

Full Length Research Paper

Variation of phytochemical profiles and content among root morphotypes of American ginseng: Economic implications for the grower

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Quality perception and hence price value of ginseng is often influenced by its root shape (morphotype). In this study, the profiles and content of six ginsenosides (Rg1, Re, Rb1, Rc, Rb2, and Rd) were compared among the three common root morphotypes ['man-like' (ML), 'bulb' (BLB), and 'stick' (STK)] of American ginseng. Also, analysis of a marketing strategy was done to ascertain if pre-sorting ginseng roots to respective morphotypes was a viable option to boost revenue returns for the grower. The results showed that ginsenosides profiles, specifically Rg1 and Re were inverse to each other. ML roots exclusively had a low Rg1/high Re profile whereas BLB and STK roots had mixed Rg1/Re profiles. The content of the evaluated ginsenosides varied significantly among root morphotypes, except for Rb2, Rc, and Rd. The sum of ginsenosides content was significantly higher in ML roots ($2.19 \pm 0.07\%$, w/w) compared to BLB or STK roots ($1.86 \pm 0.07\%$ or $1.79 \pm 0.07\%$, respectively). Based on tested ginsenoside content alone, ML roots could be inferred to be of higher quality. Analysis of a marketing strategy where roots are pre-sorted to respective morphotypes prior to selling indicated a potential for a grower to increase revenue even with just a modest price mark-up on ML roots, and given that the price value of the other root morphotypes (BLB and STK) is not drastically lowered due to sorting.

Key words: *Panax quinquefolius*, ginsenosides, marketing medicinal plants, root shapes, High-performance liquid chromatography (HPLC).

INTRODUCTION

American ginseng (*Panax quinquefolius* L.) is an economically important medicinal plant, which earns over 100 million US dollars annually (Ren and Chen, 1999). This perennial herb belongs to the Araliaceae family and is native to the eastern deciduous woodlands of North America (Catling et al., 1994). For over 300 years, it has been harvested from the wild for export to Asia where ginseng is widely used in traditional Chinese medicine (TCM) (Persons, 1994).

American ginseng and Asian ginseng (*Panax ginseng*) are the two most commonly used species of the genus

Panax for medicinal purposes (Kitts et al., 2000). The two species grow in different continents but occupy similar habitats (Pritts, 1995). They are genetically different, have different phytochemical profiles, and exert opposite therapeutic effects (Sengupta et al., 2004). Asian ginseng is purported to have stimulant properties and therefore used to energize the body, whereas American ginseng is purported to have calming properties and thus used as an adaptogen and a mild tonic to relax the body (Pritts, 1995).

The phytochemical constituents of ginseng are called ginsenosides. They are available in small quantities and are believed to be responsible for most of ginseng's pharmacological activity (Attele et al., 1999). More than 40 different ginsenosides have been identified in the

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genus *Panax* (Teng et al., 2003). Their basic structure is similar, consisting of a gonane steroid nucleus with 17 carbon atoms arranged in four rings with a modified side chain at C-20 (Radad et al., 2006). Based on their structural differences, ginsenosides can be classified into three groups including: the protopanaxadiol group (for example, Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, Rs1), the protopanatriol group (for example, Re, Rf, Rg1, Rg2, Rh1), and the oleanic acid group (for example, Ro) (Attele et al., 1999; Vanisree et al., 2004). Ginsenosides have been reported to have many pharmacological properties. For instance, ginsenosides Rg1 and Rb1 have been reported as effective neuroprotective agents promoting neural growth and protecting neurons against ischemic injury (Liao et al., 2002; Zou et al., 2002; Shen and Zhang, 2003). Also ginsenosides have been reported to enhance learning ability and preventing memory loss (Wen et al., 1996; Mook-Jung et al., 2001; Shen and Zhang, 2003), and have anti-cancer, anti-diabetic, and anti-hyperlipidemic properties (Attele et al., 2002; Chang et al., 2003; Xie et al., 2005; Cho et al., 2006).

The root is the most used plant part of ginseng, and often its value and desirability among its consumers is influenced by many factors; most important being the root shape (morphotype) (Guo et al., 1996; Pritts, 1995). Ginseng root has been classified into 3 to 5 morphotypes. Sokhansanj et al. (1999) classified fresh roots into three morphotypes: "pencil", "chunky", and "complex". A "pencil" root resembles a carrot, it has a slender body and lacks major lateral roots; a "chunky" root has 3 to 4 large lateral roots giving it a man-like shape; and a "complex" root has a single central body with lateral roots giving it a chicken claw or spider shape. Roy et al. (2003) classified dried roots into five categories: "chunky", "forked", "pencil or carrot", "spider", and "fiber". Following this classification, a "chunky" root is described as bullet or bulb shaped; a "forked" root has a humanoid appearance, and a "pencil" root has a main taproot equal to or greater than 5 cm in length. "Spider" root has no distinct taproot present or if present is less than 2 cm in length and has several secondary and tertiary roots radiating from the main root. "Fiber" roots comprise of secondary and tertiary roots with diameters of 1 to 2 mm or less.

The classification by Roy et al. (2003) is typical of that employed by ginseng wholesalers, who often prune and separate dried roots to attain different grades. For example, "fiber" roots are obtained by pruning of secondary and tertiary roots off of the main roots. A "spider" root is a very rare morphotype of ginseng and is often times regarded as a variant of a "chunky" root. Therefore only three categories are truly representative of the common ginseng root morphotypes encountered at the farm level. They are: "forked" or "man-like" (ML) – a branched root exhibiting a man-like shape; "chunky" or "bulb" (BLB) – compact, round or bullet shaped root; and "pencil" or "stick" (STK) – slender, elongated taproot without lateral roots (Figure 1). The underlying causes of

variations in root morphotypes have not been extensively studied (Li, 1997). Soil texture and bulk density have been attributed to influencing the shape of ginseng root (Li, 1997; Park et al., 2005; Roy et al., 2008), however, the effect of underlying genetics cannot be discounted given that this species has been reported to be genetically heterogeneous, and in the light that influence of genotype on root morphology has been documented on Radish (*Rhapanus sativus* L.) (Bai et al., 1997; Schluter and Punja, 2002; Tsuru et al., 2007).

Variations in content of individual and total ginsenosides among ginseng roots are frequently reported in literatures. Older roots have higher ginsenoside content than younger roots (Court et al., 1996; Smith et al., 1996; Lim et al., 2005). Wild growing roots are reported to have higher ginsenoside content than cultivated roots (Lui and Staba, 1980; Assinewe et al., 2003). Also significant variations in individual ginsenoside content and profiles have been reported among ginseng populations (Assinewe et al., 2003; Lim et al., 2005; Schlag and McIntosh, 2006). For instance, the profiles and hence content of ginsenosides Rg1 and Re are inverse to each other, and varies even within a single population (Lim et al., 2005; Schlag and McIntosh, 2006). The term "chemotype" has been used to describe the inverse relationship between Rg1 and Re ginsenosides (Schlag and McIntosh, 2006). Roots with low Rg1 but high Re content are described as "low Rg1/high Re chemotype" which is commonly observed in American ginseng, whereas roots with high Rg1 but low Re content are described as "high Rg1/low Re chemotype" which is rarely observed. Ginsenosides Rg1, Re, and Rb1 are the most abundant in American ginseng root and have been extensively investigated for their medicinal properties (Murphy and Lee, 2002).

Despite numerous reports on ginsenosides profiles and content in ginseng root, little information is available on the profiles and content of individual ginsenosides among different root morphotypes. For the reason that the root shape significantly affects the price of ginseng root, we sought to compare the profiles and content of six main ginsenosides (Rg1, Re, Rb1, Rc, Rb2 and Rd) among three American ginseng root morphotypes (ML, BLB and STK). Also, we conducted an economic analysis to evaluate the potential for a ginseng grower to increase revenue by pre-sorting roots into respective morphotypes before selling.

MATERIALS AND METHODS

Plant

Fresh roots of cultivated American ginseng aged 4, 8 and 10 years were collected from a single farm in Western Maryland, USA. These plants were cultivated in raised soil beds of similar soil composition and relatively similar environmental conditions. The ages of roots were provided by the grower and were independently confirmed by counting the scars on the rhizome, which typically indicates the



Figure 1. Different root morphotypes of American ginseng. A through C; 3-months-old roots grown in Sunshine mix in a greenhouse: (A) STK morphotype; (B) BLB morphotype; (C) ML morphotype; (D) 8-year-old woods cultivated roots showing three morphotypes, left to right; STK, BLB and ML morphotypes, respectively. The scale bar in C applies to all 3-months-old roots.

root's age. From each age group, roots were separated into three distinct morphotypes (ML, BLB, and STK) and frequency distribution of each morphotype in the entire sample size was determined. The roots were washed with tap water then blot dried with paper towels and placed in individual paper bags.

Ginsenosides extraction

The roots were freeze-dried for 72 h and grind into a fine powder to pass through 1 mm mesh in a Thomas model 4 Wiley® mill (Thomas Scientific, Swedensboro, NJ). Extraction procedure followed that outlined in Schlag and McIntosh (2006) with slight modifications. One hundred milligrams of root powder was transferred into 10 ml glass vials with vented cap, and 5 ml of 80% methanol was added and briefly vortexed before the mixture was incubated in a water bath at 70°C with constant sonication for 1 h. Thereafter, the vials were centrifuged at 2000 × g for 5 min using a Mistral 3000i centrifuge (Curtin Matheson Scientific, Inc., Houston, TX), and the supernatants were transferred into clean tubes. The residues were re-extracted once and the supernatants from both extractions were combined and concentrated by drying them under a stream of nitrogen at 38°C, and then re-suspended in 2 ml solvent of 20 : 20 : 60 (methanol: acetonitrile: water). The concentrated

extracts were filtered through 0.45 μm membrane filters into clean 2 ml polypropylene tubes (Spin-X® 8162, Corning Inc., Corning, NY) and stored at 4°C until High-performance liquid chromatography (HPLC) analysis (< 24 h). There were three separate extractions per root.

Chemicals and solvents

All chemicals used were of analytical grade (Fisher Scientific, Pittsburgh, PA). The water used for extraction and HPLC analysis was ultra purified by Milli-Q® water purification system, (Millipore, Billerica, MA). Ginsenoside standards Rb1, Rb2, Rc, Rd, Re and Rg1 (purity > 99%) was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ). All solvents were filtered through 0.45 μm Autovial® PVDF membrane filters (Whatman, Inc., Clifton, NJ) before use.

HPLC analysis

The HPLC system used was a Waters model 2695 Alliance HPLC separation module (Milford, MA) equipped with a photodiode array

detector (Waters 996 PDA), an in-line degasser, an auto sampler, and Waters Millennium 32 software. Ginsenoside separation was carried out in Atlantis[®] T3 column (5 μ m, 250 \times 4.6 mm) with Atlantis[®] T3 guard cartridge (5 μ m, 4.6 \times 20 mm) (Waters Inc, Milford, MA). Twenty micro liters of sample or standard was injected into the column and eluted at room temperature at a constant flow rate of 1.2 ml/min. The mobile phase consisted of solvent A (100% water), and solvent B (100% acetonitrile). A mobile phase gradient was based on that of Wang et al. (2006) with slight modifications to ensure good separation with our machine. The gradient elution started with 80% solvent A and 20% solvent B for the first 20 min, changed to 74% A and 26% B from 20 to 29 min; changed to 66% A and 34% B from 29 to 43 min; changed to 64% A and 36% B from 43 to 47 min; changed to 57% A and 43% B from 47 to 54 min; and lastly to 5% A and 95% B from 54 to 59 min. The UV detection wavelength was set at 203 nm.

Identifying and quantifying ginsenosides

Individual ginsenosides standards were serially diluted and injected into HPLC machine, and corresponding peak areas from each standard concentration were used to generate standard curves that were used to quantify individual ginsenosides in root samples. Presence of individual ginsenosides in each sample was confirmed by presence of peaks at retention times corresponding to those obtained from a chromatogram of mixed standards solution. Content of individual ginsenosides in each sample were calculated by integrating their peak areas with their standard curves ($r^2 > 0.99$).

Experimental design and analysis

The experiment was designed as a randomized complete block, with the entire root treated as an experimental unit. Since roots were of different ages, blocking was used to account for age effects. There were nine replicates for each morphotype in 4 and 8 year old roots, and six replicates for each morphotype in 10 year old roots. The data was analyzed using analysis of variance and GLM procedure in SAS (SAS Institute, Inc., Cary, NC), and mean separations was done using Tukey's HSD test ($P \leq 0.05$).

RESULTS

Ginsenosides profiles and content

Representative chromatograms of ginsenosides standards and samples are shown in Figure 2. All six ginsenosides were present in all root samples analyzed. Ginsenoside Rg1, Re, and Rb1 varied significantly among root morphotypes but Rc, Rb2 and Rd ginsenosides did not (Figure 3). The most abundant ginsenoside in all roots was Rb1, which accounted for 51.60, 51.08, and 45.25% of the total ginsenosides content (sum of six quantified ginsenosides) in ML, BLB and STK roots, respectively (Table 1). Rb2 was the least abundant ginsenoside accounting for 1.83, 2.69, and 3.91% of total ginsenoside contents in ML, BLB, and STK morphotypes, respectively (Table 1). There was no correlation between root age and content of Rg1 or Re ginsenosides across all root morphotypes (Figure 4a), however the content of Rb1 ginsenoside mostly

increased with root age (Figure 4b), and was positively correlated with total ginsenosides content (Figure 3). Total ginsenoside content ranged from 0.94 to 3.47% w/w for ML roots, 1.12 to 2.55% w/w for BLB roots, and 1.37 to 2.65% w/w for STK roots. There was a gradual increase in total ginsenosides content with age independent of root morphotype except for STK roots where 4 and 8 year old roots had almost equal amount of total ginsenosides content despite the age difference.

Two distinct ginsenoside profiles (low Rg1/ high Re, and high Rg1/low Re) were evident in the roots (Figure 4a). ML roots consistently exhibited low Rg1/high Re profile, whereas BLB and STK roots exhibited both profiles (Figure 4a). The proportion of roots with low Rg1/high Re profile was 50.00 and 62.50% in BLB and STK roots, respectively. Re was the most abundant ginsenoside of the protopanaxtriol group constituting 29.68, 18.82 and 22.91% of the total ginsenosides content in ML, BLB, and STK morphotypes, respectively (Table 1). Rg1 and Re ginsenosides accounted for 2.74 and 29.68% of total ginsenoside, respectively in ML roots, 11.83 and 18.83% in BLB roots, 10.06 and 22.91% in STK roots (Table 1). The relative abundance of Rg1 was low in roots of ML roots (2.74%), almost five fold less compared to that in BLB or STK roots (Table 1). In a sharp contrast, Re content was significantly higher in ML roots than in BLB or STK roots (Figure 1 and Table 1). Overall, ML roots had significantly higher total ginsenosides content (adjusted mean, $2.19 \pm 0.07\%$ w/w) than those of BLB ($1.86 \pm 0.07\%$ w/w) or STK ($1.79 \pm 0.07\%$ w/w) roots, however there was no significant difference in total ginsenosides content between BLB and STK roots (Figure 3).

DISCUSSION

In this study, the total content of six ginsenosides was lower compared to those previously reported (3 to 6% w/w) for cultivated American ginseng populations in North America (Court et al., 1996; Li et al., 1996; Assinewe et al., 2003). However these results are consistent with those reported for American ginseng populations in Maryland (2.3% w/w) and New York (2.5 % w/w) for roots in the same age range (Schlag and McIntosh, 2006; Lim et al., 2005). Variability in ginsenoside content is expected for American ginseng because this species is genetically heterogeneous and naturally grows in a broad geographic region with distinctly different ecological conditions (Bai et al., 1997; Assinewe et al., 2003).

In this study, Rb1 was the most abundant ginsenosides in all root morphotypes, which concurs with other previous published reports on field and in vitro grown American ginseng (Li et al., 1996; Mallol et al., 2001; Schlag and McIntosh, 2006; Obae et al., 2011). There was a positive correlation between age and total ginsenoside content in roots, which concurs with other

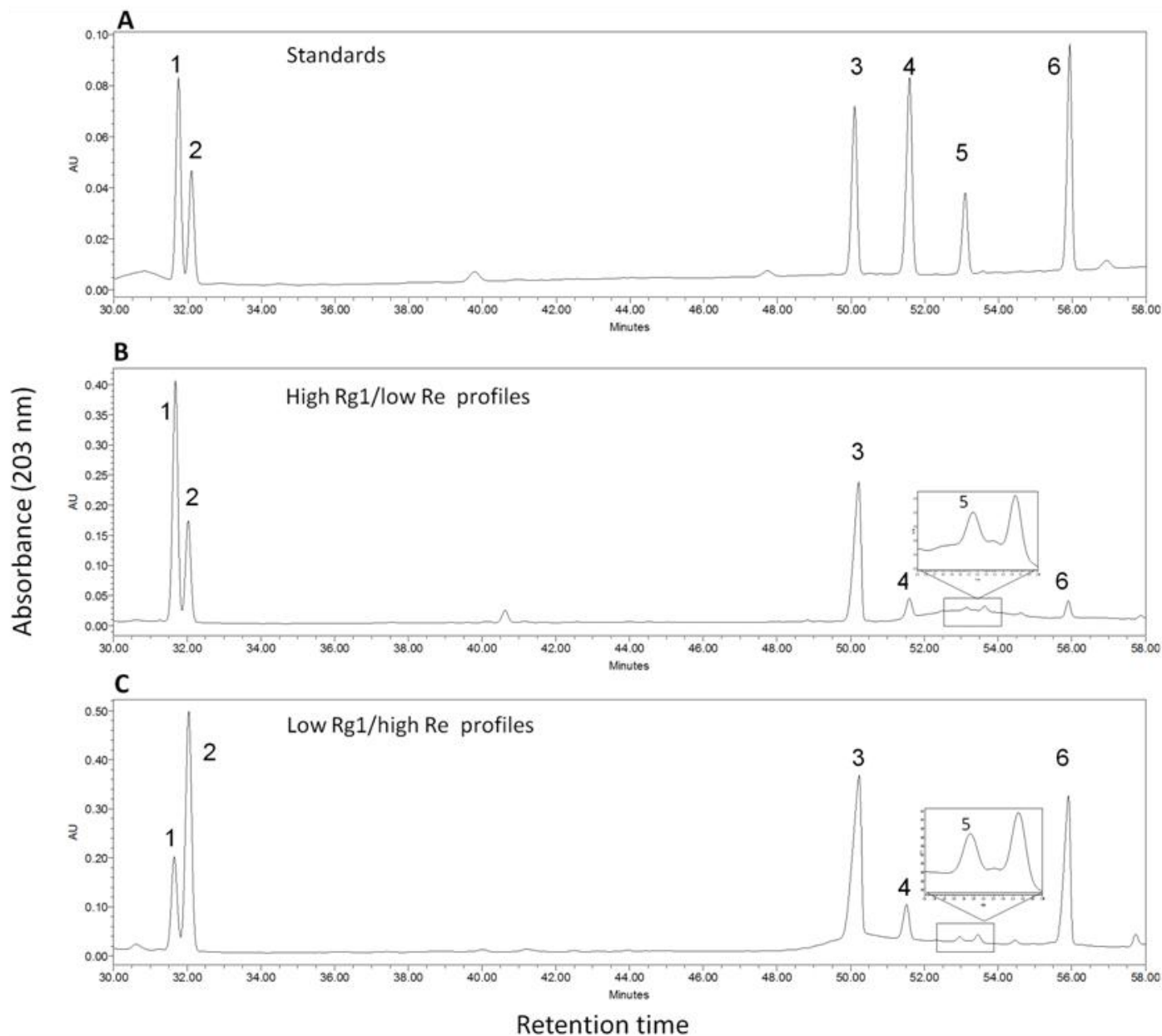


Figure 2. HPLC chromatograms of six ginsenosides in American ginseng root samples: (A) ginsenoside standards; (B) chromatograms showing high Rg1/low Re profile; (C) low Rg1/high Re profile in root samples. Ginsenoside peaks; 1 (Rg1), 2 (Re), 3 (Rb1), 4 (Rc), 5 (Rb2), and 6 (Rd).

published reports (Tani et al., 1981; Court et al., 1996; Smith et al., 1996) and further reaffirms the widely accepted practice of harvesting older roots as they are regarded to be more potent and highly priced than younger roots.

The inverse relationship between Rg1 and Re ginsenosides varied among roots. Roots of ML morphotype exclusively exhibited low Rg1/high Re profile, but those of BLB and STK morphotypes exhibited mixed Rg1/ Re profiles. The inverse relationship between

Rg1 and Re ginsenosides has been reported before (Schlag and McIntosh, 2006), but assessment of this relationship in American ginseng roots of different morphotypes is presented here for the first time and therefore provides an important aspect of consideration in herbal formulations and root choice for targeted herbal therapeutic purposes. For instance Rg1 has been reported as an effective neuroprotective agent (Liao et al., 2002), whereas Re has been shown to have anti-diabetic and anti-hyperlipidemic properties (Attele et al.,

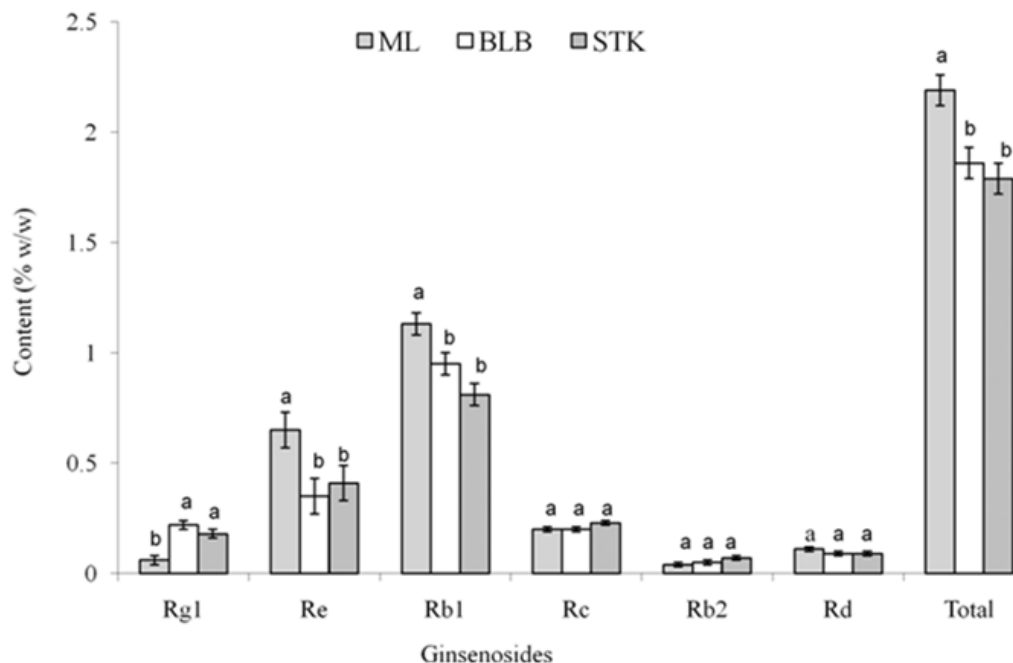


Figure 3. Concentration (Adjusted means \pm SEM) of individual and total ginsenosides in American ginseng roots of different morphotypes. Mean concentrations of individual and total ginsenosides accompanied by same letter are not significantly different among root morphotypes ($P \leq 0.05$).

Table 1. Relative abundance of individual ginsenosides to total content by root morphotype.

Root morphotype	Percentage of Individual ginsenosides relative to total content ²					
	Rg1	Re	Rb1	Rc	Rb2	Rd
ML	2.74	29.68	51.60	9.13	1.83	5.02
BLB	11.83	18.82	51.08	10.75	2.69	4.84
STK	10.06	22.91	45.25	12.85	3.91	5.03

² Sum of the six ginsenosides quantified in this study (Rg1, Re, Rb1, Rc, Rb2, and Rd).

2002; Xie et al., 2005; Cho et al., 2006). Therefore, roots with higher levels of Rg1 would be preferred for use in herbal formulations intended to improve learning, memory, and preventing or slowing down neural degeneration. Roots with higher Re content would be preferred for use in herbal formulations intended to prevent and manage high blood glucose and cholesterol levels in diabetic and hypercholesterolemia patients, respectively.

ML roots contained significantly higher total number of ginsenosides content than either BLB or STK roots. Ginsenosides are reported to be located in the periderm and cortex regions of the root (Tani et al., 1981), and root hairs (fibers) are reported to contain high ginsenosides content than main roots (Tani et al., 1981; Christensen et al., 2006). Therefore, the significantly higher total ginsenoside content in ML roots observed in this study could be due to the more abundance of root hairs and

lateral roots in this morphotype compared to the other two root morphotypes (BLB and STK). However, this could also be due to underlying genetics given the substantial genetic variability of this species.

Economic analysis

Biased quality assessment and pricing of ginseng in the market based on root morphotype has significant implications on ginseng returns for the grower. A simple marketing analysis was conducted to evaluate potential revenue implications of pre-sorting roots into respective morphotypes after harvesting. The analysis calculations were based on estimates of root yield per acre and frequency distribution of each root morphotype in a mixed batch (MXD) based on their respective frequency distributions as observed from field tabulations (15% ML,

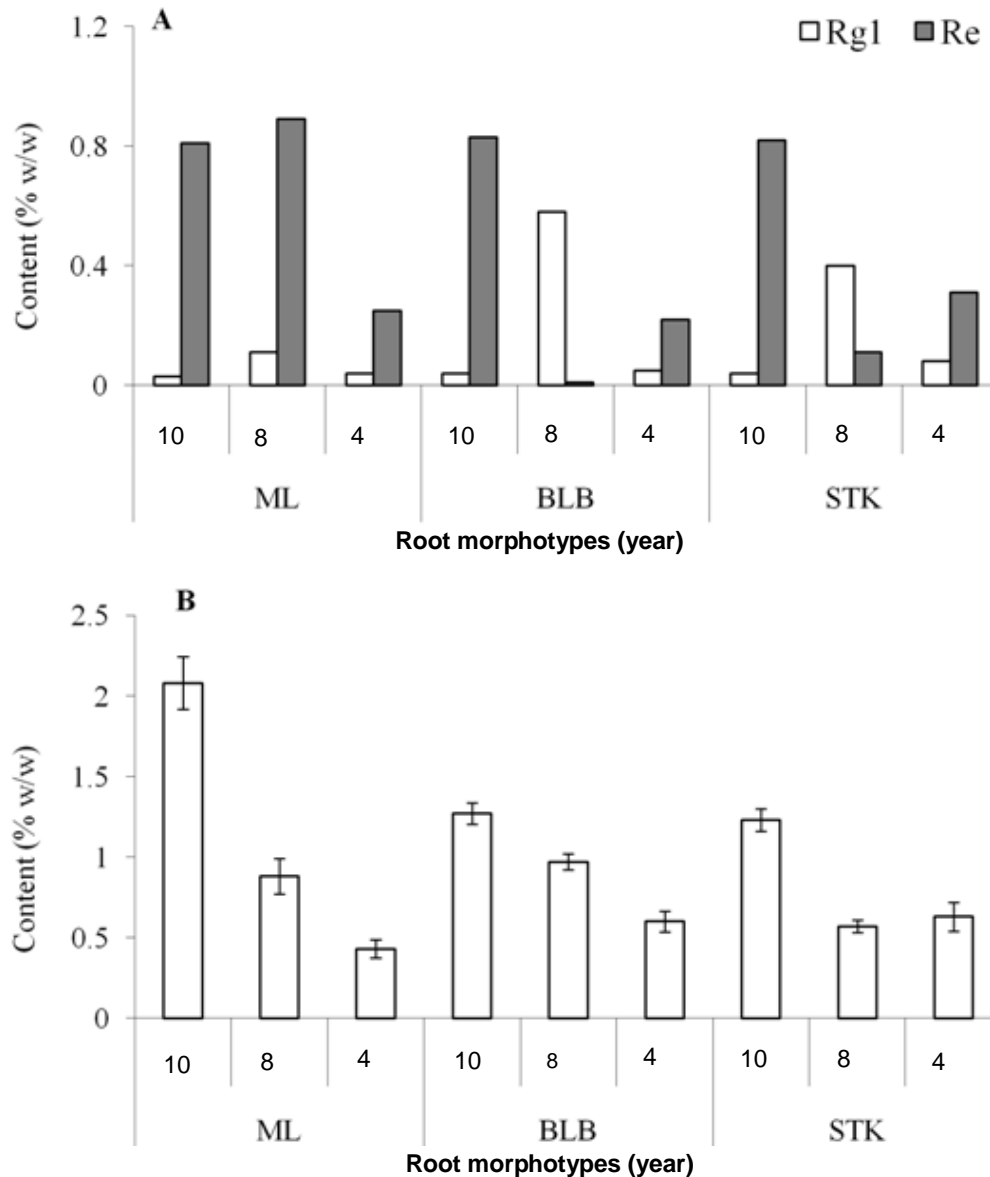


Figure 4. Profiles of ginsenosides Rg1 and Re, in American ginseng roots of different morphotypes and age (A); and concentration of ginsenoside Rb1, in roots of different morphotypes and age (B).

22% BLB, and 63% STK, n = 353). Assuming roots of ML morphotype to be of high quality, based on their higher total ginsenosides content as revealed in this study, returns due to pre-sorting of roots before selling was assessed at two different price mark ups (5 and 30%) for ML roots over the average price for one pound (2.2 kg) of MXD of roots (\$60) of woods cultivated ginseng.

Yield of roots per acre based on different planting densities is shown in Table 2. Root yield calculations in this study were based on planting density of 6 x 6 inches. Under these specifications, number of roots per acre was determined to be 238,032 upon assuming a 20% root loss (Table 2). Root weight yields per acre for MXD roots (current sale system) and pre-sorting to different root

morphotypes (for value addition) are shown in Table 3. Estimated gross revenue per acre from sale of non-sorted roots (MXD) (current system) is \$238,967 (Table 3). Revenue increase per acre due to pre-sorting of roots to their respective morphotypes before selling (factoring in sorting cost \$1,000 per acre) with 5% and 30% price premium mark up for ML roots was determined to be \$2,112 and \$17,672, respectively (Table 3). However, if labor cost for sorting is higher than \$1,000 per acre, the returns from 5% price mark up for ML roots over MXD will break even if sorting labor cost reaches \$3,112 per acre (Table 3). One potential caveat to pre-sorting roots is that despite increasing the price value of ML roots, it will likely lower the price value of BLB and STK roots. If that

Table 2. Root yield per acre of American ginseng under different planting densities.

Plant spacing (inches) ^z	Plants per square foot	Plants per acre	Plants per acre assuming 20% root loss	Root weight
1 × 6	24	793,440	634,752	Increases with plant spacing
3 × 6	12	396,720	317,376	
6 × 6	9	297,540	238,032	
9 × 6	6	198,360	158,688	

^z Adapted from Persons (1994).

Table 3. Projected returns from pre-sorting of roots to different morphotypes at farm level prior to selling under current American ginseng cultivation system.

Root type	Percentage of root morphotype per acre ^z	No. of roots per acre	Roots per pound	Pounds per acre	Price Per Pound (\$) ^y		Gross return per acre (\$) after 8 years of cultivation		Gross return per acre (\$) after 8 years of cultivation	
					5%	30%	5%	30%	5%	30%
ML	15	35,705	34	1,037	63	78	65,352	80,911	65,352	80,911
BLB	22	52,367	63	828	60	60	49,691	49,691	49,691	49,691
STK	63	149,960	71	2,117	60	60	127,037	127,037	127,037	127,037
Gross returns from sorting (total of ML, BLB, and STK)							242,076	257,639	242,079	257,639
Less sorting labor ^x							1,000	1,000	3,112 ^v	3,112
Net return ^w							241,079	256,639	238,967	254,527
MXD				3,983	60	60	238,967	238,967	238,967	238,967
Value added return due to sorting (over MXD)							2,112	17,672	0	15,560

^z Based on frequency distribution of morphotypes in our sample. ^y Price based on market price range for cultivated American ginseng (\$30 to 120) with a 5 or 30% premium mark up for ML morphotype based on its quality (high ginsenoside content). Price varies with age, production system, and market. ^x Sorting labor based on cost for picking berries per acre (grower's estimates). ^w Returns only accounts for sorting costs. All other production costs up to harvesting are not included. ^v Sorting labor cost to break even at 5% premium mark up for ML root.

occurs, then pre-sorting will not result in any increase of revenue return even at the lowest sorting labor cost.

Conclusion

The results presented in this study show that roots of ML morphotype have higher total ginsenosides content than roots of BLB or STK morphotypes, but there is no significant difference in total ginsenosides content between BLB and STK roots. Using total ginsenoside as a criterion for assessing quality of roots and hence pricing, it could be inferred from this study that roots of ML morphotypes are of higher quality and thus, will be priced higher than those of BLB or STK morphotypes. Estimates from the economic analysis show that pre-sorting roots to different morphotypes as a strategy to leverage returns will result in revenue increase for the grower, however the level of return due to sorting will be dependent upon the cost of sorting roots to respective morphotypes and the grower's price mark up for the high quality roots.

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