Assessment of Ontario-grown Ginseng (*Panax quinquefolius* L.) for Nutritional Quality and Food Safety

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Abstract

The natural health product industry is in need of standard criteria to assess the quality of herbals/botanicals for the international market. Presently, grading and pricing of the ginseng roots are exclusively determined based on the physical appearance of the roots. To initiate development of standardization criteria for ginseng quality and safety, twenty representative samples of three- and four-year-old ginseng roots (*Panax quinquefolius* L.) were collected from Ontario, Canada and were analysed for a wide range of nutritional and food safety parameters (to assess of their phytochemical qualities and for determination of concentrations of constituents such as ginsenosides, polysaccharides, macro- and micro-nutrients, microbial populations, and fungicide and pesticides levels) in an ISO 9001:2000 registered laboratory. Proximate analysis revealed that ginseng roots contained 8 to 12% proteins, 0.3 to 1.2 % fat, 67 to 80 % carbohydrate, and 3 to 8 % ash on dry weight basis. Ginseng contained 16 to 30 % dietary fibre. The calculated average energy value of ginseng was 342 kcal. Total ginsenosides concentration in roots ranged from 53 to 94 mg/g dry weight while ginsenosides Rb_1 (54.8 %) and Re (16.3 %) contributed up to 70 % of the total ginsenosides. Rf was absent from all the Ontario ginseng roots. Among the micronutrients K (12.6 to 15.7 mg/g DW), Ca (1.6 to 6.4 mg/g DW), P (2.3 to 3.8 mg/g DW) and Mg (1.3 to 1.9 mg/g DW) were predominant. Several other elements such as Na > Fe > Mn > Zn > B > Cu > Se were present in minor amounts as indicated descending order. Metalloid or heavy metals Cd, Cr, Hg, Mo, Ni, and Pb were not detectable in ginseng roots. The pesticides quintozene, iprodione and zineb were detectable in some samples and the levels were variable among samples. However, chlorothanil and metalaxyl were not detectable. The *E. coli* and total *Coliform* counts of roots were in acceptable range. The aerobic bacteria, yeast and mold counts did not exceed the levels for self-stability. A comprehensive standardization procedure to assess the quality of ginseng roots is being developed.

INTRODUCTION

American ginseng (*Panax quinquefolius* L.) is one of the economical important medicinal plants grown in Canada, primarily in the provinces of Ontario and British Columbia. Pharmacological investigations have shown that ginseng has many health promoting benefits including effects on cardiovascular, immune and nervous systems, activity as an antidote, anti-tumour, anti-aging and anti-diabetic agent, prevention of Alzheimer's disease**,** stress conditions and anti-aging effects (Konno et al., 1987 ; Tomoda et al., 1994; Vuksan et al., 2000; and Yun, 1996). The major chemical constituents of ginseng have been characterized. Ginsenosides (glycosides derivatives of triterpene

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dammarane saponins) are the major constituents, and the minor components include amino acids, peptides, polysaccharides, and minerals (Tang and Eisenbrand, 1992). To-date, more than 30 ginsenosides have been extracted from roots, leaves, and flower buds of ginseng. In general, ginsenosides composed of two basic chemical structures (aglycones), which are conjugated with sugar side-chains. The glycosides Rb_1, Rb_2, Rc, and Rd are 20(S)-protopanaxadiol derivatives, and Re, Rf, Rg_1 and Rg_2 are 20(S)-protopanaxatriol derivatives. It has been suggested that minor components of ginseng also contribute towards health benefits. As an example ginseng polysaccharides are shown to have immunological, anti-tumour, and hypoglycemic effects providing benefits to diabetics (Tomoda et al., 1994; Konno, 1987).

Export of Ontario-produced ginseng to the countries in Pacific Rim accounts for 85-90 % of the province's ginseng production. However, the standardization criteria for the assessment of quality and safety of Ontario ginseng have not yet been established. Presently, grading and pricing of the ginseng roots are exclusively determined based on the physical appearance of the roots. Determination of nutritional components and assurance of food safety by analyses of pesticide residues and microbial contaminants would help to ensure the production of high quality ginseng for the export market. The purpose of this study was to establish a scientifically sound basis for standardization and optimization of the Ontario-grown ginsengs for the high quality and food safety.

MATERIALS AND METHODS

Seven samples of 3-year old and 10 samples of 4-year old Ontario-grown ginseng roots were collected from commercial ginseng growers. The samples were dried, ground, passed through a 0.5mm particle size sieve, and homogenised. The sub-samples were taken for the following analyses:

Trace Elements

B, Ca, Fe, K, Mn, Mg, Na, P, and Zn were determined by inductively coupled plasma (ICP) method and As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, and Se by using atomic absorption spectroscopy (AAS).

Microbial Contamination

Aerobic bacterial counts, *E. coli* counts, and total *Coliform* counts and yeast and mold count were determined by standard plate count methods.

Ginsenosides

Ten ginsenosides, Ro, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, gypenoside XVII, and pseudoginsenoside F11 were determined using a reverse phase high performance liquid chromatography (RP-HPLC) with a photo diode array (PDA) detector (Court et al. 1996). Three standards, Ro, gypenoside XVII, and pseudoginsenoside F11 were generously provided by the Southern Crop Protection and Food Research Centre, Agriculture and Agri-food Canada London, Ontario. The remaining seven standards were purchased form Indofine Chemical Company, Inc., Somerville, NJ.

Agro-chemical Residues

Chlorothalonil, Ridomil™ (metalaxyl), quintozene, and iprodione were analyzed using a gas chromatography coupled with nitrogen phosphorous and mass selective detection. The fungicide ethylene-bis-dithocarbamate (EBDC) and dimethildithiocarbamate were determined by total dithiocarbamates as EBDC equivalent carbon disulphide (CS_2) in various substrates.

Proximate Analysis

Moisture content was ascertained by measuring loss of weight on drying at 135 ^{o}C for 2 hours in an oven. Ash content was estimated as weight of residue on ignition in a furnace at 550 ^{o}C overnight. Total protein analysis was carried out by means of the

Dumas combustion method (AOAC method #992.15), and total fat was determined by the Soxhlet extraction procedure (AOAC method #960.39). The soluble or insoluble fibre fraction was collected on a celite filter, rinsed several times, and dried. Finally, the ash and residual protein content and blank values were used to correct the fibre content.

1. Total Fiber and Polysaccharide Analyses. Total dietary fibre analyses were carried out by the enzymatic-gravimetric method (AOAC method #991.43). The technique for quantitative determination of non-starch polysaccharides (NSP) in ginseng fibre was validated by means of a gas-liquid chromatographic method developed by modification of the method of Englyst et al. (1994). Starch was removed from the samples enzymatically, and soluble polymers were precipitated with alcohol. The precipitated insoluble polysaccharides were then hydrolyzed with acid, and the fractionated neutral sugars were determined quantitatively by gas chromatography after conversion to their alditol acetates. Uronic acids were determined by colorimetric methods, and lignin was determined gravimetrically. The total dietary fibre was defined as the enzyme-resistant polysaccharides plus the Klason lignin.

RESULTS AND DISCUSSION

The total ginsenoside concentrations of 3- and 4- years old ginseng roots were 71.6±10.8 and 70.3±12.3 mg/g dry weight (DW), respectively (Table 1). There was no difference between 3- and 4-year old roots on total ginsenosides. The distribution of concentrations of major ginsenosides of the samples were 48 to 57 % Rb1, 20-23 % Re, and 6 to 8 % Rd. Ginsenoside Rf was detected only trace amount (0.2 mg/g) ginseng roots. In the 4-year old ginseng roots, Rb1 and Re were the two most abundant ginsenosides, contributing about 70 % of the total ginsenosides. Similarly, Li, et al. (1996) reported aggregated contribution of Rb1 and Re as >75 % of the total ginsenosides.

The results of the proximate analyses indicated that dried ginseng roots contain residual moisture content of 9.3 to 12.9 %, protein content of 8.7 to 13.6 %, fat content of 0.4 to 1.2 %, ash content of 3.2 to 8.0 %, total carbohydrate content of 67 to 77 %, and estimated energy level of 333 to 349 kcal (Table 2). The total fibre components, which are defined as non-starch polysaccharides (NSP) of ginseng, are polymers of sugars, uronic acids, and lignins. The sugars in the NSP (hydrolysed non-starch sugar profile) of the ginseng samples included small amounts of rhamnose, arabinose, xylose, mannose, galactose, and glucose. The concentrations of NSP sugars ranged from 16 to 26 %, with a mean value of 0.55 % for the all-17ginseng samples. The lignin content varied widely from a minimum of 3.8 % to a maximum of 13.5 %.

Among the “micronutrient” (trace element), ginseng contained relatively high concentrations of Ca (1.6 to 3.7 mg/g) and K (12.6 to 29 mg/g) but contained moderate levels of Mg (1.3 to 2.6 mg/g) and P (2.3 to 3.8 mg/g) (Table 3). The heavy metals Cd, Hg, Pb, Cr, Mo, and Ni and the metalloid were not detected in any of the samples, and small quantities of the following elements were found: B (8.79-16.1 µg/g), Cu (5.0-12.0 µg/g), Fe (45.9-128 µg/g), Mn (20.5 - 62.4 µg/g), Na (71.1 - 774 µg/g), Se (0.0-0.8 µg/g), and Zn (9.5-24.7 µg/g).

The fungicides chlorothalonil and metalaxyl were not detected in all samples tested. Iprodione was not detected in 7 of the 17 samples, the other 10 samples had iprodione levels ranging from 0.05 to 0.34 µg/g. Zineb equivalent to ethylene bis-dithio-carbamates (EBDC) was not detected in 12 of 17 samples, the remaining 5 samples containing this compound at levels ranging from 0.2 to 0.3 µg/g. Quintozene concentrations ranged from 0.07 to 2.1 µg/g. The maximum residue limits (MRL) designated by Crop Protection Institute of Canada for chlorothalonil, total EBDC, iprodione, and metalaxyl are 0.1, 0.1, 0.1, and 3.0 µg/g, respectively.

The aerobic counts, yeast and mold counts indicate the quality of the products in terms of self-stability. On the other hand, *E. coli* (<3 CFU/g tissue) and total *Coliform* (<1000 CFU/g tissue) counts provide information on the food safety of the products. Overall, the microbial profile of Ontario ginseng roots were within the range of the

microbiological limit guideline for the “ready-to-eat foods” established by the Health Product Branch under the Ministry of Health Canada.

CONCLUSIONS

According to our findings, the concentrations of nutrient ional and bioactive components in ginseng vary considerably from farm to farm within Ontario. This could be due to the influence of various environmental conditions such as weather, soil nutrition and moisture and different farm management practices. In general, the medicinal value of ginseng is discussed on the basis of ginsenosides content, but the content and concentration of other constituents such as polysaccharides and micronutrients could also have an important contribution for the health benefits of ginseng.

Presently, grading and pricing of ginseng is solely decided based on the physical appearance of the ginseng roots. To satisfy the expanding global market for ginseng, it is necessary to establish the standardization of ginseng roots considering nutritional/ nutraceutical components and food safety concerns in addition the physical appearance. In conclusion, standardization of ginseng roots based on scientifically sound analytical criteria is proposed to assure the quality and food safety of Ontario-grown ginseng.

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Tables

Table 1. Distribution of 10 ginsenosides (mg/g DW) in 3- and 4-year old roots harvested from 17 farms in Ontario.

Farm ID	Rg1	Re	Ro	Rf	Rb1	Rc	Rb2	Rd	XVII	F11	Total
3-Year old											
AT 1	1.06	14.10	3.66	tr	31.60	3.22	0.48	6.10	1.61	0.40	62.2
AT3	1.38	15.80	5.28	tr	43.60	3.86	0.59	4.90	0.97	0.20	76.6
CO-OP 3	0.82	14.20	4.31	0.21	36.70	2.11	0.20	4.23	4.30	1.10	68.1
CO-OP 4	1.25	13.90	4.92	tr	33.90	2.91	0.36	5.64	1.11	1.70	65.7
JV 1	1.04	15.50	4.30	0.21	36.10	2.77	0.42	3.68	3.38	0.36	67.7
KR1	1.20	15.30	4.60	tr	35.50	2.92	0.43	6.32	0.77	0.21	67.2
KR2	1.95	19.90	3.38	tr	42.40	11.00	1.47	12.90	0.64	0.29	94.0
Mean	**1.24**	**15.53**	**4.35**		**37.11**	**4.11**	**0.56**	**6.25**	**1.83**	**0.61**	**71.6**
SD	**0.36**	**2.07**	**0.67**		**4.37**	**3.08**	**0.42**	**3.09**	**1.43**	**0.57**	**10.8**
4-Year old											
AT 2	1.28	19.80	4.89	tr	50.30	3.70	0.73	6.51	1.61	0.28	89.1
CO-OP 1	0.91	12.50	4.71	tr	32.80	2.47	0.25	3.49	1.62	tr	58.8
CO-OP 2	0.88	16.50	4.36	tr	43.00	2.53	0.25	4.39	3.84	1.44	77.3
FARM A	0.83	12.50	4.00	tr	28.00	2.37	0.28	3.52	0.96	nd	52.5
FARM B	1.43	16.40	5.39	0.22	37.20	2.74	0.36	4.11	2.53	0.27	70.6
FARM C	0.84	13.50	4.48	tr	30.90	2.10	0.24	4.67	1.81	1.36	59.9
FARM D	1.08	16.90	5.55	0.21	40.20	2.74	0.38	4.23	0.93	0.31	72.5
FARM E	1.12	12.90	3.94	tr	29.70	3.00	0.41	4.92	2.58	0.41	58.9
GB1	1.00	16.60	6.35	0.28	46.80	2.34	0.38	5.27	2.96	0.42	82.4
SP2	1.78	17.70	4.13	tr	39.60	5.98	0.94	7.21	3.32	0.65	81.4
Mean	**1.12**	**15.53**	**4.78**		**37.85**	**3.00**	**0.42**	**4.83**	**2.22**	**0.64**	**70.3**
SD	**0.31**	**2.52**	**0.78**		**7.51**	**1.14**	**0.23**	**1.22**	**0.99**	**0.48**	**12.3**

Note: The values are reported as the mean of duplicate analysis with units of mg/g dry weight (DW) sample as received.
SD, standard deviation; tr, trace (trace is defined as less than 0.2 mg/g); nd, not detectable

Table 2. Proximate analysis (%), calculated energy (kcal), and total dietary fibre (%) of ginseng roots grown for 3 and 4 years.

Farm ID	Proximate Analysis (%)					Energy	Total Dietary
	Moisture	Protein	Fat	Ash	Carbohydrates	(kcal)	fibre (%)
3-Year old							
AT-1.	11.2	11.7	0.4	3.8	72.9	342	17.8
AT3	12.9	11.4	0.4	4.3	71.0	334	20.9
CO-OP 3	10.0	10.2	0.5	3.8	75.5	347	18.3
CO-OP 4	10.7	10.9	0.6	3.9	73.9	345	16.1
JV 1	9.6	10.9	0.6	3.8	75.1	349	17.8
KR1	11.8	11.4	0.6	4.1	72.1	340	16.8
KR2	10.0	13.6	1.2	8.0	67.2	334	21.6
Mean	**10.9**	**11.5**	**0.6**	**4.5**	**72.5**	**341**	**18.5**
SD	**1.16**	**1.07**	**0.25**	**1.56**	**2.84**	**6**	**2.05**
4-Year old							
AT 2	10.5	11.8	0.6	4.0	73.2	345	18.5
CO-OP 1	11.0	10.0	0.5	4.2	74.3	342	16.1
CO-OP 2	9.3	10.2	0.7	3.4	76.5	353	19.4
FARM A	11.9	12.3	0.6	4.1	71.2	339	16.7
FARM B	11.5	12.3	0.6	4.3	71.3	340	16.5
FARM C	11.7	8.7	0.4	3.2	76.0	342	17.0
FARM D	10.1	11.1	0.6	4.1	74.1	346	16.8
FARM E	10.2	12.1	0.6	4.3	72.9	345	17.0
GB1	10.8	12.0	0.5	4.0	72.8	343	16.5
SP2	10.6	12.1	0.5	5.4	71.6	339	25.6
Mean	**10.7**	**11.3**	**0.5**	**4.1**	**73.4**	**343**	**18.0**
SD	**0.81**	**1.23**	**0.09**	**0.59**	**1.85**	**4**	**2.85**

Table 3. Presence of trace elements and heavy metal in Ontario-grown ginseng roots (ug/g).

Farm ID	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Se	Zn
3-Year old											
AT1	14.0	1,960	9.6	97.2	14,400	1,560	34.0	372.0	3,410	0.5	24.3
AT3	15.6	3,660	6.8	64.9	15,300	1,970	20.5	774.0	3,440	0.2	16.6
CO-OP 3	10.9	2,030	6.6	94.0	13,800	1,390	41.8	138.0	3,020	0.3	23.7
CO-OP 4	13.0	2,360	5.0	81.5	14,500	1,880	32.3	94.5	2,720	0.4	16.7
JV1	11.6	2,440	5.8	87.3	15,300	1,390	34.2	270.0	2,630	0.7	21.7
KR1	13.1	2,410	nd	72.0	14,900	1,440	23.7	388.0	2,340	0.4	12.2
KR2	16.1	3,730	5.8	287.0	29,600	2,640	48.4	112.0	2,290	0.2	22.0
Mean	**13.5**	**2,656**	**6.6**	**112.0**	**16,829**	**1,753**	**33.6**	**306.9**	**2,836**	**0.4**	**19.6**
SD	**1.92**	**734**	**1.6**	**78.0**	**5,657**	**456**	**9.6**	**238.7**	**470**	**0.2**	**4.5**
4-Year old											
AT2	14.0	2,650	12.0	80.3	13,000	1,580	24.9	565.0	2,920	0.6	19.3
CO-OP 1	12.0	2,880	nd	45.9	15,900	1,770	20.5	118.0	2,730	0.5	9.5
CO-OP 2	10.6	2,210	5.0	62.9	12,600	1,374	25.9	146.0	3,070	nd	14.3
Farm A	13.4	2,950	5.5	67.0	15,100	1,750	47.2	121.0	2,910	0.4	23.5
Farm B	12.8	2,040	5.0	79.9	15,700	1,620	21.5	71.1	2,670	0.3	22.6
Farm C	8.8	1,610	6.9	54.2	13,300	1,260	32.8	127.0	2,780	nd	20.7
Farm D	9.0	2,180	nd	106.0	15,500	1,820	54.9	112.0	2,910	0.4	17.5
Farm B	9.4	1,960	6.9	97.9	16,200	1,470	38.4	139.0	2,960	0.2	23.5
GB3	10.4	2,090	6.3	102.0	13,700	1,320	26.5	550.0	3,230	0.6	18.5
SP2	15.1	3,350	5.8	128.0	19,700	1,880	62.5	545.0	3,760	0.8	24.7
Mean	**11.6**	**2392**	**6.7**	**82.4**	**15070.0**	**1584**	**35.5**	**249.4**	**2994**	**0.5**	**19.4**
SD	**2.23**	**540**	**2.28**	**25.85**	**2091.81**	**221**	**14.77**	**210.72**	**314**	**0.19**	**4.74**

Note: As, Cd, Co, Cr, Hg, Mo, Ni and Pb were below the detectable limit.