

**PROFILLING OF GLUCOSINOLATE AND MYROSINASE
ACTIVITY IN *CARICA PAPAYA* (PAPAYA) VS
EKSOTIKA UNDER DIFFERENT CONDITIONS**

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**THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR
THE DEGREE OF MASTER OF SCIENCE**

**FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2015**



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UNIVERSITI MALAYSIA SABAH

ABSTRACT

Glucosinolates are sulfur-containing secondary metabolites found largely in Brassicaceae family. Glucosinolates undergo hydrolysis readily upon cell rupture (such as cutting and heating) by the naturally-occurring enzyme myrosinase to form mainly isothiocyanates and/or nitriles. Isothiocyanates are known to possess anticarcinogenic properties while nitriles are largely inactive. Benzyl isothiocyanate (BITC), a hydrolysis product of benzyl glucosinolate (BGSL), is one of the most potent anticancer agents. Papaya (*Carica papaya*) is known to contain has high amount of BITC. In Malaysia, beside ripe fruit, young leaves and young or unripe fruit of papaya are widely consumed as vegetables. Also, flower and seeds of papaya are used as traditional therapeutic remedies for various ailments. Although many studies have been done on BITC content and other medicinal properties of papaya, but very few reports on the distribution and concentration of BGSL in papaya. Thus, the first objective of this study is to profile BGSL in different parts of papaya namely seeds, leaf and unripe fruit pulp. The papaya (*C.papaya*) plant studied here was of Eksotika variety grown in the district of Kota Belud, Sabah. Because BITC is only formed when its precursor, BGSL, being enzymatically hydrolyzed by the endogenous myrosinase, therefore the second objective of the current study is to study enzymolysis and myrosinase activity under different conditions. The motivation to carry out myrosinase activity is the facts that agricultural and food processing may have varied effects on the myrosinase-GSL breakdown mechanism in which ultimately influenced the formation of the health-promoting compound, BITC. The conditions studied in enzymolysis (with sinigrin as a substrate) was the effect of hydrolysis time (10, 20, 30, 40 and 50 min). While for the myrosinase activity the condition studied were temperature (30 °C, 40 °C, 60 °C and 80 °C), pH (3.0, 4.0, 5.0, 6.0, 7.0 , 8.0 and 9.0) and concentration of ascorbic acid (2.0, 4.0, 6.0, 8.0 and 10.0 mM), ferrous and ferric ions (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 mM for both irons). The BGSL was extracted using deionized water and then analyzed using HPLC. For the enzymolysis and myrosinase activity, crude myrosinase extracted from seed, leaf and unripe fruit pulp of papaya was mixed with a known concentration of standard sinigrin (as substrate). After a predetermined reaction time, the unreacted (remaining) sinigrin was then extracted and analyzed using HPLC in the same manners as for BGSL. The results showed that seed has the highest content (in mg/100g dry weight) of BGSL (i.e. 490 ± 14.14) compared to leaf (14.7 ± 3.56) and unripe fruit pulp (12.5 ± 0.71). The optimum conditions for the activity of crude myrosinase extract from the different papaya parts are as follows: hydrolysis time, seed at 20 min (99.16%), leaf at 60 min (100 %) and unripe fruit pulp at 30 min (83.30%) ; temperature, 30 – 40 °C (all parts); pH, 8 (seed), 7 (leaf), 9 (unripe fruit pulp); concentration of ascorbic acid,

2.0 mM (all parts); no effect for all the iron concentrations tested. The findings show that myrosinase activity could occur in optimally in mild temperature within a short time and in neutral condition. As for the additives, higher concentration of ascorbic acid inhibit the activity while presence of iron does not affect myrosinase activity. Overall, this work shows that papaya seed is a rich source of BGSL, the precursor for the anticancer BITC. To ensure optimum uptake of BITC, food preparation methods must be performed in optimum conditions for the endogenous myrosinase to take place.