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Optimization of Extraction Conditions for the 6-Shogaol-rich Extract from Ginger (*Zingiber officinale* Roscoe)

− Research Note −

Seon Ok[1,2] and Woo-Sik Jeong[1†]

[1]*Department of Food and Life Sciences, College of Biomedical Science and Engineering, Inje University, Gyeongnam 621-749, Korea*
[2]*Department of Pharmacy, Kyungsung University, Busan 808-736, Korea*

Abstract

6-Shogaol, a dehydrated form of 6-gingerol, is a minor component in ginger (*Zingiber officinale* Roscoe) and has recently been reported to have more potent bioactivity than 6-gingerol. Based on the thermal instability of gingerols (their dehydration to corresponding shogaols at high temperature), we aimed to develop an optimal process to maximize the 6-shogaol content during ginger extraction by modulating temperature and pH. Fresh gingers were dried under various conditions: freeze-, room temperature (RT)- or convection oven-drying at 60 or 80°C, and extracted by 95% ethanol at RT, 60 or 80°C. The content of 6-shogaol was augmented by increasing both drying and extraction temperatures. The highest production of 6-shogaol was achieved at 80°C extraction after drying at the same temperature and the content of 6-shogaol was about 7-fold compared to the lowest producing process by freezing and extraction at RT. Adjustment of pH (pH 1, 4, 7 and 10) for the 6-shogaol-richest extract (dried and extracted both at 80°C) also affected the chemical composition of ginger and the yield of 6-shogaol was maximized at the most acidic condition of pH 1. Taken together, the current study shows for the first time that a maximized production of 6-shogaol can be achieved during practical drying and extraction process of ginger by increasing both drying and extracting temperatures. Adjustment of pH to extraction solvent with strong acid also helps increase the production of 6-shogaol. Our data could be usefully employed in the fields of food processing as well as nutraceutical industry.

Key words: ginger, *Zingiber officinale*, 6-shogaol, 6-gingerol, 6-shogaol-rich extract, optimization, nutraceuticals

INTRODUCTION

The rhizome of ginger (*Zingiber officinale* Roscoe) originated in Southeast Asia and widely spread to all around the world (1), being used not only as a food ingredient but also as a traditional medicinal herb to treat many diseases such as gastrointestinal, stomachic, rheumatic disorders and muscular discomfort for thousands of years (2). Several recent studies have reported antioxidant and anti-inflammatory effects of ginger (3-5). In addition, the non-volatile pungent compounds of oleoresin from ginger including gingerol, paradol, shogaol, and zingerone, reportedly to have chemotherapeutic effects (6).

Among the representative bioactive compounds in ginger, gingerols are known as homologous phenolic ketones and exist as 6, 8, and 10-gingerols with different lengths of their unbranched alkyl chains (Fig. 1A) (7). Gingerols have prominent cancer preventive effects against gastric and colon cancer *in vitro* (8,9) and skin cancer *in vivo* (10). Of the gingerols, 6-gingerol has been found to possess various pharmacological effects including anti-inflammatory, analgesic, antipyretic, chemopreventive, angiogenesis, and antioxidant application (11-13).

Shogaols, the dehydrated form of the gingerols, are homologous phenolic alkanones (Fig. 1B) (14). Of the

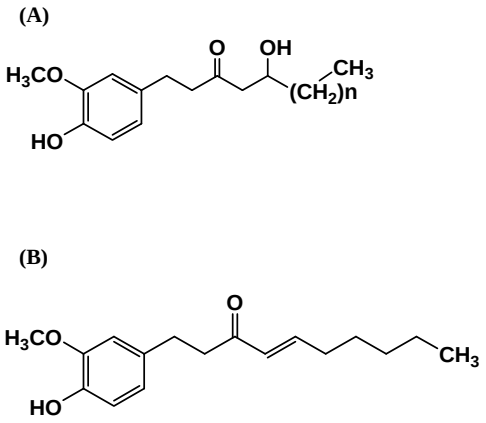

Fig. 1. Structures of the major bioactive compounds in ginger. (A) n= 6, 8 and 10; 6, 8, and 10-gingerol, respectively; (B) 6-shogaol.

†Corresponding author. E-mail: jeongws@inje.ac.kr
Phone: +82-55-320-3238, Fax: +82-55-321-0691

shogaols, 6-shogaol has many biological effects such as antibacterial, antifouling and anti-oxidative properties *in vitro* and *in vivo* (5,8,11). Recent studies have demonstrated that 6-shogaol is more potent than 6-gingerol in anti-inflammatory, antioxidant and chemopreventive effects (5,11,15).

Various extraction methods have been performed to obtain bioactive compounds from ginger, such as reflux (16), shaking at room and warm temperatures (17), sonication (18), and high-pressure Soxhlet extraction (19) etc. However, until now, studies on ginger have focused on the gingerols due to their abundant amount rather than the shogaols. Yonei et al. (20) showed that 6-gingerol content in ginger was largest, amounting to about 15 wt %, while 6-shogaol accounted for less than 2 wt %. Shogaols are more pungent than gingerols in dried ginger but are scarcely present in fresh ginger (21). The effects of drying and extraction conditions on content of 6-gingerol and 6-shogaol have conversely not yet been studied. Also, a recent study has been reported that 6-gingerol can isomerize to 6-shogaol in a model system such as an acidic condition with high temperature (22). Accordingly, we tried to apply this model system to a practical extraction environment to maximize the production of the potent 6-shogaol from the abundant but less active 6-gingerol in ginger. This paper presents the effect of drying conditions including freeze-drying and extraction conditions by modulating temperature and pH on the extract yield of 6-shogaol and 6-gingerol.

MATERIALS AND METHODS

Materials

Fresh gingers (*Zingiber officinale* Roscoe) were acquired from a local farm in Busan, Korea on March 2008. The standards (6, 8, 10-gingerols and 6-shogaol) for high performance liquid chromatography (HPLC) analysis were obtained from Chromadex Inc. (Irvine, CA, USA). All other reagents used in this study were of the highest grade available.

Extraction procedures

Overall process for drying and extracting ginger is illustrated in Fig. 2.

Drying conditions: After washing each ginger sample to remove debris, samples were sliced using a mechanical slicer and spread onto drying trays under various conditions including either freeze-drying (Biotron, Gyeonggi, Korea), room temperature (RT) drying or convection oven drying (VISION, Gyeonggi, Korea) at 60°C and 80°C. The dried samples were ground with a mortar and pestle prior to extraction. The moisture contents of dried ginger were about 6.95±1.55 wt % (n=10).

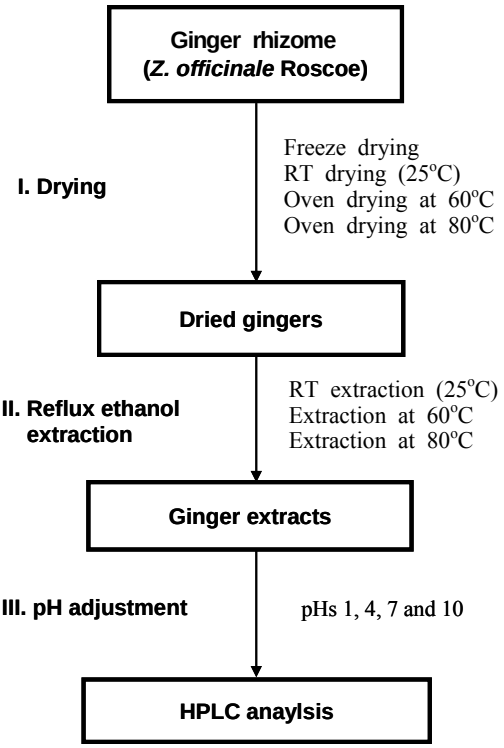

Fig. 2. Overall process for drying & extraction of ginger.

Extraction conditions: Each ginger powder (10 g) was extracted with 100 mL of 95% ethanol (EtOH) either at RT or refluxing at 60 and 80°C for 24 hr. The extracts were filtered by Whatman No. 1 filter paper and the filtrates were centrifuged (Supra22K, Hanil, Incheon, Korea) at 5,500 rpm for 10 min at 4°C. The supernatant was subsequently filtered through a 0.2 μm nylon membrane filter (Corning, Baden Bath, Germany). Two mL of each sample was transferred into Eppendorf tubes and dried by speed vacuum from Biotron. For HPLC analysis, the remained volumes were concentrated by evaporator from EYELA (Tokyo, Japan) under 40°C.

pH adjustment: EtOH was adjusted to pH 1, 4, 7, or 10 with 1 N NaOH or 1 N HCl before the above extraction process. A hundred mL of EtOH extraction at 80°C after ginger dried at 80°C was added into a mixture of water and dichloromethane (DCM) (each 100 mL) and partitioned. The DCM layer was collected from the mixture and solvent was removed by vacuum evaporation.

HPLC analysis: The ginger extracts were analyzed by a HPLC system (Agilent, Foster, CA, USA) consisting of a quaternary pump, photodiode array detector, and autosampler. Chromatographic analysis was performed on a 250×4.6 mm, 5 μm Alltima HP C18 reversed phase column (Grace, Deerfield, IL, USA) with acetonitrile-water (v/v) as the mobile phase. The HPLC operating parameters were: injection volume, 5 μL; column flow

rate, 1.0 mL/min; chromatographic run time, 48 min; UV spectra recording, 230 nm. The mobile phase consisted of water (A) and acetonitrile (B). The gradient elution was as follows; 0 min 45% B, 8 min 50% B, 17 min 65% B, 32 min 100% B, 38 min 100% B, 43 min 45% B, 48 min 45% B.

Calibration of 6-gingerol and 6-shogaol for quantitative analysis: The quantification was carried out using the external standard method. The contents of 6-gingerol and 6-shogaol from gingers were determined using a calibration graphic established with dilutions of each standard at concentrations ranging from 0.1 mM to 1 mM injected into the HPLC system (correlation coefficient ≥ 0.996). Each concentration was measured in triplicate. The corresponding peak areas were plotted against the concentration of the peak amount injected and identification of peaks was achieved by comparison of both the retention time and UV absorption spectrum with standards.

Statistical analysis

Data were expressed as the means $\pm$ SD. The statistical analyses were performed using the SPSS 10.0 program (Systat Software Inc., Chicago, IL, USA). Values were compared to control using analysis of variance (ANOVA) followed by Duncan's post hoc test. P values <0.05 were considered significant.

RESULTS AND DISCUSSION

Analysis of compounds from ginger extraction

Many qualitative and/or quantitative methods have been reported for analysis of fresh (16-18,23) and dried ginger (24). A variety of solvents such as methanol (16, 17), ethanol (18,25), DCM (23), acetone (25), and pressurized CO_2 (19,20) have been used for ginger extraction. Extraction methods to obtain bioactive compounds from ginger also include reflux (16), sonication (18,26), and high-pressure Soxhlet extraction (19,26) etc. Based on the literature, the most effective method for extracting gingerol compounds might be the reflux method (26). In the present study, therefore, we employed reflux method for the extraction of ginger. After the extraction followed by concentration process, we analyzed the extracts by HPLC with wavelength scanning. The HPLC analysis of each ginger extract was carried out with standard compounds of 6, 8, and 10-gingerols and 6-shogaol. HPLC (16) and Gas Chromatography (GC) (27) have been typically used to analyze gingerols and shogaols in ginger. In the present study, we chose HPLC analysis since the GC method might cause a conversion of 6-gingerol to 6-shogaol through the high temperatures (28). HPLC chromatograms of standard compounds and some representative ginger extracts (GEE80, GEE8080 or GEE8080-1) are listed in Fig. 3.

Effects of drying and extraction conditions on 6-gingerol and 6-shogaol contents

6-Gingerol content was analyzed after applying various drying and extraction conditions and the results are shown in Fig. 4A. When ginger was extracted at RT, 6-gingerol content tended to decrease as the drying temperature increased up to 60°C while drying at 80°C (GEE80) resulted in an increase in 6-gingerol yield. 6-Gingerol seems to degrade as the drying temperature increases to 60°C. The reason for the highest 6-gingerol

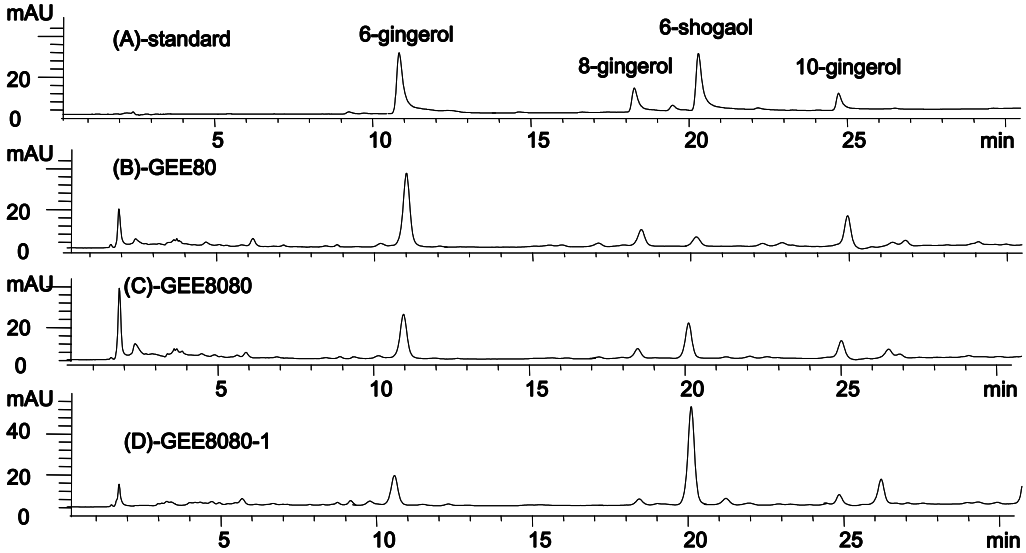

Fig. 3. HPLC chromatograms of standard ginger compounds and representative ginger extracts. (A) standards components of ginger, (B) GEE80, ginger ethanol extract after drying at 80°C, (C) GEE8080, ginger ethanol extract dried and extracted at 80°C, (D) GEE8080-1, ginger ethanol extract dried and extracted at 80°C (pH 1).

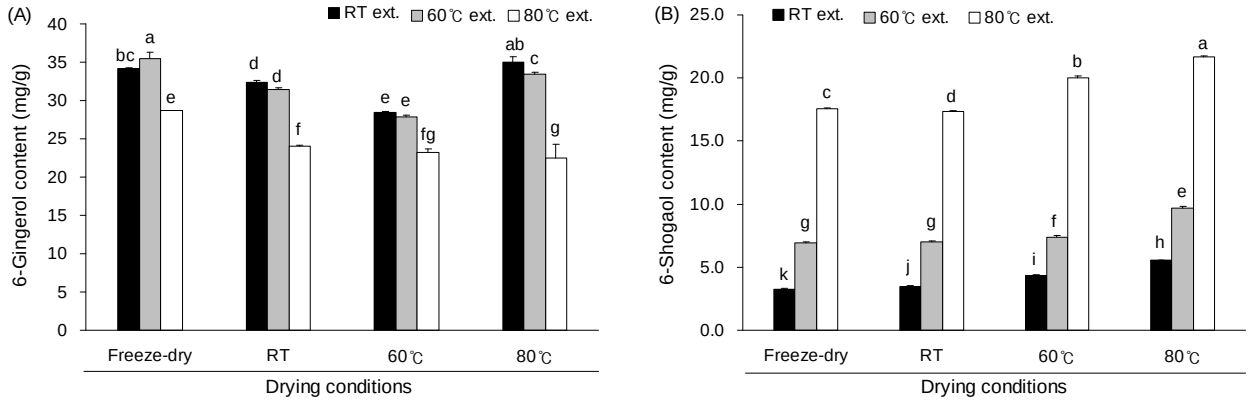

Fig. 4. Contents of 6-gingerol (A) and 6-shogaol (B) after drying and extraction. Values are represented as mean±SD (n=5). Means with the different superscripts (a-k) are significantly different at $p<0.05$ by Duncan's multiple range test.

content drying at 80°C is not clear at this moment. The extraction temperature also affected the 6-gingerol content. In all drying conditions, the highest extraction temperature of 80°C resulted in the lowest 6-gingerol content, probably due to the degradation of 6-gingerol at high temperature.

Effect of drying and extraction temperature on 6-shogaol content seems more apparent than on the 6-gingerol. As shown in Fig. 4B, 6-shogaol content increased with both drying and extraction temperatures. The lowest 6-shogaol content was achieved when the freeze-dried ginger was extracted at RT while the highest 6-shogaol content was obtained when the ginger was dried and extracted at 80°C. Between the drying and extraction conditions, the extraction condition was more critical for 6-shogaol production.

6-Shogaol content of the ginger extract at RT (GEERT) in the present study ranged from 3 to 5 mg/g and our result is similar to the results from previously reported studies (about 2～2.5 mg/g in EtOH extraction) (26,29). However, drying and extraction at 80°C (GEE 8080) resulted in 5- to 7-fold increase in the 6-shogaol content (about 22 mg/g) compared to the 6-shogaol content of drying and extraction at RT. These results imply that the drying and extraction temperature of ginger directly affects the yield of 6-shogaol.

Effect of pH extraction conditions on 6-gingerol and 6-shogaol contents

To examine the effect of pH on 6-shogaol content during extraction, the extraction solvent was adjusted to pH 1, 4, or 10 with HCl or NaOH. For the pH adjustment experiment, we used only GEE8080 because it displayed the highest 6-shogaol content (Fig. 4).

Among the pH-adjusted GEE8080s, GEE8080-4 showed the highest 6-gingerol content (about 28 mg/g) due to its stability at pH 4 than at pH 1 and 7 (22) and the lowest 6-gingerol content (about 14 mg/g) was observed at GEE8080-1 (Fig. 5). Conversely, GEE8080-1 exhibited the highest 6-shogaol content (about 57 mg/g), which was more than a 2-fold increase than GEE8080 (about 22 mg/g). The lowest 6-shogaol content was obtained

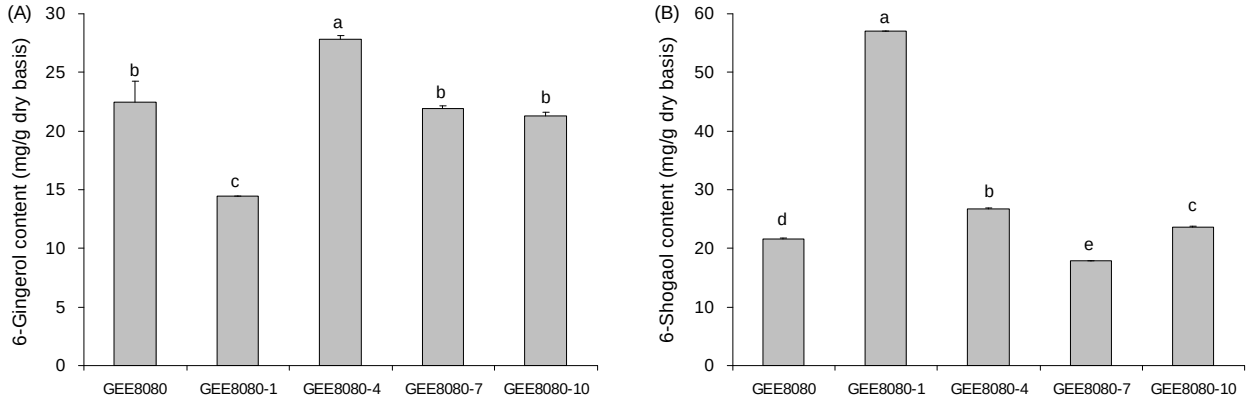

Fig. 5. Contents of 6-gingerol (A) and 6-shogaol (B) after pH adjustment of GEE8080. GEE8080, ginger ethanol extract dried and extracted at 80°C; GEE8080-1, 4, 7 and 10, GEE8080 adjusted to pH 1, 4, 7 and 10, respectively. Values are represented as mean±SD (n=5). Means with the different superscripts (a-e) are significantly different at $p<0.05$ by Duncan's multiple range test.

with GEE8080-7 (about 18 mg/g). 6-Gingerol can convert to 6-shogaol with strong acid or base due to dehydration of 6-gingerol either by protonation with strong acid or deprotonation by a strong base, while 6-gingerol is more degradable in acid than in alkaline solution (22).

Overall, the current study shows for the first time that a maximized production of 6-shogaol can be achieved during a practical drying and extraction process of ginger by increasing both drying and extracting temperatures. Adjusting the pH of the extraction solvent with strong acid also help increase the production of 6-shogaol. Our data could be usefully employed in the fields of food processing as well as nutraceutical industry.

ABBREVIATIONS

GEE, ginger ethanol extract; GEERT, GEE extracted at RT; GEE60, GEE extracted at 60°C; GEE80, GEE extracted at 80°C; GEE60FD, GEE60 after freeze-drying; GEERTFD, GEERT after freeze-drying; GEE8080, GEE80 after drying at 80°C; GEE80-1, GEE80 adjusted by pH 1; GEE8080-1, 4, 7 and 10, GEE8080 adjusted by pH 1, 4, 7 and 10, respectively.

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