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Carbohydrate and Ginsenoside Changes in Ginseng Roots Grown in the Bay of Plenty, New Zealand

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Abstract : Ginseng is traditionally cultivated worldwide in cold continental climates. It is now also being cultivated in maritime environments such as New Zealandís. This paper reports a number of growth and quality parameters for plants grown under those conditions over two growing seasons and the intervening winter dormant period. While shoot biomass peaked mid-summer, in contrast, root biomass peaked late autumn/early winter. Starch, sucrose, fructose, glucose and inositol were detected in the roots. Starch concentrations were highest in early autumn (mean 470 mg g^{-1} dry weight) and lowest in mid spring (218 mg g^{-1} dry weight). Sucrose concentrations were low during early summer until late autumn but increased rapidly with the onset of winter and peaked during mid spring (168 mg g^{-1} dry weight). Fructose and glucose concentrations were similar and peaked in late spring (5.3 and 6.2 mg g^{-1} dry weight). Inositol concentrations peaked in mid summer (1.7 mg g^{-1} dry weight). Starch/sugar ratios were high during summer and autumn and low during winter and spring. Ginsenoside concentrations and profiles showed that the six major ginsenosides, Rg1, Re, Rb1, Rc, Rb2 and Rd, were present, but Rf was absent. Concentrations did not vary with sampling date. The most abundant ginsenosides were Re (15.9 to 17.5 mg g^{-1} dry weight) and Rb1 (10.7 to 18.1 mg g^{-1} dry weight). Combined, they accounted for > 75% of total ginsenoside concentrations. Limited taste tests indicated that highest root quality occurred during late autumn, after the shoots had senesced. However, quality could not be related to plant chemistry.

Key words : North American ginseng, *Panax quinquefolius*, growth rate, ginsenosides, starch, sugar, sucrose, fructose, glucose, *myo*-inositol

INTRODUCTION

Considerable research[1-5] has been undertaken over the last 15 years into the potential of producing both *Panax ginseng* C. A. Meyer (Asian ginseng) and *Panax quinquefolius* L. (American ginseng) in New Zealand. Commercial production of both species has now begun based on published guidelines developed from this research.[6] New Zealandís maritime climate with its mild winters and cool summers is different to the continental climate of North America where ginseng is native.[7] As a consequence, many growers in New Zealand are able to adopt different management practices to those used in North America. For example, in areas in the North Island of New Zealand, where ginseng is being developed commercially, the ground does not freeze permanently during winter, thus allowing growers to harvest roots throughout the winter. This harvest opportunity is not available to growers in continental climates, but raises the question of whether winter or early spring harvesting influences root quality. An indication that root quality may change over this period was found in a preliminary taste panel assessment in which ginseng roots harvested in late winter were down-graded on quality because they tasted too sweet[8]. Cold-induced sweetening has also been reported in potato tubers.[9-10] In North America, ginseng growers have only a narrow harvest window between the plant senescence and the ground freezing and so harvesting generally

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occurs in mid autumn.[11] Reynolds[12] reported that ginseng quality could be improved by harvesting earlier in autumn, when total ginsenoside concentrations were higher. However, in that study the sampling was not continued into winter.

This work aims to define the monthly ginseng root and shoot dry weights over 18 months in a maritime environment and determine the changes in the starch and sugar concentrations in the roots over this period. Ginsenoside concentrations were assessed at four harvest dates while taste panel tests were undertaken at three harvest times to measure consumer acceptance.

MATERIALS AND METHODS

Three-year-old *P. quinquefolius* plants growing at a commercial property in the Bay of Plenty, North Island, New Zealand (176°05'E, 38°50'S), were harvested monthly from 15 February 1999 to 25 August 2000. At each harvest date, four plants were randomly selected and removed from production beds, chilled, and transported to Ruakura Research Centre in Hamilton. After washing, the plants were separated into the component parts (roots, shoots and seeds depending on season) and weighed. Shoot growth and seeds were oven-dried at 75°C, the roots lyophilised, and dry weights (DW) recorded. The roots were then ground to a fine powder using a ring grinder and stored at -17°C until analysed for starch and sugars using techniques described by Cranswick and Zabkiewicz[13] and Smith *et al.*[14]

Ginsenoside concentrations were determined for samples collected in February and October 1999 and February and July 2000. Analyses were carried out at 25°C on a C18 column (Phenomenex Luna C18(2) 250×2.0 mm) with a 2×4 mm C18 guard column. Peaks were detected at 205 nm and also monitored at 254 nm. A gradient programme was used; the initial solvent mix was 100% 15 : 85 MeCN : H_2O changing linearly to 100% 35 : 65 at 70 min, returning to 100% 15 : 85 at 71 min with a 9 min hold for equilibration prior to the next analysis. The flow rate was 0.5 mL/min, with an injection volume of 50 μL. The HPLC was controlled by Millennium32 (version 4.00, 2001, Waters Corporation) software. The HPLC component system was a 717 auto sampler, 600 controller and a 2487 programmable dual wavelength detector. A 0.5 mg/mL solution of C5-anilide was prepared in acetonitrile for use as the internal standard. A subsample (0.5 g) of finely ground (passed through 40 mesh seive) plant material was taken for analysis. This sample was accurately weighed into a 100 mL volumetric flask. About 50 mL of 15 : 85 MeCN : H_2O was added with 1.0 mL internal standard solution. The solution was mixed to disperse the solid and sonicated for 30 min. The solution was allowed to cool and made to volume with 15 : 85 MeCN : H_2O. An aliquot of this solution was filtered through a PTFE filter (0.45 μm, Phenomenex) into a vial for HPLC analysis. The compositions of the analytes were calculated using Millennium32, using the relative reponse factors given by Chromadex.[15] The method was calibrated using Ginsenoside Rb1 (Aldrich).

On 6 September 2000, after the carbohydrate analysis, lyophilised root samples from each of three harvest dates

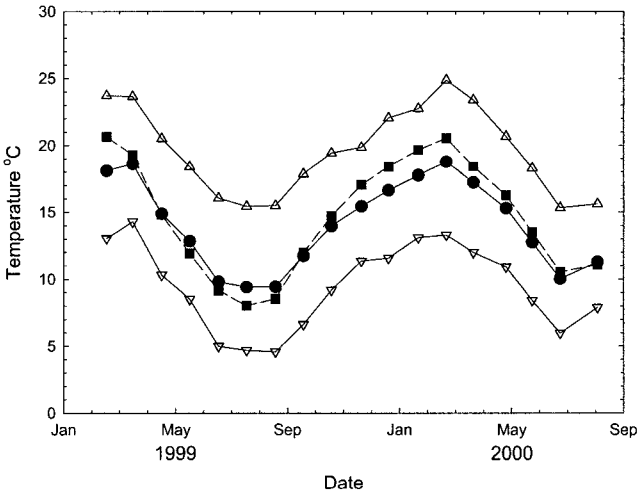

Fig. 1. Monthly mean temperatures for Te Puke during the trial period [air maximum (△), air minimum (▽), air mean (●) and soil 10 cm deep (■)].

Table 1. Comparison of climate data between Te Puke, New Zealand, and Simcoe, Ontario

Site	Mean annual rainfall (mm)	Mean mid winter temperature (°C)	Mean mid summer temperature (°C)	Degree days (>10°C)	Air frost-free days
New Zealand					
Te Puke	1754	9.3	18.7	1451	360
Canada					
Simcoe, Ontario	888	-5.6	20.6	1239	155

(April, May and August 1999) were presented to three experienced Asian medicinal herbalists for a double blind taste evaluation. The herbalists were asked to grade and comment on the individual taste characteristics of each individual sample. The April and May samples were chosen to represent the usual autumn harvest (during leaf senescence), while the August sample was representative of a late winter harvest.

Mean climate data for Te Puke, New Zealand (176° 19'E, 37°49'S, the national meterological site nearest the experimental site), and Simcoe, Ontario, Canada (80° 20'W, 42°50'N) are presented in Table 1 for comparison. Mean monthly air and 100 mm deep soil temperatures collected at Te Puke over the trial period are presented in Fig. 1.

RESULTS

Plant Development

In February 1999, the mean shoot dry weight was 1.75 g plant^{-1} (Fig. 2A). However, at this stage the foliage had begun to senesce for winter. From May through to September 1999 the plants were dormant (no above ground components). The first sign of new growth was at the October 1999 sampling. At that time, the mean DW of the foliage was 0.5 g plant^{-1}. The mean DW of the foliage increased until January, 2000 (5.0 g plant^{-1}), after which time senescence commenced, with a resultant decline in mean DW. The plants were again dormant by May 2000.

Mean root DW oscillated through the year. It peaked in June of both 1999 and 2000 (early winter), 8.9 and 14.6 g plant^{-1}, respectively, and was at a minimum in October, 1999 (mid spring) at the commencement of spring growth (Fig. 2B). Between June 1999 and June 2000 the net increase in root DW was 3.3 g plant^{-1}. Root dry matter remained at 31% of harvested wet weight (SD = 2.7) throughout the trial period except for a period between August 1999 and November 1999 (late winter and spring) when it dropped to 22.5% (SD = 1.3).

Although shoot senescence started in mid summer, total plant DW remained relatively constant from mid-summer until dormancy due to the increases in root biomass (Fig. 2C).

Seed Development

The mean seed head fresh weight in February 1999, immediately prior to harvest, was 4.6 g plant^{-1}. During the following season, seed head development was first observed in November 2000 and the mean fresh weight of seed heads was 0.7 g plant^{-1}. That weight steadily increased over the growing season until it was 12.1 g when harvested in February 2000. Seed was returned to the grower for propagation so DW was not determined.

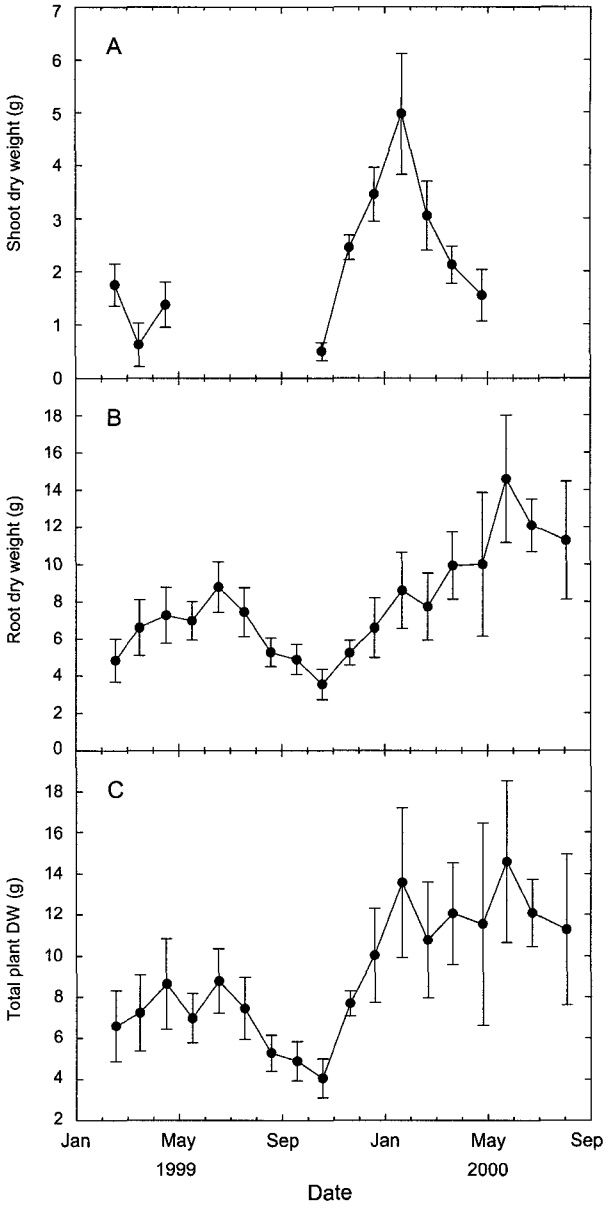

Fig. 2. Shoot, root and total dry weight changes in three- and four-year old ginseng, 1999 and 2000 respectively. Error bars indicate the standard error of the mean.

Sugars and Starch Concentrations

The concentrations of sugars and starch varied throughout the year.

Mean sucrose concentrations in the root were low from December until May (summer and autumn) (approximately

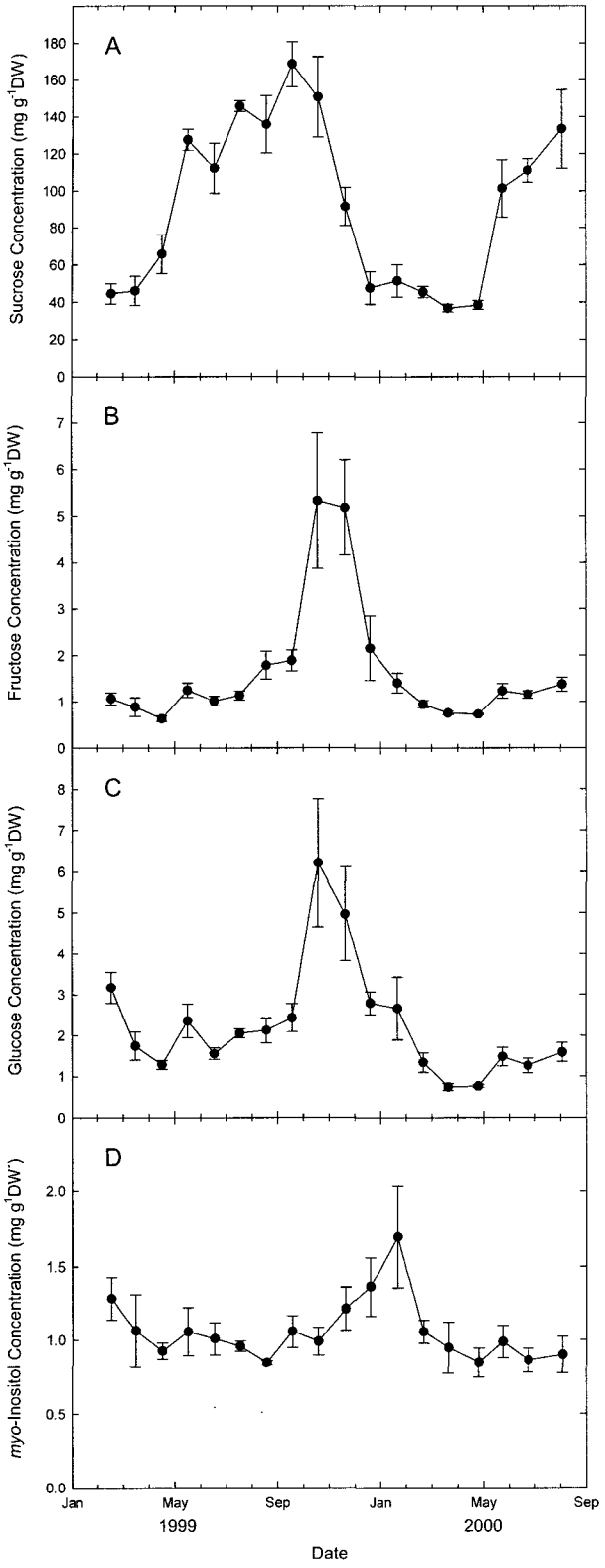

Fig. 3. Seasonal fluctuations in sucrose, fructose, glucose and *myo*-inositol concentrations in three- and four-year old ginseng roots, 1999 and 2000 respectively. Error bars indicate the standard error of the mean.

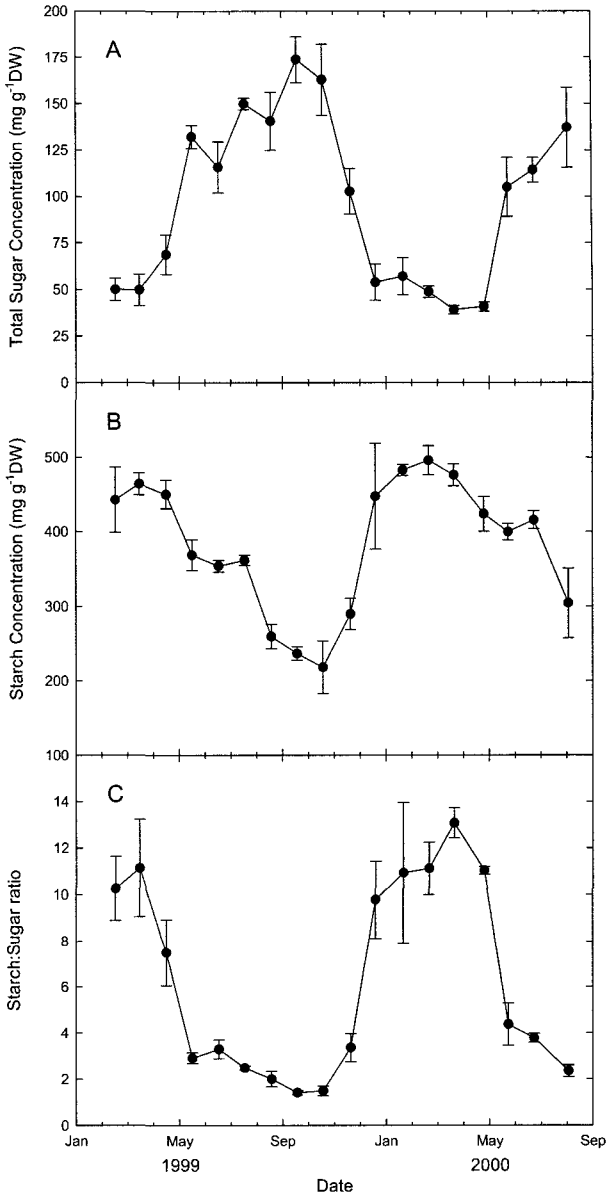

Fig. 4. Seasonal fluctuations in starch and sugar concentrations and the starch sugar ratio in three- and four-year old ginseng roots, 1999 and 2000 respectively. Error bars indicate the standard error of the mean.

40 mg g^{-1} dry weight), but rapidly increased with the onset of winter and stayed high, peaking in October (mid spring) (168 mg g^{-1} DW) and then returning to low summer/autumn concentrations (Fig. 3A). Mean fructose and glucose concentrations in the root followed a similar seasonal pattern with the concentrations remaining between 1 and 2 mg g^{-1} DW, except in the October-November spring period when they rose rapidly to 5.3 and 6.2 mg g^{-1} DW respectively (Fig. 3B and 3C, respectively). These fructose and glucose peaks

occurred approximately one month later than the sucrose peak. The mean inositol concentrations remained at a concentration of 0.9-1.1 mg g^{-1} DW for most of the year but rose to 1.7 mg g^{-1} DW in January (mid summer), before returning to the lower level (Fig. 3D).

The seasonal pattern of total sugars (sum of sucrose, fructose, glucose and inositol values) followed that of sucrose with a peak value of 173.8 mg g^{-1} DW in September (early-spring) then declining to a concentration of 39.2 mg g^{-1} DW until the following April (mid autumn) when it began to increase again (Fig. 4A).

Starch concentrations in the roots were highest (460-480 mg g^{-1} DW) during March (early autumn) in both seasons, and decreased gradually to be lowest (218 mg g^{-1} DW) during October 1999 (mid spring), before rapidly increasing again in November (Fig. 4B).

Starch/Sugar Ratios

The ratios of starch and total sugars concentrations were lowest during winter and spring (< 2 : 1) and highest during the summer/autumn period (10 to 12 : 1) (Fig. 4C).

Ginsenoside Concentrations

Concentrations of five of the major ginsenosides, Rg1, Re, Rb1, Rb2 and Rd, did not differ significantly for the four sampling dates (Table 2). No Rf ginsenoside was detected. The most abundant ginsenosides were Re and Rb1, accounting for 75% of the total (range 79.9 to 87.2% of total ginsenoside concentrations). Only ginsenoside Rc varied significantly with sampling date; with variation greatest in the spring sample.

Taste Evaluations

The sample harvested in May 1999 was judged to be of very good quality with strong flavour. The sample harvested in April 1999 was judged to be of poor quality while the sample harvested in August 1999 was judged to be of medium quality.

DISCUSSION

In the Bay of Plenty, New Zealand, ginseng emerged in mid spring and achieved maximum shoot biomass in mid-summer. Shoot senescence started in mid summer and continued until autumn with the plant completely dormant by late autumn. In contrast to the shoots, root biomass steadily increased from late spring until peaking in late autumn/early winter. Root biomass declined during winter to a low point in mid spring when shoot growth commenced. Total plant biomass was greatest from summer until autumn dormancy with approximately half the seasonal dry matter accumulation in the roots occurring after mid summer when leaf senescence had commenced. The slight decline in root weight during winter is likely to be the result of respiration. Proctor *et al.*[16] showed that ginseng roots have relatively high respiration rates. In this study, soil temperatures were well above freezing (ranging from 8.0 to 14.7°C) during the winter.

Root dry mass for plants reported here (in their third growing season from planting) increased from 4.8 to 8.8 g dry weight and is similar for ginseng plants growing in southern Ontario, Canada.[16] The ratio of shoot to root dry weight was 1 : 1.7, similar to the ratio of 1 : 2 reported by Proctor *et al.*,[16] supporting their contention that this ratio is constant for *P. quinquefolius* plants irrespective of their age or growing location. As new shoot development does not occur until after leaf senescence and chilling temperatures during winter,[17] any shoot loss during the growing season (to either disease or grazing), is likely to cause major reductions in root yield.

Peak starch concentrations (late summer through autumn) of the plants growing in this trial were higher than those for plants of an equivalent age and stage of growth grown in the Northern Hemisphere.[12] However, total sugar concentrations were lower in New Zealand plants than in Northern Hemisphere plants; total carbohydrate concentrations (sum of starch and sugars) were sim-

Table 2. Ginsenoside concentrations from roots harvested in February 1999 and 2000, October 1999 and July 2000 (mg g^{-1} DW)

Harvest Date	Rg1	Re	Rf	Rb1	Rc	Rb2	Rd	Total
February 1999	1.3	17.5	0.0	10.7	2.8	0.3	1.8	34.4
October 1999	1.1	17.5	0.0	17.9	5.0	0.2	2.0	43.3
February 2000	2.3	16.1	0.0	12.1	2.3	0.3	2.2	35.3
July 2000	1.3	15.9	0.0	18.1	2.2	0.1	1.5	39.0
SED	0.47ns	3.49ns	0.0	3.41ns	0.64**	0.22ns	0.86ns	7.08ns

ns means not significantly different
** means significantly different at 1% level.

ilar, between 490 and 496, and 486 to 467 mg^{-1} g dry weight, respectively.

Individual sugar concentrations for *P. quinquefolius* grown in New Zealand are similar to those for *P. ginseng*.[18] However, total sugar concentrations for New Zealand grown plants were lowest in early autumn (March), while for *P. ginseng* grown in Korea[18] they were lowest in late spring (May).

Ginseng, like many plants, stores high concentrations of carbohydrates, such as starch, in the root during summer[19] to provide an energy source when growth is reactivated during spring.[20] During autumn and winter, starch is slowly hydrolysed and sucrose accumulated (as seen in other root crops, including potato),[9,10] so that sucrose concentrations peak at the time of shoot emergence in spring. The conversion of starch to sugar is reported to protect the root from freezing.[12,21] Increased sucrose in the roots during winter has also been shown to occur in Asian ginseng.[22] Further, starch concentrations in ginseng roots have also been found to decrease in North America if autumn harvesting is delayed.[12] Yun and Lee[10] found that starch concentrations decreased and sucrose, fructose and glucose concentrations increased when *P. ginseng* was cool stored after harvesting during late summer and autumn, prior to processing.

Although the relative concentrations of glucose and fructose in New Zealand-grown plants were small compared to sucrose, their concentrations peaked in October, when growth recommenced, suggesting an important role during this time. Inositol concentrations remained relatively constant throughout the growing season except for a small peak in mid-summer. The role of inositol in the root is unclear but that peak may have an osmoprotective function[23] during periods of drought stress. However, the relationships between the sugars are complex, with sugars being continually converted from one to another depending on the plantís biochemistry and circumstances[23]. Consequently, the exact roles of the sugars in the ginseng plant remain unclear.

The total ginsenoside content of the roots, 34.4 to 43.3 mg g^{-1} DW is consistent with that reported for *P. quinquefolius* field-grown roots in Ontario, Canada,[24] and in Australia.[28]

The uniformity of ginsenoside content found between the winter, spring and summer sampling dates is consistent with the patterns observed in plant growth stages in Australia.[28] The consistent concentrations of total ginsenosides between summer and winter differ from those found in Canada by Reynolds,[12] who reported that levels declined in autumn. The findings suggest that harvesting during winter would not lower root quality based on ginsenoside concentrations.

The predominance of the Re and Rb1 in New Zealand roots is in agreement with reports from Ontario and British Columbia.[25] The higher level of ginsenoside Rc in spring has also been reported[22] and is explained by new hair root development as the plant begins to grow. In Australia hair roots had much higher ginsenoside Rc concentrations than lateral or main roots.[28] Non-detection of ginsenoside Rf in *P. quinquefolius* roots is consistent with results from multiple growing locations in Ontario and British Columbia, Canada.[25] This supports the suggestion that presence or absence of Rf could be used as a chemical marker to distinguish between roots of *P. quinquefolius* and *P. ginseng*.[26,27]

Our limited taste assessment provided no clear relationships between the subjective taste panel assessments and the measured parameters. A comparison of the root composition between the poor quality sample in April and the very good quality in May reveals that the total sugar concentration is increasing rapidly over this period, while there is a small decline in starch concentration giving a steep decline in starch:sugar ratio. If the increase in sugar concentration or the decline in starch:sugar ratio is related directly to taste quality the expectancy would be for the August sample to be of equal quality to the May sample as the sugar levels and starch:sugar ratios are similar. This, however, was not the case. It should be noted that these quality assessments be used powdered, lyophilised ginseng root rather than whole root. Ginseng quality is often determined as much or more by the appearance of the root than by its chemical constituents and this is unlikely to change in the short term.

CONCLUSIONS

This research has shown there is a strong seasonal pattern in the sugar and starch concentrations in ginseng roots but that the ginsenoside concentrations show little change. From these results it is evident that ginseng roots high in sugars can be harvested in the late autumnñearly spring period or low sugar and high starch roots in summerñearly autumn. The relationship of these changes to consumer preference has not been established and more research is needed to enable the use of seasonal component changes to guide harvest strategies. Ginseng quality is complex with many constituents contributing to the overall quality. Components other than sugars and starch,

which are important in affecting root quality based on taste and flavour, are likely to be involved. Where ginsenoside concentration is the basis of quality we have shown that harvest time is unimportant and to achieve the highest root yields in our maritime environment harvesting should take place in the late autumn-early winter period when the roots are at their maximum dry weight.

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