The yield of isothiocyanates in wasabi rhizomes stored at different temperatures

Article *in* Journal of Food Agriculture and Environment · August 2003

CITATIONS
10

READS
345

4 authors, including:

Tamanna Sultana
Trent University
43 PUBLICATIONS **1,177** CITATIONS
SEE PROFILE

Geoffrey Peter Savage
Lincoln University
313 PUBLICATIONS **9,740** CITATIONS
SEE PROFILE

David Mcneil
Government of Western Australia
167 PUBLICATIONS **6,084** CITATIONS
SEE PROFILE

WFL **Publisher**
Science and Technology
Food, Agriculture & Environment Vol.1(3&4) : 39-45. 2003
www.world-food.net

The yield of isothiocyanates in wasabi rhizomes stored at different temperatures

Tamanna Sultana[1]*, Geoffrey P. Savage[1], David L. McNeil[2] and Noel G. Porter[3]

*[1]Food Group, Animal and Food Sciences Division, PO Box 84, Lincoln University, Canterbury, New Zealand. [2]DNRE, Victoria Institute of Dryland Agriculture, Melbourne University, Horsham, Victoria, Australia. [3]New Zealand Institute for Crop & Food Research Ltd, Private Bag 4707, Christchurch, New Zealand. *email: sultanat@lincoln.ac.nz*

Received 20 February 2003, accepted 30 September 2003.

Abstract

Wasabi rhizomes are commonly kept for varying lengths of time in cold storage as harvesting occurs at set times of the year and manufacturing into products, for instance, mayonnaise and sauces, occurs throughout the year. Six isothiocynates (ITCs) (*iso*propyl ITC, *sec*-butyl ITC, allyl ITC, 3-butenyl ITC, 4-pentenyl ITC and 5-hexenyl ITC) were measured in the fresh and stored rhizomes; allyl ITC was the most abundant one, comprising 94% of the total ITC concentration. The total ITC content in the fresh rhizome was 1857.8 was mg kg^{-1}. The yields of individual and total ITC remained stable when rhizomes were stored at -10, -20 and -80°C over a period of 8 weeks, except for 3-butenyl ITC which showed a significant fall after 3 weeks storage. The mean yield of total ITC after 8 weeks of storage of rhizomes at ≤-10°C was 1859.7 mg kg^{-1}. There was no significant advantage to rapidly freezing the rhizomes with liquid nitrogen when compared to slower conventional freezing techniques. Defrosting and then refreezing the rhizomes at -15°C led to very significant losses of allyl and total ITC. Defrosting led to a 99.7% loss of allyl ITC when previously frozen rhizomes were stored at 4°C for 16 days. These data will considerably assist the efficient storage of this valuable material.

Key words: *Wasabia japonica*, storage temperature, storage time, freezing techniques, allyl isothiocyanate, *iso*propyl isothiocyanate, *sec*-butyl isothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, 5-hexenyl isothiocyanate, total isothiocyanate.

Introduction

Wasabi (*Wasabia japonica* (Miq.) Matsum), known as Japanese horseradish, is a perennial herb mainly used for flavouring foods. This crop is cultivated primarily for its rhizomes, which is used to prepare high-quality wasabi paste [1]. Due to its characteristic flavour, sharp hot taste and pungent-green smell [2], wasabi is a popular condiment in Japanese cuisine and more recently developing as a new flavour in western cuisine[3]. The unique flavour of wasabi comes from volatile isothiocyanates (ITCs) which are evolved from precursor glucosinolates [4, 5, 6] by enzyme myrosinase when plant tissues are mechanically disrupted or injured by any means. Recently, considerable interest has focused on ITC study due to the variety of medicinal properties of ITCs e.g. chemo-prevention, inhibition of platelet aggregation, anti-asthmatic and anti-inflammatory and antibiotic properties [7, 8, 9, 10, 11, 12, 13, 14]. After harvest, freshly cut wasabi plant parts need to be stored for varying lengths of time before being used in two major ways- processed into food products like flavoured mayonnaise or sauces or sold as fresh or frozen raw material (packed or loose) for preparation into a fresh paste before each meal. Sultana et al.[15] investigated six ITCs (*iso*propyl ITC, *sec*-butyl ITC, allyl ITC, 3-butenyl ITC, 4-pentenyl ITC and 5-hexenyl ITC) in all the plant parts of wasabi and among them rhizomes yielded highest levels of all ITCs. Thus, wasabi rhizomes are the most useful part of the plant for extracting flavour compounds. Allyl ITC was the most abundant ITC, contributed 89-94% of the total ITC in all pant parts [5, 15]. Sultana et al.[3] found that New Zealand soil-grown wasabi rhizomes contained between 1564 and 3366 mg of allyl ITC/kg and can be affected by different soil fertilisations. Since ITCs are volatile and chemically labile, the stability of these flavour components in storage can be influenced by a range of factors, e.g. temperature, storage conditions and time. A 60% loss of ITC concentration was reported by heating crambe (*Crambe abyssinica*) at between 100–110°C for 30-60 minutes[16]. Sekiyama et al. [17] recommended that processing wasabi at high temperature between 170 and 180°C was not feasible due to the decomposition of the main flavour component, allyl ITC. It was also reported that, ITCs disappeared following autoclave cooking of cabbage root samples prior to extraction [18]. From a storage study of wasabi powder in airtight containers Kojima and Nakano [19] noticed that ITC content decreased with increased moisture content. The hydrolysates showed a reduced total ITC content when stored at 5 to 30°C but were stable in cold storage (-15C). About half of the concentration of a variety of ITCs (*sec*-butyl, n-butyl, allyl and 3-butenyl ITC) was lost during four weeks storage of wasabi powder in sealed vessels at 30°C [20]. However, *iso*propyl ITC level decreased more rapidly than all the other ITCs; with its concentration reduced by 50% in one week. Tseng et al.[21] suggested that hot air drying at 45°C (3363 mg allyl ITC kg^{-1} dry wt) was better than freeze drying (3030 mg kg^{-1} dry wt) and sun drying (357 mg kg^{-1} dry wt) for preparing wasabi powder. Depree and Savage [22] reported that the level of total ITC fell 15% and 25% in wasabi mayonnaise when stored at 4 to 20°C and 30°C, respectively. They also stated that in mayonnaise ITC appeared to decrease with time but when dissolved in oil 99% of the original level of ITC was observed after storage for 57 days at 4°C, which indicated that the decrease was not significant during long-term storage. Shasrabudhe and Mullin [23] recommended that processing horseradish roots to a dried product must be carried out on cleaned roots immediately after dicing or crushing, and at a temperature below 65°C, to retain the viability of myrosinase enzyme and to yield a product with the desired flavour intensity and acceptable odour. As a consequence, cool temperature is important during processing wasabi otherwise ITC loss could be related with the time of processing, especially at higher temperatures. However,

no scientific investigation has ever been reported on potential loss of ITCs in raw material (rhizomes) when stored at cool or cold temperatures for either short or longer times. Therefore, a storage experiment was set up with a primary objective of investigating the yield of individual ITCs in wasabi rhizomes under a range of conditions. The variables imposed and considered during storage included different storage times (up to eight weeks), different temperatures (4, -4, -10, -20 and -80ºC), two different freezing methods, (slow conventional freezing and fast initial freezing) and defrosting effects.

Materials and Methods

Collection of samples: Freshly harvested wasabi rhizomes (3 kg) of cultivar 'Daruma' grown at Stillwater on the West Coast (42^0 25ˊS, 171^0 20ˊE, 23 m above sea level) in the South Island, New Zealand were used in this study. Plants were grown in normal soil without manure or any fertiliser application and the mature plants were harvested at 18 months of age in May 2001. Leaves, petioles and roots were removed from the rhizomes and cleaned by water spray.

Treatments applied: 20 to 70 g rhizomes were randomly selected for the two treatments.

1) For slow freezing no pre-storage treatment was applied.
2) For fast initial freezing the rhizomes were cut into 20 g pieces and then quick frozen in liquid nitrogen. The samples were then put into previously labelled snaplock^R plastic bags and placed in different temperatures.

Experimental design for storage: The whole experiment was divided into three parts.

1) Control: The fresh rhizomes were analysed on the day of harvest (0 day of storage).
2) Cool storage: A refrigerator was set to 4ºC and used to store both treated and untreated samples for 6 weeks. Extractions were carried out on days 2, 5, 7, 10, 16, 28, 35 and 42.
3) Frozen storage: Five freezers were set to -4, -10, -15, -20 and -80ºC and used to store both fast frozen and untreated samples for 6 (at -4ºC) and 8 weeks (for the other temperatures).

Extractions were carried out weekly for all the temperatures but for -4ºC additional extractions took place on days 2 and 7. The samples stored in the -15ºC freezer were used for the defrosting experiment.

Extraction: Wasabi rhizome (50 g) was homogenised into a Braun kitchen blender (MR 430 CA, Braun, Spain). Four g of ground sample was weighed into a 40 ml Beckman plastic centrifuge tube (Beckman, Palo Alto, CA, USA) containing 7 ml of distilled water and 5 ml of dichloromethane (Hipersolv grade, BDH Laboratory Supplies, Poole UK) was added into it. The samples were mixed in a Hybaid oven (MK II, Hybaid Ltd, Ashford, UK) at 20ºC for 2 hours and then separated from the paste and water phase by centrifugation (121,000 g, 20°C for 5 minutes) in a Beckman J2 M1 centrifuge. The dichloromethane extract was stored at -20ºC prior to GC analysis of ITCs. The moisture contents were determined in accordance with AOAC methods [24].

Gas chromatography analysis: Samples (2 µl) of dichloromethane extract were injected (splitless mode) onto a Hewlett-Packard (HP) INNOwax capillary column (30 m, 0.25 mm, i.d. and 0.25 ìm film thickness) in a HP 6890 gas chromatograph fitted with a flame ionisation detector (FID), and a HP 6890 automatic sampler (Hewlett Packard, Palo Alto, CA, USA). The inlet and detector temperatures were 160°C and 250°C, respectively. Hydrogen was used as a carrier gas at an inlet pressure of 85 kPa, flow rate of 2.3 ml min^{-1}. Separations were performed under the following temperature program: 50 to 100°C at 5°C min^{-1}, 100 to 200°C at 10°C min^{-1}, then held at 200°C for 2 minutes. Peaks areas were recorded and calculated using HP Chemstation software (Version A.06.03). N-butyl ITC (Eastman Organic Chemical, Rochester, NY, USA) and phenyl ITC (Merck, Darmstadt, Germany) were used as external standards and demonstrated significant differences in FID response to ITCs of different carbon to sulphur ratios. N-butyl ITC calibration curve was used to quantify the levels of each ITC and expressed as mg kg^{-1} fresh weight of wasabi rhizomes in each Table.

Gas chromatography-mass spectroscopy (GCMS): Allyl ITC, 3-butenyl ITC, 4-pentenyl ITC, 5-hexenyl ITC, isopropyl ITC and sec-butyl ITC were identified by GCMS on a Carlo Erba MFC500 gas chromatograph (split ratio 30:1, HP DB5MS 30 m, 0.25 mm i.d., 0.25 µm film column, He carrier gas flow 2 ml min^{-1}; Carlo Erba, Milan, Italy) and a Kratos MS80RFA mass spectrometer (4 kV accelerating potential, 70 eV ionization energy, source temperature 250°C, magnet scan 500-30 AMU; Kratos, Manchester, UK). The identity of individual ITC was confirmed by comparing spectra with published spectra from Kjœr et al. [25] and Wiley's database (using Mach 3 database system).

Statistical analysis: The effects of storage temperatures, treatments, time and their interactions on the yield of individual ITCs from wasabi rhizomes were analysed with factorial analysis of variance (ANOVA) and least significant difference of means (LSD at 5%)

Table 1. Comparison of the isothiocyanate yield from fresh rhizomes and frozen rhizomes given different initial freezing treatments and then stored for up to 8 weeks at ≤ -10ºC.

Isothiocyante	Isothiocyanate yield (mg $kg^{-1}$of rhizome)			
		Treatments		
	Fresh (N = 3)	No initial treatment-Slow freezing (N=72)	Fast initial freezing liquid nitrogen (N=72)	*P* value (DF = 2) LSD for min-max replication
Isopropyl ITC	14.0	14.2	13.7	0.980, NS, 11.7
Sec-butyl ITC	21.3	21.1	21.3	0.997, NS, 19.9
Allyl ITC	1741.7	1763.4	1724.4	0.935, NS, 797.6
3-butenyl ITC	37.6	37.9	37.3	0.964, NS, 16.9
4-pentenyl ITC	34.1	36.8	29.6	0.185, NS, 28.2
5-hexenyl ITC	5.1	5.2	4.9	0.893, NS, 3.6
Total ITC	1857.8	1881.4	1833.9	0.918, NS, 862.1

NS= not significantly different at LSD of 5% level.

Table 2. The effect of low temperature storage on the yield of isothiocyanates from wasabi rhizomes between 1and 8 weeks.

Isothiocyanate	Yield of isothiocyanate (mg kg^{-1} of rhizome) at different freezing temperatures (ºC) (N = 48)			*P* value DF = 2
	-10	-20	-80	
Isopropyl ITC	12.9	13.3	15.9	NS
Sec-butyl ITC	21.7	19.9	22.0	NS
Allyl ITC	1698.7	1837.3	1695.6	NS
3-butenyl ITC	36.9	39.7	36.4	NS
4-pentenyl ITC	31.8	34.9	32.4	NS
5-hexenyl ITC	5.0	5.5	4.7	NS
Total ITC	1811.9	1955.3	1811.9	NS

NS= not significant different at LSD of 5% level.

using rhizomes samples as blocks with the Genstat statistical package [26]. The total ITC yield was calculated from the sum of individual ITCs. Three replications were used for extraction of each sample. Significant and non-significant responses are presented in Tables in association with *P* values to give an indication of trends. Graphical presentations were prepared for the mean values from the three extraction replications using Sigmaplot graphics software [27].

Results

The yield of total ITC and individual ITCs from the freshly harvested rhizomes used in this study are shown in Table 1. Six ITCs (*iso*propyl ITC, *sec*-butyl ITC, allyl ITC, 3-butenyl ITC, 4-pentenyl ITC and 5-hexenyl ITC) have been investigated in rhizomes, among them allyl ITC composed of 94% of the total ITC concentration.

Storage at low temperatures (-10, -20 and -80ºC): The effects of three different temperatures (-10, -20 and -80ºC) on ITCs yield from the stored frozen rhizome are presented in Table 2. The mean *iso*propyl ITC yields observed at -10, -20 and -80ºC storage were 12.9, 13.3 and 15.9 mg kg^{-1} respectively and were not significantly different from each other (*P* = 0.294). Similarly *sec*-butyl, allyl, 3-butenyl, 4-pentenyl and 5-hexenyl ITCs also gave no significant differences of mean yields after eight weeks storage at -10, -20 and -80ºC. The total ITC yields measured in rhizomes stored at -10, -20 and -80ºC were 1811.9, 1955.3 and 1811.9 mg kg^{-1} of rhizome respectively.

Table 1 also showed the effect of slow and fast freezing treatments on ITC yield from rhizomes when stored at ≤ -10ºC relative to the unfrozen fresh rhizomes. The freezing treatments did not have any significant effect on any ITCs (*P* = 0.185 to 0.997), nor on total ITC yield measured (*P* = 0.918) in the rhizomes.

The effect of time on individual and total ITC yields from rhizomes stored at ≤ 10ºC and colder temperatures are presented in Table 3. No ITC (*iso*propyl, *sec*-butyl, allyl, 4-pentenyl and 5-hexenyl ITCs) other than 3-butenyl ITC showed any significant variation of yield with storage time of up to 8 weeks from the ANOVA analysis (*P* = 0.101 to 0.694). These ITCs also showed no significant yield responses when analysed using linear regression analysis. However, 3-butenyl ITC behaved differently with a significant difference of *P*< 0.05. At week 1 and 2 the yields of 3-butenyl ITC found were 47.7 and 46.8 mg kg^{-1}, which was reduced by 27% on average from week 3 to 8. However, the total ITC yield did not show significant losses (*P* = 0.117) with changes in storage times. No significant interactions of temperature (-10, -20 and -80ºC) versus week (1-8); temperature versus freezing treatment (slow freeze and initially fast frozen) and freezing treatments versus week (1-8) were found for any ITCs from stored rhizomes at ≤ -10ºC.

Comparison of --4ºC with storage at low temperatures (-10, -20 and -80ºC): All the ITCs showed no significant loss with time (*P*= 0.121-0.735) when stored at -4ºC for 6 weeks except *iso*propyl ITC. *Iso*propyl ITC reduced by 43.5% (*P* = 0.009) comparing the mean levels from week 1 and 2 (16.2 and 15.9 mg kg^{-1} of rhizomes, respectively) with week 3 to 6. There was no significant effect of the two freezing treatments (slow freeze and fast initially frozen) and freezing treatments versus time interaction on individual and total ITC yields when stored at --4ºC for 6 weeks.

Comparison of ITC yields between rhizomes stored just below freezing (-4ºC) and those stored well below freezing (-10, -20 and -80ºC) for 6 weeks are presented in Table 4. The mean allyl ITC yield at -4ºC was 1297.5 mg kg^{-1}, which was significantly (*P* = 0.002) lower than the yield obtained from storage at -10ºC and lower temperatures (1759.8 mg kg^{-1}). Similarly, 3-butenyl ITC and 5-hexenyl ITC also gave significant differences when the -4ºC storage (27.8, 3.3 mg kg^{-1} respectively) was compared to the samples stored at ≤ -10ºC (38.4, 4.7 mg kg^{-1} respectively). However, *iso*propyl, *sec*-butyl and 4-pentenyl ITC were not significantly affected but tended to give lower yields at -4ºC than ≤ -10ºC.

Table 3. The effect of time of storage on the isothiocyanate yield of stored wasabi rhizomes at ≤ -10ºC.

Isothiocyanate	Mean value of ITC yield (mg kg^{-1}of rhizome) in each week (N=18)								P value, DF = 7
	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8	
Isopropyl ITC	19.3	15.8	15.0	11.1	11.5	14.8	10.5	14.2	0.192, NS
Sec-butyl ITC	27.3	22.0	21.7	17.9	17.9	19.2	20.2	23.7	0.694, NS
Allyl ITC	2102.9	2073.8	1686.9	1596.9	1505.6	1592.7	1808.2	1584.2	0.101, NS
3-butenyl ITC	47.7a	46.8a	37.3b	32.7b	32.4b	33.6b	37.4b	33.3b	0.020, *
4-pentenyl ITC	34.1	25.9	37.9	30.9	31.5	27.4	39.5	38.4	0.530, NS
5-hexenyl ITC	4.8	5.9	4.7	4.1	4.1	4.7	6.2	6.1	0.244, NS
Total ITC	2242.0	2194.6	1807.9	1697.8	1607.4	1696.8	1926.7	1705.1	0.117, NS

NS= not significantly different; *= Significantly different at P<0.05, Values with different letters are significantly different in storage weeks, based on 5% LSD.

Table 4. Comparison of the isothiocyanate yields from rhizomes stored for 6 weeks at -4ºC with the mean from storage at colder temperatures (-10, -20 and -80ºC).

Isothiocyanate	Mean isothiocyanate yield (mg kg^{-1} of fresh rhizome)		P value
	Storage at - 4ºC N= 36	Storage at -10, -20, -80ºC N=108	
Iso-propyl ITC	11.9	14.6	0.166, NS
Sec-butyl ITC	15.1	20.9	0.054, NS
Allyl ITC	1297.5	1759.8	0.002, **
3-Butenyl ITC	27.8	38.4	0.001,***
4-Pentenyl ITC	22.5	31.1	0.072, NS
5-Hexenyl ITC	3.3	4.7	0.031, *
Total ITC	1379.6	1874.3	0.002, **

NS= Not significant; *= Significantly different at P<0.05; **= Significantly different at P<0.01;
***= Significantly different at P<0.001

Cool storage at 4ºC: Trends for individual and total ITC yields during refrigerated storage at 4ºC are presented in Figure 1. Allyl ITC and total ITC showed significant (P = 0.041, P = 0.039 respectively) reductions in yields with storage time. The loss of AITC yield ranged from 12 to 24% with 4 weeks of storage and increased to 33-59% loss in the following two weeks. Similarly, total ITC level fell from 1360.1 mg kg^{-1} at day 0 to 1032.2 mg kg^{-1} after 2 weeks storage at 4ºC. The overall mean loss of total ITC was 46% after weeks 5 and 6. However, *sec*-butyl, 3-butenyl and 4-pentenyl ITC did not show significant reductions. The moisture content remained steady at between 82 and 89% during cool storage and the regression trend on the moisture content of the stored rhizomes was not significant.

1) Effect of two days defrosting on the frozen rhizomes: The samples stored at -15ºC were allowed to defrost over a 2-day period and then refrozen to determine the effect on ITC yield, as intermittent defrosting may be a problem during poor storage conditions. The -15ºC data was then grouped as before (week 1and 2), and after (week 3 to 8) defrosting and presented in Table 5. *Iso*propyl and *sec*-butyl ITCs could not be detected after defrosting while allyl, 3-butenyl and total ITC were significantly reduced to half of their pre-defrosting yields (51.6%, 54.3% and 50.6% reduction respectively). For 4-pentenyl and 5-hexenyl ITCs the reductions in yields were 37% and 28% respectively.

2) Effect of 16 days defrosting of frozen samples: Samples that were rapidly frozen by liquid nitrogen (initial freezing treatment) and stored in a refrigerator at 4ºC were defrosted and ITC yields were measured with time. The effect of this defrosting for up 16 days on allyl ITC yield is presented in Figure 2. On the first day the allyl ITC found in fresh samples was 1636.15 mg kg^{-1}. After quick freezing, defrosting and cool storage of the samples, the allyl ITC yield had fallen to 536.97 mg kg^{-1} after 2 days of storage. On the 5th day of cool storage the yield continued to fall to 70.81 mg kg^{-1}, which was a 95.7% loss compared to the original value. All the other ITCs could not be detected from the 5th day onwards and they are thus not included in the Figure 2. Further reductions continued after 5 days of defrosting, and the levels observed were 62.21, 22.77 and 4.64 mg kg^{-1} of rhizome after 7, 10 and 16 days of storage respectively. The loss was 99.7% on day 16 and after that the rhizomes were totally spoiled. The decay of allyl ITC with defrosting followed an exponential decay curve.

Discussion

The yields of individual and total ITCs reported in the present study are comparable with the previously published values [5, 3, 15] for New Zealand grown wasabi. This study was also in agreement with

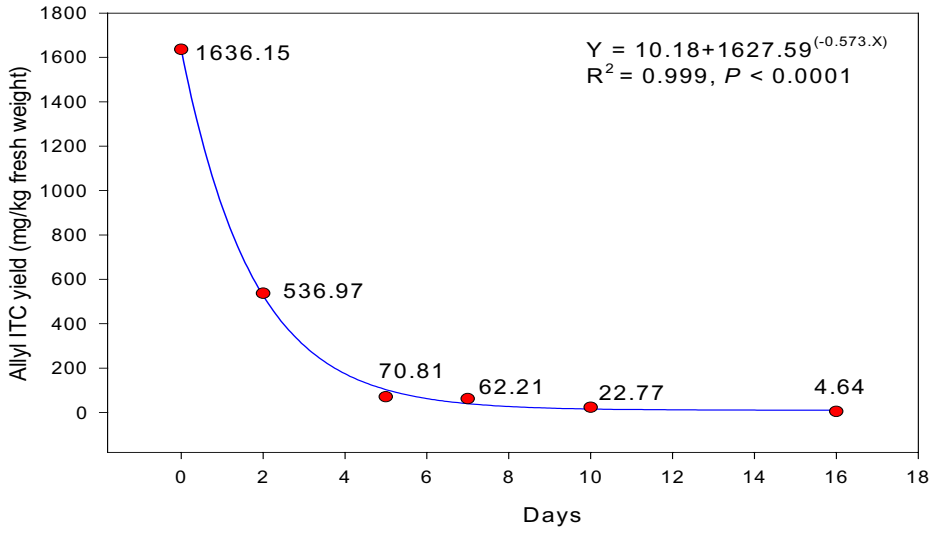

Figure 2. The effect of defrosting over 16 days on the allyl isothicyanate yield of previously frozen rhizomes stored at a cool temperature (4ºC).

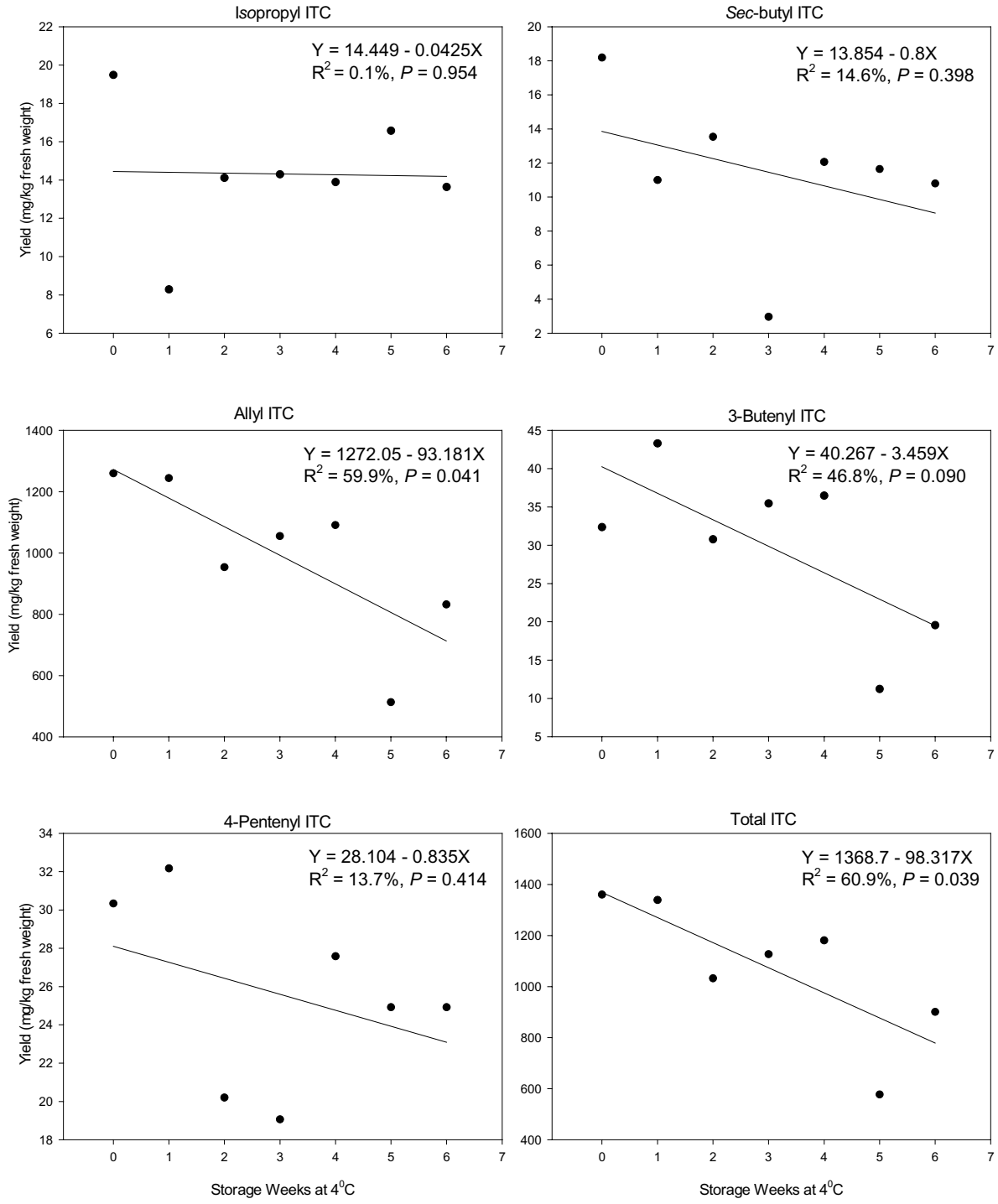

Figure 1. Individual isothiocyanate decay pattern with storage time at 4ºC.

Table 5. The effect of a brief period of defrosting from -15ºC on the yields of individual isothiocyanates from wasabi rhizomes.

Isothiocyanate	Yield of isothiocyanates (mg kg^{-1} of rhizome)		Probabitily (P)
	Before defrosting (Week 1 and 2) N=12	After defrostingand refreezing. (Week 3 to 8) N=36	DF = 1
Isopropyl ITC	10.5	-	-
Sec-butyl ITC	13.7	-	-
Allyl ITC	1718.3	831.5	0.002, **
3-butenyl ITC	38.7	17.7	<0.001, ***
4-Pentenyl ITC	22.7	14.3	0.055, NS
5-Hexenyl ITC	3.9	2.8	0.066, NS
Total ITC	1807.5	863.3	0.002, **

-= Not detected, NS= not significant; *= Significantly different at P<0.05; **= Significantly different at P<0.01;
***= Significantly different at P<0.001

previously reported observations [2, 5, 28] that, allyl ITC is the most abundant ITC and, therefore, a primary contributor to the total ITC concentration. Compared to the mean published value of total ITC yield of wasabi grown in Japan (1659 mg kg^{-1}, summarised in Sultana et al. [5]), this study showed that the total ITC yield in fresh rhizomes (1857.8 mg kg^{-1}) from New Zealand grown wasabi are acceptable in quality. When the individual ITC and total ITC yields in the frozen rhizomes were compared between the storage temperatures (-10, -20 and -80ºC), between the storage times (up to 8 weeks) and between the freezing treatments (initially liquid nitrogen treated fast frozen, untreated slow freeze samples) no significant differences were observed either for storage temperatures, storage weeks or treatments. Exceptionally, 3-butenyl ITC showed a negative trend with storage time when stored at ≤ -10ºC. The use of liquid nitrogen to snap freeze the fresh rhizomes is an expensive treatment and it appeared to give no additional advantages over untreated conventional freezing techniques because, no significant differences of ITC yields were also noticed by comparing freshly harvested rhizomes with liquid nitrogen fast frozen or slow frozen rhizomes. Therefore, it is an economical option to avoid the fast freezing treatment and to store the raw rhizomes at ≤ -10ºC to retain the original levels of ITCs. Again, no significant interactions on ITCs yields were observed from any two of the three variables (temperature, storage time and treatment) which obviously supported that overall ITCs were stable when stored at ≤ - 10ºC for up to eight weeks onwards. This statement agrees with the report of Kojima and Nakano [19] who found ITCs were remained stable on storage of wasabi powder at -15ºC.

The difference in levels noticed for the main component allyl ITC and other minor ITCs (3-butenyl ITC, 5-hexenyl ITC) by comparing -4ºC storage with ≤ -10ºC temperatures indicating a point that, there may be a difference in time requirement to completely freeze the samples at -4ºC and ≤-10ºC that needs to be investigated further. Samples stored at -4ºC might have taken a longer time to freeze than the samples stored at ≤ -10ºC. This was suggested because a (non-significant) difference on ITC yields observed between the mean values for initially fast frozen samples relative to the untreated rhizomes when stored at -4ºC. The trend showed 15, 30, 26, 39 and 35% reduction of *sec*-butyl, allyl, 3-butenyl, 4-pentenyl and 5-hexenyl ITC yields respectively in fast frozen samples relative to slow freeze samples when stored at -4ºC for 6 weeks. A 41% reduction of *iso*propyl ITC was noticed at week 6 compared to week 1 when stored at -4ºC. Specifically this indicates that *iso*propyl ITC behaves differently from other ITCs in wasabi during storage just below the freezing temperature (-4ºC) might be because of the highly volatile nature of *iso*propyl ITC, which has a lower boiling point 29-30ºC/10 mm compared to other ITCs. This statement was supported by the observation of Kojima et al.[20] that *iso*propyl ITC behaved differently when wasabi powder was stored at 20 to 30ºC in a sealed vessel. They reported that while other ITCs were reduced by 50% after storage of 4 weeks *iso*propyl ITC was reduced by 50% of its initial concentration after one week of storage. Defrosting is highlighted as an important factor from this study for unwanted ITC loss from frozen rhizomes. When the rhizomes were defrosted the myrosinase began to break down the glucosinolates and as the isothiocyanates are volatile they were then lost from the tissue. However, the loss of ITC content was affected by the time of defrosting. The allyl ITC and 3-butenyl ITC yields were reduced by 50% within 2 days of defrosting from frozen rhizomes at -15ºC. However, the losses of 4-pentenyl (37%) and 5-hexenyl ITCs (28%) were comparatively less than the loss of allyl and 3-butenyl ITCs. This indicates that the physical properties (volatility and boiling point, chemical stability) of the ITCs might be responsible for the relative loss during defrosting.

The defrosting time was related to the degree of ITCs yield reduction. This was apparent following defrosting of frozen rhizomes during storage at 4ºC in the refrigerator for 16 days, and gave a 99% loss of allyl ITC yield. After 2 days of defrosting, all minor ITCs disappeared and by day 5 a 96% loss of allyl ITC was observed. Overall, this study has shown that defrosting of frozen rhizomes leads to unwanted losses of ITCs. The yield of individual ITCs were not stable in the stored rhizomes at 4ºC for 6 weeks and after 6 weeks most ITCs showed considerable losses when compared to the levels in the fresh rhizomes (shown in Figure 1). About half of the allyl and total ITC yield was lost after 6 weeks storage at 4ºC. The moisture content was not correlated with the potential loss of ITCs at 4ºC but the ITCs were unstable at 4ºC. *Iso*propyl ITC may behave differently at 4ºC, which needs to be investigated further.

Conclusions

There was no loss of individual and total isothiocyanate (ITC) yields from storage of wasabi rhizomes at -10, -20 and -80ºC temperatures for 8 weeks. No significant differences were found in the mean ITC yields between fast initial freezing treatment (1833.9 mg total ITC kg^{-1} of rhizome) and slow freezing rhizomes (no initial treatment, 1881.4 mg total ITC kg^{-1} of rhizome) when stored at -10ºC and lower temperatures. Moreover, no difference on total ITC yields between fresh (1759.7 mg kg^{-1}) and frozen rhizomes (1859.7 mg kg^{-1}) at ≤ -10ºC were noticed. But storage just below freezing temperature (-4ºC) resulted in losses of ITCs compared to storage at lower temperatures (-10, -20 and -80ºC) over 6 weeks. Temporary storage of the whole rhizomes at a cool temperature (4ºC) for a few days leads to a very minor loss of ITCs but the total ITC yields were negatively affected by further storage for more than 4 weeks. Allowing frozen rhizomes to defrost, even for a short time, substantially reduced the total ITC yield. There was a total loss of allyl ITC when the rhizomes were allowed to defrost for 16 days. During defrosting individual ITCs behaved slightly differently. Data from this research will assist growers and processors to understand the stability of isothiocyanates under different storage conditions with time and thereby provide valuable knowledge on how to store harvested plant parts to retain their full commercial value as a high quality flavouring material.

Acknowledgements

We thankfully acknowledge Bruce Clark (University of Canterbury) and Dr Nigel Perry (Crop & Food Research, Dunedin) for their assistance in GCMS analysis of individual isothiocyanates in Wasabi. The authors wish to thank Mr G. Kitson of Jatra Corp. Ltd and Mr M. Kitson of Hi Tech Foods Ltd, Christchurch for providing samples and their encouragement and financial support to carry out this work.

References

[1]Chadwick, C.I., Lumpkin, T.A. and Elberson, L.R. 1993. The botany, uses and production of *Wasabia japonica* (Miq.) (Cruciferae) Matsum. Economic Botany. **47**(2): 113-135.

[2] Masuda, H., Harada, Y., Tanaka, K., Nakajima, M., Tebeta, H., Takeoka, G.R., Teranishi, R., Williams, P.J. and Kobayash, I A. 1996. Characteristic odorants of wasabi (*Wasabia japonica* Matum) (*Eutrema wasabi*), Japanese horseradish, in comparison with those of horseradish (*Armoracia rusticana*). Biotechnology for Iimproved Foods and Flavours. ACS Symposium Series No. 637 **25:** 67-78.

[3] Sultana, T., Savage, G.P., McNeil, D.L., Porter, N., Martin, R.J. and Deo, B. 2002. Effects of fertilisation on the allyl isothiocyanate profile of the above-ground tissues in New Zealand grown wasabi. Journal of the Science of Food and Agriculture. **82**:1477-1482.

[4] McGregor, D.I., Mullin, W.J. and Fenwick, G.R. 1983. Analytical methodology for determining glucosinolate composition and content: Review of analysis of glucosinolates. Journal of the Association of Official Analytical Chemists. **66**(4): 825-849.

[5] Sultana, T., Savage, G.P., McNeil, D.L., Porter, N. and Martin, R.J. 2000. Flavour compounds in the rhizome of soil-grown Wasabi. Proceedings of the Nutrition Society of New Zealand. **25:** 95-106.

[6] Fahey, J.W., Zalcmann, A.T. and Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. **56:** 5-51.

[7] Dorsch, W., Adam, O., Weber, J. and Ziegeltrum, T. 1985. Antiasthmatic effects of onion extracts- detection of benzyl and other isothiocyanates (mustard oil) as antiasthmatic compounds of plant origin. European Journal of Pharmacology. **107:** 17-25.

[8] Kumagai, H., Kashima, N., Seki, T., Sakurai, H., Ishii, K. and Ariga, T. 1994. Analysis of volatile components in essential oil of upland wasabi and their inhibitory effects on platelet aggregation. Bioscience Biotechnology and Biochemistry. **58** (12): 2131-2135.

[9] Depree, J.A., Howard, T.M. and Savage, G.P. 1999. Flavour and pharmaceutical properties of the volatile sulphur compounds of wasabi (*Wasabia japonica*). Food Research International. **31:** 329-337.

[10] Verhoeven, D.T.H., Goldbohm, R.A., van Poppel, G., Verhagen, H. and van den Brandt, P.A. 1996. Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiology and Biomarkers Prevention. **5:** 733-748.

[11] Lin, H.J., Probst-Hensch, N.M., Louie, A.D., Kau, I.H., Witte, J.S., Ingles, S.A., Frankl, H.D., Lee, E.R. and Haile, R.W. 1998. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. Cancer Epidemiological and Biomarkers Prevention. **7:** 647-652.

[12] Hasegawa, N., Matsumoto, Y., Hoshino, A., Iwashita, K. 1999. Comparison of effects of *Wasabia japonica* and allyl ITC on the growth of four strains of *Vibrio parahaemolyticus* in lean and fatty tuna meat suspensions. International Journal of Food Microbiology. **49:** 27-34.

[13] Soledade, M., Pedras, C. and Sorensen, J.L. 1998. Phytoalexin accumulation and antifungal compounds from the crucifer Wasabi. Phytochemistry, **49:** 1959-1965.

[14] Chung, F.L. 2002. Chapter 7 In Phytochemicals in Nutrition and health, Edited by Meskin, M.S., Bidlack, W.R., Davies, A.J. and Omeaye, S.T., CRC Press, USA.

[15] Sultana, T., McNeil, D.L., Porter, N. and Savage, G.P. 2003. Investigation of isothiocyanate yield from flowering and non flowering tissues of wasabi grown in a flodded system. Journal of Food Composition and Analysis. Accepted for publication.

[16] Yong-Gang, L., Steg, A., Smits, B. and Tamminga, S. 1994. Crambe meal: removal of glucosinolates by heating with additives and water extraction. Animal Feed Science and Technology. **48**(3-4): 273-287.

[17] Sekiyama, Y., Mizukami, Y., Takada, A. and Numata, S. 1994. Vapour pressure and stability of allyl isothiocyanate. Journal of the Food Hygienic Society of Japan. **35**(4): 365-370.

[18] Vierheilig, H. and Ocampo, J.A. 1990. Effect of isothiocyanates on germination of spores of *G. mosseae*. Soil Biology and Biochemistry. **28**(8): 1161-1662.

[19] Kojima, M. and Nakano, Y. 1980. Studies on evaluation of quality of japanese horseradish (wasabi) powder by gas chromatography. X. Studies to change in pungent components during storage of horseradish, mastard and wasabi powder. Journal of Japanese Society of Food Science and Technology. **27**(2): 86-88.

[20] Kojima, M., Hamada, H. and Yamashita, M. 1982. Studies on the evaluation of quality of Japanese horseradish (wasabi) powder by gas chromatography. XI. Studies on the stability of dried wasabi flour. Japanese Society of Food Science and Technology. **29**(4): 232-237.

[21] Tseng, C.Y., Tsai, T.C. and Tseng, Y.K. 1986. Studies on the processing of wasabi produced in Taiwan. I. Production of Wasabi powder. Food Science, China. **13**(1/2): 60-70.

[22] Depree, J.A. and Savage, G.P. 1996. Storage properties of a wasabi flavoured mayonnaise. Proceedings of the Nutrition Society of New Zealand. **21:** 142-151.

[23] Sahasrabudhe, M.R. and Mullin, W.J. 1980. Dehydration of horseradish roots. Journal of Food Science. **45:** 1440-1443.

[24] AOAC. 2000. Official Methods of Analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD, USA.

[25] Kjœr, A., Ohashi, M., Wilson, J.M. and Djerassi, C. 1963. Mass spectra of isothiocyanates.Acta Chemica Scandinavica. **17:** 2143-2154.

[26] Genstat. 2000. Genstat release 4.2 reference manual, Genstat committee, Lawes Agricultural Trust, Harpenden, Hertfordshire, UK.

[27] Sigmaplot. 1999. Sigmaplot version 5.0, SPSS Inc., Canal Blvd. Suite F, Richmond, USA.

[28] Ina, K., Ina, H., Ueda, M., Yagi,A. and Kishima, I. 1989. w-Methylthioalkyl isothiocyanate in wasabi. Agricultural and Biological Chemistry. **53(**20): 537-538.