Comparison of flavour compounds in wasabi and horseradish

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Summary

The Japanese horseradish (*Wasabia japonica* (Miq) Matsum) and European horseradish (*Amoracia rusticana*) are aromatic herbs used as spices and condiments due to their characteristic flavour. The flavour of both comes from the liberation of volatile isothiocyanates (ITCs) by the hydrolysis of precursor glucosinolates. Seven ITCs were measured in this study in order to compare the flavour compounds of wasabi rhizomes and horseradish roots. New Zealand grown horseradish contained 1900.7 mg total isothiocyanate/kg (on a fresh weight basis) while the level of total ITC in wasabi was 2067.55 mg/kg. Allyl ITC (AITC) was the main ITC in both of the plants (1937.8 and 1658.1 mg/kg respectively in wasabi and horseradish). 2-phenylethyl ITC (2-PEITC) was present as a major component after AITC (185.2 mg/ kg on a fresh weight basis) only in horseradish and therefore the radish-like flavour of 2-PEITC is likely to have a characteristic role in the overall flavour of horseradish. However, all minor ITCs (_-alkenyl ITCs, alkyl ITCs) occurred at higher levels in wasabi rhizomes. The differences in ITC observed between wasabi and horseradish could well explain the difference in taste between these two herbs.

Introduction

The flavour of a dish determines the choice of spices used in cooking. Traditionally, condiments are used with cooked foods to turn an ordinary dish into an extra special one by using a freshly prepared paste from plant tissues that provides a hot taste with a pungent smell. Although spices and condiments have no nutritional value other than to offer flavour, some of the flavour compounds have effective chemopreventive roles with important pharmacological properties (Dorsch *et al*., 1985; Steinmetz and Potter, 1991; Isshiki *et al*., 1992; Fuke *et al*., 1994; Kumagai *et al*., 1994; Delaquis and Mazza 1995; Hecht, 1995; Verhoeven *et al*., 1996; Lin *et al*., 1998; Ono *et al*., 1998; Soledade *et al*., 1998 and Depree *et al*., 1999). A number of different plants are specially cultivated to supply a hot spicy flavour, for instance, the European horseradish (*Amoracia rusticana*) and the Japanese horseradish (*Wasabia japonica* (Miq) Matsum). Both of these plants belong to the *Cruciferae* family, which also includes cabbage, cauliflower, broccoli and mustard. Wasabi and horseradish both liberate isothiocyanates (ITCs) as flavour compounds and so possess a hot taste with a similar flavour (Palmer *et al*., 1990). However, Masuda *et al*. (1996) reported that the main difference between them is the green odour of wasabi as both of them possess a strong pungent flavour. Horseradish is a distant cousin of wasabi and is sometimes used as a substitute for wasabi with an added green food colour in it (Chadwick *et al*., 1993).

Horseradish is a taproot with many lateral roots that penetrates deeply into the soil. It is grown mainly for the white flesh of the roots, which is grated fresh or made into a sauce to use as a condiment with roast beef (Langer & Hill, 1991). However, wasabi is cultivated primarily for the rhizomes (stems) because they possess more flavour than other plant parts. The mature wasabi plants produce thickened rhizomes, which are used to make a hot green paste, eaten as a sauce with other foods or as an ingredient in a range of Japanese sauces.

Wasabi is also used to decorate foods because of its bright green colour and also to flavour traditional and modern foods e.g. raw fish, noodles, sushi, and mayonnaise. Although rhizomes are the most valuable part of the wasabi plant, other parts such as petioles and leaves also contain ITCs and give these tissues some pungency (Sultana *et al*., 2002). Wasabi leaves and petioles are used to make a variety of food products including pickles in sake brine or soysauce, ice cream, cheese and crackers (Chadwick *et al*., 1993).

The flavour of both wasabi and horseradish comes from ITCs, the volatile sulphur compounds, which are liberated from the precursor glucosinolates (GSLs) when plant tissues become damaged (McGregor *et al.*, 1983). GSLs are a group of glucosides, stored within the cell vacuoles of all *Crucifereae* plants (Delaquis & Mazza, 1995) and are biosynthesised from protein amino acids (Fahey *et al*., 2001). GSLs coexist in plants, but are not in contact, with the hydrolytic enzyme myrosinase (thioglucoside glycohydrolase EC 3.2.2.1). When plant tissues are mechanically disrupted or injured (e.g. by chewing, crushing or grating in the preparation of food), myrosinase is released from the cell wall, and in the presence of adequate moisture it rapidly hydrolyses the GSLs to yield glucose and an aglucone (Fenwick *et al*., 1983; McGregor, 1993). The organic aglucone is unstable and undergoes lossen rearrangement to produce sulphate and a variety of other products. Under neutral and alkaline conditions ITCs are formed from GSLs. However, once formed ITCs are more stable under acidic conditions.

Most of the sulphur-containing end products formed by enzymatic and non-enzymatic reactions of GSLs are volatile (Delaquis & Mazza, 1995). Among these compounds, ITCs have been recognised as the characteristic flavour compounds of plants in the *Cruciferae* family because of their pungency (Masuda *et al*., 1996). Table 1 shows a summary of ITC content of wasabi and horseradish roots from previously reported investigations. Each ITC is found at different levels in the two plants. The taste or aroma of a paste made from each plant depends on the relative abundance of the ITCs in the original tissue, as each ITC has a different flavour profile. For instance, the more radish-like aroma of the horseradish roots may occur because of the higher content of 2-phenylethyl ITC. The typical wasabi flavour is derived from the high content of allyl ITC (2-propenyl ITC) but other ITCs contribute to the characteristic fresh 'green' notes (Ina *et al*., 1989; Masuda *et al*., 1996). However, allyl ITC (AITC) is the compound found at the greatest amounts in both of wasabi and horseradish (Kojima & Ichikawa, 1969; Ina *et al*., 1981; Masuda *et al*., 1996) while 2-phenylethyl ITC is only present in horseradish roots.

The objectives of this study were to compare isothiocyanate (total and individual ITC) concentration between New Zealand grown wasabi rhizomes and horseradish roots and to compare these with previously published data for plants grown and analysed in other countries.

Materials and methods

Sources of wasabi and horseradish

The current commercial cultivar of wasabi (Daruma) grown at Stillwater on the West Coast (42 25ˉS, 171 20ˉE, 23 m above sea level) in South Island, New Zealand was used in this trial. The plants were 18 months old and the weight of rhizomes ranged from 60 to70g. Horseradish was collected from Yaldhurst, Christchurch (43 3ˉS, 172 29ˉE, 11 m above sea level) New Zealand. Horseradish roots were one year old and weight ranged from 50 to 60g. Both of the plant species were grown in soil without manure or any fertiliser application.

Extraction

Approximately 50 g of fresh wasabi rhizome or horseradish roots were cut into small pieces and then homogenised into a Braun multi quick grater (MR 430 CA) separately. Five g of ground sample was weighed into a 40 ml Beckman plastic centrifuge tube containing 5 ml of dichloromethane (Hipersolv grade, BDH) and 7 ml of distilled water. The samples were tumbled in a Hybaid oven at 20ºC for an hour and then separated from the paste and water phase by centrifugation (12100 g, 20°C for 5 minutes). The dichloromethane extract was stored at –20ºC prior to GC analysis. The moisture contents were determined in accordance with AOAC methods (AOAC, 1995).

Gas chromatography analysis

Samples (2 µl) of dichloromethane extract were injected (split less mode) onto a Hewlett-Packard (HP) INNO wax capillary column (30 m, 0.25 mm i.d. and 0.25 _m film thickness) in a HP 6890 gas chromatograph fitted with a flame ionisation detector (FID), and a HP 6890 automatic sampler. 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 and flow rate of 2.3 ml/minute. Separations were performed under the following temperature program: 50 to 100°C at 5°C/min, 100 to 200°C at 10°C/min, then held at 200°C for 2 minutes. Peaks areas were recorded and calculated using HP Chemstation software (Version A.06.03).

Butyl and phenyl ITCs were used as external standards and demonstrated significant differences in FID response to ITCs of different carbon to sulphur ratios. Calibration curves were prepared with butyl and phenyl ITCs and the butyl ITC calibration curve was used to quantify the levels of each ITC.

Gas chromatography- mass spectroscopy (GCMS)

Allyl ITC, 3-butenyl ITC, 4-pentenyl ITC, 5- hexenyl ITC, *iso*propyl ITC, *sec*-butyl ITC and 2-phenylethyl ITC were identified by GCMS on a Carlo Erba MFC500 GC (split ratio 30:1, HP DB5MS 30 m, 0.25 mm i.d., 0.25 _m 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). Minor peaks beyond 5-hexenyl ITC are under investigation.

Statistical analysis

The extraction was replicated three times for each plant species. Data were analysed with the Descriptive statistics and One-way Analysis of Variance of the MINITAB statistical package (Minitab, 1996). Mean values of concentration for each ITC in both plants are presented with SE of Means in Table 2. The total ITC concentration was calculated from the sum of individual ITCs. Probability (*P*) is also reported to compare individual concentrations between wasabi rhizomes and horseradish roots.

Results

Seven isothiocyanates (ITCs) were identified and measured as flavour compounds in New Zealand grown wasabi rhizomes and horseradish roots (Table 2). These were *iso*-propyl ITC, *sec*-butyl ITC, allyl ITC (AITC), 3-butenyl ITC (3-BITC), 4-pentenyl ITC (4-PITC), 5-hexenyl ITC (5-HITC) and 2-phenylethyl ITC (2-PEITC). The concentration of each ITC except 3-BITC was different in the two plant species and their relative values are given in Table 2.

AITC was the highest concentration ITC in both wasabi (1937.8 mg/kg of fresh rhizome) and horseradish (1658.1 mg/kg fresh root). Its concentration was significantly lower in horseradish (14%) than in wasabi. However, AITC comprised a higher proportion of the total ITC concentration of wasabi (93.7%) than of horseradish (87.2%).

The level of 4-PITC in wasabi was 47.97 mg/kg of rhizome, (2.32% of the total ITC concentration in wasabi) whereas in horseradish the level was only 8.99 mg/kg of the root, which was significantly lower (81%) than for wasabi. The proportion of 4-PITC of the total ITC concentration in horseradish was also very small (0.47%).

2-phenylethyl ITC was only detected in horseradish (185.2 mg/kg of root) and was the second highest ITC in horseradish root; contributing 9.74% to the total ITC content.

The concentrations of 3-BITC in wasabi and horseradish were not significantly different (43.13 and 39.43 mg/kg of the sample, respectively). Thus its proportion of the total ITC concentration was very similar between wasabi and horseradish (2.09% and 2.07% respectively).

Sec-butyl ITC was present at 18.78 mg/ kg of wasabi rhizome but was only 2.77 mg/kg in horseradish root. The proportion of *sec*-butyl ITC of total ITC concentration in wasabi and horseradish was very small (0.91% and 0.15% respectively).

The levels of *iso*-propyl ITC in wasabi and horseradish were 12.81mg/kg and 3.57 mg/kg respectively. Very small amounts of 5-HITC were found in wasabi (7.06 mg/kg of rhizome) and horseradish (2.77 mg/kg of root).

The total ITC content in wasabi was 2067.55 mg/kg of rhizome while the level in horseradish was 1900.7 mg/kg of roots. There was a significant difference (8%) ($P = 0.04$) in total ITC concentration between wasabi and horseradish.

Discussion

The differences in the flavour between wasabi and horseradish depend on differences in the flavour compounds, which occur in the two plants. 2-phenylethyl ITC was the accept biggest component after AITC and was detected only in horseradish. It is likely to have a significant role in the overall aroma and taste profile of horseradish. Gilbert & Nursten, (1972) suggested that a small change in the proportions of ITCs would be sufficient to significantly alter the overall aroma. As a consequence, 2-phenylethyl ITC may play a major role in distinguishing the flavour profile of horseradish from wasabi. Masuda *et al*. (1996) and Gilbert & Nursten, (1972) reported that 2-PEITC imparted a strongly radish-like, fresh watercress aroma and gave a tingly sensation to the mouth. However, 2-PEITC had no pungency and lachrymatory role at all, like AITC, and therefore was distinctively different from AITC. Masuda *et al*. (1996) suggest that 2-PEITC was responsible for the obvious difference in odour between wasabi and horseradish. Hence the analytical and organoleptic data are consistent in attributing the radish-like aroma in horseradish to the contribution of 2-PEITC.

This study has identified several differences in ITC concentrations among the common ITCs found in wasabi and horseradish that could lead to the changes in perceived flavour.
In wasabi, every ITC was found in significantly greater amounts when compared to horseradish except for 3-BITC. Although AITC was the primary ITC in both of the plants, there was only a 14% difference in concentration between them. In contrast, the secondary

ITCs (_-alkenyl and alkyl ITCs) showed greater variations (3-7 times higher in wasabi). Among the secondary (minor) ITCs, the _-alkenyl ITCs (4-PITC, 5-HITC) were present at 63 – 81% lower concentration in horseradish and also contributed 2.7–4.9 times greater proportion in the total ITC concentration in wasabi relative to horseradish. Thus, these variations may be expected to exert some effect on the overall aroma of these plants. Masuda *et al.* (1996) stated that _-alkenyl ITCs are responsible for the difference between wasabi and horseradish by their 'green' odour. However the results of this study showed only a small difference in 3-B ITC concentration (9%) between wasabi and horseradish, which was different from the observations made by Masuda *et al*. (1996). So the aroma of _-alkenyl ITCs, specifically 4-PITC, 5-HITC could be stronger in wasabi.

The two alkyl ITCs (*iso*-propyl and *sec*-butyl ITCs) are reported to have a chemical odour but no role in producing a pungent smell, thus making only a small contribution to the characteristic odour of wasabi and horseradish (Masuda *et al*., 1996). However, Gilbert and Nursten, (1972) reported a pungent but not distinctive odour for synthesized *iso*-propyl ITC while Ina *et al.* (1981) reported weak mustard-like odour for them. This experiment measured 72% and 85% lower concentrations for *iso*-propyl and *sec*-butyl ITCs respectively in horseradish. Their greater contributions to the total ITC in wasabi suggests they may have a characteristic role in overall flavour and need to be investigated.

AITC was the main flavour compound because of its high concentration in both wasabi and horseradish and this result is in agreement with previous investigations carried out by Ina *et al*. (1989); Kumagai *et al*. (1994) and Masuda *et al*. (1996). Therefore, AITC was the primary contributor into total ITC concentration of wasabi (94%) and horseradish (87%) and this suggests it will provide the most important character to the total flavour profile in both species. AITC is reported to be a lachrymatory compound, bitter in taste with a strong, pungent and mustard like smell (Masuda *et al*., 1996). Consequently, pungency is the common characteristic of both wasabi and horseradish flavours. However, this study found a 14% lower concentration of AITC in horseradish (significant at P = 0.007). As a major contributor this difference could be sufficient to easily differentiate the total flavour of wasabi and horseradish.

From this experiment the difference in total concentrations of ITCs were small between wasabi and horseradish. The reported total ITC concentration in different publications on wasabi and horseradish (Table 1) were 4-35% and 8-35% lower respectively than the results reported in this work. A number of reasons could be responsible for the variation with previous investigations like e.g. method of extraction, application of fertilizer and other treatments to the grow plants (Wang *et al*., 1999; Sultana *et al*., 2001), age of the plants used, soil and environmental factors (Wang *et al*., 1999). This study has showed higher concentrations of individual ITC's in horseradish than observed by Masuda *et al*. (1996). Although they used New Zealand grown horseradish but did not mention where or how it was grown. This study also shows that it is possible to grow high quality wasabi and horseradish in New Zealand.

Conclusions

In this study wasabi was shown to contain higher levels of all ITCs other than 2-phenylethyl ITC. Thus, 2-PEITC is likely to be the most important component responsible for distinguishing horseradish flavour from wasabi flavour. Thus, the radish-like aroma of 2-phenylethyl ITC may have a major role in horseradish. There are significant differences in common ITCs concentrations, which could also be responsible for distinctive differences between horseradish and wasabi flavours. Among the common ITCs the minor ITCs (4-PITC, 5-HITC, isopropyl and sec-BITC) provided larger variation (63-85%) than the major AITC (14%) between wasabi rhizome and horseradish root. Consequently, minor ITCs are probably responsible for differentiating the flavour between wasabi and horseradish. Compared to the reported values for ITC in wasabi and horseradish New Zealand grown raw material appears to contain good levels of flavour compounds which is important for making high quality products.

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Table 1. Comparison of flavour compounds between wasabi and horseradish with a description of the odour of each compound

Isothiocyanates (ITCs)	*Concentration mg/kg fresh weight*					*Odour description*[b,d,e]
	Wasabi rhizome[a]	*Wasabi rhizome*[b]	*Wasabi root*[c]	*Horse radish root*[b]	*Horse radish root*[c]	
*Iso*propyl ITC	-	7.6	-	Tr	-	Chemical, weak mustard like
Allyl ITC	1282 ± 123	1880	1110	1570	966	Strongly pungent, mustard-like, lacrymatory, bitter
n- butyl ITC	-	-	17.4	-	4.2	---
Sec-butyl ITC	11.4 ± 1.2	13	-	27	-	Chemical, weak mustard like
Iso butyl ITC	0.6 ± 0.1	3.9	-	0.6	-	Sweet, chemical
3-butenyl ITC	123.5 ± 8.8	25	18.3	5.5	8.1	Green, pungent, aroma
4-pentenyl ITC	65.2 ± 3.8	31	39.0	1.7	1.0	Green, pungent, acrid, fragrant leaf
5-hexenyl ITC	16.9 ± 1.0	8.0	10.2	-	1.8	Green, pungent, fatty
6-heptenyl ITC	1.0 ± 0.1	0.6	-	-	-	Green, pungent, fatty
3-methylthio propyl ITC	2.4 ± 0.5	Tr	-	1.5	-	Strongly raddish-like, pungent
Benzyl ITC	-	0	-	1.6	-	Chemical, pungent
2-phenylethyl ITC	-	Tr	-	133	225	Strongly radish-like, pungent, strong watercress aroma, tingling sensation
4-methylthio butyl ITC	0.2 ± 0.0	-	-	-	-	-
5-methylthio pentyl ITC	9.9 ±1.2	1.5	4.8	Tr	-	Radish-like, pickle-like
6-methylthio hexyl ITC	35.0 ± 3.3	4.8	18.9	Tr	-	Radish-like, sweet, fatty
7-methylthio heptyl ITC	3.2 ± 0.2	0.9	14.4	Tr	-	Sweet, fatty, radish-like, pickle-like
5-methyl sulphinyl pentyl ITC	-	-	21.7	-	8.1	-
6-methylsulphinyl hexyl ITC	-	-	78.0	-	9.0	-
7-methylsulphinyl heptyl ITC	-	-	14.1	-	7.8	-
Total ITC	1653.9	1976.3	1346.8	1740.9	1231	

[a]Data from Kumagai *et al*., 1994; [b]Data from Masuda *et al*., 1996; [c]Wasabi root presumably refers to either the rhizome or the total root plus rhizome mass, data from Etoh *et al*., 1990; [d]Data from Fenwick *et al*., 1983; [e]Ina *et al*., 1981; Tr, less than 0.5mg/kg; -, not reported

Table 2. Isothiocyanates in wasabi and horseradish from New Zealand grown material

Isothiocyanates (ITCs)	*Concentration (mg of ITCs/kg fresh weight) Mean ± SEM*		*p-value*
	Wasabi	*Horseradish*	
*Iso*propyl ITC	12.81 ± 0.75	3.57 ± 0.01	0.000 ***
Sec-butyl ITC	18.78 ± 0.49	2.77 ± 0.24	0.000 ***
Allyl ITC	1937.80 ± 47.80	1658.10 ± 25.80	0.007 **
3-butenyl ITC	43.13 ± 0.92	39.43 ± 1.94	0.160 NS
4-pentenyl ITC	47.97 ± 0.84	8.99 ± 0.53	0.000 ***
5-hexenyl ITC	7.06 ± 0.09	2.64 ± 0.15	0.000 ***
2-phenylethyl ITC	†	185.20 ± 2.11	-
Total isothiocyanates	2067.55 ± 50.60	1900.70 ± 26.90	0.04 *
% composition	%	%	-
Moisture content	81	66	-

†, not detected, NS, not significant, *P<0.05, **P<0.01 and ***P<0.001