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ARTICLES

Simultaneous Determination of Ginsenosides and Polyacetylenes in American Ginseng Root (*Panax quinquefolium* L.) by High-Performance Liquid Chromatography

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A method for simultaneous determination of ginsenosides and polyacetylenes in *Panax quinquefolium* L. (American ginseng) roots was developed. The ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1, Ro, malonyl-Rb1, malonyl-Rc, and malonyl-Rd and the polyacetylenes falcarinol and panaxydol were extracted from fresh ginseng roots in a sequential extraction process with 100% methanol followed by 80% aqueous methanol and quantified simultaneously in extracts by high-performance liquid chromatography using diode array detection. Separations were achieved with a phosphate buffer−acetonitrile gradient system using an RP-C_{18} column. Except for Rd, the present extraction method resulted in similar or significantly higher concentrations of both ginsenosides and polyacetylenes in comparison to commonly used extraction methods for these compounds. The contents of polyacetylenes and ginsenosides were determined in the root hairs, lateral roots, and main roots of 6 year old ginseng plants. The total mean concentrations of ginsenosides and polyacetylenes in root hairs were 31.0 g/kg fresh weight (FW) and 2.6 g/kg FW, respectively, whereas the concentrations of these bioactive compounds in the main roots were significantly lower with total mean concentrations of 17.8 g/kg FW for ginsenosides and 0.6 g/kg FW for polyacetylenes. The concentration of individual and total ginsenosides and polyacetylenes did not differ significantly between main roots of different sizes. Consequently, it is possible to do quantitative screening for ginsenosides and polyacetylenes to breed ginseng roots with higher levels of bioactive compounds.

KEYWORDS: ***Panax quinquefolium*; roots; ginsenosides; falcarinol; panaxydol; extraction; quantification; HPLC analysis**

INTRODUCTION

American ginseng (*Panax quinquefolium* L.), which is native to North America, is one of the most widely used medicinal herbs in the world. The popularity of American ginseng among consumers has led to an extensive cultivation of the plant in many parts of the world, including the United States, Canada, and China. The alcoholic extract of the roots of the plant has been widely used as a tonic against cancer. However, many other pharmacological effects have also been associated with American ginseng and other ginseng species (*1*−*4*). Apart from its anticarcinogenic activity, it has been claimed that the ginseng roots are effective in improving several disorders and general health functions including diabetes, cardiovascular disorders, immune functions, vitality, sexual function, and cognitive and physical performance (*1*−*4*).

The active principles of ginseng roots appear to be polyacetylenes and dammarane saponins, commonly referred to as ginsenosides. Ginsenosides in American ginseng roots include the ginsenosides Rb1, Rb2, Rc, and Rd and their corresponding malonyl ginsenosides (m-Rb1, m-Rb2, m-Rc, and m-Rd) having (20*S*)-protopanaxadiol as an aglycon, ginsenosides Re and Rg1 having (20*S*)-protopanaxatriol as an aglycon, and the oleanolic acid-type ginsenoside Ro (*5*−*7*) (**Figure 1**). Although much of the research focuses on crude ginseng root extracts and preparations, combinations of ginsenosides as well as individual ginsenosides have been shown to have a diverse range of pharmacological activities. Individual ginsenosides occurring in American ginseng roots such as Rc have demonstrated inhibitory effects on the proliferation of human breast cancer cells (*8*). Rb1 has been shown to act as a phytoestrogen in breast cancer cells (*9*, *10*). Neuroprotective effects have been demonstrated for Rb1

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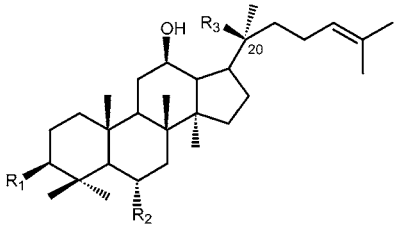
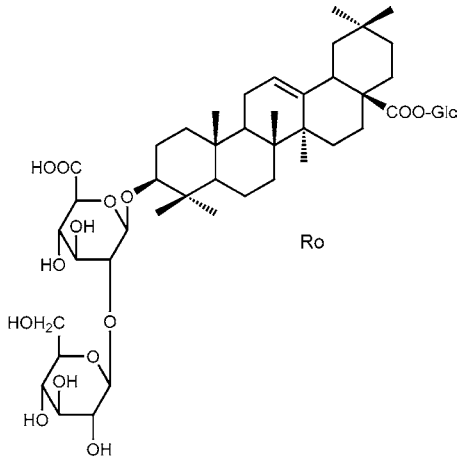


Ginsenoside (name)	R_1	R_2	R_3
Rb1	O–Glc(2→1)Glc	H	O–Glc(6→1)Glc
Rb2	O–Glc(2→1)Glc	H	O–Glc(6→1)Ara(p)
Rc	O–Glc(2→1)Glc	H	O–Glc(6→1)Ara(f)
Rd	O–Glc(2→1)Glc	H	O–Glc
Re	OH	O–Glc(2→1)Rha	O–Glc
Rg1	OH	O–Glc	O–Glc
m-Rb1	O–Glc(2→1)Glc–malonyl	H	O–Glc(6→1)Glc
m-Rc	O–Glc(2→1)Glc–malonyl	H	O–Glc(6→1)Ara(f)
m-Rd	O–Glc(2→1)Glc–malonyl	H	O–Glc



Glc = β-D-glucose; Ara(p) = α-L-arabinopyranose; Ara(f) = α-L-arabinofuranose; Rha = α-L-rhamnose

Figure 1. Chemical structures of ginsenosides identified and quantified in the roots of American ginseng (*P. quinquefolium*).

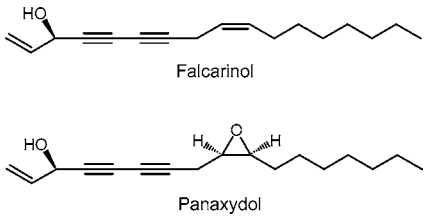


Figure 2. Chemical structures of the major polyacetylenes (falcarinol and panaxydol) identified and quantified in the roots of American ginseng (*P. quinquefolium*).

and Rg1 (*11*), and Rg2 was found to inhibit nicotine acetylcholine receptor-mediated Na^+ and channel activity, also indicating an effect on the human nervous system (*12*). Furthermore, Rg1 has shown to serve as a functional glucocorticoid receptor (*13*) and as a suppressor of oxidative stress (*14*). Many other pharmacological properties have been reported for ginsenosides in American ginseng and other ginseng species, including anti-inflammatory activity (*15*, *16*), immunosuppressive effects (*17*), and platelet inhibition activity (*3*, *18*−*21*).

Several polyacetylenes have been isolated from ginseng roots, including American ginseng (*22*−*27*). Some of these polyacetylenes exhibit anti-platelet-aggregatory action (*3*, *20*, *21*) and an anti-inflammatory effect (*28*−*30*), including inhibition of lipoxygenases (*29*) and 15-hydroxyprostaglandin dehydrogenase (*30*). In particular, the anticarcinogenic activity of some ginseng polyacetylenes is interesting (*23*, *31*−*37*). The most abundant polyacetylenes in American ginseng and other ginseng species are falcarinol (=panaxynol) and panaxydol (**Figure 2**), which are also some of the most bioactive polyacetylenes isolated from ginseng and hence may be very important in relation to the potential anticarcinogenic effect of ginseng preparations.

The contents of total and individual ginsenosides and polyacetylenes vary in ginseng roots and depend on the species, such as *Panax ginseng* (Korean or Chinese ginseng), *P. quinquefolium*, *Panax japonicus*, and *Panax notoginseng* (*38*), growing conditions (*39*, *40*), age of the roots (*40*−*42*), plant organs (*40*, *43*, *44*), and extraction methods (*40*, *45*−*47*). Several analytical methods for the quantitative determinations of ginsenosides have been described. The most commonly employed methods are based on high-performance liquid chromatography (HPLC) coupled to photodiode array or UV detection (*5*, *38*, *40*, *43*, *46*, *47*) or mass spectrometric detection (*45*, *47*, *48*), although gas chromatography−mass spectrometry (GC−MS) (*47*, *49*) and enzyme-linked immunosorbent assays (ELISAs) (*47*, *50*) have also been used. Despite the importance of the polyacetylene compounds in ginseng and related plant species, only a few quantification methods have been described for these secondary metabolites, which include a few HPLC methods (*38*, *51*−*53*) and GC methods (*53*−*56*). Analyzing both ginsenosides and polyacetylenes is very time-consuming by the existing methods using different extraction techniques and quantitative methods. This fact may explain why most investigations only have focused on the quantification of either the ginsenosides or the polyacetylenes in ginseng root extracts. An analytical method that is able to determine the contents of both ginsenosides and polyacetylenes simultaneously in ginseng roots would therefore be a large step forward in the effort to determine the different factors that may have an impact on the formation of both these groups of bioactive compounds in ginseng roots. Hence, it will make it easier to produce high-quality ginseng roots and ginseng preparations with optimized levels of these bioactive constituents.

In the present study a method for simultaneous determination of ginsenosides and polyacetylenes in American ginseng roots by reversed-phase (RP) HPLC is described. The method is based on simultaneous extraction of ginsenosides and polyacetylenes from fresh ginseng roots by a sequential extraction first with 100% methanol (MeOH) followed by extraction with 80% aqueous MeOH and analysis of both groups of bioactive compounds in the same HPLC analysis. The efficiency of the present extraction method for extraction of both ginsenosides and polyacetylenes was determined and compared to those of commonly used extraction methods for these compounds. Furthermore, the method was used to determine the content of polyacetylenes and ginsenosides in different root sections, i.e., the root hairs, lateral roots, and main roots of 6 year old American ginseng to investigate the possibility for selection and breeding of high-quality ginseng roots and to produce high-quality and differentiated herbal ginseng remedies with an optimized level of bioactive compounds.

MATERIALS AND METHODS

Plant Material. Roots of 5 and 6 year old American ginseng (*P. quinquefolium* L.) were collected in early October 2005 from a small ginseng field at Research Centre Aarslev, Denmark. The ginseng plants

were grown organically without use of any pesticides. The collected roots were washed under running tap water, drained, and gently air-dried at room temperature before analysis.

Chemicals. Ethyl acetate (EtOAc), MeOH, *n*-hexane, and acetonitrile (MeCN) of Ratborn HPLC grade (99.9% HPLC grade) and anhydrous potassium dihydrogen phosphate (KH_2PO_4) were obtained from Sigma-Aldrich (Steinheim, Germany). The water used for HPLC analysis was ultrapure generated by an Elgastat Maxima Analytica water purification system (Elga Ltd., United Kingdom). All eluents for HPLC were filtered through a 0.45 μm Cameo 25P syringe filter (nylon) (Bie & Berntsen, Rødovre, Denmark) and degassed with ultrasound for 20 min before use. Authentic samples of the ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 (purity >99%) were purchased from Extrasynthese (Lyon, Genay, France).

Polyacetylene Standards. Fresh roots of 5 year old (550 g) American ginseng were ground and extracted twice with 1.5 L of EtOAc for 24 h at 8 °C. The combined extracts were filtered, dried over anhydrous Na_2SO_4, and concentrated in vacuo (30 °C) under dim light to 15 mL. The concentrated extract was chromatographed on silica gel (silica gel 60, 230−400 mesh, Merck, Darmstadt, Germany), eluting with *n*-hexane−EtOAc ((v/v) 20:1, 10:1, 20:3, 5:1, 4:1, 7:3, 6:4, 11:9, 1:1, 4:6, 3:7; 200 mL at each step) and finally with MeOH (250 mL). Fractions containing crude falcarinol and panaxydol, as shown by thin-layer chromatography (TLC) and analytical RP HPLC, were combined and purified by preparative HPLC on a Dionex SemiPrep system (Dionex Denmark A/S, Denmark) consisting of an HPLC pump (P 680P HPG-2), a diode array detector (DAD) (UVD 340U) operating from 200 to 595 nm, and a fraction collector (Foxy Jr. FC 144). The DAD was employed at 205 nm, and absorption spectra of the eluted compounds were recorded between 200 and 595 nm. Separations were performed on an RP Develosil ODS-HG-5 HPLC column (RP-18, 250 × 20 mm i.d., Nomura Chemical Co., Seto, Japan), at 25 °C, using the following solvent gradient: MeOH−H_2O [0 min (40:60), 10−40 min (75:25), 80−100 min (100:0), 115 min (40:60)]. The gradient was linearly programmed. The flow rate was 5 mL/min, and the injection volume was 20 mL per separation. Falcarinol and panaxydol eluted at approximately 85 and 70 min, respectively, and finally, 70 mg of falcarinol and 95 mg of panaxydol were obtained as colorless oils with a purity of >98%, as shown by analytical RP-HPLC and TLC. Falcarinol and panaxydol were identified by UV (falcarinol, λ_{max} 231, 244, 256 nm; panaxydol, λ_{max} 220, 231, 243, 256 nm), MS [GC−MS (EI, 70 eV)], NMR (^{1}H and ^{13}C NMR and ^{1}H−^{1}H and ^{1}H−^{13}C correlation spectroscopy recorded in *d*-$CDCl_3$ with TMS as the internal standard), and optical rotation, and the complete spectral data set corresponded fully with literature values for falcarinol (*24*, *26*, *52*, *55*, *56*) and panaxydol (*22*, *23*, *26*, *52*).

Extraction of Ginsenosides and Polyacetylenes. To investigate the effectiveness of different extraction methods to extract ginsenosides and polyacetylenes from fresh ginseng roots, a whole fresh ginseng root (225 g) from a 5 year old American ginseng plant was cut into small pieces (1−3 mm^2) that were mixed carefully to obtain a homogeneous and representative sample. From this root sample, 8 g of freshly cut root material was weighed into a 100 mL flask with a screw cap and homogenized with an Ultra-Turrax T25 for 60 s with 30 mL of solvent. Three extraction methods (methods A−C) were used. Method A: The roots were extracted twice with 30 mL of EtOAc under stirring overnight at room temperature (approximately 22 °C) in the dark. Method B: Root material from method A was extracted twice with 30 mL of 80% MeOH overnight under the same conditions. The extracts were carefully filtered between each extraction. The combined EtOAc extracts (method A) and the combined aqueous MeOH extracts (method B) were transferred into a 100 mL volumetric flask, adjusted to a final volume of 100 mL with EtOAc and MeOH, respectively, and then carefully shaken. The extracts were filtered through 0.45 μm Cameo 25P syringe filters into 2 mL brown vials for analysis of ginsenosides and polyacetylenes by analytical RP-HPLC. Method C: In a second extraction experiment 8 g of freshly cut roots was extracted twice with 30 mL of MeOH overnight at room temperature followed by extraction once with 30 mL of 80% MeOH overnight by the same procedure as described above. The MeOH extracts were combined (method C), transferred to a 100 mL volumetric flask, and adjusted to a final volume of 100 mL with MeOH. The extracts were prepared for HPLC analysis of ginsenosides and polyacetylenes as described above. All extractions were performed in triplicate.

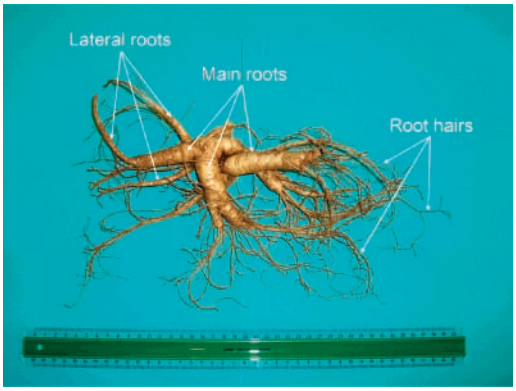


Figure 3. A typical ginseng root from a 6 year old American ginseng plant (*P. quinquefolium*) grown in Denmark with root hairs, lateral roots, and main roots.

The concentrations of ginsenosides and polyacetylenes in sections of ginseng roots that differ in root diameter were also determined. The following root sections were investigated: diameter 0.5−2.5 mm (root hairs), 5.0−10.0 mm (lateral roots), and 15.0−20.0 mm and >20.0−38.0 mm (main roots) (**Figure 3**). The root sections were obtained by cutting off the different root parts (according to size) of four whole fresh roots of 6 year old ginseng with masses of 68.8 g (root I), 104.1 g (root II), 120.3 g (root III), and 188.0 g (root IV). The roots were cut separately. The different root sizes were cut into small pieces (0.5−3 mm^2) and mixed carefully to obtain homogeneous and representative samples within the individual root size classes. From the different root size samples, 4 g (root hairs) or 8 g (lateral roots and main roots) of freshly cut root material was weighed into a 100 mL flask with screw cap, homogenized with an Ultra-Turrax T25 for 60 s with 30 mL of MeOH, and extracted as described above for extraction method C. The MeOH extracts were combined, transferred to a 100 mL volumetric flask, and adjusted to a final volume of 100 mL with MeOH. The extracts were prepared for HPLC analysis of ginsenosides and polyacetylenes as described above. Extractions were performed in triplicate for lateral roots and main roots and in duplicate for root hairs.

Analysis of Ginsenosides and Polyacetylenes by Analytical RP-HPLC. Analytical RP-HPLC was carried out on a Dionex HPLC system (Dionex Denmark A/S) consisting of an HPLC pump (P580), an autosampler (ASI-100), an oven (STH 585), and a DAD (PDA 100) operating between 200 and 700 nm. The DAD was employed at 203 nm, and UV spectra of ginsenosides and polyacetylenes were recorded between 200 and 600 nm. Separations were performed on a Purospher STAR RP-18 end-capped column (5 μm, 250 × 4 mm i.d., Merck, Darmstadt, Germany) protected with an RP-18 guard cartridge (5 μm, 15 × 4 mm i.d., Merck). The column temperature was maintained at 35 °C, and the mobile phases consisted of solvent A (10% MeCN−90% 10 mM KH_2PO_4, pH 5.82 (v/v)) and solvent B (75% MeCN−25% H_2O (v/v)). Separations were performed by the following solvent gradient: 0 min, 0% B; 5−15 min, 15% B; 26 min, 20% B; 36 min, 22% B; 45 min, 33% B; 50 min, 35% B; 55 min, 40% B; 75 min, 80% B; 90−105 min, 100% B; 115 min, 0% B. All increases and decreases of solvent B were linearly programmed. The flow rate was 1 mL/min and the injection volume 20 μL. The samples were analyzed within 12 h by RP-HPLC while they were kept refrigerated (5 °C). Otherwise the samples were kept at −20 °C until analysis within a few days. Identification of the individual ginsenosides and polyacetylenes in the extracts was performed by spiking with authentic standards to a ginseng root extract sample, and quantification of the individual compounds was made by external calibration curves of authentic standards. The malonyl ginsenosides m-Rb1, m-Rc, and m-Rd were identified by basic hydrolysis according to the method described by Court et al. (*5*), and the oleanolic acid-type ginsenoside Ro was identified by comparing the retention times with published data and by varying the detection wavelength (*57*). Since standards of malonyl ginsenosides and Ro were

Table 1. Content of Ginsenosides and Polyacetylenes (mg/kg FW) in a Whole Root from a 5 Year Old American Ginseng Plant Grown in Denmark Using Different Extraction Methods (Methods A–C)

extraction method[a]	Rg1	Re	Rb1	Rc	Rb2	Rd	Ro	m-Rb1	m-Rc	m-Rd	total ginsenosides	falcarinol	panaxydol	total polyacetylenes
A	ND[b] b[c]	ND b	ND b	ND c	ND b	ND c	ND c	ND c	ND c	ND c	ND c	350 a	270 a	620 a
B	720a	4310 a	3500 a	370 b	290 a	360 a	370 b	2860 b	170 b	770 b	13700 b	<10 b	<10 b	<10 b
C	750a	4240 a	3880 a	510 a	330 a	320 b	630 a	3920 a	270 a	1100 a	15900 a	340 a	250 a	590 a

[a] Extraction methods A and B: roots extracted with 2 × EtOAc (A) followed by 2 × 80% MeOH (B). Extraction method C: roots extracted with 2 × MeOH followed by 1 × 80% MeOH. Extractions performed in triplicate. For further details, see the Materials and Methods. [b] ND = not detected. [c] Means within a column followed by different letters are significantly different ($P \leq 0.05$).

Table 2. Content of Ginsenosides and Polyacetylenes (mg/kg FW) in Different Root Sections [Diameter 0.5–2.5 mm (Root Hairs), 5.0–10.0 mm (Lateral Roots), and 15.0–20.0 and >20.0–38.0 mm (Main Roots)] of Fresh Roots from 6 Year Old American Ginseng Plants Grown in Denmark[a]

root size class according to root diameter (mm)	Rg1	Re	Rb1	Rc	Rb 2	Rd	Ro	m-Rb1	m-Rc	m-Rd	total ginsenosides	falcarinol	panaxydol	total polyacetylenes
0.5–2.5	800 a[b]	7420 a	8070 a	3170 a	910 a	2650 a	310 c	4850 a	520 a	2290 a	31000 a	1880 a	680 a	2560 a
5.0–10.0	500 a	4450 b	6260 b	900 b	280 b	1250 b	470 bc	4420 ab	260 b	1280 b	20100 b	450 b	460 b	910 b
15.0–20.0	510 a	4290 b	5910 b	530 c	180 c	740 c	540 b	3820 b	170 b	920 c	17600 b	260 c	340 c	600 c
>20.0–38.0	650 a	4390 b	6230 b	380 c	160 c	550 c	780 a	3780 b	200 b	790 c	17900 b	230 c	300 c	530 c

[a] Extractions (method C) performed on four different ginseng roots in triplicate for lateral and main roots and in duplicate for root hairs. For further details, see the Materials and Methods. [b] Means within a column followed by different letters are significantly different ($P \leq 0.05$).

not commercially available, the contents of m-Rb1, m-Rc, m-Rd, and Ro were determined from the external calibration curve of Rb1. Further, the molecular weight (MW) of the individual malonyl ginsenosides was included in the calculations of their concentration, taking into account their different MWs compared to that of Rb1 (MW 1108). Consequently, different conversion factors for m-Rb1 (MW 1194), m-Rc (MW 1164), and m-Rd (MW 1032) were used. The HPLC method was validated with regard to linearity, precision, and accuracy. Linearity for ginsenosides and polyacetylenes was determined in the concentration range occurring in the plant material (**Tables 1** and **2**) with the following correlation coefficients: $R^2 > 0.995$ (ginsenosides) and $R^2 > 0.993$ (polyacetylenes). Mean recovery rates (~accuracy) for the individual neutral ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) and polyacetylenes (falcarinol and panaxydol) were determined by spiking a known amount of authentic standard (from stock solutions) of each compound to a ginseng root extract sample with a known content of the individual ginsenosides and polyacetylenes. Mean recovery rates for the neutral ginsenosides and the polyacetylenes were in the range 94.5−100.6% and 95.5−98.2%, respectively, with a relative standard deviation (RSD) of <5%. Repeatability was determined by four injections of a ginseng root sample in 1 day (intraday variability) and intermediate precision was determined by analyzing a ginseng root sample on four different days (interday variability) with an RSD < 5% for all neutral ginsenosides and polyacetylenes.

Statistics. For statistical analysis of variances, the general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, NC) was used. The data were checked for outliers and ln transformed when necessary to fit the normal distribution and uniform variances. The sources of variances were the extraction method and root size. The least significant difference (LSD) at $P \leq 0.05$ was used to assess the location of the significant differences.

RESULTS AND DISCUSSION

Analysis of Ginsenosides and Polyacetylenes in American Ginseng Root Extracts. The analysis of the hydrophilic ginsenosides and lipophilic polyacetylenes in ginseng root are usually performed separately due to the large differences in their chemical structure and hence physical properties (**Figures 1** and **2**). Consequently, different extraction and analytical methods for the determination of these compounds in various ginseng roots and products have been developed (*6*, *38*, *42*, *43*, *45*, *46*, *48*, *49*, *57*−*60*). However, to the best of our knowledge no method has been described that simultaneously determines the contents of both ginsenosides and polyacetylenes in roots of American ginseng or other ginseng species, i.e., a method that is able to combine both an optimal extraction and analysis of these important bioactive compounds.

The majority of methods described in the literature for the quantitative analysis of ginsenosides or polyacetylenes from ginseng roots and/or ginseng herbal remedies are based on RP-HPLC (*5*, *6*, *38*, *43*, *46*, *47*, *52*, *53*, *56*−*60*). In the present study we describe an RP-HPLC method that can be used to determine the contents of both polyacetylenes and ginsenosides in the same analysis. Ginsenosides and polyacetylenes were separated from root extracts on a Purospher STAR RP-18 column using a gradient consisting of different proportions of MeCN and water with the presence of KH_2PO_4 in the mobile phase (see the Materials and Methods). The addition of 10 mM KH_2PO_4 in the mobile phase was found to be important for the resolution of the ginsenosides, in particular malonyl ginsenosides (data not shown), which is in accordance with previous investigations (*5*, *47*). Furthermore, the addition of KH_2PO_4 to the mobile phase did not have any influence on the chromatographic behavior of the polyacetylenes (data not shown). The compounds were detected at 203 nm, where both ginsenosides and polyacetylenes have noncharacteristic but relatively strong UV absorptions. Typical RP-HPLC chromatograms of American ginseng root extract are shown in **Figure 4**. The ginsenosides eluted between 30.0 and 70.0 min and included the neutral protopanaxadiols Rb1, Rb2, Rc, and Rd, the neutral protopanaxatriols Re and Rg1, the malonyl ginsenosides m-Rb1, m-Rc, and m-Rd, and the oleanolic acid-type ginsenoside Ro. The neutral ginsenosides were identified by the use of authentic standards (see the Materials and Methods), with the neutral protopanaxatriols and protopanaxadiols eluting between 33.0 and 35.0 min and between 57.5 and 65.0 min, respectively. The malonyl ginsenosides m-Rb1, m-Rc, and m-Rd that are expected to occur between the neutral protopanaxatriol and protopanaxadiol ginsenosides on RP-HPLC columns (*38*, *40*) eluted between

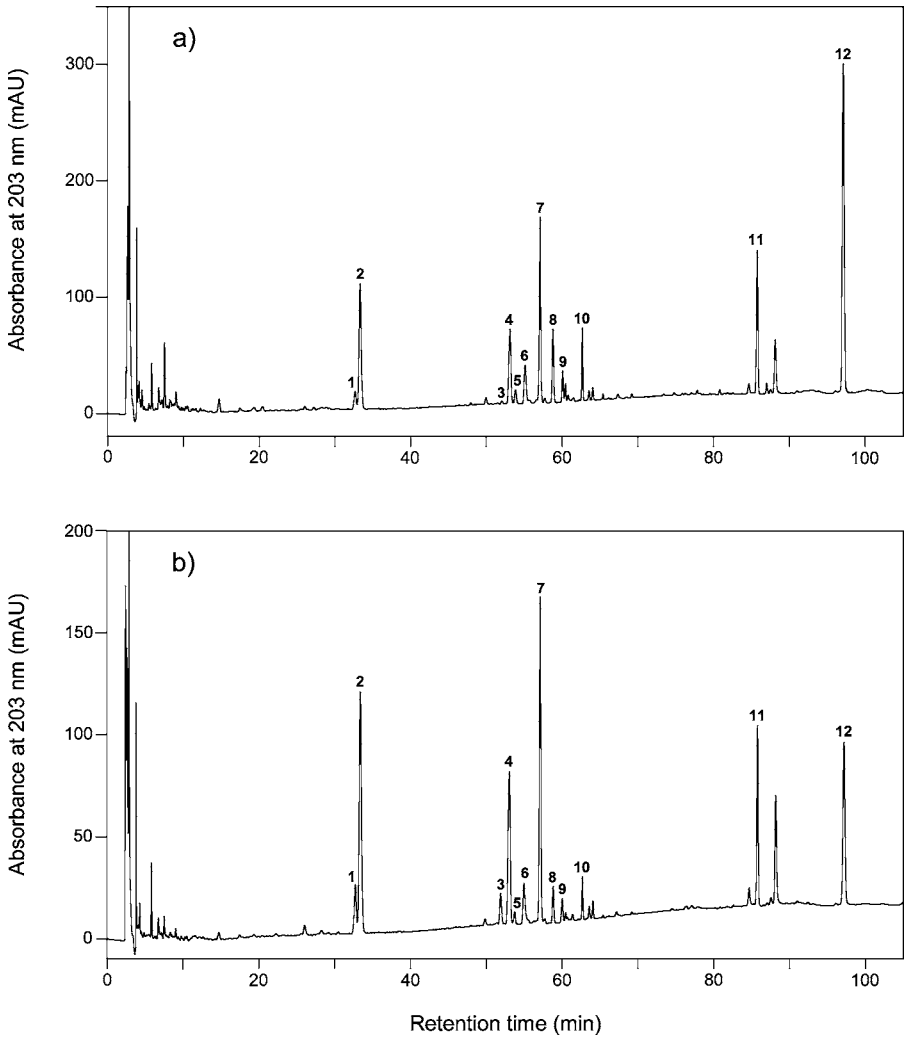


Figure 4. Typical HPLC chromatograms at 203 nm showing the separation of ginsenosides and polyacetylenes in an aqueous methanolic extract (extraction method C) of (**a**) root hairs (root diameter 0.5–2.5 mm) and (**b**) main roots (root diameter 15.0–20.0 mm) from a fresh root of a 6 year old American ginseng (*P. quinquefolium*) plant grown in Denmark. Ginsenosides: **1** = Rg1; **2** = Re; **3** = Ro; **4** = m-Rb1; **5** = m-Rc; **6** = m-Rd; **7** = Rb1; **8** = Rc; **9** = Rb2; **10** = Rd (**Figure 1**). Polyacetylenes: **11** = panaxydol; **12** = falcarinol (**Figure 2**). The extraction procedure and chromatographic conditions are described in the Materials and Methods.

53.5 and 57.0 min just before the neutral protopanaxadiols (**Figure 4**). The malonyl ginsenosides were tentatively identified by basic hydrolysis and HPLC retention times according to the method described by Court et al. (*5*). The oleanolic acid-type ginsenoside Ro eluting at approximately 53.0 min was identified by comparing the retention times with published data and by varying the detection wavelength (*57*). The identified ginsenosides constituted approximately 98% of the total ginsenoside content in the American ginseng root extracts, with Re, Rb1, Rc, Rd, and m-Rb1 being the major ginsenosides in accordance with previous investigations (*5*, *6*, *40*, *42*, *57*). A few minor ginsenosides that constituted less than 2% of the total ginsenoside content were not identified. These ginsenosides occurred mainly in part of the RP-HPLC chromatogram where the neutral protopanaxadiols were eluted (**Figure 4**). It is therefore likely that some of these minor unidentified ginsenosides are either other neutral ginsenosides such as 24(*R*)-pseudoginsenoside F_{11} that have previously been reported to be present in root extracts of American ginseng (*5*, *42*, *48*) or other protopanaxadiols such as Rb3, Rg3, and Rh2 that were recently detected in the leaves and berries of American ginseng (*61*).

The two major peaks eluting at approximately 86.0 and 97.5 min in the HPLC chromatograms (**Figure 4**) had distinctive HPLC-DAD UV absorbance maxima at λ_{max} 220, 231, 243, and 256 nm and λ_{max} 231, 244, and 256 nm, respectively. These UV spectra are characteristic UV spectra for polyacetylenes with a diyne chromophore (*53*). The compounds were isolated by column chromatography and identified by NMR spectroscopy and mass spectrometry to be panaxydol and falcarinol (see the Materials and Methods), respectively. These two compounds constituted above 97% of the total polyacetylene content in the fresh roots. This is also in accordance with previous investigations of American ginseng roots for polyacetylenes (*38*, *52*, *53*). A third major peak with λ_{max} at 224 and 278 nm occurred at 88.2 min in the polyacetylene area of the HPLC chromatograms (**Figure 4**). However, careful investigation of this peak by HPLC-DAD revealed that this compound was not a polyacetylene.

Extraction of Ginsenosides and Polyacetylenes. A wide variety of procedures have been employed for the extraction of ginsenosides from plant material. Most methods used MeOH−water mixtures (*47*). However, the optimal extraction of ginsenosides from plant material appears to be extraction with 80% aqueous MeOH, at room temperature, under stirring/sonification (*60*) or extraction with 100% MeOH by refluxing at 60−65 °C (*46*). Extraction with 80% MeOH is, however,

not compatible with an optimal extraction of the lipophilic polyacetylenes that are normally extracted with 100% EtOAc or 100% MeOH (*53*). Furthermore, extraction with 100% MeOH by refluxing is not optimal, since the polyacetylenes are sensitive to heat (*53*). In addition, refluxing will also result in some changes in the ginsenoside profile of the extracts, since the malonyl ginsenosides are partially degraded to their corresponding neutral ginsenosides when subjected to heat (*5*, *47*). A method that simultaneously ensures optimal extraction of neutral and malonyl ginsenosides as well as polyacetylenes was developed and its efficiency compared to those of optimal extraction methods for ginsenosides and polyacetylenes.

Extraction twice of fresh ginseng roots with EtOAc (method A, **Table 1**) followed by extraction twice with 80% MeOH (method B, **Table 1**) at room temperature overnight under stirring clearly showed that EtOAc is an effective solvent for extracting polyacetylenes but as expected not the ginsenosides. Extraction twice of homogenized fresh roots with 100% MeOH followed by extraction with 80% MeOH at room temperature under stirring overnight (method C) was shown to be an efficient method for extracting both ginsenosides and polyacetylenes from the fresh roots (**Table 1**). However, as the water content in ginseng roots was determined to be approximately 75% on a fresh weight basis (data not shown), the MeOH concentration in the first extraction was approximately 83% due to the water from the fresh roots. Hence, in the second extraction with 100% MeOH the water content was relatively low, resulting in an effective extraction of most of the remaining polyacetylenes. The final extraction with 80% MeOH primarily ensured an optimal extraction of the remaining ginsenosides. Accordingly, extraction method C resulted in significantly higher concentrations of total ginsenosides and of several individual ginsenosides (Rg1, Rb1, Rc, m-Rb1, m-Rc, m-Rd, and Ro) compared with the concentrations obtained by extraction method A plus method B, although the differences were relatively small as shown in **Table 1**. For the polyacetylenes, there was a tendency to obtain higher concentrations of these bioactive compounds by extraction method A compared to extraction method C. However, since the differences were nonsignificant, it can be concluded that extraction method C is comparable with extraction method A for the polyacetylenes (**Table 1**). Further extraction of the ginseng root material from extraction method C with 100% MeOH followed by 80% MeOH showed that the extractions of ginsenosides and polyacetylenes were almost complete using extraction method C as >98% of the bioactive constituents were extracted by this method (data not shown). It can therefore be concluded that extraction method C is efficient for simultaneous extraction of both polyacetylenes and ginsenosides from fresh plant material.

Extractions of ginseng roots are normally performed on dried root material. Significant discrepancy can be noticed in several papers reporting quantitative analysis of whole roots of American ginseng for polyacetylenes and/or ginsenosides. In general, the content of the major polyacetylenes falcarinol and panaxydol was found in amounts between 150 and 560 mg/kg dry weight (DW) and between 180 and 950 mg/kg DW (*23*, *37*, *62*), respectively, whereas the total content of ginsenosides was found in amounts between 17 and 60 g/kg DW (*6*, *38*, *40*, *41*, *43*, *46*), with Re and Rb1 being the major ginsenosides in most investigations. The concentrations of polyacetylenes and ginsenosides found in the present investigation when converted to dry weight data corresponded to a total polyacetylene content of approximately 2400 mg/kg DW and a total ginsenoside content of over 60 g/kg DW (**Tables 1** and **2**). The higher concentrations of both types of bioactive compounds found in the fresh American ginseng roots in the present investigation compared to other studies may be due to the loss of these compounds during processing, e.g., drying (*40*, *63*), and/or due to other factors such as genotype, cultivation practice, plant age, etc. (*39*−*44*). However, by extracting fresh plant material compared to dried root material time-consuming drying methods such as freeze-drying and possible degradation of bioactive compounds are avoided. Therefore, the analysis of fresh ginseng roots gives more accurate quantitative data on the content of these bioactive compounds in whole ginseng roots. The developed extraction and HPLC method may, however, find use for the determination of ginsenosides and polyacetylenes in all types of ginseng plant material and ginseng herbal remedies.

Ginsenosides and Polyacetylenes in Different Root Sections of American Ginseng. The contents of ginsenosides and polyacetylenes in American ginseng roots of different sizes (root hairs, lateral roots, and main roots) were investigated using extraction method C and the described HPLC method. The concentration of total ginsenosides and polyacetylenes decreased significantly with increasing root size/diameter (**Table 2**). Total ginsenosides decreased significantly from 31.0 g/kg FW in root hairs to 20.1 g/kg FW in lateral roots and approximately 17.8 g/kg FW in main roots. Total polyacetylenes decreased significantly from 2560 mg/kg FW in root hairs to 910 mg/kg FW in lateral roots and to an average concentration of 570 mg/kg FW in main roots (**Table 2**). For individual ginsenosides the largest decreases in concentration from root hairs to lateral and main roots were observed for Rc, Rb2, and Rd and the malonyl ginsenosides m-Rb1, m-Rc, and m-Rd, whereas the content of Rg1 did not change significantly in the different root sizes, although a tendency for a decrease in the content of Rg1 from root hairs to lateral and/or main roots was observed. On the other hand, the content of Ro was significantly lower in root hairs compared to lateral and main roots (**Table 2**). The results are in accordance with previous investigations on root hairs and lateral and main roots for ginsenosides of American ginseng and other ginseng species (*43*, *64*, *65*). The significantly higher concentrations of ginsenosides found in root hairs compared to the other root types have led to the proposal that ginsenosides are mainly distributed in the periderm and cortex root tissues (*64*).

The polyacetylenes also decreased significantly in concentration from root hairs to lateral and main roots. Falcarinol showed 4-fold and 7-fold decreases in concentration from root hairs to lateral and main roots, respectively. On the contrary, increasing the root diameter had a minor effect on the concentration of panaxydol with only a 2-fold decrease in concentration from root hairs to main roots (**Table 2**). This is in accordance with a recent study by Baranska et al. (*52*), who found that the polyacetylenes falcarinol and panaxydol decreased with increasing root size/diameter in American ginseng roots and that the content of the polyacetylenes were mainly concentrated in specific areas of the ginseng root in the outer sections as observed by using Raman spectroscopy. A similar distribution pattern of polyacetylenes has also been observed in carrot roots (*66*, *67*). Kidmose et al. (*66*) demonstrated that the concentration of the polyacetylenes falcarindiol and falcarindiol 3-acetate decreased significantly with increasing carrot root size whereas the content of falcarinol was almost the same regardless of the root size. Since falcarinol is mainly located in the phloem tissue in the carrot root (*67*, *68*), an increase of the root diameter had only a minor effect on the falcarinol content. Falcarindiol on

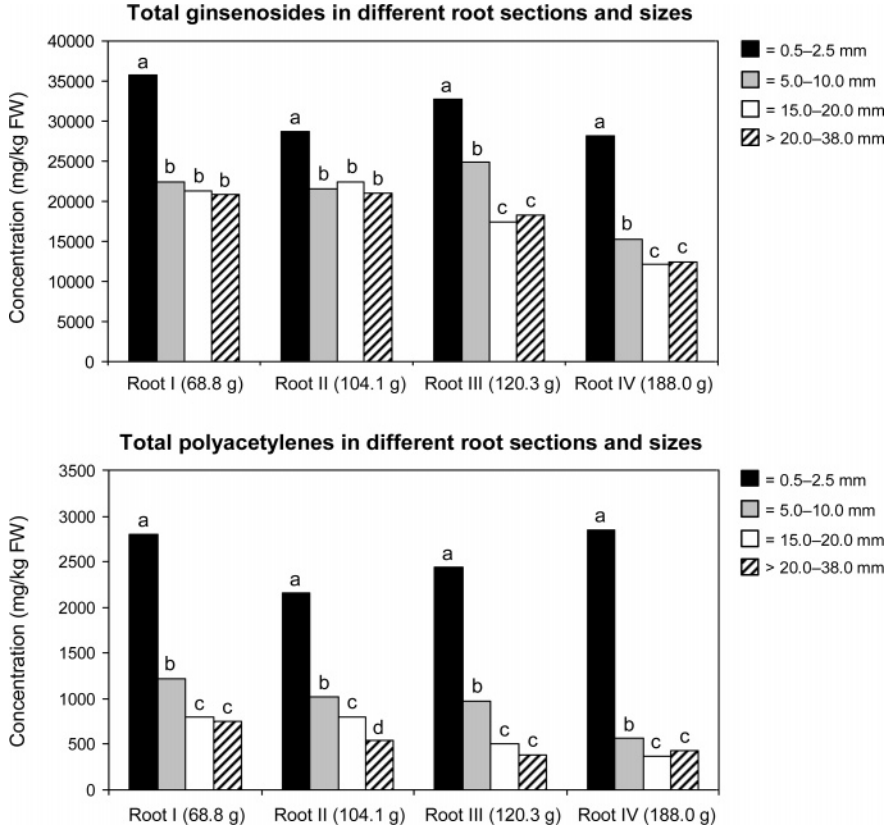


Figure 5. Total content of ginsenosides and polyacetylenes in different root sections and sizes, root diameter 0.5–2.5 mm (root hairs), 5.0–10.0 mm (lateral roots), and 15.0–20.0 and >20.0–38.0 mm (main roots), of four individual ginseng roots from 6 year old American ginseng (*P. quinquefolium*) plants. Bars for the individual roots marked by different letters are significantly different ($P \leq 0.05$). See **Table 2** for the mean content of individual ginsenosides and polyacetylenes. FW = fresh weight.

the other hand is primarily located in the periderm tissue, so during the increase in root size this polyacetylene is "diluted" (*66*). This dilution effect may also explain to some extent the higher content of polyacetylenes in root hairs compared to lateral and main roots. The decrease in concentration of falcarinol compared to panaxydol from root hairs to the other root types seems to indicate that falcarinol is located in more external tissues than panaxydol (*52*).

The decrease in the content of most of the individual ginsenosides with increasing root size (**Table 2**) clearly indicates a distribution of the ginsenosides in the ginseng root similar to that for the polyacetylenes. The minor decrease in the content of Rg1, Re, Rb1, and m-Rb1 compared to Rc, Rb2, Rd, m-Rc, and m-Rd with increasing root size/diameter indicates a more external accumulation of the latter ginsenosides compared to the former and that the major ginsenosides may be more uniformly distributed in the ginseng root. However, further research and more detailed investigations are needed to establish the exact distribution of the individual ginsenosides and polyacetylenes in the ginseng root.

It has previously been shown that the content of bioactive compounds in ginseng roots may vary among locations, growing conditions, age of the roots, and genotype (*39*−*44*). **Figure 5** shows the content of total ginsenosides and polyacetylenes in the different root parts of the individual ginseng roots investigated in the present study. **Figure 5** clearly indicates that the production of bioactive ginsenosides and polyacetylenes in ginseng roots depends on the genotype. This information may be used for selection/breeding of high-quality roots with regard to bioactive constituents. It is particular interesting to note that differences in the total concentration of ginsenosides and polyacetylenes in the main roots of different diameters were nonsignificant in nearly all roots investigated (**Figure 5**) and that this was also the case for individual polyacetylenes and ginsenosides (data not shown). It therefore appears that the main roots regardless of the root diameter/size in general represent the content in the other main roots of the ginseng root. The ginseng plant can easily be cultivated even if one of the smaller main roots is cut off for chemical analysis. This may be used to select roots with the highest concentration of bioactive constituents and hence the production of high-quality ginseng roots. The present investigation further indicates that there is no direct relationship between the content of bioactive constituents and the root weight, i.e., selection of larger roots may result in higher root yields without resulting in a direct decrease in the concentration of bioactive compounds. However, a larger number of roots need to be investigated to establish this relationship with certainty. Further, such an investigation may also be able to establish whether selection for a high content of ginsenosides will result in a lower concentration of polyacetylenes and vice versa.

Conclusions. The presented results have shown that it is possible to simultaneously extract and to quantify ginsenosides and polyacetylenes in ginseng root extracts by RP-HPLC, which clearly has made it much easier to analyze for both these groups of bioactive compounds in ginseng roots compared to existing methods. This provides a good opportunity to investigate the content of ginsenosides and polyacetylenes in ginseng roots and to generate data on various factors that may influence the content of these compounds in the roots. Furthermore, this information may be used to improve the quality of ginseng roots with respect to bioactive constituents and in general to improve the quality

of herbal remedies based on ginseng. The present investigation of different sections of ginseng roots, i.e., root hairs, lateral roots, and main roots, showed that the content of bioactive compounds depended on the root type/diameter and further that the different sizes of the main roots are not significantly different with respect to polyacetylenes and ginsenosides. This information may be used to make differentiated ginseng herbal remedies and/or to select and improve the genotypes of ginseng with respect to a higher yield and content of bioactive constituents. The developed extraction and HPLC method may find use as a quality control of fresh ginseng roots as well as dried root material and ginseng herbal remedies.

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