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Comparison of Isothiocyanate Yield from Wasabi Rhizome Tissues Grown in Soil or Water

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The isothiocyanate (ITC) yield of wasabi, the Japanese horseradish (*Wasabi japonica*), was measured on its release from glucosinolates in the rhizomes of plants grown in two traditional ways. Mature plants of 18 months old were harvested from two different commercial farms located in the South Island of New Zealand. At one farm, the plants were grown in raised soil beds, while the plants at the other farm were grown in gravel irrigated by river water. Following harvest, the rhizomes from each growth medium were divided into five size groups based on the weight and length of the rhizomes. The different sized rhizomes were also subdivided into proximal, medial, and distal portions of the rhizomes and each portion was further subdivided into epidermis plus cortex, and vascular plus pith. The individual and total ITC contents of each portion (proximal, medial, and distal) of the rhizomes were measured using dichloromethane extraction followed by the GC−FPD. The total ITC content of the rhizomes grown in soil increased (13 times) linearly from 6 to 114 g of rhizome weight, while the mean ITC content of the water-grown wasabi increased (10 times) nonlinearly for similar sized rhizomes. Water-grown rhizomes in the weight range from 18 to 45 g gave significantly ($P = 0.030$) higher total ITC (1−2 times) than similarly sized soil-grown rhizomes. Analysis of the tissues showed that the total and the individual ITCs were found in significantly higher levels (73 and 64%, respectively) in the skin and cortex tissue compared to the vascular and pith tissues. Analysis of the ITC content of the different locations of the wasabi rhizome showed that the distal portion of the rhizome contained significantly higher levels of both total and individual ITCs compared to the medial and proximal portions of the rhizome.

KEYWORDS: ***Wasabi japonica*; rhizome; hydroponic wasabi; epidermis; cortex; vascular tissues; pith; glucosinolates; isothiocyanate; allyl isothiocyanate; 3-butenyl isothiocyanate; 4-pentenyl isothiocyanate**

INTRODUCTION

Wasabi, the Japanese horseradish (*Wasabia japonica* (Miq.) Matsum), is a perennial native to Japan. It is widely grown in Japan, Taiwan, and China and its production in New Zealand is increasing. In Japan, wasabi is grown in two traditional ways, upland or soil grown, and water grown in specially built flooded beds. Japanese farmers use upland soil production principally to produce leaf and petiole products, while hydroponic methods are used to produce large rhizomes (*1*, *2*). Japanese publications suggest that hydroponic systems can produce larger rhizomes, which are highly regarded and, consequently, highly valued compared to stems grown in soil-based systems. In a review in 1993, the optimal environmental parameters to produce high quality water-grown wasabi in Japan were investigated (*2*). The unique environmental requirements to grow wasabi and the shortage of cultivable land limits its production area to 880 hectares in Japan and 400 hectares in Taiwan, but its demand has spread from Japanese cuisine to modern Western food. Therefore, the increasing demand for wasabi products and the difficulties in expanding production have seen prices rise steadily since 1970 (*2*). High prices have stimulated research into soil production methods and the investigation of production areas outside Japan. Cultivation of wasabi has been investigated in New Zealand since 1982 (*3*). New Zealand's climate (air temperature, water quality, high sunlight hours) appears to meet the requirements for growing quality wasabi. In New Zealand, both hydroponic and soil cultivation are used, but most wasabi is grown in soil in shade houses. The effects of fertilization on plant yield and ITC in different plant tissues of soil-grown wasabi in New Zealand has been reported by Sultana et al. (*4*). However, the amount of wasabi grown in soil has increased not only in New Zealand but also in Taiwan, as the costs of production are much lower than the costs of establishing flooded field structures.

The unique taste of wasabi comes from isothiocyanates (ITCs), the volatile sulfur compounds evolved from precursor glucosinolates (GSLs) by the action of myrosinase. Various

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GSLs have been detected in the family C*ruciferae* (*5*). They are stored in cell vacuoles, co-localized with the enzyme myrosinase but not in direct contact with each other (*6*). Myrosinases are mainly localized in idioblastic myrosin cells scattered in most plant parts (*7*). It is also reported that GSLs in vacuoles are stored together with ascorbic acid (*8*); high levels of ascorbic acid inhibits myrosinase activity, while low levels activate this enzyme (*9*). The function of ascorbic acid indicates the possibility of co-localization of GSLs and myrosinases. Only one plant *Koeberlinia spinosa* has been reported to contain myrosin cells but lack GSLs (*10*). The physical separation of the substrate and the enzyme suggests a role in the prevention of the formation of products in vivo. Cell disruption triggers the enzymatic degradation of GSL to release the ITCs which gives the unique flavor of wasabi. Details of the biosynthesis of GSLs, their enzymatic degradation process and products are discussed in publications (*5*, *11*, *12*). The myrosinase−GSL system is usually regarded as a defense system against herbivores, but other functions have been suggested in different species (*13*).

In mature wasabi plants, the rhizome is the most valuable plant part, as it possesses the highest yield of flavor compounds (ITCs) compared to other plant parts (*4*). The rhizome is made up of four major tissues: epidermis, cortex, vascular and pith. The epidermis is the outer layer of rhizome, and it consists mainly of dead cells which provide physical protection and some mechanical support. Underneath the epidermis, the cortex tissue is a layer of greenish colored tissue a few millimeters wide. It is a metabolically active tissue that carries out a variety of functions in the plant including storage and some photosynthesis (*14*). A thin, incomplete cylinder of vascular strands separates the cortex tissue from the substantial inner pith.

Considerable debate about the location of GSL synthesis has occurred. It has been reported that the leaves are the primary site of benzyl-GSL synthesis in garden nasturtium (*Tropaeolum majus*), but high amounts accumulate in other tissues such as the developing seeds, indicating that transport of benzyl-GSL from the leaves does occur (*15*). Other investigations have showed that glucosinolates have physicochemical properties that allow phloem mobility (*16*). It was also proposed that GSLs synthesized in mature leaves are readily loaded into and transported by the phloem in the osmotically driven translocation stream from leaf sources to major sinks (*17*). For example, young leaves were found to contain more GSLs than mature leaves in black mustard (*Brassica nigra*). However, although translocation is a major contributor to GSL accumulation in seeds, data in the literature (*18*) demonstrated that seeds of *Sinapis alba* contain all the enzymes required for GSL synthesis, unlike the seeds of *Brassica napus*. The possibility that GSLs can be transported from their synthesis site(s) and accumulated in other tissues must be considered in wasabi.

It has been claimed that flooded systems can produce large, high quality, premium priced rhizomes but data to support these claims cannot be found in published reports (*2*). Thus, it is also important to establish whether soil-grown wasabi contains similar levels of ITCs. The objectives of this study were to compare ITC levels obtained from wasabi plants grown in two ways and to establish the levels of ITCs in the different sized rhizomes and tissues. This information will help growers, food processors, and consumers to gather information on quality differences between soil- and water-grown wasabi rhizomes and their potential to yield flavor compounds important in the manufacture of traditional condiments.

MATERIALS AND METHODS

Collection of Plants. Soil-grown wasabi was collected from Brighton, Dunedin (45° 54⁻ S, 170° 20⁻ E), and water-grown wasabi from Rapaura, Blenheim (41° 30⁻ S, 173° 50⁻ E), in the South Island, New Zealand. Both groups of plants were grown in a cool temperate maritime climate. Twelve wasabi plants (cultivar Daruma) were collected from both systems. The plants were 18 months old at the time of harvesting. Water-grown wasabi was harvested on August 8, 2001, and soil-grown wasabi was harvested on November 19, 2001. Both of the water and soil-grown plants were grown without any fertilizer application, but water-grown wasabi plants were irrigated from the Wairau river by controlled water flow.

Experimental Design and Preparation of Plant Tissues. A randomized block design of 2 × 5 × 3 × 2 factorial with three extraction replicates were used to compare the two cultivation methods (water and soil grown), five rhizome sizes, three portions in rhizomes and two types of tissues. Leaves, petioles, and roots were removed from rhizomes of the fresh wasabi plant and then soil and dirt were removed using a cold tap water spray. The rhizomes from both media were then separated into five groups according to size measured by lengths and weights of the rhizomes: extra large (150−173 mm and 94−114 g), large (129−150 mm and 62−80 g), medium (90−104 mm and 35−45 g), small medium (84−89 mm and 18−29 g), and very small (47−52 mm and 6−13 g). Each rhizome group was then divided into three subgroups by sectioning the rhizomes into three equal portions by length, and labeled proximal, medial, and distal. Finally, each portion was further divided into epidermis + cortex and vascular tissue + pith using a knife.

ANALYTICAL METHODS

Extraction. Fresh tissue (25 g) from each portion, size, and media were homogenized separately in a Braun kitchen blender (MR 430 CA). Ground rhizome (4 g) was weighed into 40 mL Beckman centrifuge tubes containing Beckman centrifuge tubes containing 7 mL of chilled distilled water and 5 mL of dichloromethane (Hipersol grade, BDH) was added into it. The samples were mixed continuously for 2 h, by inversion, in an incubator at 20 °C. The solid and water phases were separated by centrifugation in a Beckman centrifuge at 12100*g*. The solvent extract was stored at −20 °C until gas chromatography (GC) analysis of ITCs. The remainder of the wasabi paste was used for moisture content determination using AOAC method (*19*).

Gas Chromatography Analysis. Samples of dichloromethane extract (1 μL) were injected into a Hewlett-Packard (HP) 6890 gas chromatograph fitted with flame photometric detector (FPD). A HP 6890 automatic injector was used with the following operating conditions: column: HP INNOWax, 30 m, 0.25 mm internal diameter, 0.25 μm film thickness, capillary column. Carrier gas: hydrogen, 85.9 kPa inlet pressure, flow rate 75 mL/min, linear velocity 58 cm/s. Injection: Splitless mode, 1 μL of dichloromethane extract. Temperature (°C): inlet 160 °C, detector 250 °C, column 50−100 °C at 5 °C/min, 100−240 °C at 10 °C/min. Chromatograms were recorded and peak areas were calculated using HP Chemstation software (version A.06.03). Pure butyl ITC was used as an external standard, and this response curve was used to measure individual ITC levels from peak areas from different samples.

Gas Chromatography Mass Spectroscopy. Allyl ITC, 3-butenyl ITC, and 4-pentenyl ITC were identified by GCMS on a Carlo Erba MFC500 GC (split ratio 30:1, HP DB5MS 30 m, 0.25 um film column, He carrier gas flow 2 mL/min) and a Kratos MS80RFA mass spectrometer (4 kV accelerating potential, 70 ev ionization energy, source temperature 250 °C, magnet scan 500−30AMU) from wasabi rhizome extract of this study. Other ITCs (*iso*propyl, *sec*-butyl, *iso*butyl, 5-hexenyl ITCs) were also observed in the tissue in minor amounts and were below the detection limit (≤8 mg/kg) in the small rhizomes. Three ITCs (allyl, 3-butenyl, and 4-pentenyl ITCs) were summed together to measure the total ITC concentration. The mean yields of total and individual ITCs are expressed as mg/kg fresh weight basis.

Statistical Analysis. The effects of growing media, rhizome size, tissue types, and portions on the yield of different ITCs from wasabi rhizomes were analyzed across the whole experiment by analysis of variance using a completely randomized factorial ANOVA model in

Table 1. The Yield of Isothiocyanates (mg/kg fresh weight basis) from Rhizomes of Wasabi Grown Either in Soil or Water[a]

isothiocyanates	N	soil-grown wasabi	water-grown wasabi	*P* value
allyl ITC	30	941.3	1371.0	0.127, NS
3-butenyl ITC	24	81.6	109.6	0.298, NS
4-pentenyl ITC	24	44.7	19.9	0.435, NS
total ITC	24	1042.3	1474.6	0.162, NS

[a] N = no of replicates, NS = not significant.

MINITAB (*20*), least significant difference of means (LSD at 5%) and by regression analysis. The mean value with association of *P* and 5% LSD are reported in the tables across the whole experiment. The two- or three-way interactions, which were nonsignificant from this study for all the ITCs, are not reported here. Graphical presentations were prepared based on the mean values using Sigmaplot graphics software (*21*).

RESULTS

Effects of Growing Media on Yield of ITCs. The mean yield of individual and total ITCs from rhizomes grown in water and soil are presented in **Table 1**. No significant differences were found ($P = 0.127-0.435$ at LSD of 5%) between any of the mean ITC levels (for allyl, 3-butenyl, 4-pentenyl, and total ITCs) for the soil- and water-grown rhizomes. The major ITC was allyl ITC (941.3 and 1371.0 mg/kg obtained in soil- and water-grown rhizomes, respectively), and it contributed 90−93% of the total ITC. The levels of total ITC found in soil- and water-grown rhizomes were 1042.3 and 1474.6 mg/kg, respectively.

Interaction of Size of Rhizomes and Growing Media on Yield of ITCs. There were significant increases ($P = 0.030-0.048$) in all ITC yields with the increase in rhizome size (**Table 2**). For example, allyl ITC in the soil medium was 2.2, 4.2, 7.7, and 11.4 times higher in small medium, medium, large, and extra large rhizomes, respectively, than in very small rhizomes. Similarly, from the water medium, allyl ITC yield from the extra large size rhizomes showed a 9.2-fold increase in concentration compared to the very small rhizome. 3-Butenyl, 4-pentenyl, and total ITC yield also showed similar trends of increase with increasing rhizome size. However, 4-pentenyl ITC was the only ITC that was found in significantly higher levels (4 times) in the extra large rhizome from soil medium compared to the level found in water-grown extra large rhizomes.

The relationship between the total ITC content and size of rhizome is given in **Figure 1**. There was a different response for the two growing media. The soil-grown rhizomes showed a significant linear relationship ($P < 0.001$), whereas water-grown rhizomes showed a significant curved (inverse second order) relationship ($P = 0.009$). The total ITC concentration in the water-grown wasabi rhizomes was significantly higher in the 18−45 g of rhizomes (small medium to medium, at $P = 0.030$) than in the soil-grown rhizomes. No such difference was observed for 62 g and above rhizomes (extra large, large size) or in the very small sized rhizomes (13 g and below).

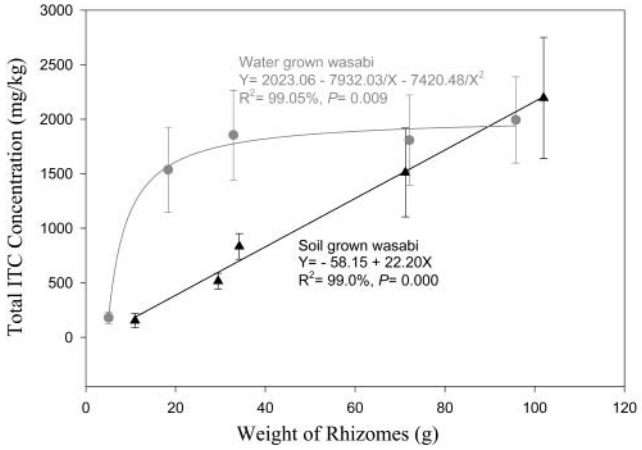

Figure 1. Effect of weight of the rhizomes on yield concentration of total ITC (mg/kg fresh weight basis). Error bars = mean total ITC concentration ± SE.

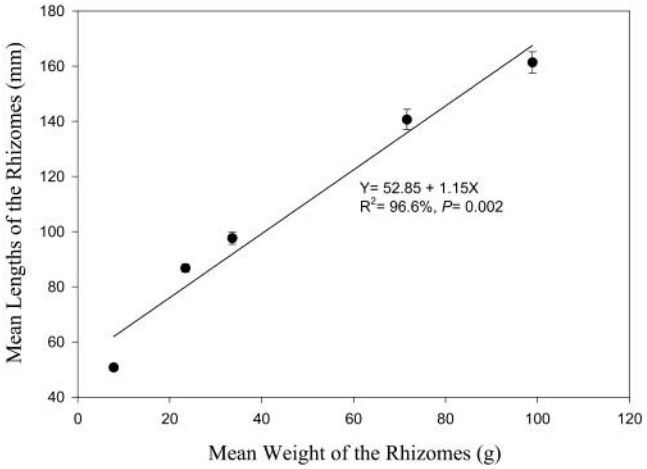

Figure 2. Relationship between mean weight and mean length of the rhizomes. Error bars = mean length ± SE.

Figure 2 showed that for both water- and soil-grown rhizomes a significant ($P = 0.002$) linear relationship exists between mean weights and mean lengths of the rhizomes indicating that either could be used to specify size-ITC yield relationships.

Effect of Rhizome Tissue Types on Yield of ITCs. There were significant differences in the yield of different ITCs from

Table 2. Yield of Isothiocyanates (mg/kg fresh weight basis) for Different Sized Rhizomes Grown in Either Soil or Water

		different sized rhizomes (mean from $N = 6$)					
isothiocyanates	growth medium	very small	small medium	medium	large	extra large	*P* value, 5% LSD
allyl ITC	soil grown	154.1c	492.8c	803.0bc	1347.4ab	1909.3a	0.034* 768.1
	water grown	179.2c	1476.7ab	1727.7a	1648.7a	1822.7a	
3-butenyl ITC	soil grown	–	14.5c	21.0c	38.6a	152.0a	0.048* 70.4
	water grown	–	48.7bc	110.1ab	135.4a	144.4a	
4-pentenyl ITC	soil grown	–	9.1b	9.8b	25.9b	133.8a	0.035* 80.0
	water grown	–	11.4b	17.1b	24.6b	26.5b	
total ITC	soil grown	154.1c	516.3c	833.9bc	1512.0ab	2195.1a	0.030* 847.3
	water grown	179.1c	1536.8ab	1854.9a	1808.7a	1993.6a	

[a] – = below detection limit, *= significant at $P < 0.05$, NS = not significant, N = no of replicates, values with a different letter are significantly different within rhizome size and growth media based on 5% LSD.

Table 3. Yield of Isothiocyanates (mg/kg fresh weight basis) from Different Tissues of the Rhizomes[a]

		tissue types		
isothiocynates	*N*	epidermis + cortex	vascular + pith	*P* and 5% LSD
allyl ITC	30	1437.1	875.2	0.001*** 103.9
3-butenyl ITC	24	149.5	41.8	0.001*** 18.9
4-pentenyl ITC	24	47.7	16.9	0.020* 23.9
total ITC	24	1594.8	922.1	0.001*** 112.6

[a] *N* = no of replicates. *** = significant at $P < 0.001$. * = significant at $P < 0.05$.

the two tissues of the rhizomes (**Table 3**, $P = 0.02$ to <0.001). The mean level of allyl ITC found in epidermis + cortex tissue was 1437.1 mg/kg, whereas in vascular + pith tissue the level observed was 875.2 mg/kg. This gave a 39% difference, which was significant at $P < 0.001$. The level of 3-butenyl ITC in epidermis + cortex tissue (149.5 mg/kg) was 2.6 times higher than the level in vascular + pith tissue (41.8 mg/kg). 4-Pentenyl ITC was 1.8 times higher in epidermis + cortex tissue than that found for vascular + pith (16.9 mg/kg). Total ITC yield was 922.1 mg/kg in vascular + pith tissue, which was 42% lower than the level found in epidermis + cortex tissue (1542.8 mg/kg).

Interaction of Rhizome Size and Tissue Type on Yield of ITCs. There was a relationship between rhizome size and tissue type for ITC yield (**Table 4**). For the very small rhizomes, no significant difference observed in allyl ITC levels between the two tissues, whereas significance differences found for the larger-sized rhizomes between the two tissues. Overall, there was 11-fold increase of allyl ITC yield in the epidermis + cortex tissue between very small (202.9 mg/kg) and extra large rhizomes (2455.9 mg/kg), but the increase was only 8.8 times in vascular + pith tissues (130.4 mg/kg, very small to extra large 1276.1 mg/kg). Similarly, total ITC increased 13 times from very small to extra large rhizomes in the epidermis + cortex tissues but only 9.5 times in the vascular + pith tissues. 3-Butenyl and 4-pentenyl ITC behaved slightly differently. For the epidermis + cortex tissue, there was a substantial increase (>5 times) in 3-butenyl and 4-pentenyl ITC yields with increasing rhizome size, whereas for the vascular + pith tissue the increase in ITC was 1−2 times, which was significantly less ($P = 0.001$).

Effect of Rhizome Portion on Yield of ITCs. Total and individual levels of all ITCs except 4-pentenyl ITC were significantly ($P \leq 0.001$) higher in the distal portion compared to the proximal portion of the rhizomes (**Table 5**). The mean allyl ITC level in the distal portion was 1636.4 mg/kg, which fell to 1230.1 and 602.1 mg/kg in the medial and proximal portions, respectively, and thereby showed a negative trend.

Interaction of Rhizome Portion and Tissue Types on Yield of ITCs. The two-way interaction between tissue types and portions indicated that the decrease in ITC levels along the length of the rhizome was greater in the epidermis + cortex tissues than in the vascular + pith tissues, although the epidermis + cortex yielded relatively higher ITC concentrations (**Table 6**). Allyl and total ITC decreased by 67 and 68.5%, respectively, in the epidermis + cortex tissue, whereas the reduction was only 57 and 56.4%, respectively, in the vascular + pith tissues from the distal to proximal portion of the rhizomes. 3-Butenyl ITC level decreased by 52% for the vascular + pith tissues but by 85% for the epidermis + cortex from proximal to distal, but the decreases for 4-pentenyl ITC were not significant.

Dry Matter Content and Relationship with Size of Rhizomes. In the soil-grown rhizomes, there was no significant relationship between regression of mean dry matter content in the epidermis + cortex tissue versus rhizome size ($P = 0.651$ by length and $P = 0.772$ by weight) (**Table 7**). In addition, the dry matter content in the vascular + pith tissue versus rhizome size ($P = 0.056$ by length and $P = 0.096$ by weight) was also not significant for soil-grown rhizomes. A comparison of the dry matter content in the two tissue types of soil growing medium showed significantly higher levels in the epidermis + cortex tissue for the extra large (6.3%) and large-sized rhizomes (3.5%) than in the vascular plus pith tissue. Other sizes of rhizomes followed this trend except for the very small sized rhizomes.

A significant linear regression of dry matter content in the epidermis + cortex tissues versus rhizome length ($P < 0.001$) and weight ($P = 0.005$) was found for water-grown rhizomes. As rhizome size (by weight or by length) decreased, there was a 17% decrease in mean dry matter % in the epidermis + cortex tissue. However, in the water-grown vascular + pith tissue no significant relationship was found ($P = 0.370$, 0.289). For water-grown rhizomes, the dry matter content in the epidermis + cortex tissue was also significantly higher for the extra large (3.1%) and large-sized rhizomes (1.1%) than for the vascular + pith tissues of the water-grown rhizomes.

No significant difference in dry matter content of epidermis + cortex tissue between two growing medium was noticed except for the very small sized rhizomes (last two rows in **Table 7**). Comparing vascular + pith tissues between two growing mediums, a marginally higher level was observed in the extra large rhizomes grown in soil.

DISCUSSION

Consistent with other reports (*4*, *12*, *22*) allyl ITC was the most abundant ITC identified in wasabi rhizomes. It contributed 89−100% of the total ITC found in this study. These data

Table 4. Interaction of Size and Tissues of Rhizomes on Yield of Isothiocyanates (mg/kg fresh weight basis)[a]

		different sized rhizomes (mean from N = 6)					
isothiocyanates	tissues	very small	small medium	medium	large	extra large	*P* value, 5% LSD
allyl ITC	epidermis + cortex	202.9 g	1086.5d	1463.7c	1976.8b	2455.9a	0.001*** 164.3
	vascular + pith	130.4 g	882.9f	1067.0e	1019.4e	1276.1d	
3-butenyl ITC	epidermis + cortex	–	34.7cd	97.9b	227.3a	237.8a	0.001*** 26.8
	vascular + pith	–	28.5d	33.2cd	46.7cd	58.7c	
4-pentenyl ITC	epidermis + cortex	–	11.1b	15.8b	33.6b	130.2a	0.035* 33.9
	vascular + pith	–	9.4b	11.0b	16.9b	30.1b	
total ITC	epidermis + cortex	202.9 g	1132.3e	1577.0c	2237.7b	2823.8a	0.001*** 178.1
	Vascular + pith	130.4 g	920.8f	1111.2ef	1083.0ef	1364.9d	

[a] N = no. of replicates, *** = significant at $P < 0.001$, * = significant at $P < 0.01$, – = not detected, values with a different letter are significantly different with in rhizome size and tissues based on 5% LSD.

Table 5. Yield of Isothiocyanate from Different Portions of the Rhizome (mg/kg fresh weight basis)[a]

isothiocyanates	N	different portions of the rhizome: proximal	medial	distal	P value 5% LSD
allyl ITC	20	602.1b	1230.1ab	1636.4a	0.001 *** 420.7
3-butenyl ITC	16	30.4b	117.0ab	139.0a	0.001 *** 43.1
4-pentenyl ITC	16	13.4	38.4	45.0	0.141 NS
total ITC	20	637.1b	1354.6a	1783.6a	0.001 *** 464.1

[a] NS = not significant. *** = significant at $P < 0.001$, N = no. of replicates, values with different letter are significantly different within rhizome segments based on 5% LSD.

Table 6. Yield of Isothiocyanates in Different Portions from Each Tissue of the Rhizomes (mg/kg fresh weight basis)[a]

isothiocyantes	N	tissues	mean yield of isothiocyantes in three portions: proximal	medial	distal	P value 5% LSD
allyl ITC	10	epidermis + cortex	687.8e	1541.5d	2082.2a	0.001*** 179.9
		vascular + pith	516.3e	918.7b	1190.5c	
3-butenyl ITC	8	epidermis + cortex	34.1b	191.9a	222.3a	0.001*** 32.8
		vascular + Pith	26.7b	42.7b	55.8b	
4-pentenyl ITC	8	epidermis + cortex	15.8	58.9	68.2	0.247 NS
		vascular + pith	11.0	17.7	21.9	
Total ITC	10	epidermis + cortex	727.8e	1742.2b	2314.5a	0.001*** 195.1
		vascular + pith	546.5e	967.1d	1252.7c	

[a] *** = significant at $P < 0.001$, NS = not significant, N = no. of replicates, values with a different letter are significantly different within rhizome sizes and tissues based on 5% LSD.

measured by GC−FPD showed very similar results to previous studies using GC-FID (*4*, *12*).

No significant differences were observed in the mean yields of total and individual ITCs from the rhizomes produced in two different growing media, soil and water. However, this overall response masked differences in the response of ITCs levels to rhizome size between the media. The water medium gave an initial rapid increase in ITC yield with increasing rhizome size (18−45 g), which leveled off (>62 g of rhizome) to give a significant asymptotic regression. The soil media produced a linear response in total ITC. However, the start and finish points were similar for both media. On the basis of this result, production of small- to medium-sized rhizomes (18−45 g) in a shorter time may be more efficient in a water medium than in a soil medium. The reasons behind these differences in response are not understood at this stage. However, the commercial importance of such differences in production (in terms of total ITC/ha/year) warrants further investigation. The greater availability of water to the water-grown plants may cause more rapid transportation of GSLs from the synthetic plant organs to the rhizomes. Larger rhizomes grown in either soil or water mediums contained higher levels of total ITC than smaller rhizomes. Overall, the data indicate that the size of rhizome should be a production parameter used to determine when to harvest and process a crop. However, when the rhizomes are utilized for processing the length of the rhizomes is unimportant (*23*). In the Japanese market, rhizomes grown in water are much higher priced than rhizomes grown in soil. It is reported that a water growing system can produce large, high quality stems though no supporting scientific data were found in English language publications (*2*).

ITC yield varied greatly between the inner and outer tissues of the rhizome. The outer layers of the rhizome, i.e., the epidermis + cortex tissue, gave significantly higher levels of all ITCs than the vascular + pith tissue. This was true across four different rhizome sizes and at the distal and medial sections of the rhizome. But no significant variation was found for very small sized rhizomes or for the proximal portion of the rhizomes. These data may suggest that the variation of yield of ITCs from precursor GSLs in tissues was related to the age and location, and maturity of the tissue. It is believed that the myrosinase−glucosinolate system is a defense system for plants (*13*), and since ITCs are anti-nutritional compounds the higher levels of ITCs in the outer tissues is consistent with protection from external attacks, which is activated at tissue disruption. In addition, the cortex tissue is more metabolically active than vascular tissue, which could be a reason for synthesis of precursor GSLs and consequently higher yield of ITCs in the outer layers. GSLs may be synthesized as plant secondary metabolites in different tissues in C*ruciferae*, but there is still no clear indication of where synthesis takes place in wasabi. Brudenell et al. reported that GSLs could be transported to where they are required by the plant (*16*). On the basis of this, it may be that cortex tissues are the storage place for the GSLs, but it is also possible that GSLs are synthesized in these tissues. Despite the variation in abundance of the precursor GSLs in different tissues, the presence of protein and amino acids may also be responsible and important in ITC yield from GSLs (*24*). It is suggested that ITCs interact irreversibly with sulfhydryl groups, disulfide bonds, and amines (*25*). All of these factors need to be investigated.

Table 7. Mean Length (mm ± SE), Weight (g ± SE), and Dry Matter (g/100 g WM ± SE) Content in Different Sized Rhizomes[a]

	sizes of the rhizomes: very small	small medium	medium	large	extra large
length (mm)	50.8 ± 1.2	86.9 ± 1.4	93.1 ± 2.2	140.4 ± 3.7	161.3 ± 3.9
weight (g)	8.0 ± 0.7	23.9 ± 1.9	33.6 ± 1.0	71.6 ± 3.3	98.9 ± 4.2
soil grown rhizomes (DM%)					
(I) epidermis + cortex tissue	30.1 ± 0.5	28.4 ± 1.2	24.8 ± 1.4	28.1 ± 0.7	27.8 ± 0.7
(II) vascular + pith tissue	32.3 ± 1.3	26.4 ± 0.4	23.8 ± 0.2	24.6 ± 0.7	21.5 ± 0.9
comparison of I versus II (*P* value)	0.193	0.197	0.494	0.022	0.006
water grown rhizomes (DM%)					
(I) epidermis + cortex tissue	23.6 ± 1.7	25.2 ± 1.1	26.0 ± 2.8	27.2 ± 0.2	28.5 ± 0.3
(II) vascular + pith tissue	24.8 ± 4.7	22.5 ± 1.4	25.1 ± 2.5	26.1 ± 0.2	25.4 ± 0.4
comparison of (I) versus (II) (*P* value)	0.626	0.196	0.820	0.021	0.002
P values for comparison					
soil (DM%) (I) versus water (DM%) (I)	0.022	0.124	0.724	0.292	0.439
soil (DM%) (II) versus water (DM%) (II)	0.309	0.058	0.581	0.102	0.018

[a] SE = standard error of mean, DM = dry matter content, WM = wet matter content.

The variation of ITCs in three portions of the rhizomes (shown in **Tables 4** and **5**) indicates a relation between ITC yield and maturity of the rhizomes may exist. The proximal portion of the rhizome gave significantly lower ITC yield than medial and distal portions of the rhizomes. It may be that the distal tissues of the rhizome are comparatively older than the proximal tissues. It is also possible that the older portion of the rhizome has been exposed to more frequent attacks than the upper portion of the rhizomes. Further work is required to determine whether these differences in ITC levels resulted from synthesis in situ or by accumulation in a particular tissue.

Data from this study will allow growers and processors to understand the factors (growth medium, size, portion, and tissues of the rhizomes), which affect the ITCs levels in the rhizomes and the location within the rhizomes. These data will allow the efficient manufacture of quality ITC-flavored food products.

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LITERATURE CITED

(1) Hodge, W. H. Wasabi, native condiment plant of Japan. *Econ. Bot.* **1974**, *28*, 118−129.

(2) Chadwick, C. I.; Lumpkin, T. A.; Elberson, L. R. The botany, uses, and production of *Wasabia japonica* (Miq) (Cruciferae) Matsum. *Econ. Bot*. **1993**, *47* (2), 113−135.

(3) Douglas, J. A.; Follett, J. M. Initial research on the production of water-grown wasabi in the Waikato. *Proc. Agron. Soc*. **1992**, *22*, 57−60.

(4) Sultana, T.; Savage, G. P.; McNeil, D. L.; Porter, N. G.; Martin, R. J.; Deo, B. Effects of fertilisation on the allyl isothiocyanate profile of the above-ground tissues in New Zealand grown wasabi. *J. Sci. Food Agric.* **2002**, *82*, 1477−1482.

(5) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5−51.

(6) Delaquis, P. J.; Mazza, G. Antimicrobial properties of isothicynates in Food Preservation. *Food Technol*. **1995**, 73−84.

(7) Höglund, A. S.; Lenman, M.; Falk, A.; Rask, L. Distribution of myrosinase in rapeseed tissues. *Plant Physiol.* **1991**, *95*, 213−221.

(8) Grob, K.; Matile, P. H. Vacuolar location of glucosinolates in horseradish root cells. *Plant Sci. Lett.* **1979**, *14*, 327−335.

(9) Bones, A. M.; Rossiter, T. J. The myrosinase-glucosinolate sysytem, its organization and biochemistry. *Physiol. Plant*. **1996**, *97*, 194−208.

(10) Ettlinger, M. G.; Kjaer, A. Sulfur compounds in plants. *Rec. Adv. Phytochem.* **1968**, *1*, 59−144.

(11) Fenwick, R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *CRC Crit Rev. Food Sci. Nutr*. **1983**, *18*, 123−201.

(12) Sultana, T.; Savage, G. P.; McNeil, D. L.; Porter, N. G.; Martin, R. J. Flavour compounds in the rhizome of soil-grown Wasabi. *Proc. Nutr. Soc. New Zealand* **2000**, *25*, 95−106.

(13) Koroleva, O. A.; Davies, A.; Deeken, R.; Thorpe, M. R.; Tomos, A. D.; Hedrich, R. Identification of a new glucosinolate-rich cell type in Arabidopsis flower stalk. *Plant Physiol.* **2000**, *124*, 599−608.

(14) Taiz, L.; Zeiger, E. Plant and cell architecture in Plant physiology, Brady, E. B.. Eds.; The Benjamin/Cummings Publishing Company, Inc.: Redwood City, California, 1991; Chapter 1, 3−13.

(15) Lykkesfeldt, J.; MØller, B. L. Synthesis of benzylglucosinolate in Tropaeolum majus L.: isothiocyanate as potent enzyme inhibitors. *Plant Physiol.* **1993**, *102*, 609−613.

(16) Brudenell, A. J. P.; Griffiths, H.; Rossiter, J. T.; Baker, D, A. The phloem mobility of glucosinolates. *J. Exp. Bot*. **1999**, *50*, 745−756.

(17) Merritt, S. Z. Within-plant variation in concentrations of amino acids, sugar, and sinigrin in phloem sap of black mustard, Brassica nigra L. Koch. *J. Chem. Ecol*. **1996**, *22*, 1133−1145.

(18) Du, L.; Halkier, B. Biosynthesis of glucosinolates in the developing silique walls and seeds of Sinapis alba. *Phytochemistry* **1998**, *48*, 1145−1150.

(19) AOAC, Official Methods of Analysis of AOAC International, 17th Edition, AOAC International, Gaitherburg, MD, 2000.

(20) Minitab, Minitab release 13.1, Minitab Inc., State College, PA, 2000.

(21) Sigmaplot, Sigmaplot version 5.0, SPSS Inc, Canal Blvd. Suite F, Richmond, USA. 1999.

(22) Sultana, T.; Savage, G. P.; McNeil, D. L.; Porter, N.; Clark, B. Comparison of flavour compounds in wasabi and horseradish. *Int. J. Food Sci. Nutr*. 2002, submitted.

(23) Palmer, J. Germination and growth of wasabi (Wasabia japonica (Miq.) Matsumara). *New Zealand J. Crop Hortic. Sci.* **1990**, *18*, 161−164.

(24) Warton, B.; Matthiessen, J. N.; Shackleton, M. A. Glucosinolate content and isothiocyanate evolution- two measures of the biofumigation potential of plants. *J. Agric. Food Chem.* **2001**, *49*, 5244−5250.

(25) Brown, P. D.; Morra, M. J. Control of soil-borne plant pests using glucosinolate-containing plants. *Adv. Agron.* **1997**, *61*, 167−231.

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