

Benzyl Glucosinolate Hydrolysis Products in Papaya (*Carica papaya*)

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ABSTRACT

Glucosinolates are sulphur-containing secondary metabolites found largely in Brassicaceae family. Glucosinolates undergo 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 for breast, lung liver and colon cancers. 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. Based on the sole presence of benzyl isothiocyanate/nitrile, the results show that its precursor, benzyl glucosinolate, is the major (if not sole) glucosinolate in papaya. The concentration of benzyl isothiocyanate was found greater in leaf with 52.2 mg/kg (dry weight), followed by 18.0 mg/kg in unripe fruit and 3.6 mg/kg 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. In these cooking experiments, both hydrolysis products were found largely leached into the cooking liquid (soup). As for the effects of pH, production of benzyl isothiocyanate was optimum at pH 6 – 7. The addition of both ferrous and ferric ions (0 – 10 mM) favours the formation of benzyl nitrile. Conveniently, adding an iron-chelating agent, EDTA, has shown an increasing trend in the formation of benzyl isothiocyanate. Small amount of ascorbic acid enhanced the formation of benzyl isothiocyanate, however, higher concentration inhibits the benzyl isothiocyanate production. Overall, this work has shown that to ensure an optimum uptake of this beneficial compound from papaya consumption, food preparation practices have to be favouring the formation of benzyl isothiocyanate. Beside, the presence of reductant agents such as iron and ascorbic acid has to be at low concentration.

1.0 INTRODUCTION

Glucosinolates are sulfur containing glycosides with variable side chains which derived from amino acids precursors (Fahey et al., 2001). Glucosinolates undergoes hydrolysis readily upon cell rupture by cutting, chewing, cooking, fermenting or freezing with the presence of naturally occurring enzyme myrosinase (β -thioglucosidase glucohydrolase) to produce isothiocyanates, nitriles, epithionitriles, oxazolidinethiones and thiocyanates depending upon the structure of glucosinolates and reaction conditions (Blazevic et al., 2010). Myrosinase stored separately from glucosinolates in cell vacuoles known as myrosin, and only after the cell is disrupted it is hydrolyses the glucosinolates with the presence of water (Pontoppidan et al., 2005). A particular glucosinolates hydrolysis product; isothiocyanates are well known for its diverse biological activities ranging from bactericidal, nematocidal, fungicidal, insecticidal, antioxidant, antimutagenic, antiproliferative and allelopathic properties (Kim et al., 2010). Isothiocyanates are potential anticarcinogen, which could inhibit the liver, lungs, colon, breast, ovary, prostate, bladder and pancreas cancer (Vig et al., 2009; Zhang et al., 2005). In vitro and in vivo studies have proven that isothiocyanates inhibits enzyme phase I which involve in the bioactivation of chemical carcinogens and induces enzyme phase II which protect cells or tissues against carcinogenenic intermediates (Herr and Buchler, 2010; Moreo et al., 2009). It is also induce apoptosis in various cancer cell lines (Kuang and Chen, 2004). One of the convincing glucosinolates hydrolysis products in cancer studies is benzyl isothiocyanate; a

hydrolysis product of benzyl glucosinolate. Benzyl isothiocyanate is proven to induce cell apoptosis in human breast cells (Xiao et al., 2008), pancreatic cancer cells (Wicker et al., 2010; Sahu et al., 2009) and reduced the growth of solid tumors (Kim et al., 2010).

Glucotropaeolin or well known as benzyl glucosinolate is proven to be present widely in *Carica papaya* (Bennett et al., 1996; Kermanshai et al., 2001). Leaves and flowers of *Carica papaya* are commonly cooked like spinach and consumed by people of Sabah. The boiled water of leaves and flowers are believed good for health. The leaves are also brewed into tea and the young fruits are cooked like curry (Klain and RD, 2010). Plant based foods are often heat treated which inactivate the enzyme myrosinase hence thermally degrade the glucosinolates to undesired hydrolysis products. The health promoting compounds are no longer formed, leads to consumption of worthless hydrolysis products rather than biologically active isothiocyanates. Isothiocyanates itself are temperature labile and volatile which makes it to vanish from heat processed foods (Volden et al., 2008; Volden et al., 2009). It is important to preserve the health promoting compounds, isothiocyanate in food until consumption and the food processing conditions should be optimized to maximize the consumption of isothiocyanates.

The aim of this study was to analyze the effect thermal processing on the benzyl glucosinolates hydrolysis product in leaf, flower and young fruit of *Carica papaya* from Sabah. Thermal processes that have been studied are boiling, blanching and slow heating. Domestic food processing conditions have been imitated in this study to evaluate the effectiveness of our food processing conditions in producing maximum benzyl isothiocyanate intake into diet. As most of the previous researches concentrated on glucosinolates studies in *Brassicaceae*, the glucosinolates study of *Carica papaya* is yet to be propagated.

2.0 MATERIALS AND METHODS

2.1 Sample preparation

All plant materials used in this research were collected from Penampang, Sabah. The samples were free from insect and mechanical damage. The plant materials were washed and stored in freezer. Later, the plant materials were freeze dried and crushed into powder prior to the analysis.

2.2 Glucosinolates profiling in leaf, flower and fruit of *carica papaya*

2.2.1 Glucosinolates hydrolysis products extraction

Freeze dried plant samples (1.0g) were left to homogenize in 10mL of deionized water for 30 minutes at room temperature. The glucosinolates hydrolysis products were extracted using 20mL HPLC grade dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2 μ m nylon filter and kept in vial (Songsak and Lockwood, 2004; Al-Gendy et al, 2010).

The extraction final product was kept at -20°C prior to the GC/MS analysis. The extraction was screened using GC/MS and the glucosinolates hydrolysis products were identified using GC/MS database. The glucosinolates hydrolysis products were evaluated to identified their respective glucosinolates (Songsak and Lockwood, 2004; Al-Gendy et al, 2010).

2.2.2 GC/MS condition

GC/MS Perkin Elmer clarus 500 series was used in this study. The 5MS column (30.0m X 0.25mm X 0.25 μ m) with helium carrier gas at constant flow of 1.0ml/min was used. The temperature program was initially 50°C for 2 minutes and gradually increased to 270°C with the rate of 10°C/min. The transfer line temperature was 250°C and the ion source was 200°C. The MS scan was carried out in the range of 50 to

400 m/z. The injector temperature was 200°C in split ratio of 1:50, and the injection volume was 1 µL (Blazevic et al., 2010). The above mentioned GC/MS condition was followed throughout this study.

2.3 Quantitative determination of benzyl isothiocyanate in leaf, flower and fruit of *carica papaya*

Benzyl isothiocyanate was extracted by homogenized 1.0g of sample into 10mL of deionized water for 30 minutes at room temperature. Later, 20mL HPLC grade dichloromethane was added and the mixture was shaken for 30 minutes. After centrifuged the mixture at 3500rpm for 5 minutes, the dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2µm nylon filter and kept in vial. The extraction final product was kept at -20°C prior to the GC/MS analysis. The quantification of benzyl isothiocyanate in leaf, flower and young fruit of *Carica papaya* was determined using the standard calibration curve (Songsak and Lockwood, 2004; Al-Gendy et al, 2010).

2.4 Effect of temperature, pH, ascorbic acid and iron concentration on formation of benzyl isothiocyanate and benzyl nitrile in leaf, flower and fruit of *carica papaya*.

2.4.1 Effect of autolysis temperature

The autolysis temperatures used for this study were 25°C, 30°C, 40°C, 50°C, 65°C and 80°C. The autolysis temperatures were controlled using a water bath. Freeze dried plant samples (1.0g) were left to homogenize in 10mL of deionized water for 30 minutes at respective temperatures. The glucosinolate hydrolysis products were extracted using 20mL HPLC grade dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for five minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2µm nylon filter and kept in vial. Two replicates were prepared for each sample. The extraction final product was kept at -20°C prior to the GC/MS analysis.

2.4.2 Effect of pH

Buffer solutions of 0.01M trishydroxymethylaminomethane (tris) solution with pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 were prepared. The pH was adjusted using 0.1M acid hydrochloric. Some variation in acid hydrochloric and tris solution was estimated, yet this will not give any significant effect on the hydrolysis process (Shen et al., 2010).

Freeze dried plant samples (1.0g) were left to homogenize in 10mL of different buffer solutions for 30 minutes at room temperature. The glucosinolate hydrolysis products were extracted using 20mL HPLC grade dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2µm nylon filter and kept in vial. The extraction final product was kept at -20°C prior to the GC/MS analysis (Vaughn and Berhow, 2005 and Shen et al., 2010).

2.4.3 Effect of ascorbic acid concentration

Ascorbic acid solutions with 2.0mM, 4.0mM, 6.0mM, 8.0mM and 10.0mM concentrations were prepared. Freeze dried plant samples (0.5g) were left to homogenize in 10mL of different concentrations of ascorbic acid solutions for 30 minutes at room temperature. The glucosinolate hydrolysis products were extracted using 20mL HPLC grade dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated

extraction was filtered using 0.2µm nylon filter and kept in vial. The extraction final product was kept at -20°C prior to the GC/MS analysis (Shen et al., 2010).

2.4.4 Effect of Iron Concentration

Fe²⁺, Fe³⁺ and Ethylenediaminetetraacetic acid (EDTA) solutions with 2.0mM, 4.0mM, 6.0mM, 8.0mM and 10.0mM concentrations were prepared from their respective salts FeSO₄·7H₂O, FeCl₃·6H₂O and C₁₀H₁₄N₂Na₂O₈.

Freeze dried plant samples (1.0g) were left to homogenize in 10mL of different concentrations of Fe²⁺, Fe³⁺ and EDTA solutions for 30 minutes at room temperature. The glucosinolate hydrolysis products were extracted using 20mL HPLC grade dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2µm nylon filter and kept in vial. The extraction final product was kept at -20°C prior to the GC/MS analysis (Williams et al., 2009; Liang et al., 2006).

2.5 Effect of processing conditions on formation of benzyl isothiocyanate and benzyl nitrile in leaf, flower and fruit of *carica papaya*

2.5.1 Sample preparation

All plant materials were washed and chopped into 2cm size prior to the analysis. Fresh plant materials were chopped each time before the experiment conducted and storage of chopped plants materials were avoided.

2.5.2 Effect of blanching

The blanching process was carried out by immerse 10g of chopped plant material into 100mL of boiling water for 3 minutes. The temperature was maintained to ranging from 94°C to 95°C. Later, the blanched plant material was drained, and cooled in 200mL of cold water for 1 minute. Then, the blanched plants material and residual water were placed at -20°C prior to further analysis (Volden et al, 2008; Volden et al., 2009b).

The blanched plant material was left to homogenize in 100mL of deionized water for 30 minutes at room temperature. The glucosinolates hydrolysis products were extracted using 50mL HPLC grade dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2µm nylon filter and kept in vial. Two replicates were prepared for each sample. The extraction final product was kept at -20°C prior to the GC/MS analysis (Volden et al, 2008; Volden et al., 2009b).

The residual blanched water also extracted using 50mL HPLC grade dichloromethane as shown previously for blanched plant material (Volden et al, 2008; Volden et al., 2009b).

2.5.3 Effect of boiling

For boiling process, 10g of plant material was placed immediately after chopping into 150mL of boiling water and the boiling was continued for 10 minutes. Then, the plant material was left at room temperature to cool. The boiled plants material and residual water was placed at -20°C prior to further analysis (Jones et al., 2010).

The boiled plant material was left to homogenize in 100mL of deionized water for 30 minutes at room temperature. The glucosinolate hydrolysis products were extracted using 50mL HPLC grade

dichloromethane. The mixture was shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2 μ m nylon filter and kept in vial. Two replicates were prepared for each sample. The extraction final product was kept at -20°C prior to the GC/MS analysis (Jones et al., 2010).

The residual boiled water also extracted using 50mL HPLC grade dichloromethane as shown previously for boiled plant material (Jones et al., 2010).

2.5.4 Effect of slow heating

For slow heating, 10g of chopped plant materials were immersed into 50mL water which was slowly heated to a desired temperature and hold for 5 minutes at the maximum temperature before left to cool at room temperature. The samples were slowly heated up to 40°C, 60°C and 80°C.

Later, the benzyl glucosinolate hydrolysis products were extracted using 50mL dichloromethane. The sample and the dichloromethane mixture were shaken for 30 minutes before centrifuged at 3500rpm for 5 minutes. The dichloromethane layer was separated and dried over anhydrous sodium sulfate. The extraction was concentrated to about 1.0mL using rotary evaporator. The concentrated extraction was filtered using 0.2 μ m nylon filter and kept in vial. Two replicates were prepared for each sample. The extraction final product was kept at -20°C prior to the GC/MS analysis.

3.0 RESULTS AND DISCUSSION

3.1 Glucosinolates profiling in leaf, flower and young fruit of *Carica papaya*.

In this study, leaf, flower and young fruit of *Carica papaya* were screened using GC/MS to profile the glucosinolates present. The samples were homogenized in deionized water at room temperature for natural hydrolysis to take place between glucosinolates and myrosinase. The hydrolysis products of the glucosinolates were analyzed in GC/MS and their *R groups* were matched using build in library in GC/MS and the standards. During the enzymatic hydrolysis of glucosinolates, the glucoside entity will undergoes rearrangement to form either isothiocyanates or nitriles except for some special conditions where epithionitriles, thiocyanate and oxazolidine-thione while the *R groups* of the glucosinolate remain unchanged. Therefore the identification of glucosinolates hydrolysis products could reveal their precursor. The hydrolysis of glucosinolate is shown in Figure 1:

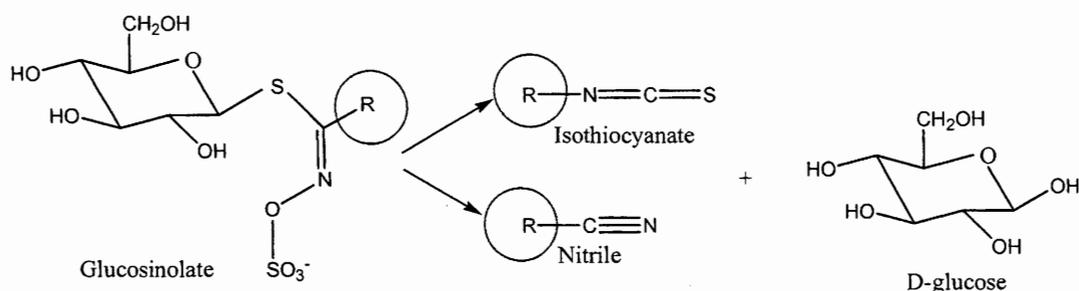
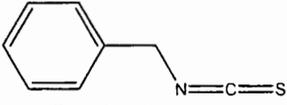
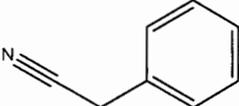
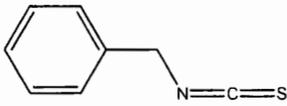
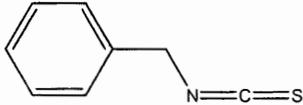


Figure 1: Enzymatic hydrolysis of glucosinolate (Vaughn and Berhow, 2005)

In this study, only two main hydrolysis products, i.e. benzyl isothiocyanate and benzyl nitrile were observed. The *R group* of these hydrolysis products (benzyl) revealed the identity of their parent glucosinolate. Table 1 shows the hydrolysis products present in leaf, flower and young fruit of *Carica papaya* and their respective GC chromatogram are shown in Figure 2.

Table 1: Glucosinolate hydrolysis products from leaf, flower and young fruit of *Carica papaya*, using GC/MS

Sample	Structure	RT	MS spectral data (m/z)
Leaf	 Benzyl isothiocyanate	12.8	149(M ⁺ , 20%), 91(100%), 77(7%), 65(17%), 63(7%) 51(7%)
	 Benzyl nitrile	9.5	117(M ⁺ , 100%), 116(42%), 90(52%), 89 (32%) 77(7%), 62 (6%), 51(14%)
Flower	 Benzyl isothiocyanate	12.8	91(99.9%), 149(18.5%), 65(13.5%), 92(8.3%), 39(5.6%), 51(4.2%), 63(4.1%), 89(4.0%), 50(2.3%), 90(2.0%)
Young fruit	 Benzyl isothiocyanate	12.8	91(99.9%), 149(18.5%), 65(13.5%), 92(8.3%), 39(5.6%), 51(4.2%), 63(4.1%), 89(4.0%), 50(2.3%), 90(2.0%)

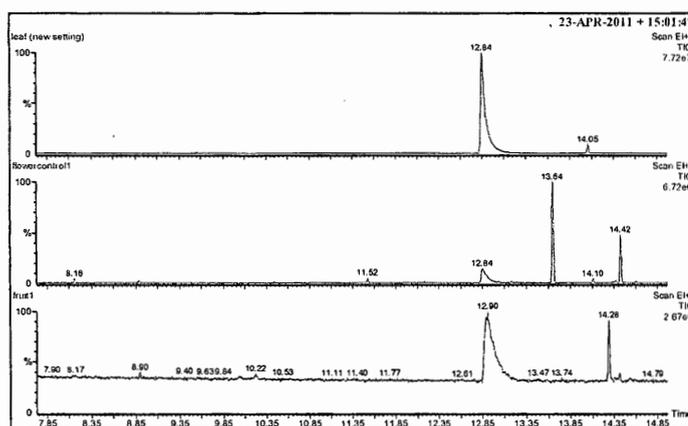


Figure 2: Chromatogram of leaf, flower and young fruit of *Carica papaya*.

The elution time recorded for benzyl nitrile was at 9.5 minutes followed by benzyl isothiocyanate at 12.8 minute. Both hydrolysis products recorded were matched using NIST library and mass spectrum

of standards. The mass spectrum of benzyl isothiocyanate and benzyl nitrile were shown in Figure 3 and Figure 4, respectively.

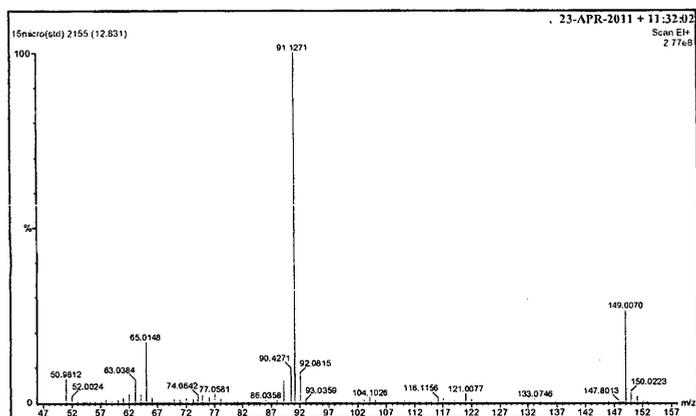


Figure 3: Mass spectrum of benzyl isothiocyanate.

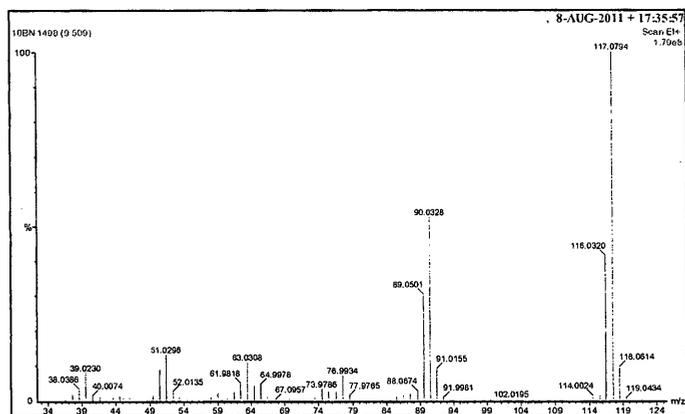


Figure 4: Mass spectrum of benzyl nitrile.

Natural autolysis of leaf gave benzyl nitrile and benzyl isothiocyanate whereas in flower and young fruit, only benzyl isothiocyanate were detected using GC/MS. The benzyl R group of these hydrolysis products proves the presence of benzyl glucosinolate or also known as glucotropaeolin (shown in Figure 5) in the samples.

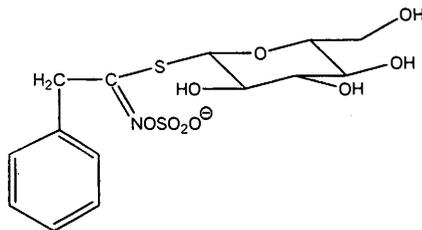


Figure 5: Structure of benzyl glucosinolate.

Throughout this report, the term benzyl glucosinolate is used. Benzyl glucosinolate is the sole glucosinolate that found in leaf, flower and young fruit of *Carica papaya*. The presence of benzyl glucosinolate in papaya flower has never been reported elsewhere. Interestingly the current finding is opposed to Bennett et al., (1997) who assumed there were no benzyl glucosinolate in any other tissues except the leaf and root of *Carica papaya*. Previously, the presence of benzyl glucosinolate has been reported in fruit (Flath and Forrey, 1977; MacLeod and Pieris, 1983; Schwab et al., 1989) and seed (Kermanshai et al., 2001) of *Carica papaya*. Probably the differences between the current and previous findings are due to the difference in papaya varieties.

Other than benzyl nitrile and benzyl isothiocyanate; benzyl thiocyanate also expected as part of the benzyl glucosinolates hydrolysis product since benzyl R group could give rise to a stable carbocation which can form into thiocyanate (Bones and Rossiter, 1996). MacLeod and Pieris (1983) and Flath and Forrey (1977) also stated the presence of methyl thiocyanate as part of the glucosinolate hydrolysis product in fruit of *Carica papaya*. However in the current study, no thiocyanate was found either in leaf, flower or young fruit. Recently Williams et al., (2009) reported that the presence of benzyl thiocyanate from benzyl glucosinolate hydrolysis in garden cress is due to Thiocyanate Forming Protein (TFP). TFP can redirect the glucosinolates hydrolysis to form thiocyanate instead of isothiocyanate. There might be two possible explanation for the absence of thiocyanate in this current study as recorded in garden cress. First, there is no TFP activity in *Carica papaya* and secondly the amount of thiocyanate present might be too low to be detected in GC/MS. However, further studies are needed to elucidate this suspicion.

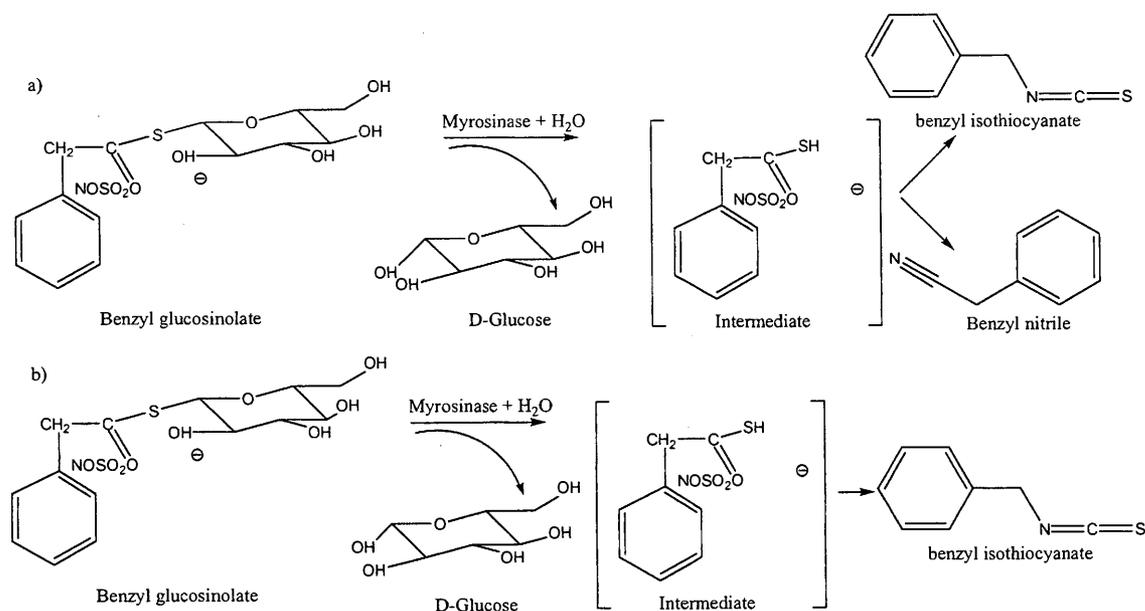


Figure 6: Benzyl glucosinolate hydrolysis in a) leaf and b) flower and young fruit of *Carica papaya*.

The presence of benzyl nitrile in leaf strongly proposes the presence of ESP, as the natural autolysis of benzyl glucosinolate should give benzyl isothiocyanate only. The ESP interacts with unstable hydroximate-O-sulphonate intermediate to produce benzyl nitrile before it undergoes Lossen rearrangement to produce benzyl isothiocyanate (Bones and Rossiter, 1996; Lambrix et al., 2001). Besides ESP, NSP also can redirect the benzyl glucosinolate hydrolysis to benzyl nitrile (Kissen and

Bones, 2008). NSP also act like ESP by rearranging the aglycone intermediate to benzyl nitrile instead of benzyl isothiocyanate (Wittstock and Burow, 2007). In flower and young fruit, the absence of benzyl nitrile could not be taken as the nonexistence of ESP and NSP, as ESP and NSP can be activated with the presence of Fe^{2+} (Wittstock and Burow, 2007; Matusheski et al., 2006; Kissen and Bones, 2009). The effect of Fe^{2+} on the benzyl glucosinolate hydrolysis is discussed in section 3.3.4.

3.2 Quantification of benzyl isothiocyanate in leaf, flower and young fruit of *Carica papaya*.

The quantification of benzyl isothiocyanate in leaf, flower and young fruit of *Carica papaya* has not been study yet. As previous studies focus on benzyl glucosinolate quantification, this study explored on benzyl isothiocyanate since benzyl isothiocyanate is biologically active as compared to benzyl glucosinolate and a potent anticancer. Apart from that, not all benzyl glucosinolates can be converted into benzyl isothiocyanate. There are several factors influencing the formation of benzyl isothiocyanate which will be discussed later in this study. So, it is important to examine the benzyl isothiocyanate production in natural autolysis. The plants were homogenized in deionized water at room temperature for the myrosinase to hydrolyse the benzyl glucosinolates naturally without influence of any external factors. The benzyl isothiocyanate quantified based upon GC peak area measurements with standard calibration curve. The concentration of benzyl isothiocyanate was greater in leaf with 52.10mg/kg, followed by young fruit, 3.59mg/kg and lesser amount in flower with 1.80mg/kg.

3.3 Analysis on glucosinolate hydrolysis products in leaf, flower and young fruit of *Carica papaya*.

Since benzyl isothiocyanate is biologically active and potent anticancer agent, the factors influencing the production of benzyl isothiocyanate at the expense of other hydrolysis products were examined in this study. As most of the previous researches focus on factors influencing level of glucosinolate, factors influencing isothiocyanate content should taken into great concern because isothiocyanate is biologically active as compared to benzyl glucosinolate.

3.3.1 Effect of autolysis temperature

The activity of myrosinase is influenced by temperature hence influence the glucosinolate hydrolysis pathway. As an enzyme, myrosinase deactivated at higher temperature. Yet, at some extent glucosinolate can be degraded thermally into nitrile rather than isothiocyanate without the myrosinase activity (Bones and Rossiter, 2006). The effect of temperature on isothiocyanate production is important to be monitored since most of food processing conditions involving heat treatment. In his study, the effects of autolysis temperature on benzyl glucosinolate hydrolysis pathway were evaluated by homogenizing the papaya samples at different temperature and left for 30 minutes.

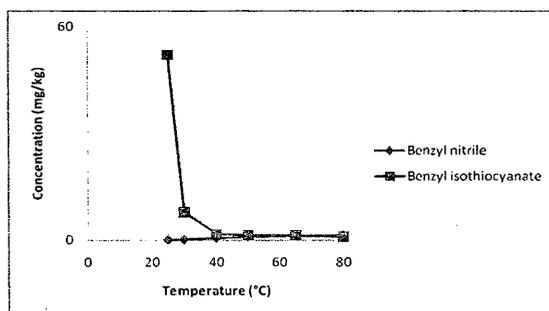


Figure 7: Effect of autolysis temperature on benzyl nitrile and benzyl isothiocyanate formation in leaf.

At room temperature (25°C), the highest amount of benzyl isothiocyanate was detected in leaf. A slight increase of temperature to 35°C in leaf, gave significant decrease in benzyl isothiocyanate production. The temperature increment decreases the benzyl isothiocyanate production and in meantime also increases the benzyl nitrile production. Eventhough the benzyl nitrile production increases as the temperature increase, but it does not able to overtake the benzyl isothiocyanate production. After 40°C, both benzyl isothiocyanate and benzyl nitrile production almost reach equilibrium as no further significant changes recorded (Figure 7).

In flower, the benzyl isothiocyanate production decreased and the benzyl nitrile production increased as the temperature increases. After 60°C, the productions of both benzyl isothiocyanate and benzyl nitrile were almost steady (Figure 8).

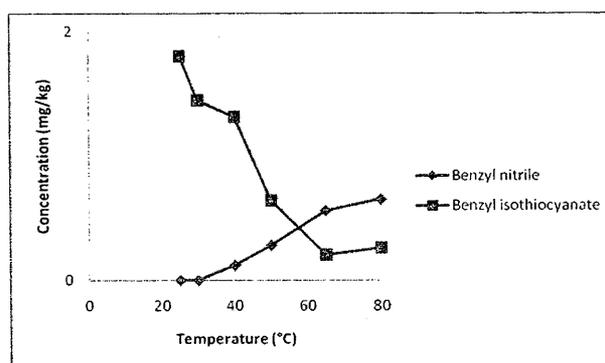


Figure 8: Effect of autolysis temperature on benzyl nitrile and benzyl isothiocyanate formation in flower.

In young fruit, the autolysis temperature increment does not influence the benzyl glucosinolate pathway eventhough significant changes recorded in the benzyl isothiocyanate production. No benzyl nitrile production was observed although the autolysis temperature increased up to 80°C. Still, the benzyl isothiocyanate production decreased gradually as the temperature increased (Figure 9).

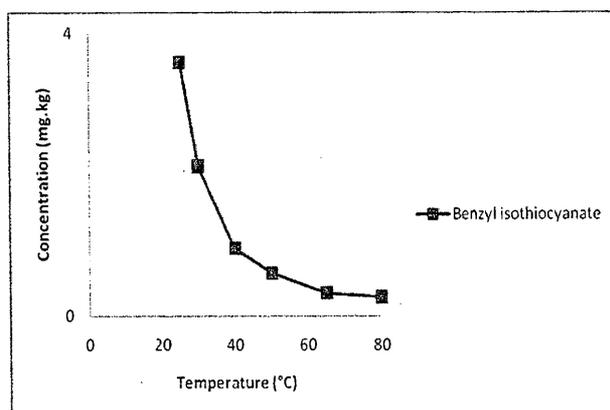


Figure 9: Effect of autolysis temperature on benzyl nitrile and benzyl isothiocyanate formation in young fruit.

Since there is no reported study on effect of autolysis temperature on the hydrolysis of benzyl glucosinolate, the trend of other glucosinolates hydrolysis towards temperature was compared with this current study. The maximum production of benzyl isothiocyanate was observed in leaf, flower and young fruit at room temperature (25°C) indicating the maximum myrosinase activity in *Carica papaya* is at room temperature. This result agrees well with Shen et al. (2010) who reported the maximum myrosinase action at 25°C in *Brassica oleracea*. Eylon et al. (2008) reported that the myrosinase in broccoli was stable until 45°C. In later study, Eylon et al. (2009) reported that the myrosinase in broccoli denatured at 100°C with the highest glucosinolate degradation was observed at this temperature. In this study, the decline in benzyl isothiocyanate production as the temperature increases in all plant part tested indicating the myrosinase inactivation. The myrosinase inactivation starts at temperature as low as 30°C might be due to the longer autolysis period, 30 minutes. The longer autolysis temperature also could release the volatile benzyl isothiocyanate produced from the samples. The increasing benzyl nitrile production as the temperature increased confirmed by Williams et al. (2009) suggesting increasing temperature favors more nitrile. A steady benzyl nitrile and benzyl isothiocyanate productions in leaf at 40°C, flower at 65°C and young fruit in 65°C showed the inactivation of myrosinase and the thermal degradation of benzyl glucosinolate at respective temperatures. As the benzyl glucosinolate thermally degraded, a stable amount of hydrolysis products were formed. The current study is the first preliminary study to report on myrosinase activity in papaya since there is no previous study has been reported on myrosinase activity in papaya.

3.3.2 Effect of pH

In this study, samples were homogenized into pH buffers range from 3 to 9 to observe the distinct effect of pH on benzyl glucosinolates hydrolysis products. The pH range chosen based on the preliminary studies which showed diverse changes in glucosinolates hydrolysis products.

In leaf, significant changes in benzyl isothiocyanate productions were observed. Lower level of benzyl isothiocyanate detected at pH 3 and gave rise as the pH increases. The optimum benzyl isothiocyanate produced at pH 7 before reduced again as the pH increased to 9. On the other hand, only slight change was observed for the benzyl nitrile production. Optimum benzyl nitrile production found at pH 4 and gradually decreased as the pH increased (Figure 10). However, abundant benzyl isothiocyanate were detected with insignificant production of benzyl nitrile in all the pH range tested. Eventhough only minor amount of benzyl nitrile production was detected in leaf, but the changes in benzyl nitrile production as the pH increases was in compatible with the studies previously reported. Vaughn and Berhow (2005) postulated that acidic conditions favoring nitrile formation and higher pH favoring isothiocyanate. Abundant production of isothiocyanate with only slight changes by pH conditions showed that the myrosinase activity in leaf of *Carica papaya* is stable and pH independent.

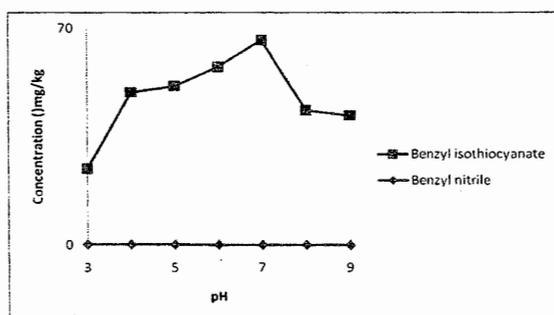


Figure 10: Effect of pH on benzyl nitrile and benzyl isothiocyanate formation in leaf.

In flower, the same pattern of benzyl isothiocyanate productions were trace as detected in leaf. Increasing pH favors more benzyl isothiocyanate with optimum yield found at pH 6. However, pH after 6 gradually decreased the benzyl isothiocyanate production (Figure 11). No benzyl nitrile was found in all the pH range tested. This showed changes in pH conditions do not influence the benzyl glucosinolate hydrolysis pathway in flower. The optimum benzyl isothiocyanate productions in leaf and flower were in agree with Bones and Rossiter, 1996 stated the optimum myrosinase activity achieved between pH 4 to 7.

In young fruit, the optimum benzyl isothiocyanate yield observed at pH 6 (Figure 12). However, the increasing pH decreased the benzyl isothiocyanate production while increased the benzyl nitrile production after pH 6. Surprisingly, no benzyl nitrile was observed before pH 7.

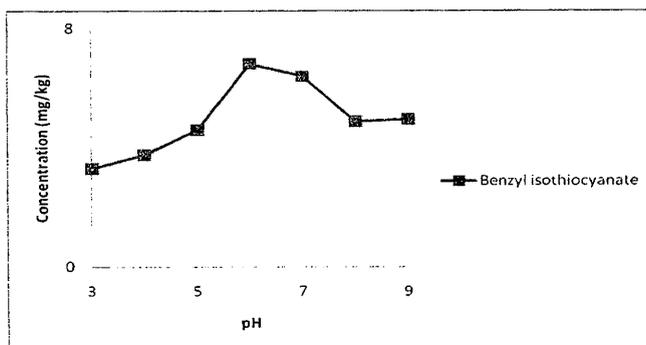


Figure 11: Effect of pH on benzyl isothiocyanate formation in flower.

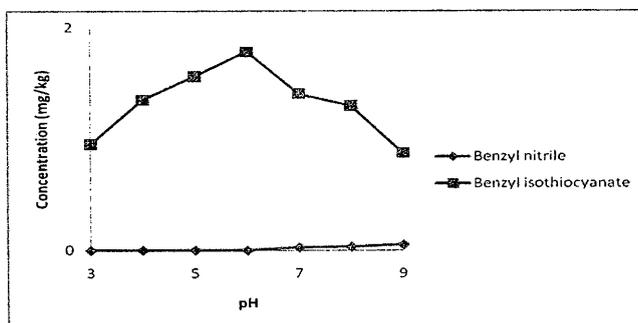


Figure 12: Effect of pH on benzyl nitrile and benzyl isothiocyanate formation in young fruit.

3.3.3 Effect of ascorbic acid

The addition of ascorbic acid to benzyl glucosinolate hydrolysis products of leaf, flower and young fruit were tested in this study. The samples were homogenized in different concentrations of ascorbic acid solutions.

In leaf, the addition of increasing ascorbic acid concentrations gradually decreased the formation of benzyl isothiocyanate. However, no significant changes were observed in benzyl nitrile production with the addition of ascorbic acid (Figure 13). This might be due to the presence of ascorbic acid that disturbed the myrosinase activity which hydrolyzed the benzyl glucosinolate into benzyl isothiocyanate. When the myrosinase activity was disturbed, lesser aglycone intermediate were produced which undergo Lossen rearrangement later to produce lesser benzyl isothiocyanate (Bones and Rossiter, 1996).

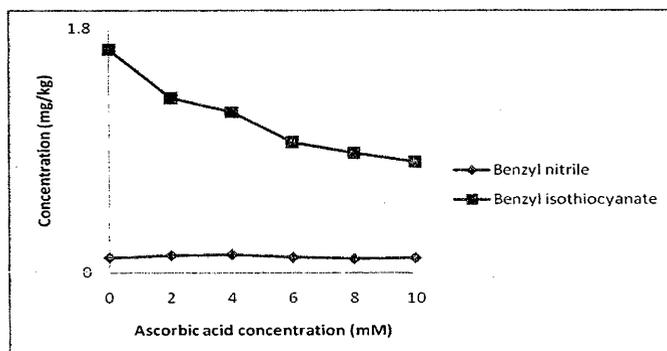


Figure 13: Effect of ascorbic acid on benzyl isothiocyanate and benzyl nitrile formation in leaf.

Bellostas et al. (2009) showed the addition of as low as 0.3mM of ascorbic acid has enhanced the nitrile production by 11-fold as compared with the sample without addition of ascorbic acid. The phenomena where higher concentration of ascorbic acid decreased the nitrile production was also recorded with the presence of ESP. This was because of the increasing myrosinase activity which in turn produced more intermediate aglycone; hence leads to the Lossen rearrangement which takes over the ESP activity which form nitrile.

The production of benzyl nitrile in leaf was almost steady possibly because, the addition of ascorbic acid did not influence the ESP activity which responsible for the benzyl nitrile production. The ESP produced constant amount of benzyl nitrile eventhough the myrosinase activity detriment by the addition of ascorbic acid; hence more benzyl glucosinolates is available for ESP activity. This showed that the ESP activity is not based on available benzyl glucosinolates but rather to the capability of ESP. Burow et al. (2006) stated that ESP works by binding to myrosinase as a cofactor or by binding to the unstable intermediate aglycone that produced from glucosinolate-myrosinase interaction before its rearrange. Since the myrosinase activity was disturbed by ascorbic acid, lesser intermediate aglycone produced, therefore lesser benzyl nitrile produced by ESP. However, in this study no such change in benzyl nitrile production was observed. The steady benzyl nitrile production suggests that the ESP activity and the myrosinase activity in leaf are independent. It is proposed here that another mode of mechanism for ESP activity which requires further studies. Another possible hypothesis for this phenomenon may be because the benzyl nitrile production was not at the expense of benzyl isothiocyanate. The benzyl nitrile and benzyl isothiocyanate formations are independent.

The result of this study is in disagreement with what have been reported by Shakita et al. (1999) where the addition of ascorbic acid was said to activate and enhance the myrosinase activity in *Raphanus sativus*. Addition of ascorbic acid as low as 0.02mg/g, significantly improved the enzymolysis process with increasing glucosinolate conversion rate in sulforaphane. However, further addition of ascorbic acid showed decrement of myrosinase activity (Shen et al., 2010). Kleinwachter and Selmar et al. (2004) reported 2mM of ascorbic acid showed maximal activation of *Tropaeolum majus* myrosinase activity with benzyl glucosinolate and further addition of ascorbic acid decreased myrosinase activity. The myrosinase activation should have stimulated more benzyl isothiocyanate production. But, the myrosinase activity in leaf of *Carica papaya* was interfered with addition of ascorbic acid. This might be because of the initial concentration of ascorbic acid, 2mM that was tested itself was high enough to interrupt the myrosinase activity hence decreased the conversion of benzyl glucosinolates into benzyl isothiocyanate.

For flower, the addition of ascorbic acid increased the benzyl isothiocyanate production with maximum benzyl isothiocyanate production was observed at addition of 6mM ascorbic acid. Further addition of ascorbic acid resulted in benzyl isothiocyanate decrement. Yet, the production of benzyl isothiocyanate after addition of more than 6mM ascorbic acid was still higher as compared to the benzyl isothiocyanate production without the addition of ascorbic acid. Shen et al. (2010) reported the further addition of ascorbic acid caused the enzymolysis to decrease below than the reference (without addition of ascorbic acid). No benzyl nitrile was observed with addition of ascorbic acid (Figure 14). This showed that the addition of ascorbic acid only influence the myrosinase activity which responsible for benzyl isothiocyanate production with no effect on benzyl nitrile production.

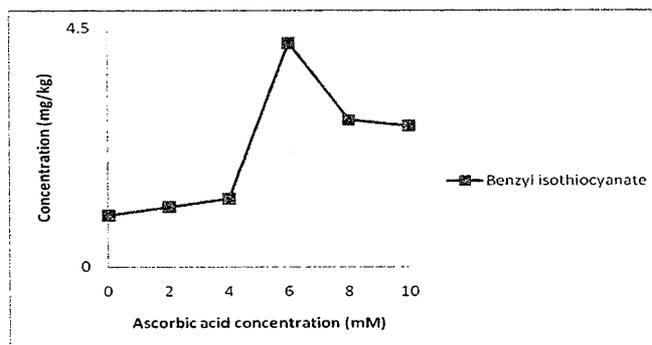


Figure 14: Effect of ascorbic acid on benzyl isothiocyanate formation in flower.

In flower, the outline of benzyl isothiocyanate production with addition of ascorbic acid was in compatible with Shakita et al. (1999), Shen et al. (2010) and Kleinwachter and Selmar et al. (2004). Addition of 0.02mg/g ascorbic acid reported to gave maximum myrosinase activity by Shen et al. (2010) whereas Kleinwachter and Selmar et al. (2004) reported myrosinase enhancement was observed with addition of 2mM ascorbic. Both Kleinwachter and Selmar et al. (2004) and Shen et al. (2010) reported the increment of myrosinase activity with slight addition of ascorbic acid and detriment with addition of higher concentration of ascorbic acid. In this study the concentration of ascorbic acid (6mM) was higher than in these previous works, but an equal detriment activity was observed with addition of higher concentration of ascorbic acid.

In young fruit, the addition of 2mM of ascorbic acid produced optimum yield of benzyl isothiocyanate and further addition decreased the benzyl nitrile production (Figure 15). The presence of benzyl nitrile observed with addition of 6mM ascorbic acid and further addition of ascorbic acid has increased the benzyl nitrile production.

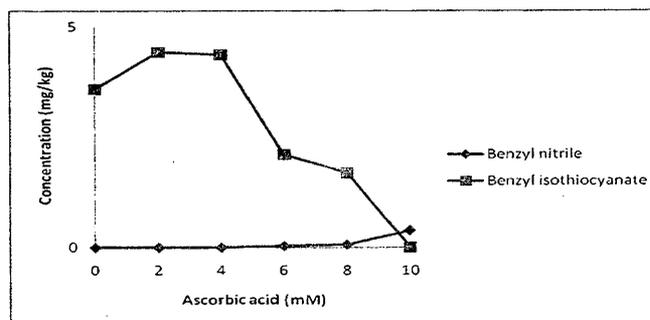


Figure 15: Effect of ascorbic acid on benzyl isothiocyanate formation in young fruit.

According to Bones and Rossiter, (1996) the ascorbic acid activation is due to some structural arrangement in substrate side of myrosinase. As an enzyme, myrosinase have two moieties on substrate side. One for the aglycone while another for the glucosinolate. As ascorbic acid occupied the aglycone moiety, glucosinolates fits better on its site, hence increase the myrosinase enzymolysis. Since both moieties on substrate side for ascorbic acid and the glucosinolate are the same, increasing concentration of ascorbic acid creates a great competition between ascorbic acid and glucosinolates in binding site. Therefore, higher concentration of ascorbic acid gave inhibitory effect on myrosinase. In all plant parts tested, the inhibitory effect of ascorbic acid was observed with the decrement in benzyl isothiocyanate productions.

The addition of 6mM and 2mM of ascorbic acid increased the benzyl isothiocyanate production in flower and young fruit respectively but no optimum production of benzyl isothiocyanate observed in leaf. This clearly suggests that the myrosinase activation is dependent on the tissue source of the enzyme (Bellostat et al., 2009). Myrosinase present as isoenzymes in many plants. Myrosinase isoenzymes are proven as organ and species specific. Different degree of glycosylation showed diverse myrosinase isoenzymes activity in each plant species and each plant organs. So, the degree of activation also influenced by type of myrosinase isoenzymes, plant species and plant part that the myrosinase isoenzyme found (Rask et al., 2000; Bones and Rossiter., 1996). However, the hydrolysis products are cannot be used to assess the myrosinase specificity in plant parts of *Carica papaya*. Other influencing benzyl glucosinolate hydrolysis factor such as specifier proteins should be evaluated as well.

3.3.4 Effect of iron concentration

Iron species, Fe^{2+} and Fe^{3+} are essential micronutrients for plant. The presence of Fe^{2+} and Fe^{3+} influence the level of benzyl isothiocyanate and benzyl nitrile produced.

i. Effect of Fe^{2+}

Addition of Fe^{2+} tremendously has increased the production of benzyl nitrile and at the same time reduced the production of benzyl isothiocyanate in leaf. As the concentration of Fe^{2+} increased, the production of benzyl nitrile also increased while the level of benzyl isothiocyanate decreased. Maximum benzyl nitrile produced with the addition of 8mM of Fe^{2+} . The benzyl isothiocyanate production was almost ceased after addition of 2mM of Fe^{2+} (Figure 16).

As ESP activity is suspected to present in papaya leaf, the addition of Fe^{2+} might have activated the ESP activity hence increased the benzyl nitrile production. ESP is said to possess a β -sheets known as Kelch motifs which able to bind with metallic cations. So, Fe^{2+} could bind with this motif at active site of ESP to operate it effectively. Raising Fe^{2+} concentration resulted in major nitrile production at the expense benzyl isothiocyanate (Bellostas et al., 2009).

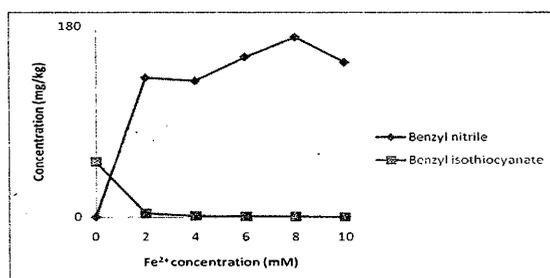


Figure 16: Effect of Fe^{2+} concentrations on benzyl isothiocyanate and benzyl nitrile productions in leaf.

In flower, the addition of Fe^{2+} produces results that are almost in same trend as obtained for leaf. Addition of Fe^{2+} produced benzyl nitrile which did not appear in the natural autolysis of flower without addition of Fe^{2+} . Increasing Fe^{2+} concentrations increased the benzyl nitrile content, at the same time decreased the benzyl isothiocyanate productions (Figure 17). Flower which was initially assumed does not possess ESP activity at the beginning of this study due to no benzyl production showed large production of benzyl nitrile with the addition of Fe^{2+} . This observation proposed that the ESP activity in flower is activated by the addition of Fe^{2+} .

Williams et al. (2009) also suggested that the ESP remains inactive without Fe^{2+} and only sufficient Fe^{2+} concentration can enhance its activity. Matusheski et al. (2006) also postulated that the hydrolysis of alkenyl glucosinolates in purified myrosinase and ESP in the absence of Fe^{2+} only produces isothiocyanate without the formation of any nitrile or epithionitrile. The nitrile was only observed after the addition of Fe^{2+} . The formation of benzyl nitrile with the presence of Fe^{2+} also leads to a possibility that the production of benzyl nitrile can be ESP independent where myrosinase and Fe^{2+} only responsible for the presence of benzyl nitrile. This possibility have been reported previously by Zabala et al. (2005) where nitrile production was still detected with the sole presence of myrosinase and Fe^{2+} without ESP. High concentration of Fe^{2+} may also derived a limited iron dependent non enzymatic nitrile formation since nitrile is the major non enzymatic product (Williams et al., 2009).

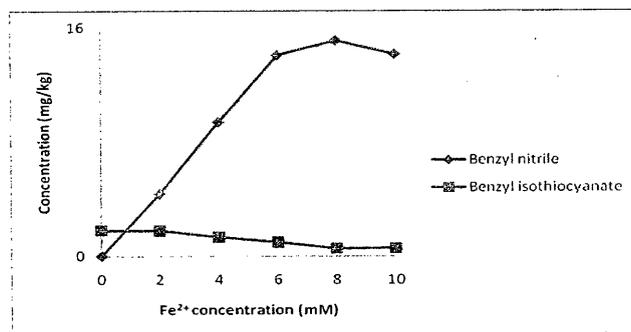


Figure 17: Effect of Fe^{2+} concentrations on benzyl isothiocyanate and benzyl nitrile productions in flower.

In young fruit, the addition of Fe^{2+} also increased the production of benzyl nitrile and at the same time, decreased the formation of benzyl isorhiocyanate (Figure 18). Zabala et al. (2005) reported, the presence of 2mM Fe^{2+} increased the corresponding nitrile up to 90% while in the absence of Fe^{2+} only isothiocyanate was detected. The study also stated that increasing concentration of Fe^{2+} increased the nitrile content. Burow et al. (2006) also proposed the Fe^{2+} promotes the nitrile formation in the presence of ESP which compatible with the result obtain in this study. All plant parts tested prove that the addition of Fe^{2+} increased the benzyl nitrile formation. This might be due to the ESP activation. The absence of benzyl nitrile in flower and young fruit during natural autolysis might be due to the insufficient endogenous Fe^{2+} content in flower and young fruit cells which leads to ESP inactivation. But leaf showed benzyl nitrile production in natural autolysis itself. Hence these show, ESPs from different plant organ sources vary in their iron requirement and ESP activity is organ specific (Burow et al., 2007).

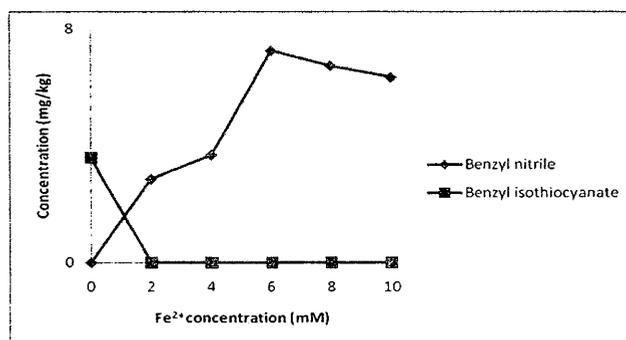


Figure 18: Effect of Fe²⁺ concentrations on benzyl isothiocyanate and benzyl nitrile productions in young fruit.

ii. Effect of Fe³⁺

In leaf, raising concentration of Fe³⁺ also increased the content of benzyl nitrile and decreased the production of benzyl isothiocyanate as observed with the addition of Fe²⁺. Reduction in benzyl isothiocyanate content almost invariable after addition of 4mM of Fe³⁺ (Figure 4.22). This result is in disagreement with Williams et al. (2009) who reported there was no effect found in benzyl glucosinolates hydrolysis with addition of Fe³⁺ or its chelator. For Liang et al. (2006), the addition of Fe³⁺ inhibits the isothiocyanate formation parallel with the addition of Fe²⁺. Moreover, they also stated that the inhibition of isothiocyanate with Fe²⁺ is greater as compared to the Fe³⁺. The same detriment effect also observed in this study where higher benzyl isothiocyanate inhibition effect was found with addition of Fe²⁺ as compared to addition of Fe³⁺. The ESP enhancement by Fe³⁺ also reported previously. The addition of Fe³⁺ resulted seven fold increment in ESP activity and no change in nitrile production upon addition of Fe³⁺ in absence of ESP (Burow et al., 2006).

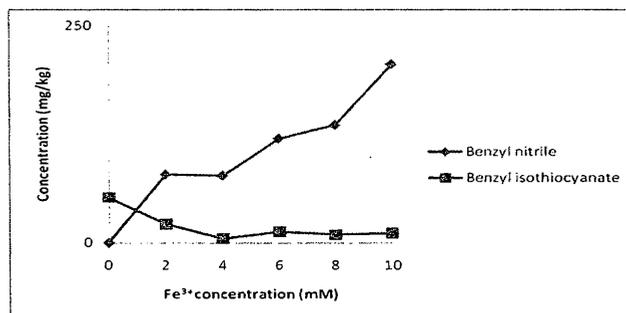


Figure 19: Effect of Fe³⁺ concentrations on benzyl isothiocyanate and benzyl nitrile productions in leaf.

In flower, addition of Fe³⁺ showed an unusual phenomena where increasing concentration of Fe³⁺ increased both benzyl nitrile and benzyl isothiocyanate production (Figure 20). The presence of benzyl nitrile with addition of Fe³⁺ could prove the presence of ESP which have been activated by Fe³⁺. However, the increasing benzyl isothiocyanate suggesting, a iron dependent non enzymatic degradation which produced benzyl nitrile. The Fe³⁺ ion formed benzyl glucosinolate-Fe³⁺ complex which leads to the formation of benzyl nitrile. Benzyl glucosinolate that did not form the benzyl glucosinolate-Fe³⁺ complex, undergo normal hydrolysis with the aid of myrosinase hence form benzyl isothiocyanate (Bellostas et al., 2009). Another theory to explain this phenomenon is that the addition of Fe³⁺ in flower might have enhanced

both ESP and myrosinase activity simultaneously which resulted in the increment of both benzyl nitrile and benzyl isothiocyanate productions.

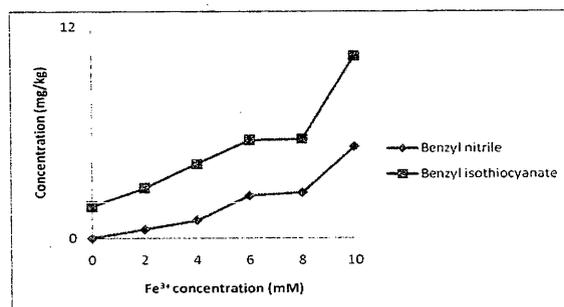


Figure 20: Effect of Fe³⁺ concentrations on benzyl isothiocyanate and benzyl nitrile productions in flower.

In young fruit, the addition of Fe³⁺ also decreased benzyl isothiocyanate formation and increased benzyl nitrile production (Figure 21). The result is in agreement with Liang et al. (2006) and Burow et al. (2006) where addition of Fe³⁺ increased the nitrile production by ESP activation. In this study all plant part tested showed a common increment in benzyl nitrile production with addition of Fe³⁺ which proves Fe³⁺ and Fe²⁺ are equally activating the ESP activity which is responsible for the benzyl nitrile production.

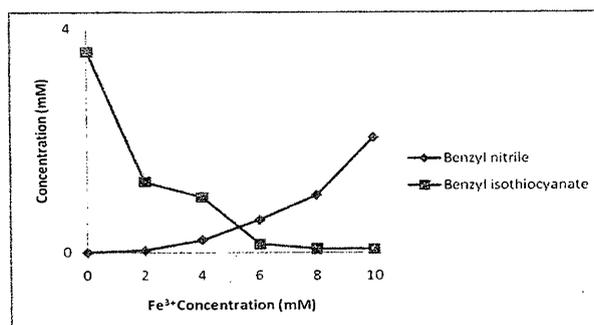


Figure 21: Effect of Fe³⁺ concentrations on benzyl isothiocyanate and benzyl nitrile productions in young flower.

iii. EDTA

EDTA is a chelator for iron ion. The chelating agent is used to evaluate the role of iron in hydrolysis of benzyl glucosinolate into benzyl nitrile. Ethylenediaminetetraacetic acid (EDTA) solution was prepared using Tris-HCl buffer at pH 7.0 in order to stabilize the pH of the reaction. The samples were dissolved into different concentration of EDTA solutions to chelated the iron ions in samples.

In leaf the increasing concentration of EDTA increased the formation of benzyl isothiocyanate and at the same time decreased the benzyl nitrile production. Overall, significant amount of benzyl isothiocyanate productions were observed whereas only small amount of benzyl nitrile were produced which can be neglected (Figure 22).

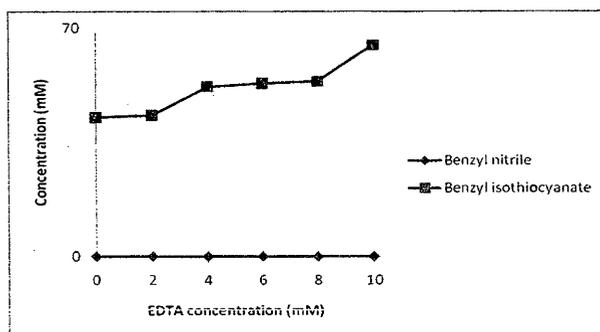


Figure 22: Effect of EDTA concentrations on benzyl isothiocyanate and benzyl nitrile productions in leaf.

In flower and young fruit, increasing concentrations of EDTA increased the benzyl isothiocyanate productions. There were no benzyl nitrile observed in both flower and young fruit (Figures 22 and 23).

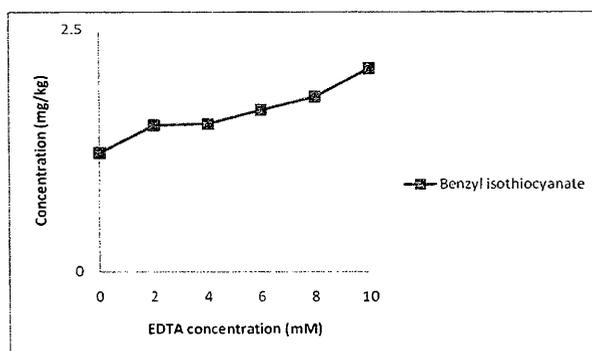


Figure 22: Effect of EDTA concentrations on benzyl isothiocyanate and benzyl nitrile productions in flower.

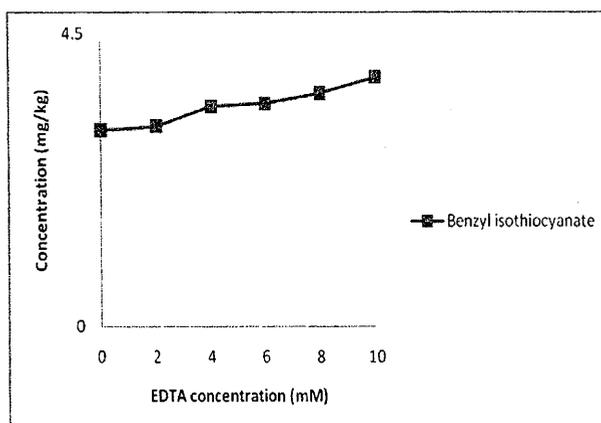


Figure 23: Effect of EDTA concentrations on benzyl isothiocyanate and benzyl nitrile productions in young flower.

The addition of EDTA into all plant parts tested showed significant increment in benzyl isothiocyanate production. This proves that the presence of iron in sample have contribute to the benzyl nitrile production thus decreased the benzyl isothiocyanate formation. Addition of EDTA chelated the iron in plant samples and this has assisted the myrosinase to hydrolyze benzyl glucosinolate into benzyl isothiocyanate. Williams et al. (2010) and Liang et al. (2006) also recorded the similar results.

3.3.5 Processing Condition

In this study, the effect of food processing conditions specifically blanching, boiling and slow heating on benzyl glucosinolate hydrolysis products in leaf, flower and young fruit have been observed. The real food processing conditions have been imitated in this study to show the influence of domestic food processing conditions in the production of health promoting compound; benzyl isothiocyanate formation. Other influencing factors such as shredding is controlled by chopped the leaf, flower and young fruit into 2cm size prior to the analysis (Figure 24). In both blanching and boiling, the plant material after processed and the residual water were tested to identify the loss of benzyl glucosinolate hydrolysis products into the processing water and the plant after processed. The term plant material referred to plant material after blanching or boiling whereas the residual water referred to the water that used to immerse the plant material during blanching or boiling process.

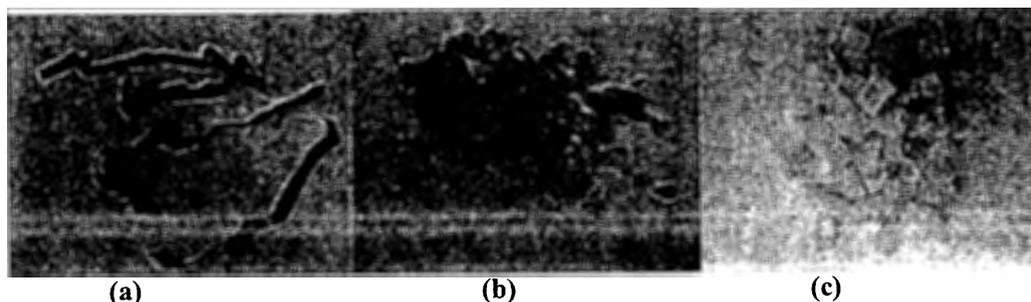


Figure 24: Chopped (a) leaf, (b) flower and (c) young fruit for blanching and boiling processes.

i. Blanching

In blanching, the plant material was immersed into water below boiling point (95°C - 96°C) for 3 minutes. The plant material and the blanched residual water were examined to evaluate the remaining benzyl isothiocyanate in plant material.

In leaf, higher benzyl isothiocyanate level was observed in residual water as compared to plant material which showed significant benzyl isothiocyanate loss into residual water. The presence of benzyl nitrile was higher at the expense of benzyl isothiocyanate in both plant material and residual water. Even so, the same outline as benzyl isothiocyanate was studied with the presence of benzyl nitrile where higher level of benzyl nitrile detected in residual water as compared to plant material (Figure 4.29). In this study, loss of 99.7% benzyl isothiocyanate was recorded in blanched leaf material as compare with before blanching.

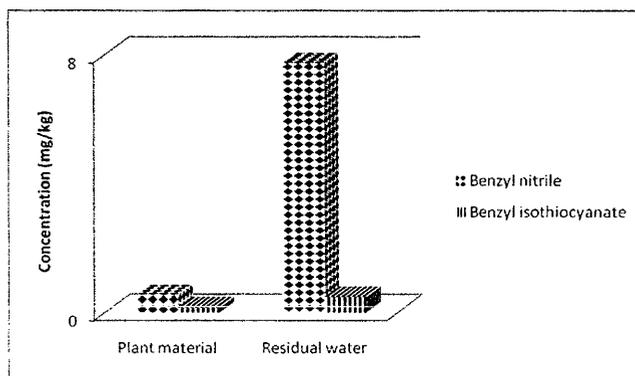


Figure 25: Effect of blanching to the production of benzyl nitrile and benzyl isothiocyanate in leaf.

Blanching flower only showed benzyl nitrile production without presence of benzyl isothiocyanate in both plant material and no compound detected in residual water (Figure 26). As previously reported in this study for the effect of autolysis temperature in section 3.4.1, formation of benzyl nitrile was already found at temperature as low as 30°C and the increasing temperature increased the benzyl nitrile formation. At high blanching temperature, the benzyl glucosinolate was thermally degraded into benzyl nitrile only and all the benzyl nitrile formed were leached out into residual water left the plant material contain none of the benzyl glucosinolate hydrolysis products.

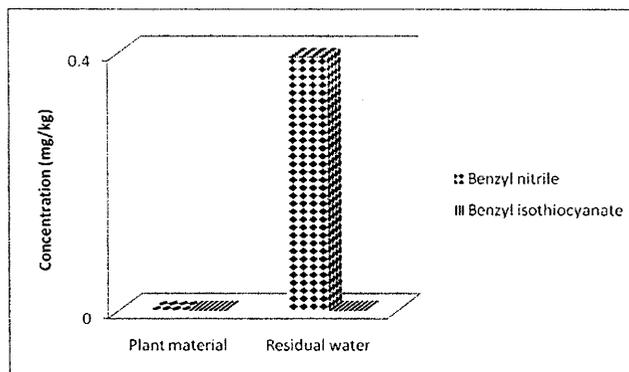


Figure 26: Effect of blanching to the production of benzyl nitrile and benzyl isothiocyanate in flower.

In young fruit, significant loss of benzyl isothiocyanate into residual water was observed. Only small amount of benzyl isothiocyanate formed was remained in plant material (Figure 27). Nearly 88.5% of benzyl isothiocyanate was lost in blanched flower material as compared to the reference.

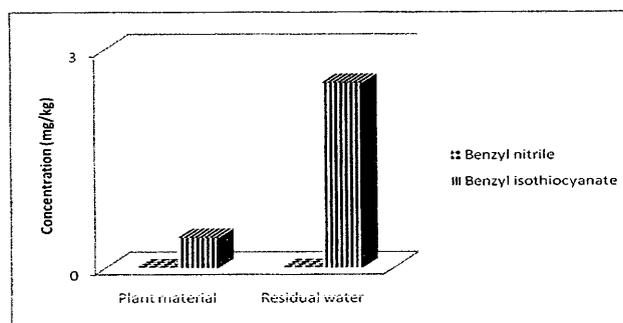


Figure 27: Effect of blanching to the production of benzyl nitrile and benzyl isothiocyanate in young fruit.

For all plant part tested, major loss of benzyl isothiocyanate content in plant materials were recorded as compared to the raw plant materials. The loss of benzyl isothiocyanate is either by leaching out into residual water or by the thermal degradation which favors the benzyl nitrile formation rather than benzyl isothiocyanate. For instant, 388% increment of benzyl nitrile level in blanched leaf plant material as compared to the raw plant material showed the thermal degradation of benzyl glucosinolates favors the benzyl nitrile formation. Leaching is the major cause for the loss of health promoting compound into residual water. In domestic cooking practices, leaching of benzyl isothiocyanate into residual water cannot be considered as a dietary lost if the culinary practices include the blanching residual water as well.

ii. Boiling

Boiling leaf showed significant loss of benzyl isothiocyanate in plant material where no benzyl isothiocyanate was observed in plant material while small amount of benzyl isothiocyanate was present in residual water. Boiling leaf only produced small amount of benzyl isothiocyanate which completely leached out into residual water. Benzyl nitrile content in residual water is higher compared to plant material (Figure 28). Complete absence of benzyl isothiocyanate in plant material showed higher temperature during boiling process absolutely deactivated the myrosinase activity in leaf hence thermal degradation produced foremost benzyl nitrile.

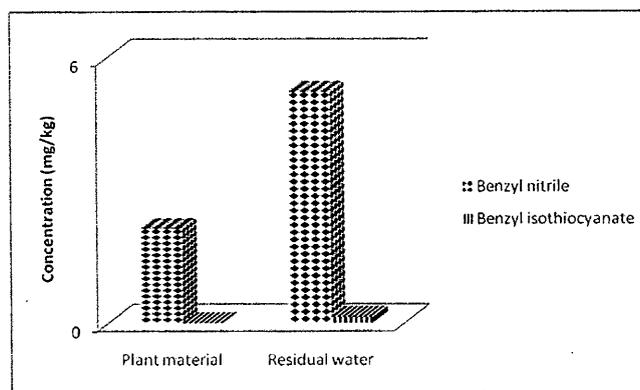


Figure 28: Effect of boiling to the production of benzyl nitrile and benzyl isothiocyanate in leaf.

In flower, no benzyl isothiocyanate was detected either in plant material or residual water whereas, benzyl nitrile only detected in the residual water. Boiled flower plant material showed no benzyl

glucosinolate hydrolysis products (Figure 29). All the benzyl nitrile produced by thermal degradation in plant material was leached out into residual water. The water volume regarded with the boiling duration could give vigorous action which leads to effective leaching process (Jones et al., 2010).

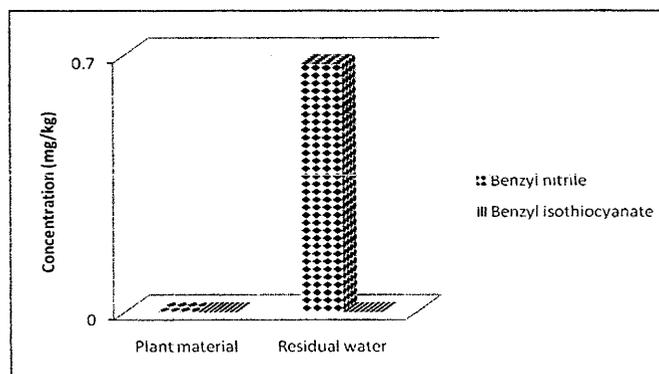


Figure 29: Effect of boiling to the production of benzyl nitrile and benzyl isothiocyanate in flower.

Surprisingly no benzyl nitrile production was observed in boiling young fruit. Benzyl glucosinolate only hydrolyzed into benzyl isothiocyanate. However, the benzyl isothiocyanate formed leached out into residual water leaving only small amount of benzyl isothiocyanate into young fruit material (Figure 30).

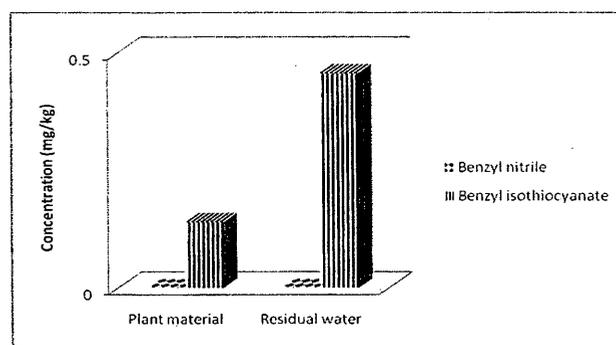


Figure 30: Effect of boiling to the production of benzyl nitrile and benzyl isothiocyanate in young fruit.

iii. Slow heating

In slow heating, three different temperature conditions were tested mainly; 40°C, 60°C, and 80°C. The temperatures were slowly increased from room temperature to the desired temperature and hold there for 5 minutes. This is to imitate the exact domestic cooking method where the vegetables are put into water first before heat it.

In leaf and flower, the slow heating process decreased the benzyl isothiocyanate production and increased the benzyl nitrile production as the temperature increased (Figures 31 and 32). For young fruit, the increasing temperature also decreased the benzyl isothiocyanate production but no benzyl nitrile was observed (Figure 3.7).

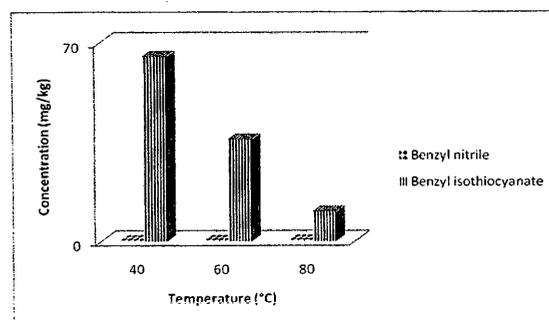


Figure 31: Effect of boiling to the production of benzyl nitrile and benzyl isothiocyanate in leaf.

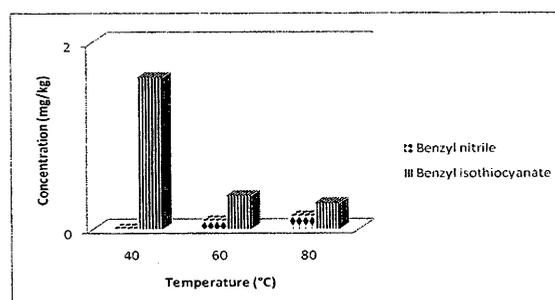


Figure 32: Effect of boiling to the production of benzyl nitrile and benzyl isothiocyanate in flower.

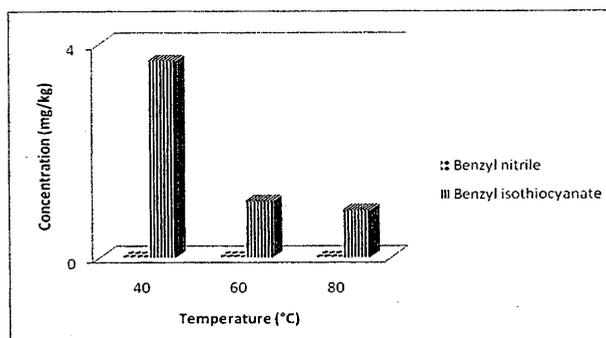


Figure 33: Effect of boiling to the production of benzyl nitrile and benzyl isothiocyanate in young fruit.

In all plant part tested, increasing temperature has detriment the myrosinase activity where lesser benzyl isothiocyanate produced at higher temperature. The increasing benzyl nitrile production as the temperature increased might be due to the ESP activation. However, Matusheski et al. (2004) showed mild heat treatment at 60°C to broccoli floret and broccoli sprouts could increase the production of isothiocyanate hence detriment the ESP activity. This showed, the ESP in leaf and flower of *Carica papaya* is more stable and heat treatment could activate their activity since no benzyl nitrile were observed at room temperature.

4.0 CONCLUSIONS

The presence of benzyl glucosinolate in *Carica papaya* as the sole glucosinolate in papaya has been confirmed in this study. The hydrolysis of benzyl glucosinolate gave benzyl isothiocyanate and benzyl nitrile only. The highest benzyl isothiocyanate was reported in leaf with 52.10 mg/kg, followed by young

fruit, 3.59 mg/kg and flower, 1.80 mg/kg. It was found that an optimum benzyl isothiocyanate production is at room temperature (~25°C) and pH range 6-7 in all plant parts tested. Addition of small amount of ascorbic acid gave optimum benzyl isothiocyanate production whereas, further addition of ascorbic acid inhibits benzyl isothiocyanate production. Optimum benzyl isothiocyanate production was observed with the addition of 2mM ascorbic acid in flower and 6mM ascorbic acid in young fruit. In contrast, addition of ascorbic acid only decreased benzyl isothiocyanate production in leaf. This might be due to the initial concentration of ascorbic acid tested itself was high enough to interrupt the benzyl isothiocyanate production.

The effect of the addition of either iron species on benzyl glucosinolate hydrolysis in *Carica papaya* favored the benzyl nitrile production and at the same time decreased the benzyl isothiocyanate production tremendously. The results of chelator addition confirmed this iron dependency by increased the benzyl isothiocyanate production. Heating or cooking of *Carica papaya* plant reduced human health benefits as benzyl nitrile production increased many fold at high temperature. In boiling and blanching processes, leaching was found as the major cause for the loss of health promoting compound into residual water. The water volume, temperature and the time interval should have considered in cooking processes to preserve the health promoting compound until consumption.

This study has provided valuable information regarding the hydrolysis products of benzyl glucosinolate in *Carica papaya*. As we gain more information about the health promoting benzyl isothiocyanate, it becomes increasingly important that we understand the mechanisms that control their formation or the production of any alternative compounds so that food processing parameters or guidelines for human consumption can be developed to offer greater health benefits. More research are needed to acquire better understanding on myrosinase activity, amount of benzyl glucosinolate and specifier proteins in different parts of papaya.

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