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# Endogenous and exogenous enzymolysis of vegetable-sourced glucosinolates and influencing factors

Lianqing Shen, Guangyao Su *, Xiangyang Wang, Qizhen Du, Kuiwu Wang

*College of Food and Biology Engineering, Zhejiang Gongshang University, Hangzhou 310035, China*

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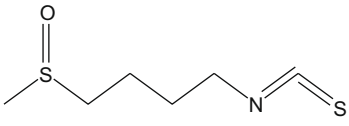
ABSTRACT

Glucosinolates are naturally abundant in many vegetable sources. These compounds have limited health benefit in their original forms, however their derived product, sulforaphane, has been shown to be hugely health beneficial in protecting against certain types of cancer. This work investigated the conversion of glucosinolates (glucoraphanin) to sulforaphane using either an endogenous myrosinase or an exogenous myrosinase under various enzymolysis conditions. It was found that an optimum degradation of glucosinolates to sulforaphane by the endogenous method was achieved under the following conditions: a liquid–solid ratio of 3 ml/g, an enzymolysis time of 8 h, at 25 °C, at pH 4.0, and with the addition of ascorbic acid 0.02 mg/g. This gave 35% conversion rate of glucosinolates to sulforaphane. However, the exogenous approach appeared to be much more efficient in converting glucoraphanin to sulforaphane. At a combined condition of a liquid–solid ratio of 1000 ml/g, 3 h enzymolysis, at 35 °C and pH 5.0, and in the presence of 0.02 mg/g ascorbic acid, as much as 68% of glucoraphanin was found to be degraded to form sulforaphane.



## 1. Introduction

Sulforaphane has recently received great attention from food scientists and nutritionists because of its reported bioactivity in enhancing detoxification of carcinogens and in blocking the initiation of chemically-induced carcinogenesis in animal models (Gills et al., 2006; Zhang, 2004). It is generally believed that the mechanism by which sulforaphane protects cells is through Nrf2-mediated induction of phase two detoxification enzymes that elevate cell defence against oxidative damage and promote the removal of carcinogens (Juge, Mithen, & Traka, 2007; Levi, Borne, & Williamson, 2001). Sulforaphane is a type of isothiocyanates with a molecular weight of 177.29 Da ($C_6H_{11}S_2NO$). It is easily water soluble but rather unstable in an aqueous environment. Its molecular structure is shown below.




Sulforaphane is unfortunately not directly available from food sources, but is a derived product via enzymatic hydrolysis from glucosinolates, a group of compounds naturally abundant in some plant source vegetables (Hecht, 1999). Glucosinolates themselves have limited health benefit, but after the enzymatic reaction, they could be degraded to form sulforaphane together with some other toxic compounds. The key enzyme for this conversion process is the so-called myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1). Interestingly, even though myrosinase co-exist with glucosinolates in plant cells, enzymolysis reaction is only possible when plant cells are disrupted during chopping and cooking. It is believed that myrosinase breaks up the β-thioglucoside bond of glucosinolate molecules, producing glucose, sulphate and a diverse group of aglycon products. The resultant aglycones then undergo non-enzymatic intramolecular rearrangement to yield nitriles, epithionitriles, thiocyanates and isothiocyanates (Liang, Li, Yuan, & Vriesekoop, 2007; McGregor & Mullin, 1983).

Fig. 1 shows the general sequences of glucosinolates enzymolysis (Steven & Mark, 2005), where glucoraphanin, a typical glucosinolate compound, is used as an example. The formation of hydrolysis products will of course depend on the –R group of glucosinolate and reaction conditions. Under acidic conditions (pH 2–5), epithionitrile, thiocyanates, and nitriles will be the main enzymolysis products, but at or close to a neutral environment (pH 5–8), sulforaphane and oxazolidine-thione will be dominating. However, at above pH 8 and if –R group is indole or contains benzenoid structure, isothiocyanates may rearrange to become thiocyanates. In the presence of –OH group, spontaneous cyclization to generate oxazolidine-thione occurs (Serkadis, Getahun, & Fung-Lung, 1999). It has also been known that glucosinolates could also be degraded via

* Corresponding author. Tel.: +86 571 28877624; fax: +86 571 28877605.
*E-mail address:* suguangyao@yahoo.com.cn (G. Su).

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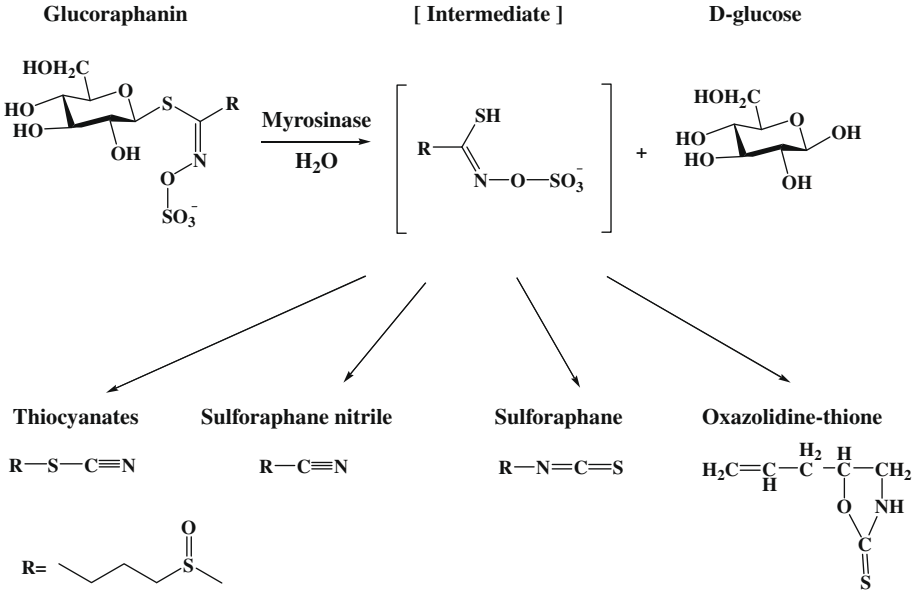


**Fig. 1.** The sequences of glucosinolate hydrolysis by myrosinase and its products.

non-enzymatic method. For example, a combined high pressure and heating treatment could lead to glycerinate degradation. But, surprisingly, a non-enzymatic treatment appeared to produce little amount of isothiocyanate (or sulforaphane) (Jiang, Zhang, & Li, 2005).

Enzymatic activity of myrosinase and its capability in glucosinolates degradation could be influenced by a number of factors. For example, it was observed that the myrosinase sourced from cabbage could be activated in the presence of ascorbic acid (vitamin C) and had its optimum performance at pH 8.0 and 60 °C, but its enzymatic activity ceased to function after 30 min exposure at 70 °C (Yen & Wei, 1993). A separate study indicated that the myrosinase from *Brassica juncea Coss* had its optimum enzymatic performance at pH 6.0–7.0 and at 70 °C (Zhao & Yang, 1998). It was also found that the presence of ascorbic acid enhanced the enzyme activities, however salt (NaCl) tended to suppress such activities. This repression effect was most significant once NaCl concentration was higher than 0.5% and myrosinase almost completely lost its activity once NaCl concentration exceeded 4% (Zhao & Yang, 1998).

This work investigated the use of two different types of myrosinases (endogenous and exogenous) in glucosinolates degradation to sulforaphane. Broccoli seeds were used for the investigation because of their rich content of glucoraphanin (20–50 mg/g) (West et al., 2004). The optimum enzymolysis conditions for the two methods were determined. It is hoped that findings from this work could enhance our understanding of glucosinolates conversion to sulforaphane during food preparation and consumption and to provide useful guidance for possible industrial applications in using enzymatic methods for the production of sulforaphane from various plant sources. It should also be noted that, in this work, only one type of glucosinolates (glucoraphanin) was investigated for its enzymolysis. Therefore, terms of glucoraphanin and glucosinolates were interchangeable in this paper.

## 2. Materials and methods

### 2.1. Materials

Luxiong 90 broccoli seeds were obtained from Hangzhou Seeds Company (Hangzhou, China); sulforaphane standard was purchased from Sigma (St. Louis, Mo); glucoraphanin was provided by Prof. Q. Du of Zhejiang Gongshang University and was stored at −20 °C.

Methanol (HPLC grade) was purchased from Huadong Chemical Reagents Ltd. (Hangzhou, China). Acetone, hexane, ascorbic acid, trishydroxymethylaminomethane (Tris), ammonium sulphate, sodium dihydrogen phosphate, disodium hydrogen phosphate, citric acid, hydrochloric acid, and ethanol were all AR grade and supplied by the same company.

### 2.2. Methods

#### 2.2.1. Preparation of the calibration curve

A HPLC standard curve of sulforaphane was prepared as follow: adding 0.5 ml methanol into 5 mg sulforaphane to make a sulforaphane solution with a concentration of 10 mg/ml. This solution was further diluted with methanol to make a set of sulforaphane solutions with concentration of 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2 mg/ml. Each standard solution was analysed using a HPLC (Shimadzu LC-20AT, Shimadzu, Japan). The peak area for each solution was recorded and plotted against sulforaphane concentration to form a standard curve. This standard curve was then used for the quantification analysis of sulforaphane formation from various enzymolysis reactions.

#### 2.2.2. Controlled enzymolysis using endogenous myrosinase

Two grams of broccoli seeds powder and a controlled amount of distilled water were mixed into a 100 ml beaker. The beaker was then covered with clingfilm to prevent evaporation and dust contamination, and left undisturbed for a controlled length of time and at a controlled temperature. The amount of distilled water was weighed accurately to give a precise liquid/solid ratio.

For the effect of pH condition, 2 g of broccoli seed powder together with 6 ml distilled water were mixed into a 100 ml beaker. The pH was adjusted to 1.0, 2.0, 3.0, 4.0, 6.0, 7.0 and 8.5 respectively, using 1 M HCl and 1 M trishydroxymethylaminomethane (Tris) solution. So, some variations of HCl molarity in these solution were expected, however this shouldn't have had any significant effect on the hydrolysis process. The beakers were covered with clingfilm and left still for 8 h at a controlled temperature for hydrolysis.

To determine the effect of ascorbic acid, a set of beakers each containing 2 g broccoli seeds powder and 6 ml distilled water were

prepared. Ascorbic acid was further added to each beaker to give an acid/solid ratio of 0, 0.02, 0.2, 2, 4, 6.0, 8.0, 10.0, and 20.0 mg/g, respectively. Samples were thoroughly stirred for complete mixing before been sealed with clingfilm and left for enzymolysis for 8 h.

*2.2.3. Separation of hydrolysed products*

Hydrolysed samples were freeze dried overnight. The powder obtained was dispersed into 40 ml acetone and treated with an ultrasonic bath for 60 min before being filtered. The filtrate was carefully evaporated to remove solvent. The raw product collected after evaporation was further treated with (3 × 2 ml) distilled water and centrifuged at 12,000 rpm/min at 4 °C for 10 min. The collected supernatant was filtered using 0.22 μm membrane and made ready for HPLC analysis.

*2.2.4. Quantification of enzymolysis yield*

The efficiency of glucosinolates enzymolysis was quantified by the rate of conversion of glucoraphanin to sulforaphane and is expressed as the percentage of glucoraphanin converted to sulforaphane to the total amount of glucoraphanin as shown by the following equation:

$$\text{Conversion rate}(\%) = \frac{\text{Amount of glucoraphanin converted to sulforaphane} \times 436}{\text{Total amount of glucoraphanin} \times 177} \times 100,$$

where 436 is the molecular weight of glucoraphanin and 177 is the molecular weight of sulforaphane. It should be noted, that even though experimental results have been analysed quantitatively, no statistical analysis has been applied.

*2.2.5. Preparation of exogenous myrosinase*

Twenty grammes of broccoli seeds were first milled using a mill grinder in the presence of liquid nitrogen and then mixed with 60 ml 0.2 M tris–HCl buffer (pH 7.5). The mixture was filtered and then centrifuged at 4 °C and at 12,000 g for 20 min. The supernatant was added with 80% ammonium sulphate to lead to protein precipitation. The suspension was centrifuged again under the same condition and the collected precipitate was added with a minimal amount of 0.2 M tris–HCl buffer and was dialyzed overnight at 4 °C in a dialysis bag with molecular weight cut off 14,000 Da. The obtained myrosinase was kept in a fridge of 4 °C for further use.

*2.2.6. Controlled enzymolysis using exogenous myrosinase*

Glucoraphanin solution of 10 mg/ml was prepared as a stock. Carefully transfer 50 μl this stock solution into 2 ml centrifuge tubes and, into each tube, a certain amount of crude myrosinase solution (125, 250, 375, 500, 625, 875, and 1000 μl) was added. Mixtures were left undisturbed for enzymolysis for 2 h. After that, distilled water was added to make up a total volume to 2 ml and samples were centrifuged at 12,000 g at 4 °C for 10 min. The supernatant was filtered through a 0.22 μm membrane before HPLC analysis.

For the effects of hydrolysis time, samples of 50 ml glucoraphanin solution plus 500 ml myrosinase solution were enzymolysed at room temperature for a set time of up to 8 h. For the effects of temperature, similar systems were studied at a temperature between 5, 15, 25, 35, 45, 55, and 65 °C for 2 h. After enzymolysis, samples were treated further to obtain enzymolysed product as discussed above.

Similarly, pH effect on exogenous myrosinase hydrolysis has also been conducted. The procedure was the same as discussed above, except that disodium hydrogen phosphate–citric acid buffer solution was used to adjust the pH to 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 respectively. For the investigation of the effect of ascorbic acid, 0.04, 0.08, 0.16, 0.4, 0.8, 4.0, 8.0, and 16.0 mg of ascorbic acid was added and the enzymolysis was conducted at room temperature for 2 h.

*2.2.7. HPLC and GC-MS analysis*

Sulforaphane was analysed using a Shimadzu HPLC system (Shimadzu LC-20AT, Shimadzu Cooperation, Tokyo, Japan) equipped with Shimadzu model LC-20AT pumps. A Shimadzu reversed-phase C18 column (250 mm × 4.6 mm, 5 μm) was used at column temperature of 25 °C and the injection volume was 10 μl. A binary solvent mixture of water–methanol (60:40, v/v) was used as the mobile phase and measurements were performed at a flow rate of 1.0 ml/min and a Shimadzu SPD-20A detector set at 201 nm was used.

Experiments were conducted with a Trace 2000 GC–DSQ MS instrument (Thermo Electron, USA) with the NIST (V2.0) MS library. A solution of the sample in $CH_2Cl_2$ was injected directly into the GC inlet for analysis. The sample was separated on a 30 m × 0.25 mm × 0.25 μm HP-5 ms capillary column (Agilent). Helium (>99.9%) was used as carrier gas at a constant flow of 1.0 ml/min. The column temperature was maintained at 50 °C for 2 min then programmed at 10 °C/min to 250 °C which was maintained for 8 min. The GC inlet temperature was 250 °C and the transfer line temperature was 250 °C. Mass spectra were obtained by electron-impact (EI) ionization over the mass range 35–500 amu at a scan rate of 2 scans/s. The ion-source temperature was 200 °C, and the electron energy was 70 eV.

## 3. Results and discussion

### 3.1. Determination of sulforaphane

HPLC method was used to determine sulforaphane both qualitatively and quantitatively. A standard sulforaphane solution was first analysed using HPLC technique and results are shown in Fig. 2a, where an elution time of about 9.635 min is clearly observed. This elution time will be used for quantification of sulforaphane from glucosinolates hydrolysis. Fig. 2b is the HPLC analysis of the enzymolysed mixture of glucosinolates using the endogenous myrosinase enzyme. The peak of sulforaphane is clearly identifiable (at 9.827 min). In addition to sulforaphane, a number of other by-products have also been identical between 2 and 8 min elution time. In comparison, the enzymolysis by using exogenous myrosinase produced relatively less number of by-products, with the peak of sulforaphane also distinctively observable (at 9.992 min) (see Fig. 2c). The reason for longer elution time for sulforaphane in the enzymolysed mixture than that of a pure system is not yet known, but one may speculate that the presence of by-products may be the main cause for such a delay. Results shown in Fig. 2 demonstrate that HPLC analysis is a reliable and effective technique for the determination of sulforaphane from a mixed system.

The existence of sulforaphane in enzymolysed mixture has also been determined by using a GC-MS method and results are shown in Fig. 3. A characteristic peak at a retention time of 20.20 min and 20.60 min (with relative abundance of 42.23% and 34.36%) was observed respectively for samples from using the endogenous myrosinase (Fig. 3a) and using the exogenous myrosinase (Fig. 3b). From fragmentation analysis, it was confirmed that both peaks were the identification of sulforaphane. The above HPLC and GC-MS analysis evidently demonstrated that the presence of sulforaphane in the enzymolysed mixture from glucosinolates, using both endogenous myrosinase and exogenous myrosinase.

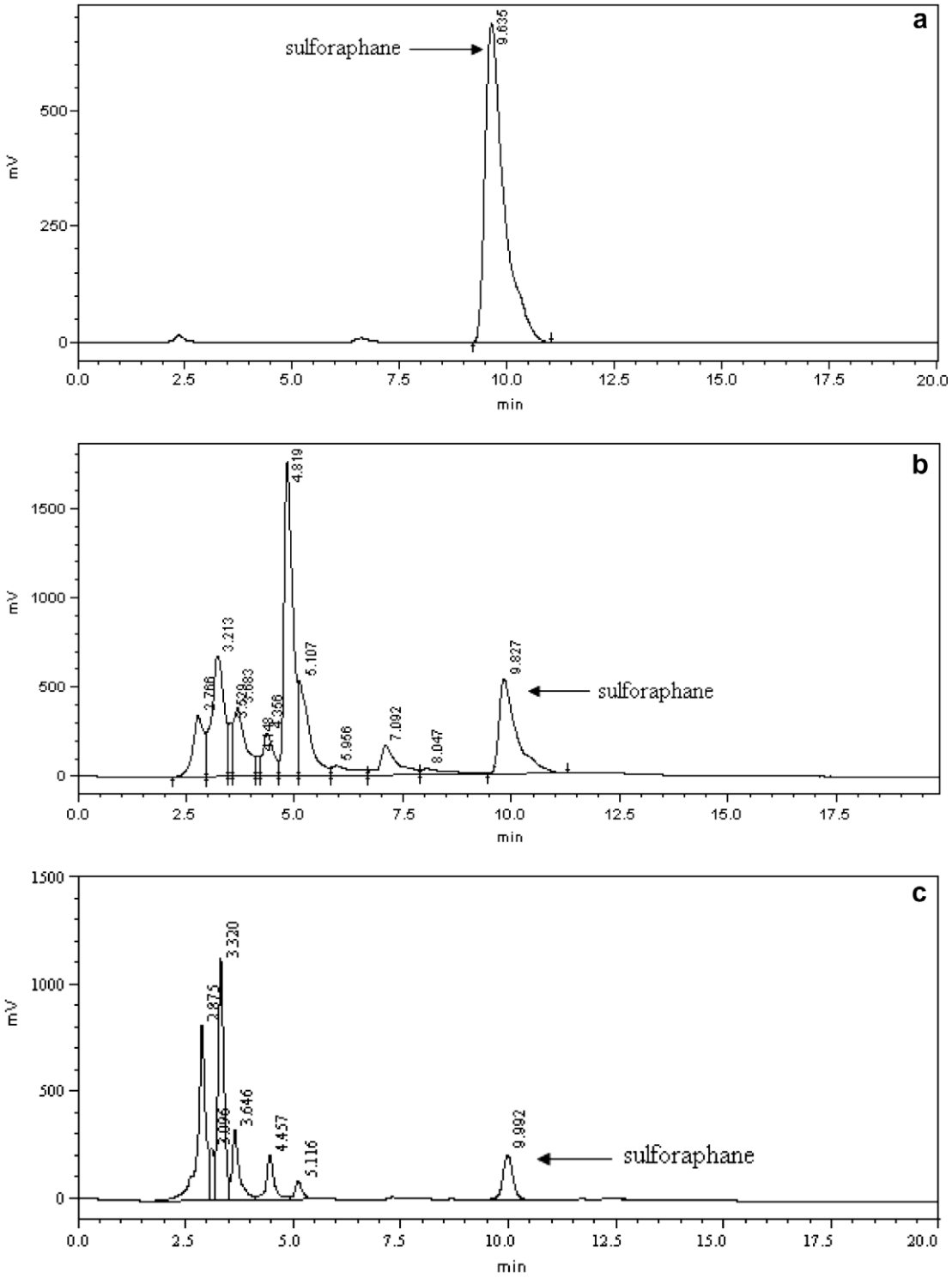


**Fig. 2.** The HPLC analysis of sulforaphane: (a) a pure sulforaphane solution; (b) from hydrolysed mixture by using endogenous myrosinase and (c) from hydrolysed mixture by using exogenous myrosinase.

In the following work, HPLC analysis will be applied further for the quantification of sulforaphane from various enzymolysis processes.

### *3.2. Effects of enzymolysis conditions on glucosinolates conversion by using endogenous myrosinase*

Glucosinolate degradation carried out by using endogenous myrosinase has been investigated under various controlled conditions, including liquid/solid ratio, time, temperature, pH, and the presence of ascorbic acid, and the results are shown in Fig. 4. The liquid/solid ratio was controlled by mixing broccoli seed powder with a varied amount of distilled water. The enzymolysis was conducted at room temperature for 8 h. After the completion of an enzymolysis process, the sample was freeze dried and went through a number of separation processes to extract enzymolysed products. Fig. 4a shows that the enzymolysis rate of glucosinolates gradually increases with the liquid/solid ratio, from around 19% at

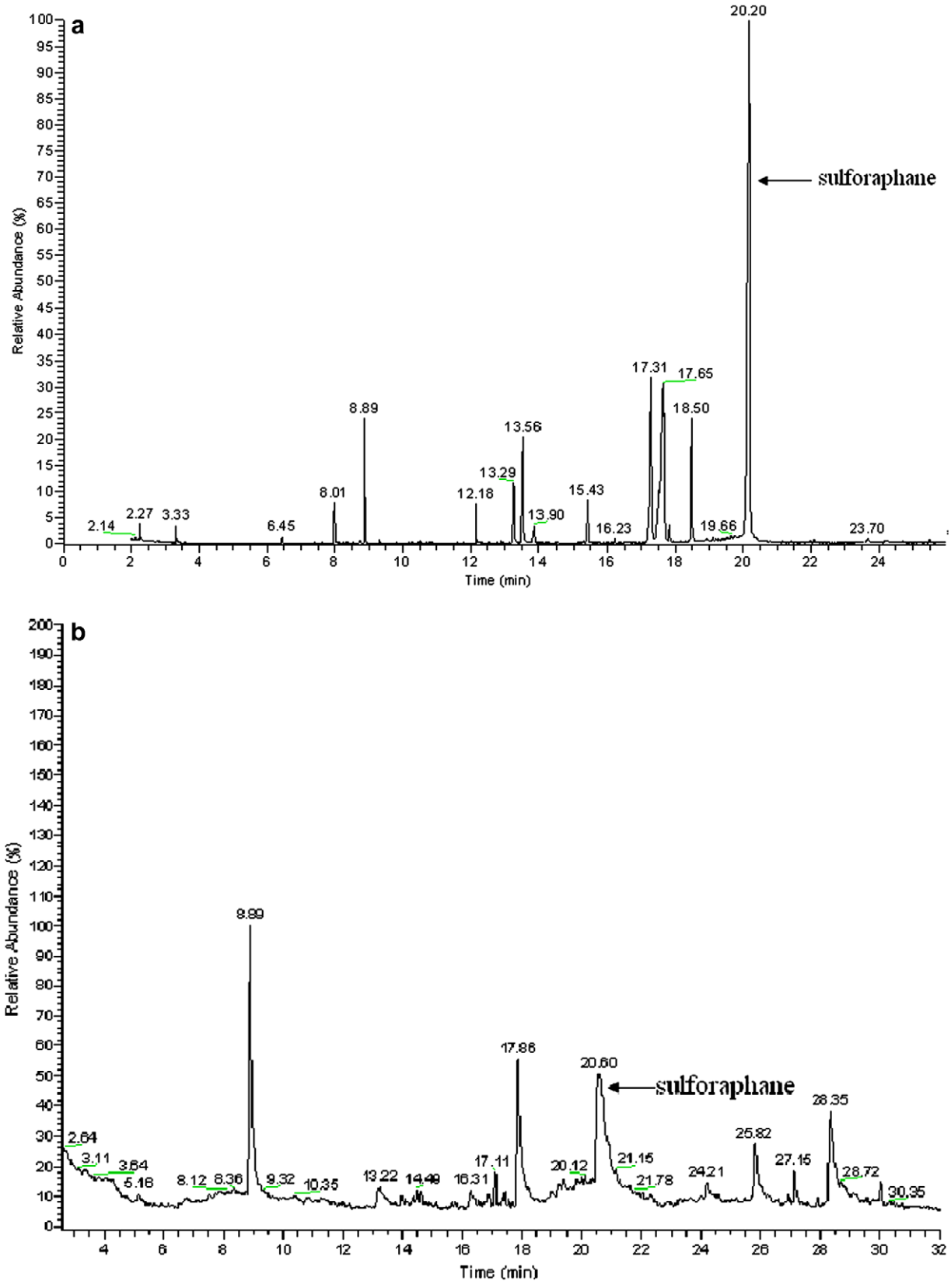


**Fig. 3.** The GC chromatograph of glucosinolate degradation products. (a) Enzymolysis by endogenous myrosinase and (b) enzymolysis by exogenous myrosinase.

the liquid/solid ratio of 1 ml/g to higher than 30% at the ratio of 6 ml/g. However, when considering the need of solvent removal to be carried out by freeze drying, it was decided that a liquid/solid ratio of 3 ml/g should be used, where the enzymolysis rate was 27%. A similar range of liquid/solid ratios have also been reported in literature for enzymolysis studies (Matusheski et al., 2001).

The time effect on glucosinolates enzymolysis is shown in Fig. 4b. For this set of experiments, the liquid/solid ratio was

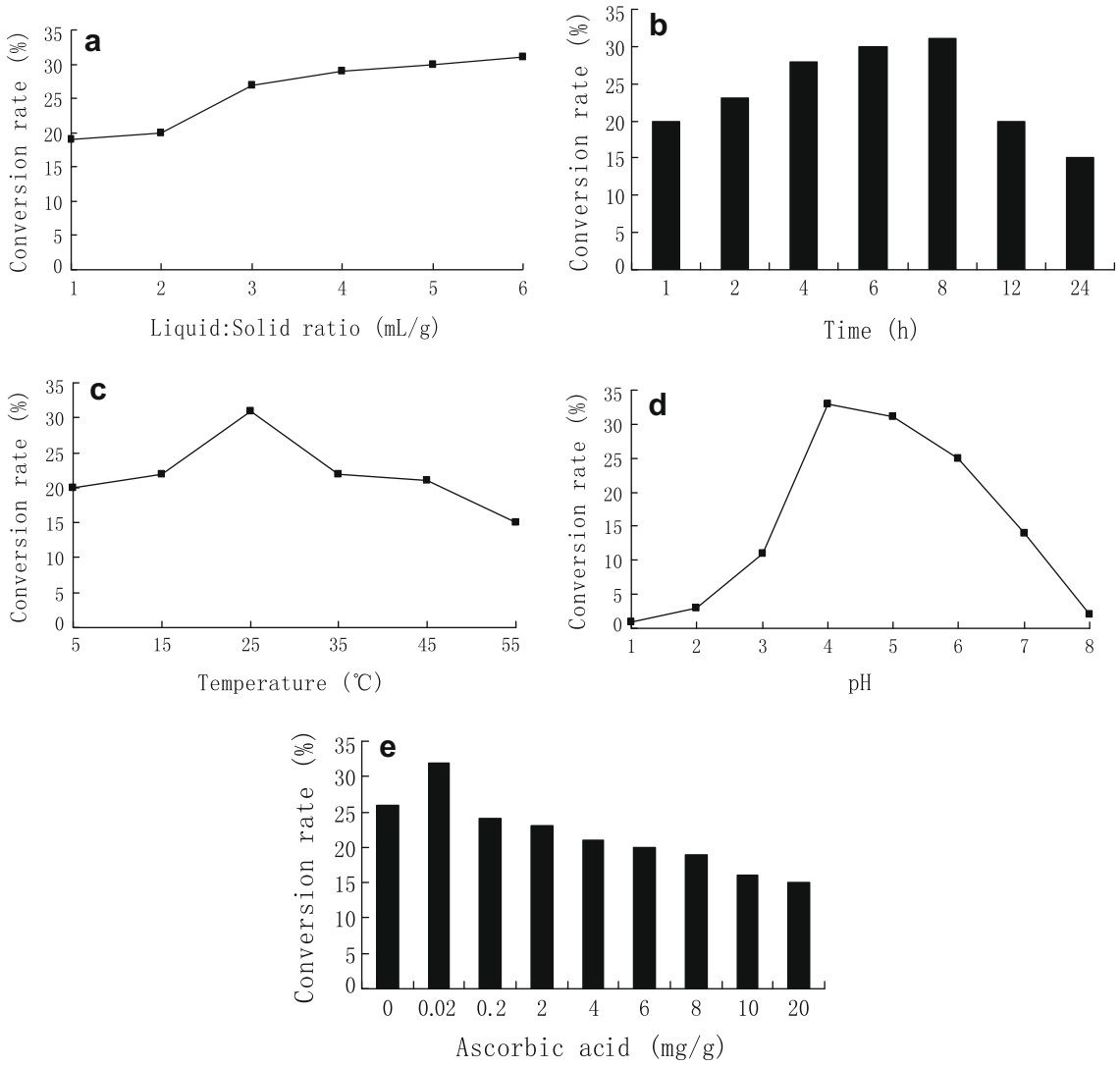


**Fig. 4.** Effects of various conditions on the enzymolysis of glucosinolates using endogenous myrosinase: (a) effects of liquid/solid ratio; (b) effects of time; (c) effects of temperature; (d) effects of pH condition; and (e) effects of the presence of ascorbic acid.

controlled at 3 ml/g and room temperature was applied. The enzymolysis time was investigated for up to 24 h, as previous reported by Matusheski et al. (Matusheski et al. (2001)). It was found that, with the increase of time, enzymolysis showed initial favoured increase of sulforaphane yield, but turned out to be counter-productive during the extended enzymolysis. The highest rate (31%) of glucosinolates enzymolysis was observed at 8 h treatment. This optimum enzymolysis time may be due to the balance between the formation and degradation of sulforaphane. Where enzymolysis is a kinetic process that requires time, and the yield of sulforaphane increases with the time. However, for a further extended time, there could be an increased risk of degradation of sulforaphane, which is known to be highly unstable in an aqueous environment (Cejpek, Urban, Velisek, & Hrabcova, 1998).

Temperature is an important factor for the activities of any enzyme action. Fig. 4c shows temperature effects on the enzymolysis of glucosinolates using endogenous myrosinase, tested over a range between 5 and 55 °C. This temperature range has been confirmed to be feasible for enzymolysis experimentation by many independent researches (Bertelli, Plessi, Braghiroli, & Monzani, 1998; Kore, Spencer, & Wallig, 1993; Matusheski et al., 2001). As we generally believed, too low or too high temperature is not beneficial for enzyme actions. For this particular case, the optimum temperature for myrosinase action appeared to be at 25 °C, when a maximum rate of 31% was observed for glucosinolates enzymolysis.

The most dramatic effect on glucosinolates enzymolysis was found to be the pH condition (Fig. 4d). Both low and high pHs are not favoured for this enzymolysis process. At a pH below 2 and at a pH above 8, the conversion of glucosinolates was almost completely ceased. The most significant increase of the conversion rate was observed between pH 3 and pH 4. Once pH value exceeded 5, the enzymolysis rates showed a relentless decrease with the increase of pH. Therefore, it is clear that a pH at 4 gives the best performance in enzymatic degradation of glucosinolates using an endogenous approach. This agrees well with those previously reported results (Bertelli et al., 1998; Kore et al., 1993).

The presence of ascorbic acid has been shown to have some impacts on myrosinase activity (Yen & Wei, 1993; Zhao & Yang, 1998). In this work, the glucosinolates degradation was monitored with the addition of ascorbic acid over a wide range of concentration (between 0 and 20 mg/g of seed powder) and results are shown in Fig. 4e, where the glucosinolates conversion rate is plotted against the ascorbic concentration. We can see that the presence of a small amount of ascorbic acid could significantly improve the enzymolysis process, with the conversion rate of glucosinolates from only 26% without the ascorbic addition to over 32% in the presence 0.02 mg/g ascorbic acid. The optimum amount of

ascorbic acid obtained in this work was lower than that reported (1 mM) by Ohtsuru and Hata (1979). However, it was noticed that the further addition of ascorbic acid showed no benefit but caused glucosinolates enzymolysis to decrease to a level below that of the reference (without ascorbic acid addition). The stimulating effect of ascorbic acid is believed to be the configuration change it causes to the enzyme (Ohtsuru & Hata, 1979), but it is unclear why higher concentration of ascorbic acid lead to inhibition of myrosinase activity.

### 3.3. Effects of enzymolysis conditions on glucosinolates conversion by using exogenous myrosinase

Investigations into the enzymolysis conditions effect on glucosinolates conversion to sulforaphane has also been conducted for exogenous myrosinase, instead of endogenous enzyme. In this case, glucosinolates solutions were mixed with lab-extracted myrosinase solution. Fig. 5a shows that the enzyme/glucosinolates ratio, expressed as their volume ratio, generally has a positive effect in enhancing the enzymolysis process. The conversion rate of glucosinolates showed increase with the increase of volume ratio (2 h enzymolysis time and at room temperature). However, the most significant enhancing effect was observed for the volume ratio increase from initially 250–1000 ml/g, where the conversion rate of glucosinolates more than doubled from 32% to 65%. Further increase of volume ratio showed limited benefit. Also considering the cost involved in following removal of solvent, it was recommended that a volume ratio of 1000 should be applied. The enzymolysis time was investigated over a period between 1 and 8 h and results are shown in Fig. 5b. We can see that a certain time length (3 h) is required for an optimum production of sulforaphane (65% conversion of glucosinolates).

For exogenous myrosinase, its activity appeared to be maintained to a relatively wide temperature range (Fig. 5c) compared to that of endogenous myrosinase (Fig. 4c). Its enzyme activity more than doubled for the temperature increase from 5 °C to 35 °C. Higher temperatures were not desirable, where a decrease of glucosinolates conversion became evident. The effect of pH on the enzymolysis of glucosinolates was equally dramatic for both exogenous myrosinase and endogenous myrosinase (see Fig. 5d). Between pH 3 and 4, there was a sharp increase of glucosinolate conversion, from 10% to 60%. This increase continued to reach a maximum conversion rate of 75% at pH 5, but a further increase of pH became detrimental. In previous literature, the enzymatic

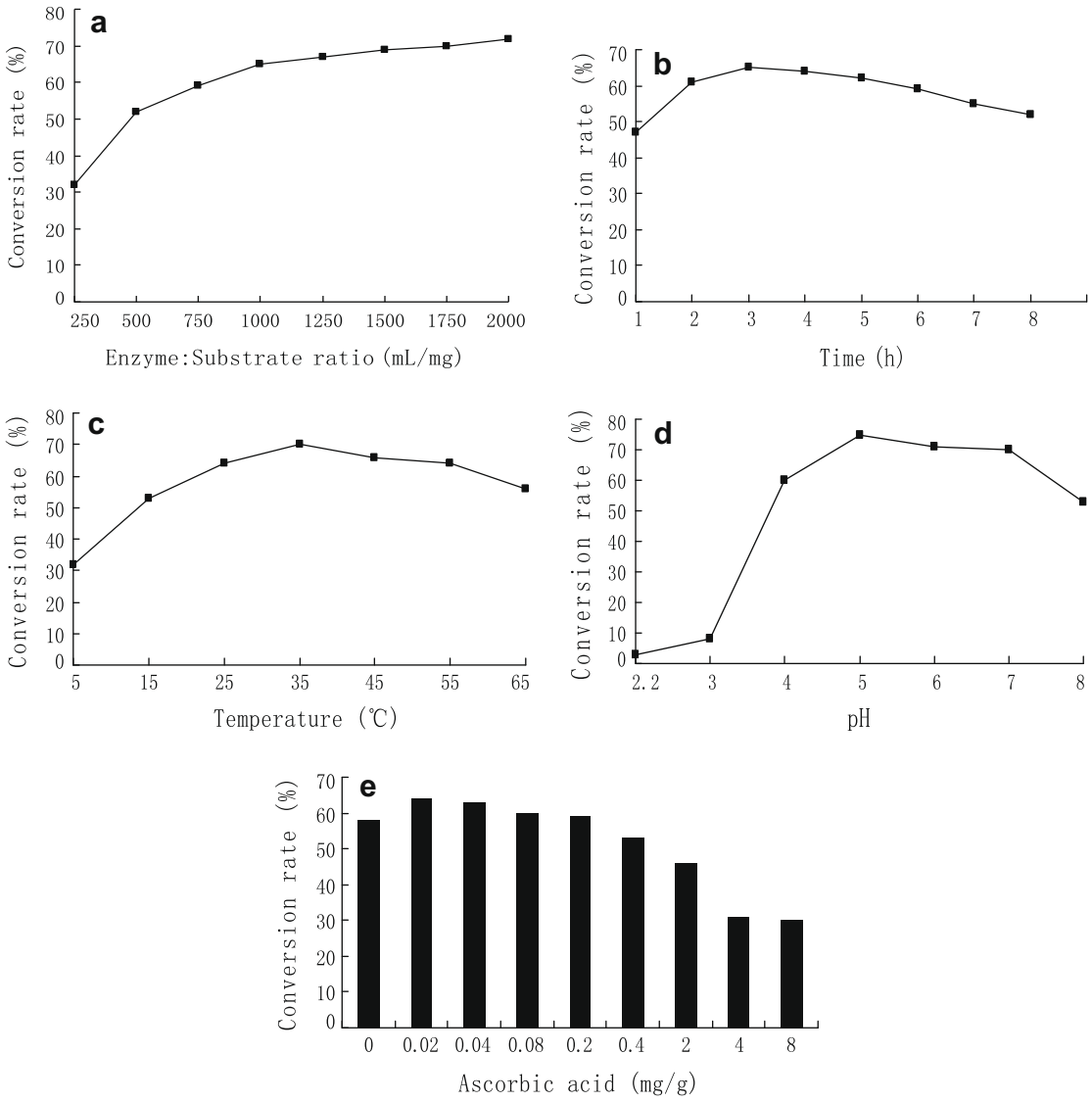


**Fig. 5.** Effects of various conditions on the enzymolysis of glucosinolates using exogenous myrosinase: (a) effects of liquid/solid ratio; (b) effects of time; (c) effects of temperature; (d) effects of pH condition; and (e) effects of the presence of ascorbic acid.

**Table 1**
Optimum conditions for glucosinolates enzymolysis using endogenous and exogenous myrosinase.

| Enzymolysis | Optimum conditions | | | | | |
|---|---|---|---|---|---|---|
| | Enzyme/substrate ratio | Time (h) | Temperature (°C) | pH | Presence of ascorbic acid (mg/g) | Enzymolysis rate of glucosinolates (%) |
| Endogenous method | 3 (liquid/solid ratio, ml/g) | 8 | 25 | 4 | 0.02 | 35 |
| Exogenous method | 1000 (volume ratio) | 3 | 35 | 5 | 0.02 | 78 |

hydrolysis products were produced using free or immobilized myrosinase in 0.1 M phosphate buffer pH 6.5 at 37 °C reported by Leoni et al. (1997). However, unlike the process involving endogenous enzyme, the conversion rate of glucosinolates remained at a satisfactory level even up to pH 8. This behaviour was different from that observed when endogenous myrosinase was used, where at pH 8 sulforaphane was hardly detectable (see Fig. 4d). The effect of ascorbic acid was similar for both endogenous and exogenous enzymolysis processes. As shown in Fig. 5e, the presence of a small amount of ascorbic acid (0.02 mg/ml) shows an enhanced effect on the enzymolysis of glucosinolates, with an enzymolysis rate of 68%, significantly higher than that of the reference (61% without the ascorbic acid addition). This enhancement was also observable for when ascorbic acid concentration was increased to 0.2 mg/ml. However, significant weakening effects were evident when the ascorbic acid concentration exceeded 2 mg/ml.

### *3.4. Comparison of enzymolysis of glucosinolates using the endogenous and exogenous methods*

Above results show that conversion of glucosinolates to sulforaphane is achievable using either endogenous enzymolysis method or exogenous enzymolysis method. The efficiency of both approaches are influenced by a number of factors, including liquid/substrate ratio, time, temperature, pH, and the presence of ascorbic acid. The optimum enzymolysis conditions for both methods are summarised in Table 1.

By comparing the results from the two approaches, one can see that exogenous enzymolysis has obvious advantages over the endogenous approach, such as its shorter time, a wider range of working pH, and more importantly a much higher rate of glucosinolates conversion to sulforaphane. The exogenous method has a conversion rate of 78% compared to only 35% for the endogenous method. On the other hand, the exogenous method appeared to produce less by-products (see Fig. 2), which makes it easier for the following extraction and purification of sulforaphane. However, it has to be realised that endogenous method is much simpler to apply in real application. It requires a huge amount of efforts to extract myrosinase and glucosinolates from plant sources for exogenous approach. Therefore, the cost of exogenous enzymolysis of glucosinolates to produce sulforaphane will be certainly much higher.

## 4. Conclusions

This work investigated the enzymolysis of glucosinolates and the formation of sulforaphane. HPLC and GC-MS were used for the identification and quantitative analysis of sulforaphane from enzymolysis products. It was confirmed that both endogenous and exogenous approaches were applicable for the enzymolysis of glucosinolates. However, efficiency of glucosinolates enzymolysis to sulforaphane was strongly influenced by various reaction conditions. It was found that, using an endogenous approach, an optimum enzymolysis of glucosinolates could be achieved at a liquid/solid ratio of 3 mg/g, at 8 h reaction time, at a temperature of 25 °C, pH 4.0, and in the presence of ascorbic acid 0.02 mg/g. The maximum conversion rate for endogenous method was 35%. However, an exogenous approach produced a much higher yield of sulforaphane. It was found that a conversion rate of 68% was achievable for glucoraphanin enzymolysis at combined conditions of enzyme/substrate ratio 1000 ml/g, 3 h enzymolysis, 35 °C, pH 5, and in the presence of 0.02 mg/ml ascorbic acid.

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