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Myrosinase Activity and Total Glucosinolate Content of Cruciferous Vegetables, and Some Properties of Cabbage Myrosinase in Taiwan

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Abstract: The myrosinase activity and total glucosinolates of 10 different cruciferous vegetables and some properties of cabbage (*Brassica oleracea* L var *capitata* L) myrosinase were investigated. Radish, cabbage and broccoli showed higher myrosinase activity than other samples. No correlation ($P > 0{\cdot}05$) was found between myrosinase activity and total glucosinolates of cruciferous vegetables tested. Myrosinase activity for white cabbage was higher than red cabbage. An optimal pH of myrosinase activity was observed at pH 8·0 for both white and red cabbages. The myrosinase extracted from cabbage was more stable in neutral or alkaline pH. The optimal temperature of myrosinase activity for both cabbages was about 60°C, but the myrosinase activity was destroyed after heating at 70°C for 30 min. The myrosinase activities of both white and red cabbages were activated by 10 mM and 5 mM ascorbic acid, respectively.

Key words: myrosinase, total glucosinolates, cruciferous vegetables.

INTRODUCTION

Glucosinolates (thioglucosides) are sulphur-containing compounds present in *Brassica* plants (Kjaer 1976). The characteristic flavour and aroma of *Brassica* vegetables are due, in part, to the presence of volatile compounds arising from the enzymic hydrolysis of glucosinolates (Fenwick *et al* 1983). High intake levels of certain glucosinolates are associated with toxic effects, such as goitre development from ingested progoitrin (Fenwick *et al* 1983). However, indole glucosinolates have been shown to inhibit experimental chemical carcinogenesis (McDannell *et al* 1987). Therefore, the glucosinolate content of cruciferous vegetables has been extensively studied (Van Etten *et al* 1976; Daxenbichler *et al* 1979; Heaney and Fenwick 1980; Sones *et al* 1984).

Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) is responsible for the development of the pungent flavour of many *Brassica* products, because it hydrolyses the glucosinolates. Myrosinase appears to be important with regard to the quality and the safety of cruciferous food and feed materials. Myrosinase seems to occur in all plants that contain glucosinolates (Ettlinger and Kjaer 1968). Wilkinson *et al* (1984) investigated the myrosinase activity of 12 cruciferous vegetables, but did not determine the glucosinolate content. Furthermore, data concerning the relationship between glucosinolate content and myrosinase activity of cruciferous vegetables is not available in the literature.

The properties of myrosinase have been investigated in some cruciferous vegetables, such as yellow mustard (*Brassica juncea*) (Ohtsuru and Hata 1972), rapeseed (*Brassica napus* L) (Lonnerdal and Janson 1973), wasabi (*Wasabia japonica*) (Ohtsuru and Kawatani 1979) and Brussels sprouts (Springett and Adams 1989). However, little information is available on the myrosinase properties of cabbage (*Brassica oleracea* L var *capitata* L). Since cabbage is consumed in large amounts as either cooked or processed products in Taiwan, it is important to establish the properties of myrosinase and, in particular, those factors which control activity of the enzyme.

The objectives of this study were to determine the

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relationship between myrosinase activity and glucosinolate content of various cruciferous vegetables, and to investigate some of the properties of cabbage myrosinase.

MATERIALS AND METHODS

Samples and chemicals

Cruciferous vegetables, including Chinese mustard (*Brassica chinensis*), Chinese cabbage (*B pekinensis*), Chinese kale (*B oleracea* L var *alboglabra*), broccoli (*B oleracea* L var *italica* Plenca), cabbage (*B oleracea* L var *capitata* L), cauliflower (*B oleracea* L var *botrytis* L), kohlrabi (*B caulorapa* Pasq), leaf mustard (*B juncea* L), and radish (*Raphanus sativuns* L), were purchased from local supermarkets in Taichung (Taiwan).

Sinigrin, myrosinase (200 units g^{-1}), aryl sulphatase (300 units mg^{-1}) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Protein assay reagent kit was obtained from Bio-Rad Co. (Richmond, CA, USA). Glucose test kit was obtained from Boehringer Mannheim GmbH (Germany). Sephadex A-25 was purchased from Pharmacia LKB Biotechnology AB (Sweden). All other chemicals were reagent grade from E. Merck (Germany).

Sample preparation and total glucosinolate analysis

Each of two samples of each cruciferous vegetable was ground with a Waring blender at −20°C and stored in the powdered form at −40°C until extracted. The powder (10 g) was extracted with boiling methanol (100 ml) and the extract concentrated under vacuum below 40°C. The crude extracts were stored at −20°C until analysed. Total glucosinolate content was determined by the method of Heaney and Fenwick (1981). A control test was conducted for endogenous glucose without the addition of myrosinase. Two replicate experiments were performed, and analyses of all samples were run in duplicate and averaged.

Preparation of crude enzyme extract

Ten grams of sample powder was homogenised with 30 ml 0·1 M sodium phosphate buffer (pH 7·0) containing 0·01 M mercaptoethanol. The homogenate was filtered and centrifuged at 10000 × *g* at 0°C, and the supernatant was made to 90% saturation (on a weight basis) at 0°C with ammonium sulphate. The resultant precipitate was centrifuged at 10000 × *g* at 0°C for 30 min and the pellet resuspended in 0·01 M sodium phosphate buffer (pH 7·0) containing 0·001 M mercaptoethanol. After passed through an Avidchrom desalting cartridge (Bioprobe International, Inc., Tustin, CA, USA), the extract was held at 4°C and determined immediately.

Determination of myrosinase activity

The myrosinase activity was determined according to the procedure previously reported by Wilkinson *et al* (1984) except that the enzymes and cofactors of the Boehringer text kit were used directly. One millilitre of nicotinamide adenine dinucleotide phosphate (NADP)/adenosine triphosphate reagent, 0·02 ml hexokinase/glucose-6-phosphate dehydrogenase reagent, 0·5 ml 10 mM sinigrin in 0·1 M sodium phosphate buffer (pH 7·0) and 1 ml 10 mM ascorbic acid in 0·1 M sodium phosphate buffer were placed in each of two 3-ml glass cuvettes (1 cm light path). After equilibration for 5 min at 30°C, 0·1 ml enzyme extract was added to the sample cuvette and the absorbance increase due to the formation of NADPH measured at 340 nm. Myrosinase activity was expressed as the initial, linear rate of increase in NADPH absorbance (A_{340} nm min^{-1}) (Wilkinson *et al* 1984). A buffer blank was tested for background presence of glucose or to verify that no glucose was produced non-enzymically. All tests and analyses were run in duplicate and averaged.

Effect of ascorbic acid on cabbage myrosinase activity

Sinigrin concentrations were 1, 2·5, 5, 10, 15 and 20 mM, and ascorbic acid concentrations were 0, 1, 5, 10, 20 and 50 mM. Crude enzyme extract (0·2 ml, prepared from red or white cabbage) and 0·4 ml ascorbic acid in 0·1 M sodium phosphate buffer (pH 7·0) were mixed and equilibrated at 30°C for 5 min. The reaction was initiated by adding 0·4 ml 20 mM sinigrin and terminated by vigorously mixing in 0·2 ml 180 g $litre^{-1}$ metaphosphoric acid after 10 min. Glucose concentration was determined by means of the Boehringer glucose test kit.

Effect of pH on cabbage myrosinase activity

The crude enzyme extract (1 ml) and 0·4 ml 0·01 M ascorbic acid in buffer solutions at various pH values were mixed and equilibrated at 30°C for 5 min. The pH was adjusted within the range 3–9 using 0·025 M sodium borate (pH 9·0), 0·1 M sodium phosphate (pH 6·0, 7·0, 8·0) and 0·1 M sodium citrate (pH 3·0, 4·0, 5·0) buffers. The reaction was initiated by adding 0·4 ml 0·02 M sinigrin in the same buffer at 30°C and stopped after 30 min by vigorously mixing in 0·2 ml 180 g $litre^{-1}$ metaphosphoric acid. Glucose concentration was determined for each pH using the Boehringer glucose test kit. Myrosinase activity was taken as the slope of the regression line of glucose concentration against time (1 mM glucose min^{-1}).

For determining the pH stability in the range 3–9, the crude myrosinase extract was incubated in buffers at

each of the chosen pH values for 1 h at 30°C. The myrosinase activity at pH 7 was then measured as described above.

Effect of temperature on cabbage myrosinase activity

The crude myrosinase extract (0·2 ml), 0·4 ml 0·01 M ascorbic acid and 0·8 ml 0·1 M sodium phosphate buffer (pH 7·0) were mixed and equilibrated at various temperatures (20–70°C) for 5 min. The reaction was then started by the addition of 0·4 ml 0·02 M sinigrin at the same temperature and stopped after the desired time, by adding 0·2 ml 180 g litre^{-1} metaphosphoric acid. The myrosinase activity was then measured as described above.

For determining the thermal stability between 30 and 70°C, the crude myrosinase extract (0·2 ml) in 0·8 ml 0·1 M sodium phosphate buffer (pH 7·0) was incubated at each of the chosen temperatures for 30 min. After cooling the resulting enzyme solutions to 4°C, the myrosinase activity at 30°C was then measured as before.

Protein determination

Protein concentration was measured by the method of Bradford (1976) using the Bio-rad protein assay. A standard curve was constructed using bovine serum albumin in the concentration range 0·2–1·4 mg ml^{-1}.

Statistical analysis

Statistical analysis involved used of the SAS (1985) software package. Significant differences between means were determined by Duncan's multiple range test.

TABLE 1
Myrosinase activity and total glucosinolates content of various cruciferous vegetables

Species	*Myrosinase activity*[a] (*μmol min*$^{-1}$ *mg*$^{-1}$ *protein*)	*Total glucosinolates*[a] (*mg kg*$^{-1}$ *fresh weight*)
Chinese mustard	3·41 ± 0·34	572 ± 166
Chinese cabbage	2·45 ± 0·35	89 ± 33
Cauliflower	2·84 ± 0·32	117 ± 39
Chinese kale	2·02 ± 0·18	622 ± 110
Broccoli	9·87 ± 1·95	408 ± 107
Cabbage (white)	6·08 ± 0·52	229 ± 52
Cabbage (red)	4·05 ± 0·41	642 ± 103
Radish	22·64 ± 3·19	1724 ± 192
Kohlrabi	3·06 ± 0·19	524 ± 115
Leaf mustard	3·47 ± 0·27	2815 ± 355

[a] Mean ± SD of two replicate experiments.

RESULTS AND DISCUSSION

Relationship between myrosinase activity and total glucosinolates of some cruciferous vegetables

Table 1 shows the myrosinase activity and total glucosinolates of some cruciferous vegetables produced in Taiwan. Among 10 different cruciferous vegetables, radish, broccoli and white cabbage showed higher myrosinase activity than that of other vegetables. Radish had the highest myrosinase activity (20·64 μmol min^{-1} mg^{-1} protein), while Chinese kale had the lowest myrosinase activity (2·02 μmol min^{-1} mg^{-1} protein). This result is in agreement with the report of Wilkinson *et al* (1984), suggesting that radish (12·8 μmol min^{-1} mg^{-1} protein) has the greatest myrosinase activity among the 12 cruciferous vegetables. The myrosinase activities of white and red cabbages were 6·08 and 4·05 μmol min^{-1} mg^{-1} protein, respectively. These values were higher than the report of Wilkinson *et al* (1984) which showed 5·2 and 2·3 μmol min^{-1} mg^{-1} protein for white and red cabbages, respectively. The results also showed that leaf mustard had the highest content of total glucosinolates (2·815 g kg^{-1}), however, its myrosinase activity was only 3·47 μmol min^{-1} mg^{-1} protein. The Chinese cabbage contained the lowest level of total glucosinolates (89 mg kg^{-1}) but its myrosinase activity (2·45 μmol min^{-1} mg^{-1} protein) was greater than that of Chinese kale (2·02 μmol min^{-1} mg^{-1} protein) which had a total glucosinolate content of 622 mg kg^{-1}. Although radish had the highest myrosinase activity, its total glucosinolates (1·724 g kg^{-1}) was not the highest among the test samples. Based on these results, it can be concluded that myrosinase coexisted with glucosinolates in cruciferous vegetables, however, there is no clear relationship between the myrosinase activity and the content of total glucosinolates ($r = 0·3178$, $n = 10$, $P < 0·05$).

The white cabbage was consumed all year round in Taiwan, therefore, the property of myrosinase in white cabbage was investigated in the following study and compared with the red cabbage.

Effect of pH on myrosinase activity from cabbages

The effect of pH on activity of myrosinase from white and red cabbages is shown in Fig 1. The results indicate an optimum pH at 8·0 for both white and red cabbages, which show significant difference from other pH values ($P < 0·05$). West *et al* (1977) have shown two pH optima at 5·0 and 8·0 for myrosinase in crude extracts from cabbage. Bjorkman and Janson (1972) reported that the optimum for white mustard and rapeseed myrosinases ranged from pH 4·5 to 4·9. Two optima were reported for Brussels sprout thioglucosidase, pH 6·0–6·5 and pH 8·0 (Springett and Adams 1989). However, the optimum of

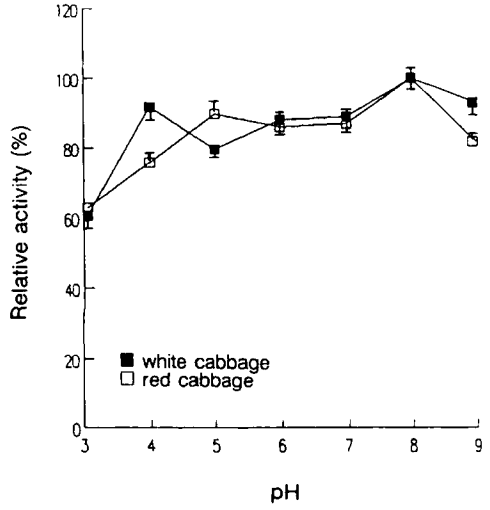

Fig 1. pH profiles of myrosinase from white and red cabbages. The sample solution was incubated in 0·1 M citrate buffer (pH 3–5), or 0·1 M phosphate buffer (pH 6–8), or 0·025 M borate buffer (pH 9) at 30°C for 30 min before measurement of the enzymic activity.

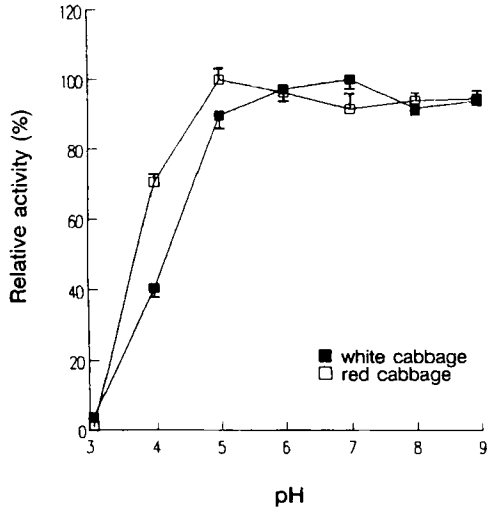

Fig 2. pH stability curves of myrosinase from white and re cabbages. The sample solution was incubated in various pH buffers at 30°C for 1 h before measurement of the enzymic activity.

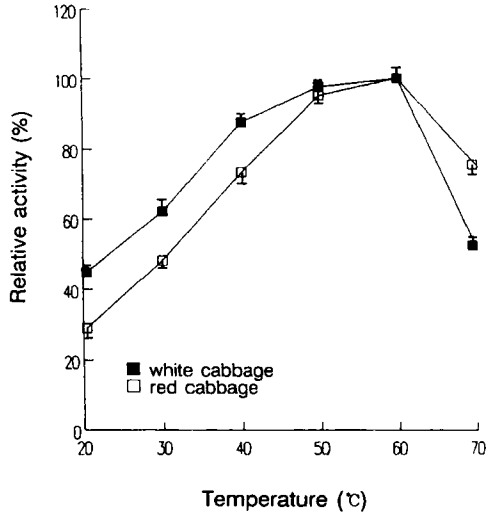

Fig 3. Temperature profiles of myrosinase from white and red cabbages. The sample solution was incubated in 0·1 M phosphate buffer, 0·01 M ascorbic acid, pH 7·0, at each temperature before measurement of the enzymic activity.

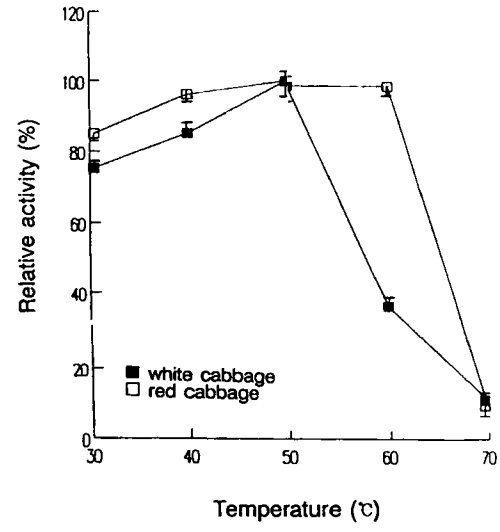

Fig 4. Heat stability curves of myrosinase from white and red cabbages. The sample solution was incubated at various temperatures for 30 min before measurement of the enzymic activity.

wasabi myrosinase is about pH 6·5–7·0 (Ohtsuru and Kawatani 1979).

The pH stability in myrosinase from cabbage is shown in Fig 2. The myrosinase from both cabbages was stable at pH values between 6·0 and 9·0 (no significant difference at $P > 0{\cdot}05$) but stability declined markedly at pH < 5·0. Bjorkman and Janson (1972) indicated that the activity of myrosinase decreases rapidly at pH 3·0, while the activity is stable at pH 9·0.

Effect of temperature on myrosinase activity from cabbages

Figure 3 shows the temperature dependency of myrosinase activity. Myrosinase activity increased with increasing temperature up to 60°C; and decreased above 60°C. This result is similar to those obtained for myrosinase from white mustard and rapeseed (Bjorkman and Janson 1972). However, the optimal temperature of myrosinase from Brussels sprouts is 50°C (Springett and Adams 1989).

The thermal stability of myrosinase from cabbage is shown in Fig 4. It shows that red cabbage myrosinase is substantially more stable than white cabbage myrosinase at 60°C ($P < 0{\cdot}05$). However, about 90% of myrosinase activity for both white and red cabbages was destroyed after heating at 70°C for 30 min. Springett and Adams (1989) reported that the myrosinase activity of Brussels sprouts was stable below 50°C, but the activity was lost when heated at 60–90°C.

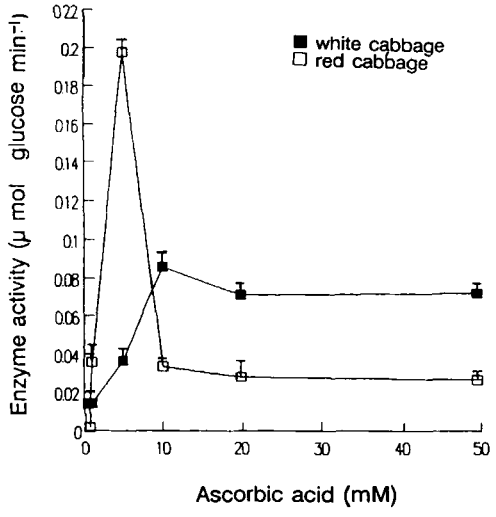

Fig 5. Effect of ascorbic acid concentration on myrosinase activity from white and red cabbages. The sample solution was incubated in various ascorbic acid concentrations at 30°C, pH 7·0 for 30 min before measurement of the enzymic activity.

Effect of ascorbic acid on myrosinase activity from cabbages

The myrosinase activities of red and white cabbages were enhanced by 5 mM and 10 mM ascorbic acid, respectively (Fig 5). Cabbage myrosinase activity was low in the absence of ascorbic acid. Ohtsuru and Kawatani (1979) reported that the activity of wasabi myrosinase is enhanced by ascorbic acid at 2 mM but at higher concentrations the enhancement fell away.

CONCLUSIONS

Based on the results of this study, the characteristics and activity of myrosinase varied between different cruciferous vegetables. No correlation was found between myrosinase activity and total glucosinolate content. These results may have important implications for food industry produce and provide the basis for controlling the glucosinolate hydrolysis during food preparation and processing.

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