Effects of Post-harvest Storage and Drying Temperatures on Four Medicinal Compounds in the Root of Chinese Licorice (*Glycyrrhiza uralensis*)

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The root of Chinese licorice (*Glycyrrhiza uralensis*) is used worldwide as a medicinal herb. The goal of this study was to understand changes in the concentrations and compositions of four medicinal compounds—glycyrrhizic acid (GL), liquiritin (LQ), liquiritigenin (LG), and isoliquiritigenin (ISLG)—in the root of Chinese licorice during post-harvest treatment. The effects of post-harvest storage temperatures (−30, −13, 4, and 25°C) and drying temperatures (30, 40, 50, and 60°C) on concentrations of the four medicinal compounds were investigated. GL and LQ concentrations in roots stored at −30 and −13°C for 1–2 weeks tended to be 4% to 13% higher than GL and LQ concentrations in roots dried directly in a vacuum freeze dryer (controls). LG concentrations in roots stored at 4°C for 2 weeks were nearly 60-fold higher and ISLG concentrations at 25°C for 1 week were 10-fold higher than LG and ISLG concentrations in the controls. In addition, low temperature (30 and 40°C) drying compared to vacuum freeze drying (controls) increased LG and ISLG concentrations without decreasing GL and LQ concentrations. This study provided an approach to increase the target compound concentrations in Chinese licorice for different market demands (drugs, cosmetics, and food).

Keywords: flavonoids, glycyrrhizic acid, herbal medicine, secondary metabolites

INTRODUCTION

Licorice (*Glycyrrhiza*) belongs to the family Leguminosae, and licorice root is the most common ingredient in traditional Japanese *Kampo* medicines. The principal pharmacologically active compound in licorice root is glycyrrhizic acid (GL). GL is a triterpenoid that has been shown to exhibit antiviral, immunomodulatory, and antitumorigenic activities (Pompei et al., 1979; Chavali et al., 1987; Agarwal et al., 1991). Several studies also suggest that GL is highly active in inhibiting replication of the human immunodeficiency virus type 1 (HIV-1) and the severe acute respiratory syndrome (SARS)-associated virus (Hattori et al., 1989; Cinatl et al., 2003). According to the Japanese Pharmacopoeia (2011), licorice roots used in Japanese *Kampo* medicines are required to have GL concentrations above 2.5% dry weight (DW). GL is also a powerful natural sweetener (50–170 times sweeter than sucrose) used in food (Mukhopadhyay and Panja, 2008).

In recent years, liquiritin (LQ), liquiritigenin (LG), and isoliquiritigenin (ISLG) have been the major flavonoids studied in licorice roots. LQ from *Glycyrrhiza uralensis* has been reported to exert antioxidant-like effect in animal studies (Zhao et al., 2008). LG shows immunomodulatory and antioxidant activities (Pan et al., 2000; Lee et al., 2009). ISLG can be used as an oxidase inhibitor and shows anticancer activities (Pan et al., 2000; Kanazawa et al., 2003). Additionally, licorice root extracts are frequently used in cosmetics, because the flavonoids (including LQ, LG, ISLG, and others) have been shown to promote skin whitening and prevent skin pigmentation and aging (Wang et al., 2004), and as antioxidants added to foods including oil, ham, snacks, and instant noodles (You, 2001). Mixtures of compounds or single compounds from licorice root extracts are both used in different applications (Hayashi and Sudo, 2009; Zhao et al., 2010). Therefore, not only GL concentration but also concentrations of the other three major flavonoids should be controlled for various market demands (drugs, cosmetics, and food).

Licorice does not grow in the wild in Japan. Most licorices for the market demand were imported from other countries such as China, Afghanistan, and Turkmenistan (Hayashi and Sudo, 2009). However, with the increasing demands for licorice throughout the world market, wild licorices have been harvested excessively, leading to serious desertification of the grassland, and as a result, the exportation of wild licorice has been limited in these countries. Therefore, cultivated licorice is expected to increase as the principal source for licorice demands.

Until now, many studies concentrated on the effects of environmental factors and stress on the growth and GL concentration of Chinese licorice (*Glycyrrhiza uralensis*) (Afreen et al., 2005; Wang et al., 2008; Hou et al., 2010;...
Sun et al., 2012). Generally, reports show that the concentrations of secondary metabolites in harvested medicinal plants change during the storage and drying process (Du et al., 2004; Policegoudra and Aradhya, 2007). Little research has been conducted on the concentrations of medicinal compounds in Chinese licorice during the post-harvest process. Hayashi (2010) reported that GL concentrations increased in harvested Chinese licorice roots stored in the incubator at −5°C for 4 weeks and in the storehouse at ambient temperatures (~15 to 8°C) for 8 weeks; however, the ambient temperatures (~15 to 8°C) were not controlled. Concentrations of LQ, LG, and ISLG as secondary metabolites in Chinese licorice root may change depending on storage temperature.

Although it is generally known that compound concentrations in medicinal plants dried in a vacuum freeze dryer can be steadily maintained, the cost of vacuum freeze drying is much higher than that of oven drying or natural air drying. Natural air drying is the most common method for drying medicinal plants during the production process because of the low cost, but drying time is long and drying temperature cannot be controlled. In contrast, oven drying offers short drying time and ease of temperature control. Therefore, oven drying was used in our study. Drying temperature may affect compound compositions in medicinal plants. Du et al. (2004) reported that increasing the drying temperature (40, 55, and 70°C) of American ginseng (Panax quinquefolium) decreased the concentration of total ginsenosides but increased neutral ginsenosides and decreased malonyl ginsenosides. Therefore, the compositions of the four compounds in Chinese licorice root studied here may change depending on drying temperature.

To understand the changing concentrations and compositions of four medicinal compounds in Chinese licorice root during post-harvest treatment for different market demands, the effects of post-harvest storage and drying temperatures were investigated in this study.

MATERIALS AND METHODS

Plant materials and sampling method

Chinese licorice plants (2-month-old, line No. 2, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Japan) from cutting propagation were grown in a hydroponic system for 10 months under artificial environmental conditions. Main root propagation were grown in a hydroponic system for 10 months under artificial environmental conditions. Main root blocks (0.4 × 0.7 cm diameter) were separated from lateral roots and were cut into 1.0 to 1.5 cm lengths directly. Main root blocks (0.4–0.7 cm diameter) were collected from different Chinese licorice plants for use in the different treatments.

Temperature treatments

Post-harvest storage temperature treatments

Root blocks were cut vertically into two parts (Fig. 1). One part was used as a control and was dried directly in a vacuum freeze dryer for 3 days. The other part was used in the treatments; treatment parts were dried in the oven at different drying temperatures (30, 40, 50, and 60°C) for 5 days. Each drying temperature treatment consisted of 4–5 repetitions.

Drying temperature treatments

Root blocks were cut vertically into two parts (Fig. 1). One part was used as a control and was dried directly in a vacuum freeze dryer for 3 days. The other part was used in the treatments; treatment parts were dried in the oven at different drying temperatures (30, 40, 50, and 60°C) for 5 days. Each drying temperature treatment consisted of 4–5 repetitions.

Extraction and determination of the four medicinal compounds

The dried root blocks from all treatments and controls were powdered for extraction. According to the extract method described by Li et al. (2008) with some modifications, the root powder was extracted with 60% (v/v) ethanol for 30 min in an ultrasonic bath, and the ratio of solid to liquid was 1 g DW/100 ml. The recoveries of GL, LQ, LG, and ISLG were 94%, 94%, 96%, and 97%, respectively. The solid matter was removed by centrifugation at 15,000 rpm for 10 min. Then, the supernatant was filtered through a 0.2 μm syringe filter. Finally, the filtered supernatant was injected into the high-performance liquid chromatography (HPLC) column, and concentrations of the four compounds were calculated from the peak areas.

The HPLC quantification method for GL, LQ, LG, and ISLG described by Li et al. (2008) was modified and was used for the present experiment. In brief, quantification was performed on a Shimadzu 10AD HPLC system equipped with an SCL-10A system controller, SIL-10A auto-injector, and CTO-10A column oven with ultraviolet (UV) detection at 254 nm for GL, 276 nm for LQ and LG, and 372 nm for ISLG in an SPD-M10AV photodiode array detector (Shimadzu Co., Ltd., Japan). The four compounds were separated on a TSK-Gel ODS-100V reversed-phase column (4.6 mm diameter × 250 mm length) (Shimadzu Co., Ltd., Japan). The mobile phase consisted of acetonitrile and 1% acetic acid. Baseline separation of GL, LQ, LG, and ISLG was achieved with gradient elution. Flow rate was 0.9 ml/min, injection volume was 20 μl, and column temperature was maintained at 40°C. Standards of GL (PMRJ, Japan) and LQ, LG, and ISLG (ChromaDex, Inc., USA) were dissolved in methanol to obtain different solution concentrations. Chromatographic peaks of the four compounds were confirmed by comparing their retention times and UV spectra with those of the standards. Standard curves based on the standards showed good linearity over ranges of 37.5–600.0 μg/ml for GL, 15.6–250.0 μg/ml for LQ, 37.5–600.0 μg/ml for LG, and 37.5–600.0 μg/ml for ISLG.

Root blocks were cut vertically into two parts (Fig. 1). One part was used as a control and was dried directly in a vacuum freeze dryer for 3 days. The other part was used in the treatments; treatment parts were dried in the oven at different drying temperatures (30, 40, 50, and 60°C) for 5 days. Each drying temperature treatment consisted of 4–5 repetitions.
μg/ml for LQ, 0.3–10.0 μg/ml for LG, and 0.05–1.56 μg/ml for ISLG. Amounts of the four compounds in every unknown sample were determined using standard curves.

**Calculation**

To elucidate concentration trends in the four medicinal compounds between the storage treatment or drying temperature and the controls, the increment percentage in the compounds with respect to controls (\(P_{\text{inc}}\), %) was calculated according to the following equation:

\[ P_{\text{inc}} = \left( \frac{C_T - C_C}{C_C} \right) \times 100 \]

\(C_T\): the compound concentration in each storage or drying temperature treatment (mg/g DW).
\(C_C\): the compound concentration in each control (mg/g DW).

**Statistical analysis**

All data presented in the figures are mean values. Significant differences between temperature treatments (storage and drying temperatures) and controls for the same periods were determined by t-test (\(P \leq 0.01\) or 0.05) using Excel Statistics ver. 5.0 software (ESUMI Co., Ltd., Japan).

**RESULTS AND DISCUSSION**

The four medicinal compounds were completely separated from impurities under HPLC conditions (Fig. 2). Reported concentration ranges of GL, LQ, LG, and ISLG in wild Chinese licorice are 14.8–33.4, 4.1–26.6, 0.7–3.5, and 0.3–1.2 mg/g DW, respectively (Chen et al., 2009; Zhao et al., 2006). GL and LQ concentrations in our study were consistent with reported concentrations in wild Chinese licorice, but LG and ISLG concentrations were one-tenth of those reported. In our previous studies, the four compounds in the line (No. 2) used in our study may have been different from that used in the above reports.

**Effects of post-harvest storage temperature on concentrations of the four medicinal compounds**

Generally, most enzyme activities decrease by decreasing the amount of liquid in plants stored below the freezing point (about 0°C). Therefore, the synthesis or decomposition of enzyme activity for the four medicinal compounds in this experiment may change under below- or above-zero temperature conditions via liquid movement. The results of the below-zero (−80, −30, and −13°C) and above-zero (4 and 25°C) treatments in this experiment are discussed in the following sections.

**Below-zero treatments**

Concentrations and increment percentages (\(P_{\text{inc}}\)) of the four medicinal compounds in roots stored at −80°C for 1–4 weeks were not changed significantly compared to those in the controls, except for LQ at 3 weeks and ISLG at 2 weeks (Fig. 3). Little liquid seemed to exist in the roots of Chinese licorice stored at −80°C. Therefore, plant biosynthesis and most enzyme reactions also seemed to be stopped at −80°C.

Hayashi (2010) reported that GL concentrations in harvested Chinese licorice roots stored in the incubator at −5°C for 4 weeks and in the storehouse at ambient temperatures in winter (−15 to 8°C) for 8 weeks were 7% and 12% higher, respectively, than GL concentrations in roots dried directly. In our study, GL and LQ concentrations in roots stored at −30 and −13°C for 1–2 weeks tended to be 4–13% higher than GL and LQ concentrations in roots dried directly in the vacuum freeze dryer (controls) (Fig. 3A and 3B). The \(P_{\text{inc}}\) of GL and LQ concentrations fluctuated in roots stored at −30 and −13°C for 1–4 weeks. For Chinese licorice, GL and LQ were the two secondary metabolites in the high-concentration group. Generally, secondary metabolites have a key role in protecting plants from environmental pressures or controlling plant growth (Harborne, 1999). When roots were stored at −30°C, additional GL and LQ were produced to resist the low temperature and then were consumed gradually. GL and LQ concentrations in roots stored at −30°C increased in the...
second week and decreased in the third week (Fig. 3A and 3B). Then, the plants continued to produce more GL and LQ to resist the low temperature (\(-30^\circ\text{C}\)). GL and LQ concentrations began to increase again in the fourth week. However, when roots were stored at \(-13^\circ\text{C}\), additional GL was produced in the second and third weeks and was consumed in the fourth week, and additional LQ was produced in the first to third weeks and was consumed in the fourth week (Fig. 3A and 3B). Probably because \(-13^\circ\text{C}\) is warmer than \(-30^\circ\text{C}\), consumption rates of GL and LQ in roots stored at \(-13^\circ\text{C}\) were slower than those in roots stored at \(-30^\circ\text{C}\). These results suggest that fluctuations in GL and LQ concentrations in Chinese licorice stored at \(-30^\circ\text{C}\) and \(-13^\circ\text{C}\) were related to plant protection.

LG and ISLG concentrations in roots stored at \(-30^\circ\text{C}\) for 1–4 weeks were not significantly changed compared to those in the controls, except for LG at 4 weeks (Fig. 3C and 3D). The \(P_{\text{inc}}\) of LG and ISLG concentrations tended to be higher in roots stored at \(-13^\circ\text{C}\) than in roots stored at \(-30^\circ\text{C}\) (Fig. 3C and 3D). However, the \(P_{\text{inc}}\) of LG and ISLG concentrations also fluctuated like the \(P_{\text{inc}}\) of GL and LQ concentrations, although LG and ISLG were the two secondary metabolites in the low-concentration group.

**Above-zero treatments**

LG and ISLG concentrations in roots stored at 4 and \(-25^\circ\text{C}\) were significantly \((P \leq 0.01 \text{ and } 0.05)\) higher than those in the controls, except for ISLG concentration in roots stored at \(-4^\circ\text{C}\) for 2 weeks and at \(-25^\circ\text{C}\) for 4 weeks (Fig. 3C and 3D). The \(P_{\text{inc}}\) of LG concentration was highest in roots stored at 4 and \(-25^\circ\text{C}\) for 2 weeks, and LG concentration was nearly 60-fold (6000%) higher than that in the controls (Fig. 3C). The \(P_{\text{inc}}\) of ISLG concentration was higher in roots stored at 4 and \(-25^\circ\text{C}\) for 1 week than in roots stored for 2–4 weeks, and ISLG concentration was nearly 10-fold (1000%) higher than that in the controls (Fig. 3D). However, LQ concentration decreased when roots were stored at 4 and \(-25^\circ\text{C}\) (Fig. 3B). LQ, LG, and ISLG are flavonoids. Generally, certain flavonoids occur in combination with glucoses as glucosides, and free flavonoids are released when exposed to certain enzymes or stimulation (Fu et al., 2008). The flavonoids LQ and isoliquiritin (ISLQ) in Chinese licorice as glucosides possibly could have decomposed to free flavonoids (Fig. 4), LG and ISLG, when roots were stored at 4 or \(-25^\circ\text{C}\) (Fig. 3D). However, LQ concentration decreased when roots were stored at 4 and \(-25^\circ\text{C}\) (Fig. 3B). LQ, LG, and ISLG are flavonoids. Generally, certain flavonoids occur in combination with glucoses as glucosides, and free flavonoids are released when exposed to certain enzymes or stimulation (Fu et al., 2008). The flavonoids LQ and isoliquiritin (ISLQ) in Chinese licorice as glucosides possibly could have decomposed to free flavonoids (Fig. 4), LG and ISLG, when roots were stored at 4 or \(-25^\circ\text{C}\). This would explain the decrease in LQ concentration and the increase in LG and ISLG concentrations in roots stored at 4 and \(-25^\circ\text{C}\) for 1–4 weeks.

GL is a triterpenoid. GL and the other three flavonoids (LQ, LG, and ISLG) are synthesized in Chinese licorice through the action of certain enzymes by different
biosynthetic pathways (Fig. 4) (Winkel-Shirley, 2001; Hayashi et al., 2003; Taiz and Zeiger, 2010). Secondary metabolites are known to be derived from primary metabolites through the activity of enzymes. Acetyl coenzyme A (acetyl-CoA) is the most important enzyme for the synthesis of secondary metabolites in plants. Therefore, a possible reason for the decreased GL concentration in roots stored at 4 and 25°C was that more acetyl-CoA in the plant was used to synthesize the flavonoids (LG and ISLG) instead of the triterpenoid (GL). However, plant biosyntheses are complex, and this mechanism needs to be studied further.

**All treatment ranges**

Most of the enzyme reactions in the roots of Chinese licorice stored at −80°C were stopped without liquid movement; therefore, the $P_{nm}$ of the four medicinal compounds in roots stored for 1–4 weeks was stable (Fig. 3). At −30 and −13°C, some enzyme activity seemed likely; therefore, the $P_{nm}$ of GL and LQ concentrations in roots stored at −30 and −13°C fluctuated, and the $P_{nm}$ of LG and ISLG concentrations in roots stored at −13°C tended to increase. At above-zero temperatures, because most of the enzyme still maintained activity, the $P_{nm}$ of GL and LQ concentrations tended to decrease (Fig. 3A and 3B) and the $P_{nm}$ of LG and ISLG concentrations increased (Fig. 3C and 3D). According to these results, the concentrations of medicinal compounds in roots stored at different temperatures changed. Roots with stable concentrations of the four medicinal compounds were obtained at −80°C, and roots with high concentrations of the four medicinal compounds were obtained at −30°C for 2 weeks for GL, −13°C for 1 week for LQ, 4°C for 2 weeks for LG, and 25°C for 1 week for ISLG. In our previous studies in which we used different-aged seedlings for different periods, the results were consistent with the above results in this study.

**Effects of drying temperature on concentrations of the four medicinal compounds**

Compound concentrations in the medicinal parts of plants dried in a vacuum freeze dryer can be steadily maintained; however, oven drying is a popular method for the medicinal plant production industry because of short drying time and ease of temperature control. Drying temperature affects the quality and concentration of active compounds in medicinal plants. In commercial operations in Canada, ginseng roots are dried at low temperatures (32–38°C) to achieve a high-quality product (Davidson et al., 2004). Lin et al. (2011) reported that hot drying temperatures (40, 55, or 70°C) decreased caffeic acid derivatives and total phenolics contents, and a cool drying temperature (30°C) tended to increase cichoric acid and total phenolics contents in the roots of *Echinacea purpurea*.

In our study, drying temperature also affected the concentrations of medicinal compounds in the roots of Chinese licorice. GL and LQ concentrations in roots dried in the oven at 60°C were significantly ($P \leq 0.01$ and 0.05) lower than those in roots dried in the vacuum freeze dryer (controls) (Fig. 5A and 5B), and the $P_{nm}$ of GL concentration in roots dried at 50 and 60°C tended to decrease (Fig. 5A). These results indicate that GL and LQ in roots dried at high temperatures (50 and 60°C) decomposed to other related compounds. However, because LG and ISLG concentrations in roots dried in the oven at 30–60°C were significantly ($P \leq 0.01$) higher than those in the controls (Fig. 5C and 5D), LG and ISLG in roots dried at 30–60°C probably decomposed to LG and ISLG (Fig. 4). In addition, the $P_{nm}$ of LG and ISLG concentrations tended to be lower in roots dried at 60°C than in roots dried at 30–50°C (Fig. 5C and 5D), which indicates that LG and ISLG also decomposed when roots were dried at temperatures above 60°C. These results suggest that drying Chinese licorice root in...
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The oven at 30 and 40°C with low energy consumption could increase LG and ISLG concentrations without decreasing GL and LQ concentrations. In our previous studies in which roots were dried in the oven at 50°C, the results were consistent with the above results in this study, although other drying temperature treatments (30, 40, and 60°C) were not repeated.

CONCLUSION

In this study, concentrations and compositions of GL, LQ, LG, and ISLG were changed using different storage temperature treatments. Concentrations of the four medicinal compounds were stable at 80°C. GL and LQ concentrations in the root of Chinese licorice can be increased by below-zero storage temperature treatments (−30 and −13°C), and LG and ISLG concentrations can be increased by above-zero storage temperature treatments (4 and 25°C). In addition, after above-zero storage temperature treatments, LG and ISLG concentrations can be increased more by low-temperature drying (30 and 40°C). This study provides an approach to increase the concentration of each target compound in Chinese licorice for different market demands (drugs, cosmetics, and food).

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