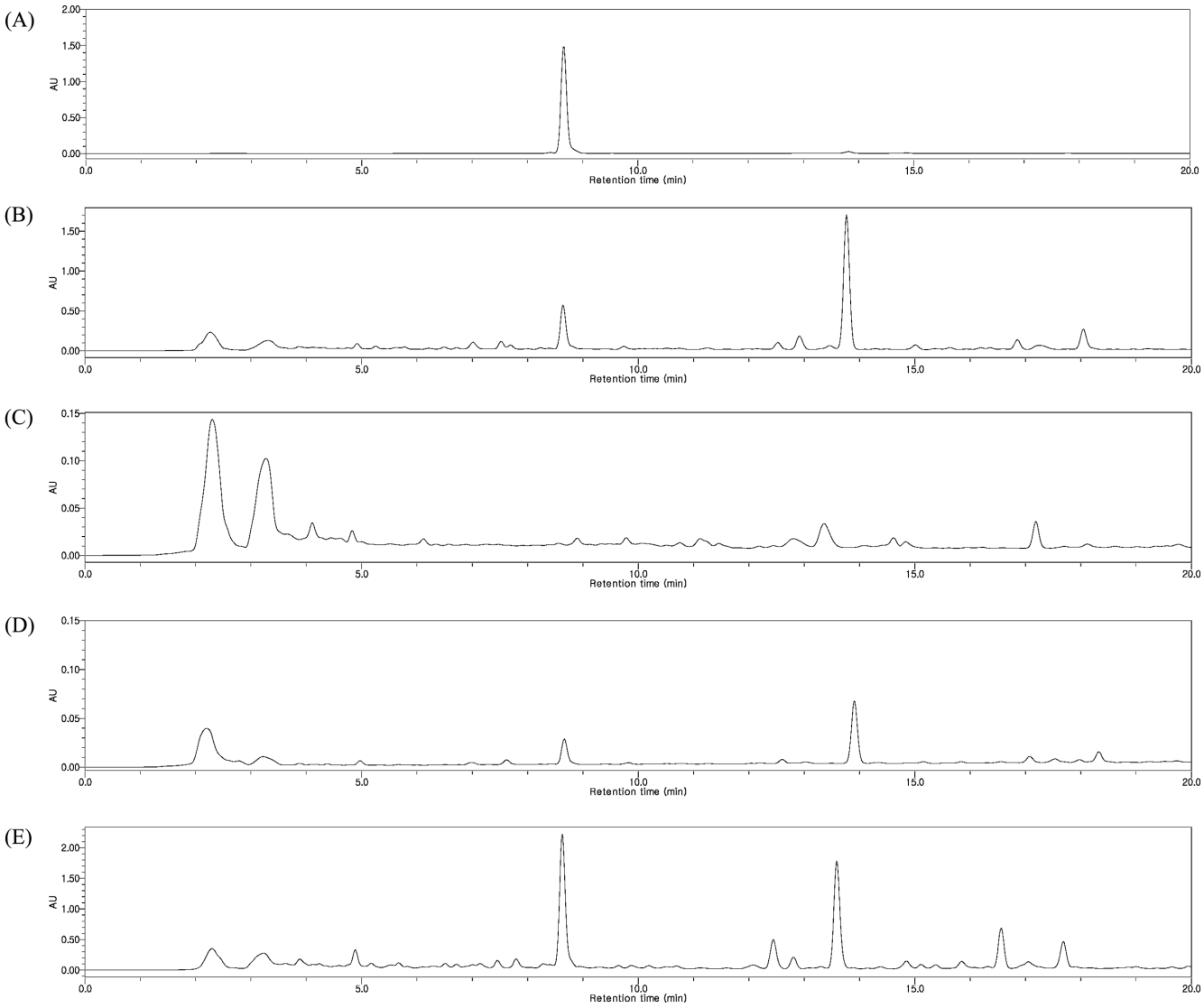

Fig. 2 HPLC chromatograms of 6-gingerol (A), the MeOH extract of *Z. officinale* root from KRIBB (B), *Z. mioga* root from KRIBB (C), ginger tea C (D), and ginger powder B (E).

Table 1 6-Gingerol contents of the MeOH extracts of *Zingiber* spp.

Sample	Content (mg/g extract)
Z. officinale root [a]	13.24±0.55
Z. officinale (aerial part)	0.83±0.05
Z. officinale root [b]	17.09±0.10
Z. mioga (whole plant)	0.28±0.01
Z. mioga root	0.16±0.01

Data are represented as the mean ± SD (n =3) in mg/g extract.
[a]Root of *Z. officinale* was purchased from Omniherb.
[b]Root of *Z. officinale* was purchased from KRIBB.

Table 2 6-Gingerol contents of the MeOH extracts of ginger teas

Sample	Content (mg/g extract)
Ginger tea A	0.11±0.01
Ginger tea B	0.09±0.01
Ginger tea C	0.78±0.09
Ginger tea D	0.07±0.01
Ginger tea E	0.01±0.01

Data are represented as the mean ± SD (n =3) in mg/g extract.

Table 3 6-Gingerol contents of the MeOH extracts of ginger powders

Sample	Content (mg/g extract)
Ginger powder A	1.34±0.01
Ginger powder B	15.92±0.35

Data are represented as the mean ± SD (n =3) in mg/g extract.

of the ginger powders were higher than those of the ginger teas. In a previous study, the 6-gingerol contents of three ginger beverages (in granule form), five ginger tea bags (dried rhizome

Table 4 LOD and LOQ of 6-gingerol

Compound	Calibration equation[a]	r^{2} [b]	Linear range (mg/mL)	LOD (mg/mL)	LOQ (mg/mL)
6-gingerol	Y=156911X+236.8	0.9999	0.016-1	0.048	0.164

[a]Y=peak area, X = concentration of standard (μg/mL).
[b]r^2=correlation coefficient for three data points in the calibration curve.

and leaves), and three ginger powders (dried ginger rhizome) were determined. Among them, the 6-gingerol contents of the ginger powders were almost ~35−80 times higher than those of the ginger beverages (Shao et al., 2010).

Therefore, ginger powders are the best source of 6-gingerol, and they are important natural medicinal foods. The 6-gingerol content of ginger powders is associated with various pharmacological and physiological effects, which include cholagogic, anti-cancer, apoptotic, anti-oxidant, cardiotonic, and anti-tumor activities (Shoji et al., 1982; Suekawa et al., 1984; Hiking et al., 1985).

The 6-gingerol content of *Zingiber* spp. extracts and commercial foods, such as ginger teas and powders, was quantified using the linear regression equation. The linear regression data from the extracts had a good linear relationship and the resulting equation was valid over the relevant concentration range. The linear calibration equation was Y=10889X+61399, where Y is peak area and X is concentration of 6-gingerol. It had a correlation coefficient (r^2) of 0.9996. The LOD and LOQ under our chromatographic conditions were determined at signal to noise ratios (S/N) of 3 and 10, respectively. The LOD and LOQ values for 6-gingerol were determined to be 0.048 and 0.164 mg/mL, respectively (Table 4).

In this work, the 6-gingerol contents of *Zingiber* spp. and their commercial foods were determined qualitatively and quantitatively using an HPLC method. This HPLC analysis provided useful information accurately, rapidly, and easily. The results demonstrated that this method was simple and reliable for the quality control of *Zingiber* commercial foods.

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