

## Study on the Relevance Between Beany Flavor and Main Bioactive Components in Radix Astragali

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Beany flavor is a traditional sensory indicator for evaluating the quality of Radix Astragali (RA or “Huangqi” in Chinese). A RA root with a strong beany flavor is considered to be good quality in Chinese medicine. However, there is neither a study reporting volatile compounds contributing to RA beany flavor nor the relevance between beany flavor and the quality of RA. In this study, we assessed the quantification of beany flavor substance and main bioactive metabolites. The results showed that hexanal was a major volatile component contributing to the beany flavor in RA. The value of hexanal was significantly related to the origin and growth age of RA, indicating that the component could be used as a volatile indicator for the distinction of RA. Statistical analysis further demonstrated that hexanal, astragaloside IV, and total polysaccharides were primary indicators and total isoflavonoids, astragalosides, calycosin, and formononetin were the secondary indicators for quality control of RA. Correlation analysis showed that the level of hexanal was positively associated with the concentration of astragaloside IV and total polysaccharides. Our study demonstrated that aroma is one of the most important quality attributes of RA and will help to understand the role of aroma in quality assessment of traditional Chinese medicines.

**KEYWORDS:** Radix Astragali; beany flavor; quality; hexanal; sensory evaluation

### INTRODUCTION

Radix Astragali (RA or “Huangqi” in Chinese) is an important traditional Chinese herb in the Leguminosae family. The dried roots of RA have been used as a tonic and diuretic. RA has a wide range of immunopotentiating effects and has proven efficacious as an adjunct cancer therapy (1). The beany flavor is a traditional sensory indicator for evaluating the quality of RA. It is widely accepted that RA with a strong beany flavor is in relatively good quality in Chinese medicine. However, the studies about chemical analysis of RA are mainly focused on non-volatile bioactive compounds. These studies have showed that isoflavonoids, saponins, and polysaccharides are three major groups of beneficial compounds of RA, and the contents of these main constituents change according to the origin and age of the plants (1–3). Among them, calycosin and formononetin are two isoflavones widely distributed in the Leguminosae family (4). In addition, astragaloside IV has been commonly used as a standard substance for the quality control of the herb. To our knowledge, there is no study showing that volatile compounds contributed to the beany flavor of RA or the relationship between the volatile beany flavor components and the non-volatile bioactive components in RA.

Volatile compounds can be derived from fruits and vegetables, such as soybean, green pea, lentil, peanut, and tomato (5–9). Studies have showed that the typical flavor-causing compounds were volatile aldehydes, such as *n*-hexanal, *cis*-3-hexenal,

and *trans*-2-hexenal (6, 10, 11). Most findings derived from these studies indicated that lipoxygenase mediated the conversion of polyunsaturated fatty acids to hydroperoxides and that their degradation was responsible for off-flavor (12, 13). On the one hand, researchers are trying to reduce the concentration of the beany-flavor-producing compounds in raw or processed products, in an attempt to enhance the potential of use in the formulation of food systems (5, 7, 13, 14). On the other hand, it is necessary to understand the role that aldehydes have played in the physiological functioning of plants. Gardner et al. demonstrated that undamaged soybean seedlings and normal commercial soybean cultivars produced *n*-hexanal in trace amount, inhibiting the germination and subsequent growth of soybean (15). The study from Lanciotti et al. showed that hexanal had a significant inhibitory effect against the pathogen microorganisms frequently isolated from raw materials in both model and real systems (16). However, no studies have shown the possible roles of volatile flavor compounds in traditional Chinese medicine.

In this study, we isolated and determined the major volatile components from raw RA materials using gas chromatography (GC) techniques. Quantitative determination and statistical analysis were also applied to study the correlation between hexanal, beneficial compounds, and standard substances for quality control of RA.

### MATERIALS AND METHODS

**Plant Material.** Dried roots of *Astragalus membranaceus* and *A. membranaceus* var. *mongholicus* (numbers 1–6 in Table 1) were collected respectively from Shanxi, Shaanxi, Sichuan, and Heilongjiang in China.

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**Table 1.** Plant Materials Examined and Their Basic Facts

number	species	source	age of growth (year)	collection time
1	<i>A. membranaceus</i>	Heilongjiang; planting	2	Dec 2007
2	<i>A. membranaceus</i> var. <i>mongholicus</i>	Lixian, Sichuan; planting	2	Dec 2007
3	<i>A. membranaceus</i> var. <i>mongholicus</i>	Shaanxi; natural	unknown	Dec 2007
4	<i>A. membranaceus</i> var. <i>mongholicus</i>	Guanglin, Shanxi; planting	5	Dec 2007
5	<i>A. membranaceus</i> var. <i>mongholicus</i>	Hunyuan, Shanxi; natural	unknown	Dec 2007
6	<i>A. membranaceus</i> var. <i>mongholicus</i>	Hunyuan, Shanxi; planting	5	Dec 2007
7	<i>A. membranaceus</i> var. <i>mongholicus</i>	Hunyuan, Shanxi; planting	3	Oct 2008
8	<i>A. membranaceus</i> var. <i>mongholicus</i>	Hunyuan, Shanxi; planting	4	Oct 2008
9	<i>A. membranaceus</i> var. <i>mongholicus</i>	Hunyuan, Shanxi; planting	5	Oct 2008
10	<i>A. membranaceus</i> var. <i>mongholicus</i>	Hunyuan, Shanxi; planting	6	Oct 2008

The collected samples were identified morphologically by Prof. Xue-Mei Qin of the Modern Research Center of Traditional Chinese Medicine, Shanxi University. The detailed information of the materials applied was presented in **Table 1**. Individual samples were broken into small pieces approximately 2 cm in length. A portion of the broken pieces was dried in an air-forced oven at 60 °C until a constant weight (for at least 24 h) and then stored with silica gel for dry matter determination and constituent analysis, excluding the volatile substance. The remaining part was frozen with liquid nitrogen and then stored at -80 °C for volatile substance analysis. Approximately 500 g of each sample was collected from 10 plants of the same population.

Fresh roots of the cultivated *A. membranaceus* var. *mongholicus* (numbers 7–10 in **Table 1**) that had grown for different years were collected from Hunyuan, Shanxi. The roots were sun-dried and treated as described above. The voucher specimens were deposited in the laboratory of the Modern Research Center of Traditional Chinese Medicine.

**Chemicals for Quantitative Analysis.** The authentic reference standards of formononetin (>99.0%), calycosin (>98.0%), and astragaloside IV (>98.0%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Hexanal (>99.0%) was obtained from Labor Dr. Ehrenstorfer-Schäfers (Germany). Phenylcarbinol (>98.0%) was added to the standard stock solution as an internal standard for hexanal determination. Dextran, with a molecular weight at 10 000 (Sigma, St. Louis, MO), was applied as a standard for quantitative determination of total polysaccharide. High-performance liquid chromatography (HPLC)-grade reagents were provided by Fisher Scientific (Loughborough, U.K.).

In the quantification of hexanal, isoflavonoids, and astragalosides, the standards were weighed and dissolved in 1 mL of *n*-hexane, methanol, and methanol, to give serial concentrations, respectively. Three injections were performed for each dilution. The standard curve was calibrated using a linear least-squares regression equation derived from the peak area. All of the solutions were stored at 4 °C before analysis.

**Simultaneous Steam Distillation and Extraction (SDE) of the Volatile Substance and Quantification of Hexanal.** Sub-samples of air-dried samples (2 g) in triplicate for each sample were mixed with 60 mL of distilled water and then subjected to SDE with 10 mL of *n*-hexane as a solvent for 4 h using a 523010-000 Likens–Nickerson apparatus (Kontes, NJ) (17). A total of 0.1 mL of phenylcarbinol, used as an internal standard (20.4 mg/mL in distilled water), was added to the samples before extraction. The extraction was performed 3 times, and the total extract was initially concentrated to 2 mL under a gentle stream of nitrogen gas, before being dried using anhydrous sodium sulfate at 4 °C. Finally, samples were concentrated to 1 mL under reduced pressure and at room temperature.

A HP5890 series II gas chromatograph (Hewlett-Packard Company, Avondale, PA) equipped with a capillary column with a polar resin of DB-Wax (OV1701, 30 m × 0.25 mm inner diameter, 0.25 μm film thickness) was used for hexanal determination. The GC conditions were as follows: column temperature, 160 °C; flame ionization detector (FID) temperature, 250 °C; carrier gas, nitrogen; flow rate, 30 cm/s; and inlet temperature, 230 °C.

**Dry Matter.** Sub-samples of air-dried samples (5 g) in duplicate for each sample were analyzed for residual water content using a proven method (18). Samples were oven-dried at 105 °C for 12 h, before weighing.

**Extraction and Quantification of Polysaccharides.** Polysaccharides were determined using an anthrone–sulfuric acid method. Sub-samples of air-dried samples (5 g) were homogeneously ground into fine powder using a D-7319 electric hammer-mill (Tianjin, China) and refluxed

3 times with 100 mL of water for 1 h. The watered extract was filtered while hot. The filtrate was evaporated to about 10 mL *in vacuo*, and then 95% ethanol was added to the exact until the concentration of ethanol was about 80%. The solution was kept airtight for 24 h and was then filtrated under vacuum. The cake was washed with 70% ethanol 5 times and then evaporated to a constant weight at 60 °C. Polysaccharides were weighed and dissolved in water at 60 °C. The solution was then centrifuged at 2000 rpm for 5 min to remove insoluble matter. The supernatant was adjusted to 100 mL in a volumetric flask, before being stored at 4 °C for further analysis.

To calibrate polysaccharides, dextran was weighed (30 mg) and dissolved in 100 mL of water to give serial concentrations. Standard solutions (0.2 mL) or prepared samples were taken and adjusted to a volume of 2.0 mL. Then, 4.0 mL of 0.2% anthrone–sulfuric acid was added. Absorbance at 625 nm was measured after 30 min of color reaction.

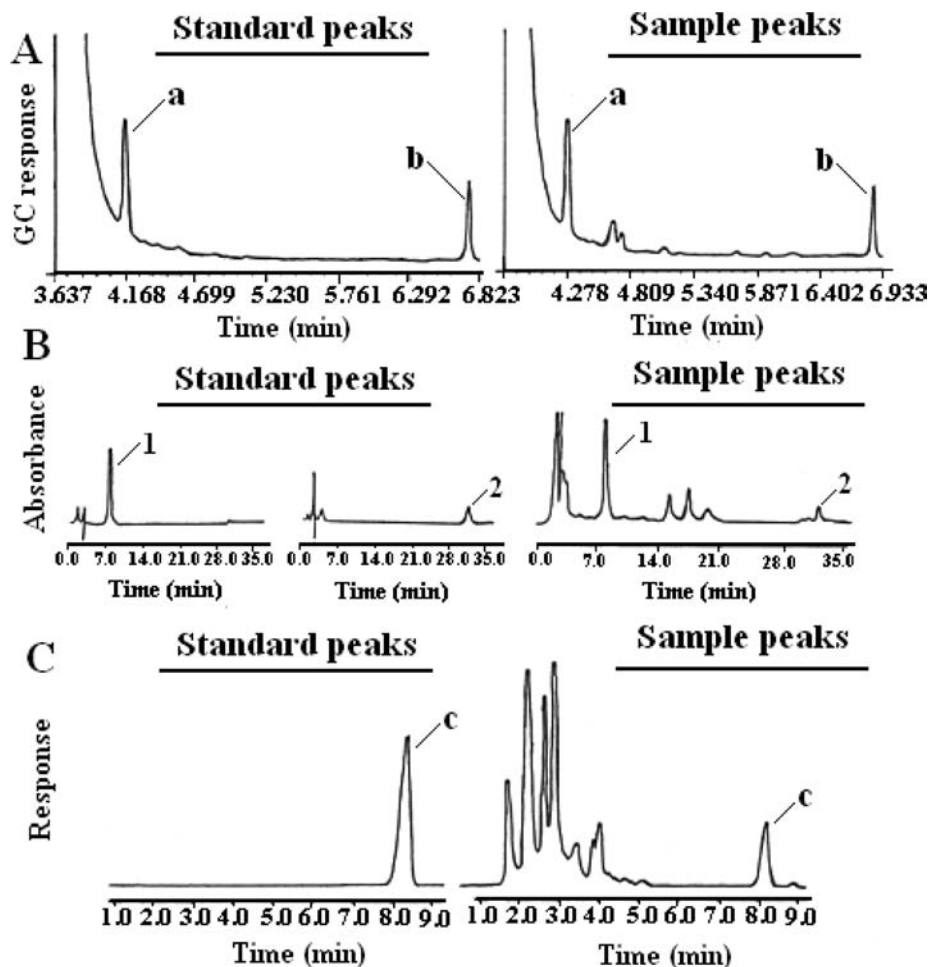
**Extraction and Quantification of Total Isoflavonoids, Saponins, Formononetin, Calycosin, and Astragaloside IV.** The extraction and quantification of isoflavonoids and saponins were carried out using a modified method based on Ma et al. (3) and Wang et al. (19). For isoflavonoids, 5 g of ground powder was extracted 3 times with 50 mL of aqueous MeOH (4:1 MeOH/H<sub>2</sub>O) under reflux for 3 h. The combined MeOH extracts were filtered and evaporated to dryness at a reduced pressure. Saponins were analogously extracted using *n*-butanol saturated with water, before the butanol extract was concentrated. Both viscous residues were dissolved in 2 mL of MeOH and then adjusted to 5 mL in a volumetric flask. Finally, they were filtered through a Millipore filter unit. A total of 20 μL of the samples was injected to HPLC.

HPLC was performed on a Waters 2487 system with UV and evaporative light-scattering detection (ELSD) 6800 detectors (ESA, Chelmsford, MA), a Hypersil ODS<sub>2</sub> C<sub>18</sub> (4.6 × 250 mm, 5 μm) column, and Waters 1525 pump. For the astragaloside IV assay, the mobile phase was CH<sub>3</sub>CN/H<sub>2</sub>O (34:66). For the determination of calycosin and formononetin, the mobile phase was also CH<sub>3</sub>CN (A)/H<sub>2</sub>O (B) but a linear gradient elution was applied from 17 to 31% A starting from 0 to 25 min, from 31 to 35% A starting from 25 to 26 min, 35% A starting from 26 to 45 min, and from 35 to 17% A starting from 45 to 60 min. The values were detected at 210 nm for astragaloside IV (ELSD) and at 230 nm for formononetin and calycosin (UV), with a flow rate of 1.0 mL/min. ELSD was set at a tube temperature of 50 °C and a gas (N<sub>2</sub>) flow rate of 1.5 mL/min.

**Data Analysis.** The data were statistically analyzed using SPSS for Windows, version 11.5.0 (SPSS, Inc., Chicago, IL), and SIMCA-P+ 11 (Umetrics, Umea, Sweden).

## RESULTS

**GC Spectra and Determination of Hexanal.** GC calibration curves for hexanal exhibited a good linearity in a range from 12 to 405 μg/mL. The relative standard deviation (RSD) was 1.87%, and the average recovery was 95.7% ( $n = 6$ ). Gas chromatographic separation of total volatile distillates was shown in **Figure 1A**. Three to six peaks were separated from each sample (see the Supporting Information). The peak of hexanal was identified by comparing the retention times of unknown peaks to the standard analyzed under identical instrument conditions. Significant differences were observed in the RA samples examined, as far as the content of hexanal was concerned. However, the percentage of hexanal was more than 66% of the volatile



**Figure 1.** GC and HPLC chromatograms of RA extracts. (A) GC chromatogram for determination of hexanal. Peaks a and b correspond to hexanal and phenylcarbinol, respectively. (B) HPLC chromatogram of methanol extracts for isoflavonoids. Peaks 1 and 2 correspond to calycosin and formononetin, respectively. The scale bar indicates the absorbance of 0.01 at 230 nm. (C) HPLC–ELSD chromatogram of butanol extracts for astragaloside IV. The indicated peak c corresponds to astragaloside IV. The scale bar indicates the absorbance of 0.01 at 210 nm.

**Table 2.** Amounts of Hexanal and Other Major Bioactive Products in RA Samples. Samples are Numbered in Line with Table 1<sup>a</sup>

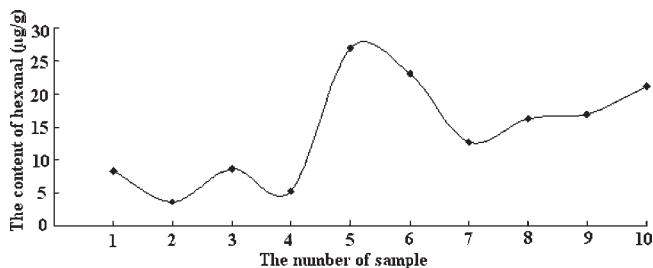
sample number	hexanal ( $\mu\text{g/g}$ )	IS1 ( $\mu\text{g/g}$ )	IS2 ( $\mu\text{g/g}$ )	TIS (mg/g)	AS1 ( $\mu\text{g/g}$ )	TAS (mg/g)	TP (%)	DM (%)
1	8.234	165.4	84.97	1.73	540	20.60	0.79	87.10
2	3.536	266.6	57.21	1.70	850	19.75	3.08	86.90
3	8.609	668.5	60.77	2.49	790	22.96	2.88	90.62
4	5.220	733.1	34.98	2.31	860	24.28	2.84	89.00
5	26.95	518.1	239	2.28	2010	20.97	6.56	91.15
6	22.95	379.1	34.87	2.05	1890	18.98	6.89	87.01
7	12.67	236.2	35.52	1.95	2070	16.63	8.90	88.66
8	16.14	108.2	37.01	1.83	1960	22.84	8.54	90.95
9	16.80	518.6	119.1	1.84	2160	17.48	5.86	90.83
10	21.02	790.7	75.97	2.26	1870	22.29	10.30	90.95
means	14.21	438.4	77.94	2.04	1500	20.68	5.66	89.32
$\pm$ SD	7.886	243.6	62.88	0.28	650	2.49	3.14	1.80

<sup>a</sup> Hexanal (X1); IS1 (X2), calycosin; IS2 (X3), formononetin; TIS (X4), total isoflavonoids; AS1 (X5), astragaloside IV; TAS (X6), total astragalosides; TP (X7), total polysaccharides; DM, dry matter; SD, standard deviation of the mean. Values are averaged, and  $n = 10$ .

substance in each sample (number 8 made an exception with only 30.5%), indicating that hexanal was a major component producing the beany flavor in RA. Hexanal concentrations in RA samples were shown in Table 2.

**Determination of Main Non-volatile Bioactive Constituents.** Astragalosides and isoflavonoids exhibited a good linearity in HPLC calibration curves ranging from 39 to 312  $\mu\text{g/mL}$  and from 0.3 to 1.0 mg/mL, respectively. Recovery tests by extracting known amounts of astragalosides and isoflavonoids showed that

the recoveries were 93.6 and 97.5%, respectively. In addition, the corresponding RSDs were 1.25 and 2.56%. Panels B and C of Figure 1 were typical chromatograms of MeOH and butanol extracts derived from different materials. The peaks of formononetin, calycosin, and astragaloside IV were identified by comparing the retention times of the unknown peaks to the corresponding standards analyzed under identical instrument conditions. Table 2 demonstrated the concentrations of formononetin, calycosin, and astragaloside IV in different RA samples. The amounts



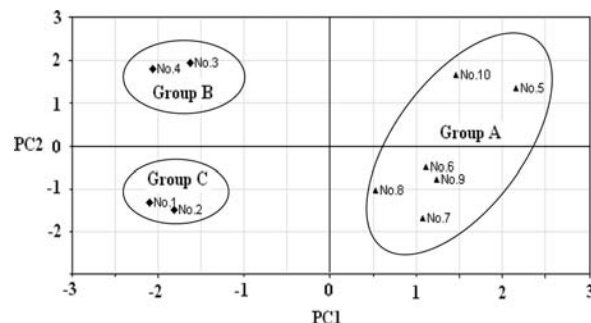
**Figure 2.** Effects of growth age and origin on the content of hexanal in RA. Samples are numbered in line with Table 1.

of dry matter, total isoflavonoids, total astragalosides, and polysaccharides were also determined and exhibited in Table 2.

**Data Analysis of Hexanal and Main Non-volatile Bioactive Constituents.** Previous studies showed that there was no obvious difference in the amount of total isoflavonoids, polysaccharides, and amino acids among different species of *Astragalus* (3). Our results indicated that the amounts of total isoflavonoids, total astragalosides, and dry matter were relatively constant in all RA samples examined, and the values of mean  $\pm$  standard deviation (SD) were  $2.04 \pm 0.28$  mg/g,  $20.68 \pm 2.49$  mg/g, and  $89.32 \pm 1.80$  g/100 g, respectively. However, the contents of hexanal, formononetin, calycosin, astragaloside IV, and polysaccharides were variable. Among them, the content of hexanal ranged from 5.22 to 26.95  $\mu\text{g/g}$ , with an average of  $14.21 \pm 7.89$  (mean  $\pm$  SD,  $\mu\text{g/g}$ ,  $n = 10$ ). The RA sample from a natural source in Hunyuan contained the highest amount of hexanal, while the RA sample from a cultivated source in Lixian contained the lowest amount of hexanal. The content of hexanal in the cultivated plant of *A. membranaceus* (Heilongjiang) was closed to that of *A. membranaceus* var. *mongholicus* (Guanglin). Moreover, the level of hexanal was age-differed, and the 6-year-old plants contained the highest value for hexanal (Figure 2). The result implied that hexanal should be considered as a volatile marker for the distinction of RA.

Calycosin registered a concentration ranging from 108.2 to 790.7  $\mu\text{g/g}$ , with an average at  $438.4 \pm 243.6$  (mean  $\pm$  SD,  $\mu\text{g/g}$ ,  $n = 10$ ), with formononetin, astragaloside IV, and polysaccharides being at 34.87–239  $\mu\text{g/g}$  with an average of  $77.97 \pm 62.88$  (mean  $\pm$  SD,  $\mu\text{g/g}$ ,  $n = 10$ ), 540–2160  $\mu\text{g/g}$  with an average of  $1500 \pm 650$  (mean  $\pm$  SD,  $\mu\text{g/g}$ ,  $n = 10$ ), and 0.79–10.3% with an average of  $5.66 \pm 3.14\%$  (mean  $\pm$  SD,  $n = 10$ ), respectively. Results (Table 2) also showed that the natural RA collected from Hunyuan contained the highest concentrations of formononetin, astragaloside IV, dry matter, as well as hexanal. The natural RA from Shaanxi recorded the highest concentration of total isoflavonoids, with the cultivated plants from Guanglin having the highest calycosin and total astragalosides and the plants cultivated in Hunyuan being the highest in total polysaccharides. Comparatively speaking, the RA plants grown in Hunyuan contained relatively higher amounts of the components examined here.

**Quality Assessment.** Principle component analysis (PCA) and SPSS analysis were introduced for RA quality assessment using the data obtained above. A full  $10 \times 7$  autoscaled data matrix was submitted to PCA analysis, and three factors [(principal components (PCs)] were identified with initial eigenvalues  $> 1$ , which explained 87.6% of the total variability of the data set. PC1, PC2, and PC3 explained 40.7, 32.4, and 14.5% of the variance in the original observations, respectively. PC1 ( $Y_1$ ) was mainly characterized by astragaloside IV ( $X_5$ ), hexanal ( $X_1$ ), and total polysaccharides ( $X_7$ ), with the corresponding eigenvectors being at 0.957, 0.913, and 0.852, respectively. PC2 ( $Y_2$ ) was correlated with total isoflavonoids ( $X_4$ ), calycosin ( $X_2$ ), and total astragalosides ( $X_6$ ) at 0.937, 0.891,



**Figure 3.** Sample distributions on a score plot. Samples are numbered in line with Table 1.

and 0.695, respectively. PC3 ( $Y_3$ ) was involved in formononetin ( $X_3$ ) with an eigenvector of 0.839. Apparently, astragaloside IV, hexanal, and total polysaccharides were the primary indicators that could be employed for quality control purposes, followed by total isoflavonoids, calycosin, total astragalosides, and formononetin as secondary indicators. The relational expressions of the three PCs were as follows (suppressed absolute values being less than 0.10):

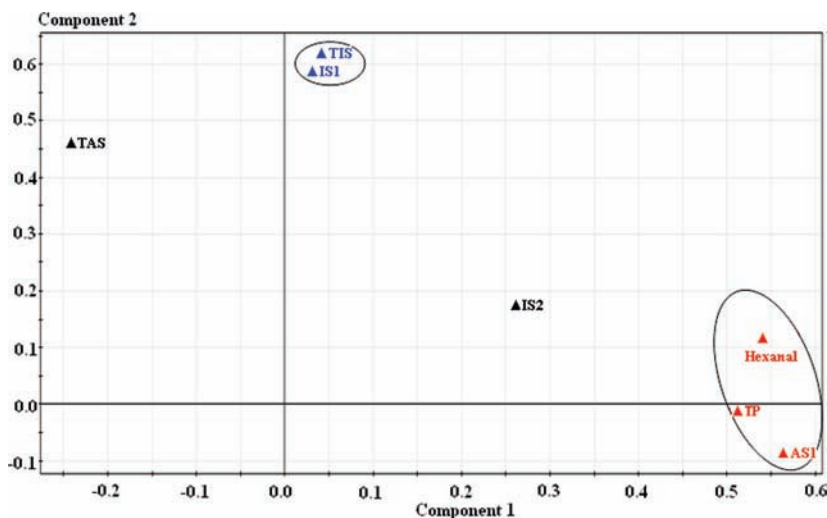
$$Y_1 = 0.913X_1 + 0.440X_3 + 0.957X_5 - 0.407X_6 + 0.852X_7$$

$$Y_2 = 0.181X_1 + 0.891X_2 + 0.266X_3 + 0.934X_4 - 0.132X_5 + 0.695X_6$$

$$Y_3 = 0.146X_1 + 0.839X_3 - 0.107X_4 - 0.159X_5 - 0.160X_6 - 0.470X_7$$

Because PC1 and PC2 accounted for 73.1% of the total variance, the multi-dimensional information stemming from the first two PCs was incorporated into a 2D data set to classify the samples. A score plot (Figure 3), obtained from measuring the data of 10 RA samples, showed a fine effect on RA quality assessment. Scores clustered samples into three major groups, namely, groups A, B, and C, corresponding to the differed chemical profiles for different regions. PC1 separated Hunyuan RA samples (group A) from the other RA samples collected from other regions, while PC2 distinguished group B. The remaining regions were clustered together as group C. Meanwhile, group A was further split into subgroups according to their chemical components. The loading plot of PC1 versus PC2 (Figure 4) showed that a range of components, including astragaloside IV, hexanal, total polysaccharides, total isoflavonoids, and calycosin, had an effect on the clusters in a top-down manner. They constituted the most important components that can be applied to discriminate Hunyuan RA samples from others. A great similarity was observed among Hunyuan RA samples (group A), the renowned original and genuine RA herbs in the country. Meanwhile, the results provided the solid evidence showing that the RA plants grown in Hunyuan were noticeably different from the one grown in Sichuan or Heilongjiang (group B).

PC1 explained more components compared to PC2 and took up the right side of Figure 3. In this context, RA samples grown in Hunyuan were supposed to be distinct from the others grown in other regions and the samples in the upper part of group A were supposed to be higher than the remaining parts in the concentration. Accordingly, it implied that the wild RA grown in Hunyuan ranked the best, followed by the cultivated one in the same area. Unfortunately, the cultivated RA grown in Sichuan or Heilongjiang (*A. membranaceus*) was the worst in the context of the concentrations of hexanal and other major bioactive constituents. Moreover, the quality of RA grown in Guangling and Shaanxi (group C) was



**Figure 4.** Loading plot of variables. Hexanal; IS1, calycosin; IS2, formononetin; TIS, total isoflavonoids; AS1, astragaloside IV; TAS, total astragalosides; TP, total polysaccharides.

**Table 3.** Correlation between Hexanal ( $\mu\text{g/g}$ ) ( $X$ ) and Other Major Bioactive Components ( $Y$ ) in RA<sup>a</sup>

chemical index	number	regression equations	related coefficient
astragaloside IV ( $\mu\text{g/g}$ )	10	$Y = 572 + 65.3X$	0.795** ( $p = 0.006$ )
TIS (mg/g)	10	$Y = 2.13 - 0.828X$	0.236 ( $p = 0.511$ )
TAS (mg/g)	10	$Y = 22.7 - 0.796 \ln X$	-0.214 ( $p = 0.553$ )
TP (%)	10	$Y = 1.79 + 0.272X$	0.684* ( $p = 0.0291$ )
formononetin ( $\mu\text{g/g}$ )	10	$Y = -51 + 34.9X - 3.05X^2 + 0.079X^3$	0.823 ( $p = 0.0635$ )
calycosin ( $\mu\text{g/g}$ )	10	$Y = 379.9 + 4.121X$	0.133 ( $p = 0.713$ )

<sup>a</sup>The components noticeably correlated to hexanal are marked with an asterisk.

relatively worse compared to the one grown in Hunyuan. Therefore, more attention should be paid to the use of the regions of RA.

#### Correlation Between Hexanal and Main Bioactive Components.

The beneficial compounds stemming from RA are saponins, isoflavonoids, and polysaccharides ( $I$ ). At present stage, a multiple attribute comprehensive appraisal is widely applied to assess the quality of RA ( $4, 20$ ), although astragaloside IV is a major marker employed for quality control purposes. In this context, it is meaningful to analyze the relationship between the two: (i) one index component controls another; (ii) determine the least index component to understand the comprehensive quality of RAs. Further analysis indicated that both astragaloside IV ( $X_5$ ) and calycosin ( $X_2$ ) agreed with total polysaccharides ( $X_7$ ) and total isoflavonoids ( $X_4$ ) in concentration, suggesting that astragaloside IV and calycosin could be borrowed to sit in place of total polysaccharides and total isoflavonoids for concentration. The relationship can be expressed as follows:

$$X_5 = 473 + 181X_7 \quad (r = 0.879^{**}, p = 0.001)$$

$$X_2 = -1034 + 720.5X_4 \quad (r = 0.816^{**}, p = 0.004)$$

As mentioned above, hexanal is a lead compound producing the raw bean flavor in RA and has an effect on the quality of the plant as well. The correlation between hexanal and other major bioactive components was exhibited in **Table 3**. Results showed that hexanal was positively correlated to astragaloside IV and total polysaccharides in concentration.

#### DISCUSSION

It is well-known that the levels of biologically active products vary widely in Chinese traditional medicinal herbs, depending

upon plant genotype and the environment where plants are grown ( $21$ ). Previous studies showed that a range of factors, including processing, temperature, storage time, additive, genotype, and lacking of lipoxygenase, could affect the yields of the beany-flavor-producing compounds in legumes ( $22-25$ ). The present study suggested that hexanal is a major volatile component responsible for the beany flavor. The level of hexanal can be noticeably different, depending upon the origin and age of the plants, suggesting that hexanal can be a volatile marker to assess the quality of RA. PCA analysis showed that astragaloside IV, hexanal, and total polysaccharides can be primary quality control indicators for RA. Data analysis further indicated that hexanal is correlated to astragaloside IV and total polysaccharides in concentration, with both of them normally used as indicators for the quality control of RA. Our research suggests that aroma is one of the most important quality attributes to RA. Moreover, RA samples contain relatively higher contents of bioactive components if they have a high hexanal level.

The pharmacological effects of RA are believed to be derived from isoflavonoids and saponins. Among them, astragaloside IV exerts a protective effect on porcine-serum-induced hepatic fibrosis in rats and increases the rate of peripheral nerve regeneration across a wide gap ( $26, 27$ ). Both calycosin and formononetin possess a range of beneficial effects, including antioxidation, neuroprotection, and endothelial cell protection ( $28, 29$ ). In addition, polysaccharides, one of major bioactive ingredients in RA, have been proven to stimulate immune response ( $30$ ). Although hexanal is identified as a major volatile compound contributing to beany flavor and positively correlated to the quality of RA, its biological role remains unclear in the herb. In conclusion, this study provides evidence that sensory indicators play an important role in the quality assessment of traditional Chinese medicine.

#### ACKNOWLEDGMENT

We are thankful to Wansheng Radix Astragali Development Co. Ltd. in Hunyuan (Shanxi, China) for providing natural RA samples grown in Shanxi and Shaanxi.

**Supporting Information Available:** Chromatograms of GC for the determination of volatile compounds contributing to beany flavor in RA (from sample 1 to sample 10). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Received for review December 3, 2009. Revised manuscript received March 23, 2010. Accepted March 25, 2010. Thanks are given to the Ministry of Science and Technology of the People's Republic of China (Grant 2006BA106A15-7) and Shanxi Provincial Department of Science and Technology (Grant 2005091016-0502) for their valuable and generous support.