ANALYSIS OF GLUCOSINOLATES IN *BRASSICA* VEGETABLE AND NON-*BRASSICA* VEGETABLE

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TABLE OF CONTENTS

TABLE OF CONTENTS

Page Number

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOS

LIST OF SYMBOLS AND ABBREVIATIONS

D-glucose	Dextrose glucose
GS	Glucosinolates
g	Gram
mAU	Milliabsorbance units
mg	Milligram
µg	Microgram
µL	microliter
µmol	micromol
µm	Micrometer
min	minute
mL	Milliliter
mm	Millimeter
nm	nanometer
PAPS	3`-phosphoadenosine-5`-phosphosulphate
PEITC	Phenethyl isothiocyanate
ppm	Part per million
R^2	coefficient of correlation
rpm	Revolutions per minute
UDPGlc	Uridine Diphosphoglucose
%	Percentage
^0C	Degree Celsius

ANALISIS GLUCOSINOLATES DALAM SAYURAN BRASSICA DAN SAYURAN BUKAN-BRASSICA

ABSTRAK

Glucosinolate merupakan sebuah kumpulan proses metabolik tumbuhan sekunder bagi tumbuhan cruciferous yang kebanyakan adalah dalam sayur-sayuran brassica. Ia menjalankan fungsi yang penting dalam diet manusianya. Satu endogenus enzim tumbuhan, mirosinase akan memangkinkan glucosinolates yang menjalani hidrolisis dan seterusnya akan menjadi satu julat sebatian-sebatian biologi aktif. Kajian ini melibatkan satu sayuran brassica, brokoli dan satu sayuran bukan brassica, kangkung. Methanol digunakan bagi kedua-dua sayur-sayuran tersebut untuk mengektrak glucosinolates di dalamnya. Sebelum itu, sayur-sayuran tersebut disejuk-beku kering selama 48 jam. Ekstrak dituraskan dengan manggunakan Watman no.1 (0.2nm) sebelum ia dikeringkan menggunakan gas nitrogen. Air ultra-tulen di campurkan dengan ekstrak kering tersebut dan dituraskan untuk kali yang kedua untuk dianalisis dengan HPLC. Piawaian sinigrin adalah dianalisis dengan suntikan sebanyak 10 µL, kadar aliran 1.0 mL/min, colum Agilent C-18 dan 100% acetonitril. Bagi analisis sampel, kaedah yang digunakan adalah sama tetapi sumtikan telah diubah kepada 20 µL, kadar aliran 0.5 mL/min dan kaedah kecerunan dengan air HPLC dan acetonitril. Keputusan menunjukkan kedua-dua ekstrak mempunyai glucosinolates (sinigrin) dengan kemunculan puncak yang tinggi dan tajam pada 229nm. Spectrum bagi sinigrin piawaian dengan spectrum sample menunjukkan terdapat persamaan homogeniti. Kandungan sinigrin pada ekstrak kankung didapati adalah lebih banyak daripada sinigrin pada ekstrak brokoli, akan tetapi perbezaan antaranya tidak besar. Kepekatan sinigrin pada brokoli adalah 38.03 x 10^3 mg/Kg manakala ekstrak kankung menunjukkan 40.74 x 10^3 mg/Kg.

ABSTRACT

Glucosinolates constitute a well-defined group of secondary plant metabolites in cruciferous plants which occur most commonly in *brassica* vegetables that play its part in human diet. An endogenous plant enzyme, myrosinase will catalysed glucosinolates that undergo hydrolysis and become a range of biological active compounds. The studied involve a *brassica* vegetables, broccoli and a non-brassica vegetables, water spinach. Methanol was use for the extraction process to extract glucosinolates from the vegetables after the vegetables had been freeze-dried and lyophilized for 48 hours. Extract was filtered with syringe filter Watman no.1 (0.2nm) before dried with nitrogen gas. Reconstituted deionized water and filtered for the second time for HPLC analysis. Sinigrin standard was injected with 10 µL, 1.0 mL/min flowrate, with Agilent C-18 Column and 100% acetonitrile. For sample analysis, all the condition was similar with sinigrin standard analysis but the flowrate is 0.5 ml/min and the injection was 20 µL with gradient of HPLC water and acetonitrile. Result showed both vegetables contain glucosinolates (sinigrin) with the high sharp peak at 229nm. Peak spectrum for sinigrin standard and sample did shown homogeneity. Sinigrin content in water spinach extract was more when it compared with broccoli extract, but the difference was not big. Sinigrin concentration in broccoli extract was 38.03×10^3 mg/Kg while water spinach showed 40.74×10^3 mg/Kg.

CHAPTER 1

INTRODUCTION

1.1 Background of Glucosinolates

The glucosinolates (GS) are a class of organic compounds that contain sulfur, nitrogen and a group derived from glucose. Every glucosinolate contains a central carbon atom which is bond via a sulfur atom to the glycone group, and via a nitrogen atom to a sulfonated oxime group. In addition, the central carbon is bond to a side group; different glucosinolates have different side groups. Glucosinolates are naturally occurring β-D-thioglucosides found in genus *Brassica* of *Cruciferae* family (Rangkadilok *et al.*, 2002). They are found exclusively in dicotyledenous plants, with highest concentrations in the Brassicaceae families.

At the present time the diets of people in many parts of the world include considerable amounts of Cruciferous crops and plants. These range from the consumption of processed radish and wasabi in the Far East to that of cabbage and traditional root vegetables in Europe and North America. Other crops, such as rapeseed, kale, swede and turnip may also contribute indirectly to the human food chain since they are extensively used as animal feed stuffs.

Glucosinolates can be grouped into three chemical classes, aliphatic, indolyl, and aromatic glucosinolates, according to whether their amino acid precursor is methionine, tryptophan or an aromatic amino acid (tyrosine or phenylalanine) (Padilla *et al* .,2006). The most important glucosinolates are methionine-derived glucosinolates which are found in Brassica vegetables (Padilla *et al*., 2006). Glucosinolates are β-thioglucosideN-hydroxysulfates containing a side chain and a β-D-glucopyranose moiety. Many wild members of the *Brassica oleracea* species complex (chromosome number, *n* 5 9) have high levels of individual aliphatic glucosinolates.

About 120 different glucosinolates are known to occur naturally in plants. Glucosinolates release biologically active products such as isothiocyanates, organic cyanides, oxazolidinethiones, and ionic thiocyanate (Moreno *et al*, 2006). Upon enzymatic degradation by myrosinase in the presence of water and these substances are also responsible for the bitter or sharp taste of many common foods such as mustard,

horseradish, cabbage and Brussels sprouts but this appear to have little biological impact on themselves. When plant cell tissues are damaged, as occurs during cutting or chewing, the enzyme myrosinase initiates rapid hydrolysis of glucosinolates to yield glucose, sulphate and either isothiocyanates, thiocyanates, nitriles or oxazolindine-2-thiones.

The formation of specific hydrolysis products is dependent on a variety of factors. These factors include the side chain, pH, metal ions, and the presence of protein cofactors (Rangkadilok *et al.*, 2002). Isothiocyanates may also have important roles in plant defence system against insect, fungi and microbial infections (Rangkadilok *et al.*, 2002). Propenylisothiocyanate, released from glucosinolate sinigrin in B. nigra and B. juncea, is an effective fumigant to suppress soil-borne fungal pathogens such as Rhizoctonia solani, Fusarium graminearum, Bipolaris sorokiniana, Sclerotinia sclerotiorum and Fusarium culmorum (Rangkadilok *et al.*, 2002).

Recent research has focused on the anti-carcinogenic activity of several glucosinolates and their breakdown products. In addition these breakdown products of certain glucosinolates have been shown to protect against lung, colon, liver and stomach cancer (Rangkadilok *et al.*, 2002). A good source of glucoraphanin is 3-day-old broccoli sprouts with a concentration 10–100 times greater than in mature plants or florets (Rangkadilok *et al.*, 2002). Sinigrin was found in high levels in mustard greens (B. juncea

var. rugosa), and B. oleracea (Rangkadilok *et al.*, 2002). Glucoraphanin was found to be the predominant glucosinolate in broccoli (Rangkadilok *et al.*, 2002).

Glucosinolates and their breakdown products are known to have important biological activity. The range of activities of these compounds is wide; some are beneficial while others are detrimental for human and animal consumption (Padilla *et al* ., 2006). Progoitrin has been shown to be potentially goitrogenic in animals. However, there is no evidence for any goitrogenic effect on humans from Brassica consumption (Padilla *et al* ., 2006). Some of these compounds have a chemoprotective effect related to a reduction in the risk of certain cancers in humans (Padilla *et al.*, 2006).

Considerable research has been conducted on the nutritional value of isothiocyanates found in other crops, such as watercress (*Rorripa nasturtium aquaticum*), and cancer prevention. Phenethyl isothiocyanate (PEITC) is a derivate of the glucosinolate gluconasturtiin, which occurs in large quantities in watercress showed that extracts of broccoli and watercress inhibit the invasive potential of the human breast cancer cell line in vitro and suggested that their phytochemical constituents, the isothiocyanates, are a new class of invasion inhibitors (Padilla *et al.*, 2006).

1.2 Objectives of the study

1. To extract glucosinolates from broccoli (*Brassica oleracea var. borytis*) and water spinach (*Ipomoea aquatica*)

2. To identify the type of glucosinolate (Sinigrin) in broccoli (*Brassica oleracea var. borytis*) and water spinach (*Ipomoea aquatica*).

1.3 Scope of study

A type of *brassica* vegetables and a type of non-*brassica* vegetable that were selected in the study. These were broccoli for *brassica* and water spinach for non-*brassica* vegetables. These *brassica* and non-*brassica* vegetables were originated from Kundasang and local farm that can be found at the centre of Kota Kinabalu market. In this study leaves and stem will be extract from these vegetables. Leaves are mostly that will be used because most of the glucosinolates can be found at there. HPLC will be used to determine and analyse the glucosinolates in the extract.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy Classification of Broccoli

2.1.1 Scientific classification of brocccoli

The scientific classification of broccoli is shown in Table 2.1.

Table 2.1 The taxonomy of *Brassica oleracea var. botrytis*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Brassicaceae
Genus	*Brassica*
Species	*Brassica oleracea var. botrytis*

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2.1.2 Morphology of broccoli

Broccoli (Photo 2.1) is grown for the clustered green (or purple) flower buds that are picked before they open and eaten raw or cooked. There are three main types of broccoli. The typical green or purple broccoli with one large, central head is a "calabrese". "Romanesco" broccolis have flower buds grouped in numerous small cone-shaped heads, arranged in spirals; the "sprouting broccolis" (sometimes placed in a different group or variety within *B. oleracea*) produce a succession of small flowering heads over an extended season.

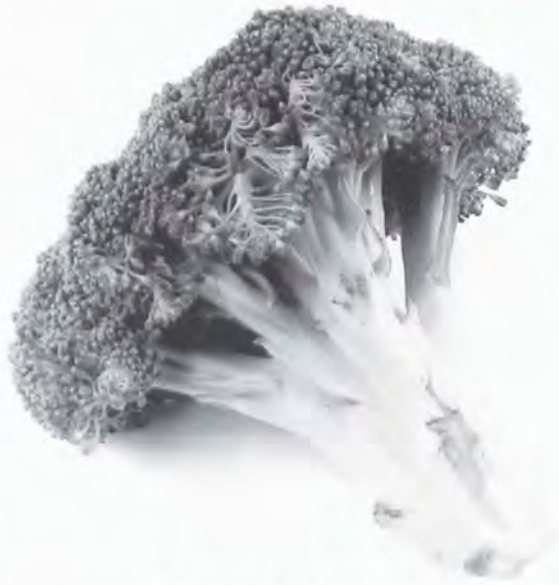

Photo 2.1 Broccoli

2.1.3 Usage of broccoli

Broccoli have impressive nutritional profile that includes beta carotene, vitamin C, calcium, fiber, and phytochemicals, specifically indoles and aromatic isothiocynates, broccoli and its skin may be responsible for boosting certain enzymes that help to detoxify the body (Moreno *et al.*, 2006). These enzymes help to prevent cancer, diabetes, heart disease, osteoporosis, and high blood pressure. high levels of antioxidant and anticancer compounds. One unusual phytoterapic role of broccoli is for skin diseases, where the juices from leaves are used against warts (Moreno *et al*, 2006).

Its vitamins and nutrients typically are more concentrated in flower buds than in leaves. These makes broccoli a better source of vitamins and nutrients than cole crops in which only the leaves are eaten (Moreno *et al*, 2006). The anti-cancer properties of these vegetables are so well established that the American Cancer Society recommends that Americans increase their intake of broccoli and other Cole crops. It effectively suppresses proliferation of cancer cells in culture and in vivo by causing apoptosis induction. Sulforaphane is one of several compounds that protect cells from injury found in cruciferous veggies (Moreno *et al*, 2006, Pascale *et al.*, 2006, Rochfort *et al.*, 2006).

Recent studies have shown that broccoli sprouts may be even higher in important antioxidants than the mature broccoli heads (West *et al.*, 2002). Other research has

REFERENCE

Betz, J.M and Fox, W.D. 1994. High Performance Liquid Chromatographic Determination of Glucosinolates in Brassica Vegetables. *Food phytochemicals I: Fruits and vegetables* ***14***, 18-21

Dekker, M., Verkerk, R., Jongen, W. M. F. 2000. Predictive modeling of health aspects in the food production chain: a case study of glucosinolates in cabbage. *Trends in Food Scienceand Technology* **11,** 174-181.

Fahey, J. W., Wade, K. L., Stephenson, K. K., Chou, F. E. 2003. Separation and purification of glucosinoalates from crude plant homogenates by high-speed counter-current chromatography. *Journal of Chromatography* **996,** 85-93.

Fréchard, A., fabre, N., Péan, C., Montaut, S., Fauvel, T. S., Rollin, P., Fourasté. 2001. Novel indole-type type glucosinolates from woal. *Tetrahedron Letter* **42**, 9015-9017.

Faulkner, K., Mithen, R., Williamson, G. 1998. Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolates in broccoli. *Carsinogenesis* **19**, 605-609

Huang, D.J., Chen, H.J., Lin, C. D., Lin, Y. H. 2005. Antioxidant and antiproliferative of water spinach (*Ipomoea aquatica*) constituent. *Bot. Bull. Acad. Sin* **46**, 99-106

Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G., Knasmüller, S., 1996. Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. *Chemico Biological Interaction* **102,** 1-16.

Mellon, F. A., Bennett, R. N., Holst, B., Willianson, G. 2002. Intact glucosinolate analysis in plant extracts by programmed cone voltage electrospray LC/MS: Performance and comparison with LC/MS/MS Methods. *Analytical Biochemistry* **306,** 83-91

Moreno, D. A., Carvajal, M., Lopez-Berenguer, C., García-Viguera. C. 2006. Chemical and biological characterization nutraceutical compounds of broccoli. *Journal of Pharmaceutical and Biomedical Analysis* **41,** 1508–1522.

Padill, G., Cartea, M., Velasco, P., Haro, A., Ordás, A. 2006. Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry* **68**, 536-545.

Pascale, S. D., Maggio, A., pernice, R., Fogliano, V., Barbieri, G. 2006.Sulphur fertilizationmay improve the nutritional value of Brassica rapa L.subsp. sylvestris. *Europ. J. Agronomy* **26,** 418-424.

Rangkadilok, N., Nicolasa, M. E., Bennettc, R. N., Premierb, R. R., Eaglingb, D. R., Taylora, P. W. J. 2002. Determination of sinigrin and glucoraphanin in Brassica species using a simple extraction method combined with ion-pair HPLC analysis. *Scientia Horticulturae* **96**, 27-41.

Rochfort, R., Caridi, D., Stinton, M., Trennery, V. C., Jones, R. 2006. The isolation and purification of glucoraphanin from broccoli seeds by solid phase extraction and preparative high performance liquid chromatography. *Journal of Chromatography A* **1120,** 205-210.

Song, L. J., Morrison, J. J., Botting, N. P., Thornalley, P. J. 2005. Analysis of glucosinolates, isothiocynates, and amine degradation products in vegetables extracts and blood plasma by LC-MS/MS. *Analytical Biochemistry* **347,** 234-243.

Surugau, N and Self , R. 2001. Effets of temperature ad addition of commercial myrosinase on the compositions of glucosinolatesdegradation products in broccoli, *Brassica Oleracea L. Borneo Science* **9**, 1-18.

Tian, Q., Rosselot, R. A., Schwartz, S. J. 2005. Quantitative determination of intact glucosinolate in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry. *Analytical Biochemistry* **343**, 93-99.

Troyer, J. K., Stephenson, K. K., Fahey, J. W. 2001. Analysis of glucosinolates from broccoli and other cruciferous vegetables by hydrophilic interaction liquid chromatography. *Journal of Chromatography A* **919,** 299-304.

Vang, O., Mortensen, J., Andersen, O. 2001. Biochemical effects of dietary of different Broccoli samples. II. Multivariate of contribution of specific glucosinolates in modulating cytochrome P-450 and antioxidant defensive enzyme activities. *Metabolism* **50**, 1130-1135.

Verkerk, R., van der Gang, M. S., Dekker, M., Jongen, W. M. F. 1997. Effects of processing on glucosinolates in cruciferous vegetables. *Cancer Letter* **114,** 193-194

West, L., Tsui, I., Haas, G. 2002. Single column approach of the liquid chromatographic of polar and non-polar glucosinolates from broccoli sprouts and seeds. *Journal of Chromatography A* **966,** 227-232