Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 86 (2004) 155–159
Food Chemistry
www.elsevier.com/locate/foodchem

Rapid Communication

Changes in neutral and malonyl ginsenosides in American ginseng (*Panax quinquefolium*) during drying, storage and ethanolic extraction

X.W. Du [1], R.B.H. Wills *, D.L. Stuart

School of Applied Sciences, University of Newcastle, P.O. Box 127, Ourimbah NSW 2258, Australia

Received 3 October 2003; accepted 3 November 2003

Abstract

Changes in the neutral and malonyl ginsenosides of American ginseng roots (*Panax quinquefolium*) were examined during drying, storage and extraction. Freshly harvested roots dried at 40, 55 or 70 °C in a hot air drier showed a marked decrease in the time taken to dry as the air temperature increased but the colour of roots dried at 70 °C was darker than the desired cream colour. Increasing the drying temperature decreased the concentration of total ginsenosides but with an increase in the neutral ginsenosides and a decrease in malonyl ginsenosides. Dried root powder, stored at 5, 20 and 30 °C in air of low humidity, showed a decrease in the concentration of total ginsenosides at all temperatures with the rate of loss increasing at higher temperatures. The concentration of malonyl ginsenosides showed the same trend but the concentration of neutral ginsenosides did not significantly change during storage at any temperature. Extraction of ginsenosides, from dried root powder with aqueous ethanol, varied with the ethanol content. Maximum extraction of neutral ginsenosides was obtained with 70% ethanol while, for malonyl ginsenosides, it was 40% ethanol and 60% ethanol for total ginsenosides. The ratio of neutral to malonyl ginsenosides in the extract increased as the proportion of ethanol in the solvent increased. The findings show that the relative proportions of neutral and malonyl ginsenosides vary considerably during postharvest handling and reinforce the need to measure both types to obtain the true level of total ginsenosides.

1. Introduction

Ginseng, a traditional medicinal herb in Asia, has become internationally popular in recent years. The traditional source of ginseng root has been Asian ginseng (*Panax ginseng* C.A. Meyer) but American ginseng (*Panax quinquefolium* L.), a plant native to North America, is now also cultivated and used in many countries. While the full pharmaceutical activity of ginseng is due to a range of compounds, the triterpene saponins, known as ginsenosides, are widely considered to be the most important components contributing to the multiple medicinal properties of both Asian and American ginseng (Court, 2000). The importance of the saponins in Asian ginseng has been known for about 100 years and, with the advent of modern chromatographic techniques, about 30 triterpene saponin glycosides, designated as neutral ginsenosides, have been identified (Sticher, 1998). The presence of neutral ginsenosides in American ginseng was first demonstrated by Ando, Tanaka, and Shibata (1971) with later studies confirming that a range of neutral ginsenosides exists in this species similar to the previously studied Asian ginseng (*Panax ginseng*) (Hou, 1978).

About 20 years ago, Kitagawa, Taniyama, Hayashi, and Yoshikawa (1983) reported the presence of four acidic ginsenosides in the Asian ginseng and 5 years later Yamaguchi, Kasai, Matsuura, Tanaka, and Fuwa

* Corresponding author. Tel.: +61-2-43-48-4140, fax: +61-2-43-48-4565.
E-mail address: ron.wills@newcastle.edu.au (R.B.H. Wills).
[1] Present address: Pharmaceutical College, Heilongjiang University of Chinese Medicine, 24 Heping Road, Harbin 150040, China.

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.11.003

(1988) found the same malonyl ginsenosides in American ginseng root. The compounds contain a malonyl residue attached to a glucose unit of the corresponding neutral ginsenosides, Rb_1, Rb_2, Rc and Rd to create the corresponding mRb_1, mRb_2, mRc and mRd. Both authors reported the malonyl ginsenosides to be relatively unstable and to demalonylate to the corresponding neutral ginsenosides. In a review, Awang (2000) commented that the true ginsenosides content of ginseng was being underestimated by ignoring malonyl ginsenosides, as they can constitute a substantial proportion of the total ginsenosides content.

While there is some trade in fresh Asian ginseng root, the local and international trade in American ginseng is almost exclusively as a dried product. Where quality was determined by colour and/or root shrinkage, the most common methods used to accelerate the drying of ginseng is by ventilating roots with hot air, with or without dehumidification (Li & Morey, 1987; Sokhansanj, Bailey, & van Dalfsen, 1999; Wilhelm, 1990). Chemical assessments of the effect of drying on the medicinal quality of Asian ginseng found lower ginsenoside concentrations at 60 °C than 45 °C (Yoshikawa et al., 1993). Studies on the effect of drying of American ginseng, based on changes in the neutral ginsenosides, resulted in recommended drying temperatures in the range 20–50 °C (Wang, Jia, Liu, & Ren, 1990). However, Reynolds (1998) examined the effect of forced air drying, at 32, 38 and 44 °C, on both neutral and malonyl ginsenosides and found increasing loss of both with increasing temperature and recommended an optimal drying temperature of 38 °C.

In commercial practice, dried ginseng is stored for some time during marketing of the root or before further processing into food or therapeutic products. However, the published data on changes in ginsenosides during the storage of dried ginseng are only on Asian ginseng and on changes in the neutral ginsenosides (Choi, Byun, & Park, 1983; Noh, Do, Kim, & Oh, 1983). There are no published data on the effects of storage on the malonyl ginsenosides in either species.

An increasingly common method of processing ginseng is to obtain an extract containing the ginsenosides by mixing dried ginseng powder with aqueous ethanolic solution, then removing the solid residue (Liu & Song, 1992; Xu, Zheng, Zhou, & Zhao, 1996; Yang, Lin, & Ma, 1989). These studies have recommended 40–50% ethanol for optimal extraction but they have only examined the effect on neutral ginsenosides.

In this study, changes in the neutral and malonyl ginsenosides were determined in fresh American ginseng roots dried at various temperatures in a hot air drier, in dried ginseng powder stored under different environmental conditions for 12 weeks, and in extracts of ginseng root powder obtained with aqueous ethanolic solutions.

2. Materials and methods

2.1. Ginseng treatments

Freshly harvested 4-year-old American ginseng roots, that had been grown in Victoria, Australia were used in all experiments. The three studies on drying, storage and extraction were conducted in different seasons and hence used different batches of ginseng roots but, in each study, all roots were of a uniform size and appearance.

For the drying study, 27 roots were washed, then separated into nine groups of three roots and the weight of each group determined. The roots were placed in a hot air drier (GTD, Sydney) and ventilated with air at 40, 55 or 70 °C, with three groups held at each temperature to provide three replicate samples at each temperature. An additional group of three roots was dried in a vacuum oven at 70 °C to determine the water content of the fresh root. Roots in the hot air drier were weighed at periodic intervals and drying was terminated when the water content remaining in the roots was 10 g/100 g, the level found in commercially dried roots. Each group of roots was then ground to a powder and analysed for neutral and malonyl ginsenosides.

For the storage study, sufficient fresh roots were obtained to generate 130 g of dried material. The roots were dried at 40 °C in a hot air drier, then ground to pass through a 250 μm mesh sieve. Analysis of neutral and malonyl ginsenosides was performed on a sample (1 g) of dried root powder. Dried root powder was then spread across 12 Petri dishes at 10g/dish and covered with the Petri dish lid. Four Petri dishes containing the dried root powder were then placed in the dark in a room held at temperatures of 5, 20 and 35 °C in air of $<10\%$ relative humidity. Ginsenosides analysis was conducted on two samples of root powder (1 g) taken from a Petri dish at each temperature after storage for 3, 6, 9 and 12 weeks.

For the extraction study, sufficient fresh roots were obtained to generate 25 g of dried material. Extraction of ginsenosides by aqueous ethanol was studied over the full range of 0–100% ethanol. The extracting solvent (50 ml) was mixed with dried ginseng root powder (1 g) and stirred with a magnetic stirrer at room temperature for 30 min, followed by filtering through paper (Whatman #1) to remove the ginseng residue. The extracts were made up to 100 ml with the appropriate solvent and the concentration of ginsenosides in the extracts determined. An extraction run with all ethanol:water ratios was replicated on three different days.

2.2. Analysis of ginsenosides

Dried ginseng powder (1 g) was extracted with 80% methanol (15 ml) for 3 min in a high speed blender (Sorvall Omni-Mix, Waterbury, CT) then sonicated

(Mey Dental, Yucaipa, CA) for 15 min and filtered (Whatman #1). The solid residue was subjected to an additional extraction by sonication for 15 min in 10 ml methanol and the combined extracts made up to 50 ml. The extracts were analysed for neutral and malonyl ginsenosides by an HPLC method developed by Court, Hendel, and Elmi (1996). The method involved analysis of the extracted solution for neutral ginsenosides, hydrolysis of the extract to convert the malonyl ginsenosides to the corresponding neutral ginsenosides, analysis of the hydrolysed extract for total neutral ginsenosides, and calculation of the malonyl ginsenosides by the differential increases in the relevant neutral ginsenosides.

The HPLC system was a Shimadzu (Kyoto) 10AT pump, 10AXL autosampler, SPDM10A photodiode array detector, and CTO-10A column oven, 5 μm Lichrosorb column (250×4.6 mm^2) (Alltech, Deerfield IL) and a C_{18} guard column, at 40 °C. The eluted peaks were detected at 203 nm and quantified against external standards of the ginsenosides Rb_1 (Indofine, Somerville, NJ), Rb_2, Rc, Rd (Apin, Oxon, England), Re and Rg_1 (National Institute of Quality Control of Pharmaceutical and Biological Products, Beijing). The mobile phase was a gradient elution of water and acetonitrile (B), commencing with 20% B, rising to 22% B after 20 min then to 46% B after 45 min and 55% B after 50 min.

Aliquots (20 μl) of the initial extract were analysed for six neutral ginsenosides, Rb_1, Rb_2, Rc, Rd, Re and Rg_1. The total ginsenosides was determined by summing the levels of the six ginsenosides. All values were calculated on a dry weight basis. The extract solution (10 ml) was then taken to dryness by vaporisation of the methanol in a fume cupboard at room temperature. The residue was mixed with water (10 ml); 5% KOH solution (1 ml) was added and the resulting solution allowed to stand for 2 h at room temperature. The hydrolysed solution was neutralized by adding 14% KH_2PO_4 solution (1 ml) and made up to 25 ml with acetonitrile. An aliquot (20 μl) was then re-analysed for neutral ginsenosides. The chromatograms showed an increase in four neutral ginsenosides and quantification of the four corresponding malonyl ginsenosides, mRb_1, mRb_2, mRc and mRd, was obtained by subtracting the relevant neutral ginsenosides concentration in the original extract from that of the hydrolysed solution. Ginsenosides values in each study were statistically examined by analysis of variance and regression analysis.

3. Results

The data in Table 1 show that the time taken to dry roots in the hot air drier markedly decreased as the air temperature increased ($P < 0.001$) with the time to dry at 70 °C being 20% of that at 40 °C. The relationship followed a significant quadratic equation of $y = 0.04x^2 - 4.87x + 171.9$ ($P < 0.01$), where y = drying time (h) and x = drying temperature (°C). However, the colour of the roots dried at 70 °C was darker than that of roots dried at 55 and 40 °C, an undesirable characteristic in the industry (Sticher, 1998). Increasing the drying temperature showed differing effects on the levels of ginsenosides. Regression analysis showed that, with increasing drying temperature (x), the concentration of neutral ginsenosides (y) increased linearly ($y = 0.40x + 30.3$; $P < 0.01$), while that of malonyl ginsenosides decreased linearly ($y = -0.82x + 71.4$; $P < 0.001$). The overall effect was that the concentration of total ginsenosides also linearly decreased with increasing temperature ($y = -0.42x + 101.7$; $P < 0.05$).

The data in Table 2 show changes in the concentrations of neutral, malonyl and total ginsenosides during storage at 5, 20 and 30 °C in air of low humidity. Regression analysis, conducted for each type of ginsenosides at each storage temperature, showed a highly significant decrease in the concentration of total ginsenosides during storage for ginseng powder stored at all temperatures. Regression analysis of the rate of decrease in total ginsenosides (i.e., the slope of the equations in Table 2) (y) with storage temperature (x) showed a significant increase in the rate of loss with increasing temperature, ($y = -0.01x - 0.16$; $P < 0.01$). The concentration of malonyl ginsenosides was found to follow a similar trend involving a significant loss during storage at each temperature, with the rate of loss increasing as the temperature was increased ($y = -0.02x - 0.04$; $P < 0.01$). The concentration of neutral ginsenosides, however, did not significantly change during storage at any temperature.

The extraction of neutral and malonyl ginsenosides with aqueous ethanol varied with the proportion of ethanol in the extracting solvent (Table 3). Regression analysis showed that the concentration of neutral, malonyl and total ginsenosides followed a quadratic relationship with % ethanol in the solvent. Maximum extraction of neutral ginsenosides was obtained with about 70% ethanol and with 40% ethanol for the malonyl

Table 1
Drying time, appearance and ginsenoside levels in American ginseng roots dried in a hot air drier at different temperatures

Drying temperature (°C)	Drying time (h)	Root colour	Ginsenosides concentration (mg/g)		
			Neutral	Malonyl	Total
40	35	Cream	46.0[a]	38.1	84.1
55	12	Cream	53.1	26.5	79.6
70	7	Light brown	58.0	13.3	71.3

[a] Each value is the mean of three replicated root samples.

Table 2
Concentration of ginsenosides in dried root powder during storage over 12 weeks at 5, 20 and 35 °C

Temperature (°C)	Ginsenosides concentration (mg/g)					Regression equation[a]	Significance level[b]
	0	3	6	9	12 week		
Neutral ginsenosides							
5	45.4[c]	46.0	45.6	46.2	45.0	–	ns
20		46.1	46.1	45.6	46.0	–	ns
35		44.4	47.9	48.1	48.1	–	ns
Malonyl ginsenosides							
5	24.4	23.4	23.3	22.1	21.6	$y = -0.23x + 24.3$	***
20		23.3	22.7	22.0	19.9	$y = -0.34x + 24.5$	***
35		23.3	17.3	16.0	14.8	$y = -0.88x + 24.4$	***
Total ginsenosides							
5	69.8	69.4	68.8	68.4	66.6	$y = -0.25x + 70.0$	***
20		69.4	68.8	67.5	65.9	$y = -0.32x + 70.2$	***
35		67.7	65.1	64.1	62.9	$y = -0.58x + 69.4$	***

[a] Equation coordinates are: y = ginsenosides concentration (mg/g); x = storage time (weeks).
[b] (**) and (ns) indicate a significant relationship at $P < 0.001$ and no significant relationship at $P = 0.05$, respectively.
[c] Each value is the mean of two replicates.

Table 3
Concentration of ginsenosides in ethanolic extracts obtained from dried ginseng root powder

Solvent (% ethanol)	Concentration of ginsenosides (mg/g[a])		
	Neutral	Malonyl	Total
100	39.0[b]	11.8	50.8
90	48.5	16.0	64.5
80	51.1	17.2	68.2
70	50.4	19.5	69.9
50	50.4	20.9	71.3
30	47.9	19.8	67.7
0	44.0	17.8	61.8
$LSD_{(5\%)}$	±1.4	±1.7	±1.9
	Regression equation		
Neutral ginsenosides	$y = -0.003x^2 + 0.35x + 42.8$ ($P < 0.01$)		
Malonyl ginsenosides	$y = -0.002x^2 + 0.18x + 17.4$ ($P < 0.01$)		
Total ginsenosides	$y = -0.006x^2 + 0.53x + 60.2$ ($P < 0.01$)		

[a] Concentration was standardised against the weight of ginseng powder used.
[b] Each value is the mean of three replicates.

ginsenosides, resulting in 60% ethanol giving the maximum extraction of total ginsenosides. Consequently, there was a slightly higher proportion of neutral ginsenosides in the extract as the proportion of ethanol in the solvent increased, ranging from 71% in 0–50% ethanol to 77% in 100% ethanol.

4. Discussion

The findings have demonstrated the importance of analysing both neutral and malonyl ginsenosides to determine the true ginsenosides content of a ginseng product. First, it would seem that only estimating the neutral ginsenosides can underestimate the total ginsenosides by up to 40%. Neutral ginsenosides analysis can therefore give a misleading impression of the efficacy of a handling or processing treatment. This was illustrated in the drying trial, where the concentration of neutral ginsenosides increased as the drying temperature increased, whereas this was undoubtedly due to hydrolysis of malonyl ginsenosides to neutral ginsenosides (Kitagawa et al., 1983) so that the total ginsenosides were actually decreasing as the drying temperature increased. Similarly in the storage trial, analysis of neutral ginsenosides only would suggest that storage at temperatures up to 30 °C for 12 weeks was an acceptable practice as there was no decrease in neutral ginsenosides. However, the total ginsenosides were decreasing with increasing storage time and temperature due to substantial loss of malonyl ginsenosides, which off-sets the actual loss of neutral ginsenosides. Measurement of malonyl ginsenosides also alters the solvent mixture, giving optimum extraction of total ginsenosides to a lower ethanol proportion, due to their greater solubility in slightly more polar solvents relative to the neutral ginsenosides.

Findings from the drying trial indicate that increasing the drying temperature above 40 °C results in a loss of total ginsenosides, due to the loss of malonyl ginsenosides. Reynolds (1998), who examined drying at 32, 38 and 44 °C, found a similar effect and recommended 38 °C as a drying temperature. However, the drying time at 40 °C was about thrice that at 55 °C. Since the loss of total ginsenosides at 55 °C was only about 5% of that obtained at 40 °C, the industry may consider the greater operational efficiency of drying at the higher temperature to be worthwhile.

The storage trial found that there was some loss of total ginsenosides at all storage temperatures with a greater rate of loss at higher temperatures, hence the recommended storage temperature would be as low as

possible. The rate of loss at 20 °C would, however, result in a loss of about 4.5% of total ginsenosides after 10 weeks storage and at 30 °C it would be about 8%. The lower cost of non-refrigerated storage could mean that some higher rate of loss of ginsenosides would be acceptable. The extraction trial found that there was some variation in the proportion of neutral and malonyl ginsenosides extracted by the different solvent ratios, and while the differences were not large, the recommended solvent for optimum total ginsenosides would seem to have about 10% more water than for extracting only neutral ginsenosides.

Acknowledgements

We thank the Australian Ginseng Growers Association and the Rural Industries Research and Development Corporation for funding support and Gembrook Organic Ginseng Pty Ltd for helpful advice and the generous supply of ginseng roots.

References

Ando, T., Tanaka, O., & Shibata, S. (1971). Chemical studies on the oriental plant drugs. XXV. Comparative studies on the saponins and sapogenins of ginseng and related crude drugs. *Svoyakugaku Zasshi, 25*, 28–32.

Awang, D. V. C. (2000). The neglected ginsenosides of North American ginseng (*Panax quinquefolius* L.). *Journal of Herbs Spices and Medicinal Plants, 7*, 103–109.

Choi, J. H., Byun, D. S., & Park, K. D. (1983). Study on stability of red and white ginseng by storage years. *Korean Journal of Food and Nutrition, 12*, 350–356.

Court, W. A., Hendel, J. G., & Elmi, J. (1996). Reversed-phase high-performance liquid chromatographic determination of ginsenosides of *Panax quinquefolium*. *Journal of Chromatography A, 755*, 11–17.

Court, W. E. (2000). *Ginseng. The Genus Panax*. Amsterdam: Harwood Academic.

Hou, J. P. (1978). *The Myth and Truth about Ginseng*. New York: A.S. Barnes.

Kitagawa, I., Taniyama, T., Hayashi, T., & Yoshikawa, M. (1983). Malonyl-ginsenosides Rb_1, Rb_2, Rc and Rd, four new malonylated dammarane-type triterpene oligosaccharides from ginseng radix. *Chemical and Pharmaceutical Bulletin, 31*, 3353–3356.

Li, Y., & Morey, R. V. (1987). Thin-layer drying rates and quality of cultivated American ginseng. *Transactions of the American Society of Agricultural Engineers, 30*, 842–847.

Liu, J., & Song, A. (1992). Studies of industrial extraction technology on total saponins in aerial part of Asian ginseng. *Zhongguo Yaoxue Zazhi, 27*, 291–293.

Noh, H. W., Do, J. H., Kim, S. D., & Oh, H. I. (1983). Effect of relative humidities on the qualities of white ginseng during storage. *Korean Journal of Food Science and Technology, 15*, 32–36.

Reynolds, L. B. (1998). Effects of drying on chemical and physical characteristics of American ginseng (*Panax quinquefolius* L.). *Journal of Herbs Spices and Medicinal Plants, 6*, 9–21.

Sokhansanj, S., Bailey, W. G., & van Dalfsen, K. B. (1999). Drying of North American ginseng roots (*Panax quinquefolius* L.). *Drying Technology, 17*, 2293–2308.

Sticher, O. (1998). Biochemical, pharmaceutical and medical perspectives of ginseng. In R. B. Larry & D. Lawson (Eds.), *Phytomedicines of Europe, chemistry and biological activity* (pp. 221–240). Washington, DC: American Chemical Society.

Wang, T., Jia, Z., Liu, C., & Ren, G. (1990). The studies of processing technology and technique on American ginseng. *Zhongguo Yaoxue Zazhi, 15*, 24–27.

Wilhelm, L. R. (1990). Ginseng drying – The effect of drying air temperature and humidity upon quality. *Transactions of the American Society of Agricultural Engineers, 6*, 635–639.

Xu, W., Zheng, Q., Zhou, H., & Zhao, Q. (1996). Studies of extraction technology on saponins in Asian ginseng residue. *Xiandai Yingyong Yaoxue, 13*, 34–35.

Yamaguchi, H., Kasai, R., Matsuura, H., Tanaka, O., & Fuwa, T. (1988). High-performance liquid chromatographic analysis of acidic saponins of ginseng and related plants. *Chemical and Pharmaceutical Bulletin, 36*, 3468–3473.

Yang, X., Lin, S., & Ma, J. (1989). Probe into an extraction technology for ginseng preparations. *Zhong Cao Yao, 20*, 209–210.

Yoshikawa, M., Fukuda, Y., Hatakeyama, S., Murakami, N., Yamahara, J., Tanitama, T., Hayashi, T., & Kitagawa, I. (1993). Chemical fluctuation of the constituents during the drying of ginseng radix and ginseng radix rubra. Crude drug processing by far-infrared treatment. *Yakugaku Zasshi, 113*, 460–467.