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EVALUATION OF QUALITY CHANGES IN ANGELICA SINENSIS (DANGGUI) DURING STORAGE PERIOD

Dong Gao[1]*, Wang Li[1] and Yanyuan Chen[1]

*Corresponding Author: Dong Gao, ✉ gaodong5211@126.com

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Storage is the key link for keeping the quality of Chinese herbal medicines. In order to better understand the role of storage, we evaluated the content of major constituents of Danggui during natural and factitious storageconditions. The content of ferulic acid and Z-ligustilide was less than 0.5 and 6 mg/g, respectively, in most two-years-stored Danggui under natural condition. The order of the percent of pass is tail > body > head. Under the factitious conditions, the content of the two were lessened. Under dry condition (4 or 25 °C, 50% RH), ferulic acid basically met the standard, however, Z-ligustilide was below standard after 5-6 months; if moist (25 °C, 90% RH), the two were rapidly below standard. Mild condition (30 °C + 75% RH) is unhelpful to hold the two. Taken altogether, the dry condition is essential, if cold together is preferred for maintaining the quality of Danggui.

Keywords: *Angelica sinensis*, Ferulic acid, Z-ligustilide, Storage condition, Quality

INTRODUCTION

Angelica sinensis (AS; Apiaceae; syn. Danggui) is popularly used as health food and traditional Chinese medicine for its immunoenhancement, hematinic and antioxidant (Zheng *et al.*, 2015). The quality control of Danggui is more difficult due to its multiple chemical constituents and geographic origins. Danggui contains more than 80 composite formulae, and is planted in more than 8 provinces in China (Chen *et al.*, 2013). Moreover, substitute herbs, such as *A. uchiyamana* (Taiwan), *A. gigas* (Korea), *A. acutiloba* (Japan), and *Levisticum officinale* (Europe) have also been used in medical practice (Yi *et al.*, 2009). Among multitudinous chemical constituents, ferulic acid and Z-ligustilide are mainly associated with the bioactivities and are usually chosen as chemical markers to assess the quality of Danggui and its products (Zhao *et al.*, 2003; and Yi *et al.*, 2009). The former exhibits immunostimulatory and anti-inflammatory activities; the latter exerts anti-cancer, -inflammatory, -hepatotoxic, and neuroprotective activities (Zhao *et al.*, 2003; and Chao and Lin, 2011). The amounts of the two constituents within Danggui could vary significantly in geographical variations (Zhao *et al.*, 2003), plant anatomy (head, body, and tail parts) (Wei *et al.*, 2008), or storage method and period. It is generally known that many components are unstable in traditional Chinese medicines (Meng *et al.*, 2013).

It's widely assumed that Danggui cultivated in Gansu, Yunnan provinces of China, is the authentic herb (Yi *et al.*, 2009; and Zheng *et al.*, 2015); and the post-harvested Danggui dried under the sun is standard commodity of herb preparation (Zhan *et al.*, 2014). Storage of post-harvested dry Danggui at room temperature and in light (natural condition), as common practice, is detrimental to its shelf life and quality. These traditional experiences have been spread for thousands of years, but substantial or convincing data are few yet. Therefore, it is important to reveal the

[1] Shenzhen Hornetcorn Biotechnology Co., Ltd., Shenzhen, 518045, China.

quality of different parts of Danggui exposed to natural condition to enrich quality standard and to draft optimizational storage method.

MATERIALS AND METHODS

Plant Materials and Chemicals

Cultivated biennial Danggui were collected from the main cultivation areas in China (Table 1). The fresh samples were identified morphologically during the field collection and then dried under the sun. Parts of them were stored for two years under natural condition until the isolation of ferulic acid and Z-ligustilide from them. Each plant was dissected into head, body, and tail parts as described in literature (Wei *et al.*, 2008), then chemical constituents were extracted, respectively. The rest of them were immediately stored under four factitious storage conditions (Figure 2) and were evaluated every two months. Ferulic acid and Z-ligustilide with purities of >98% were purchased from Sigma (St. Louis, MO) and TLCM (Hong Kong, China), respectively.

Table 1: The Samples Up to Standard

Source of Samples (n=5)	Ferulic Acid			Ligustilide			Total
	Body	Head	Tail	Body	Head	Tail	
YX-GS (Yanxian of Gansu)	*		*			#	2* + 1#
WX-GS (Weixian of Gansu)			*				1*
ZX-GS (Zhangxian of Gansu)	*	*	*				3*
MX-GS (Minxian of Gansu)	*		*	#		#	2* + 2#
ZY-YN (Zhanyi of Yunnan)	*	*	*		#	#	3* + 2#
QJ-YN (Zhanyi of Qujing)	*	*	*	#		#	3* + 2#
BS-LJ-YN (Baisha of Lijiang of Yuannan)				#		#	2#
JS-LJ-YN (Jinshan of Lijiang of Yuannan)							
CF-LJ-YN (Chufeng of Lijiang of Yuannan)	*	*	*				3*
XZ-LJ-YN (Xinzhu of Lijiang of Yuannan)	*	*	*				3*
HS-LJ-YN (Huangshan of Lijiang of Yuannan)	*	*	*				3*
WX-YN (Weixi of Yuannan)							
DL-YN (Dali of Yuannan)	*		*				2*
ZJ-SC (Zhongjiang of Sichuan)			*				1*
Total	9*	6*	11*	3#	1#	5#	26* + 9#
	64%	43%	79%	21%	7%	36%	(62% + 21%)

Note: *The sample contains more than 0.05% of ferulic acid (Pharmacopoeia of the People's Republic of Chi-na), or #the sample contains more than 0.6% of Z-ligustilide (Hong Kong Chinese Materia medica Stan-dards), calculated with reference to the dried substance.

Sample Preparation

Powdered samples were accurately weighed for 0.5 g, soaked overnight in methanol (10 mL), and extracted twice under ultrasonic conditions for 30 min. The mixture was centrifuged at 4000 rpm for 15 min. The supernatant was filtered by a Millipore filter unit (0.2 μm), and then subjected to high-performance liquid chromatography (HPLC) analysis.

HPLC Analysis

HPLC was performed on the analytical column (Ultimate XB-C18, 250 × 4.6 mm i.d., 5 μm) in Agilent 1260 Infinity HPLC system. The mobile phase were (A) water (pH 4.0 adjusted with phosphoric acid) and (B) acetonitrile. The injection volume was 10 μL. The gradient of V_A:V_B was initial 80:20; 0–15 min, 80:20; 15–25 min, 48:52; 25–50 min, 50:50. The flow rate kept constant at 1.0 mL/min and the peak was detected at 316 nm.

Quantitative Analysis

Standards (ferulic acid, 1 mg/mL) and (Z-ligustilide, 2 mg/mL) were serially diluted, injected triplicately to HPLC at each dilution. Calibrated standard curve was derived from the peak area using the linear least-squares regression equation. The content of ferulic acid and Z-ligustilide in the samples was calculated according to the respective standard curves.

Graph

The graphs were performed by using Prism software version 4.0c (GraphPad, San Diego, CA).

RESULTS AND DISCUSSION

Plant Part vs Chemical Constituent

The therapeutic activities were not in complete accord of three parts of Danggui according to empirical observations of thousands of years (Ang-Lee *et al.*,2001; and Wei *et al.*, 2008). Head is usually used to stop bleeding; body to nourish blood for pregnant women; tail to promote blood circulation for puerpera. However, the background knowledge of its chemical composition is few yet. Quantitative analysis data showed that the order of the content of ferulic acid and Z-ligustilide was in tail > body > head of Danggui in most cases (Figure 1A and 1B). There seem to be some correlation between therapeutic activities and content of ferulic acid and Z-ligustilide in three parts of Danggui. This result may be helpful for further research on accuracy of quality control.

Cultivated Region vs Chemical Constituent

Profuse literature has reported the variation in content of active constituents depending on (i) growth period (Sa *et al.*, 2012; and Qian *et al.*, 2013), (ii) cultivated region (Wang *et al.*, 2013; Zhang *et al.*, 2013), (iii) plant part (Lu *et al.*, 2005; and Wei *et al.*, 2008). In contrast with plant part, our result showed that there are no significant variation of Z-ligustilide and ferulic acid from different sub-regions of China (Figure 1A and 1B). The results are inconsistent with previous reports, which may be caused by different freshness of samples. We evaluated the stored samples and we speculated that there exists considerable variation in concentrations of active constituents in fresh samples collected from different places, but the variation is narrowed during stored time.

The Quality of Long-Term-Stored Danggui

According to the quality standards, the sample should contain not less than 0.05% of ferulic acid (Pharmacopoeia of the People's Republic of China, 2010), and 0.6% of ligustilide (Hong Kong Chinese Materia medica Standards, 2005), calculated with reference to the dried substance. The concentrations of ferulic acid were seriously lessened in two-years-stored Danggui under natural condition (Figure 1A), more seriously for Z-ligustilide (Figure 1B). We sum up the standard samples in Table 1. The order of the percent of pass is tail > body > head, for ferulic acid as 79 > 64 > 43%, add up to 62%; for Z-ligustilide as 36 > 21 > 7 %, add up to 21%.

Storage Condition vs Quality Control

In order to understand the variation of Z-ligustilide and ferulic acid during stored period, we imitate four storage conditions (Figure 2) to survey their variation every two months during 18 months. The concentrations of Z-ligustilide and ferulic acid are, on the whole, lessened during stored period under any factitious storage conditions; the content of Z-ligustilide reduced more easily than ferulic acid (Figure 2A and 2B). This result consisted with an all-known fact that Z-ligustilideis more unstable than ferulic acid (Lu *et al.*, 2004). Under dry condition (4 or 25 °C, 50% RH), the concentrations of ferulic acid basically met the standard, however Z-ligustilide was below standard after 5-6 months. Under moist condition (25 °C, 90% RH), the concentrations of ferulic acid and Z-ligustilide were rapidly below standard. Mild condition (30 °C + 75% RH) is unhelpful

Figure 1: Content of (A) Ferulic Acid and (B) Z-ligustilid in Two-Years-Stored *A. sinensis* of Different Sources, Red Dashed Line is the Limit Line of Ferulic Acid (Pharmacopoeia of the People's Republic of China), or of Z-ligustilide (Hong Kong Chinese Materia Medica Standards)

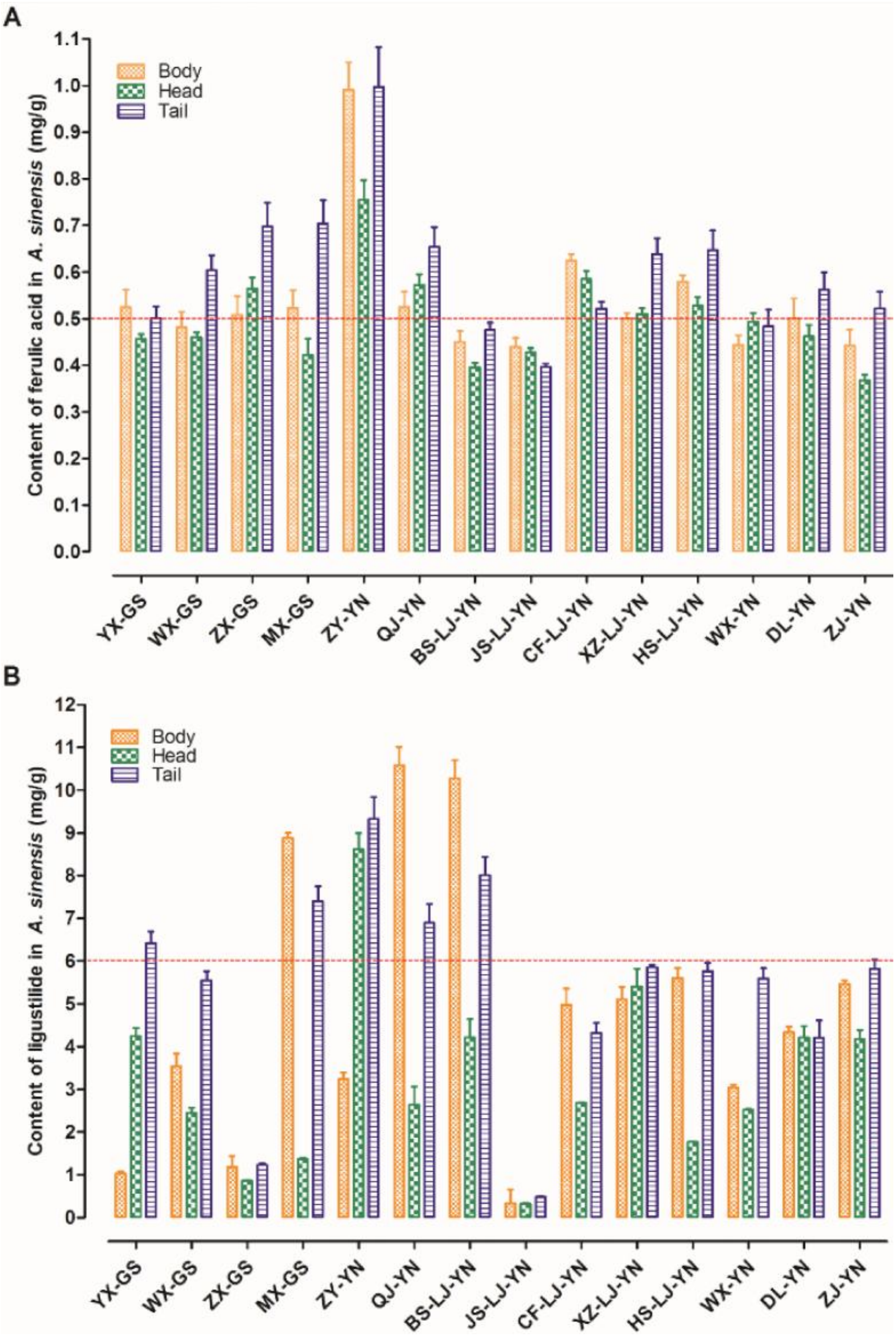
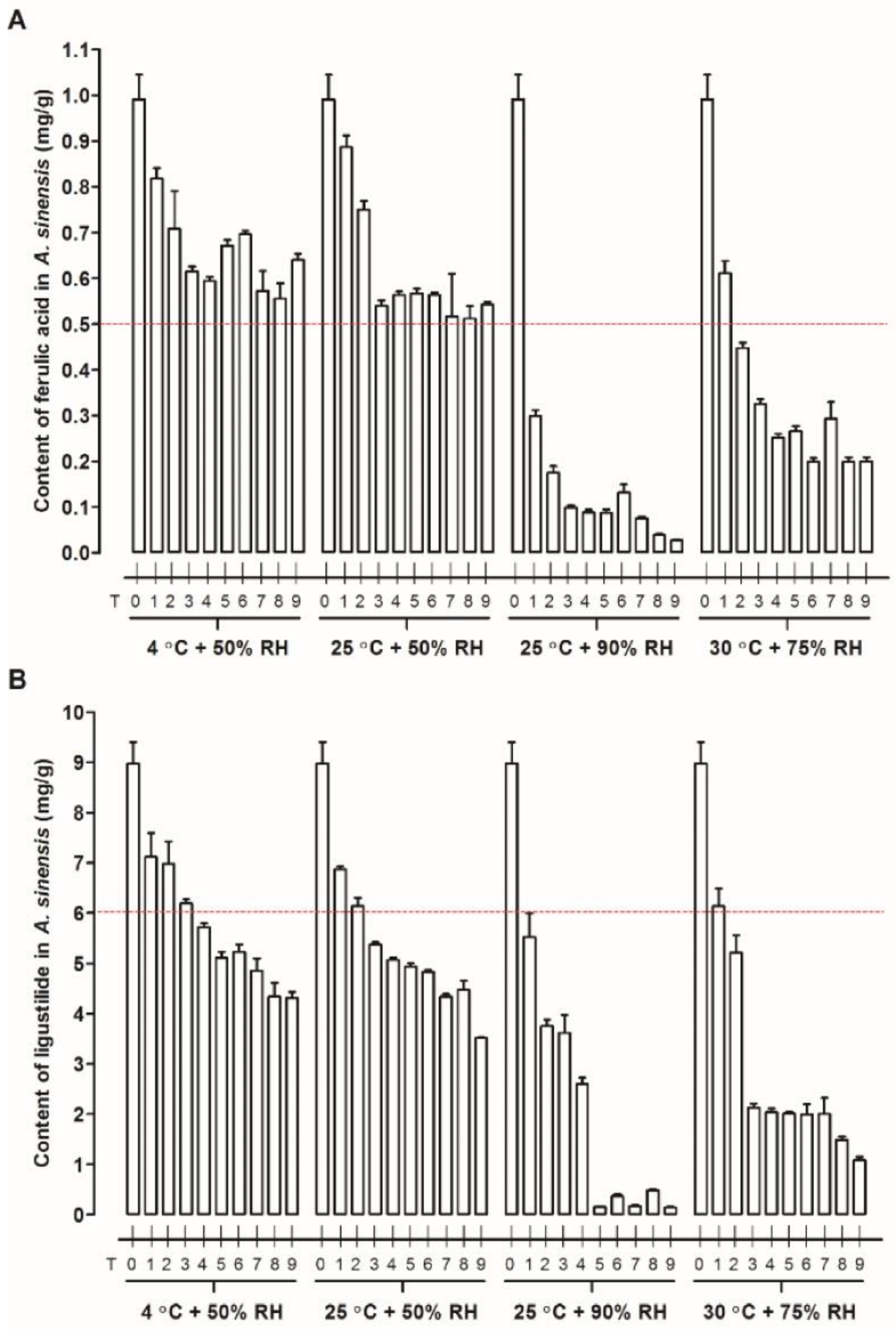


Figure 2: Content of (A) Ferulic Acid and (B) Z-ligustilid in *A. sinensis* Stored Under Different Conditions, Red Dashed Line is the Limit Line of Ferulic Acid (Pharmacopoeia of the People's Republic of China), or of Z-ligustilide (Hong Kong Chinese Materia Medica Standards)



to hold the two chemical constituents. Taken altogether, the dry condition is essential, if cold together is preferred for maintaining thequality of Danggui.

CONCLUSION

In summary, this study investigated the content of main chemical constituents in Danggui, ferulic acid and Z-ligustilide, and their changes during storage period. The data obtained indicate that the content of ferulic acid and Z-ligustilide were lessening after post-harvested Danggui. Dry condition is indispensable for storage and distribution of Danggui, if cold together is preferred. It is recommended that the post-harvested Danggui be stored at 4°C, 50% RH, and the shorter the better. To say the least of it, under current regulation surveillance in China, herbal quality control should be done after storage and distribution, not before. The standard of storage and distribution should be established as soon as possible to meet expectations in safety, quality, and efficacy of Chinese herbal medicines.

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