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

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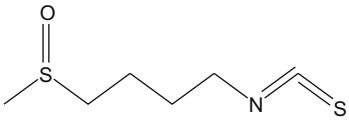
ABSTRACT

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

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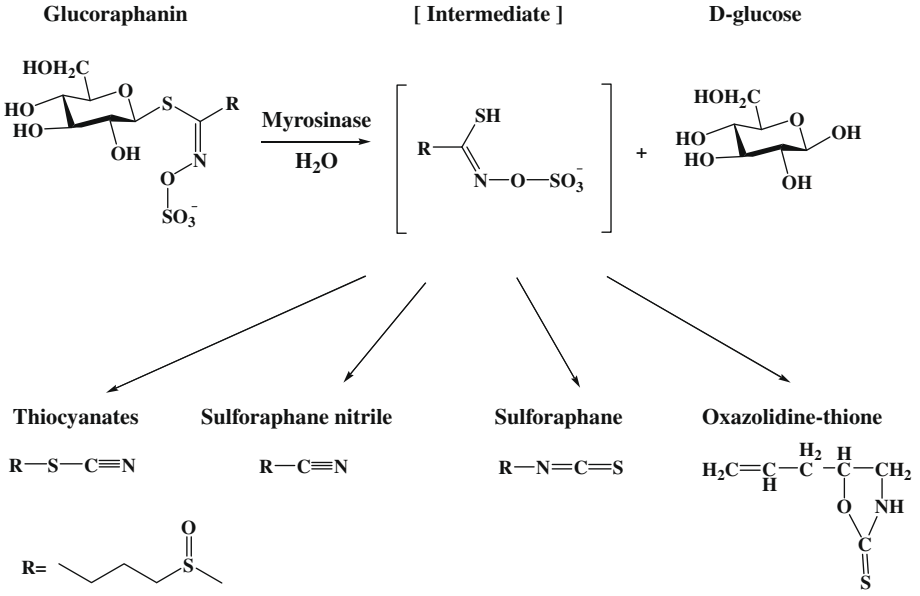

Fig. 1. The sequences of glucosinolate hydrolysis by myrosinase and its products.

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