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Pungent Components from Thioglucosides in *Armoracia rusticana* Grown in China, Obtained by Enzymatic Hydrolysis

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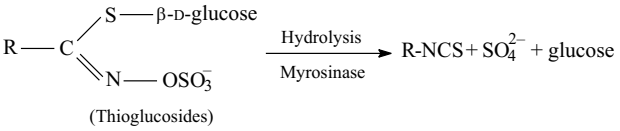
Summary

The conditions of enzymatic hydrolysis of thioglucosides, which are the precursors of the pungent components in *Armoracia rusticana* grown in China, were studied. The effects of incubation time, temperature, pH and the addition of ascorbic acid on the hydrolysis of thioglucosides were determined. The optimum hydrolytic conditions for the pungent components from thioglucosides were time, 120 min; temperature, 65 °C; pH=4.0 and ascorbic acid, 2 mg/g. The mixture of pungent components in a pale-yellow liquid and a yield of 0.85 % were isolated and analyzed by GC/MS. Nine constituents were identified, representing 92.1 % of the pungent components. The major constituents were allyl isothiocyanate (78.4 %), 3-butenyl isothiocyanate (1.5 %), 2-pentyl isothiocyanate (2.1 %) and β-phenylethyl isothiocyanate (9.4 %).

Key words: *Armoracia rusticana*, enzymatic hydrolysis, thioglucosides, isothiocyanates

Introduction

Armoracia rusticana (horseradish) is a perennial crop belonging to the genus *Armoracia* of the *Cruciferae* family. It has a widespread distribution in Europe and Asia including England, Hungary, Japan and China. In China, this plant is mainly grown in Liaoning Province. Its roots are popularly used as a pungent spice and an indispensable material for producing horseradish paste and pungent sauces. The intact or dried horseradish does not exhibit much aroma, but while cutting, shredding, and especially grating it or in contact with water, a very strong pungent and lachrymatory odour is released, which is enzymatically produced from thioglucosides (TGOs). TGOs exist naturally in horseradish as the precursors of the pungent components including sinigrin for allyl ITC, gluconasturtiin for β-phenylethyl ITC, and methylthiopropyl TGO for methylthiopropyl ITC. The hydrolytic reactions of TGOs in *A. rusticana* are as follows (*1*):



Scheme 1. Hydrolysis of TGOs in *A. rusticana* under enzymatic condition

Isaac and Kohlstaedt (*2*) reported allyl isothiocyanate (ITC) and β-phenylethyl ITC as the main pungent components of horseradish grown in Japan. Gilbert and

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Nursten (*3*) made a comparison of the pungent components of horseradish grown in England, Hungary and Japan. The authors identified five components including allyl ITC, 4-pentenyl ITC, β-phenylethyl ITC, 2-butyl ITC and allyl thiocyanate in British, Hungarian and Japanese horseradish. Kishima *et al.* (*4,5*) and Kojima *et al.* (*6–12*) reported on the pungent components of wasabi (*Wasabia japonica* Matsum.), which is not the same plant as horseradish, but it is used in Japan as horseradish. They found that allyl and β-phenylethyl ITCs were the major components. In addition, eight minor components such as methyl, isopropyl, 2-butyl, 3-butenyl, 4-pentenyl, 5-hexenyl, hexyl and 6-heptenyl ITCs were also identified in wasabi. Wasabi is nearly indistinguishable from horseradish in taste. Moreover, Hansen (*13*) found ITC contents in horseradish grown in Europe to range from 1.2 to 2.0 %. In recent years the pharmacological effects of horseradish have been receiving a great interest. Depree *et al.* (*1*) have reviewed the pharmaceutical properties of horseradish. ITCs inhibit the growth of food poisoning bacteria and fungi (*14*). In addition, these compounds also exhibit antioxidative and superoxide scavenging potency and antimutagenic activity (*14*). Further, ITCs might be used as cancer chemopreventive agents (*15–17*).

The differences in climatic and geographic conditions usually result in qualitative differences of horseradish such as chemical compositions and pharmacological effects. The purpose of this paper is to present the conditions of enzymatic hydrolysis for TGOs and a detailed analysis of the composition of pungent components in *A. rusticana* grown in China.

Materials and Methods

Plant material

The dried roots of *A. rusticana* were collected from Dalian (N39°, E122°), Liaoning Province, China. The sample was ground into 40–60 mesh powders before analysis. The level of glucosinolates in horseradish powders was 88.2 μmol/g prior to enzymatic hydrolysis, which was determined according to the official HPLC ISO method (*18*).

Reagents

All chemicals were of analytical reagent grade or better. Milli-Q water (Millipore Company, Bedford, Mass, USA) was used throughout the experiment. ITC standard compounds were obtained from Aldrich (Aldrich Chemical Company Inc., Milwaukee, WI, USA) or were prepared according to the literature (*3*).

Ascorbic acid stock solution of 10 mg/mL was prepared by dissolving an appropriate mass of ultra pure grade ascorbic acid (Beijing Chemical Company Inc., Beijing, China) in Milli-Q water. Working solutions of 1 mg/mL and 100 μg/mL were prepared by serial dilution, respectively.

The piperidine standard solution of 0.1 mol/L was prepared by dissolving 4.8 mL of piperidine (Aldrich) in an appropriate volume of acetone and diluting to 500 mL with acetone. Finally, the piperidine solution was standardized titrimetrically with a standard solution of 0.1 mol/L of hydrochloric acid and a mixed indicator of 0.1 % bromcresol green (3 parts) and 0.2 % methyl red (1 part).

Buffer solutions for pH ranges of 1.0–11.0 were prepared by mixing the solutions of sodium citrate and hydrochloric acid for pH ranges of 1.0–4.0, the solutions of acetic acid and sodium acetate for pH ranges of 4.0–6.5 and the solutions of sodium hydroxide and potassium dihydrogen phosphate for pH ranges of 6.5–11.0.

Selection of the optimum hydrolytic conditions of TGOs

To each of different 250-mL stoppered conical flasks, 2 g of the powdered roots of *A. rusticana*, 10 mL of buffer solution with different pH value and an appropriate volume of ascorbic acid solution were added successively. The mixture was diluted to 100 mL with Milli-Q water. The flask was stoppered and the temperature of the mixture was maintained by keeping it in a hot water bath at fixed temperature for 120 min to make TGOs in *A. rusticana* hydrolyzed. The incubation mixture was cooled quickly with an ice water bath, and then the pungent components were extracted with a mixture of 20 mL of petroleum ether (boiling point 30–60 °C) and 1 mL of butanol. After filtering with defatted cotton, the filter residue was washed with a little petroleum ether 3–4 times. The filtrates were collected and the ether layer was separated in a separating funnel. A volume of 10 mL of the piperidine standard solution was added to the ether layer, which was stoppered, mixed up and stood for 50 min, then the excess piperidine was titrated against the standard hydrochloric acid using a mixed indicator of 0.1 % bromocresol green (3 parts) and 0.2 % methyl red (1 part). The solution was titrated from blue-green to the milky yellow end-point (*19*).

Isolation of the pungent components

A mass of 50 g of the powdered roots of *A. rusticana*, 10 mL of the buffer solution (pH=4.0), 10 mL of 10 mg/mL ascorbic acid solution and 80 mL of Milli-Q water were added to a 250-mL stoppered round-bottomed flask. After the flask was stoppered, the mixture was hydrolyzed at 65 °C for 120 min and subjected to steam distillation. The distillate was extracted with ethyl ether as an extractant. Triplicate extractions were carried out for each distillate. The extractant was evaporated by vacuum distillation at about 25 °C and the oily residue was dried over anhydrous sodium sulphate, and filtrated into a 1-mL sample vial through a syringe filter of 0.2-μm pore size (Alltech Associates Inc., Deerfield, IL, USA) prior to GC/MS analysis.

GC/MS analysis

GC/MS analysis of the pungent components was performed on a Hewlett-Packard chromatograph, model 6890 Series II, equipped with a Hewlett-Packard 6890 Series auto-injector and a mass spectrometer selective detector 5973 (MS) (Hewlett-Packard Corporation, GA, USA). GC conditions: the capillary column (HP-5MS, cross-lined 5 % PH ME Siloxiane, 30 m × 0.25 mm i.d., 0.25 μm film thickness) was coupled directly to the MS; the flow rate for the helium carrier gas was 1.0 mL/min;

the injector temperature was 250 °C; a volume of 1.0 μL of sample of the pungent components was injected in the split mode at a ratio of 12:1; the temperature program was 35 °C for 3 min, 35–150 °C at a rate of 5 °C/min, isothermal for 2 min, 150 to 250 °C at a rate of 4 °C/min, and finally held isothermally for 6 min. MS conditions: ionization voltage (E_i) was 70 eV; ion source temperature was 280 °C; scan mass range was 25–400 m/z; solvent delay time was 1 min.

Identification of the pungent components

The identification of the pungent components was based on the comparison of their mass spectra with those of authentic standards and of a mass spectra library (Wiley Database) and the literature (*20*), and the explanation and certification of spectra. The relative amounts of the individual components were based on peak areas obtained without FID response factor correction (*i.e. F*=1.0 for all compounds).

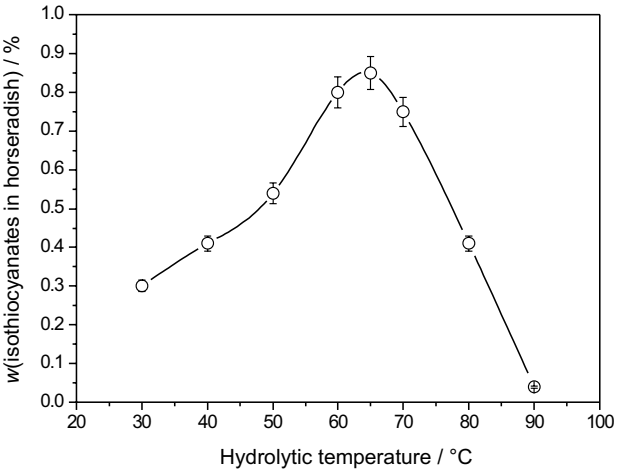

Fig. 2. Effect of hydrolytic temperature on the pungent components
Incubation time 120 min, pH=4.0, ascorbic acid 2 mg/g

Results and Discussion

The influence of incubation time on enzymatic hydrolysis of TGOs, when run at pH=4.0, ascorbic acid 2 mg/g and 65 °C, was determined. As shown in Fig. 1, TGOs are completely hydrolyzed in 120 min.

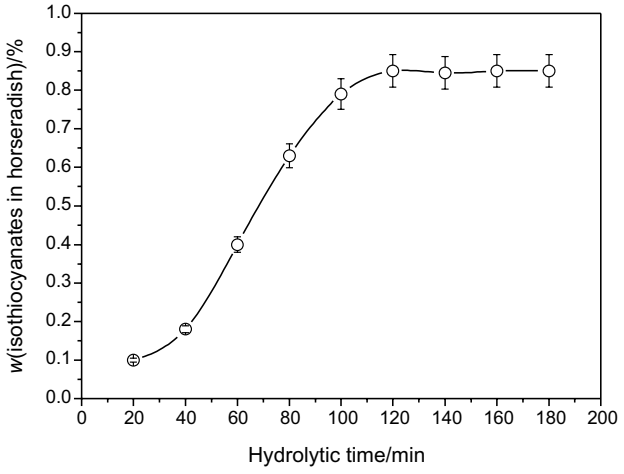

Fig. 1. Effect of hydrolytic time on the pungent components
Incubation temperature 65 °C, pH=4.0, ascorbic acid 2 mg/g

The influence of incubation temperature on enzymatic hydrolysis of TGOs was studied at incubation time 120 min, pH=4.0 and ascorbic acid 2 mg/g. The optimum temperature for release of ITCs from enzymatic hydrolysis of TGOs is 65 °C (Fig. 2). At lower temperature, the activity of glucohydrolase is insufficient. At higher temperature, the activity of the enzyme was lost. Myrosinase was completely inactivated when the incubation temperature exceeded 90 °C.

The influence of pH on enzymatic hydrolysis of TGOs was studied under the conditions of incubation time 120 min at 65 ºC and the addition of 2 mg/g ascorbic acid. The result is shown in Fig. 3. TGOs are completely hydrolyzed at pH=4.0. With the increase in pH value, the hydrolytic capacity of TGOs is reduced. We found that pH is a very important factor in enzymatic hydrolysis of TGOs to ITCs. In addition, we also found that further degradation of ITCs was also affected by pH.

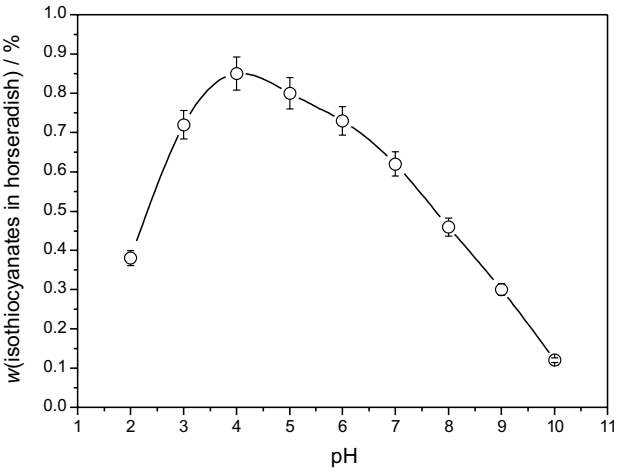

Fig. 3. Effect of pH on the pungent components
Incubation time 120 min, temperature 65 °C, ascorbic acid 2 mg/g

The influence of ascorbic acid on enzymatic hydrolysis of TGOs was determined under the conditions of incubation time 120 min at 65 ºC and pH=4.0. As shown in Fig. 4, the enzymatic hydrolysis of TGOs increases with the addition of ascorbic acid. Maximum hydrolysis of TGOs is achieved when ascorbic acid concentration reaches 2 mg/g and beyond. The result is identical to that of Appelqvist *et al.* (*21*), who reported that ascorbic acid can increase the activity of myrosinase in rape and turnip rape. This enhancing effect is an advantage for the cleavage of thioglucosides in the *Cruciferae* plants. The mixture of pungent components, obtained by the enzymatic hydrolysis of TGOs in *A. rusticana* and the steam distillation, was a pale-yellow oily liquid in a

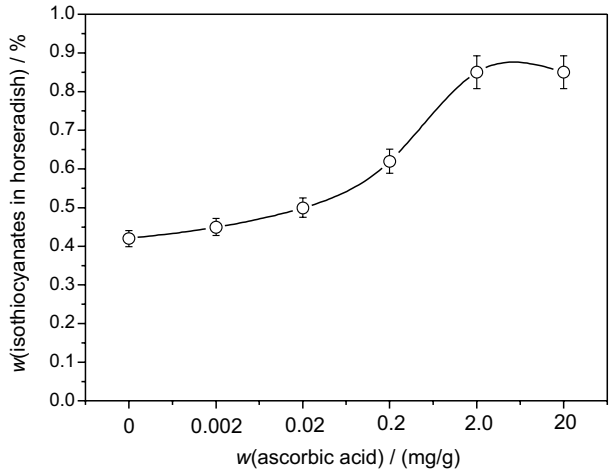

Fig. 4. Effect of ascorbic acid on the pungent components Incubation time 120 min, temperature 65 °C, pH=4.0

yield of 0.85 %. Nine constituents were identified, all of which belong to ITCs. The results are shown in Table 1. As far as the GC/MS results, the main components of the hydrolysates of TGOs in *A. rusticana* grown in China are allyl ITC (78.4 %), 3-butenyl ITC (1.5 %), 2-pentyl ITC (2.1 %) and β-phenylethyl ITC (9.4 %). Among these sulphur-containing compounds, allyl ITC is the most abundant.

Table 1. Chemical composition of the pungent components of *A. rusticana* grown in China

Peak No.	Retention time	Compound	*w*/%
1	6.09	Isopropyl isothiocyanate	0.1
2	10.69	Allyl isothiocyanate	78.4
3	11.29	Butyl isothiocyanate	0.1
4	11.96	3-Butenyl isothiocyanate	1.5
5	14.29	2-Pentyl isothiocyanate	2.1
6	16.76	Phenyl isothiocyanate	0.1
7	19.79	3-Methylthiopropyl isothiocyanate	0.3
8	21.39	Benzyl isothiocyanate	0.1
9	25.72	β-Phenylethyl isothiocyanate	9.4
		Total	92.1

Conclusions

Comparing our results of analysis with those of Gilbert and Nursten (*3*), Kishima *et al.* (*4,5*) and Kojima *et al.* (*6–12*) we found that the major pungent components of horseradish cultivated in England, Hungary, Japan and China were basically similar, but there was a little difference in the contents of allyl and β-phenylethyl ITCs, whose contents in English, Hungarian and Japanese horseradishes were 44.3–55.7 % and 38.4–51.3 %, respectively (*3*). Furthermore, the microamount of pungent components such as 3-butenyl and 2-pentyl ITCs only existed in Chinese horseradish. 4-Pentenyl ITC and allyl thiocyanate were not found in the pungent components of Chinese horseradish, but the compounds existed in English, Hungarian and Japanese horseradishes (*3,10*). Our results are identical to those of Hara *et al.* (*22*), who analyzed the changes in pungent components of green-stem and red-stem Japanese horseradish cultivars during the cultivation period. Hara found that the contents of allyl ITC did not differ between the two cultivars, but there is a little difference in the microamount of isopropyl ITC.

Acknowledgements

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References

1. J.A. Depree, T.M. Howard, G.P. Savage, Flavour and pharmaceutical properties of the volatile sulphur compounds of Wasabi, *Food Res. Int. 31* (1998) 329–337.
2. O. Isaac, E. Kohlstaedt, Essential horseradish oils, *Arch. Pharm. 295* (1962) 165–173.
3. J. Gilbert, H.E. Nursten, Volatile constituents of horseradish roots, *J. Sci. Food Agric. 23* (1972) 527–539.
4. I. Kishima, K. Fukuzawa, S. Imai, K. Ina, A checking method of quality of Japanese horseradish and black mustard in »Wasabi powder«: Studies on the volatile components in »Wasabi powder«, Part II, *J. Jpn. Soc. Food Sci. Technol. 27* (1980) 591–596.
5. I. Kishima, Y. Shibata, K. Ina, Volatile components of horseradish and black mustard, *J. Jpn. Soc. Food Sci. Technol. 17* (1970) 361–363.
6. M. Kojima, Y. Akahori, I. Ichikawa, M. Mochizuki, Gas chromatographic studies on the acrid components of Japanese horseradish (Wasabi) powder, *J. Agric. Chem. Soc. Jpn. 44* (1966) 177–184.
7. M. Kojima, Studies on the check of quality of Japanese horseradish (»Wasabi«) powder by gas chromatography, Part I. Application of isothermal temperature column, *J. Jpn. Soc. Food Sci. Technol. 17* (1970) 309–314.
8. M. Kojima, Evalution of the quality of Japanese horseradish powder by gas chromatography, IV. Investigation of sample headspace gas chromatographic method for checking quality, *J. Food Sci. Technol. (Japan), 20* (1973) 316–320.
9. M. Kojima, Volatile components other than isothiocyanates of *Wasabia japonica*, *J. Ferment. Technol. 51* (1973) 670–676.
10. M. Kojima, M. Uchida, Y. Akahori, Studies on the volatile components of *Wasabia japonica*, *Brassica juncea* and *Cochlearia armoracia* by gas chromatography-mass spectrometry, I. Determination of low mass volatile components, *Yakugaku Zasshi, 93* (1973) 453–459.
11. M. Kojima, H. Hamada, M. Yamashita, Studies on the stability of dried Wasabi flour, *J. Jpn. Soc. Food Sci. Technol. 29* (1982) 232–237.
12. M. Kojima, H. Hamada, N. Toshimitsu, Quantitative determination of β-phenylethyl isothiocyanate, *J. Jpn. Soc. Food Sci. Technol. 32* (1985) 368–371.
13. H. Hansen, Content of glucosinolates in horseradish, *Tidsskr. Planteavl. 78* (1974) 408–410.
14. N. Kinae, H. Masuda, I. S. Shin, M. Furugori, K. Shimoi, Functional properties of wasabi and horseradish, *Biofactors, 13* (2000) 265–269.

15. C.C. Conaway, Y.M. Yang, F.L. Chung, Isothiocyanates as cancer chemopreventive agents: Their biological activities and metabolism in rodents and humans, *Curr. Drug Metab. 3* (2002) 233–255.

16. Y. Morimitsu, K. Hayashi, Y. Nakagawa, F. Horio, K. Uchida, T. Osawa, Antiplatelet and anticancer isothiocyanates in Japanese domestic horseradish, wasabi, *Biofactors, 13* (2000) 271–276.

17. N. Tanida, A. Kawaura, A. Takahashi, K. Sawada, T. Shimoyama, Suppressive effect of wasabi (pungent Japanese spice) on gastric carcinogenesis induced by MNNG in rats, *Nutr. Cancer, 16* (1991) 53–58.

18. Rapeseed – Determination of glucosinolates content – Part 1: Method using high-performance liquid chromatography, *ISO Standards: ISO 9167-1* (1992) 1–9.

19. K.S. Chikkaputtaiah, M.L. Shankaranarayana, C.P. Natarajan, Volumetric determination of allyl isothiocyanate in black mustard (*Brassica nigra*), *Flavour Ind. 10* (1971) 591–593.

20. R.P. Adams: *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry*, Allured, Carol Stream (2001) pp. 389–406.

21. L.A. Appelqvist, E. Josefsson, Method for quantitative determination of isothiocyanates and oxazolidinethiones in digests of seed meals of rape and turnip rape, *J. Sci. Food Agric. 18* (1967) 510–519.

22. M. Hara, K. Mochizuki, S. Kaneko, T. Iiyama, T. Ina, H. Etoh, T. Kuboi, Changes in pungent components of two *Wasabia japonica* Matsum. cultivars during the cultivation period, *Food Sci. Technol. Res. 9* (2003) 288–291.

Izdvajanje sastojaka oštra mirisa kineskoga hrena enzimskom hidrolizom tioglukozida

Sažetak

Ispitani su uvjeti enzimske hidrolize tioglukozida koji daju oštar miris hrenu (*Armoracia rusticana*) uzgojenom u Kini. Ispitano je kako vrijeme inkubacije, temperatura, pH i dodatak askorbinske kiseline utječu na tijek hidrolize tioglukozida. Optimalni uvjeti za dobivanje oštra mirisa iz tioglukozida su: vrijeme 120 min, temperatura 65 °C, pH=4,0 i maseni udjel askorbinske kiseline od 2 mg/g. Dobivena smjesa tvari oštra mirisa u bljedožutoj tekućini, iskorištenja 0,85 %, analizirana je plinskom kromatografijom/masenom spektrometrijom. Utvrđeno je 9 sastojaka koji čine 92,1 % tvari mirisa. Glavni su sastojci alil izotiocijanat (78,4 %), 3-butenil izotiocijanat (1,5 %), 2-pentil izotiocijanat (2,1 %) i β-feniletil izotiocijanat (9,4 %).