

STUDY OF BREAKDOWN PRODUCT (ISOTHIOCYANATE)
OF GLUCOSINOLATES IN *BRASSICA* VEGETABLES
(*BRASSICA CAMPESTRIS* & *BRASSICA JUNCEA* VAR. *RUGOSA*)

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in Brassica Vegetables (Brassica Campestris & Brassica Juncea Var. Rugosa)

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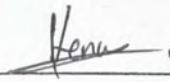
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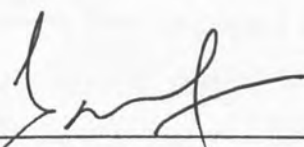


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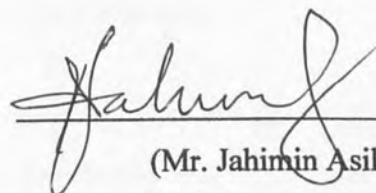
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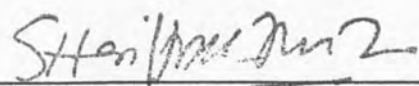
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ABSTRACT

A study was carried out on the effect of pH and temperature on the degradation of glucosinolates in Brassica vegetables namely *Brassica juncea* var. *rugosa* (Gai Choy) and *Brassica campestris* (Pak Choy). The pH used in these experiments is pH 0, pH 2, pH 7 and pH 10. Whereas, the three different temperatures used for these experiments 25°C, 40°C and 60°C. The products are extracted with dichloromethane. Standard of β -phenylethyl isothiocyanates is used for the identification of peaks in the samples. Analysis for the products was done by using GC-FID. The results show that degradation of the glucosinolates can be influenced by the pH and the temperature. Isothiocyanates can be detected in every pH and every temperature but at different quantities. The ideal pH and temperature for the degradation of glucosinolates to give isothiocyanates in these experiments is at pH 7 and 25°C.



**KAJIAN PENGURAIAN PRODUK-PRODUK GLUKOSINOLAT
DALAM SAYUR-SAYURAN BRASSICA**

ABSTRAK

Kajian yang dijalankan adalah untuk mengetahui pengaruh pH dan suhu dalam penguraian glukosinolat bagi sayur-sayuran Brassica. Penguraian glukosinolat akan member isotiosianat, nitril dan tiosianat. Sayur Brassica juncea var. rugosa (Gai Choy) dan sayur Brassica campestris (Pak Choy) diambil untuk menjalankan eksperimen. Pelbagai pH digunakan untuk eksperimen ini seperti pH 0, pH 2, pH 7 dan pH 10. Tiga jenis suhu juga digunakan untuk eksperimen ini seperti 25°C, 40°C dan 60°C. Produk tersebut diekstrak dengan menggunakan dikloromethana. Larutan piawai β -feniletill isotiosianat digunakan untuk menganalisis produk-produk tersebut dengan menggunakan kromatografi gas – pengesan pengionan nyalaan (GC-FID). Keputusan dalam eksperimen ini menunjukkan penguraian glukosinolat dapat dipengaruhi oleh pH dan suhu. Isotiosianat dapat diperolehi dalam pelbagai pH dan suhu, hanya kuantiti adalah berbeza. pH dan suhu yang unggul bagi penguraian glukosinolat dalam eksperimen ini adalah pH 7 dan 25°C.



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LIST OF SYMBOLS

%	Percentage
GC/MS	Gas chromatography and mass spectrometry
GC-FID	Gas chromatography and flame ionization detector
LC/MS	Liquid chromatography and mass spectrometry
&	And
APCI	Atmospheric pressure chemical ionization
R	Variable side chain
PAPS	3'-phosphoadenosine 5'-phosphosulphate
Cys	Cysteine
UDPG	Thiohydroximate glucosyltransferase
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
β -Pe ITC	β -phenylethyl isothiocyanates
DNA	Deoxyribonucleic acid
O ₂	Oxygen
YAF	Yeast artificial chromosome
RFLP	Restriction-fragment length polymorphism
GSL	Glucosinolate
QTL	Quantitative trait locus
ELONG	Elongation
OXID	Oxidation
ALK	Alkylation
OH	Hydrolysis
>	More than
<	Less than
g	Gram
L	Liter
M	Concentration (mol/L)



V	Volt
min	Minute
mM	Milimole
ng	Nanogram
μm	Micro meter
ml	Milliliter
mm	Millimeter
μL	Micro liter
$\mu\text{mol/g}$	Micromole per gram
g/cm^3	Gram per centimeters cube
g/ml	Gram per milliliter
g/L	Gram per liter
mol/L	Mole per liter
MV	Concentration times volume
w/v	Weight per volume
rpm	Rotation per minute
$^{\circ}\text{C}$	Degree Celsius
t_R	Retention Time



CHAPTER 1

INTRODUCTION

1.1 Background of Study

Centuries ago, people use plants and herbals to cure illness. Some of them play a protective role for various diseases, such as cancer. Epidemiological studies have demonstrated that the regular consumption of fruits and vegetables may reduce the risk of developing chronic diseases, including cardio-vascular disease and cancer (Pascale *et al.*, 2007). A large number of potentially anticarcinogenic agents have been suggested in fruits and vegetables so far, e.g. fiber, vitamins C and E, carotenoids, flavonoids, phenols, phytoestrogens, diallylsulfides, and limonene. The mechanisms by which these agents may act include dilution and binding of carcinogens in the digestive tract (fiber), antioxidant effects, inhibition of nitrosamine formation, inhibition of activation of promutagens/procarcinogens, induction of detoxification enzymes, alteration of hormone metabolism, and others (Verhoeven *et al.*, 1997).



1.2 Types of *Brassica* Vegetables

Cruciferae of *Brassica* genus also have cancer preventive potential. Cruciferous vegetables are the major dietary source of naturally occurring thioglucosides or glucosinolates in diet which distinguishes them from other vegetables. *Brassica* vegetables, including all cabbage-like vegetables are a genus of the family *Cruciferae* and contribute most to our intake of glucosinolates (Verhoeven *et al.*, 1997). They are the *Brassica* crops - Brussels sprouts, cabbage, broccoli, spinach, cauliflower, watercress, oilseed rape and mustard.

1.3 Glucosinolates in *Cruciferae* Species

Glucosinolates comprise the major secondary metabolites in many *Cruciferae* species. Glucosinolates and their hydrolysis products can affect human health significantly. Some of the hydrolysis products have anti-nutritional and toxic properties (e.g., goitrin and cyanoeithioalkanes), whereas others have potential beneficial effects (e.g., sulforaphane and indole-3-carbinol) (Mellon *et al.*, 2002).

Glucosinolates and myrosinase are segregated in intact plants. Glucosinolates-myrosinase system forms a part of the plant's natural defense system or pre-formed chemical defenses against fungal disease and pest infection. Glucosinolates and myrosinase are stored separately, but when tissues are disrupted, the enzyme will hydrolyse the glucosinolates. Glucosinolates are usually present in the leaves of *Brassica* spp. at concentrations that can yield bioactive catabolites in amounts sufficient to prevent the development of the pathogens under bioassay conditions



(Doughty *et al.*, 1996). The total content of the glucosinolates was highest in broccoli (~67 $\mu\text{mol/g}$ fresh weight) and lowest in green cabbage (~10 $\mu\text{mol/g}$ fresh weight) (Song *et al.* 2005).

1.4 Hydrolysis of Glucosinolates

Glucosinolates are characterized by their ability to be hydrolysed by the enzyme myrosinase to yield glucose and a labile aglucone which spontaneously rearranges with the loss of sulfate to give rise to an unstable thiohydroximate-*O*-sulphonate which can rearranged to an isothiocyanate as the major product. The nature of the product depends on the hydrolysis conditions and on the particular glucosinolates. Glucose has invariably been found as the sugar moiety attached through a β -thioglucosidic linkage. Myrosinase, a natural-occurring enzyme in vegetables will catalyze the breakdown or degradation of glucosinolates to give isothiocyanate, thiocyanate, nitrile and indole.

1.5 Isothiocyanates in Glucosinolates

The decrease risk of cancer linked to a diet rich in the *Brassica* vegetables is linked to isothiocyanates absorbed the ingestion of glucosinolates (Verhoeven *et al.*, 1997). It is generally thought that dietary glucosinolates are converted to isothiocyanates and other products by bacteria of the gastrointestinal tract and that isothiocyanates are absorbed to produce associated cancer-preventive activity.



Isothiocyanates have a pleasant appetite-stimulating flavor at low concentration whereas nitriles have a garlic-like flavor. The less pleasing flavor of cooked frozen Brussels sprouts compared with the flavor of the cooked fresh vegetables is related to the low concentration of isothiocyanate in the frozen material as a result of myrosinase inactivation during blanching, and to the high concentration of allyl cyanide in the frozen vegetables due to autolysis or non-enzymic degradation of allylglucosinolate (Hasapis & MacLeod, 1982).

1.6 Factors Affecting Degradation of Glucosinolates

There are several factors for the processing of degradation of glucosinolates. Temperature, pH and enzymatic conditions are the main and important factors for the processing. With these factors, the products of the degradation glucosinolates will be increase especially isothiocyanates and indole which both products will decrease the risk of cancer. For example, only 17% of glucobrassicin was degraded after acidic hydrolysis (Tiedink *et al.*, 1988). Effect of various cooking procedures, such as heating and autolysis will lead the breakdown of glucosinolates increases (Slominski & Campbell, 1988). Indole glucosinolates showed fourfold increase after chopping by Verkerk *et al.* (1997).

1.7 Methodologies for the Analysis Glucosinolates Degradation Products

Gas chromatography with flame ionization detector (GC-FID) is used in the assay of desulfated glucosinolates with a chemical derivatization step to enhance analyte volatility (Song *et al.*, 2005). LC-MS was used to identify desulfated glucosinolates

using atmospheric pressure chemical ionization (APCI) (Kim *et al.*, 2001). This ionization method gives poor sensitivity for glucosinolate, isothiocyanate, and amine detection, most of the researcher chooses to use GC/MS than LC-MS (Song *et al.*, 2005). Glucosinolates degradation products will be extracted using standard method and then analyzed with GC-FID.

1.8 Objectives of Study

The objectives of the study are:

- To analyze qualitatively the breakdown product (isothiocyanates) of the glucosinolates in *Brassica* vegetables.
- To evaluate the effects of temperature and pH on the glucosinolates degradations.

1.9 Scope of Study

Two types of *Brassica* vegetables are chosen for the experiments namely *Brassica campestris* (Pak Choy) and *Brassica juncea* var. *rugosa* (Gai Choy). The degradation of glucosinolates in *Brassica* vegetables will be analyzed using GC-FID instrument.

Based on the previous works reported elsewhere, there are many types of glucosinolates in these *Brassica* vegetables. The main focus of this present study is to identify the glucosinolates presence in these vegetables based on the glucosinolates degradation products. The glucosinolates chain (R) chemical structures of the



degradation products have similar structure to the parent glucosinolate (as indicated in Table 2.1).

In addition, due to difficulty in obtaining commercial standard compounds for all the known glucosinolates in *Brassica*, therefore quantitative analysis is not included in this study.



CHAPTER 2

LITERATURE REVIEW

2.1 Glucosinolates

2.1.1 Introduction

Glucosinolates are naturally constituents of cruciferous vegetables (Bending & Lincoln, 2000). They are formerly termed the mustard oil glucosides – are amino acid-derived secondary plant products that contain a sulphate and a thioglucose moiety, and occur in the order of Capparales and a few other unrelated families of dicotyledons (Halkier & Du, 1997). Glucosinolates are thioglycosidic compounds found in various quantities and ratios in the seeds, roots, stems, and leaves (Leoni *et al.*, 1997). They are also a uniform class of naturally occurring anions which have been found exclusively in the plant kingdom, and occur in eleven families of dicotyledon. They have been detected in practically every species studied of the *Cruciferae*, *Capparaceae*, *Tovariaceae*, *Resedaceae* and *Moringaceae*. Other families in which they occur in one or more taxa include *Limnanthaceae*, *Caricaceae*, *Tropaeolaceae*, *Gryostemonaceae*, *Salvadoraceae*, and *Euphorbiaceae* (Bones & Rossiter, 1996).



2.1.2 Types of glucosinolates

There are seven major glucosinolates in *Brassica* vegetables: the methylsulfinylalkyl glucosinolates (glucoiberin, glucoraphanin, and glycoalyssin), the olefinic glucosinolates (sinigrin, gluconapin, and progoitrin), and the aromatic glucosinolates (gluconasturtiin) (Song *et al.* 2005). Glucoraphanin and glucoiberin were the major glucosinolates in broccoli, and sinigrin was the major glucosinolates in the other *Brassica* vegetables (Song *et al.*, 2005).



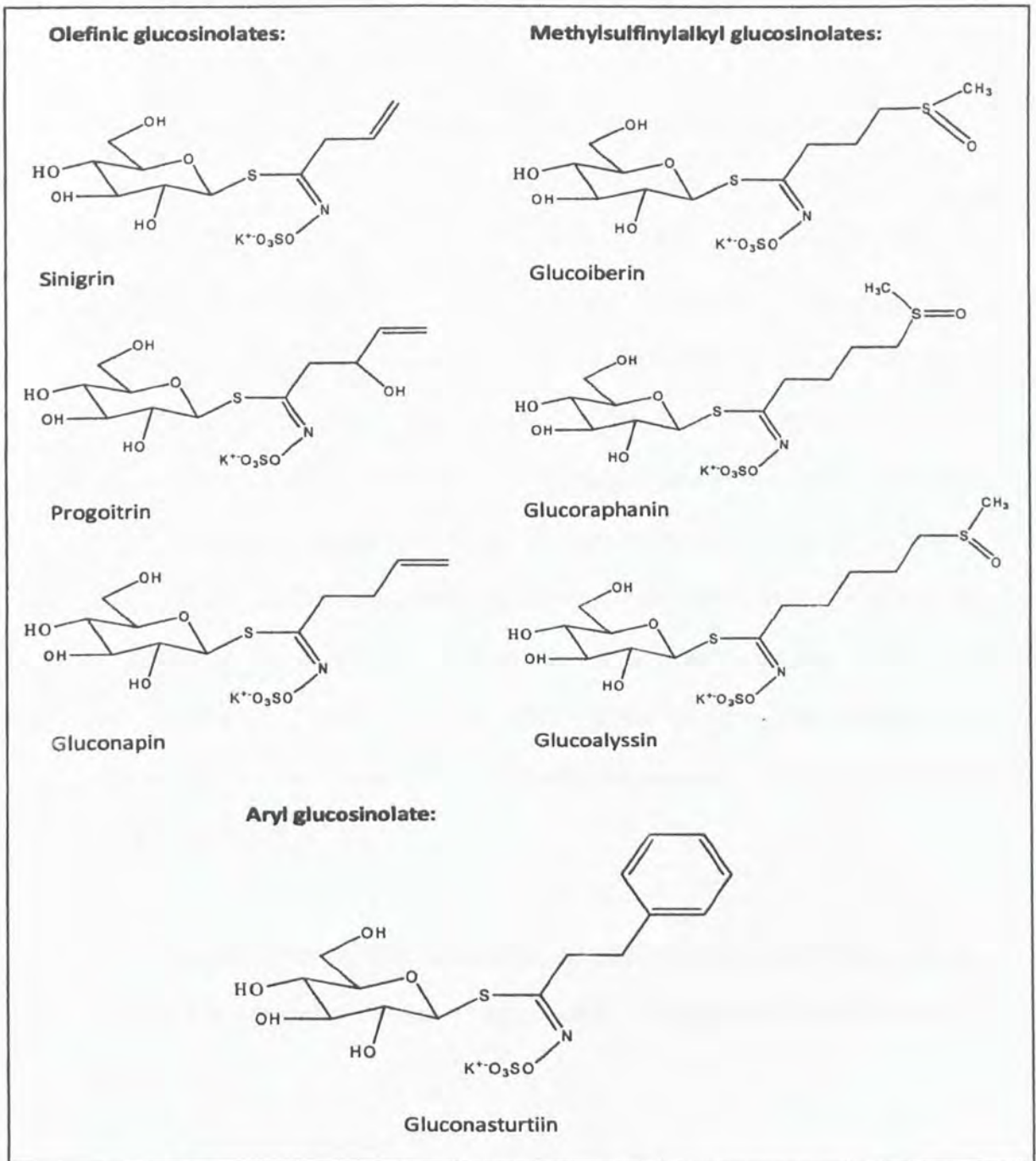


Figure 2.1 Molecular structures and classification of glucosinolates (Song & Thornalley, 2006).

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