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EFFECTS OF DRYING CONDITIONS ON DRYING KINETICS, PRODUCT QUALITY AND RETENTION OF CARPAINE IN PAPAYA LEAVES (*CARICA PAPAYA* LINN.)

By

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ABSTRACT

Dengue fever causes mortality and morbidity worldwide which is a major concern to governments and the World Health Organization (WHO). It was reported that carpaine, the major active compound extracted from papaya leaves, contributes to the anti-thrombocytopenic activity. Hence, carpaine plays an important role in treating dengue patients by raising the platelet count in the patients' blood. However, carpaine in papaya leaves could degrade easily when exposed to heat and sunlight during processing and storage. There is no literature reporting on the processing aspects (e.g., preparation, drying and storage) of papaya leaves and the leaves extract to date. This could possibly be due to papaya leaves have no commercial value and the leaves are usually treated as waste. Therefore, this thesis will focus on the effects of drying conditions on drying kinetics, product quality and retention of carpaine in papaya leaves.

Extraction and quantification of carpaine from different parts of papaya leaves (young and old) and stalks were carried out. Pale yellow carpaine crystalline powders were successfully extracted and confirmed to have high purity (>95%) by ^{1}H and ^{13}C NMR analyses. Unblended freeze dried samples from young papaya leaves extract showed the highest amount of carpaine, total polyphenol content and DPPH free radical scavenging activities. It is thus recommended to use young leaves to extract carpaine for future drug development in dengue treatment.

The drying kinetics and drying rates of papaya leaves using different drying techniques such as hot air drying (60°C, 70°C and 80°C), shade drying

and freeze drying were investigated. Typical exponential falling trends were observed, which can be best explained by Fick's second law of diffusion. The fastest drying rate was observed at high temperature (80°C), followed by at lower temperatures (hot air drying at 60°C and 70°C, and shade drying at averagely 27°C). By using the general solution of Fick's second law and Arrhenius equation, the effective diffusivities were determined in the range of 2.09×10^{-12} m^2/s to 2.18×10^{-12} m^2/s, and the activation energy required to initiate moisture diffusion was determined at 2.11 kJ/mol in hot air drying within 60°C to 80°C.

Besides, the effects of drying techniques on carpaine retention and antioxidant properties of papaya leaves were investigated to develop a preparation protocol that produces papaya leaves extract with high carpaine retention. Hot air drying at 60°C, 70°C and 80°C are not recommended for the preservation of carpaine in papaya leaves as carpaine is a heat sensitive bioactive compound. Results showed that the carpaine retention in hot air dried samples were significantly lower ($p < 0.05$) than freeze dried and shade dried samples. It is recommended to use freeze drying to remove the moisture in the papaya leaves as it showed the highest retention of carpaine and antioxidant activities.

The knowledge on the stability of carpaine during storage is vital as the raw materials used would be mainly in dried form which will be stored in bulk quantity during commercial operation. Studies show that the Weibull model produced the best graphical fit in describing the degradation kinetics of carpaine in all dried samples (freeze dried samples, hot air dried samples at 60°C and 70°C, and shade dried samples), except for hot air dried samples at 80°C, which could be best described by the first order model. Freeze dried samples showed

the highest half-life (51.20 months) among all the dried samples. It is thus recommended to select freeze dried samples for extended storage purpose as it is more stable as indicated by the lowest rate constant and the highest half-life.

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PUBLICATIONS

Part of the studies in this dissertation has been submitted for publications.

1. Yap, J.Y., Hii, C.L., Ong, S.P., Lim, K.H., Abas, F. and Pin, K.Y., 2021. Quantification of Carpaine and Antioxidant Properties of Extracts from Carica Papaya Plant Leaves and Stalks. *Journal of Bioresources and Bioproducts*, 6(4), pp.350-358.

2. Yap, J.Y., Hii, C.L., Ong, S.P., Lim, K.H., Abas, F. and Pin, K.Y., 2020. Effects of Drying on Total Polyphenols Content and Antioxidant Properties of Carica Papaya Leaves. *Journal of the Science of Food and Agriculture*, 100(7), pp.2932-2937.

3. Hii, C.L., Yap, J.Y., Ong, S.P., Lim, K.H., Abas, F. and Pin, K.Y., 2019. Drying Characteristics of Carica Papaya Leaves and Quantification of Carpaine. *The 10th Asia Pacific Drying Conference, Vadodara, India, December 14-17.*

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NOMENCLATURE

MR	moisture ratio (dimensionless)
k_s	drying constant (1/s)
t	time (s)
k_p	drying constant of Page model (1/s)
m	moisture content (g water/g dry solid)
D_e	effective diffusion coefficient (m^2/s)
R	universal gas constant (J/mol K)
T	temperature (K)
x	position (m)
L	slab thickness (m)
β	roots of the Bessel function (dimensionless)
r_c	cylinder radius (m)
r_s	sphere radius (m)
W_i	weight of leaf (g)
W_{bd}	bone-dry weight (g)
E_a	activation energy (kJ/mol)
D_o	diffusivity constant (m^2/s)
L^*	lightness (dimensionless)
a^*	greenness/redness (dimensionless)
b^*	blueness/yellowness (dimensionless)
ΔE^*	total colour difference (dimensionless)
C	bioactive compound concentration (µg/g)
k	degradation rate constant (1/month)
b	degradation rate constant for Weibull model (1/month)
$t_{1/2}$	half-life (month)
χ^2	chi-square (dimensionless)
E	average error (%)
N	number of experimental data points (dimensionless)
z	number of constants (dimensionless)

Acronyms

ABE	acid-base extraction
ABTS	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)
AEAC	ascorbic acid equivalent antioxidant capacity
CIES	Commission Internationale de l'Eclairage
CTLC	centrifugal thin-layer chromatography
CYD-TDV	Dengvaxia® vaccine
DENV	dengue virus
DF	dengue fever
DHF	dengue haemorrhagic fever
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSS	dengue shock syndrome
ESI	electrospray ionisation
ESR	electron spin resonance
FD	freeze drying
GAE	gallic acid equivalent
HD6	hot air drying at 60 °C
HD7	hot air drying at 70 °C
HD8	hot air drying at 80 °C
IC_{50}	half-maximal inhibitory concentration
LC	liquid chromatography
MRM	multiple reaction monitoring
MS	mass spectrometer
NMR	nuclear magnetic resonance
PLE	papaya leaves extract
R^2	coefficient of determination
RMSE	root mean square error
ROS	reactive oxygen species
SD	shade drying
TEAC	Trolox equivalent antioxidant capacity
TLC	thin-layer chromatography
TPC	total polyphenol content
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Dengue fever is caused by an arthropod-borne flavivirus named dengue virus (DENV), transmitted by female mosquitoes mainly of the species *Aedes aegypti* (Figure 1-1) (CDC, 2019). Dengue patients are diagnosed with non-specific symptoms including frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, internal bleeding due to their low platelet count, weakness and rash (Ono, et al., 2003; Gubler, 2006). Most of the patients recover after two to seven days (WHO, 2020), but it may also develop into dengue haemorrhagic fever (a severe form of the illness) and causes organ damage, severe bleeding, dehydration and even death. Dengue haemorrhagic fever causes approximately 18,000 deaths each year (Leyssen, et al., 2000; Ocazionez, et al., 2010). However, there is no specific antiviral treatment or vaccine to treat it to date except only for some standard treatments for fever management, i.e., nursing care, fluid balance, electrolytes and blood clotting parameters (Abd Kadir, et al., 2013).

Figure 1-1: *Aedes aegypti* **mosquito**

Carica papaya Linn. is an economically important fruit crop grown locally and Malaysia is one of the top 7 major countries exporting papaya fruits (FAOSTAT, 2019). Papaya is generally known as kepaya, papaw or paw paw in different languages according to the cultivation regions. Papaya is previously ranked as the top 5 nutritionally beneficial fruits (with guava, watermelon, grapefruit and kiwifruit) among 38 common fruits based on nutritional scores and the percentage recommended daily allowance (RDA) for pro-vitamin A, ascorbic acid, potassium, folate and fibre (Ikram, et al., 2015). The fruit is not just delicious and healthy, but the whole plant, including fruit, root, bark, peel, seeds, and pulp, is also known to have medicinal properties and have been used to treat various diseases such as digestive disorder, gastric ulcers and malarial (Mello *et al*. 2008; Muñoz *et al*. 2000). Meanwhile, the production of papaya in Malaysia had been reported to reach 98,500 tonnes in 2018, and the papaya leaves are normally not harvested but discarded as waste because papaya fruit is the main crop product (FAOSTAT, 2019).

An increase in blood platelet counts of albino mice with dengue fever was observed after administering powdered papaya leaves mixed with palm oil (Sathasivam, et al., 2009). Yunita, et al. (2012) stated that a significant increase in blood platelet counts up to the optimum level in dengue patients after oral administration of papaya leaves capsules. Carpaine, the main alkaloid present in papaya leaves, was reported as the bioactive compound that exhibited anti-thrombocytopenic activity in which the platelet count was found increased in busulfan induced thrombocytopenic Wistar rats (Zunjar, et al., 2016). This is a breakthrough in the treatment of dengue fever, as the patients normally experience dehydration due to severe internal bleeding before death. Therefore,

this has paved the way for the development of carpaine extracted from papaya leaves in tablets or capsules for dengue treatment.

1.2   Problem Statement

Papaya leaves have no commercial value and are usually discarded as agricultural waste. Up to date, literature reports on processing aspects of papaya leaves are scarce (e.g., preparation, drying and storage). This research aims to extract and quantify carpaine from different parts of papaya leaves. Besides, the drying kinetics of papaya leaves should be investigated because the transport properties such as effective moisture diffusivity of papaya leaves are important parameters in the design and development of industrial dryers. Different period of drying rates also affects moisture mass transfer and drying efficiency. Besides, different drying processes could also have different impacts on the final product characteristics such as the retention of carpaine and the antioxidant properties of dried papaya leaves. Besides, the degradation kinetics of carpaine and antioxidant properties of papaya leaves during storage remains an under explored area. Hence, it is essential to conduct further studies and developments in these areas to improve carpaine retention and enhance its final product quality.

1.3   Research Objective

The main objective of this research was to investigate the quantification and stability of carpaine in papaya leaves during processing as the retention of this compound could be affected by preparation, drying and storage due to quality

degradation. Hence, studies were carried out to investigate the operating parameters that could enhance retention of carpaine in papaya leaves extract, with the following specific objectives:

i. To extract and quantify the amount of carpaine present in the leaves (young and old) and stalks.

ii. To investigate the drying kinetics of papaya leaves during hot air, shade and freeze drying.

iii. To determine the effects of different drying techniques on the retention of carpaine and antioxidant properties in papaya leaves.

iv. To determine the degradation kinetics of carpaine and antioxidant properties in dried papaya leaves extract during extended storage.

1.4   Contribution of Research

Currently, there are no published reports on processing aspects of papaya leaves and the extracts. Primarily, papaya fruit is still the main harvested crop from commercial farms and harvesting the leaves while growing the fruits at the same time would affect its production. Nevertheless, recent papers have reported the nutraceutical potential of papaya leaves in therapeutic and medicinal applications besides for dengue fever treatments (Santana, et al., 2019; Shubham, et al., 2019; Singh & Bhatnagar, 2019). Therefore, results from current research will contribute knowledge and information on industrial scaling up operations to process papaya leaves for the production of carpaine. This will help to generate revenue for the nation by expanding the local pharmaceutical and

nutraceutical industries and also to provide a remedy option that can be derived from natural product (carpaine) to combat dengue fever.

CHAPTER 2

LITERATURE REVIEW

2.1 Dengue Fever

2.1.1 Background

In recent decades, there has been a dramatic increase in dengue cases worldwide from 0.5 million in 2000 to 5.2 million in 2019 as reported by the World Health Organization (WHO, 2020). WHO estimated about 390 million dengue virus infections worldwide every year and more than 100 countries have been seriously affected, including South-East Asia (Ahmad, et al., 2018). In 2019 alone, about 124,777 cases have been recorded in Malaysia, 11,560 cases in Laos, 105,000 cases in Vietnam, 13,000 cases in Cambodia and 20,700 cases in Thailand (WHO, 2020). In Malaysia, it was forecasted that dengue incidences could increase to almost six times higher in the year 2040 compared to the baseline year 2010 (Bujang, et al., 2017). Factors contributing to increased infections include serotype shift of dengue virus, warmer climate, increased rainfall, human movement and massive infrastructure development (Mudin, 2015).

Dengue fever is caused by the arthropod-borne *Flavivirus* named dengue virus (DENV), transmitted by the *Aedes aegypti* mosquito (Talarico, et al., 2007). To date, four antigenically related but distinct virus serotypes (DENV-1, 2, 3 and 4) have been identified as belonging to the genus *Flavivirus* in the *Flaviviridae* family (Klawikkan, et al., 2011; Guzman & Isturiz, 2010; WHO, 2020). Infection with one DENV serotype produces only a specific antibody against that serotype. When the antibody from the first infection is neutralised,

secondary infections by other serotypes can cause more serious infections (Leardkamolkarn, et al., 2012). Although DENV-2 is known to be more lethal than other serotypes (Goel, et al., 2004), some studies have revealed that primary infection with DENV-1 or DENV-3 always results in more dangerous disease than infection with DENV-2 or DENV-4 (Guzman & Isturiz, 2010; Tang, et al., 2012). In recent years, the current dengue epidemic has become a focus of international public health awareness globally. Unlike malaria, which is more prevalent in remote areas, dengue cases are distributed mostly in urban and suburban areas (Parida, et al., 2002; Ahmad, et al., 2011). This has made the epidemic more lethal, as an outbreak is difficult to control due to highly populated areas in the cities.

Types of DENV infection include mild fever, known as dengue fever (DF), which constitutes about 95% of the total cases. A more serious type of infection is known as dengue haemorrhagic fever or dengue shock syndrome (DHF/DSS, 5% of total cases) (Sanchez, et al., 2000; Jain, et al., 2008). Recovery from the first type of infection provides lifelong immunity. However, it provides only half of the protection from a subsequent viral infection that ultimately results in the risk of DHF. Most dengue infections are characterised by symptoms including frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, weakness and rash (Ono, et al., 2003; Gubler, 2006).

International travel, increasing human population (Kyle & Harris, 2008; Qi, et al., 2008) and urbanisation create suitable conditions for the mosquito vector *Aedes aegyti*, and thus spreading the virus to new areas, causing major epidemics (Gubler, 2006; SNR, et al., 2011; Amarasinghe, et al., 2011). Dengue epidemics are endemic in over 100 countries in Africa, America, Eastern

Mediterranean, Southeast Asia and Western Pacific, with Asia representing about 70% of the global burden of disease (Gubler, 2006; Grzybowski, et al., 2012; WHO, 2020). The first case of DHF was discovered in the 1950s in Thailand and the Philippines (WHO, 2020), where the first two DENV serotypes were identified, followed by the third and fourth serotypes in 1954 (Kyle & Harris, 2008). Since then, DHF has been recorded as major cases resulting in hospitalisation and death among children in most Asian and Latin American countries (WHO, 2020). Approximately 3.9 billion people, or half the world's population, are now at risk of dengue, and 390 million infections occur globally and annually (Kyle & Harris, 2008; WHO, 2020). Over 390 million cases of DF and at least 96 million of DHF and approximately 25,000 deaths may occur each year (Leyssen, et al., 2000; Ocazionez, et al., 2010; WHO, 2020; Monash, 2020). Despite its lethal consequences, the staggering numbers of those affected still increase as there is no specific antiviral treatment or vaccine for DF (Guzman & Isturiz, 2010). Early diagnosis and strict hospitalisation often save patients' lives with DHF (Guzman & Isturiz, 2010; Sanchez, et al., 2000; WHO, 2020). Regulatory bodies have undertaken efforts to combat the vector and tackle this problem via awareness campaigns and vector control (SNR, et al., 2011). Other strategies include using plants with bioactive substances with toxic properties to the vector or insecticidal properties (Grzybowski, et al., 2012). Clearly, the development of antiviral drugs and vaccines is needed to support these programs. Moreover, a safe, low-cost, and effective vaccine to control DENV would also be needed, especially in the most affected and developing countries (Klawikkan, et al., 2011; SNR, et al., 2011). Therefore, a search of highly selective but non-

toxic antiviral compounds is urgently needed because of the wide spread of dengue. (Ocazionez, et al., 2010).

2.1.2   Fatality rate in Malaysia

Dengue fever outbreak has been a serious issue globally and 70% of the dengue cases were recorded in Asia. In 2019, there were 5.2 million dengue cases reported to WHO and Malaysia was listed in the top 10 countries with the most infection cases (Figure 2-1) and fatality (Figure 2-2) in 2019 (WHO, 2020).
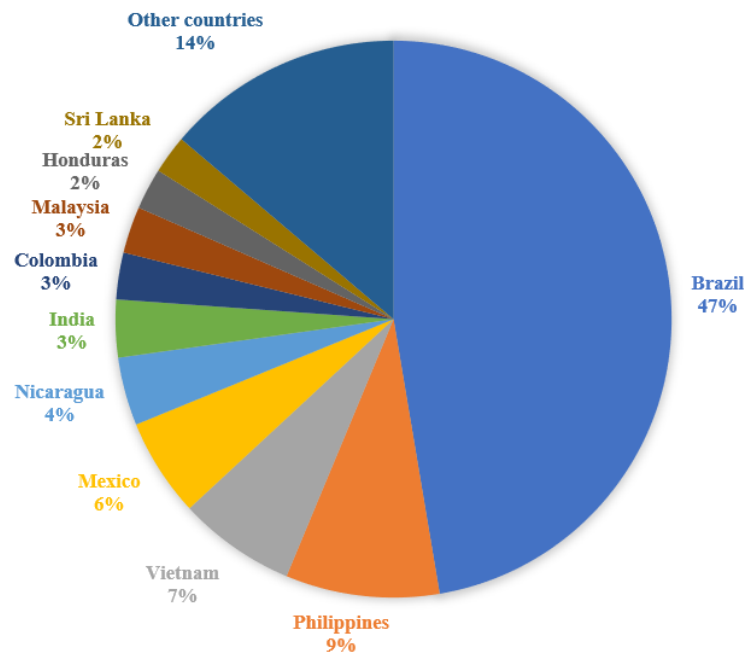
Figure 2-1: Infection percentage of dengue fever across the world
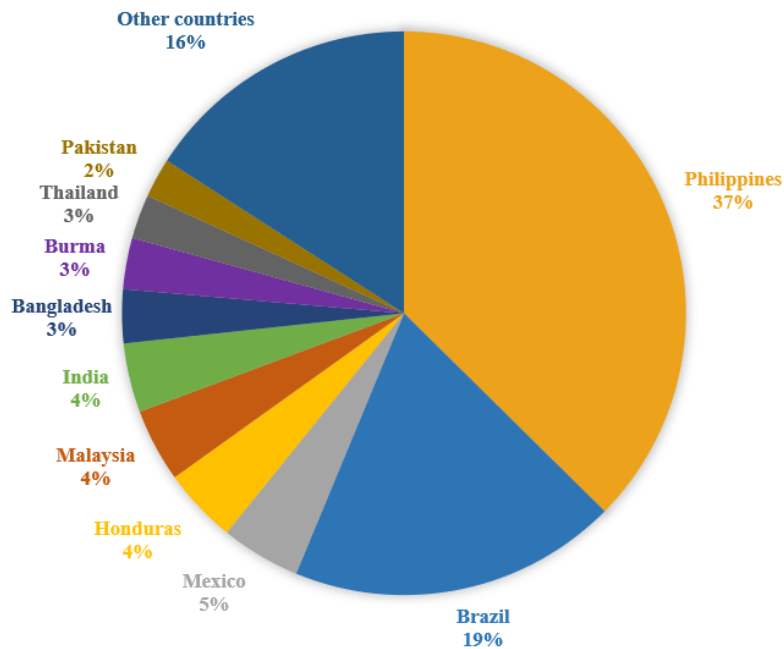
Figure 2-2: Mortality percentage of dengue fever across the world

In Malaysia, with a population of 32.7 million and a population density of 99 per km^2 (Department of Statistics Malaysia, 2020), outbreaks of dengue cases are endemic, with increasing dengue cases over the past two decades. The first case was documented in 1902 (SNR, et al., 2011; Lam, 1994; Chen, et al., 2006). During the period 1973–1982, 12,077 dengue cases were reported, with a fatality rate of 3.38%. The number of cases rose in the following decade with 26,361 cases (Lam, 1994). Between 2004 and 2005, dengue was reported causing 13,558 and 15,862 incidence rates, respectively, per 100,000 population. An increase of 16.99% of cases and 107 deaths were recorded in 2005 compared to 102 cases in 2004 (Chen, et al., 2006). In 2009–2011, the number of dengue cases decreased to 21,602 cases with the peak recorded in 2010 (Abd Kadir, et al., 2013). According to Health Facts 2019 from the Ministry of Health Malaysia, the incidence rates of DF and DHF were 397.71 and 1.61 per 100,000 population,

respectively, with a mortality rate of less than 0.01 (DF) and 0.56 (DHF). The number of dengue cases from 2012-2019 is summarised in Table 2-1 (KKM, 2020). In 2020, 88,074 dengue cases (141 deaths) were reported as of 12 December 2020 and it showed a decrease as compared to 124,777 cases (174 deaths) in 2019.

Table 2-1: Number of dengue cases from 2012-2019 in Malaysia

Year	Incidence/ mortality rate of dengue fever (per 100,000 population)	Incidence/ mortality rate of dengue haemorrhagic fever (per 100,000 population)
2012	72.20 / 0.00	2.45 / 0.12
2013	143.27 / 0.00	2.60 / 0.31
2014	357.49 / 0.00	3.66 / 0.71
2015	392.96 / 0.00	3.41 / 1.10
2016	318.13 / 0.00	2.01 / 0.75
2017	257.60 / 0.00	1.25 / 0.55
2018	244.07 / 0.00	1.23 / 0.45
2019	397.71 / 0.00	1.61 / 0.56

2.1.3   Potential cure for treatment

There is currently no specific treatment for dengue fever (Grzybowski, et al., 2012). Only standard treatment for fever management is given, i.e., nursing care, fluid balance, electrolytes and blood clotting parameters (Abd Kadir, et al., 2013). Patients with dengue fever are treated symptomatically, e.g., sponging, acetaminophen, bed rest and oral rehydration therapy. If signs of dehydration or bleeding occur, the patients are usually hospitalised or admitted for intensive care (Goel, et al., 2004; Ahmad, et al., 2011). Aspirin should be avoided because it may cause bleeding. Platelet count and haematocrit should be measured daily from the suspected day of illness until 1–2 days after defervescence (Ahmad, et al., 2011). The current prevention of dengue by potential dengue vaccine and vector control is too costly (Ocazionez, et al., 2010;

Suaya, et al., 2009). In addition, mosquito control programs are the most important preventive method (Goel, et al., 2004) but these are difficult to implement and maintain.

Development of the dengue vaccine is difficult since there are four closely related but antigenically distinct serotypes of the virus that could cause the disease (Muhamad, et al., 2010). Infection by one serotype does not ensure the protection of the patients from the other three serotypes (Qi, et al., 2008). Therefore, if vaccines were produced for only one or two serotypes, the other serotypes would increase the risk of more serious illness (Rees, et al., 2008). The first and the only dengue vaccine that had been licenced was in Mexico in December 2015 known commercially as Dengvaxia® (CYD-TDV), which was developed by Sanofi Pasteur and currently five additional dengue vaccines are still pending for further clinical testing. CYD-TDV is now licensed in 20 countries and for use in individuals between 9 - 45 years of age living in high dengue-endemic areas (Scott, 2016). The vaccine efficacy varied according to serotype, which the efficacy against serotypes 1, 2, 3 and 4 were 54.7%, 43.0%, 71.6% and 76.9%, respectively (Scott, 2016). CYD-TDV is currently not prequalified as WHO is still awaiting submission of an application from the manufacturer to prequalify this vaccine (WHO, 2018).

According to a WHO fact sheet dated December 2008, 80% of the infected population in some Asian and African countries depends on traditional medicine as their primary health care due to economic and geographical constraints (Abd Kadir, et al., 2013). Natural products have become the main source of test materials in developing antiviral drugs based on traditional medical practices (Meneses, et al., 2009). Traditional medicines are developed based on

knowledge, experience and practices relied on indigenous cultural beliefs. They are used to maintain health, prevent, treat and diagnose physical or mental illness (Abd Kadir, et al., 2013). Traditional medicinal plants have been reported to have antiviral activity (Betancur-Galvis, et al., 1999; Kudi & Myint, 1999), and some have been used to treat viral infections in animals and humans.

2.2   Carpaine as Potential Cure to Dengue Fever

2.2.1   *Carica papaya* plant

2.2.1.1   Botanical Description

Carica papaya Linn., which originated from the lowlands of eastern Central America (Nakasone & Paull, 1998), is a perennial plant belonging to the Caricaceae family. The first reference to papaya in Malaysia was made by a Dutch traveller Linshoten that the papaya was introduced from the Philippines in 1610 (Popenoe, 1934). It can be found in the whole tropical and subtropical areas. The *Carica papaya* plant has a weak, hollow and unbranched soft stem, which is light green to tan brown and can grow up to 20 m tall (Figure 2-3). There are large and long-stalked leaves grown on the stems, which can produce copious white latex.

Carica papaya is generally known for its fruit, which has high nutritional values at a reasonable price. It is also rich in natural minerals and vitamins as well as comparatively low in calories content (32 kcal/100 g of ripe fruit) (Yogiraj, et al., 2014). Papaya is a lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp (Figure 2-3). It is commonly known as papaw, lapaya, kates and betek in the United States,

Philippines, Indonesia and Malaysia, respectively. *Carica papaya* is one of the most consumed tropical fruits in Malaysia (Addai, et al., 2013) and Malaysia is also one of the top 7 papaya exporting countries in the world (FAOSTAT, 2019).
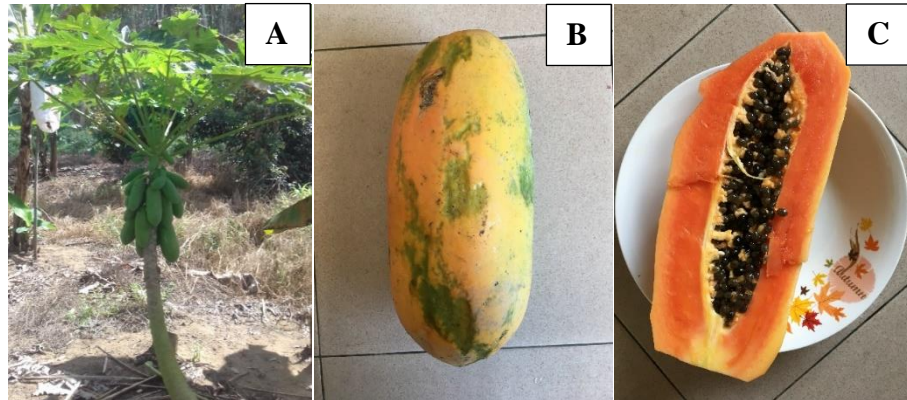
Figure 2-3: *Carica papaya* plant and fruit (plant: A, exterior: B; interior: C)

The papaya fruits are sometimes called pepo-like berries because of their oval shape with central seed cavity. Papaya is a climacteric fruit that exhibits a sudden burst of high respiration, causing it to be ripe within a few days (Zhu & Zhou, 2007). The colour of the fruit flesh changes from green (immature) to yellow (ripe) (McGrath & Karahadian, 1994). The fruit is normally grown on the main stem of the plant, and the appearance of the unripe fruits is green, turning to yellow or red-orange when ripe. The flesh surrounds a large central seed cavity. Typically, a papaya plant will mature in 5 - 9 months, depending on the cultivator and temperature, and the plant begins to bear fruits in 6 - 12 months.

Successful commercial cultivation of papaya plants is normally located in Africa, India, Hawaii, Ceylon, Philippines, Australia and Malaysia. There are smaller-scale productions in Latin America and South Africa. The variety and characteristics of *Carica papaya* are as listed in Table 2-2 (Parle M, 2011).

Table 2-2: The varieties and characteristics of *Carica papaya* fruits

Variety	Characteristic
Mexican Red	• Shape: elongated • Appearance: reddish to red-orange • Flesh: red
Solo	• Shape: pear-shaped • Flesh: reddish-orange • Remark: most common variety, does not cultivate male tree
Sunrise Solo	• Shape: pear-shaped • Appearance: smooth skin, red-orange • Flesh: sweet, high sugar content
Sunset Solo	• Shape: pear-shaped • Appearance: Small to medium size, red-orange
Waimanalo Solo (X-77)	• Shape: round fruit with neck, cavity star-shaped
Kamiha	• Shape: round • Remark: genetically engineered fruit, carries more flesh, most resistant to viruses

2.2.1.2 *Benefits of Carica papaya*

Carica papaya plants are widely reported to contain metabolites with antioxidant, antibacterial and anticancer activities. It can treat numerous diseases such as warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumours, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many (Aravind, et al., 2013). Therefore, *Carica papaya* can be regarded as a valuable nutraceutical fruit plant. The medicinal properties of different parts of *Carica papaya* are summarised in Table 2-3.

Table 2-3: Summary of medicinal uses of *Carica papaya*

Part	Medicinal use
Fruit	• Contains plenty of nutrients • Antifungal • Helps in regular bowel movement • Reduce the risk of cardiovascular diseases
Leaf	• Increase white blood cells, haematocrit level and platelets, normalise clotting and repair the liver • Potential cure for dengue viral infections • Antimalarial and antiplasmodial • Inhibit cancer cell growth • Promote digestion and treat ailments (chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart) • Antiseptic (green leaves) and tonic and blood purifier (brown leaves)
Seed	• Used as a substitute for black pepper in cooking • Nephroprotective activity and antibacterial properties are shown • Inhibit cancer cell growth
Latex	• Treat arthritis as it exhibits anti-inflammatory effects • Involved to treat commercial beer, degum natural silk, tenderise meat and help in the production of chewing gums • Used in anthelmintic, reliving dyspepsia, curing diarrhoea, pain of burns and topical use, bleeding haemorrhoids, stomachic and whooping cough
Peel	• Involved in the manufacture of cosmetics • Used as a sunscreen, soothing slave and skin lightening agent
Root	• Consuming the juice made from papaya root can ease urinary troubles

Papaya fruit is a rich source of different nutrients such as carotenoids, provitamin A, vitamin B, vitamin C, lycopene, dietary fibre and dietary minerals (Fauziya & Krishnamurthy, 2013; Rivera-Pastrana, et al., 2010). Danialone (a type of phytoalexin) has high antifungal activity against pathogenic fungus of papaya named *Colletotrichum gloesporioides* (Ademe, et al., 2013). Ripe

papaya, which is laxative, supporting in healthier bowel movements. It is also proven that the papaya fruit can prevent heart attack or stroke, as the fruit contains bioactive compounds, especially vitamin C, phenols and carotenoids, which help to reduce the risk of cardiovascular diseases (Sancho, et al., 2011). The folic acid found in papayas can convert homocysteine into amino acids such as cysteine or methionine. This prevents direct damage of blood vessel walls caused by unconverted homocysteine, which could pose a significant risk for heart attack and stroke (Aravind, et al., 2013). The fermented papaya fruit is also a promising nutraceutical as an antioxidant. It improves the antioxidant defence in elderly patients even without any obvious antioxidant deficiency state at the dose of 9 g/day orally (Krishna, et al., 2008).

Papaya leaves juice is proven to increase white blood cells, haematocrit level and platelets, normalise clotting, repair the liver, treat patients with dengue viral infections and inhibit cancer cell growth (Subenthiran, et al., 2013; Yunita, et al., 2012; Dev & Iqbal, 2015; Yunita, et al., 2012). Green papaya leaves are antiseptic, whereas brown and dried papaya leaves are the best tonic and blood purifier (Atta, 1999). In some parts of Asia, the young leaves are steamed and eaten like spinach. Green papaya leaves also can be made into tea to treat malaria. Antimalarial and anti-plasmodial activity has been described in some of the plant preparations. The papaya leaves tea helps to promote digestion and treat ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Ayoola & Adeyeye, 2010).

The seeds of the papaya are edible and have a pungent smell and peppery taste. The appearance of dried papaya seeds looks very similar to black pepper. They are sometimes ground and used to substitute for black pepper in flavouring

(Krishna, et al., 2008). The seeds were reported showing nephroprotective activity on a test on male Wistar rats where it prevented toxin-induced kidney failure as improved kidney architecture was shown after evaluating the concentration of urine and creatinine of the Wistar rats (Madinah, et al., 2015). Papaya seeds have antibacterial properties and are effective against *E. coli*, *Salmonella* and *Staphylococcus* infections (Peter, et al., 2014). It is reported that papaya seeds are a rich source of biologically active isothiocyanate with strong anti-cancer effects (Nakamura, et al., 2007).

The latex from the papaya plant aids the digestive system. It can be used to treat arthritis, as it exhibits anti-inflammatory effects, which may be attributed to the presence of papain and chymopapain (Gupta, et al., 1992; Gupta, et al., 1999). Papain can be found in a good amount in unripe papaya. It is an enzyme found in the milky juice used to prepare different remedies for indigestion (Marotta, et al., 2006). The milky juice is extracted, dried and used to treat commercial beer, degum natural silk, tenderise meat and chewing gums (Aravind, et al., 2013). It is also used in anthelmintic, relieving dyspepsia, curing diarrhoea, burns, bleeding haemorrhoids, stomachic and whooping cough (Krishna, et al., 2008). The papaya lipase, a hydrolase enzyme tightly bonded to the water-insoluble fraction of crude papain, is considered a "naturally immobilised" biocatalyst (Marotta, et al., 2006).

Papaya peel is often used in cosmetics and it is one of the ingredients used in sunscreen and soothing slave. It can also be used as a skin-lightening agent, as it contains vitamin A, which helps to restore and rebuild damaged skin. The peel can be used as soothe and moisturiser when mixed with milk and honey (Maran & Prakash, 2015).

Juice from the papaya roots is used in some countries in Asia to ease urinary troubles. A decoction is formed by boiling the outer part of the papaya tree roots to cure dyspepsia (Pal & Mazumder, 2013).

2.2.2   Carpaine

Carpaine is one of the major alkaloids of *Carica papaya* which can be found in all the green parts of the plant and the seeds. It can also be found in other members of the family Caricaceae, *Vasconcellosia hasta*, and certain *Apocynaceae* (Burdick, 1971). The chemical structure of carpaine is shown in Figure 2-4 (Wang, et al., 2015) and its formula is $C_{28}H_{50}N_2O_4$ which consists of two identically substituted piperidine rings linked together by two ester groups.
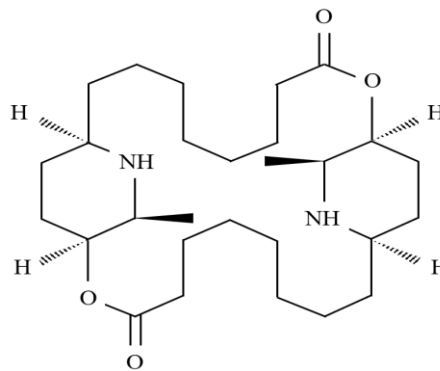
Figure 2-4: Chemical structure of carpaine

Almost all parts of the papaya plant can be utilised for food or medicinal purposes. There are many active components that can increase the total antioxidant ability in the blood and reduce lipid peroxidation. Alkaloid carpaine is the main active constituent in papaya leaves (Sato, et al., 2003; Tang, 1979; Govindachari, et al., 1965; Wang, et al., 2015). Carpaine has plenty of benefits,

e.g., reducing blood pressure and heart rate, promoting the movement of intestinal strips, and also causing uterus relaxation and bronchioles dilatation (Tuffley & Williams, 1951). It was also proven to be the major antiplasmodial compound, possessing significant *in vitro* antiplasmodial activity (IC_{50} of 0.2 µM) and high selectivity towards the parasite, i.e., *Plasmodium falciparum* (Julianti, et al., 2014). A recent study demonstrated that alkaloid carpaine showed antithrombocytopenic activity when tested on busulfan induced thrombocytopenic Wistar rats (Zunjar, et al., 2016).

Untested herbal medicines could be potentially harmful to human health. Many plants used as traditional and folk medicines are potentially toxic, mutagenic, and carcinogenic (Dharmarathna, et al., 2013). Recent studies showed that papaya leaves extract has potential anti-sickling (inhibition of sickle cell formation) properties (Imaga, et al., 2009). A protective effect against gastric ulcers had been reported in rats (Indran, et al., 2008). Besides, it was reported that papaya leaves extract can increase platelet and red blood cell in healthy mice with no adverse effects (Zunjar, et al., 2016; Halim, et al., 2011). Papaya leaves juice was also proven to significantly accelerate the rate of increase in platelet count among patients with DF and DHF (Subenthiran, et al., 2013). Oral toxicity study of papaya leaves extract in Sprague Dawley rats was repeated for 28 days and the study suggested that papaya leaves extract could be considered relatively non-toxic as medicine with dosage up to fourteen times the dose consumed empirically in traditional medicine in Malaysia (Afzan, et al., 2012).

2.3 Drying Techniques

2.3.1 Background of drying process

In recent years, the need for high quality dried food products has increased globally due to increasing health awareness, resulting in the growing interest in consuming natural food products rich in antioxidants. Drying is one of the most widely used methods to preserve foods for storage and preservation purposes. During drying, the water activity in foodstuff is reduced to minimise the rate of deterioration due to respiration, insects, microbial activity and biochemical reactions, leading to better preservation of the quality of the stored product (Ahmed, et al., 2013; Papu, et al., 2014).

Dried foods have many benefits such as extended product shelf-life and reduced packaging, storage, handling and transportation costs, as well as extending the possibility of out-of-season availability and providing a wider range of products for consumers (Moses, et al., 2014). Many centuries ago, fruits and vegetables were mainly dried using sun and solar drying owing to simplicity and low cost. However, these techniques require a long drying time and exposure to sunlight, thus resulting in poor product quality in terms of nutritional, functional and sensorial attributes and even product contamination (Bezyma & Kutovoy, 2005; Banga & Singh, 1994). Therefore, it is necessary to develop other drying techniques that are more efficient in terms of drying time, energy and nutrients preservation in food products.

2.3.2   Hot air drying

Hot air drying is a common process for food preservation in which the material to be dried is exposed to a continuous flow of hot air (Ratti, 2001). This drying technique can significantly reduce drying time compared to sun drying by utilising convective hot air with elevated temperature to remove moisture through diffusion and evaporation. Generally, a hot air dryer can be operated from ambient temperature to 300°C. Depending on the design, it can be operated in either batch (Figure 2-5) or continuous (Figure 2-6) mode (Perry, et al., 1997).
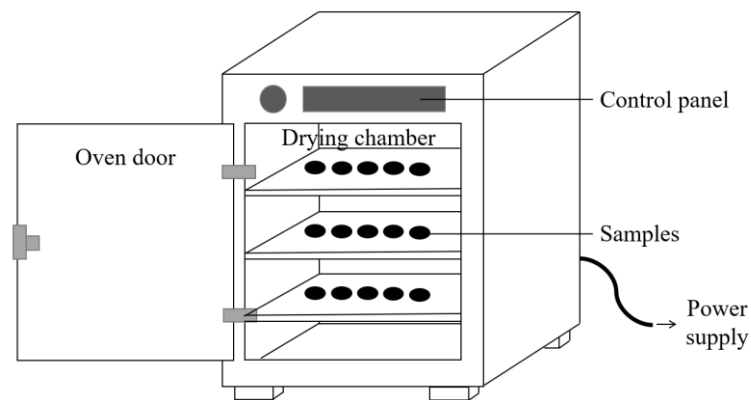
Figure 2-5: Hot air dryer (batch mode)
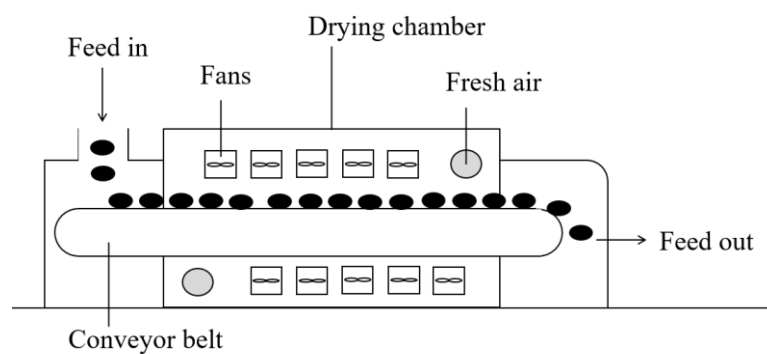
Figure 2-6: Hot air dryer (continuous mode)

Hot air drying could overcome problems encountered by sun-drying that would affect the quality of the dried products. It is common to dry leafy products by using air temperature ranging from 40°C to 60°C (Babu, et al., 2018). It was also found that 50°C to 60°C range was the most suitable drying temperature in drying of several medicinal plants (Rocha & Melo, 2011) as no microbial proliferation was observed (Sagrin & Chong, 2013). A study reported that hot air drying at 60°C was efficient for curry, mint and coriander leaves, and minimum loss in nutrients was observed (Vyankatrao, et al., 2014).

Although hot air drying produces dried food with extended shelf life, other quality attributes are found to reduce drastically, and in some cases the drying time is long even at high temperature due to low energy efficiency (Papu, et al., 2014). The temperature of hot air is crucial, as it will greatly influence the final product quality, especially for heat sensitive food materials (Lewicki, 2006). Reported literature have cited that physical, structural, chemical and nutritional attributes could be greatly affected during hot air drying, especially when the air temperature is excessively high (Poomsa-ad, et al., 2011; Hussein, et al., 2015). The loss of active ingredients could be due to the evaporation of low volatile compounds at elevated temperature.

2.3.3   Shade drying

Shade drying utilises ambient air for the drying process in a shaded area away from exposure to direct sunlight under ambient conditions. It is also known as room drying, shadow drying, indoor dehydration and sheltered air drying (Babu, et al., 2018). Shade drying of fruits and vegetables is still practised in

many tropical and subtropical countries, mainly because of the lower cost of operation. It employs natural air circulation and is highly dependent on climatic conditions. The desired moisture content of dried products could be achieved in a shorter time in hot and dry climatic conditions than in moist climatic conditions.

The disadvantages of shaded drying are time-consuming, space-consuming, labour intensive, weather dependent, low process throughput and high risk of product contamination (Xiangyang, et al., 2010). Theft, mould growth and damage by animals (birds, insects or rats) might happen during drying. The fluctuating sunlight (ambient temperature) and laminar air flow over the product could lead to non-uniformity in final product quality (Arslan & Özcan, 2008).

Despite the above disadvantages, shade drying is still used as it is a lower-cost alternative for drying non-UV sensitive food products than hot air drying and freeze drying. Besides, the products dried using shade drying process are translucent in appearance and superior in retaining colour and flavour (Thamkaew, et al., 2020). Some literature also reported that shade drying is the most effective technique for preserving primary nutrients and antioxidant capacities of leafy products (Babu, et al., 2018).

2.3.4   Freeze drying

Freeze drying, also known as lyophilisation, relies on the principle of sublimation whereby ice held under conditions of partial vacuum (611 Pa) and low temperature (less than 0°C) will evaporate without going through a liquid phase. A freeze dryer (Figure 2-7) consists of a refrigerated chamber, vacuum

chamber and vacuum pump. It can be operated either in batch or continuous mode. The food products are placed in a refrigerated chamber, followed by lowering the temperature and pressure of the chamber. The moisture in the food products is thus sublimated from solid to gaseous form, which is then collected in a separated chamber so that the food products are allowed to slowly equilibrate to room temperature for collection upon drying.
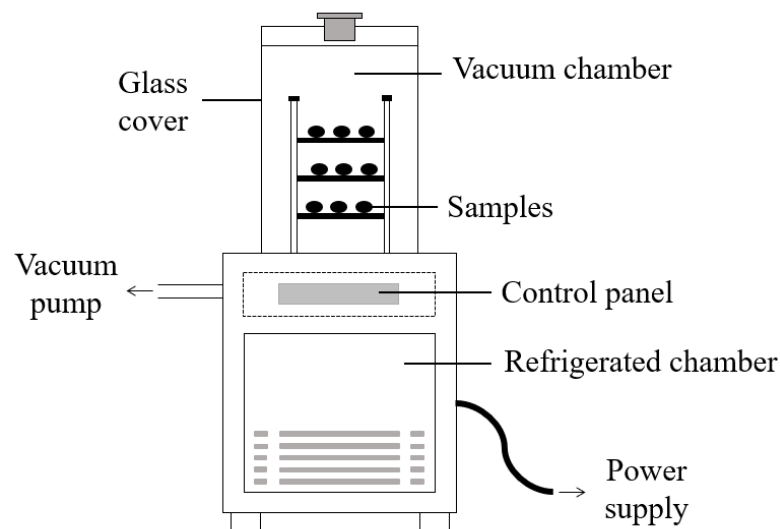
Figure 2-7: Freeze dryer

Freeze drying is one of the best techniques to remove water from biological materials while resulting in the highest final product quality (Ahmed, et al., 2013). It is superior in retaining the nutritional values in the dried leafy products, e.g., antioxidant properties and total phenolic content (Chan, et al., 2009). This is because all the microbiological and deterioration activities are inhibited under low temperature (Sagar & Suresh Kumar, 2010) and there is also no thermal degradation in freeze drying, hence, not allowing degradative enzymes to function.

The capital and operating costs of freeze drying are expensive as a vacuum pump must be installed to lower the pressure, and the drying process is time-consuming, taking up to 2 days (Dilta, et al., 2011). Besides, the freeze drying process can only be done on a small scale due to limited space in the drying chamber. Therefore, it is recommended to use freeze drying on high-value and low-volume products such as herbs, spices and medicinal plants (Chen & Mujumdar, 2014).

Table 2-4: Summary of advantages and disadvantages of different drying techniques

Drying technique	Advantage	Disadvantage
Hot air drying	- Simple - Short drying time - Eliminate contamination by droppings of birds and insects, dust, rain and fungal growth	- Drying temperature is limited to the heat sensitivity of the material - Reduced product quality due to exposure to high temperature - Operate on a smaller scale than shade drying - Energy consuming
Shade drying	- Simple - Low capital cost - Superior retention of colour and flavour - Good in preserving primary nutrients and antioxidant properties	- Time-consuming - Space consuming - Labour intensive - Weather dependent - Higher chance to be polluted by droppings of birds and insects, dust, rain and fungal growth - Non-uniformity of product quality due to the fluctuation of ambient temperature
Freeze drying	Superior retention of product quality (sensory,	High operating and capital cost

| microstructure and nutritional values) | • Volatile compounds can be removed by high vacuum |
| • Eliminate contamination by droppings of birds and insects, dust, rain and fungal growth | • Time-consuming |

2.4 Drying Theory and Diffusivity

2.4.1 Drying mechanism

The drying process involves simultaneous heat and mass transfer, whereby heat is transferred to the food products by hot air for moisture diffusion and evaporation. After preheating period (I), the drying of food products occurs in two stages (Figure 2-8), which are the constant rate period (II) follows by falling rate periods (III and IV) (Özbek & Dadali, 2007; Panchariya, et al., 2002). During the constant rate period (II), evaporation occurs at the food product's surface to remove unbound moisture. This period is not seen in the drying of leafy materials due to insignificant presence of unbound moisture in these materials. The constant rate period ends at critical moisture content, where the falling rate periods (III and IV) commence, and it will end when drying eventually reaches the desired final moisture percentage. The drying rate of food products in the falling rate period (III and IV) is controlled by diffusion and capillary effects. The moisture inside the food is slowly transported to the surface by a gradual increase of food temperature before evaporating from the surface. The drying operation stops once it reaches the equilibrium drying period (V). At this point, there is no increase in the exchange of moisture between the food products and the surrounding air unless a higher drying air temperature or

lower relative humidity is introduced into the drying chamber (Panchariya, et al., 2002).
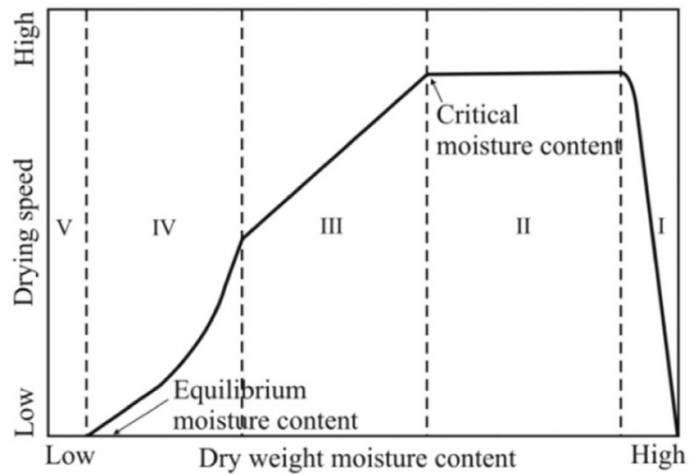
I – Preheat period
II – Constant rate period
III – First falling rate period
IV – Second falling rate period
V – Equilibrium drying rate period

Figure 2-8: Typical drying rates periods for food products

2.4.2   Theoretical models in drying process

Modelling of drying processes is one of the most important aspects of equipment design. It is used to simulate and optimise the drying processes under various operating conditions (airflow, temperature and relative humidity). Drying curves are usually modelled by defining drying rate constants based on first order kinetics thin layer model. The basic model is known as the simple exponential model (Equation 2.1).

$$MR = \exp(-k_s t)$$ (2.1)

where MR = moisture ratio, k_s = drying constant (1/s) and t = time (s).

This model is also known as the Newton model, and it tends to over predict the early stage and under-predict the later stage of drying. To overcome the shortcomings of this model, the Page model (Equation 2.2) is applied with an empirical modification to the time term by introducing an exponent 'n' (Madamba, et al., 1996; Wongwises & Thongprasert, 2000).

$$MR = \exp\left(-k_p t^n\right) \qquad (\ 2.2\ )$$

This model has been successfully used to model the experimental moisture content of beans, peas, grapes and potatoes (Afzal & Abe, 1999; Sawhney, et al., 1999).

The drying of fruits and vegetables normally occurs in the falling rate period. The moisture or vapour migration during this period is controlled by diffusion. The diffusion could include molecular diffusion, liquid diffusion through solid pores, vapour diffusion in air-filled pores, Knudsen flow and all other factors which affect drying. Since it is difficult to separate among these individual mechanisms, the rate of moisture movement is described by an effective diffusivity, which is a lumped parameter (Sablani, et al., 2000). In most situations, Fick's second law of diffusion is used to describe a typical moisture diffusion process during drying (Equation 2.3).

$$\frac{\partial m}{\partial t} = D_{eff} \frac{\partial^2 m}{\partial x^2} \qquad (\ 2.3\ )$$

where m = moisture content (g water/g dry solid), D_{eff} = effective diffusion coefficient (m^2/s), x = position (m) and t = time (s).

Fick's second law can be solved for the simple analytical solutions when shrinkage is negligible or not considered. The internal diffusion of moisture is the dominant mode of transfer (negligible external transfer resistance is

assumed), and it also neglects the initial thermal transient effect (Sablani, et al.,
2000). The analytical solutions can be used for various standard geometries.

2.4.3 Effective diffusivity for uni-dimensional moisture movement

When the food product is assumed as one-dimensional and has uniform
initial moisture content and isothermal condition, the solutions of the Fickian
equation for different geometrics are described by Equation 2.4 to 2.6 (Rossello,
et al., 1992).

2.4.3.1 Infinite slab

$$MR = \frac{m-m_e}{m_0-m_e} = \frac{8}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{(2n-1)^2}\exp\left[-(2n-1)^2\frac{\pi^2 D_{eff}t}{L^2}\right] \quad (\quad 2.4 \quad)$$

where, m = moisture content (g water/g dry solid), the subscripts o and e
indicate the moisture content (g water/g dry solid) at initial moisture content and
equilibrium moisture content, respectively, D_{eff} = effective diffusion
coefficient (m^2/s), t = time (s), L = slab thickness (m), MR = dimensionless
moisture ratio and n = positive integer.

2.4.3.2 Infinite cylinder

$$MR = \frac{m-m_e}{m_0-m_e} = \sum_{n=1}^{\infty}\frac{4}{\beta_n^2}\exp\left[-\frac{\beta_n^2 D_{eff}t}{r_c^2}\right] \quad (\quad 2.5 \quad)$$

where, m = moisture content (g water/g dry solid), the subscripts o and e
indicate the moisture content (g water/g dry solid) at initial moisture content and
equilibrium moisture content, respectively, β = roots of the Bessel function,
D_{eff} = effective diffusion coefficient (m^2/s), t = time (s), r_c = cylinder radius
(m) and n = positive integer.

2.4.3.3 Sphere

$$MR = \frac{m - m_e}{m_0 - m_e} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left[-n^2 \frac{\pi^2 D_{eff} t}{r_s^2}\right]$$ (2.6)

where, m = moisture content (g water/g dry solid), the subscripts o and e indicate the moisture content (g water/g dry solid) at initial moisture content and equilibrium moisture content, respectively, D_{eff} = effective diffusion coefficient (m^2/s), t = time (s), r_s = sphere radius (m), n = positive integer.

For long drying times (MR < 0.6), when L, r_c and r_s values are small and t value is large, simplified forms of equations are obtained for the slab, cylindrical and spherical geometries by considering only the first term of the solutions (Senadeera, et al., 2003) as shown in Equation 2.7 to 2.9.

$$MR = \frac{m - m_e}{m_0 - m_e} = \frac{8}{\pi^2} \exp\left[-\frac{\pi^2 D_{eff} t}{L^2}\right]$$ (2.7)

$$MR = \frac{m - m_e}{m_0 - m_e} = \frac{4}{\beta_1^2} \exp\left[-\frac{\beta_1^2 D_{eff} t}{r_c^2}\right]$$ (2.8)

$$MR = \frac{m - m_e}{m_0 - m_e} = \frac{6}{\pi^2} \exp\left[-\frac{\pi^2 D_{eff} t}{r_s^2}\right]$$ (2.9)

A general form of Equation 2.7 to 2.9 can be written in the logarithmic form upon linearization (Equation 2.10):

$$\ln MR = A - Bt$$ (2.10)

where constant B is $\pi^2 D_{eff}/L^2$ for a slab, $\beta_1^2 D_{eff}/r_c^2$ for a cylinder and $\pi^2 D_{eff}/r_s^2$ for a sphere.

The slope (B) is calculated by plotting $\ln MR$ versus time according to Equation 2.10, and the effective diffusivity can be determined from the slope. The effective diffusivities of various leafy materials are summarised in Table 2-5.

Table 2-5: Effective diffusivities of different plant leaves materials from hot air drying

Plant leaves material	Effective diffusivity (m^2/s)	Temperature range (°C)	Reference
Black Tea	1.14×10^{-11} – 2.98×10^{-11}	80 – 120	Panchariya, et al. (2002)
Wormwood leaves	7.10×10^{-8} – 3.19×10^{-7}	50 – 70	Beigi (2017)
Rosemary	9.74×10^{-11} – 1.48×10^{-10}	50 – 80	Mghazli, et al. (2017)
Gale of the Wind	5.87×10^{-11} – 9.63×10^{-11}	50 – 70	Sousa, et al. (2018)
Spider plant leaves	1.03×10^{-6} – 1.77×10^{-5}	50 – 70	Omolola, et al. (2019)
Peppermint leaves	1.81×10^{-9} – 39.56×10^{-9}	50 – 70	Torki-Harchegani, et al. (2016)

2.5   Antioxidants Capacity and Properties

2.5.1   Total polyphenol content

Oxidation is a necessity for many living organisms to produce energy for biological processes. Biological combustion from the respiration process produces harmful intermediates called oxygen-centred free radical, also known as reactive oxygen species (ROS) (Bloknina, et al., 2003). The ROS plays an important role in the degenerative or pathological processes of various serious human diseases such as ageing, cancer, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataract, and inflammation (Burns, et al., 2001; Diaz, et al., 1997; Aruoma, 1998). It has been reported that there is an inverse association between the consumption of some fruits and vegetables and mortality from age-related diseases, partly due to the presence of antioxidant compounds (e.g., phenolic compounds), which are the most abundant hydrophilic antioxidants in the diet and the most active

antioxidant compounds (Scalbert, et al., 2005). Antioxidants are substances that can prevent or inhibit oxidation processes in the human body and food products. It was proven that polyphenols are good antioxidants that could effectively prevent cardiovascular and inflammatory diseases, and they can also be used as chemopreventive agents for cancer (Shahidi & Naczk, 2003). Hence, total polyphenol content (TPC) should be determined to study the antioxidant properties of papaya leaves.

The amount of antioxidants in papaya leaves can be determined by investigating the TPC, which may vary due to different drying techniques applied on the leaves. The antioxidant capacities of papaya leaves can be evaluated by DPPH and ABTS free radical scavenging assays. The TPC was determined because of its strong correlation with the antioxidant activity in various fruits and vegetables as reported in many literature (Scalbert, et al., 2005; Gorinstein, et al., 2004; Sellappan, et al., 2002).

2.5.2   Free radical scavenging activity based on ABTS assay

ABTS is known as 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate). ABTS assay, also known as Trolox equivalent antioxidant capacity (TEAC) assay, measures the relative ability of antioxidants to scavenge the ABTS generated in the aqueous phase. The ABTS is generated by reacting a strong oxidising agent (potassium persulfate) with the ABTS salt. The reduction of blue-green ABTS radical coloured by hydrogen-donating antioxidant is measured by suppressing its characteristic long wave (734 nm) absorption spectrum (Miller & Rice-Evans, 1997). The ABTS method is more flexible than

the DPPH method because it can be used at different pH levels, whereas the DPPH assay is sensitive to acidic conditions (Ou, et al., 2002; Shalaby & Shanab, 2013). Additionally, the ABTS method requires a shorter reaction time, reaching a steady state within 30 minutes after mixing the ABTS solution with an aqueous buffer solution.

2.5.3   Free radical scavenging activity based on DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was introduced 50 years ago by Blois (1958). It is a measurement of antioxidant capacity, which could yield the amount of a heterogeneous mixture of antioxidants, reacting together to produce the total or net scavenging ability of the sample.

The DPPH assay is popular in the antioxidant studies of natural products. One of the reasons is that this method is simple and sensitive. Sanchez-Moreno (2002) considered this assay an easy and accurate method for fruit and vegetable juice extracts. It is a stable nitrogen synthetic radical assay. In addition, DPPH assay is unaffected by certain side reactions of phenolic compounds, including metal ion chelation and enzyme inhibition (Babbar, et al., 2011). Originally, it was monitored by Electron Spin Resonance (ESR) spectroscopy and relied on the signal intensity of the DPPH being inversely related to the antioxidant concentration and the reaction time. More recently, this reaction has been measured by the decolouration assay, where the decrease in absorbance was measured at 515–528 nm by adding the antioxidant to the DPPH in methanol or ethanol (MacDonald-Wicks, et al., 2006). This assay is not suitable for

measuring the antioxidant capacity of plasma, as proteins are precipitated in the presence of the ethanol/methanol solvent (Sanchez-Mareno, 2002).

2.6 Colour Analysis

Colour is one of the most important indicators used by consumers to assess the quality of food products. It may be defined as the individual response to the visual signals generated by the light on a product which is measurable in terms of intensity and wavelength. Colours, either natural or synthetic, have inherent properties and applications. Applications dictate if the measurement of colour becomes critical to monitor in-process samples for colour degradation or conformance to a standard (Giri, 2014). Colour is an indicator of ripeness or spoilage. For example, colour is one of the judging criteria during the endpoint of a cooking process, and also for food flavours such as berries are associated with red colour, whereas for beef flavour is brown colour (Parker & Pace, 2016). On the other hand, colour is an indicator of heat treatment severity and can predict the corresponding quality deterioration caused by exposure to the heat source (Pathare, et al., 2013). For example, the colour of leafy materials tends to be less greenish due to chlorophyll pigment degradation and browning reaction after drying (Mohapatra, et al., 2014; Sagrin & Chong, 2013; Poomsa-ad, et al., 2011). Therefore, the colour of a food product must be monitored and standardised to ensure good quality.

The colour of an object can be described by several colour coordinate systems (Clydesdale & Ahmed, 1978; Francis, 1980; Hunter & Harold, 1987) such as Hunter L a b, International Commission on Illumination (CIE) $L^*a^*b^*$

and CIE *XYZ*. According to the CIE concept, the human eye consists of three colours namely red, green and blue, thus forming all the combinations of colours. Tristimulus values are the amounts of red, green and blue needed to form any colour, and they are denoted as *X*, *Y* and *Z*, respectively. The most common notation used is the CIE *XYZ* colour space devised in 1931. It is based on the trichromatic principle, whereby imaginary positive primaries *X*, *Y* and *Z* are used. It uses the chromaticity diagram to designate various colours (Figure 2-9). The *X*, *Y* and *Z* values are used to calculate the chromaticity coordinates, designated by lowercase letters *x* (red), *y* (green) and *z* (blue). The value for x can be calculated as $x = X/(X+Y+Z)$. The values for *y* and *z* can be calculated by replacing *X* with *Y* and *Z*, respectively, in the numerator (Sahin & Sumnu, 2006).
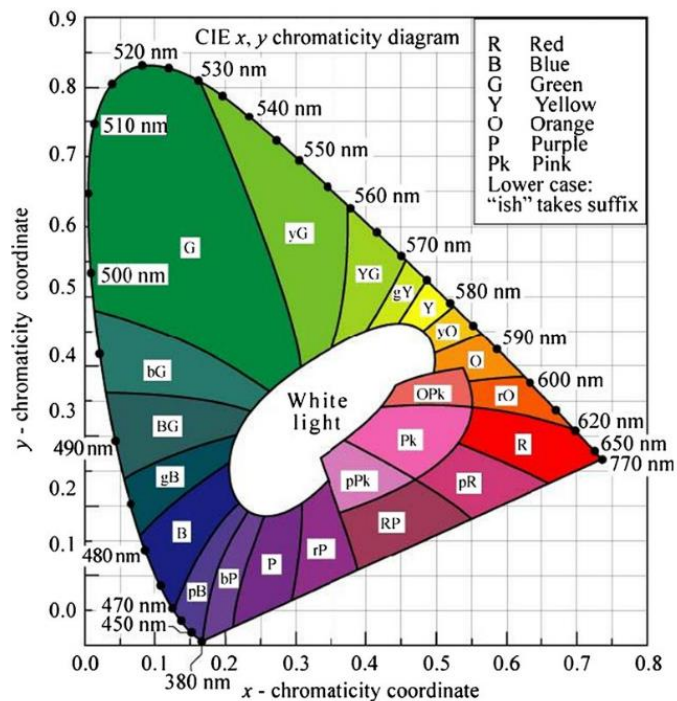
Figure 2-9: CIE chromaticity diagram

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The Hunter *L a b* was developed in 1948 for photoelectric measurement, whereas the CIE $L^*a^*b^*$ colour space (Figure 2-10) was developed in 1976 (Pathare, et al., 2013). They provide more uniform colour differences with human perception of differences (Leon, et al., 2006; Wu & Sun, 2013; Pathare, et al., 2013). The Hunter *L a b* and CIE $L^*a^*b^*$ colour scales were opponent-type systems widely used in the food industry. The CIE $L^*a^*b^*$ coordinates (L^*, a^*, b^*) can be read directly. The L^* value represents the luminosity of the leaves, which ranges from 0 (black) to 100 (white). The a^* value ranges from the negative (green) to the positive (red) scale, whereas the b^* value ranges from negative (blue) to positive (yellow) (Segnini, et al., 1999; Konica Minolta, 2018).
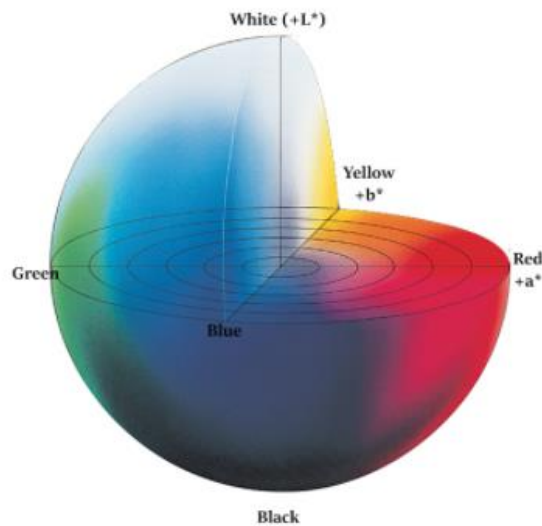
Figure 2-10: CIE *L*a*b colour space**

Colour is subject to perception and the expressions of colour are interpreted differently by different individuals. The instrumental measurement was developed to standardise the colour measurement and expression, improving the accuracy of colour expressions and communication. There are

two types of instrumental methods for colour measurement, which are colourimeter and spectrophotometer. Colourimeter gives measurements correlated with human eye-brain perception by obtaining the tristimulus values X, Y and Z directly. Colourimeter is easy to use and portable and it is thus widely used for colour measurement especially in the food industry (Pathare, et al., 2013). A spectrophotometer measures colour by providing wavelength-by-wavelength spectral analysis of the reflecting and transmitting properties of objects. Hence, it is more commonly used in research and development works in laboratories. It can provide adequate information in the calculation of colour values for any illuminant and metamerism, and automatically detect colour measurement at different angles (Sahin & Sumnu, 2006).

2.7 Storage Study

2.7.1 Degradation kinetics

The degradation kinetics of bioactive and antioxidant compounds in food products is important in understanding the degradation reaction during storage. This is to estimate the shelf life of nutritional and physicochemical properties in the food products (Taoukis, et al., 1997). The degradation kinetics of vitamins, pigments and other desirable food components during storage has been widely studied and reported in literature (Peleg, 2019). The bioactive compounds lost during storage could be due to various reasons, e.g., the type of compounds, storage condition (exposure to UV or oxygen) and drying technique. For example, rapid degradation of carotenoids in freeze dried carrots was observed, and air drying was more efficient in preserving carotene when stored at room

temperature (Kaminski, et al., 1986). This is mainly attributed to the porous structure of freeze dried products, which facilitates oxygen transfer, thus promoting rapid oxidation of carotene.

The concentration of bioactive compounds in food products against storage time was plotted to obtain the degradation kinetics, which could be used to calculate the reaction's rate constant. The degradation kinetics of bioactive compounds during food storage can be described using zero, first or higher order reaction kinetics (Corradini & Peleg, 2004). The Weibull model has an interesting potential in describing microbial and enzymatic inactivation and chemical degradation kinetics (Cunha & Oliveira, 2000). It is also important to determine the shelf life of food products by predicting the reaction's progress over time (Kim, et al., 2018).

2.7.2   Mathematical models of degradation kinetics

The kinetics models of zero and first order reactions are as shown in Equation 2.11 and 2.12, respectively.

$$C_t = C_0(-kt) \qquad\qquad (\quad 2.11 \quad)$$

$$C_t = C_0 exp(-kt) \qquad\qquad (\quad 2.12 \quad)$$

where C_t = bioactive compound concentration at time t (µg/g), C_0 = initial bioactive compound concentration (µg/g), k = degradation rate constant (1/ month). The higher the k value, the lower the stability of the bioactive compound (Rawson, et al., 2012).

The Weibull model can also be used to describe the degradation kinetics of bioactive compounds as shown in Equation 2.13.

$$C_t = C_0 exp(-bt^n) \hspace{4cm} (\ 2.13 \ )$$

where C_t = bioactive compound concentration at time t (μg/g), C_0 = initial bioactive compound concentration (μg/g), b = degradation rate constant (1/month). The b and n values are the shape and scale factors of the distribution curve, respectively.

The half-life ($t_{1/2}$), which is the time required to achieve 50% degradation of nutrients in the food products, can be calculated using Equation 2.14.

$$t_{1/2} = \frac{\ln(2)}{k} \hspace{4cm} (\ 2.14 \ )$$

where k = degradation rate constant (1/month).

2.8 Knowledge Gap

From the literature review, it can be seen that there are not many literature reporting on the effects of processing on papaya leaves and quantification of carpaine. Studies reported in literature are mainly conducted on fresh materials (fresh papaya leaves and juice extracts) but not on dried or processed materials. Therefore, some of the commonly used drying techniques (e.g., hot air drying, shade drying and freeze drying) can be utilised to dry papaya leaves and retain carpaine in the dried products. In addition, different parts of papaya leaves should be studied to maximise retention of carpaine before further processing (e.g., extraction and purification). Besides, the drying kinetics of papaya leaves using different drying techniques is vital for the production of carpaine in bulk

during commercial operations. The impact of different drying techniques on the quality of papaya leaves in terms of antioxidant properties and carpaine retention could be further investigated. Last and not least, after drying processes, the degradation kinetics of carpaine in papaya leaves should be investigated for its stability during extended storage.

The aforementioned information is particularly important when it comes to bulk production and industrial equipment design. However, these information are scarce, and if it can be made known, it could play an important role in bridging the knowledge gap between laboratory experiments and industrial operation.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Chemicals

Folin & Ciocalteu's phenol reagent, 99% tartaric acid, 98% formic acid, 99% ammonium formate, 99.9% acetonitrile, 99.8% chloroform-d ($CDCl_3$), 99.9% tetramethylsilane (TMS), 99% diethyl ether, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Trolox and Dragendorff reagents were purchased from Sigma-Aldrich (USA). Sodium carbonate, potassium persulfate, 95% ethanol and 25% ammonia solution were purchased from R&M Chemicals (Malaysia). Chloroform, Kieselghur, 99.8% ethanol, methanol, gallic acid and thin-layer chromatography (TLC) plate (Silica gel 60) were purchased from Merck (Germany). All chemicals used in this study were of analytical grade, except for the 99.8% ethanol, 98% formic acid, 99% ammonium formate and 99.9% acetonitrile, which were of LC-MS grade.

3.2 Sample Collection

Papaya leaves and stalks (Figure 3-1) were collected from a fruit farm at Titi, Jelebu. The young (green colour) and old (yellow colour) leaves were used in various parts of experimental works. The selection of the leaves was based on colour parameters (CIE $L^*a^*b^*$) as described in Section 3.9.
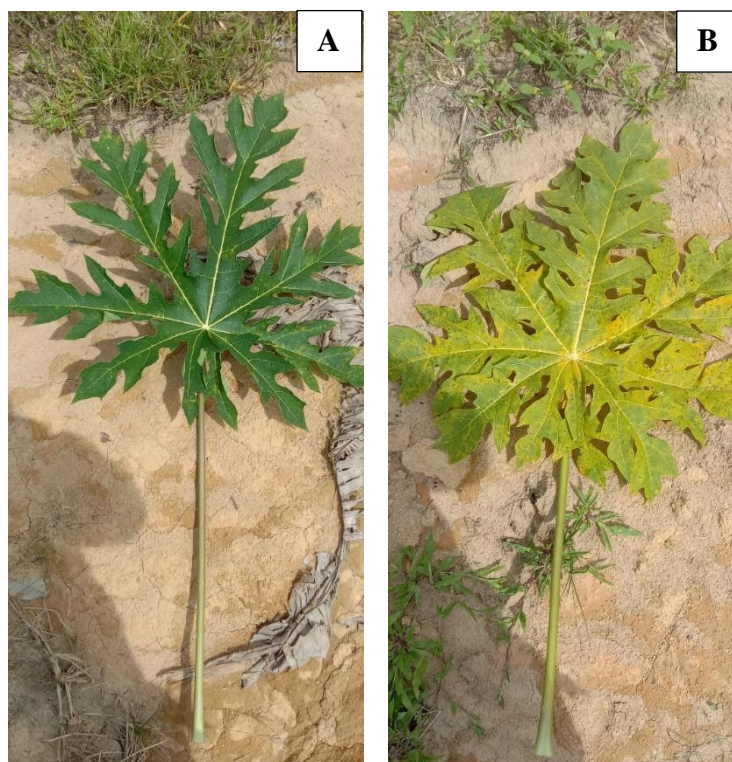
Figure 3-1: Papaya leaves samples (A) young leaves and (B) old leaves with stalks

The L^*, a^*, and b^* values recorded for the young leaves were 34.4 ± 0.7, -2.5 ± 0.4 and 9.7 ± 0.3, whereas for the old leaves, the measured values were 51.2 ± 0.2, -3.5 ± 0.5 and 35.8 ± 0.6 for the respective parameters. Stalks from the young leaves were also separated and used for part of the studies. The samples were then thoroughly cleaned under running tap water and followed by rinsing with distilled water.

3.3 Moisture Content

Moisture content (m_i) of papaya leaves was determined using the standard oven method (Equation 3.1) (Hii, et al., 2009). The bone-dry weight of the

sample was obtained by heating the sample overnight (duration > 24 h) in an air-ventilated oven at 105°C.

$$m_i = \frac{W_i - W_{bd}}{W_{bd}}$$

(3.1)

where W_i and W_{bd} indicate the weight of the sample and bone-dry weight (g), respectively.

The moisture contents were then converted into moisture ratios (MR) as shown in Equation 3.2.

$$MR = \frac{m_i - m_e}{m_o - m_e}$$

(3.2)

where MR = moisture ratio, the subscripts i, o and e indicate the moisture content (g water/g dry solid) at any time i, initial moisture content and equilibrium moisture content, respectively.

3.4   Quantification of Carpaine

A 1260 Infinity liquid chromatography (LC) system coupled to a 6420 triple quadrupole mass spectrometer (MS) with electrospray ionisation (ESI) interface was used. Data were processed with Mass Hunter Workstation software version B.06.00 (Agilent Technologies, USA). The 1260 Infinity LC system consisted of a binary capillary pump, an autosampler and a thermostatted column compartment. The separation was performed at 45°C on a Zorbax Eclipse Plus C18 (100 × 4.6 mm i.d., 3.5 μm particle size; Agilent Technologies, USA). The mobile phase consisted of 0.1% formic acid and 5 mM ammonium formate in H_2O (solvent A) and 0.1% formic acid and 5 mM ammonium formate in acetonitrile (solvent B). The following gradient profile was used: 5% solvent

B for 0.1 minutes, linear gradient to 98% solvent B in 6 minutes and stay to 9 minutes. Finally, the gradient profile returned back to the equilibrium condition of 5% solvent B for 1 minute. The flow rate was 0.6 mL/min and the sample injection volume was 1 μL. MS parameters were automatically set and then optimised manually. The following final settings were used: N_2 drying gas temperature 350 °C at a flow rate of 11 L/min, nebuliser pressure of 40 psi and capillary voltage of 3.5 kV. Quantification was performed using Multiple Reaction Monitoring (MRM) in the positive ionisation mode. Quantifier was set at 479.4 – 240.2 m/z and qualifier at 479.4 – 222.2 m/z with collision energy of 34 and 42 V, respectively. Standard solutions were prepared by a serial dilution of carpaine stock solution of 1 μg/mL in ethanol. The carpaine used as the stock solution was extracted and purified from papaya leaves in the laboratory, followed by the confirmation using nuclear magnetic resonance (NMR) spectroscopy (described in Section 4.2.3, Chapter 4). Calibration curves were obtained with carpaine solutions of 1, 5, 10, 50, 100 ppm in ethanol.

3.5   Sample Preparation for Total Polyphenol Content and Antioxidants Activities Tests

Dried leaves powder (0.1 g) was added into 10 mL of distilled water (in 15 mL centrifuge tube), followed by ultrasonic bath at an operating frequency of 35 kHz (Elma; Transsonic TI-H-15) at room temperature for 30 minutes. The samples were then centrifuged at 5000 rpm for 10 minutes. The supernatant (papaya leaves extract) was used as the samples for the testing of total polyphenol content and antioxidant activities.

3.6   Total Polyphenol Content

The total polyphenol content (TPC) in papaya leaves extract was determined using the Folin-Ciocalteu spectrophotometric method (Gogna, et al., 2015). The extract (100 µL) was mixed with distilled water (7.9 mL) followed by adding 500 µL of Folin-Ciocalteu's phenol reagent. The mixture was mixed well and left for about 30 s before adding 1.5 mL of 25% (w/v) sodium carbonate solution. The absorbance was measured at 765 nm after a 2-hour incubation period at room temperature. The standard curve was linear between 100 and 500 ppm gallic acid. The TPC for the plant extract was expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dried weight. All measurements were performed thrice and the mean values were obtained.

3.7   Free Radical Scavenging Activity based on ABTS assay

ABTS assay was conducted by following the method of Thaipong et al. (2006). The stock solutions included 7.4 mM $ABTS^{·+}$ solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 to 16 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{·+}$ solution with 25 mL methanol to obtain an absorbance of 1.17 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{·+}$ solution was prepared for each assay. The sample (10 µL) was allowed to react with (190 µL) of the $ABTS^{·+}$ solution for 2 hours in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was linear between 0.391 and 25 ppm Trolox. Results were expressed as milligrams of

Trolox equivalent (TE) per 100g of dried weight. All measurements were performed thrice and the mean values were obtained.

3.8 Free Radical Scavenging Activity based on DPPH assay

The DPPH free radical scavenging of the sample was measured by measuring the decrease in absorbance of DPPH solution at 517 nm in the presence of the extract (Krings & Berger, 2001). Methanolic DPPH solution (used as control) with an initial concentration of 0.2 mM was prepared and incubated in the dark for 2 hours at room temperature. Fresh DPPH solution was prepared for each assay. The DPPH solution (100 µL) was then mixed with the sample (50 µL). The absorbance was measured spectrophotometrically at 517 nm after a 30-minute incubation period at room temperature. The capability of the sample to scavenge the DPPH radical was calculated according to Equation 3.3. The antioxidant activity was expressed as IC_{50}, representing the sample's concentration required to decrease 50% of the initial DPPH concentration. The IC_{50} value was determined by locating the point along with the absorbance (A) vs sample's concentration curve where the absorbance was reduced by 50%.

$$Scavenging\ effect\ (\%) = \left[\frac{A_{control}-A_{sample}}{A_{control}}\right] \times 100\% \qquad (\quad 3.3\quad)$$

The results were also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using Equation 3.4 (Leong & Shui, 2002).

$$AEAC = \left(\frac{IC_{50(AA)}}{IC_{50(sample)}}\right) \times 10^5 \qquad (\quad 3.4\quad)$$

where AA = ascorbic acid. All measurements were performed thrice and the mean values were obtained.

3.9 Colour Measurement

The colour of leave samples was determined by using a handheld colourimeter (Precision, China). The results were expressed in CIE $L^*a^*b^*$ colour parameters, where L^*, a^* and b^* denote lightness/darkness, redness/greenness, and yellowness/blueness, respectively. The total colour difference (ΔE^*) was calculated from the L^*, a^*, and b^* values measured (Equation 3.5)

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b)^2]^{1/2} \qquad (\quad 3.5 \quad)$$

where ΔL^*, Δa^* and Δb^* are the differences of the individual colour parameter with reference to the fresh papaya leaves samples. Differences in perceivable colour can be classified as shown in Table 3-1 (Chen, 2008):

Table 3-1: Classification of colour based on total colour difference (ΔE^*)

Level	ΔE^* range
Trace level difference	0 – 0.5
Slight difference	0.5 – 1.5
Noticeable difference	1.5 – 3.0
Appreciable difference	3.0 – 6.0
Large difference	6.0 – 12.0
Very obvious difference	> 12.0

3.10 Statistical Analysis

All the experiments were carried out in three replicates. The experimental data were analysed using one-way ANOVA and mean comparison by Duncan's Multiple Range Test at 95% confidence level using SPSS version 26 (IBM, USA).

CHAPTER 4

QUANTIFICATION OF CARPAINE AND ANTIOXIDANT PROPERTIES OF EXTRACTS FROM DIFFERENT PARTS OF PAPAYA PLANT

4.1   Introduction

Significant increase in dengue cases have been recorded worldwide every year and South East Asian countries have been badly affected. The antiviral drug to treat dengue is still not available but papaya leaves extract (PLE) has been successfully used in treating dengue patients. Carpaine in PLE is the major active compound that contributes to the anti-thrombocytopenic activity (raising platelet count in the patients' blood) during medical treatment. Therefore, studies were carried out to extract and quantify carpaine from young leaves, old leaves and stalks of the papaya plant.

Besides, PLE also contains polyphenols that contribute to antioxidant properties. Thus, the antioxidant properties of young leaves, old leaves and stalks of papaya plants in terms of total polyphenol content and DPPH scavenging activities were also investigated. The reason for using the stalks is to avoid wastage as the leaves are usually harvested with the stalks in the farm. Typically, the leaves constitute about 57% of the combined weight of the leaves and stalks. Furthermore, this enables the comparison of the amount of carpaine that could be quantified from these samples as affected by the age (young versus old leaves) and plant parts (leaves versus stalks).

4.2   Materials and Methods

4.2.1   Sample collection and preparation

The sample collection was carried out as described in Section 3.2 (Chapter 3). Half of the cleaned samples were blended for 15 minutes with distilled water (ratio 300 g sample: 200 ml distilled water) using a low-speed electrical blender (Panasonic, Malaysia), whereas the other half were not blended. Both types of samples (blended and unblended) were placed in a deep freezer overnight at -12°C. Upon freezing, the samples were then dried in a freeze dryer (Alpha 1-2 LDplus, Christ, Germany) set at 0.012 mbar for 24 hours. Upon freeze drying, the dried samples were ground into powder form (Figure 4-1) for further chemical analysis. Freeze drying was used due to its ability in preserving the bioactive ingredients effectively during drying (Sagar & Suresh Kumar, 2010).
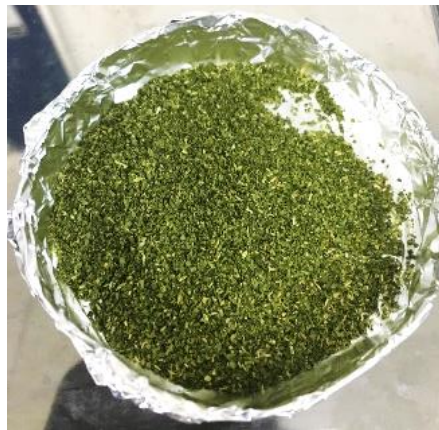
Figure 4-1: Dried papaya leaves powder (without blending treatment before freeze drying)

4.2.2 Moisture content

The moisture content of papaya leaves samples was determined as described in Section 3.3 (Chapter 3).

4.2.3 Extraction and qualification of carpaine

Freeze dried papaya leaves and stalk powders were soaked in 95% ethanol to extract the alkaloid at $60°C$ for 1 hour in a heated water bath (Figure 4-2). The mixture was then decanted and filtered to separate the ethanol extract from the plant residues. The resulting extract was then concentrated by using a rotary evaporator (BÜCHI, Switzerland). Then, acid-base extraction (ABE) was conducted to extract the alkaloids and ethanol was recovered for further use. To increase its solubility in water, acidification was conducted using tartaric acid (3%) and then filtration through the Kieselghur layer using the Büchner funnel. The acidic filtrate was then basified using ammonia solution until the pH increased to 10. Total alkaloids were obtained after extraction by using chloroform.
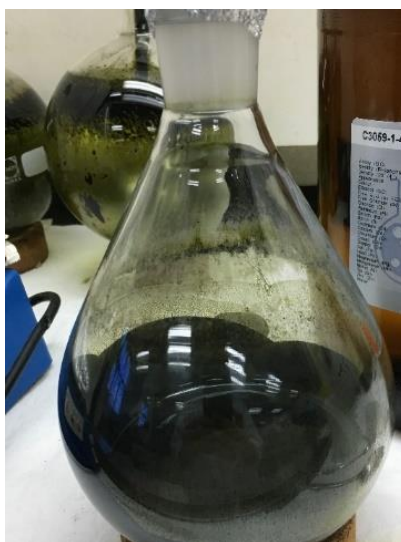
Figure 4-2: The concentrated papaya extract

Centrifugal thin-layer chromatography (CTLC) was used to separate the alkaloids in order to get pure carpaine by manipulating the solvent systems based on the polarity. Alkaloid crude extract (1.8 g) was chromatographed using centrifugal preparative thin layer chromatography (Chromatotron, silica gel 60, Et$_2$O-MeOH 20:1 $\rightarrow$ 5:1, saturated with ammonia) to give carpaine (500 mg). The presence of carpaine could be visualised by using Dragendorff reagent, which monitored the fractions by spotting the dissolved carpaine solution on TLC plates, followed by spraying the Dragendorff reagent onto the spotted plates. The occurrence of an orange-brownish precipitate confirmed the presence of carpaine.

The ^{1}H and ^{13}C NMR spectra of carpaine were acquired by dissolving the carpaine (15 mg) in chloroform-d (CDCl$_3$) containing 0.01% of tetramethylsilane (TMS) as internal standard on a JEOL 400 MHz spectrometer. The pure carpaine extracted was also used as the standard in liquid

chromatography-mass spectrometry (LC-MS) analysis. Please refer to Section 3.4 (Chapter 3) for the method to quantify the carpaine extracted.

4.2.4 Quantification of carpaine

The method to quantify carpaine from the papaya leaves samples is described in Section 3.4 (Chapter 3).

4.2.5 Total polyphenol content and DPPH free radical scavenging assay

The methods to determine total polyphenol content and DPPH free radical scavenging activity of papaya leaves samples are described in Section 3.6 and 3.7 (Chapter 3), respectively.

4.2.6 Statistical analysis

The statistical analysis was carried out as described in Section 3.10 (Chapter 3).

4.3 Results and Discussion

4.3.1 Moisture content before and after freeze drying

Table 4-1 shows the moisture contents before and after freeze drying for all the samples. The initial moisture contents of the plant samples ranged from 1.9 g water/g dry solid to 3 g water/g dry solid, whereas the final dried samples

showed moisture contents in the range of 0.03 g water/g dry solid to 0.08 g water/g dry solid.

Table 4-1: Moisture contents of samples before and after freeze drying

Sample	Moisture content (g water/g dry solid)	
	Before freeze drying	After freeze drying
Young leaves	2.8 ± 0.1	0.06 ± 0.01
Old leaves	1.9 ± 0.1	0.03 ± 0.01
Stalks	3.1 ± 0.1	0.08 ± 0.01

4.3.2   Extraction and quantification of carpaine

Carpaine was obtained as pale-yellow crystalline solids (Figure 4-3), which were soluble in ethanol and chloroform. The ^{1}H and ^{13}C NMR spectra of carpaine, which were acquired in $CDCl_3$ using TMS as internal standard on a JEOL 400 MHz spectrometer, are shown in Figure 4-4 and Figure 4-5, respectively. The ^{1}H and ^{13}C NMR data are in excellent agreement with those reported by Sato et al. (2003).
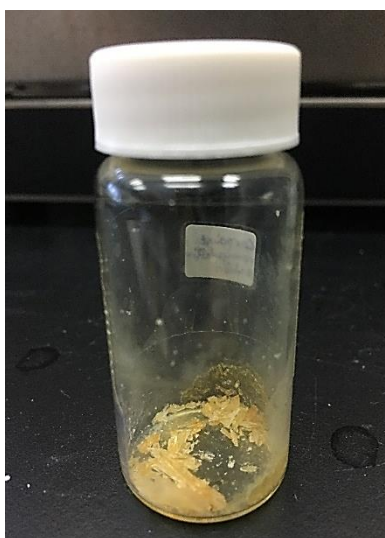
Figure 4-3: Carpaine (pale-yellow crystalline solids) extracted from *Carica papaya* leaves

Carpaine: mp 119°C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.05 (d, J = 6.6 Hz, 6H), 1.11–1.75 (m, 30H), 2.02 (dq, J = 14.3, 3.4 Hz, 2H), 2.33 (dt, J = 15, 6.9 Hz, 2H), 2.43 (dt, J = 15, 7.4 Hz, 2H), 2.59 (br m, 2H), 2.87 (qd, J = 6.6, 1.7 Hz, 2H), 4.77 (q, J = 2.6 Hz, 2H); ^{13}C NMR (100 MHz, CDCl$_3$) 18.8, 25.5, 25.6, 26.5, 28.77, 28.82, 29.2, 29.8, 34.7, 37.5, 53.7, 56.1, 70.4, 173.6.

The ^{1}H and ^{13}C NMR spectra of carpaine showed the following resonances: ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.05 (d, J = 6.6 Hz, 6H), 1.11–1.75 (m, 30H), 2.02 (dq, J = 14.3, 3.4 Hz, 2H), 2.33 (dt, J = 15, 6.9 Hz, 2H), 2.43 (dt, J = 15, 7.4 Hz, 2H), 2.59 (br m, 2H), 2.87 (qd, J = 6.6, 1.7 Hz, 2H), and 4.77 (q, J = 2.6 Hz, 2H); ^{13}C NMR (100 MHz, CDCl$_3$) 18.8, 25.5, 25.6, 26.5, 28.77, 28.82, 29.2, 29.8, 34.7, 37.5, 53.7, 56.1, 70.4, and 173.6.
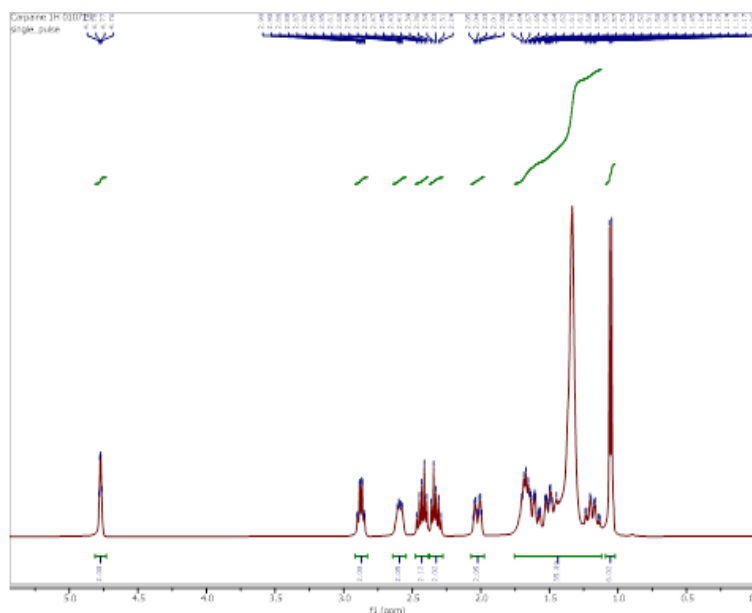
Figure 4-4: ^{1}H NMR spectrum of carpaine (400 MHz, CDCl3)
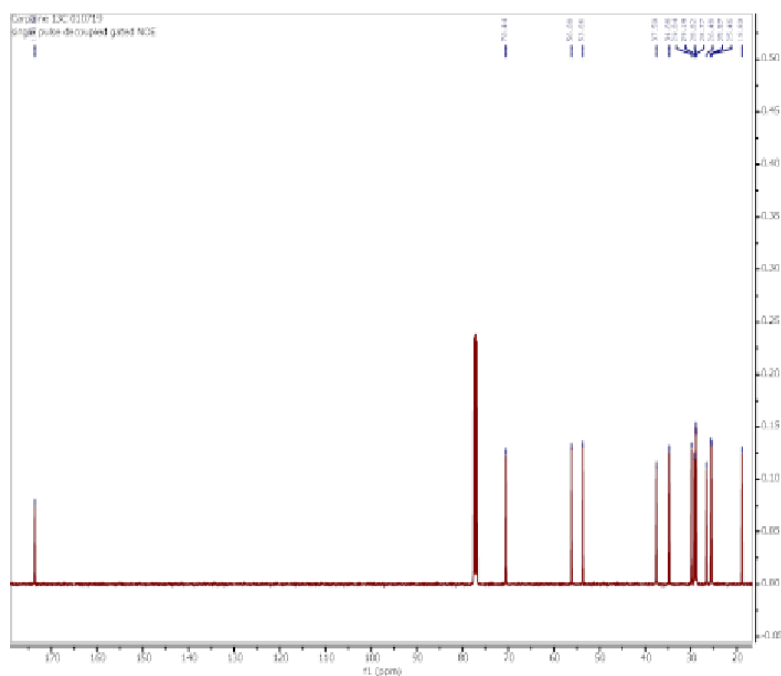
Figure 4-5: ^{13}C NMR spectrum of carpaine (100 MHz, CDCl$_3$)

4.3.3 Amount of carpaine quantified

Table 4-2 shows the amount of carpaine quantified from the plant samples after freeze drying. It can be seen that young leaves contained the highest amount of carpaine (333 μg/g and 176 μg/g) as compared to the old leaves (279 μg/g and 113 μg/g) and stalks (9 μg/g and 6 μg/g) in both blended and unblended samples. Although no significant difference was found between old and young leaves in the unblended and blended samples (p>0.05), respectively, there was tendency for the young leaves to show higher carpaine concentration than the old leaves.

Table 4-2: Carpaine in dried young leaves, old leaves and stalks

Treatment	Dried sample	Carpaine (μg/g)
	Young leaves	333 ± 68^a
Unblended	Old leaves	279 ± 25^a
	Stalks	9 ± 0^c

Blended	Young leaves	176 ± 9^{b}
	Old leaves	113 ± 38^{b}
	Stalks	6 ± 2^{c}

Note: mean having a common letter within the same column are not significantly different at 5% level.

However, all the unblended samples except for the stalks showed a significantly higher amount of carpaine ($p < 0.05$) as compared to the blended samples, and unblended young leaves obtained the highest amount (333 µg/g). This could be due to the friction-induced heat when blending the leaves into smaller sizes (Karam, et al., 2016) , and the local temperature increase can cause important losses of aroma, nutrients and flavour components (Pesek, et al., 1985; Singh & Goswami, 1999; Murthy, et al., 1999). No significant difference was observed ($p > 0.05$) between the stalk samples (unblended and blended), but there was tendency for the unblended samples to show slightly higher values. Comparatively, the concentration of carpaine in the stalks was the lowest compared to the young and old leaves.

4.3.4   Total polyphenol content

Table 4-3 shows the total polyphenol content (TPC) from the plant samples after freeze drying. The TPC determined from the various plant samples ranged from 559 to 2174 mg GAE/100g DW and 547 to 2129 mg GAE/100g DW for the unblended and blended samples, respectively. Upon comparison with other reported plant products (Table 4-4), young papaya leaves (2129 mg GAE/100g DW to 2174 mg GAE/100g DW) contain higher TPC than rutabaga, boxthorn branches, plums, shengma plant and zaaroura plant.

Table 4-3: Total polyphenol contents in dried young leaves, old leaves and stalks

Treatment	Dried sample	Total polyphenol content (mg GAE/100g DW)
Unblended	Young leaves	2174 ± 32^a
	Old leaves	2001 ± 99^b
	Stalks	559 ± 16^c
Blended	Young leaves	2129 ± 6^a
	Old leaves	2015 ± 33^b
	Stalks	547 ± 18^c

Note: mean having a common letter within the same column are not significantly different at 5% level

Table 4-4: Comparison of TPC values with other plant materials

Plant material	Total polyphenol content (mg GAE/100g DW)	Reference
Rutabaga	1070 – 1810	Stefanucci, et al. (2020)
Boxthorn branches	595 – 1336	Truong, et al. (2019)
Plums	125 – 373	Kim, et al. (2003)
Shengma plant	307 – 507	Qin, et al. (2017)
Zaaroura plant	98 – 368	Khalid, et al. (2021)
Papaya leaves	2129 – 2174	Current work

From Table 4-3, it can be seen that young leaves (both unblended and blended samples) contained a significantly higher amount of TPC ($p<0.05$) followed by the old leaves and stalks. This agrees with results reported by Do and Hwang (2014) where the level of phenolic content in younger leaves was higher than older leaves in the Aronia plant. This could be due to the degradation of secondary metabolites including phenolic compounds in the old leaves (Anwar, et al., 2017). The varying concentrations of TPC are also associated with differential cytological and physiological activities within the plant organs (Itidel, et al., 2013). No significant difference was observed between unblended and blended samples ($p>0.05$). However, there was a tendency for the blended samples to show lower TPC compared to the unblended samples, which could be due to the heat generated during the blending process, thus resulting in slight degradation of the polyphenols (Becker, et al., 2016).

4.3.5　DPPH free radical scavenging activity

Table 4-5 shows the DPPH scavenging activities in terms of IC_{50} and AEAC for the various dried samples analysed. It can be observed that both young and old leaves showed significantly higher ($p < 0.05$) DPPH scavenging activities than the stalks in both unblended and blended treatments. This is in agreement with results from published literature where leaves have higher antioxidant activity than stalks (Ketsuwan, et al., 2017; Shih, et al., 2011). Therefore, it is recommended to exclude stalks for the extraction of carpaine and antioxidants.

Table 4-5: DPPH scavenging activities (IC_{50} and AEAC)

Treatment	Dried sample	IC_{50} (µg/mL)	AEAC (mg/100g DW)
Unblended	Young leaves	319 ± 42^{cd}	3428 ± 100^{a}
	Old leaves	298 ± 12^{d}	3629 ± 155^{a}
	Stalks	1619 ± 70^{a}	668 ± 30^{c}
Blended	Young leaves	293 ± 6^{d}	3688 ± 80^{a}
	Old leaves	382 ± 11^{c}	2827 ± 82^{b}
	Stalks	1361 ± 23^{b}	794 ± 13^{c}

Note: mean having a common letter within the same column are not significantly different at 5% level.

However, no significant difference was observed ($p > 0.05$) when comparing the unblended young and old leaves with blended young and old leaves. Nevertheless, blended young leaves tend to show higher DPPH scavenging activities (IC_{50} = 293 µg/mL and AEAC = 3688 mg/100 g DW) as compared to the unblended young leaves (IC_{50} = 319 µg/mL and AEAC = 3428 mg/100 g DW) and old leaves (IC_{50} = 298 µg/mL and AEAC = 3629 mg/100 g DW). Hence, blending treatment contributes positively to the antioxidant activities of the samples. This is in agreement with literature where the antioxidant properties could be enhanced with grinding treatment on the food

materials due to smaller particle sizes and bigger surface area (Li, et al., 2020; Zhang, et al., 2014). According to Table 4-3 and Table 4-5, the blending process contributed negatively to the TPC values of papaya leaves but positively to the antioxidant activities. This could be due to the presence of other compounds which contributed to the antioxidant activities in papaya leaves that were liberated during the blending process, e.g., saponins and flavonoids (Vuong, et al., 2013).

4.3.6   Relationship between TPC and free radical scavenging activity based on DPPH assay

The DPPH scavenging activities are found to be highly correlated with TPC with the coefficients of determination (R^2) estimated at 0.9743 and 0.9581, respectively, for IC_{50} and AEAC (Figure 4-6 and Figure 4-7). This shows that the polyphenolic compounds in papaya leaves are mainly responsible for the antioxidant activity, which agrees with the results reported by Yap et al. (2020). Della Valle et al. (2020) also reported that the major polyphenol content of hot pepper is responsible for elevating the antioxidant activities and showed a high correlation (Pearson's correlation coefficient, r = 0.99) with DPPH scavenging activity.
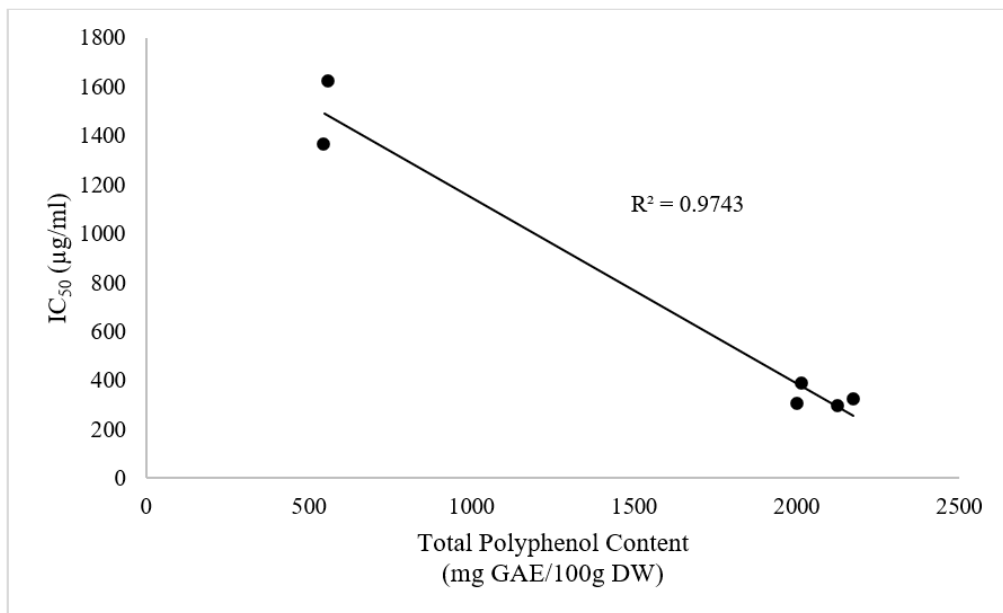
Figure 4-6: Plot of IC_{50} versus TPC of papaya plant samples
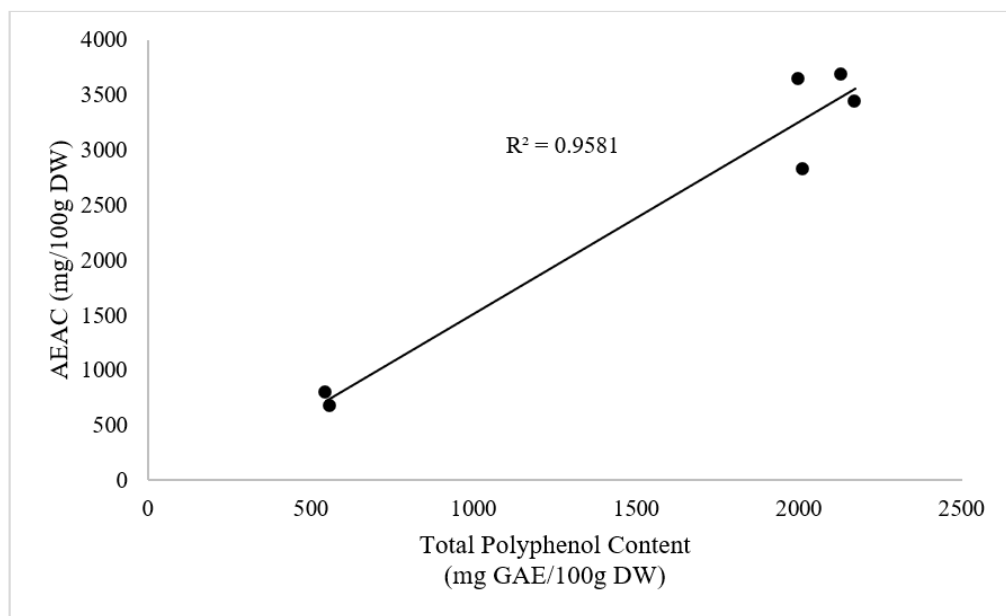
Figure 4-7: Plot of AEAC versus TPC of papaya plant samples

4.4 Conclusion

Studies were carried out to extract and quantify carpaine from papaya leaves (young and old) and stalks. Pale yellow carpaine crystalline powders were successfully purified and confirmed by ^{1}H and ^{13}C NMR. It was found that the young leaves contained the highest amount of carpaine, followed by old leaves and stalks in both blended and unblended samples. Unblended young leaves showed the highest amount of carpaine (333 µg/g) and significantly higher than all other samples ($p < 0.05$). Young leaves also contained a significantly higher amount of total polyphenol content ($p < 0.05$), followed by the old leaves and stalks. In terms of antioxidant activities, both young and old leaves showed significantly higher ($p < 0.05$) DPPH scavenging activities than the stalks in both unblended and blended samples. Therefore, it is recommended to use young papaya leaves as a source of material to extract carpaine for future drug development in dengue treatment. Significant correlations were found between the DPPH scavenging activities and TPC ($R^2 = 0.9743$ and 0.9581 for IC_{50} and AEAC, respectively), indicating the presence of phenolic compounds in papaya extracts contributes significantly to their antioxidant potential.

CHAPTER 5

DRYING KINETICS AND EFFECTIVE DIFFUSIVITY OF PAPAYA LEAVES

5.1 Introduction

Papaya is widely grown in various states in Malaysia, and typically only the fruits are sold in the market and consumed. Other parts of the plant such as leaves, roots, barks, peels, seeds and pulps are discarded after harvesting the fruits. However, these discarded parts are also known to have medicinal properties and have been used to treat various diseases. For example, papaya leaves contain flavonoids, alkaloids, phenolic compounds and cyanogenetic compounds, which are reported able to treat dengue fever.

The objective of this chapter is to study the drying kinetics of papaya leaves, which is important in processing papaya leaves (e.g., drying) in a scaled-up production. The drying process can reduce the water activity in foodstuff to minimise wastage and spoilage due to respiration, insects and pests attack, biochemical reactions and microbial activities, thus maximising the retention of nutrients and bioactive compounds in the foodstuff. Therefore, drying studies were carried out on the papaya leaves using hot air (60°C, 70°C and 80°C), shade and freeze drying. Effective diffusivities and activation energy of the drying processes were also determined and reported in this chapter.

5.2 Materials and Methods

5.2.1 Sample collection

The sample collection was carried out as described in Section 3.2 (Chapter 3). The samples used were only young (green) leaves.

5.2.2   Drying techniques

Drying was conducted using hot air, shade and freeze drying techniques. In hot air drying, the leaves samples were dried at 60°C, 70°C and 80°C using an air-ventilated oven (Memmert, Germany) (Figure 5-1). The samples (6.0–9.5 cm × 7.0–9.0 cm) were spread thinly on a meshed tray (30 cm × 30 cm) with square openings (0.4 cm x 0.4 cm). The samples were weighed every hour using an analytical balance (Sartorius, Germany).

Figure 5-1: Hot air dryer; (A) outer view; (B) inner view

In shade drying, the leaves were dried by spreading thinly on plastic trays and placed under a shaded area at the university campus (2°56'47.14"N and 101°52'31.14"E) from 9 am until 5 pm. The samples were kept in a closed area (on the table in the laboratory at room temperature) after 5 pm to avoid

contamination by rain, dew droplets and damage caused by animals. The samples were weighed consecutively at 9 a.m., 1 p.m. and 5 p.m. for five days, using an analytical balance (Sartorius, Germany).

In freeze drying, the samples were frozen at -12°C (Sharp, Japan) for 24 hours, followed by drying in a freeze dryer (Alpha 1-2 LDplus, Christ, Germany) set at 0.012 mbar for 24 hours (Figure 5-2). The samples were weighed at the beginning and the end of the experiment due to the equipment's limitation, i.e., not able to take out samples for weighing intermittently, using an analytical balance (Sartorius, Germany).

Figure 5-2: Freeze dryer

5.2.3   Moisture content

The moisture content of papaya leaves samples was determined according to the procedures as described in Section 3.3 (Chapter 3).

5.2.4 Effective diffusivity and activation energy

A general solution of Fick's second law of diffusion was used (Equation 5.1) (Rossello, et al., 1992; Crank, 1975). Effective diffusivity was only calculated for the hot air dried samples. The shape of the leaves was assumed in a slab geometry with an average thickness (L) of about 0.18 mm measured by using a digital micrometer (S_Mike PRO IP67 SIS, Czech Republic).

$$\text{MR} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp\left[-(2n-1)^2 \frac{\pi^2 D_e t}{L^2}\right] \qquad (\quad 5.1 \quad)$$

where MR = dimensionless moisture ratio, D_e = effective diffusivity (m^2/s), t = time, L = half slab thickness (m) and n = positive integer.

By using only the first term of the general solution, Equation 5.1 can be linearised by applying natural logarithm at both sides as shown in Equation 5.2, where the slope of ln MR versus t was used to determine the effective diffusivity (D_e).

$$\ln \text{MR} = \ln \frac{8}{\pi^2} - \frac{\pi^2 D_e}{L^2} t \qquad (\quad 5.2 \quad)$$

where $\pi^2 D_e / L^2$ = gradient of the graph (1/s).

The effective diffusivities were expressed in the form of Equation 5.3 using the Arrhenius temperature dependency model:

$$D_e = D_o \exp\left[\frac{-E_a}{R(T+273.15)}\right] \qquad (\quad 5.3 \quad)$$

where D_o = diffusivity constant (m^2/s), E_a = activation energy (kJ/mol) and R = universal gas constant (8.314 J/mol K).

Equation 5.3 can be linearised by applying natural logarithm at both sides as shown in Equation 5.4, where the slope of $\ln D_e$ versus $1/T$ was used to determine the D_o and E_a.

$$\ln D_e = \ln D_o - \left(\frac{E_a}{R}\right)\left(\frac{1}{T+273.15}\right) \qquad (\quad 5.4 \quad)$$

where E_a/R = gradient (K) and $\ln D_o$ = y-intercept.

5.3 Results and Discussion

5.3.1 Drying kinetics

Figure 5-3 and Figure 5-4 show the moisture content profiles of papaya leaves during hot air drying at 60°C, 70°C and 80°C (HD6, HD7 and HD8), shade drying (SD) and freeze drying (FD), respectively. In hot air drying, the exhibited trends show the characteristic exponential decay which is typically observed in many bio-products (Santhanam Menon, et al., 2017; Rumaisa, et al., 2018; Adom, et al., 1997; Sousa, et al., 2018; Samejima & Yano, 1985; Panchariya, et al., 2002). This trend can be best explained by Fick's second law of diffusion, where the moisture diffusion process is internally controlled and drying mainly takes place at the epidermis and cut portion of the leaves (Samejima & Yano, 1985; Panchariya, et al., 2002). Drying time was recorded between 1.25 hours to 7 hours for drying temperatures of 60°C to 80°C.
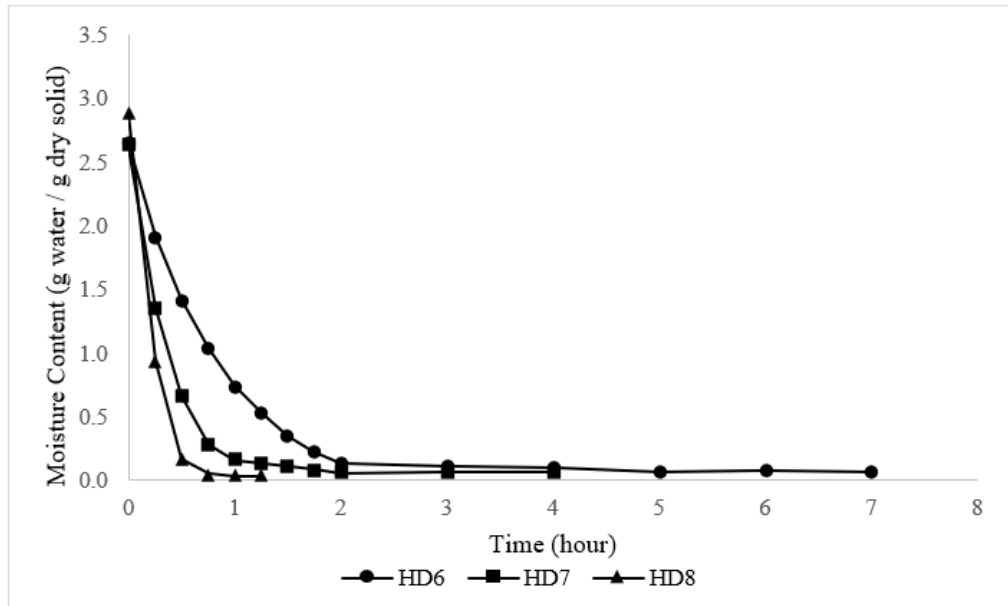
Figure 5-3: Moisture content profiles for hot air drying (60°C, 70°C and 80°C)
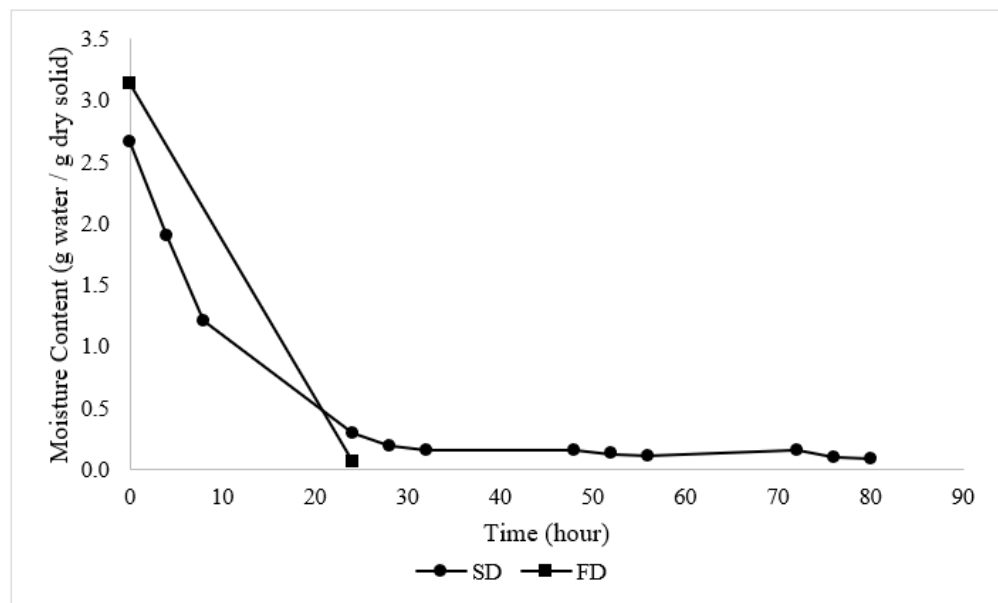
Figure 5-4: Moisture content profiles for shade drying (SD) and freeze drying (FD)

A similar trend was observed in shade drying but drying time was much longer (80 hours) due to the shaded conditions that resulted in a lower driving force for mass transfer. Nevertheless, the conditions (shaded from direct sunlight

and natural wind movement) were still conducive for drying leafy materials. The dried leaves were observed free from any visible moulds and signs of rotting.

In freeze drying, overnight drying for 24 hours ensured all the ice crystals were fully sublimed, and by visual observation, the colour of the dried samples was similar to the fresh green leaves. Colour preservation was better owing to the low temperature and vacuum drying condition used, which is conducive in preserving colour pigments as well as bioactive compounds (Santhanam Menon, et al., 2017).

Table 5-1 shows that final moisture contents were recorded at 0.07 g water/g dry solid, 0.06 g water/g dry solid, 0.04 g water/g dry solid, 0.09 g water/g dry solid and 0.06 g water/g dry solid for hot air drying at 60°C, 70°C and 80°C, shade drying and freeze drying, respectively. The initial moisture content of papaya leaves was 2.8 g water/g dry solid. The higher the hot air drying temperature resulted in a much lower final moisture content due to the greater driving force for heat and mass transfer between the drying air and product. Shade drying showed the highest final moisture content as compared to hot air and freeze dried samples due to the lower drying temperature (average temperature around 27°C) and higher RH (average RH around 80%) which fluctuated with ambient conditions.

Table 5-1: Final moisture contents of samples after drying processes

Sample	Moisture content (g water/g dry solid)
FD	0.06 ± 0.00
HD6	0.07 ± 0.00
HD7	0.06 ± 0.00
HD8	0.04 ± 0.01
SD	0.09 ± 0.02

5.3.2   Drying rate

Drying rate curves during hot air drying at 60°C (HD6), 70°C (HD7), 80°C (HD8), and shade drying (SD) are presented in Figure 5-5. It shows that the drying rate at high temperature (80°C) is the fastest, which agrees with the results reported by Ee et al. (2019), followed by lower temperatures (HD6, HD7 and SD). This is because higher temperature could provide a greater driving force for heat and mass transfer, mainly attributed to the enhanced temperature and moisture content gradients (Wu, et al., 2007).
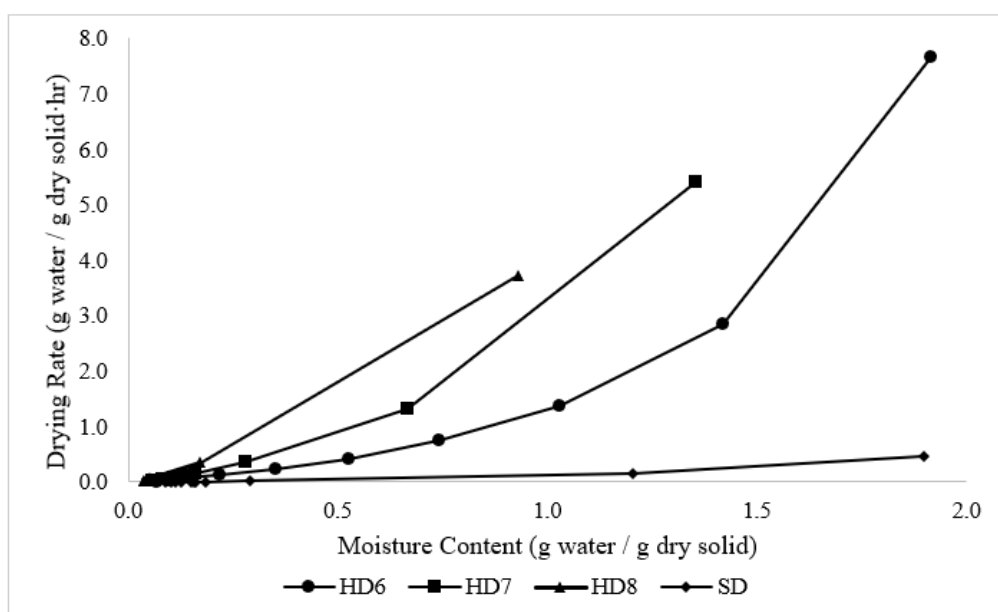
Figure 5-5: Drying rate curves during hot air drying at 60°C (HD6), 70°C (HD7), 80°C (HD8) and shade drying (SD)

5.3.3   Effective diffusivity and activation energy

Table 5-2 shows the effective diffusivities determined for hot air drying at 60°C, 70°C and 80°C. The values are in the range of 2.09×10^{-12} m^2/s to 2.18×10^{-12} m^2/s, which are within the order of magnitudes (10^{-12} to 10^{-8} m^2/s)

reported for drying of agricultural and food materials (Zogzas, et al., 1996). A graph of ln D against 1/T was plotted (R^2=0.7659) to determine the activation energy of papaya leaves (Figure 5-6).

Table 5-2: Effective diffusivities for hot air drying (60°C, 70°C and 80°C)

Drying technique	Temperature (K)	Diffusivity (m^2/s)
HD6	333.15	2.087×10^{-12}
HD7	343.15	2.177×10^{-12}
HD8	353.15	2.178×10^{-12}
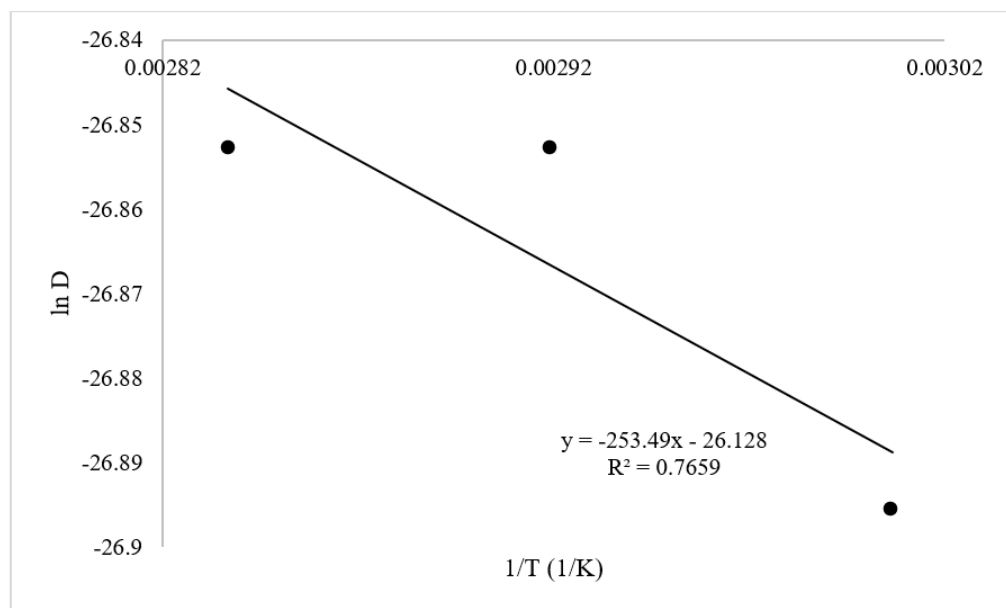
Figure 5-6: Graph of ln D versus 1/T

The Arrhenius relationship (Equation 5.5) derived from the effective diffusivities obtained from hot air drying (60°C, 70°C and 80°C) showed activation energy (E_a) and exponential factor (D_o) determined at 2.11 kJ/mol and 4.50×10^{-12} m^2/s, respectively. The activation energy indicates the minimum barrier that needs to be overcome in order to initiate the moisture diffusion process during drying. The activation energy is within the general range reported

for food materials, which ranged from 1.27 kJ/mol to 110 kJ/mol (Aghbashlo & Samimi-Akhijahani, 2008).

$$D_e = 4.50 \times 10^{-12} \exp\left[-\frac{2.11}{R(T+273.15)}\right] \qquad (\quad 5.5\quad)$$

Table 5-3 shows the comparison of activation energy with other leave materials using hot air drying. The activation energy to initiate moisture diffusion in papaya leaves is relatively lower than other leave materials. This could be due to the relatively thin leave and the cut layer (about 0.18 mm) that facilitates diffusion of moisture within the leaves to the surrounding.

Table 5-3: Comparison of activation energy (E_a) with other plant leaves materials using hot air drying

Plant leaves material	Activation energy (kJ/mol)	Temperature range (°C)	Reference
Black Tea	406.02	80 – 120	Panchariya, et al., 2002
Wormwood leaves	69.33	50 – 70	Beigi, 2017
Rosemary	54.37	50 – 80	Mghazli, et al., 2017
Gale of the Wind	43.13	50 – 70	Sousa, et al., 2018
Spider plant leaves	24.46	50 – 70	Omolola, et al., 2019
Peppermint	12.46	50 – 70	Torki-Harchegani, et al., 2016
Papaya leaves	2.11	60 – 80	Current work

5.4   Conclusion

The drying kinetics of papaya leaves dried using hot air (60°C, 70°C and 80°C), shade and freeze drying techniques were determined. Typical exponential falling trends were observed, which can be best explained by Fick's

second law of diffusion. It can be concluded that higher temperature could be a greater driving force for heat and mass transfer, thus resulting in a shorter drying time. The drying time of hot air drying (60°C, 70°C and 80°C) ranged from 1.25 to 7 hours, whereas the shade drying time was much longer (80 hours). The total drying time of freeze drying was 48 hours due to 24 hours were taken to freeze the moisture content in the leaves to solid form, followed by another 24 hours to sublimate the ice crystals to water vapour under vacuum condition and low temperature. The fastest drying rate was observed at high temperature (hot air drying at 80°C) and followed by lower temperatures (hot air drying at 60°C and 70°C, and shade drying). By using the Arrhenius equation, the effective diffusivities were determined in the range of 2.09×10^{-12} m^2/s to 2.18×10^{-12} m^2/s, and the minimum energy required to initiate moisture diffusion was determined at 2.11 kJ/mol in hot air drying within 60°C to 80°C.

CHAPTER 6

EFFECTS OF DRYING TECHNIQUES ON CARPAINE RETENTION AND ANTIOXIDANT PROPERTIES OF PAPAYA LEAVES

6.1   Introduction

Papaya is a prevalent fruit in Malaysia due to its high nutritional values and affordable price. With increasing public health awareness, more and more researches focus on the medicinal properties not only on the fruit products but also on other parts of the plants as well. Carpaine, a major bioactive compound found in papaya leaves, was proven to increase blood platelet counts and normalise blood clotting. Thus, it is a potential cure to treat dengue patients as they usually experience dehydration due to low blood platelet count and internal bleeding.

However, literature reports on processing aspects of papaya leaves are quite scarce (e.g., preparation and drying) and this information is vital for the future production of carpaine in bulk volume. Therefore, the effects of drying techniques on carpaine retention and antioxidant properties of papaya leaves were studied and reported in this chapter.

6.2   Materials and Methods

6.2.1   Sample collection and preparation

The sample collection was carried out as described in Section 3.2 (Chapter 3). The methods used to prepare the samples using different drying processes,

namely hot air (60°C, 70°C and 80°C), shade and freeze drying, were similar to those reported in Section 5.2.2 (Chapter 5). All the samples were then freeze dried and grounded into powders prior to the chemical analyses such as TPC, ABTS and DPPH assays and colour measurement.

6.2.2   Quantification of carpaine

Quantification of carpaine from the papaya leaves was carried out as described in Section 3.4 (Chapter 3).

6.2.3   Total polyphenol content, ABTS and DPPH free radical scavenging assays

Total polyphenol content, ABTS and DPPH free radical scavenging activities of papaya leaves samples were carried out as described in Section 3.6, 3.7 and 3.8 (Chapter 3), respectively.

6.2.4   Colour measurement

The colour measurement of the papaya leaves samples was carried out as described in Section 3.9 (Chapter 3).

6.2.5   Statistical analysis

The statistical analysis was carried out as described in Section 3.10 (Chapter 3).

6.3   Results and Discussion

6.3.1   Amount of carpaine quantified

Figure 6-1 shows the amount of carpaine quantified from dried papaya leaves using various drying processes ranging from 47 µg/g to 333 µg/g. The amount of carpaine quantified from freeze dried (FD) samples was significantly higher ($p < 0.05$) than hot air dried samples at 60°C (HD6), 70°C (HD7) and 80°C (HD8), and shade dried (SD) samples. Freeze dried samples showed the highest amount of carpaine because the mechanism of freeze drying is based on sublimation at a low-temperature zone (-60°C), which is suitable for heat sensitive products (Mediani, et al., 2015). Singh et al. (2006) found that low-temperature drying process could minimise damages to leafy vegetables and thus retain more nutrients than other drying processes with higher temperatures.

In the case of hot air drying, the additional effect of heat on the carpaine could contribute to the low amount of carpaine quantified from the hot air dried samples. This could explain why the amount of carpaine quantified from freeze dried and shade dried samples was higher than hot air dried samples, also indicating that carpaine is a heat-sensitive bioactive compound. There was no significant difference ($p > 0.05$) for all the hot air dried samples (60°C, 70°C and 80°C) but there was a tendency for the amount of carpaine to reduce (from 60 µg/g to 47 µg/g) as the temperature increased from 60°C to 80°C.
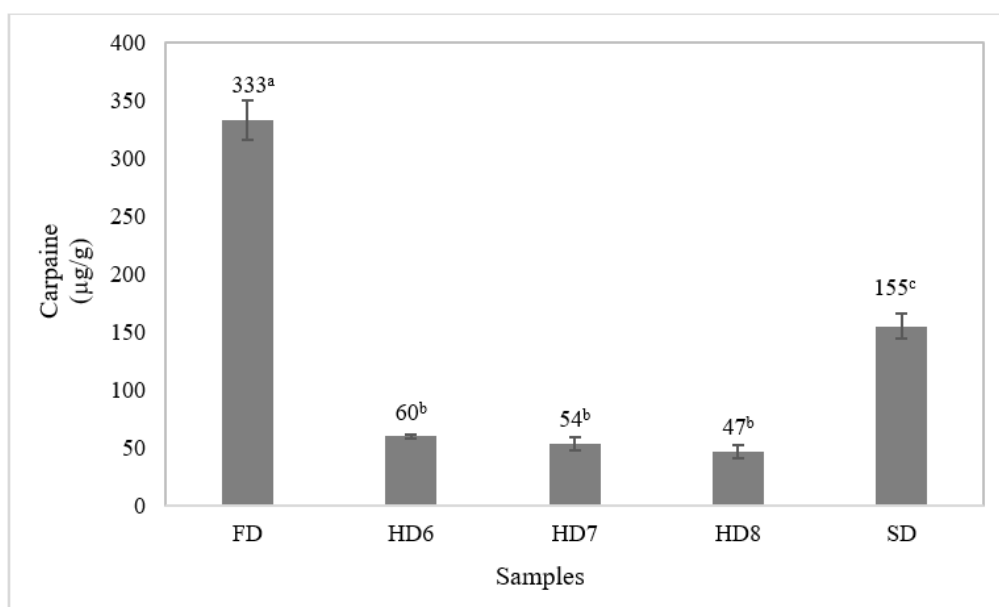
Figure 6-1: Amount of carpaine quantified from dried papaya leaves from various drying processes

6.3.2 Total polyphenol content

Figure 6-2 shows the total polyphenol content (TPC) of dried papaya leaves from various drying processes ranging from 1492 mg GAE 100g/DW to 2158 mg GAE 100g/DW. It can be seen that freeze dried samples showed significantly higher TPC ($p<0.05$) than hot air dried samples at 70°C (HD7) and 80°C (HD8), as well as shade dried samples (SD). This is in agreement with literature where freeze drying was proven to retain most nutritional and bioactive compounds, including phenolic compounds during drying attributed to the oxygenless and low/mild temperature environment inside the freeze drying chamber (Santhanam Menon, et al., 2017). The TPC obtained from the freeze dried samples (2158 mg GAE 100g/DW) is also higher than that reported by

Gogna et al. (2015) and Maisarah et al. (2013), at 318 and 425 mg GAE 100g/DW, respectively, which could be due to the origins and planting aspects of the fresh leaves.

Note: mean values having common letter within drying techniques are not significant according to the Duncan's multiple range test at 5% level.

Figure 6-2: Total polyphenol content of dried papaya leaves from various drying processes

Comparison among the hot air dried samples (range 1940 – 2093 mg GAE 100g/DW) shows that there is a tendency for higher temperature process (HD8) to record significantly lower TPC ($p<0.05$) than that at a lower temperature (HD6). This could be due to exposure of the samples to high air temperatures, leading to excessive thermal degradation of the phenolic compounds. Menon et al. (2017) reported about 25% reduction in TPC of hot air dried samples than freeze dried samples in cocoa drying.

On the other hand, shade dried samples (SD) showed the lowest TPC

(1492 mg GAE 100g/DW). This could be due to the long drying time, resulting in prolonged exposure to oxygen, which is conducive for oxidase enzymes to carry out the oxidation process (Teh, et al., 2016). Besides, prolonged exposure to UV rays from the sunlight could also result in further degradation of polyphenols.

6.3.3 ABTS and DPPH free radical scavenging activities

Figure 6-3 and Figure 6-4 show antioxidant activity analyses from ABTS and DPPH assays, respectively. DPPH/IC50 and ABTS/TEAC are better ways of comparison of antioxidant activity as these techniques are well established and have good reproducibility as recommended in various published literature (Thaipong, et al., 2006; Krings & Berger, 2001; Qin, et al., 2017; Dontha, 2016; Shofian, et al., 2011).
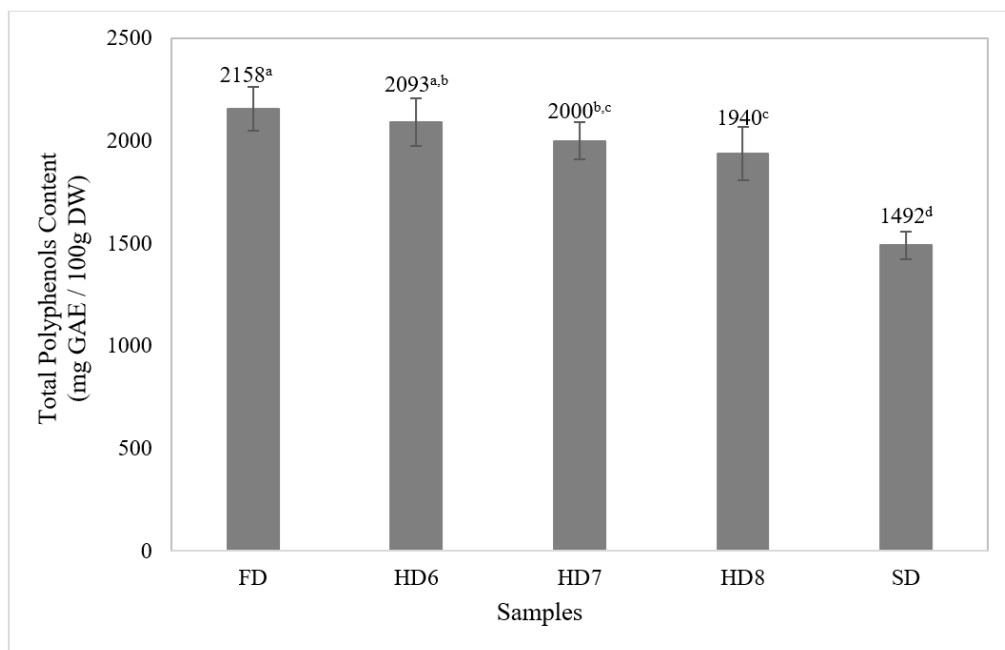

Note: mean values having common letter within drying techniques are not significant according to the Duncan's multiple range test at 5% level.

Figure 6-3: ABTS scavenging activities of dried papaya leaves from various drying processes
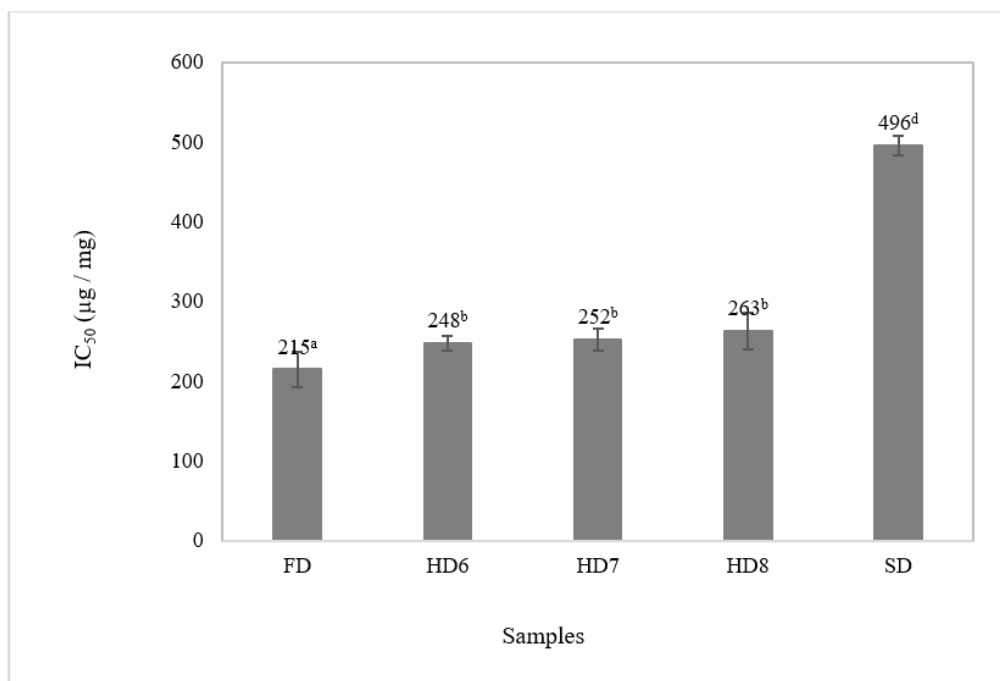
Note: mean values having common letter within drying techniques are not significant according to the Duncan's multiple range test at 5% level.

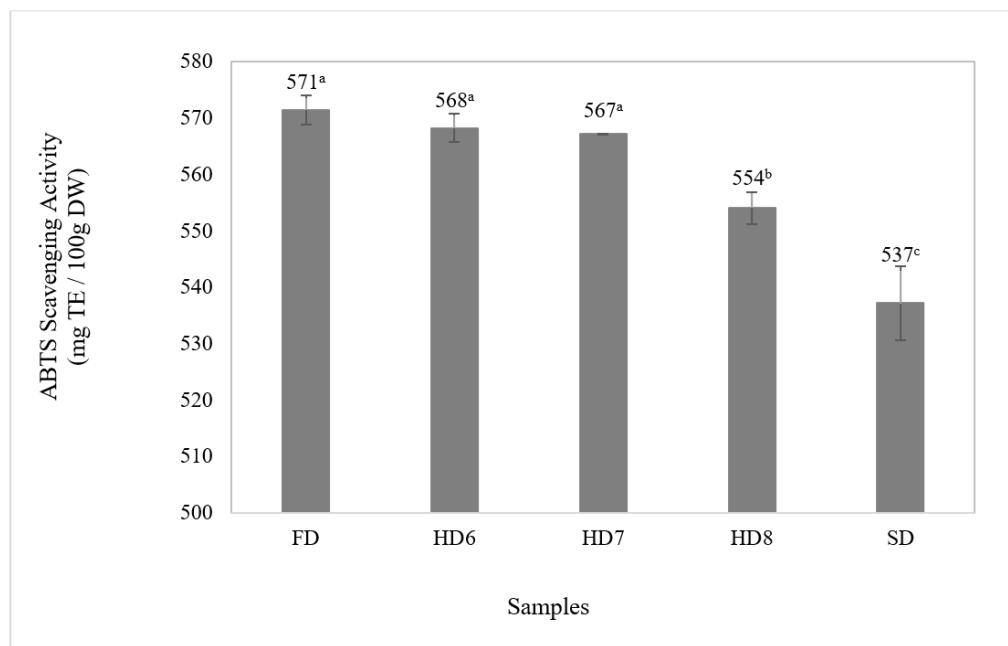**Figure 6-4: DPPH scavenging activities of dried papaya leaves from various drying processes**

Freeze dried samples (FD) showed significantly higher reading for ABTS scavenging activity ($p < 0.05$) (571 mg TE 100g/DW) as compared to hot air dried samples HD8 (554 mg TE 100g/DW) and shade dried samples (537 mg TE 100g/DW) in Figure 6-3. In terms of DPPH scavenging activity (Figure 6-4), freeze dried samples (FD) also showed significantly greater DPPH scavenging activity ($p < 0.05$) with the lowest IC_{50} value (215 µg/mg) as compared to hot air (range 248-263 µg/mg) and shade (496 µg/mg) dried samples. This is mainly attributed to the low/ mild drying and vacuum conditions during the lyophilisation process that helps in retaining high amount of bioactive compounds in dried products (Santhanam Menon, et al., 2017; Shofian, et al.,

2011; Mahn, et al., 2014). Menon et al. (2017) reported significantly low values of antioxidant activity in hot air dried cocoa samples due to severe degradation of major antioxidants. The highly aerated drying condition promotes the oxidation of polyphenols into o-quinone that subsequently condenses into a polymeric brown pigment known as melanin.

The scavenging activities from both ABTS and DPPH assays also showed a reasonably good correlation to each other ($R^2 = 0.87$) and displayed scavenging activities in the order of FD > HD6 > HD7 > HD8 > SD samples. This high correlation could be resulted from a similar mechanism between the two assay methods, which involves electron transfer and reduction of coloured oxidants, and the antioxidants are soluble in the aqueous system (Leong & Shui, 2002).

6.3.4   Relationship between TPC, ABTS and DPPH free radical scavenging activities

The antioxidant activities measured by ABTS and DPPH assays are highly correlated with TPC (Figure 6-5 and Figure 6-6) which agrees with the literature (Maisarah, et al., 2013; Vuong, et al., 2013). Typically, the higher the TPC, the higher the antioxidant activity as phenolic compounds account for a significant portion of the antioxidant activity in many plant materials. The coefficient of determinations (R^2) of ABTS and DPPH scavenging activities were determined at 0.9350 and 0.9635, respectively. These are in line with results from published studies (Maisarah, et al., 2013; Vuong, et al., 2013).
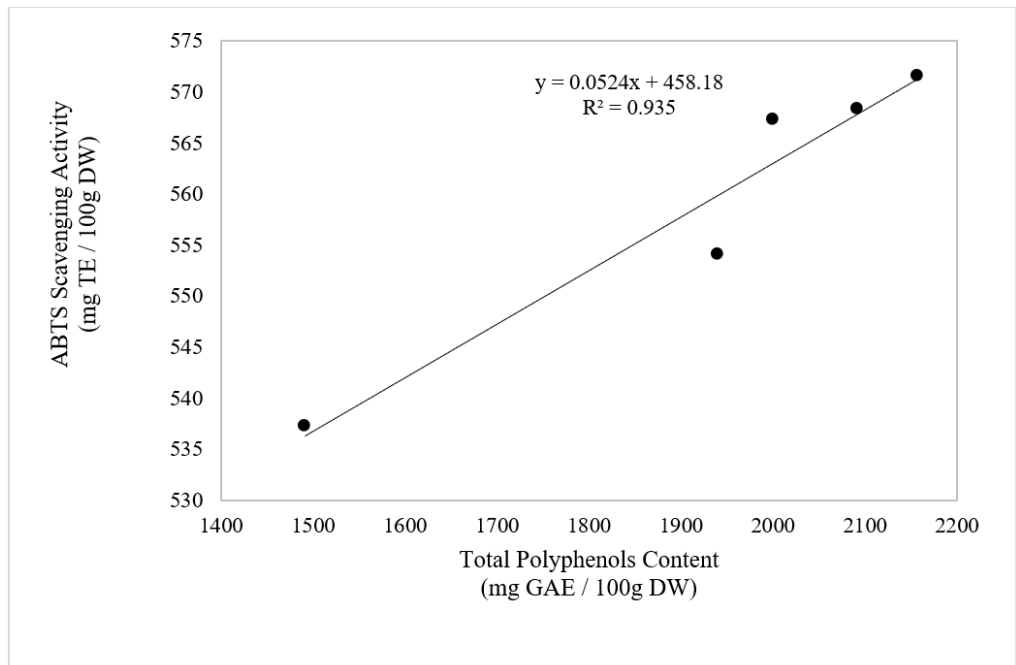
Figure 6-5: Correlation of polyphenols on ABTS scavenging activities in papaya leaves
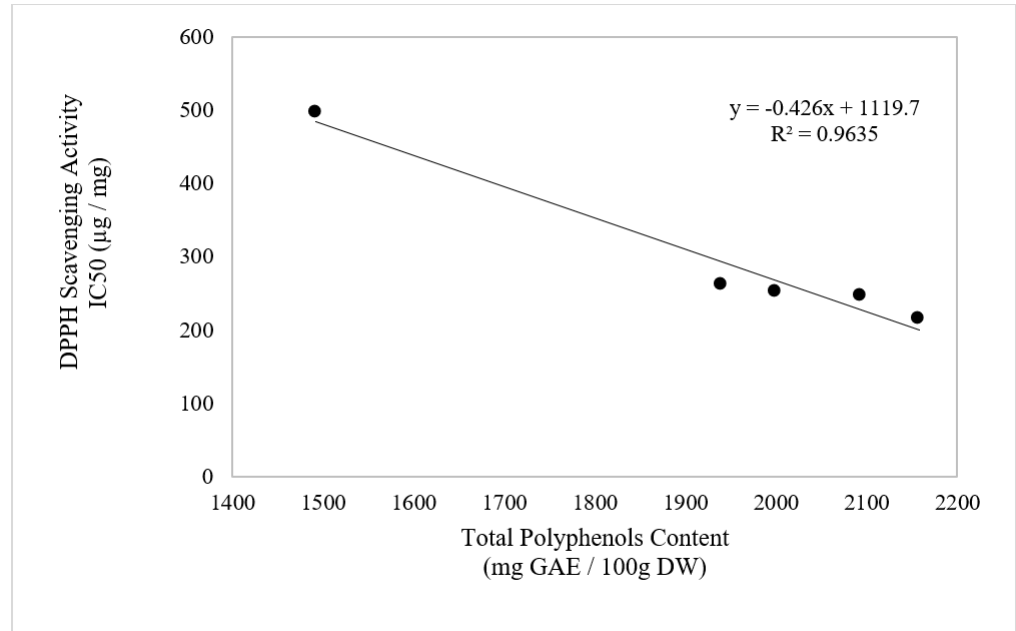
Figure 6-6: Correlation of polyphenols on DPPH scavenging activities in papaya leaves

6.3.5 Colour of dried papaya leaves

Results from colour analysis for dried papaya leaves of different drying techniques are presented in Figure 6-7, Figure 6-8 and Figure 6-9. Freeze dried samples (FD) were used as the benchmark for comparison because freeze drying removes water by sublimation of ice at low temperature, resulting in relative stability of L^*, a^* and b^* values compared to other drying techniques (Krokida, et al., 2001). There is no significant difference (p>0.05) in L^* values (lightness) in all dried samples and all the dried samples showed similar luminosity. The a^* values (greenness) of hot air dried samples at 80°C (HD8) and shade dried samples (SD) were significantly different (p<0.05) from FD samples, and they are arranged in increasing order of HD8 > FD > SD samples. SD samples showed the lowest L^* value (46.66) and highest a^* value (-3.38), indicating the lowest lightness and greenness. Visually, dull green-yellow colour was observed in the SD samples. This could be due to the longer drying time that caused chlorophyll degradation (Rudra, et al., 2008; Ali, et al., 2014). The highest b^* value (yellowness) was also observed in HD8 samples (18.35), which could be due to the degradation of chlorophyll in hot air dried samples (Ali, et al., 2014). It has been reported that many reactions could affect colour changes during the thermal processing of agricultural products, especially pigment degradation (Nyambaka & Ryley, 2004).
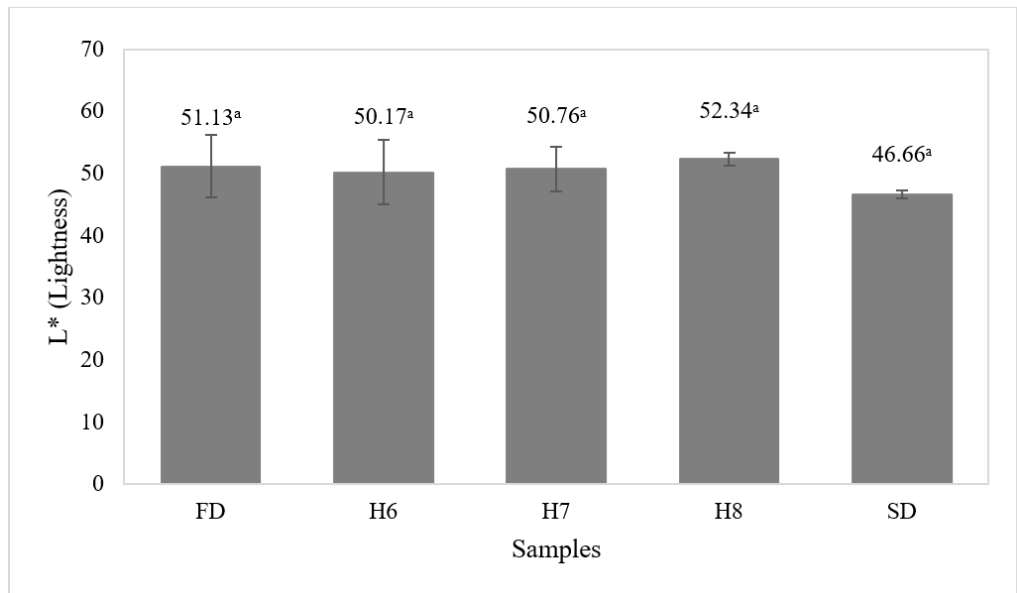
Figure 6-7: L^* **values of dried papaya leaves from various drying processes**
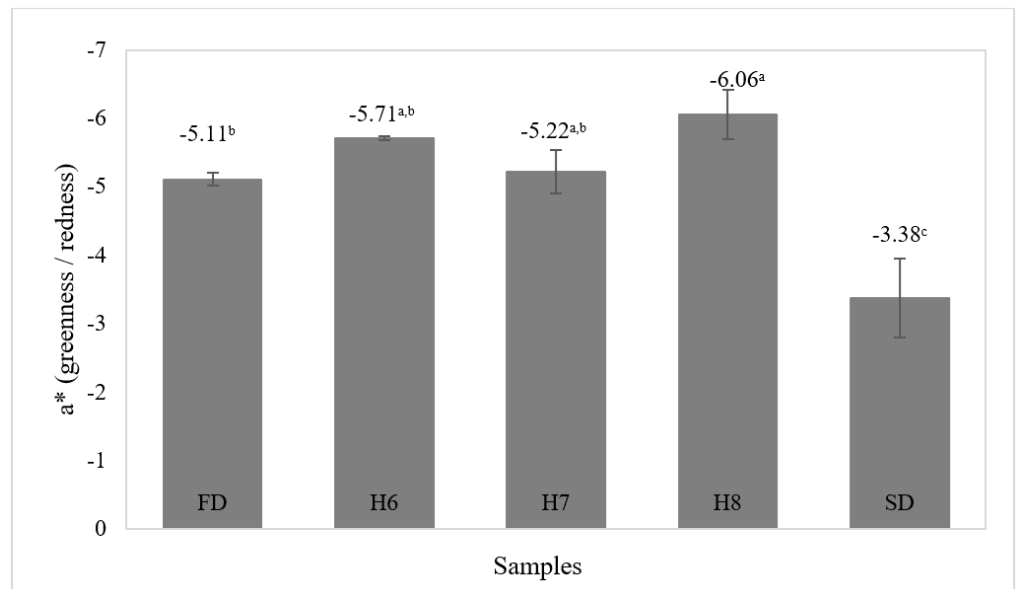
Figure 6-8: a^* **values of dried papaya leaves from various drying processes**
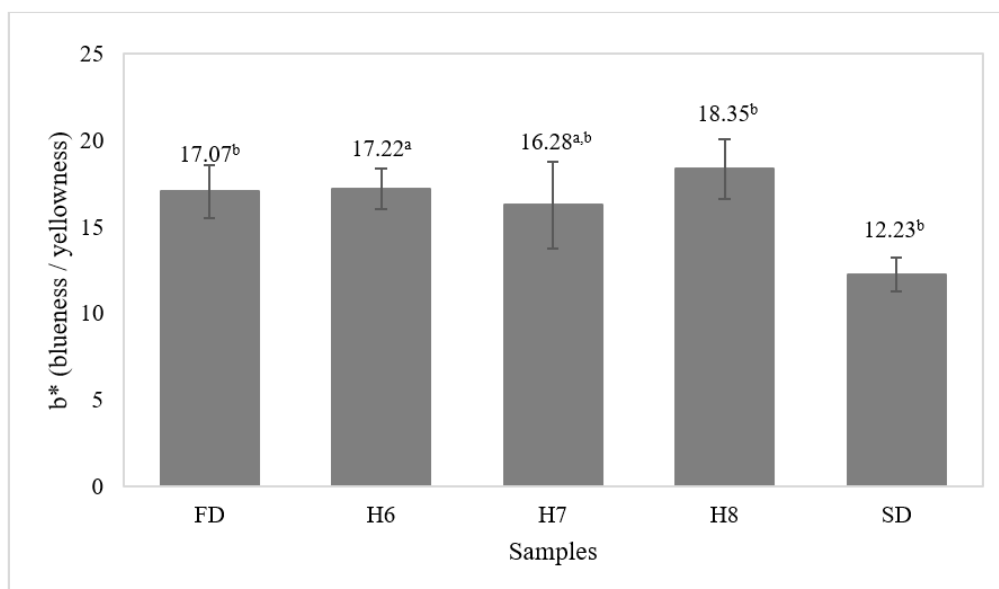
Figure 6-9: b^* **values of dried papaya leaves from various drying processes**

6.4   Conclusion

Freeze dried samples showed the highest retention of carpaine (333 µg/g), followed by shade dried samples (155 µg/g) and hot air dried samples at 60°C, 70°C and 80°C (60 µg/g, 54 µg/g and 47 µg/g, respectively). Hot air drying at 60°C, 70°C and 80°C are not recommended for the preservation of carpaine in papaya leaves as carpaine is a heat-sensitive bioactive compound. Analysis of total polyphenol content showed the highest retention of polyphenols in the freeze dried samples, followed by hot air (60°C, 70°C and 80°C) and shade dried samples. The freeze dried samples also showed the highest antioxidant activities in both ABTS and DPPH antioxidant assays. The relationship between the antioxidant activities (ABTS and DPPH scavenging activities) and TPC were found to be highly correlated ($R^2 = 0.9350$ and 0.9635, respectively). Hot air and shade drying are not conducive in preserving antioxidants owing to possible thermal degradation at elevated hot air temperatures and oxidations under

prolonged shade drying conditions. In colour measurement, there was no significant difference ($p > 0.05$) in the lightness of FD, HD6, HD7, HD8 and SD samples. However, SD samples had the lowest lightness and greenness, which could be due to the longer drying time that caused chlorophyll degradation.

CHAPTER 7

EFFECTS OF EXTENDED STORAGE PERIOD ON CARPAINE RETENTION AND ANTIOXIDANT PROPERTIES OF DRIED PAPAYA LEAVES

7.1 Introduction

Carpaine, a major bioactive compound in papaya leaves, was found to be a potential cure to treat dengue fever. Zunjar et al. (2016) reported that carpaine showed anti-thrombocytopenic activity when tested on busulfan induced thrombocytopenic Wistar rats. In the previous chapter, the effects of drying techniques on carpaine retention and antioxidant properties of papaya leaves were investigated.

In this chapter, the effects of extended storage period on carpaine retention and antioxidant properties of dried papaya leaves were discussed. This is also to further investigate on the impact of extended storage (3 months) on the stability of carpaine. Results from this study will contribute to a better understanding, especially in the extraction of carpaine at a commercial scale as the raw materials used would be mainly in dried form, which will be stored in bulk quantity.

7.2 Materials and Methods

7.2.1 Sample collection and preparation

The sample collection was carried out as described in Section 3.2 (Chapter 3). The method used to prepare the samples using different drying processes (hot air drying at 60°C, 70°C and 80°C, shade drying and freeze

drying) were carried out as described in Section 6.2.1 (Chapter 6). After drying, the powdered samples were sealed in LDPE (low-density polyethene, 0.91-0.94 g/cm^3) plastic bags and stored in dark conditions at room temperature. All the powdered samples were periodically analysed (at 0, 1, 2 and 3 months) for various physicochemical properties such as carpaine retention, total phenolic content, antioxidant properties (ABTS & DPPH radical scavenging activities) and colour as described in Section 3.4 – 3.9 (Chapter 3).

7.2.2   Quantification of carpaine

The method to quantify carpaine from the papaya leaves samples is as described in Section 3.4 (Chapter 3).

7.2.3   Degradation kinetics of carpaine

The degradation kinetics of carpaine in dried leaves samples were determined using the standard zero and first order reaction models (Equation 7.1 and 7.2):

$$C_0 + C_t = kt \qquad\qquad\qquad (\quad 7.1 \quad)$$

$$C_t = C_0 exp(-kt) \qquad\qquad\qquad (\quad 7.2 \quad)$$

where C_t = amount of carpaine at time t (µg/g), C_0 = initial amount of carpaine (µg/g), k = degradation rate constant (1/month) (Kim, et al., 2018).

The Weibull model was also used to model the degradation kinetics of carpaine (Equation 7.3).

$$C_t = C_0 exp(-bt^n) \qquad\qquad (\quad 7.3 \quad)$$

where C_t = amount of carpaine at time t (µg/g), C_0 = initial amount of carpaine (µg/g), b = degradation rate constant (1/month). The b and n parameters were the shape and scale factors of the distribution curve, respectively (Sarkis, et al., 2019). The n parameter indicated concavity (tail-forming) or convexity (shoulder-forming) of the curve when it took values below and above 1, respectively.

Statistical parameters such as chi-square (χ^2) (Equation 7.4), root mean square error (RMSE) (Equation 7.5), coefficient of determination (R^2) (Equation 7.6) and average errors between the experimental and predicted values (E) (Equation 7.7) were used to assess the goodness of fitting (Sarpong, et al., 2018).

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(C_{t_{exp,i}} - C_{t_{pre,i}}\right)^2}{(N-z)} \qquad\qquad (\quad 7.4 \quad)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(C_{t_{exp,i}} - C_{t_{pre,i}}\right)^2} \qquad\qquad (\quad 7.5 \quad)$$

$$R^2 = \frac{N\sum_{i=1}^{N}C_{t_{pre,i}}C_{t_{exp,i}} - \sum_{i=1}^{N}C_{t_{pre,i}}\sum_{i=1}^{N}C_{t_{exp,i}}}{\sqrt{\left(N\sum_{i=1}^{N}C_{t_{pre,i}}^2 - \left(\sum_{i=1}^{N}C_{t_{exp,i}}\right)^2\right)\left(N\sum_{i=1}^{N}C_{t_{exp,i}}^2 - \left(\sum_{i=1}^{N}C_{t_{exp,i}}\right)^2\right)}} \qquad\qquad (\quad 7.6 \quad)$$

$$E(\%) = \frac{100}{N}\sum_{i=1}^{N}\left|\frac{C_{t_{exp,i}} - C_{t_{pre,i}}}{C_{t_{exp,i}}}\right| \qquad\qquad (\quad 7.7 \quad)$$

where C_t = amount of carpaine at time t (µg/g) (Equation 3.2), the subscripts *exp* and *pre* indicate experimental and predicted values, respectively, whereas the subscripts i, o and e indicate the moisture content at any time i, initial moisture content and equilibrium moisture content, respectively. N = the number of experimental data points and z = the number of constant.

The half-life of degradation ($t_{1/2}$) can be determined by using Equation 7.8.

$$t_{1/2} = \frac{\ln(2)}{k}$$ (7.8)

where k = degradation rate constant (1/month).

7.2.4 Total polyphenol content, ABTS and DPPH free radical scavenging assays

Total polyphenol content, ABTS and DPPH free radical scavenging activities of papaya leaves samples were carried out as described in Section 3.6, 3.7 and 3.8 (Chapter 3), respectively.

7.2.5 Colour measurement

The colour measurement of the papaya leaves samples was carried out as described in Section 3.9 (Chapter 3).

7.2.6 Statistical analysis

The statistical analysis was carried out as described in Section 3.10 (Chapter 3).

7.3 Results and Discussion

7.3.1 Amount of carpaine quantified

Figure 7-1 shows the carpaine retention of dried papaya leaves from various drying processes during 0, 1, 2 and 3 months of storage. The initial amount of carpaine in dried samples from various drying techniques ranged from 47 µg/g to 333 µg/g. As shown in Figure 7-1, the amount of carpaine was found to decrease with storage time in all dried samples, except month 1 for FD samples which might be due to experimental variation from the samples. Various literature have reported the degradation of bioactive compounds during storage, such as anthocyanin in strawberry jam (Patras, et al., 2011), vitamin C in grapefruit (Moraga, et al., 2012) and flavonoid in jambhul (Sonawane & Arya, 2015). A total decrease of 39.5%, 38.7%, 55.7%, 59.7% and 21.0% for freeze dried samples (FD), hot air dried samples at 60°C, 70°C and 80°C (HD6, HD7, HD8), and shade dried samples (SD) were observed over the three months storage period, respectively. The results also indicated that carpaine loss was the highest for HD8 samples after three months of storage. Even though the lowest carpaine loss was recorded for SD samples (21.0%), the initial amount of carpaine (before storage) was in fact already very low as compared to other samples.

It was observed that the initial amounts of carpaine of HD6, HD7 and HD8 samples were lower than FD and SD samples because of thermal heating on the carpaine during hot air drying. Mohan and Shanmugam (2016) reported that freeze dried samples had a higher amount of bioactive compounds than hot air dried samples. Reduction in tannin, saponin and alkaloid content of

watermelon rind was also reported for hot air dried samples due to the oxidation of bioactive compounds and enzymatic degradation during hot air drying (Mohan & Shanmugam, 2016). In overall, freeze dried samples are the preferred choice for extended storage purpose as carpaine is more stable in this particular dried form (130 µg/g – 333 µg/g) over the three months storage period as compared to hot air and shade dried samples (78 µg/g – 155 µg/g).
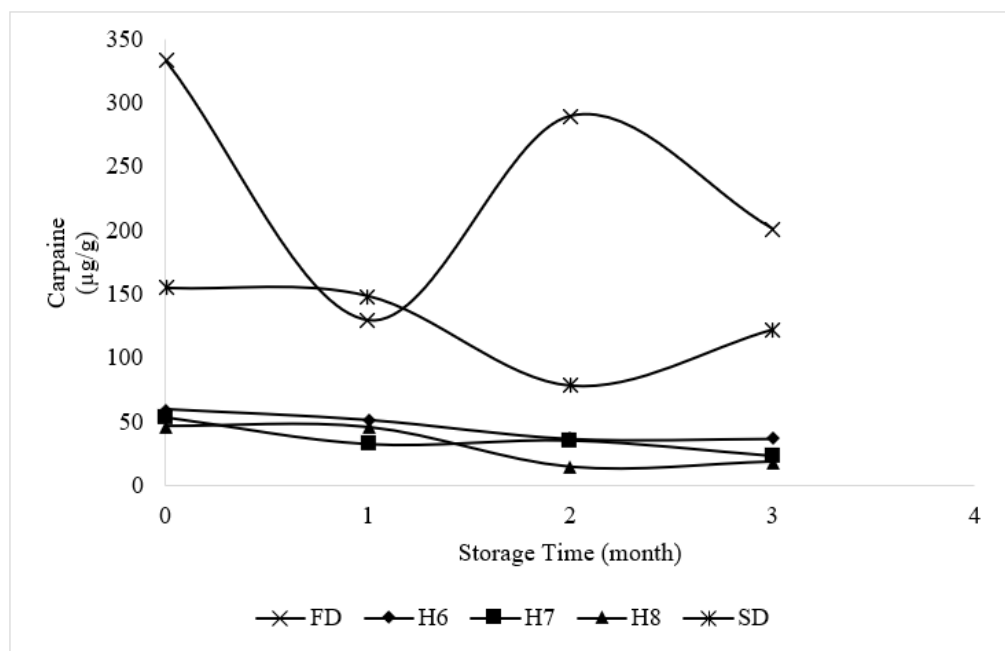
Figure 7-1: Degradation of carpaine in dried papaya leaves from various drying processes during storage

7.3.2 Degradation kinetics of carpaine

Degradation of carpaine in the dried leaves samples was further studied by analysing the kinetics with reference to the storage time (0, 1, 2 and 3 months) using three kinetic models. Four statistical parameters (R^2, χ^2, RMSE and E (%)) were used to evaluate the fitting adequacy of the models. As shown in Figure

7-1, the degradation kinetics of carpaine in FD samples can be best described by the Weibull model, which showed the highest R^2 (0.9979), and the lowest χ^2 (15.33), RMSE (2.77) and E (0.59%). Although the highest R^2 (0.9349) and lowest χ^2 (10.20) were observed in zero and first order models, the Weibull model was selected as the best fit for HD6 samples due to its lowest RMSE (2.73) and E (5.65%). The degradation kinetics of carpaine in HD7 samples can be well predicted by the Weibull model, which showed the lowest χ^2 (23.13), RMSE (3.40) and E (9.02%), despite the highest R^2 (0.8929) shown by zero order model. First order model was selected as the best fit for HD8 samples, and the deciding factor was the lowest χ^2 (80.29) and E (24.66%), although zero order model and Weibull model showed the highest R^2 (0.9076) and lowest RMSE (6.85), respectively. Weibull model was chosen as the best fit for SD samples as it gave the lowest RMSE (22.66) and E (18.70%). In overall, the Weibull model was selected to describe the degradation kinetics of carpaine in all dried samples (FD, HD6, HD7 and SD samples) except for HD8 samples, which could be best described by the first order model.

Table 7-1: Performance evaluation of selected models for carpaine degradation

Drying technique	Model	E (%)	RMSE	χ^2	R2
FD	Zero order	127.08	426.64	242690.53	0.8818
	First order	9.22	28.24	1061.43	0.7879
	Weibull	0.59	2.77	15.33	0.9979
HD6	Zero order	106.49	70.90	6702.23	0.9349
	First order	5.75	2.76	10.20	0.7610
	Weibull	5.65	2.73	14.94	0.7317
HD7	Zero order	125.05	60.17	4826.75	0.8929
	First order	9.59	4.60	28.24	0.7280
	Weibull	9.02	3.40	23.13	0.4730
HD8	Zero order	128.71	60.07	4810.41	0.9076

	First order	24.66	7.76	80.29	0.7089
	Weibull	28.63	6.85	93.72	0.8683
SD	Zero order	103.98	186.90	46576.23	0.5714
	First order	18.93	22.97	703.66	0.7767
	Weibull	18.70	22.66	1026.60	0.6602

Table 7-2 shows the kinetics parameters such as degradation rate constant (k) and half-life ($t_{1/2}$) of carpaine in dried leaves samples during storage by using the models selected. The k value shows the relationship between retention of carpaine in the dried samples and the storage time, whereas the $t_{1/2}$ value is the half-life of carpaine degradation, representing the time necessary to degrade 50% of the initial amount of carpaine. FD samples showed the lowest k value ($0.0135 \frac{1}{month}$), indicating the lowest degradation rate of carpaine with respect to the storage time, followed by SD ($0.1829 \frac{1}{month}$), HD6 ($0.2005 \frac{1}{month}$), HD8 ($0.3252 \frac{1}{month}$), and HD7 ($0.4186 \frac{1}{month}$). The lower the k value, the degradation rate of the carpaine with respect to the storage time decreases, and hence the half-life increases. Therefore, FD samples had the highest half-life (51.20 months), followed by SD (3.79 months), HD6 (3.46 months), HD8 (2.13 months), and HD7 (1.66 months) samples. This showed that FD dried samples are more stable during extended storage as compared to hot air and shade dried samples.

Table 7-2: Rate constant and half-life of carpaine degradation

Drying technique	Model	k (1/month)	$t_{1/2}$ (month)
FD	Weibull	0.0135	51.20
HD6	Weibull	0.2005	3.46
HD7	Weibull	0.4186	1.66
HD8	First order	0.3252	2.13
SD	Weibull	0.1829	3.79

7.3.3 Total polyphenol content and antioxidant activities

Figure 7-2 shows the total polyphenol content (TPC) of dried papaya leaves from various drying processes during storage for 0, 1, 2 and 3 months. The TPC of all dried samples gradually decreased as a function of storage time. During fruit processing, cell structures are disrupted, and the fruits become more prone to non-enzymatic oxidation which could be one of the main reasons for the loss of phenolic compounds (Patras, et al., 2011). Percentage degradation of 10.4%, 19.3%, 17.7%, 19.3% and 12.3% were observed for FD, HD6, HD7, HD8 and SD samples, respectively. Similar decreasing trends of TPC with storage time were also reported for grapefruit powder (Moraga, et al., 2012) and pomegranate juice (Varela-Santos, et al., 2012). It was also observed that the TPC of FD samples remained the highest among all the dried samples after three months of storage.
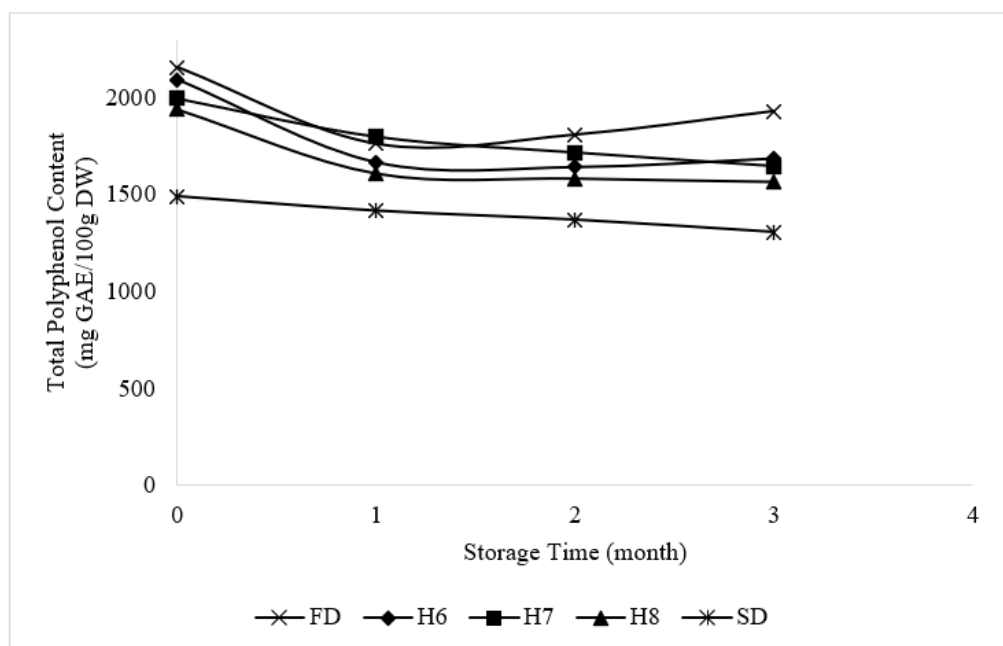
Figure 7-2: Total polyphenol content of dried papaya leaves from various drying processes during storage

ABTS radical scavenging activities of dried papaya leaves were plotted as a function of storage time as shown in Figure 7-3, and decreasing trends were observed for all dried samples. A dramatic degradation of ABTS radical scavenging activities could be observed after one month of storage where the activities degraded by 81.4%, 82.8%, 82.5%, 83.5% and 85.0% for FD, HD6, HD7, HD8 and SD samples, respectively. A similar trend was reported by Jayathunge et al. (2015), which recorded about 70% loss of ABTS radical scavenging activities in thermally processed tomato juices in the first month of storage. This similar trend in dried papaya leaves could be due to the depletion of certain antioxidants other than TPC, such as ascorbic acid (Jayathunge, et al., 2015). Besides, it was also found that the antioxidant activity of thermally processed tomato did not deplete completely after 12 months. Therefore, it can be assumed that the ABTS radical scavenging activities of dried papaya leaves would not deplete fully upon three months of storage.
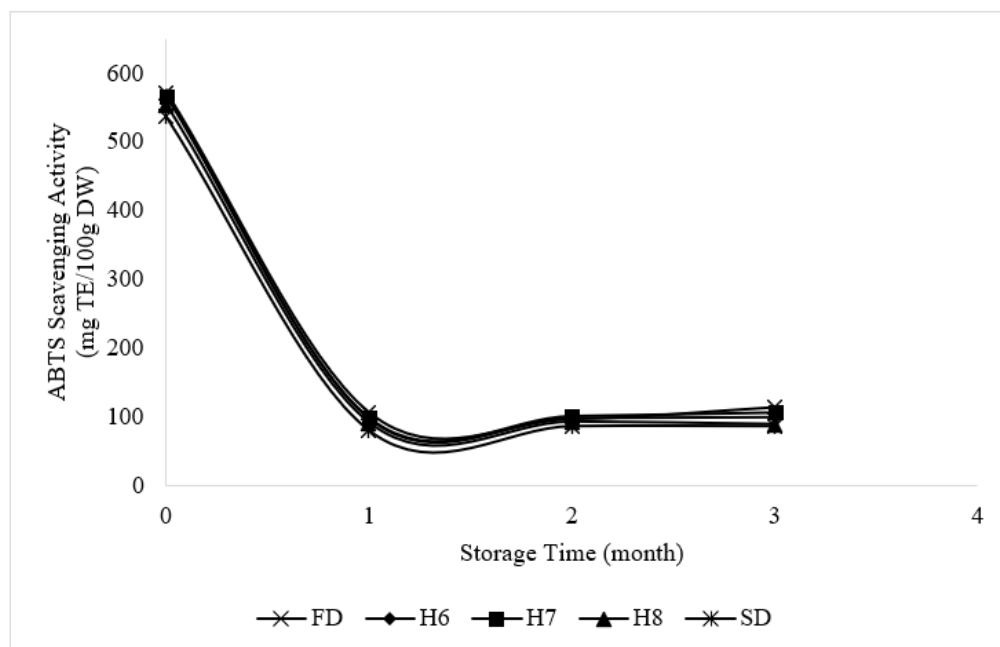
Figure 7-3: ABTS scavenging activities of dried papaya leaves from various drying processes during storage

DPPH radical scavenging activities of dried papaya leaves were determined in terms of AEAC (ascorbic acid equivalent antioxidant capacity) (Figure 7-4). The DPPH radical scavenging activities were plotted as a function of storage time and showed decreasing trends for all dried samples. The DPPH radical scavenging activities showed percentage loss of 41.3%, 37.0%, 36.7%, 29.3% and 10.5% for FD, HD6, HD7, HD8 and SD samples, respectively, over the three storage months. Some researchers have reported that reduction of DPPH radical scavenging of free radicals was attributed to the degradation of polyphenols (Amakura, et al., 2000; Maisarah, et al., 2013; Vuong, et al., 2013). However, the percentage loss of DPPH scavenging activities (10.5% – 41.3%) was not the same as the TPC (10.4% – 19.3%). This could be due to several antioxidants that may react slowly or inert to DPPH, causing the non-linear relationship between the reaction kinetics of DPPH and antioxidants, as DPPH

assay is a simple method based on electron-transfer that produces a violet solution in ethanol during analyses (Paixao, et al., 2007).
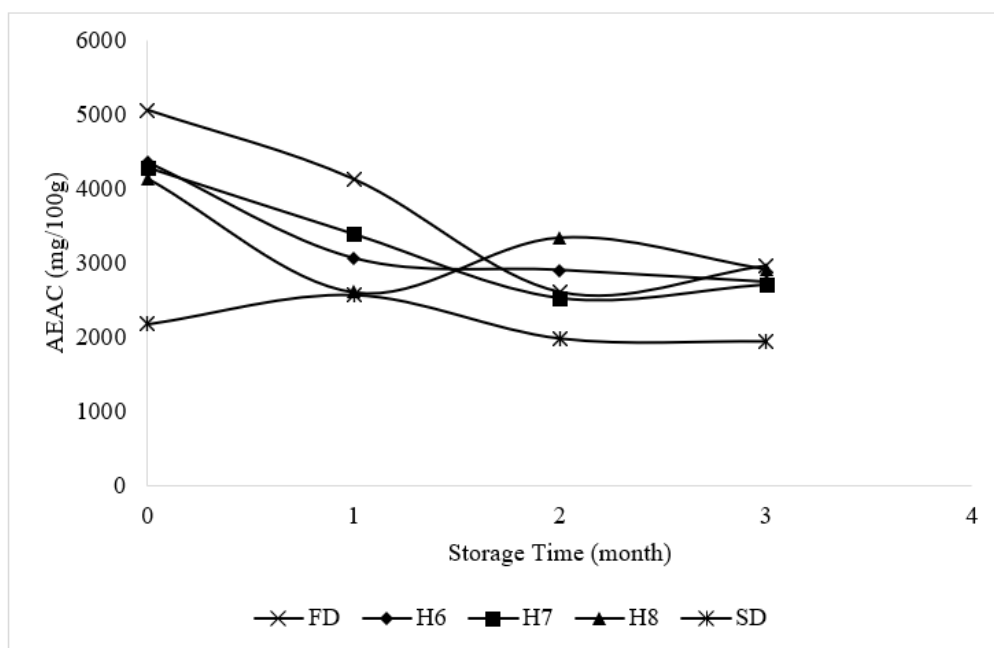
Figure 7-4: DPPH scavenging activities of dried papaya leaves from various drying processes during storage

The difference between ABTS and DPPH radical scavenging activities could be due to the low selectivity of the hydrogen donating reaction for DPPH assay, while the ABTS assay could react with any hydroxylated aromatic compound (Mareček, et al., 2017). Chen et al. (2020) reported that these factors could cause different evaluation results for similar antioxidants.

7.3.4   Colour of dried papaya leaves

Figure 7-5, Figure 7-6 and Figure 7-7 show the L^*, a^* and b^* values of dried papaya leaves, respectively, from various drying processes during storage

for 0, 1, 2 and 3 months. The drying processes included freeze drying (FD), hot air drying at 60°C, 70°C and 80°C (HD6, HD7 and HD8) and shade drying (SD).
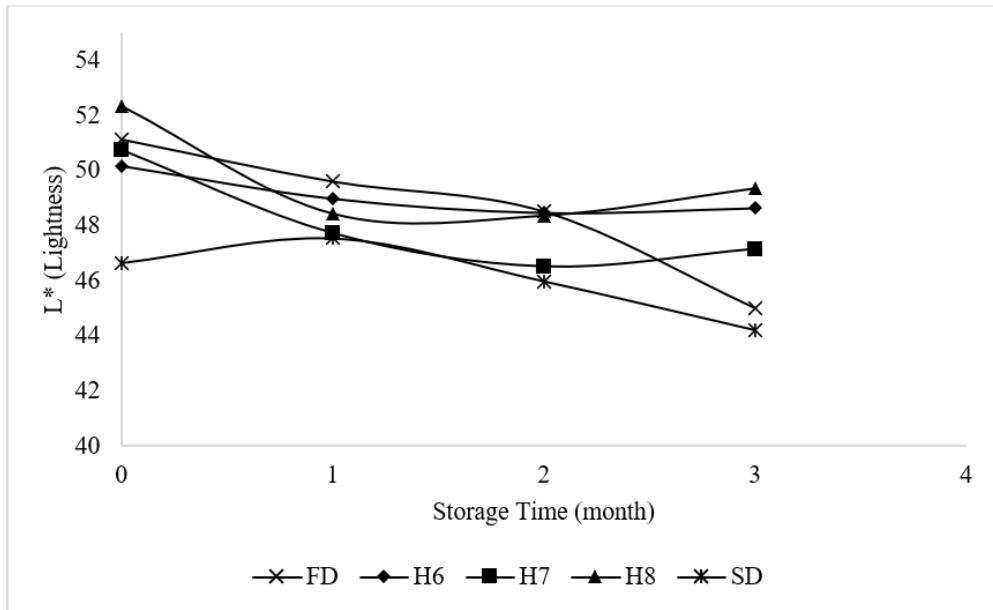
Figure 7-5: L^* values of dried papaya leaves from various drying processes during storage
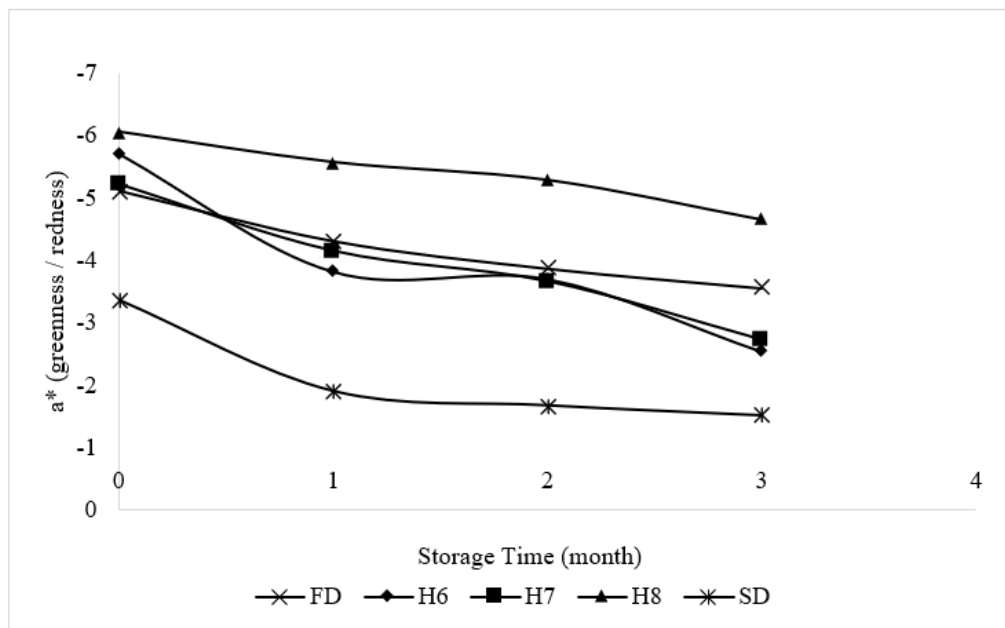
Figure 7-6: a^* values of dried papaya leaves from various drying processes during storage
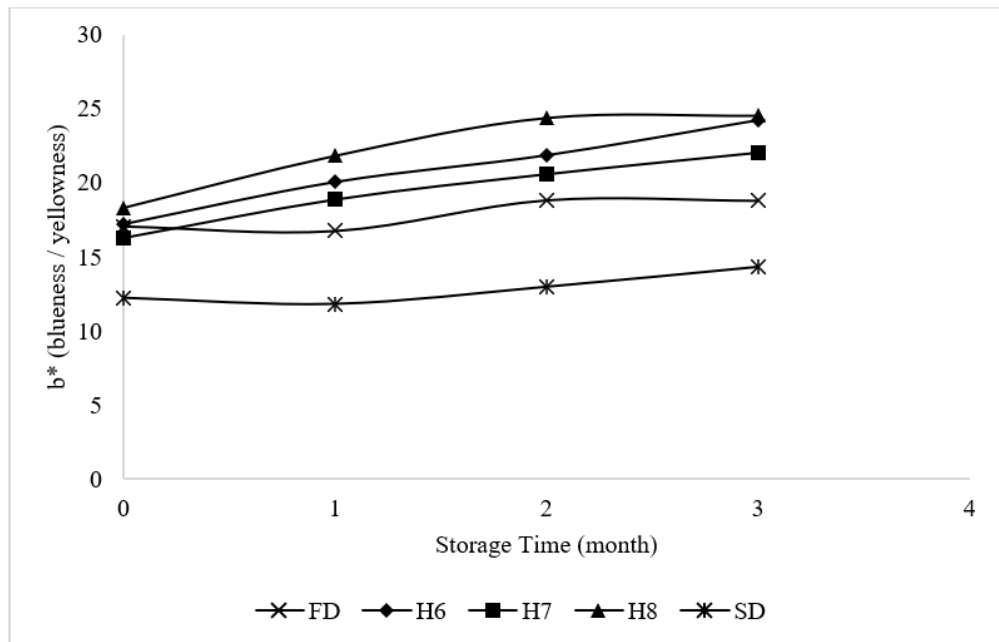
Figure 7-7: b^* values of dried papaya leaves from various drying processes during storage

There were decreasing trends observed in L^* values for all dried samples during the storage duration of three months. Changes in the L^* values (lightness) of dried leaves could be mainly due to chlorophyll degradation during storage (Ali, et al., 2014). Nyambaka and Ryley (2004) reported that colour analysis of agricultural products could be affected by many reactions during thermal processing, especially degradation of colour pigment. Visually, the colour of all dried leaves became darker, less greenish and more yellowish during storage which were consistent with the results (Figure 7-6 and Figure 7-7) where the a^* values (greenness) showed a decreasing trend, whereas the b^* values (yellowness) showed an increasing trend with storage time.

Figure 7-8 shows the ΔE^* values (total colour difference) of dried leaves after three months of storage. Differences in perceivable colour can be classified as shown in Table 3-1 (Chapter 3). Increasing trends of ΔE^* values were observed, indicating that the colour of dried leaves had changed with storage time, which could possibly be due to chlorophyll degradation in the dried leaves samples.
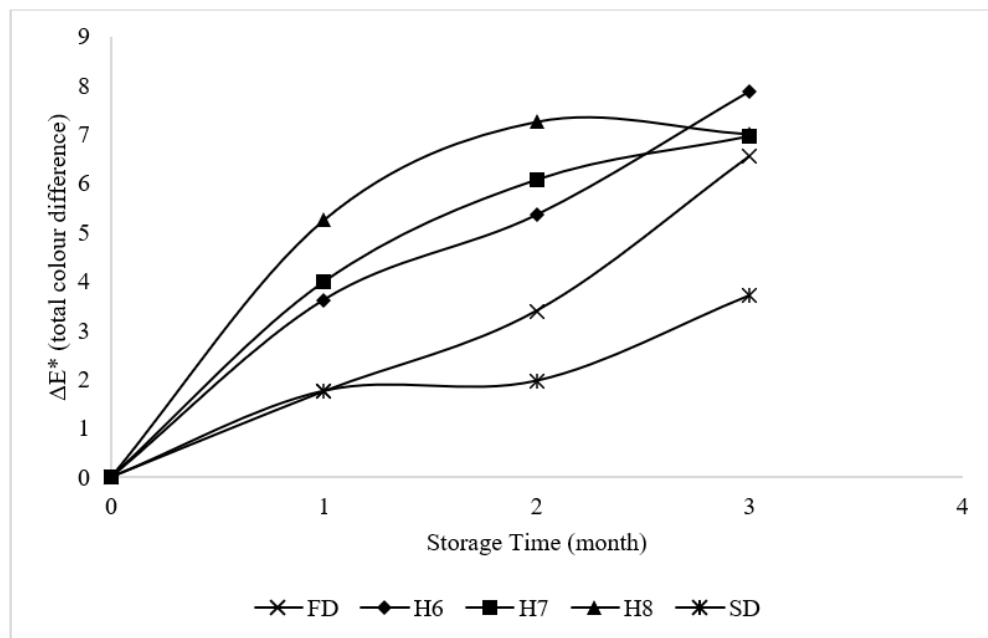
Figure 7-8: ΔE^* values of dried papaya leaves from various drying processes during storage

In the first month, the ΔE^* values of FD (1.75) and SD (1.76) samples were classified as noticeable difference, whereas the ΔE^* values of hot air dried samples (HD6, HD7 and HD8) were classified as appreciable difference (3.62, 4.13 and 5.26, respectively). In the second month, the ΔE^* values of SD samples remained in the category of noticeable difference (1.97), FD and HD6 samples were categorised as appreciable difference (3.40 and 5.36, respectively) and

HD7 and HD8 samples were categorised as large difference (6.24 and 7.27, respectively).

Finally, in the third month, the ΔE^* values of all dried samples were categorised as large difference except for SD samples which showed appreciable difference. Despite the lowest colour change in SD samples among all the dried samples, it might have already experienced the greatest colour change after drying (before storage) due to its long drying time that caused chlorophyll degradation (Rudra, et al., 2008; Ali, et al., 2014). Hence, FD samples are the preferred choice of sample to be selected for extended storage purpose as the samples showed the lowest total colour difference (ΔE^*).

7.4   Conclusion

The amount of carpaine decreased with storage time in all dried samples, and FD samples showed a greater potential for extended storage purpose for carpaine extraction. Weibull model showed the best fit in describing the degradation kinetics of carpaine in all dried samples (FD, HD6, HD7 and SD samples) except for HD8 samples, which could be best described by first order model. FD samples showed the highest half-life (51.20 months), followed by SD (3.79 months), HD6 (3.46 months), HD8 (2.13 months), and HD7 (1.66 months) samples. The TPC, ABTS and DPPH free radical scavenging activities of all dried samples gradually decreased with storage time. Besides, increasing trends of ΔE^* values were observed, indicating that the colour of dried leaves would change with the storage time, which could possibly be due to chlorophyll degradation. It can be concluded that the carpaine retention, antioxidant

properties and colour in dried samples would degrade with the storage time. Hence, it is recommended to select FD samples for extended storage purpose as it is more stable as indicated by the lowest rate constant and the highest half-life.

CHAPTER 8

CONCLUSIONS AND FUTURE WORKS

8.1 General Conclusions

The present studies investigated the effects of drying conditions on drying kinetics, product quality and retention of carpaine in papaya leaves. To date, literature reported on the processing aspects (e.g., preparation, drying and storage) of papaya leaves and the extracts are scarce. In this research, the extraction and quantification of carpaine from different parts of papaya leaves were carried out, and the drying kinetics of papaya leaves were investigated as well. Besides, the effects of different drying techniques on the retention of carpaine and the antioxidant properties of papaya leaves were also investigated. Finally, the effects of extended storage period on the retention of carpaine and antioxidant properties of dried papaya leaves were also studied. Findings from the current studies will contribute knowledge and information on industrial scaling up operations to process papaya leaves for the production of carpaine.

The studies have reported the following significant findings:

i. Pale yellow carpaine crystalline powders were successfully purified and confirmed by ^{1}H and ^{13}C NMR. Young leaves contained the highest amount of carpaine (333 µg/g), total polyphenol content (2174 mg GAE/100g DW) and DPPH scavenging activities (293 µg/mL) among all the samples (leaves and stalks). It is thus recommended to use young papaya leaves as a source of material to extract carpaine for future drug development in dengue treatment.

ii. The fastest drying rate was observed at high temperature (hot air drying at 80°C) and followed by lower temperature conditions (hot air drying at 60°C and 70°C, and shade drying). By using the Arrhenius equation, the effective diffusivities were determined in the range of 2.09×10^{-12} m^2/s to 2.18×10^{-12} m^2/s, and the minimum energy required to initiate moisture diffusion was determined at 2.11 kJ/mol in hot air drying within 60°C to 80°C.

iii. Freeze dried samples showed the highest retention of carpaine (333 µg/g), total polyphenol content (2158 mg GAE 100g/DW) and antioxidant properties (571 mg TE 100g/DW and 215 µg/mg for ABTS and DPPH scavenging activities, respectively) as compared to other dried samples obtained from hot air and shade drying.

iv. The retention of carpaine, total polyphenol content, ABTS and DPPH scavenging activities of all dried samples gradually decreased with storage time (3 months). The degradation kinetics of carpaine in FD samples can be best described by the Weibull model, which showed the highest R^2 (0.9979), and the lowest χ^2 (15.33), RMSE (2.77) and E (0.59%). Freeze dried samples also showed the greatest potential for extended storage purpose with the lowest degradation rate constant (0.0135 $\frac{1}{\text{month}}$) and the highest half-life (51.20 months).

8.2 Future Works

The studies have revealed that freeze drying is the best drying technique to retain the carpaine, total polyphenol content and antioxidant properties among

all the drying techniques (hot air drying and shade drying). However, freeze drying is expensive, energy-intensive and time-consuming. Hybrid drying techniques could be envisaged to optimise drying efficiency and energy consumption, e.g., field-based assisted freeze drying using microwave, infrared or ultrasound.

Besides, the effects of extended storage period on carpaine alone could be further studied. Such studies can be carried out by subjecting the pure carpaine sample under varying storage conditions. The studies can also be conducted for a longer period of storage (e.g., 3 years) to observe for possible product quality changes due to degradation.

Future studies can also be carried out to look into the cultivation aspects of papaya trees for carpaine extraction purpose. Variables such as soil nutrients, water uptake, weather conditions and age of trees could play vital roles in the production of carpaine during plant growth. In addition, planting materials (breed) could also have significant effect on the quantity of carpaine produced.

Finally, a pilot scale study for the production of carpaine can be conducted and with techno-economic analysis carried out using the recommended drying technique. This is to obtain more information in order to justify the commercialisation potential on a bigger production scale.

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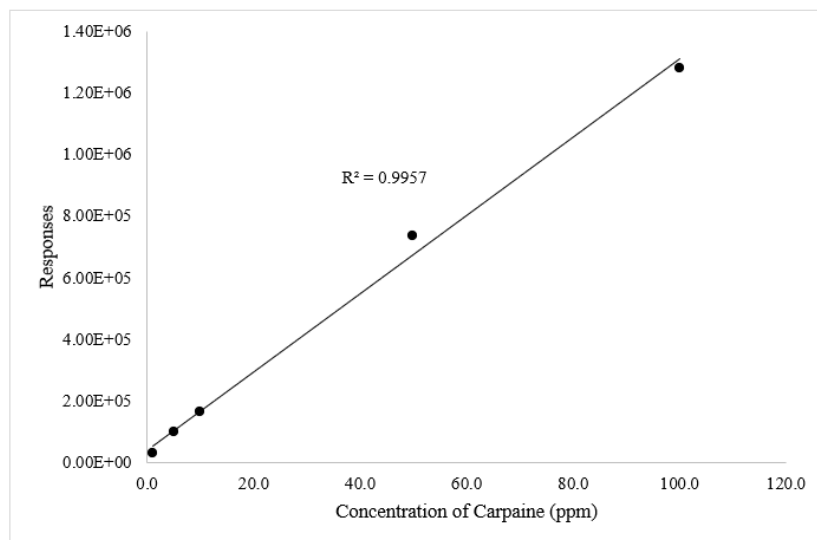
APPENDICES

APPENDIX A: Calibration curves

Figure A1: Calibration curve of carpaine solution (1, 5, 10, 50, 100 ppm in
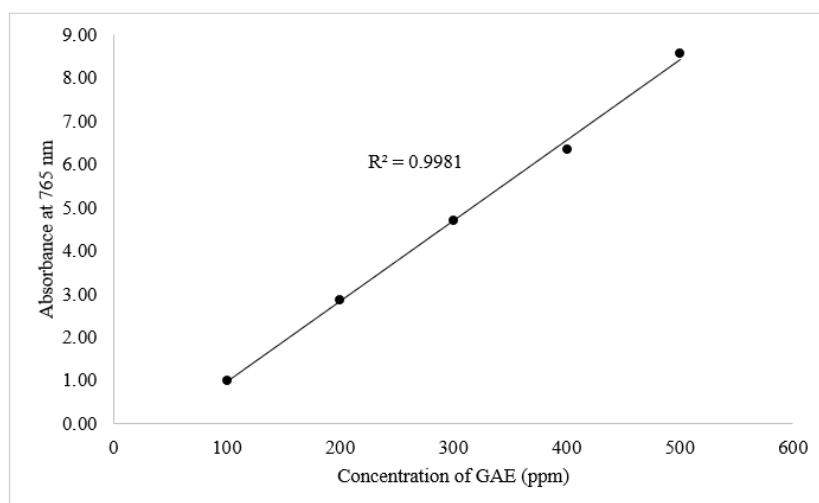
ethanol) using LC-MS

Figure A2: Calibration curve for total polyphenol content analysis in
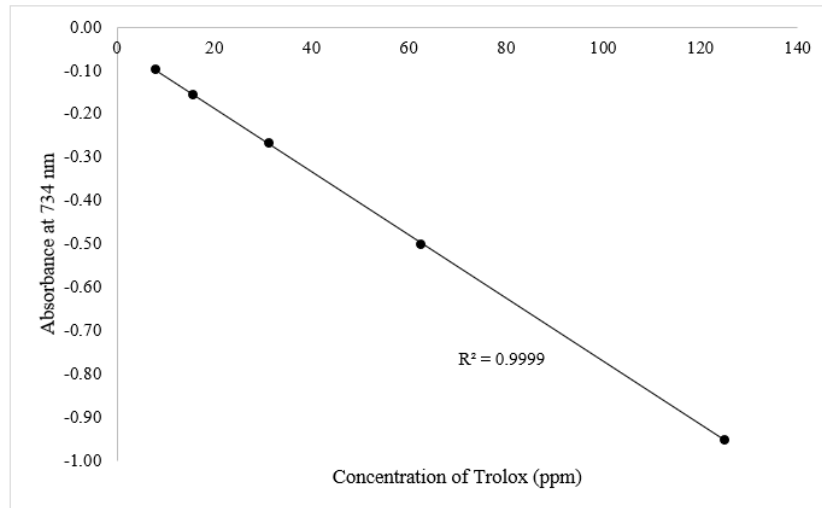
gallic acid equivalence (GAE)

Figure A3: Calibration curve for ABTS antioxidants assay in Trolox
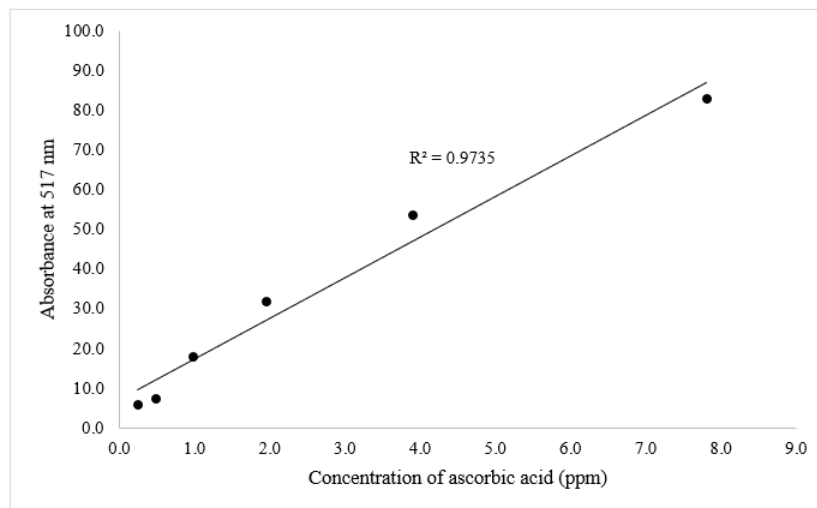
equivalence

Figure A4: Calibration curve for DPPH antioxidants assay in ascorbic

acid equivalence (AEAC)