

**EFFECT OF ADDITIVES AND FOOD
PROCESSING CONDITIONS ON BENZYL
GLUCOSINOLATE HYDROLYSIS PRODUCTS
IN *Carica papaya***



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UNIVERSITI MALAYSIA SABAH

**SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH**

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2012

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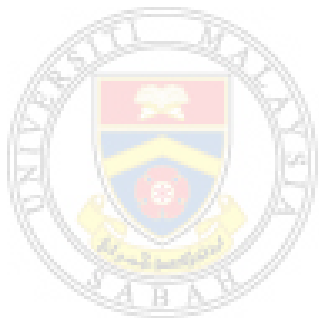
DECLARATION

I hereby declare that the material in this study is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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TITLE : **EFFECT OF ADDITIVES AND FOOD PROCESSING
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VIVA DATE : **15 JUN 2012**

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Signature



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Gayathri A/P Nagappan
15 Jun 2012



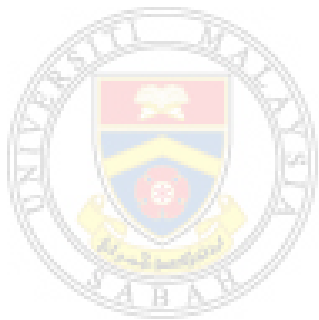
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ABSTRACT

EFFECT OF ADDITIVES AND FOOD PROCESSING CONDITIONS ON BENZYL GLUCOSINOLATE HYDROLYSIS PRODUCTS IN *Carica papaya*

Glucosinolate are sulphur-containing secondary metabolites found largely in *Brassicaceae* family. Glucosinolate undergoes hydrolysis readily upon cell rupture, such as cutting and cooking, by the naturally-occurring enzyme myrosinase to form mainly isothiocyanates and/or simple nitriles. Isothiocyanates are known to possess anticarcinogenic properties while nitriles are largely inactive. Benzyl isothiocyanate, a hydrolysis product of benzyl glucosinolate, is one of the most potent anticancer agents. However, formation of benzyl isothiocyanate is depending upon reaction conditions where benzyl nitrile is formed at the expense of benzyl isothiocyanate. Thus, the potential health benefits of benzyl isothiocyanate may be surpassed by the ineffective benzyl nitrile. In this study, the factors influencing the formation of benzyl isothiocyanate were investigated to understand further the benzyl glucosinolate hydrolysis pathway in different parts of papaya (*Carica papaya*). The results showed that the concentration (dry weight), of benzyl isothiocyanate was found greater in papaya leaf with 349 μM followed by 24 μM in unripe fruit and 12 μM in flower. The highest amount of benzyl isothiocyanate produced was observed at room temperature (25°C) where it decreases gradually as the temperature increases up to 80°C. Comparing three common domestic methods of cooking vegetable i.e. blanching, boiling and slow heating, the results show that the high temperature treatment produced mainly benzyl nitrile; while slow heating up to 40°C produced more benzyl isothiocyanate. Both hydrolysis products were found largely leached into the cooking liquid (soup). The production of benzyl isothiocyanate was optimum at pH 6 – 7. Meanwhile, the addition of both ferrous and ferric ions (0 – 10 mM) favors the formation of benzyl nitrile. Conveniently, adding an iron-chelating agent, EDTA, has shown an increasing trend in the formation of benzyl isothiocyanate. A small amount of ascorbic acid enhanced the formation of benzyl isothiocyanate, however, higher concentration inhibits its

production. Overall, this work has shown that to ensure an optimum uptake of this beneficial compound from papaya leaf, unripe fruit or flower consumption, food preparation practices have to be favoring the formation of benzyl isothiocyanate.

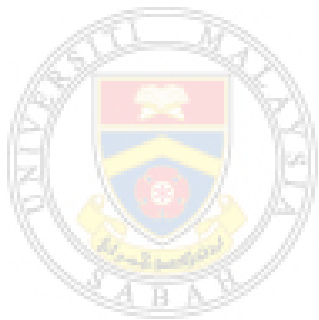


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ABSTRAK

*Glukosinolat adalah metabolit sekunder mengandung sulfur yang terdapat banyak di dalam famili Brassicaceae. Glukosinolat mengalami hidrolisis dengan mudah apabila sel dirobek, seperti dipotong dan dimasak, oleh enzim semulajadi mirosinase untuk membentuk isotiosianat dan/atau nitril. Isotiosianat diketahui mempunyai ciri-ciri antikarsinogenik manakala nitril adalah tidak aktif. Benzil isotiosianat, suatu produk hidrolisis benzil glukosinolat, merupakan salah satu agen antikanser yang paling kuat. Walau bagaimanapun, pembentukan benzil isotiosianat adalah bergantung kepada keadaan-keadaan tindak balas di mana benzil nitril yang terbentuk dan bukannya benzil isotiosianat. Oleh itu, khasiat benzil isotiosianat terhadap kesihatan mungkin akan terhalang oleh terbentuknya benzil nitril yang tidak efektif ini. Dalam kajian ini, faktor-faktor yang mempengaruhi pembentukan benzil isotiosianat telah diselidiki untuk memahami lebih lanjut laluan hidrolisis benzil glukosinolat di dalam bahagian-bahagian berbeza betik (*Carica papaya*). Keputusan kajian menunjukkan kepekatan benzil isotiosianat (berat kering), didapati lebih tinggi di dalam daun betik dengan $349 \mu\text{M}$ diikuti oleh $24 \mu\text{M}$ dalam buah muda dan $12 \mu\text{M}$ dalam bunga betik. Akaun tertinggi penghasilan benzil isotiosianat dikesan pada suhu bilik (25°C) di mana ianya semakin berkurangan apabila suhu dinaikkan sehingga 80°C . Perbandingan di antara tiga kaedah lazim memasak sayuran iaitu merebus, mendidih dan memanaskan secara perlahan menunjukkan suhu tinggi menghasilkan benzil nitril sebagai produk utama; manakala pemanasan perlahan sehingga 40°C menghasilkan lebih banyak benzil isotiosianat. Kedua-dua produk hidrolisis ini sebahagian besarnya didapati di dalam air masakan (sup). Penghasilan benzil isotiosianat adalah optimum pada pH 6 – 7. Penambahan ion-ion ferus dan ferik ($0 - 10 \text{ mM}$) cenderung menghasilkan benzil nitril. Manakala penambahan agen pengkelat besi, EDTA, menunjukkan corak meningkat dalam pembentukan benzil isotiosianat. Akaun kecil asid askorbik meningkatkan pembentukan benzil isotiosianat, walau bagaimanapun, kepekatan yang tinggi merencat penghasilannya. Secara keseluruhannya, kajian ini menunjukkan bahawa bagi memastikan pengambilan optima sebatian berguna ini*

dari pemakanan daun, buah mentah atau bunga betik, kaedah penyediaan makanan haruslah cenderung kepada pembentukan benzil isotiosianat.



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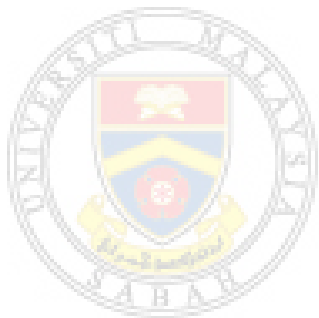
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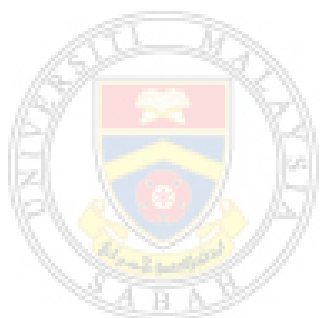
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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Glucosinolates or formally known as mustard oil are amino acid derived secondary metabolites that contain a sulphate and a thioglucose moiety with variable side chains include alkyl, alkenyl, hydroxyalkenyl, methylthioalkyl, methylsulfinylalkyl, methylsulfonylalkyl, arylalkyl and indolyl groups (Halkier and Du, 1997; Blazevic *et al.*, 2010). Glucosinolates side chains are divided according to their amino acids precursors into aliphatic, aromatic and indolic groups. There are more than 120 glucosinolates with different side chains have been identified in various edible and non edible plants (Tian *et al.*, 2005; Herr and Buchler, 2010) and reported to present in 16 families of dicotyledonous angiosperms (Fahey *et al.*, 2001; Vig *et al.*, 2009). Glucosinolates are mostly found in *Brassicaceae* family and several other plants family in the same plant order as the *Brassicaceae* such as *Capparidaceae*, *Moringaceae*, *Resedaceae*, *Stegnospermaceae* and *Tovariaceae* (Vaughn and Berhow, 2005; Kim *et al.*, 2010a). The agronomically well known and commonly consumed crucifer vegetable crops that contain abundant glucosinolates are cabbage (Oerlemans *et al.*, 2006; Wennberg *et al.*, 2006), cauliflower (Valette *et al.*, 2003; Tian *et al.*, 2005; Volden *et al.*, 2009a), broccoli (Tian *et al.*, 2005), radish (Blazevic and Mastelic, 2009), turnip (Padilla *et al.*, 2007), horseradish (Sultana *et al.*, 2003), mustard and oilseed rape (Pontoppidan *et al.*, 2005).

Glucosinolates content of a plant mainly depends on genetics and environmental factors such as climate and agronomy conditions (Cieslik *et al.*, 2007). A particular genotype will express the same ratio of glucosinolates side chains even when grown in different environment, while the level of glucosinolates might vary. Mendelin genes have been identified as the linkage which responsible to determine the length and chemical structure of glucosinolates. On the other