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EFFECT OF GAMMA IRRADIATION ON TOTAL ANTIOXIDANT CONTENTS AND TOTAL ANTIOXIDANT ACTIVITIES OF *Musa paradisiaca formetypica* L. var. Nangka *EXTRACT POWDER*

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This project entitled “Effect of Gamma Irradiation on Total Antioxidant Contents and Total Antioxidant Activities of *Musa paradisiaca formetypica* L. var. Nangka Extract Powder” was prepared by Chan Yee Yin and submitted to the Faculty of Medicine and Health Sciences as a partial fulfillment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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LIST OF ABBREVIATIONS

GAE	Gallic acid equivalenet
CE	Catechin equivalent
µ	Micro
m	Milli
g	Gram
L	Litre
M	Molarity
k	Kilo
Gy	Gray
ROS	Reactive oxygen species
SD	Standard deviation
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	Diphenyl-1-picrylhydrazyl
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
$NaNO_2$	Sodium nitrite
$AlCl_3$	Aluminium chloride
FRAP	Ferric Reducing Antioxidant Potential
ANOVA	Analysis of variance
r	Correlation coefficient

ABSTRACT

EFFECT OF GAMMA IRRADIATION ON TOTAL ANTIOXIDANT CONTENTS AND TOTAL ANTIOXIDANT ACTIVITIES OF *Musa paradisiaca formetypica* L. var. Nangka EXTRACT POWDER

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Banana with local name as *pisang* and botanical name as *Musa* (L.) spp is a popular climacteric fruit in Malaysia which is rich in antioxidant that can help to prevent diverse health problem and provide health benefits. Food irradiation is a safe food technology that has been recognized worldwide in which it involves the exposure of food to the radiation sources in order to preserve food. Present study was aimed to determine the effect of gamma irradiation on total antioxidant contents and total antioxidant activities of *Musa paradisiaca formetypica* L. var. Nangka extract powder. The antioxidant ability of the extract was determined by using total phenolic content (TPC), total flavonoid content (TFC), Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant potential (FRAP) assay. Results showed that there was no significant different ($p > 0.05$) of total phenolic content between non-irradiated and all irradiated extracts but it showed slightly increase of TPC in lower doses of irradiation. Irradiated sample extracts at dose 6 and 9 kGy showed significant decrease ($p < 0.05$) in TFC. There was no significant different ($p > 0.05$) in DPPH between non-irradiated extract and all irradiated extracts but it showed slightly decrease of EC_{50} values in irradiated extracts. There was no significant different ($p > 0.05$) of FRAP value between non-irradiated and all irradiated extracts but it showed slightly increase in FRAP values at lower dose of irradiation. This study manage to show the application of gamma irradiation by slightly increasing most of the antioxidant contents and activities of ethanol extracts of pisang nangka particularly at lower dose. Gamma irradiation has the potential to be one of the approaches to preserve the fruits and at the same time to maintain or increase antioxidant properties of the fruits.

ABSTRAK

KESAN IRADIASI GAMMA TERHADAP JUMLAH KANDUNGAN ANTIOKSIDAN DAN JUMLAH AKTIVITI ANTIOKSIDAN SERBUK EKSTRAK *Musa paradisiaca formetypica* L. var. Nangka

Chan Yee Yin

Pisang dengan nama lokal sebagai pisang dan nama botani sebagai *Musa* (L.) spp adalah buah klimaks yang popular di Malaysia yang kaya dengan antioksidan yang dapat membantu mencegah pelbagai masalah kesihatan dan memberikan manfaat kesihatan. Iradiasi makanan adalah teknologi makanan yang selamat yang telah diakui di seluruh dunia di mana ia melibatkan pendedahan makanan kepada sumber radiasi untuk mengawet makanan. Kajian ini dijalankan bertujuan untuk menentukan kesan iradiasi gamma terhadap jumlah kandungan antioksidan dan jumlah aktiviti antioksidan serbuk ekstrak *Musa paradisiaca formetypica* L. var. Nangka. Keupayaan antioksidan ekstrak ditentukan dengan penentuan jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), Diphenyl-1-Picrylhydrazyl (DPPH) uji pemulih radikal bebas dan pengujian potensi antioksidan ferrik (FRAP). Hasil kajian menunjukkan bahawa tidak ada perbezaan yang signifikan ($p > 0.05$) dari jumlah kandungan fenolik antara ekstrak yang tidak disinari dengan semua esktrak yang disinari tetapi menunjukkan sedikit peningkatan TPC pada dos iradiasi yang lebih rendah. Ekstrak sampel yang diiradiasi pada dos 6 dan 9 kGy menunjukkan penurunan yang signifikan ($p < 0.05$) dalam TFC. Ketiadaan perbezaan yang signifikan ($p > 0.05$) dalam DPPH antara ekstrak yang tidak disinari dan semua ekstrak yang disinari tetapi menunjukkan sedikit penurunan nilai EC_{50} dalam ekstrak yang disinari. Ketiadaan perbezaan yang signifikan ($p > 0.05$) dalam nilai FRAP antara ekstrak yang tidak disinari dan semua ekstrak yang disinari tetapi menunjukkan sedikit peningkatan nilai FRAP pada dos penyinaran yang lebih rendah. Kajian ini berjaya menunjukkan aplikasi iradiasi gamma dengan sedikit meningkatkan sebahagian besar kandungan antioksidan dan aktiviti ekstrak etanol pisang nangka terutama pada dos yang lebih rendah. Iradiasi gamma berpotensi menjadi salah satu pendekatan untuk mengawetkan buah dan pada masa yang sama untuk mengekalkan atau meningkatkan sifat antioksidan buah.

CHAPTER 1

INTRODUCTION

1.1 Background

Banana with local name as *pisang* and botanical name as *Musa* (L.) spp was originated in Southeast Asia including northern India, Cambodia, Sumatra, Java, Philippines and Taiwan. Banana is a climacteric fruit which has been located in approximately 120 to 130 countries especially in tropical and subtropical regions. There was around 104 million tons of annual world production of banana and was mainly from Brazil, China, Ecuador, Philippines and India, while the leading exporters were Ecuador, Colombia, Costa Rica and Philippines (Agama-acevedo et al., 2011). In Malaysia, banana is a very popular local fruit and is considered as one of the most consumed fruits in Malaysia in which Malaysians consumed 10.0 kilograms per person in 2017 (Siti and Ahamad, 2019).

Carbohydrate content in banana is mainly from glucose (3.5% of edible portion) and fructose (5.7% of edible portion). Starch is mainly present in unripe banana and the level decreases in ripe banana (Elayabalan et al, 2017). Banana is starchy fruit which provides fiber from both cell wall and resistant starch stored within the cells. Health benefits associated with fruit fiber are preventing acute or chronic colonic gastrointestinal health problems such as constipation, irritable bowel syndrome, diverticular disease as well as help in weight control (Dreher, 2018). Moreover, banana contains high amount of potassium which is important to control blood pressure (Singh et al., 2016). Diet rich in potassium can help to lower the blood pressure of patients as well as provide cardio protective effects such as reducing the risks of cardiovascular disease and cardiac arrhythmias (Houston, 2011).

Antioxidants are substances that can reduce the free radical's damage by releasing an electron and neutralize the radical (Halliwell, 1995). Previous studies showed that antioxidants provided a lot of health benefits such as disease prevention, reduce neurodegenerative diseases in elderly, maintain physiological functions of organs, prevent cardiovascular disease and cancer, delay aging and reduce chronic diseases development (Igor et al., 2017; Huang, 2018; Wilson et al., 2017). Banana is rich in antioxidant which can help to prevent diverse health problem and provide health benefits because they can trap free radicals that damage the body cells and cause cellular aging (Sidhu and Zafar, 2018). Studies showed that the antioxidant activity of banana is considered high as it contains high amount of phenolic compounds, flavonols and carotenoids which provide many health benefits. Other compounds such as vitamin C, vitamin E and beta

carotene also help to enhance the antioxidant potential (Singh et al., 2016). The antioxidant contents such as total phenolic content (TPC) and total flavonoid content (TFC) were found higher in the banana peel than in the pulp as well as higher in green banana than in ripe banana (Fatemeh, 2012).

Irradiation is considered as one of the non-thermal techniques used to preserve food in which it does not include direct heat which can act as alternative methods for conventional thermal food processing as thermal food processing can cause the loss of biological, physical and chemical functionality of food (Umaraw et al., 2015; Jan et al., 2018; Enrique and Ivan, 2014). Food irradiation is a safe food technology that has been recognized worldwide in which it involves the exposure of food to the radiation sources in order to preserve food. The sources of radiation are gamma rays, x-rays and electron beams whereas gamma rays are widely used in the food irradiation (Lacroix and Follett, 2015; Ehlermann, 2016; Kumar et al., 2015). Gamma irradiation was found to reduce and inactivate the microorganisms in food to prevent food poisoning (Sezer et al., 2019). Previous studies also showed that shelf life of mangoes can be delayed up to 14 days by gamma irradiation at dose 1kGy by delaying the ripening process without causing harm to the fruits (Iqtedar et al., 2016). In addition, the use of gamma irradiation on banana can be considered as safe and alternative method to control the fungal and pathogen contamination. Several studies found that the use of gamma irradiation can increase shelf life, delay ripening and delay peel colour change of banana (Beatriz, Gloria and Ad, 2013; Hassan, 2007).

According to International Atomic Energy Agency (2002), the different ranges of doses of irradiation are applicable for different purposes to produce various effects. The ranges of doses for common food irradiation can be categorized as low dose levels (10Gy to 1kGy), which is commonly used for delaying sprouting and ripening of fruits and vegetables, insect disinfestations and inactivation of some pathogenic parasites; medium dose levels (1kGy to 10kGy) are used to eliminate microorganisms and extend shelf life of food; and high dose levels (10kGy to 100kGy can be used to reduce total microbial load present in dried food and used for food sterilization. Collectively, this research aims to study the effect of gamma irradiation on total antioxidant contents and total antioxidant activities of banana.

1.2 Problem Statement

Foodborne illness is defined as any illness caused by the consumption of contaminated food which has been contaminated by microorganisms such as bacteria, viruses or parasites (Adley and Ryan, 2016). According to World Health Organization (2015), there were almost 1 in 10 people in the world get ill after ingesting contaminated food and 420,000 deaths each year especially among children less than 5 years old which resulting in the loss of 33 million healthy life years (DALYs). Foodborne illnesses are usually caused by bacteria, viruses, parasites or chemical substances through the ingestion of contaminated food. These pathogens can cause severe diarrhea, infections, disability and death. The most common foodborne pathogens are *Salmonella, Campylobacter* and *Escherichia coli,* while *E. coli* are associated with fresh fruits and vegetables. Foodborne disease is very common in Malaysia where the cases of foodborne

outbreaks are considered high in Malaysia, this is probably due to the temperature and humidity in Malaysia which is suitable for most of the pathogens to grow (Abdul-Mutalib et al., 2015).

Previous studies indicated that foodborne illness linked to fresh products like fruits and vegetables. Previous Centers for Disease Control and Prevention (CDC) outbreak investigations showed that microbial hazards associated with fruits and vegetables including *E.coli, Salmonella, Norovirus* and *Listeria monocytogenes*. The contamination may be occurred through agricultural and processing water, soil amendments, workers' hygiene and facility sanitation (Johnson, 2019). One study in Brazil found that foodborne outbreaks were associated with fruits and vegetables. Between years 2008 to 2014, there were a total number of 30 cases of foodborne outbreaks related to fruits and vegetables which was about 0.6 per cent of the total notified outbreaks (Elias, 2018). These conditions can lead to postharvest losses caused by the pathogens. There were approximately 25% of fruits and vegetables were spoilt by the postharvest disease (Jeong et al., 2015).

In addition, Nelson (2008) reported that crown rot and anthracnose were the two primary postharvest diseases of banana. These diseases commonly occurred on ripening fruits either at the moment of sale or after purchase. Crown rot is a postharvest disease which is mainly caused by fungus *Fusarium* spp. can cause huge negative impact on banana fruit quality. Crown rot

postharvest disease can cause significant losses in banana fruits. The infection is normally occurred at harvest period however the symptoms only appear during transportation, shipping and storage time (Kamel, Cortesi and Saracchi, 2016). Crown rot starts with a mycelium development on the crown surface. After that, it starts to infect the internal development. The whole fruit can be affected by the internal development, causing softening and blackening of the fruit tissue (Lassois et al., 2010). Crown rot has influenced export banana in all the countries which involve the production of banana and it is one of the main postharvest diseases in banana (Krauss and Johanson, 2000).

Besides that, Anthracnose disease caused by fungus *Colletotrichum musae* was the most critical postharvest disease of banana which had caused great losses of marketable fruits (Ranasinghe et al., 2005). Ripe banana can be infected by this disease and can occur at any time during the growing season which leads to great losses in the market. (Simmonds and Mitchell, 1940). Infection of banana by this fungal pathogen has resulted in severe post-harvest losses (Abd-elsalam et al, 2010). Long transport distance and storage time might increase the risk of getting anthracnose disease in banana (Thompson and Burden, 1995). Malaysia has been a net exporter of banana and the export has been increasing over the years (Siti and Ahmad, 2019).The bananas are mainly exported to Middle East, Singapore, Hong Kong and Brunei (Hassan, 2004). Previous study showed that gamma irradiation can be used in the control of postharvest disease in mango fruits by reducing the severity of the pathogen exists in the ripening process of fruits

(Maria et al., 2015). Irradiation is also a nonchemical method to eliminate the postharvest microorganisms in which no residues will be left on the fruits or vegetables (Barkai-Golan and Follett, 2017). Hence, irradiation can be applied to eliminate postharvest microorganisms at the same time it can prevent foodborne illness.

Furthermore, conventional thermal food processing technologies such as pasteurization (in the range from 70 ºC to 80 ºC), sterilization (in the range from 110 ºC to 120 ºC) and ultra-high temperature treatment (in the range from 140 ºC to 160 ºC) bring detrimental effects on many heat sensitive nutrients including vitamins, minerals and nutrients having functional properties such as pigments, antioxidant and bioactive compounds (Boekel et al., 2010). Unpleasant biochemical and nutritious changes that can affect the sensory characteristics of food products can be caused by the applied heat from thermal processing (Enrique and Ivan, 2014). Other than loss of certain nutrients, thermal processing can also cause formation of toxic compound and bring negative effects on flavor, texture and colour of food products (Boekel et al., 2010). Irradiation is one of the non-thermal techniques which does not include direct heat has emerged as the alternative method of conventional food preservation techniques due to the increased need and interest of consumer in term of taste, nutritious, natural and easy-to-handle food products (Ajlouni et al., 2006).

Moreover, public acceptance on food irradiation is still low in some of the countries. The low acceptance of food irradiation is mainly due to misconceptions and irrational fear towards the technologies which involve nuclear principle. In Malaysia, most of the industrial respondents are aware of the food irradiation. However, their level of knowledge about food irradiation is poor (International Atomic Energy Agency, 2001). The factors which contributed to the lack of interest by the food industry to utilize this technology include no demand for the benefit provided due to unawareness of the technology, competitiveness with alternative processes, uncertainty in consumer acceptance and the current restricted market for irradiated products. The findings from consumer attitude on food irradiation survey indicated low acceptance due to very limited knowledge and misunderstanding of the technology (Othman, 2001).

There was limited study related to irradiation of banana in Malaysia and its effect on antioxidant levels are important components to increase the shelf life and quality of the fruits. Hence, this study is aimed to determine the total antioxidant contents and total antioxidants activities of irradiated and non-irradiated banana. The samples will be treated with different dose of gamma irradiation such as 3, 6 and 9 kGy for further use in this study.

1.3 Significance of Study

Banana is a very popular fruit in Malaysia where it is frequently served at home or restaurant. In Malaysia, banana has been incorporated into wide variety of food such as banana fritters, banana smoothies and banana sundae. From this study, irradiation will be applied on banana and this may help to reduce the incidence of food borne illness because gamma irradiation can eliminate the potential pathogen in banana. Also, irradiation on banana may help to preserve the fruit and improve the quality of banana by overcoming the postharvest disease. Hence, the local consumption and the export of banana to other countries can be increased because irradiation can preserve the banana during the period of packaging, transportation and storage as well as extend the shelf life and improve quality of banana. Irradiation is also a nonchemical method to eliminate the postharvest microorganisms in which no residues will be left on the fruits or vegetables (Barkai-Golan and Follett, 2017). Therefore, irradiation can be applied to eliminate postharvest microorganisms at the same time it can prevent foodborne illness.

Furthermore, irradiation is one of the non-thermal techniques which does not include direct heat can be emerged as the alternative method of conventional food preservation techniques due to the increased need and interest of consumer in term of taste, nutritious, natural and easy-to-handle food products (Ajlouni et al., 2006). Conventional thermal food processing technologies can bring detrimental effects on many heat sensitive nutrients including vitamins,

minerals and nutrients having functional properties such as pigments, antioxidant and bioactive compounds (Boekel et al., 2010). Unpleasant biochemical and nutritious changes that can affect the sensory characteristics of food products can be caused by the applied heat from thermal processing (Enrique and Ivan, 2014). Other than loss of certain nutrients, thermal processing can also cause formation of toxic compound and bring negative effects on flavor, texture and colour of food products (Boekel et al., 2010).

Moreover, the public can gain more knowledge and information about food irradiation as well as the use of food irradiation technology in the food industry sectors can be enhanced. This is because public acceptance on food irradiation is still low in some of the countries. The low acceptance of food irradiation is mainly due to misconceptions and irrational fear towards the technologies which involve nuclear principle. The findings from consumer attitude on food irradiation survey indicated low acceptance due to very limited knowledge and misunderstanding of the technology (Othman, 2001). Therefore, the public will gain extra information about antioxidant contents and antioxidant activities of irradiated and non-irradiated banana from this study. Besides, the public can gain knowledge on the benefits of antioxidant in preventing chronic disease and maintain health. This study can also enhance the commercial value of banana and stimulate more intensive research on banana in future. Furthermore, this study can be a baseline study on the effect of gamma irradiation on total antioxidant contents and total antioxidant activities of banana which can provide a reference for the future research.

1.4 Objectives of Study

1.4.1 General Objective

To determine the effect of gamma irradiation on total antioxidant contents and total antioxidant activities of *Musa paradisiaca formetypica* L. var. Nangka extract powder.

1.4.2 Specific Objectives

1. To determine and compare the total antioxidant contents (total phenolic content and total flavonoid content) of ethanol extraction of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3kGy, 6kGy and 9kGy by using Folin-Ciocalteu and aluminium chloride colorimetric methods.

2. To determine and compare the total antioxidant activities of ethanol extraction of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3kGy, 6kGy and 9 kGy by using DPPH and FRAP assays.

3. To determine the correlation between total antioxidant contents and total antioxidant activities of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3kGy, 6kGy and 9kGy.

1.5 Alternative Hypothesis

1. There is significant difference in total antioxidant contents (total phenolic content and total flavonoid content) between ethanol extraction of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3kGy, 6kGy and 9kGy.

2. There is significant difference in total antioxidant activities between ethanol extraction of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3kGy, 6kGy and 9 kGy.

3. There is significant difference in correlation between total antioxidant contents and total antioxidant activities of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3kGy, 6kGy and 9kGy.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Irradiation

Food irradiation is a technology that can be used to improve food safety by removing the insects and microorganisms to extend the shelf life of food without affecting the nutritional value especially for fresh or frozen products (Maherani et al., 2016). Ehlermann (2016) concluded that food irradiation is a safe food technology which has been widely recognized by national and international expert bodies in which the irradiated food will not bring any risk but can help to maintain the nutritive and other positive properties. Food irradiation involves the treatment of food by exposing the food to radiation sources such as gamma rays, x-rays and electron beams to eliminate the bacteria that can cause damage to food (Lacroix and Follett, 2015). In Malaysia, food irradiation has been recognized as an alternative technology for improving safety and quality of food as well as overcoming barriers in food trade (Othman, 2001).

According to Malaysia Nuclear Agency (2019), food irradiation is the use of controlled dose of ionizing radiation to treat the food items for a specific time to reach a certain objective. Radiation processing of different varieties of products had begun developing in January 1989 at the SINAGAMA Irradiation Plant uses ionizing energy in the form of gamma radiation from Cobalt-60 source. SINAGAMA is the irradiation plant in which it is the first government facility to certify with ISO 9001 since 1991. Commercial irradiation in Malaysia began in 1997 at the Cobalt 60 irradiation facility (SINAGAMA) of the Malaysian Institute for Nuclear Technology Research. Approximately 70 to 80 tons of food had been processed by this plant annually since 2006. A sum of 785 tons of herbs and spices including curry powder, coriander and pepper were irradiated in year 2010. In Malaysia, irradiation of papaya, rambutan, starfruit (carambola), pineapple and jakfruit is a compulsory requirement for US importation. Examples of food and products in Malaysia which contain food irradiation components include herbs, spices, tea, grains and frozen food (Loh, 2016).

2.1.1 Thermal and Non-thermal Technique of Food Preservation

Food preservation can be defined as the physical, chemical or biological methods that used to destroy, prevent or inhibit the natural worsening processes in food as well as to increase the shelf life as edible and safe products. Food preservation methods can be classified into thermal and non-thermal methods (Corradini and Empresa, 2010). Thermal processes are conventional food preservation methods that have been widely used. Thermal processes can be categorized based on the intensity of heat treatment such as pasteurization (70 ºC to 80 ºC),

sterilization (110 ºC to 120 ºC) and ultra-high temperature treatment (140 ºC to 160 ºC). There are some advantages of thermal processing which are making the food-borne microorganisms become inactvie, shelf life extension and enhance bioavailability of nutrients. However, thermal processing can bring some unpleasant effects such as loss of certain nutrients, deposition of toxic compound and bring negative effects on flavor, texture and colour (Boekel et al., 2010).

Conventional thermal food processing technologies bring detrimental effects on many heat sensitive nutrients including vitamins, minerals and nutrients having functional properties such as pigments, antioxidant and bioactive compounds. Alternative methods for thermal processing of food are needed to prevent the loss of biological, physical and chemical functionality of food (Jan et al., 2018). Unpleasant biochemical and nutritious changes that can affect the sensory characteristics of food products can be caused by the applied heat from thermal processing. Non-thermal food preservation methods which do not include direct heat can preserve food by inactivating the microbial same as in the conventional thermal food processing. These alternative technologies produce food safe for consumption and will not affect nutritional and sensory attributes of food as much as in thermal processes (Enrique and Ivan, 2014). Thus, new non-thermal preservation technologies have emerged as the demand to replace conventional thermal processes in order to deal with the lethality of conventional methods (Corradini and Empresa, 2010).

Non-thermal technique is now the most investigated new food preservation technology which emerges as the replacement of conventional food preservation techniques due to the increased need of consumer in term of taste, nutrients, natural and easy-to-handle food products (Ajlouni et al., 2006). Irradiation is one of the non-thermal techniques used to preserve food. Other non-thermal techniques are pulse electric fields, pulse light, oscillating magnetic fields, high pressure processing and high power ultrasound waves. The advantages of non-thermal technique are energy efficient, environmental friendly, preventing harmful microorganisms and can be generated using very inexpensive machine (Umaraw et al., 2015). Non-thermal technologies produce food products that are undergoing less processing, premium quality, convenient and safe besides extend shelf life without the use of preservatives or additives, while still maintaining the colour, flavor, texture, nutrients and functional qualities (Jan et al., 2018).

2.1.2 Types of Radiation

Food irradiation involves the process of food products expose to the ionizing radiation or non-ionizing radiation for the purpose of preserving the food. High energy electron, X-rays or gamma rays (from Cobalt-60 or Cesium-137) are the sources of ionizing radiation while electromagnetic radiation such as ultraviolet rays, visible light, microwave and infrared rays are the sources of non-ionizing radiation (Alothman and Karim, 2009). Ionizing radiation has high energy power in which it has enough energy to change atoms by striking an electron to form an ion. However, it is not powerful enough to affect the atoms and make the exposed items to become radioactive. The wavelength of ionizing radiation is 2000 Aº or less. Whereas for non-

ionizing radiation, it does not have enough energy to create ions, instead it makes the molecules become excited without removing electrons. Molecules can be made to move by the non-ionizing radiation but the atom in the molecules cannot be structurally changed (Kumar et al., 2015).

The use of ionizing radiation to preserve food began in the early 1920s (Kim and Vanee, 2004). United States Food and Drug Administration (FDA) approved the use of sources from ionizing radiation such as X-rays up to 5 meV, electron beams under 10 meV, and gamma rays from the natural decay of cobalt-60 or cesium-137. X –rays are formed by making reflection on high-energy stream of electrons off a target substance into food, while electron beam is a stream of high-energy electrons moved from an electron accelerator into food. Gamma rays are released from radioactive forms of the element Cobalt-60 or the element Cesium137 (FDA, 2016). However, the use of electron beam and Cesium137 are not encouraged due to the isotope is highly soluble in water (Lung et al., 2015). Both X-ray and electron beam are machine-generated and dependent on constant electricity supply. Gamma irradiation is always the considerate method of food irradiation because of its ray emits continuously at the predictable rate (Hallman, 2017).

2.1.3 Safety of Food Irradiation

In recent years, food irradiation has emerged as a safe food processing technique and many of the countries have started to adopt it. Recent studies showed that consumption of irradiated food does not bring any health risk (Ravindran and Jaiswal, 2019). International Atomic Energy

Agency (IAEA), World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations have made a conclusion that enhanced toxicological, microbiological or nutritional hazard in the food irradiation process would not more than those produced by the conventional food processing technique (Kim and Vaneee, 2004).

Furthermore, the safety of irradiated food has been evaluated by Food and Drug Administration (FDA), World Health Organization (WHO), Centers for Diseases Control and Prevention (CDC) and U.S. Department of Agriculture (USDA). The sale of irradiated food in Malaysia had been approved by Ministry of Health (MOH) under the Food Irradiation Regulations and was enforced in 2013. Many people concerned about whether irradiation can make the food become radioactive. Regardless of how high the radiation dose used, the irradiated food would not become radioactive because the radiation energy is not powerful enough to affect the nucleus of atoms within the food. All of the radiation energy absorbed will be immediately converted to a relatively small quantity of heat and nothing will be remained in the food, hence the food will not become radioactive (Eustice, 2014). In addition, Ehlermann (2016) also concluded that any food irradiated at any high dose is safe to consume, as long as it remains palatable. This conclusion has also been approved by World Health Organization (WHO) and international expert committees.

2.1.4 Gamma Irradiation and Its Effect on Food

Gamma rays are widely used in the food irradiation by using Cobalt-60 and Cesium-137. Gamma ray can penetrate food container and eliminate the growing microorganisms without causing radioactive. Additionally, these nucleosides are cheap source of radiation. Cobalt-60 is the most common source of food irradiation. The half life of Cobalt-60 is 5.27 years while the half life of Cesium-137 is 30 years (Kumar et al., 2015).

Gamma irradiation on food can help to preserve food and sterile certain foodstuffs to ensure food security due to the high energy radiation able to eliminate the microorganisms and hence keep the quality and freshness of food (Muhammad and Hesih, 2017). Furthermore, gamma irradiation can increase the shelf life of chiffon cake up to 75 days by using dose of radiation at 4kGy (Sirisoontaralak et al., 2017). The finding of microbiological analysis from one study showed that gamma irradiation is effective to reduce and inactivate the number of microorganisms in the sample as well as to prevent food poisoning (Sezer et al., 2019). Gamma irradiation is effective to eliminate the food borne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp and *Listeria monocytogenes* from food products (Sommers et al., 2004). The irradiation process destroys bacteria by ionizing the bacteria within the food products. When the DNA molecule of bacteria is ionized, then the bacteria will be destroyed. Thus the bacteria cannot reproduce anymore (Eustice, 2014).

Additionally, previous study showed that gamma irradiation can help to improve the bacterial hygiene in Dakgalbi, a chicken based food in South Korea (Min et al., 2012). The shelf life of mango can be increased up to 14 days by gamma irradiation dose at 1kGy by delaying ripening process without harming fruit. Also, dose at 1kGy was effective in controlling the microbial load on mango and thus improving the quality of mango (Iqtedar et al., 2016). Another study showed that the fruits treated with 0.40kGy gamma rays had significantly reduced ripening per cent as well as increased maximum average days to ripening and shelf life of fruits (Yadav et al., 2017). Moreover, gamma irradiation was proved to be a safe approach to improve the nutritional properties and functional properties of canola seeds and its protein (Anwar, Ali and Nasr, 2015). Irradiation up to 10kGy is safe to be used to extend shelf life of common beans without decrease in nutrient contents. Other than that, it can reduce anti nutrient content, reduce cooking time and improve protein digestibility (Carvalho et al., 2019).

Several studies reported that irradiation will not significantly affect the nutritional value of macronutrients such as carbohydrates, proteins and fats. Some micronutrients like vitamins can be decreased by irradiation, but other food processing method can also similarly reduce the same micronutrients (Eustice, 2014). According to Taipina et al. (2011), nutrients such as macronutrient, essential amino acids, essential fatty acids, minerals, trace elements and most vitamins will not experience great loss during the food irradiation process.

2.1.5 Doses of Irradiation

Dose of radiation is usually expressed in standard international unit which is Grays (Gy) and it is equivalent to 1 Joule of absorbed energy per 1 kg of sample (Food Irradiation Regulations, 2011). According to International Atomic Energy Agency (2002), the different ranges of doses of radiation are applicable for different conditions to produce various effects. The ranges of doses for common food irradiation can be categorized as low dose levels (10Gy to 1kGy), medium dose levels (1kGy to 10kGy) and high dose levels (10kGy to 100kGy). Low dose levels of irradiation are commonly used for delaying sprouting and ripening of fruits and vegetables, insect disinfestations and inactivation of some pathogenic parasites. Medium dose levels are used to eliminate microorganisms and extend shelf life of food. High dose levels can be used to reduce total microbial load present in dried food and used for food sterilization.

Based on Food Safety and Quality Division of Ministry of Health Malaysia, there are recommended doses of irradiation allowed for 9 classes of food. The foods in class 1 consist of bulbs, roots and tubers. The recommended minimum dose of irradiation for class 1 is 0.05kGy and maximum dose is 0.2kGy and the purpose of treatment is to inhibit sprouting during storage. Class 2 consists of fresh fruits and vegetables. To delay ripening, the minimum dose is 0.2kGy and maximum dose is 1.0kGy; to extend shelf life, the minimum dose is 1.0kGy and maximum dose is 2.5kGy; for quarantine control, the minimum dose is 0.15kGy and maximum dose is 1.0kGy. Next, class 3 consists of cereals and their milled products, nuts, oil seeds, pulses and dried fruits. For insect disinfestations, the minimum dose is 025kGy and maximum dose is

1.0kGy; for reduction of microbial load, the minimum dose is 1.5kGy and the maximum dose is 5.0kGy; for sprouting inhibition, the minimum dose is 0.1kGy and maximum dose is 0.25kGy (Food Irradiation Regulations, 2011).

The foods in class 4 are fish, seafood and their products. To reduce pathogenic microorganisms, the ranges of dose are from 1.0kGy to 7.0kGy; to extend shelf life, the ranges are from 1.0kGy to 3.0kGy; to control infection by parasites, the ranges are from 0.1kGy to 2.0kGy; while for insect disinfections, the ranges of dose are from 0.3kGy to 1.0kGy. The foods in class 5 are poultry, meat and their products. The recommended ranges of dose of irradiation for class 5 are similar to class 4. In class 6, there are dry vegetables, spices, condiments, dry herbs and tea. To reduce pathogenic microorganisms, the minimum dose is 2.0kGy and the maximum dose is 10kGy. For insect disinfestations, the ranges are from 0.3kGy to 1.0kGy (Food Irradiation Regulations, 2011).

Furthermore, the foods in class 7 are coco and their products. The ranges of dose from 2.0kGy to 5.0kGy are recommended to reduce microbial load and the ranges from 0.3kGy to 1.0kGy are recommended for insect disinfestations. Class 8 consists of dried food and animal origin. The ranges of dose from 0.3kGy to 1.0kGy are recommended for insect disinfestations; ranges from 1.0kGy to 3.0kGy are recommended to control mould, while the ranges from 2.0kGy to 7.0kGy are recommended to reduce pathogenic microorganism. Class 9 consists of the

other foods. The ranges of dose of irradiation from 1.0kGy to 10kGy are recommended to reduce pathogenic microorganism and microbial load (Food Irradiation Regulations, 2011).

2.1.6 Public Acceptance on Food Irradiation

The public acceptance on food irradiation is still low in some of the countries. The reasons why the public cannot accept food irradiation are mainly because of their misconceptions and irrational fear towards the technologies which involve nuclear principle. Other than that, people are having difficulties to differentiate irradiated food from radioactive foods. Radioactive foods are the food which has become accidentally contaminated by radioactive substances, while the irradiated foods are deliberately processed with specific types of radiation energy to make some desirable changes. In Malaysia, most of the industrial respondents were aware of food irradiation but they have limited knowledge about the concept of food irradiation (International Atomic Energy Agency, 2001).

The factors which contributed to the lack of interest by the food industry to utilize this technology include no demand for the benefit provided due to unawareness of the technology, competitiveness with alternative processes, uncertainty in consumer acceptance and the current restricted market for irradiated products. The findings from consumer attitude on food irradiation survey indicated low acceptance due to poor knowledge level and misunderstanding of the technology (Othman, 2001).

However, a recent study conducted in UITM Penang, Malaysia showed that consumer's level of awareness as well as level of positive attitude and trust toward food irradiation was high after the consumer learned and understood about food irradiation (Rozekhi et al., 2018). Besides that, many of the respondents showed their interest to get more information on irradiated food products as well as on how to differentiate a treated product with a conventional product (Galati et al., 2019). Respondents were found positively influenced and increased in great acceptance after they were given information about food irradiation and many of them were willing to buy irradiated food products (Finten et al., 2017). Bruhn and Eustice (2013) stated that people who received appropriate information about food irradiation technology tend to accept food irradiation.

2.2 Antioxidants

Antioxidants are substances that can reduce the damage caused by the free radical by releasing an electron to neutralize the radical. Cellular damage can be slowed down or prevented by this free radical scavenging property (Halliwell, 1995). Hertzberg , the Nobel Prize Laureate in chemistry (1971), stated that free radicals are any chemically unstable species such as atoms, molecules or ions. Free radicals can be produced by oxidative reactions occur in our body which can destroy the body cells (Shebis et al., 2013). Too much free radicals circulating in the body can oxidize the low density lipoproteins (LDL), accelerate aging processes and cause diseases like diabetes mellitus, brain stroke, rheumatoid arthritis, cancer, Parkinson's disease and Alzheimer's disease (Campanella et al., 2006). Antioxidants work by eliminating the free radical

intermediates and inhibiting other oxidative reactions. Oxidation processes are harmful to the body cells. Spontaneous oxidation can cause food spoilage to occur (Shebis et al., 2013).

Our body is completed with different type of antioxidants that help to counterbalance the effect of oxidants (Birben et al., 2012). The first attribute to classify antioxidants is based on the functions which are primary and secondary antioxidants. Primary antioxidants are the chain breaking antioxidants which mainly work with lipid radicals by converting them into more stable products. These antioxidants are mainly phenolics. Secondary antioxidants are phenolic compounds which work by capturing the free radicals and preventing the chain reactions. They include butylated hydroxyl anisole (BHA), butylated hydroxytoleune (BHT) and propyl gallate (PG). The second attribute used to classify antioxidants is based on enzymatic and non enzymatic antioxidants (Moharram and Youssef, 2014). Enzymatic antioxidants include glutathione peroxidase, catalase, and superoxide dismutase (Anwar et al., 2012), while non-enzymatic antioxidants are mainly vitamins such as vitamin A, vitamin E, vitamin C and to lesser extent vitamin D, enzyme cofactors (Q10), peptides and some minerals like selenium and zinc (Carocho and Ferreira, 2013).

The antioxidants used can be categorized into natural and synthetic antioxidants. Natural antioxidants especially polyphenols (phenolic acids and flavonoids), carotenoids (xanthophylls and carotenes) and vitamins come from food, medicinal plants, fruits, vegetables and other plants and agriculture waste products. They provided a wide range of biological effects such as

anticarcinogenicity, antimutagenicity, anti-aging, anti-inflammatory, anticancer and anti-atherosclerosis (Xu et al., 2017; Haseeb et al., 2018).

Synthetic antioxidants are mainly used in pharmaceutical and food industry. The purposes are to increase shelf life of food and increase the stability of therapeutic agents. The most common synthetic antioxidants used are butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT). Recent data showed that synthetic antioxidants used in industry could bring carcinogenic effects on human cells resurface every year, thus researchers believe that natural antioxidants are much better to replace synthetic antioxidants (Shebis et al., 2013). Recently, food industry focus in substituting synthetic antioxidants with natural antioxidants in which the results would not have significant change compared to the synthetic one. Thus, natural additives can be good choice for consumers who prefer natural foods. In addition, natural additives are environmentally friendly and safe (Caleja et al., 2017).

Antioxidants can help to preserve food and prevent the food from oxidative deterioration on storage and processing. Antioxidants can also help to preserve the nutrients level, texture, taste, freshness, colour, functionality and aroma of the food (Griffiths et al., 2016). In addition, many studies showed that antioxidants bring a lot of health benefits such as disease prevention, reduce neurodegenerative diseases in elderly, maintain physiological functions of organs, prevent cardiovascular disease and cancer, delay aging and reduce chronic diseases development (Igor et al., 2017; Huang, 2018; Wilson et al., 2017). Fruits and vegetables are good sources of

antioxidant hence are good for health promoting and diseases preventing. The common antioxidants which can be found in vegetables and fruits are phenolic compounds, ascorbic acids, carotenoids, vitamin E and phytosterols (Ravimannan and Nisansala, 2017). Malaysian Food Pyramid recommended us to take at least 2 servings of fruits and 3 servings of vegetables everyday to maintain health. Hence, Bazzano et al (2014) stated that diet high in fruits and vegetables can reduce the risk of cardiovascular disease and lower cardiovascular disease mortality.

2.2.1 Oxidative stress and ROS

Oxidative stress is a status in which the increase of steady-state reactive oxygen species (ROS) concentration occurs, and thus cellular metabolism and its regulation and damaging cellular constituents are disturbed (Lushchak, 2011). When there is unbalance occurs between antioxidant defense and pro-oxidant load, reactive oxygen species (ROS) will be produced and free radicals will be generated (Zamora-Ros et al., 2018). Oxidative stress can increase due to too much reactive oxygen species (ROS) generated by metabolic reactions that use oxygen and thus change the balance between oxidant and antioxidant conditions in favor of the oxidants. ROS are highly reactive molecules due to the unpaired electron and are generated by cellular metabolic activities and environmental factors like air pollutants or cigarette smoke (Birben et al., 2012). Oxidative stress can bring many human diseases and lethal disease such as cancer and this is probably because of inappropriate nutrition and lacking of exercise (Shebis et al., 2013).

2.2.2 Phenolics

Phenolics are aromatic benzene ring compounds with attached hydroxyl groups found in plants. They are having important functions in plant development such as protect against stress and provide structural integrity as well as give support to plants (Bhattacharya, Sood and Citovsky, 2010). Phenolics are the secondary metabolites of plants which are involved in defense functions and they can be found in plant foods such as fruits and vegetables (Dai and Mumper, 2010). Plant secondary metabolites are the end products of primary metabolites with no important role in the metabolisms of plants. The main classification of secondary metabolites includes phenolics, terpenoids and alkaloids. One of the largest groups of secondary metabolites is phenolic compounds, while the first class of phenolics is flavonoids (Singh, Kumar and Malik, 2017). The common phenolic compounds include phenolic acids, flavonoids and tannins while stilbenes and lignans are the less common one (Dai and Mumper, 2010). Plant phenolic compounds act as antioxidants and defense responder such as anti-aging, anti-inflammatory and anti-proliferative properties. Plant foods which are high in antioxidant compound content are beneficial to consume to help to reduce chronic diseases by managing the oxidative stress in body (Lin et al., 2016).

Folin-Ciocalteu assay is commonly used to evaluate the total phenolic compound of extracts. Folin-Ciocalteu reagent is used to react with the polyphenol in plant extracts in order to form a blue complex which can be quantified by using visible-light spectrophotometry (Andressa, Gisely and Joao, 2013). The mechanism is based on electron transfer that involves the reduction

of phenols in alkaline solution. The reduction reaction turns yellow reagent into blue complex. The maximum absorption is in the range of 700 to 760 nm (Xu et al., 2017). Total phenolic content is usually calculated from calibration curve of gallic acid plotted and the result is usually expressed in milligrams of gallic acid equivalent per gram of dry mass (mg GAE/g) (Andressa, Gisely and Joao, 2013). Gallic acid is the most common phenolic acid which is normally appeared as complex sugar esters in gallotannins (Daniele et al., 2013).

2.2.3 Flavonoids

Flavonoids are phenolic compounds consist of 15 carbons with two aromatic linked to a three-carbon bridge (Daniele et al., 2013). Flavonoids can be classified into different groups depending on the position of B ring and C ring of carbon. When the B ring is attached in position 3 of C ring of carbon are called isoflavones. If the B ring is attached in position 4 of C ring are called neoflavonoids. For those in which the B ring is attached to the position 2 of C ring can be subdivided into different subgroups which are flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones (Panche, Diwan and Chandra, 2016). Flavonoids can be widely found in fruits, vegetables, tea, nuts, grains and wine (Horn et al., 2001).

Flavonoids provide health benefits and protection to human body including reduce cardiovascular disease, cancer and neurodegenerative disorders (Kozłowska and Szostak-węgierek, 2014). Many studies found that flavonoids consumption was associated with risk of

cardiovascular disease. Flavonoids can decrease the risk of cardiovascular disease, risk of stroke and reduce mortality rate. Flavonoids can help to reduce the risk factors of cardiovascular disease such as hypertension, dyslipidemia and endothelial dysfunction. This was due to the strong antioxidant capacity of flavonoids that act as cardioprotective role (Velayutham et al., n.d.) Furthermore, dietary flavonoids were found to be associated with decreased risk of various types of cancers such as breast, gastric, colorectal and prostate cancers. Flavonoids intake decreased the risk of cancers due to the strong antioxidant capacity that provided protective effect on cancers (Rodr and Cristina, 2019).

Aluminium chloride colorimetric method is used to evaluate total flavonoid content. Aluminium chloride will form acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonold. It also forms acid labile complexes with orthodihydroxyl groups the A- or B-ring of flavonoids. Quercetin is usually used as the standard to create the calibration curve. Total flavonoid content can be calculated from calibration curve of quercetin plotted and the result is expressed as mg/g (quercetin equivalent/ dry extract weight) (Bag, Devi and Bhaigyabati, 2015).

2.2.4 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging

Antioxidants act as scavenger to eliminate the free radical reactions. The enzymatic antioxidant mechanisms are used to protect body from reactive oxygen species (ROS) by reducing the levels of lipid hydroperoxide and hydrogen peroxide (H_2O_2). The antioxidant

enzymes convert the dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water in the presence of cofactors (Satish and Dilipkumar, 2015).

DPPH is long-lived organic nitrogen radical with a dark blue color. It is commercially available and does not have to be generated before the assay (Pyrzynska, and Pekal, 2013). DPPH is a stable free radical with a maximum absorbance at 517 nm. It is converted into 1, 1-diphenyl-2-2picrylhydrazine when it undergoes scavenging by an antioxidant. The degree of discoloration shows the scavenging potentials of the antioxidant extract (Nataraj, Perumal and Sellamuthu, 2013). DPPH reagent is only soluble in organic solvents such as methanol. Its stability can be influenced by the solvent used for its preparation (Pyrzynska, and Pekal, 2013).

DPPH is popular method used to evaluate the compounds' ability to inhibit free radical or act as hydrogen donors and to measure the antioxidant capacity of food (Pyrzynska and Pekal, 2013). DPPH assay was developed by Brand-Williams et al. The stable free radical DPPH has a strong purple color that can be measured spectrophotometrically. DPPH solution will become discolored if the compounds present are capable of either transferring an electron or donating hydrogen. After the addition of a test material, the change in DPPH absorbance is often used as an index of antioxidant capacity of the material (Karamac et al, 2002). When a DPPH radical solution is mixed with an antioxidant substance, the color of solution will change from purple to yellow. The decrease of DPPH absorbance indicates the reducing ability of antioxidants towards DPPH (Pyrzynska and Pekal, 2013).

2.2.5 β-carotene

β-carotene is one of the members of carotenoids and is a precursor for vitamin A. β-carotene made up of 40 carbon atoms in the core structure of conjugated double bonds attached with two β-ionone rings. As an antioxidant, β-carotene can be described as efficient singlet oxygen quencher and inhibits the formation of singlet oxygen by quenching excited triplet sensitizers. The major function of β-carotene is provitamin A (Tilman et al., 2010). β-carotene is the most abundant in food that has the highest activity of pro-vitamin A and is commonly found in edible plants such as acerola, mango, pumpkin, carrot and nuts. β-carotene is associated with protection against heart disease and cancer due to its potential protective mechanism (Mezzomo and Ferreira, 2016).

The principle of β-carotene assay is due to the discoloration of yellowish color of β-carotene solution. This is because of the breaking of π-conjugation occurs due to the addition reaction of lipid or lipid peroxyl radical to a C=C double bond of β-carotene. When the antioxidant is mixed with β-carotene solution, β-carotene and antioxidant will compete with each other with the subjected radicals and hence prevent this discoloration (Djordjevic et al., 2004).

2.2.9 Principle of FRAP assay

FRAP assay is based on the ability of test material to reduce colorless oxidized Fe^{3+} form of iron to blue color Fe^{2+} tri-pyridyl-triazine (TPTZ) reduced form. This occurs because of the action of electron donation from antioxidants. This assay evaluates a change in the absorbance at

a wavelength of 593 nm. The reagents used for FRAP assay consist of TPTZ, HCl and $FeCl_3$ (Pahune, Choudhari and Muley, 2013). The assay is carried out at pH 3.6 in order to maintain the solubility of the iron in the solvent. The absorbance is measured to evaluate the amount of iron reduced. Therefore this can be correlated with the amount of antioxidants. (Ou et al., 2002).

2.3 Solvent Extraction

Solvent extraction is defined as a process that conducted to isolate dissolved antioxidant ingredients through diffusion phenomena from a solid substance (Yu et al., 2002). Liquid material or solvent is required in the process. Solvent extraction is one of the most convenient ways for extraction because it has been spectrum used to get the active ingredient components from plants (Yu et al., 2002). The main purpose of extraction process is to obtain maximum value of required active compound that having the highest antioxidant capacity or activity of extractions (Musa et al., 2011).

Extraction is the first step and also an important step to study the antioxidants of substances. There are many factors can affect the extraction efficiency. For example, type of extraction solvent, concentration of extraction solvent, extraction time, temperature of extraction and pH. Extraction solvent is one of the most important factors that can influence the extraction efficiency. The solvents selected are based on the chemical nature and polarity of antioxidant compounds. The polar and medium polar solvents such as water, ethanol, methanol, propanol and acetone are widely used for hydrosoluble antioxidants like phenolics and flavonoids.

Organic solvents such as mixtures of hexane with acetone ethanol, methanol or mixtures of ethyl acetate with acetone, ethanol, methanol are used for liposoluble antioxidants such as β-carotene, α-carotene, lycopene, lutein and zeaxanthin (Xu et al., 2017). Ethanol is more commonly used for extraction than methanol extraction because ethanol is safe and less toxic as compared to methanol (Prasad et al., 2009).

2.4 Banana (*Musa* spp.)

Banana with local name as *pisang* and botanical name as *Musa* spp was originated in Southeast Asia including northern India, Cambodia, Sumatra, Java, Philippines and Taiwan. Banana is a climacteric fruit which has been located in approximately 120 to 130 countries especially in tropical and subtropical regions. About 104 million tons of annual world production of banana and was mainly from Brazil, China, Ecuador, Philippines and India, while the leading exporters were Ecuador, Colombia, Costa Rica and Philippines (Agama-acevedo et al., 2011). In 2017, the annual production of banana has become approximately 114 million tons and the world banana consumption was approximately 86 million tons. Banana is the world most popular fruits in which they account for approximately 75% of the tropical fruit trade as well as there is over one hundred billion are consumed every year (National Geographic, 2017).

In Malaysia, banana is a very popular local fruit and is commonly consumed by Malaysians. Banana is considered as one of the most consumed fruits in Malaysia in which Malaysians consumed 10.0 kilograms per person in 2017. The main banana production is from

Johor (32%), followed by Pahang (21%) and Sabah (16%) (Siti and Ahamad, 2019). Banana is planted in approximately 30,455 hectares of land and the production is about 330,957 metric tons which are considered as the second highest of hectare and production in Malaysia. In addition, the average yield for banana is roughly 13.67 metric ton per hectare and the value of production is about RM552,697 in 2018 (Department of Agriculture Malaysia, 2018).

Banana can be planted throughout the year in any of the season in the equatorial climate. Banana is suitable to grow at temperatures range from 12 ºC to 32 ºC with precipitation between 1000 to 3000 mm (Hanizah, 2019). Banana can grow in almost all types of soil but not in the salty soil. They grow well in nutrient rich and well-drained soils with acidic pH between 5.5 and 6.5. The thick chunky banana plant prefers to grow with large amount of water. Frost free weather for about 10 to 15 months can produce very healthy flower stalk (Elayabalan et al., 2017).

2.4.1 Taxonomy

Banana belongs to the genus *Musa* from the family *Musaceae* and order Zingiberales. The genus *Musa* is categorized into *Callimusa, Australimusa, Eumusa and Rhodochlamys* sections (Singh et al., 2016). Banana is classified into cooking banana or plantain and dessert banana (International Tropical Fruits Network, 2016). All the cultivars of bananas and plantains are hybrids and polyploids of *M. acuminata* and *M. balbisiana*. Dessert bananas are commonly the cultivars derived from *M. acuminata*, while cooking bananas are those derived from *M.*

balbisiana and hybrids of the two (Singh et al., 2016). The evolution of plantain and banana cultivars is from the natural hybridization between the two species which are *M. acuminata* (contributing genome A) and *M. balbisiana* (contributing genome B). Both plantain and banana are monocotyledonous plants which are belonging to the section Eumusa within the genus *Musa* of the family Musaceae on the order Scitamineae (International Tropical Fruits Network, 2016). There are more than 300 types of bananas are cultivated worldwide in which they are categorized based on the number of chromosome sets present and the proportion of genomes of *M. acumimata* (A) and *M. balbisiana* (B). Plantains or cooking bananas and dessert bananas are the hybrids of these two species. They are different from each other as the amount of starch and sugar produced in their fruits is different (Stover & Simmonds, 1987). There are triploid hybrids called AAA, AAB and ABB which are the most familiar and seedless cultivars of banana. Dessert bananas are mainly AA or AAA, while plantains are mainly AAB, ABB and BBB genomes. Dessert bananas are commonly consumed in raw, while plantains are usually not consumed in raw unless they are very ripen, they are normally consumed after cooking or processing (Nakasone & Paull, 1999; Rieger, 2006). Plantain is classified as *Musa paradisiana* (International Tropical Fruits Network, 2016).

2.4.2 Health Benefits

According to USDA (2019), 100 grams of banana contains 358 milligrams of potassium which are about 10% of daily value. Banana provide us high amount of potassium which is important to control blood pressure (Singh et al., 2016). Diet high in potassium can help to lower

the blood pressure of patients as well as provide cardioprotective effects such as reducing the risks of cardiovascular disease and cardiac arrhythmias (Houston, 2011).

Banana contains a lot of dietary fibers which are important for intestinal peristalsis, promote growth of beneficial gut flora and prevent colon cancer (Agama-acevedo et al., 2011). Fiber content will increase when the maturity of banana increases and the ripe banana has the highest fiber content (Ogbonn et al., 2016). In 100g of banana, it contains approximately 2.6 grams of total fiber components in which 1.8 grams are insoluble fiber and 0.8 gram is soluble fiber. Banana is starchy fruit which provide fiber from both cell wall and resistant starch stored within the cells. Health benefits associated with fruit fiber are preventing acute or chronic colonic gastrointestinal health problems such as constipation, irritable bowel syndrome, diverticular disease as well as help in weight control (Dreher, 2018).

Besides, Agama-acevedo et al (2011) also stated that banana is high in antioxidant which can help to prevent diverse health problem because this antioxidant can inhibit the free radicals that damage the body cells and cause cellular aging. Banana contains high amount of phenolic compounds, flavonols and carotenoids which provide many health benefits. Other compounds such as vitamin C, vitamin E and beta carotene also help to enhance the antioxidant potential (Singh et al., 2016). The antioxidant compounds such as total phenolic content (TPC) and total flavonoid content (TFC) were found higher in the banana peel than in the pulp as well as higher in green banana than in ripe banana (Fatemeh, 2012).

2.4.3 Pisang Nangka (*Musa paradisiaca formetypica* L. var. Nangka)

The common varieties of banana in Malaysia are *Cavendish, Berangan, Tanduk* and *Mas* (Siti and Ahmad, 2019). Pisang nangka (*Musa paradisiaca formetypica* L. var. Nangka) is one of the popular plantains or cooking bananas in Malaysia (Hassan, 2004). Plantain can be eaten raw as a dessert or sweet fruit and is often classified as the dessert or table banana or as a staple food by cooking (Singh et al., 2016). Pisang nangka is about 8 inches long and 1.5 inches width, rounded to slightly angular in cross section. The skin is green in colour and the flesh is light yellow and tastes sour-sweet. It is a popular banana for *pisang goreng* in Malaysia. When it is raw, it has the fragrance of jackfruit. The fragrance is the strongest in the inside surface of the skin. The fruit is also known as jackfruit banana (Milsum, 2015). The harvesting period of pisang nangka is about 22 weeks (Hanizah, 2019). Figure 2.4.3 shows the botanical photo of pisang nangka.

Figure 2.4.3: Botanical photo of pisang nangka

2.4.4 Nutrition Facts of Pisang Nangka

The nutrition facts of 100 grams of pisang nangka are listed in the Table 2.4.4 below. The data is based on Malaysian Food Composition Database (1997).

Table 2.4.4: Nutrition facts of pisang nangka

Calories: 121 kcal		Amount per 100 gram
Nutrients	**Unit**	**Value**
Proximates		
Water	g	68
Carbohydrate	g	28.5
Protein	g	1.4
Fat	g	0.2
Fibre	g	1.2
Ash	g	0.7
Minerals		
Calcium, Ca	mg	0
Iron, Fe	mg	0.4
Phosphorus, P	mg	3.0
Potassium, K	mg	242.0
Sodium, Na	mg	22.0
Vitamins		
Vitamin C	mg	14.1
Thiamin (B1)	mg	0.07
Riboflavin (B2)	mg	0.1
Niacin (B3)	mg	0.8
Retinol	mg	0
Carotenes	mg	197.0
Retinol Equivalents (RE)	mg	33.0

CHAPTER 3

METHODOLOGY

3.1 Sampling

Convenient sampling method was used to select sample pisang nangka (*Musa paradisiaca formetypica* L. var. Nangka) from local market in Kajang, Selangor. Sampling method was based on Nor Liyana et al. (2019). Pisang nangka was selected at green stage from local market in Kajang, Selangor. Pisang nangka was selected at green stage because we need to preserve the banana immediately after harvested. The ripening stage of banana was determined based on Beatriz et al. (2013). The sample was then sent to laboratory in Faculty of Medicine and Health Sciences, UPM.

Figure 3.1: Pisang nangka selected from local market

3.2 Chemicals and Reagents

Distilled water (Favorit W4L Genristo, Nottingham, UK), 100% ethanol solution (Scifex Cas: 64-17-5), Folin-Ciocalteu reagent (R&M PHAO160814, Essex, UK), sodium carbonate (Na_2CO_3) (R&M PHOU130716), gallic acid (Acros, New Jersey, USA), sodium nitrite ($NaNO_2$) (Sigma-Aldrich, USA), aluminium chloride ($AlCl_3$) (Sigma-Aldrich, Germany), sodium hydroxide (NaOH) (R&M PTEN220218, Selangor, Malaysia), catechin, DPPH powder (Aldrich), butylated hydroxytoluene (BHT) (Sigma, Germany), sodium acetate (Sigma, Japan), acetic acid, 2,4,6 tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, Switzerland), ferric chloride solution ($FeCl_3$) (Emsure, Merck, Germany), deionized water and iron sulphate ($FeSO_4$) (Hamburg).

3.3 Instruments

Weighing scale (AND GF300, Japan) , -20 ºC freezer (Haier Low Temperature Freezer, China), oven-dry machine (Memmert 600 Schwabach, Germany), heavy duty blender (National MX-895M, Shah Alam, Malaysia), microplate reader (ELISA E16), orbital shaker (Heidolph Unimax 1010, Germany), filter paper (Whatman, China), vortex (BV1000, Taiwan, ROC), belly dancer (Inkubator 1000, Heidolph) & rotary evaporator (Laborata 4000, Heidolph, Japan).

3.4 Preparation of Sample

First of all, the inedible portion of the sample was removed. Then, the edible portion of sample was weighed and cut into small pieces. The edible portion was put into oven-dry machine using trays. The temperature of oven was set at below 50 ºC and the sample was dried for 24 hours. After that, the dry sample was grinded into powder form using heavy duty blender. The powder was divided equally into 4 portions and was stored in sealed plastic bag. The powder was kept in chiller at 4 ºC for further use (Nor Liyana et al., 2019).

3.5 Irradiation

The sample powder was then sent to Nuclear Agency of Malaysia, Bangi to be irradiated. The sample was treated with different amount of dosage (3kGy, 6kGy and 9kGy) using the

treatment of gamma ray with Cobalt-60 source (Malaysia Nuclear Agency, 2019; Nor Liyana et al., 2019). Dose of radiation is expressed in standard international unit which is Grays (Gy) and it is equivalent to 1 Joule of absorbed energy per 1 kg of sample (Food Irradiation Regulations, 2011).

3.6 Sample Extraction

About 40 g of dried powder was homogenized with 400 ml of 100% ethanol solution on an orbital shaker at 200 rpm for 48 hours at room temperature. Then, the mixture was filtered with filter paper and then was concentrated by rotary evaporator under 48 ºC. Next, the filtrate was kept at -20 ºC for further analysis (Hijazi et al., 2015).

3.7 Determination of Total Antioxidant Contents

In this study, there were two assays carried out to determine the total antioxidant contents which were total phenolic content (TPC) and total flavonoid content (TFC).

3.7.1 Total Phenolic Content (TPC)

Folin-Ciocalteu method was used to determine total phenolic content (Ishak et al., 2018; Nor Liyana et al, 2019). Stock solution of 5 mg/ml plant extracts in ethanol was prepared. Firstly,

about 20 µl of sample extract (5 mg/ml) was mixed with 100 µl of Folin-Ciocalteu reagent (diluted 1: 10 with deionized water). After 5 minutes of incubation, 80 µl of 7% sodium carbonate (Na_2CO_3) was added and the mixture was placed in dark condition at room temperature for 30 minutes. The absorbance was measured at 770 nm by using microplate reader. The sample was analyzed in triplicate. Different concentration of gallic acid (0.98 µg/ ml to 1000 µg/ ml) was used as standard curve. The results were expressed as mg of gallic acid (GAE) per gram of extract sample. The total phenolic content was calculated as formula below:

$$\text{Total Phenolic Content (TPC)} = \frac{R \times V \times D.F}{W}$$

Where

R= x value obtained from standard curve

V= Total sample volume used

W= Sample weight used

D.F= Dilution factor

3.7.2 Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by using aluminium chloride colorimetric method (Ishak et al., 2018; Nor Liyana et al, 2019). Stock solution of 5 mg/ml of plant extracts in

ethanol was prepared. Firstly, about 25 µl of sample extract (5 mg/ ml) was mixed with 100 µl of distilled water followed by 7.5 µl of sodium nitrite (5% $NaNO_2$). After 5 minutes, about 7.5 µl of aluminium chloride solution (10% $AlCl_3.6H_2O$, 10g of $AlCl_3.6H_2O$ in 100ml) was added and incubated for 5 minutes. Then, 50 µl of sodium hydroxide (1M NaOH) and 60 µl of distilled water are added. The mixture was turned into pink. The absorbance was measured at 510 nm using microplate reader. The sample was analyzed in triplicate. Different concentration of catechin (0.98 µg/ ml to 1000 µg/ ml) was used as standard curve. The results were expressed as mg of catechin equivalent (CE) per gram of extract sample. The total flavonoid content was calculated as formula below:

$$\text{Total Flavonoid Content (TFC)} = \frac{R \times V \times D.F}{W}$$

Where

R= x value obtained from standard curve

V= Total sample volume used

W= Sample weight used

D.F= Dilution factor

3.8 Determination of Total Antioxidant Activities

In this study, DPPH free radical scavenging assay and ferric reducing antioxidant potential (FRAP) assay were used to determine total antioxidant activities.

3.8.1 Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity

DPPH free radical scavenging assay will be used to determine total antioxidant activity (NurHuda et al., 2015; Nor Liyana et al, 2019). Firstly, different concentration of diluted sample extracts which range from 0.6 µg/ ml to 2500 µg/ ml were prepared. Then, 3.9 mg of DPPH powder was added to 10 ml of ethanol to make DPPH solution in volumetric flask. The volumetric flask was wrapped with aluminium foil to reduce light exposure. Next, 60 µl of DPPH solution was added to 10 µl of each diluted sample extract and mixed well using belly dancer for 30 minutes under dark condition. Butylated hydroxytoluene (BHT) was used as standard. The absorbance was measured at 540 nm using microplate reader. The scavenging activity of sample extracts was calculated as formula below:

Scavenging Activity (%) = [1- (Absorbance sample/ Absorbance control) x 100]

Then, EC_{50} values were determined by plotting the graph of scavenging activity against the concentration of the standard. EC_{50} value indicates the concentration of extracts that are required to reduce the initial DPPH free radical concentration by 50%.

3.8.2 Ferric Reducing Antioxidant Potential (FRAP)

Ferric reducing antioxidant potential (FRAP) was used to determine total antioxidant activity (Prasad et al., 2013; Nor Liyana et al, 2019). Firstly, 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6 tripyridyl-s-triazine (TPTZ) and 20 mM of ferric chloride solution ($FeCl_3$) were prepared in proportion of 10:1:1 (v/v/v) and mixed to make working FRAP reagent. Next, about 30 ml of FRAP reagent was warmed at 37 ºC. After that, 180 µl of FRAP reagent was mixed with 20 µl of sample and the mixture was incubated for 30 minutes at 37 ºC. The absorbance was measured at 570 nm using microplate reader. Different concentration of iron sulphate ($FeSO_4$) ranging from 0.04 mM to 1.28 mM was used as standard. The results were expressed as mmol Fe^{2+}/ gram of dry weight.

3.9 Statistical Analysis

All the statistical analysis was performed using IBM SPSS Statistics 22. All the results from this experiment were expressed as mean ± standard deviation. Pearson's correlation was used to determine the correlation between total antioxidant contents and antioxidant activities with amount of radiation dose. One-way ANOVA was used to identify the significance difference between samples. Tukey post hoc test was used to further identify the significant difference groups. The significance level was set at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Total Phenolic Content (TPC)

Table 4.1 shows the total phenolic content (TPC) of non-irradiated and irradiated extracts of pisang nangka. TPC was expressed as mg equivalent gallic acid per gram extract (mg GAE/g extract).

Table 4.1: TPC of non-irradiated and irradiated extracts of pisang nangka

Sample extract	TPC (mg GAE/ g extract)
Non-irradiated	10.73 ± 1.82^{a}
3 kGy	13.42 ± 1.63^{a}

6 kGy	12.77 ± 2.06^{a}
9 kGy	9.31 ± 2.56^{a}

Values were expressed as mean ± standard deviation, SD (n=3). Mean values with different letters were significantly different at the level of p (<0.05).

In this study, the concentration used was 5 mg/ ml of crude dried extract. The formation of deep blue color of sample solution indicated that the sample contains high concentration of phenolic content, while the formation of light blue color indicated the present of low concentration of phenolic content. Based on Table 4.1, the highest level of total phenolic content (TPC) was found in irradiated extract at 3 kGy (13.42 ± 1.63 mg GAE/ g extract), followed by 6 kGy (12.77 ± 2.06 mg GAE/ g extract), non-irradiated (10.73 ± 1.82 mg GAE/ g extract) and 9 kGy (9.31 ± 2.56 mg GAE/ g extract). There was no significant different ($p > 0.05$) of total phenolic content between non-irradiated and all irradiated extracts. Besides, there was also no significant different ($p > 0.05$) of total phenolic content among all the irradiated extracts. Irradiated sample extracts at dose 3 and 6 kGy showed an increase in TPC but decrease at dose 9 kGy as compared to non-irradiated extract as shown in Table 4.1.

The antioxidant compounds found in bananas included ascorbic acid, tocopherol, beta carotene, phenolic groups, dopamine acid and gallocatechin (Qusti et al., 2010; Someya et al.,

2002). Banana contains different phenolic compounds such as catechin, tannins, gallic acid, epicatechin and anthocyanins (Singh et al., 2016). The total phenolic compounds in pisang nangka, pisang mas, pisang berangan, pisang raja and pisang nipah in Malaysia were reported ranging from 0.09 to 20.47 mg GAE/ 100 g in ripe banana (Sulaiman et al., 2011). However, the total contents of phenolic compounds can be varied due to the different in banana cultivars, the extraction method, the ripening stage and the analysis method (Aquino et al. 2016). Phenolic compounds are important as they act as antioxidants and defense responder such as anti-aging, anti-inflammatory and anti-proliferative properties by preventing or eliminating the free radicals. Plant foods which are high in antioxidant compound content are beneficial to consume to help to reduce chronic diseases by managing the oxidative stress in body (Lin et al., 2016). Fatemah et al. (2012) observed that the TPC was generally higher in the peel than the pulp and was higher in unripe banana than ripe banana.

In the present study, irradiation did not give significant changes in TPC but it showed slightly increase of TPC in lower doses of irradiation which are 3 and 6 kGy. A previous study observed that there were significant difference ($p < 0.05$) in TPC between ethanol extracts of irradiated red beet roots and leaves at dose 3, 6, 9 and 12 kGy. Irradiation treatment increased TPC of all extracts compared to non-irradiated samples (Elshiemy et al., 2019). Nilima and Kunda (2012) reported the total phenolics of *Amoora rohitaka* in aqueous, methanol and ethanol extracts was significant increases with irradiation dose from 0 to 5 kGy. Moreover, another study showed the total phenolic content of pomegranate peel powder increased after treatment of

gamma irradiation at dose 5, 10 and 25 kGy (Khedkar and Lele, 2011). A similar trend was also reported by Abolhasani et al. (2018) who observed a significant increase in the total phenolic content of irradiated pistachio green hull at dose 20 and 40 kGy and a non significant increase at dose 10 kGy as compared to non-irradiated sample. Some other previous studies also observed that there was an increase in the level of total phenolic content after ionizing irradiation (Behgar et al., 2011; Abolhasani et al., 2018; Sima et al., 2014).

According to Variyar et al. (2004), irradiation process induced an increase in total phenolic content may be attributed to an increase in the availability of phenolic compounds. This was probably because of the larger phenolic molecules was decomposed into smaller one during the gamma irradiation process, causing a release of active ingredients which were contributed to increasing TPC. Some researchers had shown that gamma irradiation is able to increase phenolic content of some of the plant source substances. However, the real mechanism for the increase in phenolic content remained unclear. There was little agreement found in the literature about the increase of total phenolic content of various plant materials. This may be linked to the difference doses of gamma rays, type of extraction method used and the identity of phenolics present (Abolhasani et al., 2018).

In addition, there was a previous study by Nor Liyana et al. (2019), who observed a significant difference ($p < 0.05$) between non-irradiated and irradiated of hot aqueous extracts of *A. bubalinum*. Total phenolic content in irradiated of hot aqueous extracts of *A. bubalinum* at dose 3, 6, 9 and 12 kGy was significantly lower than non-irradiated extract. Some previous studies also showed reduction in total phenolic content with irradiation (Kavitha et al., 2015; Moosavi et al., 2014). This can be due to the impact of higher dose irradiation which reduced the protection against the glycoside wall and also the loss of bioactive compound including phenolic (Kortei et al., 2016). A decrease in total phenolic content in irradiated extract compared to non-irradiated extract can also relate with the type of plant materials, geographical and environmental settings (Ghadi et al., 2015).

Studies involved gamma irradiation had reported different effects that were attributed to the different phenolic compounds present in the different species of plants. Some of the plants have large quantity of hydrolysable compound; hence it may be more susceptible to gamma irradiation as compared to the other compounds (Moosavi et al., 2014). Gamma irradiation had been shown to either increase or decrease the total phenolic content of different types of plant materials due to the factors such as type of plant, topographical, ecological, individual phenolic present, and extraction procedure as well as dose of gamma rays (Ghadi et al., 2015). Therefore, these factors could influence the results for total phenolic content of pisang nangka in this study as the related research studies for this plant species was very limited.

4.2 Total Flavonoid Content (TFC)

Table 4.2 shows the total flavonoid content (TFC) of non-irradiated and irradiated extracts of pisang nangka. TFC was expressed as mg equivalent catechin per gram extract (mg CE/g extract).

Table 4.2: TFC of non-irradiated and irradiated extracts of pisang nangka

Sample extract	TFC (mg CE/ g extract)
Non-irradiated	23.94 ± 2.23^{a}
3 kGy	23.89 ± 3.54^{a}
6 kGy	14.57 ± 2.58^{b}
9 kGy	12.51 ± 4.46^{b}

Values were expressed as mean ± standard deviation, SD (n=3). Mean values with different letters were significantly different at the level of p (<0.05).

In this study, the concentration used was 5 mg/ ml of crude dried extract. The formation of dark pink color indicated the present of high concentration of flavonoid content, while the formation of light pink color indicated the present of low concentration of flavonoid content. Based on Table 4.2, the result shows that the total flavonoid content (TFC) of non-irradiated extracts (23.94 ± 2.23 mg CE/ g extract) was the highest, followed by irradiated extract at 3 kGy (23.89 ±3.54 mg CE/ g extract), 6 kGy (14.57 ± 2.58 mg CE/ g extract) and 9 kGy (12.51 ± 4.46 mg CE/ g extract). There was no significant different ($p > 0.05$) between non-irradiated extract

and irradiated extract at 3 kGy. However, non-irradiated extract was significantly ($p < 0.05$) higher than irradiated extract at both 6 kGy and 9 kGy. Among the irradiated extracts of sample, the total flavonoid content of irradiated extract at 3 kGy was significantly different ($p < 0.05$) with irradiated extract at both 6 kGy and 9 kGy. There was no significant different ($p > 0.05$) between irradiated extract at 6 kGy and 9 kGy. Overall, irradiated sample extracts at dose 6 and 9 kGy showed significant decrease in TFC, however extracts at dose 3 kGy did not show significant changes but a slightly decrease in TFC as compared to non-irradiated extract based on Table 4.2.

The main classes of flavonoids found in banana are the flavonols, which are including quercetin, myricetin, kaempferol and cyanidin (Singh et al., 2016). Flavonoids are a class of secondary plant metabolites with important antioxidant and chelating characteristics. The concentration of flavonoids in plants extract can be influenced by the polarity of solvents used in the extraction (Shahzad et al., 2017). The antioxidant activity of flavonoids mainly depends on the structure and substitution pattern of the hydroxyl groups (Fariba et al., 2009). Flavonoids provide protective effect due to the mechanisms like trapping of free radicals, inhibition of enzymes and chelation of metallic ions. Those properties depend on the degree of substitution and saturation as well as the structure of flavonoids (Ioannou and Ghoul, 2012). Fatemah et al. (2012) observed that green banana had higher TFC than ripe banana. While in all types and ripening stages, it was evident that banana pulp always showed a lower TFC than the peel.

This study observed that there was a significant decrease in the total flavonoid content in irradiated extracts at dose 6 and 9 kGy as compared to the non-irradiated extract. This study was in agreement to a previous study by Nor Liyana et al. (2019) which showed that the total flavonoid content of gamma irradiated *A. bubalinum* extracts at dose 6 and 9 kGy was significantly lower as compared to non-irradiated extract. Supporting this present study in which gamma irradiated pisang nangka extracts at dose 6 and 9 kGy was significantly decrease as compared to the non-irradiated extract. Another previous study reported that gamma irradiation at dose 2, 6 and 10 kGy significantly reduced the flavonoid content of methanol extract of almond hull (Moosavi et al., 2014). Moreover, Shazad et al. (2017) also observed the decrease in total flavonoid content of irradiated *C. tubercullataextracts* as compared to the control at irradiation level of 1, 3, 5 and 7 kGy. The reduction in flavonoid content could be due to the occurrence of radiolysis chemical reaction in which the attacked of free radicals from radiation has disturbed the catechol group and hence influencing the level of flavonoids (El-Batal et al., 2013). In addition, the interaction between gamma irradiation with atoms and molecules created free radicals in the cells. Thus, this changed the important components in the cells which influenced the biochemical and morphological of the plants (Wi et al., 2005).

However, Elshiemy et al. (2019) observed a significant increase in TFC of irradiated red beet roots and leaves ethanolic extracts at dose 3, 6, 9 and 12 kGy as compared to non-irradiated samples. Sima et al. (2014) reported that there was significant increase in flavonoid content of *C.*

alismatifolia after gamma irradiation treatment at dose 15 and 20 kGy. Another study done by Cho et al. (2017) also showed a significant increase in flavonoid content of gamma irradiated persimmon leaf extract at dose 10 kGy. Gamma irradiation can interact with atoms and molecules in the cells. Free radicals generated by this interaction are able to modify the significant components of plant cells. These free radicals are able to influence the physiology, biochemistry and morphology of plants, depending on the dose of gamma rays. The effect for examples, changes in the plant cellular structure and metabolism, changes in photosynthesis, enhancement of phenolic compound and modulation of antioxidative system (Sina et al., 2011). Therefore, there were many factors could influence the results of total flavonoid content in pisang nangka in this study as the related research studies for this plant species was very limited.

In the present study, irradiation treatment increased TPC but decreased TFC of extracts of pisang nangka which was in contrast with previous studies that observed irradiation treatment either increased both TPC and TFC or decreased both TPC and TFC (Nor Liyana et al., 2019; Moosavi et al., 2014; Shazad et al., 2017). The contrast in findings was probably because of only single solvent was used as extraction. Solvent used for extraction is important to determine extraction yield and antioxidant contents. The presence of different antioxidant compounds may or may not be soluble in a specific solvent due to varies in chemical characteristics and polarities. For examples, ethanol is good solvent for polyphenol extraction; methanol is more efficient in extraction of lower molecular weight polyphenols; and aqueous acetone is suitable for extraction

of higher molecular weight flavanols (Quy et al., 2014). Ngo et al. (2017) reported that the extraction solvents had a significant influence on the extraction of flavonoid, this variation can be explained by the different polarities of compounds which were selectively more soluble in different solvents. Therefore, more than one solvent such as methanol, distilled water and acetone are suggested to use for extraction because different antioxidant compound reacts differently to different solvents and thus can affect the results (Sallam and Anwar, 2017; Elshiemy et al., 2019).

4.3 Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The DPPH free radical scavenging activity has been used to evaluate the total antioxidant activity of non-irradiated and irradiated extracts of pisang nangka. Figure 4.3 shows the DPPH free radical scavenging activity (%) of extracts of pisang nangka. Range of concentration used between 0.0024 mg/ ml until 2.5 mg/ ml. Values were expressed in mean ± standard deviation, SD (n=3). Butylated hydroxytoluene (BHT) was used as positive control.

Figure 4.3: DPPH free radical scavenging activity (%) of extracts of pisang nangka

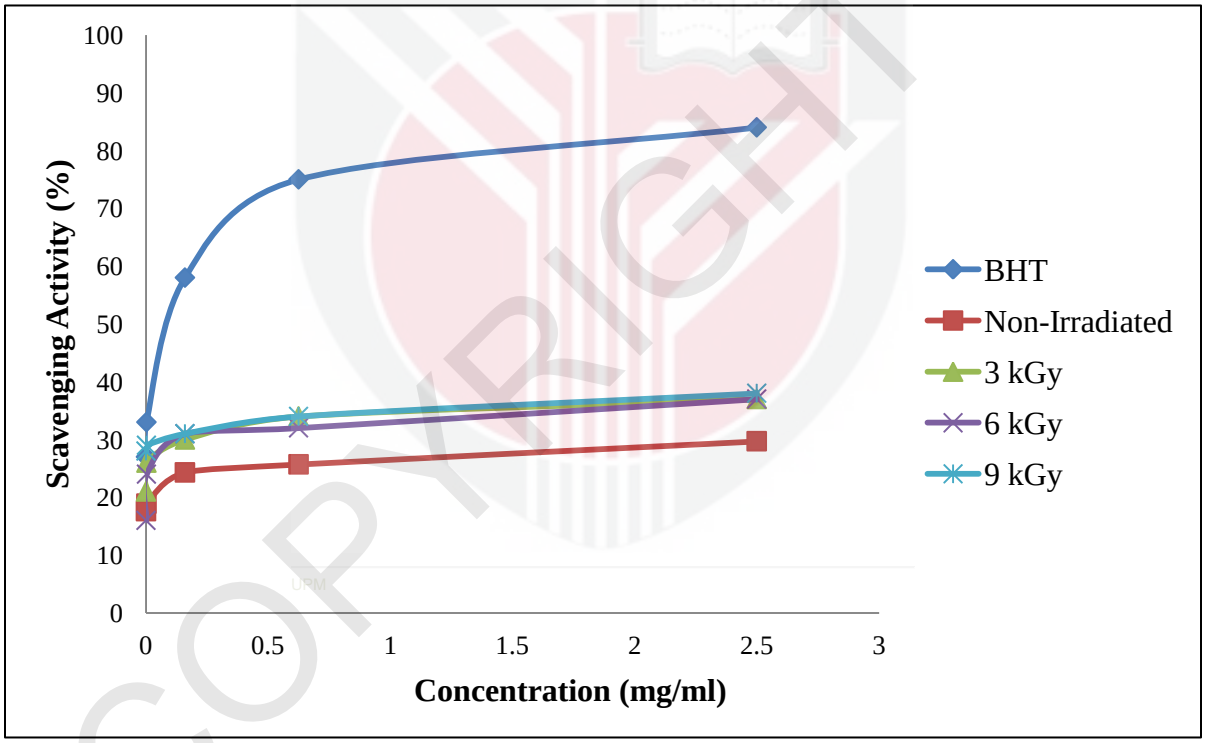

Table 4.3: EC_{50} values of extracts of pisang nangka

Sample extract	EC_{50} (mg/ ml)
Non-irradiated	7.92 ± 2.29^{a}
3 kGy	5.44 ± 2.19^{a}
6 kGy	4.73 ± 1.48^{a}
9 kGy	6.64 ± 2.18^{a}
BHT	0.36 ± 0.0065^{b}

Values were expressed as mean ± standard deviation, SD (n=3). Mean values with different letters were significantly different at the level of p (<0.05).

In this study, the concentration used was 5 mg/ ml of crude dried extract. EC_{50} value, efficient concentration of extract (mg/ ml) indicates 50% of the radicals being scavenged by the samples. A lower EC_{50} indicates the higher antioxidant activity. Butylated hydroxytoluene (BHT) was used as positive control. EC_{50} value was used to indicate the antioxidant power in which lower EC_{50} value indicates a higher antioxidant properties. DPPH solution turned from purple color into colorless indicated that the antioxidant compounds present were capable of either transferring an electron or donating hydrogen (Pyrzynska and Pekal, 2013). Based on Table 4.3, the highest level of EC_{50} was found in non-irradiated extract (7.92 ± 2.29 mg/ ml), followed by 9 kGy (6.64 ± 2.18 mg/ ml), 3 kGy (5.44 ± 2.19 mg/ ml) and 6 kGy (4.73 ± 1.48 mg/ ml). There

was no significant different ($p > 0.05$) between non-irradiated extract and all irradiated extracts. Also, there was no significant different ($p > 0.05$) among all the irradiated extracts. However, EC_{50} values of all non-irradiated and irradiated extract were significantly different ($p < 0.05$) as compared to the positive control (BHT) as shown in Table 4.3. Overall, irradiation treatment did not give significant changes in EC_{50} values but it showed slightly decrease in EC_{50} values. This also indicated that irradiation slightly increased the DPPH radical scavenging activity.

This finding was in similar to another study by Nor Liyana et al. (2019), who observed a non significant decrease in DPPH EC_{50} value between non-irradiated and irradiated at dose 3, 6, 9 and 12 kGy of hot aqueous extracts of *A. bubalinum*. Fatima and Rahman (2016) observed a significant increase in free radical scavenging activity (decrease in EC_{50}) in *Ziziphus mauritiana* leaves after gamma irradiation treatment at dose 2.5, 5.0, 7.5, 10.0 and 12.5 kGy. In addition, a similar result was also reported by Nilima and Kunda (2012) in which ethanol extract of *Amoora rohitaka* showed a significant increase in DPPH free radical scavenging activity after gamma irradiation at dose 1, 3 and 5 kGy. Several past studies also reported that gamma irradiation increased the DPPH radical scavenging activities of irradiated extracts (Abolhasani et al., 2018; Kavitha et al., 2015; Antonio et al., 2011).

The slightly increase in DPPH free radical scavenging activity could probably due to the occurrence of Maillard Reaction Products (MRP) as a result of gamma irradiation. The interruption of nutritional contents such as glucose and amino acids might occur which lead to the induction of Maillard Reaction Products (MRP) (Kim et al., 2009). MRP can be defined as non enzymatic browning reaction in foods between reducing sugars and amino acids, peptides or proteins due to the result of heating process, cooking and storage of food. This chemical reaction between amino acids and reducing sugars contributes to the flavor and brown color (Bastos et al., 2012). MRPs possess good antioxidant ability through process of chelation of metal ions, breakdown of radical chains and hydrogen peroxide, and scavenging of ROS. MRPs have antibrowning effect in fruits and vegetables and can significantly prevent food oxidation (Majid et al., 2019). These MRPs could release hydroxyl and superoxide anion radical and consequently lead to increase in DPPH free radical scavenging activity (Nilima and Kunda, 2012).

On the other hand, Suhaj et al. (2006) observed that DPPH free radical scavenging activity of black pepper decreased (higher EC_{50}) after gamma irradiation treatment between 5 kGy and 30 kGy. Moosavi et al. (2014) also observed that the DPPH free radical scavenging activity of almond hull extracts was reduced at dose of 6 and 10 kGy. Radiation process has been shown to either increase or decrease the DPPH activity of plant extracts depending on the dose of radiation used, exposure time and raw materials used (Althoman et al., 2009). Though many researchers have studied on the effect of gamma irradiation on free radical activity of fruits or

plants, limited data was reported on the effect of gamma irradiation on DPPH radical scavenging activity of banana. Previous research studies reported different results for the effect of gamma irradiation on the antioxidant properties of other plants. These differences could be due to their chemical composition, solvent used for extraction and other individual factors (Gumus et al., 2011).

4.4 Ferric Reducing Antioxidant Potential (FRAP)

Table 4.4 shows the ferric reducing antioxidant potential (FRAP) values of non-irradiated and irradiated extracts of pisang nangka. FRAP value was expressed as mmol Fe^{2+}/ g dry weight.

Table 4.4: FRAP values of extracts of pisang nangka

Sample extract	FRAP (mmol Fe^{2+}/ g dry weight)
Non-irradiated	87.62 ± 3.89^{a}
3 kGy	92.63 ± 7.31^{a}
6 kGy	103.01 ± 11.99^{a}
9 kGy	79.99 ± 10.81^{a}

Values were expressed as mean ± standard deviation, SD (n=3). Mean values with different letters were significantly different at the level of p (<0.05).

In this study, the concentration used was 5 mg/ ml of crude dried extract. FRAP assays are widely used to evaluate the ability of antioxidant compounds in plant extract to reduce ferric (Fe^{3+}) to ferrous ion (Fe^{2+}) in FRAP reagent. The formation of blue color product, TPTZ complex indicated the reduction of ferric to ferrous ion in FRAP reagent (Shian et al., 2012). The absorbance was measured to evaluate the amount of iron reduced and the increasing in absorbance indicated an increase in reducing power. Therefore this can be correlated with the amount of antioxidants. (Ou et al., 2002). Based on Table 4.4, the highest level of FRAP value

was found in irradiated extract at 6 kGy (103.01 ± 11.99 mmol Fe^{2+}/ g dry weight), followed by 3 kGy (92.63 ± 7.31 mmol Fe^{2+}/ g dry weight), non-irradiated (87.62 ± 3.89 mmol Fe^{2+}/ g dry weight) and 9 kGy (79.99 ± 10.81 mmol Fe^{2+}/ g dry weight). There was no significant different ($p > 0.05$) of FRAP value between non-irradiated and all irradiated extracts. Besides, there was also no significant different ($p > 0.05$) of FRAP value among all the irradiated extracts. Overall, irradiation treatment did not give significant changes in FRAP values but it showed slightly increase in FRAP values at lower dose of irradiation as shown in Table 4.4.

This finding was supported by Nor Liyana et al. (2019) in which there was no significant difference in FRAP values between non-irradiated and all irradiated extracts and no significant difference among all irradiated *A. bubalinum* extracts at dose 3, 6 and 9 kGy. Elshiemy et al. (2019) reported that irradiation treatment at dose of 3, 6 and 12 kGy did not show significant difference but increasing FRAP values for ethanol extracts of red beet roots as compared to non-irradiated extracts. Besides, there was also no significant difference in FRAP values between non-irradiated and irradiated methanol extracts of *B. griseipuireus* at dose 2.5, 5.0, 7.5 and 10.0 kGy in the previous study done by Chookaew et al. (2019). Another study reported that gamma irradiation at dose 5 kGy increased FRAP values in ethanol extracts of *Mursalski, Staroplaninski* and *Planinski* tea (Michal et al., 2016). This increase in FRAP value may be due to the formation of MRPs which was caused by gamma irradiation. These MRPs could release hydroxyl and superoxide anion radical and consequently lead to increase in reducing power (Nilima and

Kunda, 2012). Besides, the increase in FRAP value after irradiation could be due to the result of high total phenol as phenols are the most active ferric reducing agents (Štajner, Miloševi & Popovi, 2007). After irradiation, the enhanced antioxidant activity of a plant was mostly due to increase in enzyme activity such as Phenylalanine ammonia-lyase and peroxidase activity. Also it can be due to the increase extractability from the tissues by dissolution and depolymerization of cell wall polysaccharides by the irradiation (Alothman et al., 2009).

However, Abolhasani et al. (2018) reported that there was significant decrease in FRAP value of pistachio green hull extracts after gamma irradiation treatment at dose 10, 20, 30 and 40 kGy. Moosavi et al. (2014) also observed a significant decrease in FRAP value was found in irradiated fresh almond hull extract at dose 2 kGy. Another study reported by Nilima and Kunda (2012) showed that the FRAP value of ethanol extract of *Amoora rohitaka* were decreasing with irradiation dose at 1, 3 and 5 kGy. The methanol extract of *Amoora rohitaka* showed higher FRAP value as compared to ethanol extract of the same sample at the same dose of irradiation. Thus, the increase or decrease in FRAP value could be due to the different solvent used for extraction (Nilima and Kunda, 2012). Irradiation can affect the level of antioxidant or phytochemicals as well as the capacity of the plant to produce them at different levels. The conditions such as exposure to radiation sources, wounding and storage temperature can influence the concentration of plant phytochemicals. Dose of radiation applied, sensitivity of the

antioxidants or phytochemicals towards irradiation and the radiation effect can also influence the production or accumulation of antioxidants in the plant (Alothman et al., 2009).

Antioxidant compounds that are able to function in FRAP assay approach are categorized as secondary antioxidants in which they suppress the formation of free radical and prevent oxidative deterioration. Secondary antioxidants are also playing a role in metal chelating and oxygen scavenging (Shian et al., 2012). Bananas are known as a weak primary antioxidant source but a powerful source of secondary antioxidant (Haripyaree et al., 2010). There was no previous research study on the effect of gamma irradiation on FRAP values of pisang nangka. The difference in the results from other studies can be due to types of plants used, chemical composition, solvent used for extraction and other individual factors (Gumus et al., 2011).

4.5 Correlation between Antioxidant Contents and Antioxidant Activities

Pearson's correlation analysis was used to determine the relationship between the antioxidant contents and antioxidant activities. Table 4.5 shows the correlation coefficients (r) between TPC, TFC and antioxidant activities of ethanol extract of pisang nangka.

Table 4.5: Pearson's correlation analysis of the antioxidant contents and antioxidant activities of ethanol extracts of pisang nangka

		DPPH (EC_{50})	FRAP
TPC	r-value	-0.198	0.203
TFC	r-value	0.076	0.273

**Correlation is significant at $p < 0.01$; *Correlation is significant at $p < 0.05$. TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant potential.

According to Table 4.5, the findings indicated that there was no significant difference ($p > 0.05$) in correlation between antioxidant contents (TPC and TFC) with antioxidant activities (DPPH and FRAP assay). There was weak and negative correlation ($r = -0.198$) between DPPH (EC_{50}) with total phenolic content (TPC) of ethanol extracts of pisang nangka. This weak and negative correlation meant increase in TPC had fewer tendencies to cause increase in DPPH radical scavenging activity which was showed by lower EC_{50}. Moreover, there was no

correlation (r = 0.076) between DPPH (EC_{50}) with TFC. This indicated that there was no relationship between TFC and DPPH. It revealed that flavonoid compound in pisang nangka extracts had no influence on antioxidant activity by DPPH method. Furthermore, there was also weak and positive correlation between FRAP with TPC (r = 0.203) and TFC (r = 0.273) as shown in Table 4.5. This weak and positive correlation indicated that increase in TPC or TFC will have less tendency to cause an increase in FRAP value. This predicted that the TPC and TFC contributed to a weak antioxidant activity of this plant species that evaluated by DPPH and FRAP assay. This also indicated that TPC and TFC were not the one contributed to DPPH scavenging activity and FRAP but could be other antioxidant compounds such as ascorbic acid, tocopherol, beta carotene, dopamine or gallocatechin identified in the banana (Qusti et al., 2010).

A previous study showed significant correlation between total phenolic content, flavonoid, carotenoid content in various banana peels extracts with their IC_{50} of DPPH scavenging activities (Fidrianny, Anggraeni & Insanu, 2018). Another previous study indicated that there was high correlation between FRAP and TPC for three cultivars of banana studied which were *Berangan, Mas* and *Raja* (Shian et al., 2012). The present finding was consistent with the study by Nor Liyana et al. (2019), who obtained the similar result in which antioxidant activity assessed by DPPH free radical scavenging activity was showing a weak correlation with TPC (r = 0.309) and TFC (r = 0.254) of *A. bubalinum* extracts. Moosavi et al. (2014) observed no correlation between TPC and TFC content with DPPH of almond hull extract and this can be

explained by the effects of irradiation on other chemical present such as tannins and lipids. Moreover, another study suggested that the Pearson's correlation coefficient between TFC of *Sesbania sesban* leaves extract with EC_{50} scavenging of DPPH gave no significant correlation (Fitriansyah et al., 2017). In addition, Akbari et al. (2012) also observed low correlation coefficient between TPC and DPPH free radical scavenging activity in kernel extract. Another previous study suggested that a weak correlation of r= 0.161 was observed between TPC and FRAP of ethanol extracts of common culinary herbs and spices (Ramkissoon et al. 2012).

However, Fitriansyah et al. (2017) observed that TPC in *Sesbania sesban* leaves extract had significant and negative correlation with EC_{50} of DPPH with r= -0.943. Another study suggested that total phenolic content (TPC) showed significantly positive strong correlation with DPPH (r = 0.934*) and FRAP (r = 0.993*). This showed that TPC had the strong correlation with FRAP value and followed by DPPH (Pinsirodom et al., 2018). Also, a positive correlation between TPC and antioxidant activities in both DPPH (r = 0.728) and FRAP (r = 0.741) were observed in irradiated pistachio nuts (Akbari et al., 2018). Moreover, Nor Liyana et al. (2019) reported that FRAP assay was significantly strongly correlated with TPC (r = 0.705**) and TFC (r = 0.804**). Kavitha et al. (2015) also reported that DPPH scavenging radical activity was strongly correlated with TFC of irradiated Chinese dates. In addition, Nilima and Kunda (2012) reported that TPC was in good correlation with FRAP values and DPPH.

The inconsistent findings from the studies could be probably due to different type of plant species, different individual phenolic present, extraction procedure, dose of irradiation, chemical composition and solvents used for extraction (Gumus et al., 2011; Ghadi et al., 2015). Besides that, irradiation can influence the levels of antioxidants or phytochemicals and the capacity of a plant to produce the antioxidants at different levels. It has been shown that the concentration of antioxidants in plant might be enhanced under certain favorable conditions. These conditions can include storage at low temperature, wounding, exposure to radiation sources, dose of radiation applied, sensitivity of the antioxidants towards irradiation and the effect of irradiation on the plant species. These conditions are responsible for the production and accumulation of antioxidants in the plant (Alothman et al., 2009). In addition, extraction techniques and solvent used for extraction had been shown to play a significant role on the concentration of antioxidant determined (Ngo et al., 2017; Quy et al., 2014; Sultanana et al., 2009).

CHAPTER 5

CONCLUSION, LIMITATION AND RECOMMENDATION

5.1 Conclusion

This study was conducted to investigate the total antioxidant contents and total antioxidant activities of ethanol extraction of non-irradiated and irradiated *Musa paradisiaca formetypica* L. var. Nangka extract powder at dose 3, 6 and 9 kGy. Total phenolic content was determined by using Folin-Ciocalteu method while total flavonoid content was determined by using aluminium chloride colorimetric method. Meanwhile, total antioxidant activity was determined by using DPPH free radical scavenging assay and ferric ion reducing antioxidant potential (FRAP) assay.

In this study, there was no significant different ($p > 0.05$) of total phenolic content between non-irradiated and all irradiated extracts at dose 3, 6 and 9 kGy. The highest level of total phenolic content (TPC) was found in irradiated extract at 3 kGy. There was significant different ($p < 0.05$) of total flavonoid content between non-irradiated and irradiated extracts at dose 6 and 9 kGy. As for total antioxidant activities, there was no significant difference ($p > 0.05$) of DPPH EC_{50} between non-irradiated and irradiated extracts at dose 3, 6 and 9 kGy. The highest level of EC_{50} was found in non-irradiated extract of pisang nangka. Next, there was also no significant difference ($p > 0.05$) of FRAP value between non-irradiated and irradiated extracts at dose 3, 6 and 9 kGy. The highest level of FRAP value was found in irradiated extract at 6 kGy.

It was demonstrated that a weak and negative correlation was found between DPPH EC_{50} and TPC with r= -0.198. This correlation indicated that higher TPC level was less tendency to result in a lower DPPH EC_{50} which indicated a higher radical scavenging activity. Moreover, there was no correlation found between DPPH EC_{50} and TFC with r= 0.076. This showed that that flavonoid compound in pisang nangka extracts had no influence on antioxidant activity by DPPH method. On the other hand, a weak and positive correlation was found between FRAP and TPC with r= 0.203. This correlation indicated that higher TPC level was fewer tendencies to result in higher FRAP value. Also, there was a weak and positive correlation between FRAP and TFC with r= 0.273. This correlation indicated that higher TFC level was fewer tendencies to

result in higher FRAP value. This had predicted that the TPC and TFC contributed to a weak antioxidant activity of this plant species that evaluated by DPPH and FRAP assay.

In conclusion, this study had provided a rough estimation of total antioxidant content and activity of non-irradiated and irradiated ethanol extracts of pisang nangka at dose 3, 6 and 9 kGy. This study manage to show the application of gamma irradiation by slightly increasing most of the antioxidant contents and activities of ethanol extracts of pisang nangka particularly at lower dose of irradiation. Gamma irradiation has the potential to be one of the approaches to preserve the fruits and at the same time to maintain or increase antioxidant properties of the fruits.

5.2 Limitation

The limitation in this study was little repetition due to time constraints which resulted in big standard deviation. Also, there was occurrence of technical errors during the experiment which could probably result in big value of standard deviation. For example, limitation in the technique of using a pipette and limitation in technique of extraction probably can cause inconsistent reading. In addition, most of the antioxidant assays were sensitive to light and need to be conducted in a dark environment. However, there might be a little light exposure during the experiment such as sunlight from windows or lighting used by other users in the lab that were unavoidable. Thus, these can interference with the result of the experiment.

The result from this study cannot be generalized to overall species of pisang nangka because the use of convenient sampling can influence the demographic, origin and cultivation of plants which will result in different antioxidant properties. There was also limitation in determining antioxidant activities because there were only two assays conducted and two of these assays did not show significant effects for most of the tested samples. Furthermore, there was only one extraction method which was ethanol extraction used in this study which could probably give limited outcome for all tested samples of pisang nangka.

5.3 Recommendation

This research study will be useful to provide baseline information for the future study to explore the potential and application of gamma irradiation on antioxidant properties of plants species. The limitations of the present study revealed that TPC and TFC of the extracts did not always correlated with antioxidant activity by DPPH and FRAP assays. Therefore, a future study is needed to identify phenolic and flavonoids compounds in the pisang nangka extracts. High Performance Liquid Chromatography (HPLC) is recommended to be conducted for isolation and purification of phenolic and other antioxidant compounds in order to know the bioactive compounds present that can contribute to the increase of antioxidant properties.

Moreover, the probability sampling method is recommended to use in order to ensure the generalizability and validity of samples. Large number of repetition of experiment is recommended to provide consistent findings and reduce standard deviation. In addition, future study is recommended to use and compare other types of extraction solvent such as methanol, acetone and hexane because different polarity of solvents gives different outcomes for antioxidant properties. Other antioxidant assays such as ABTS and ORAC were recommended to determine antioxidant activity of non-irradiated and irradiated pisang nangka. Furthermore, the experiment is suggested to be carried out in the dark environment because the antioxidants and the reagents used are sensitive to light.

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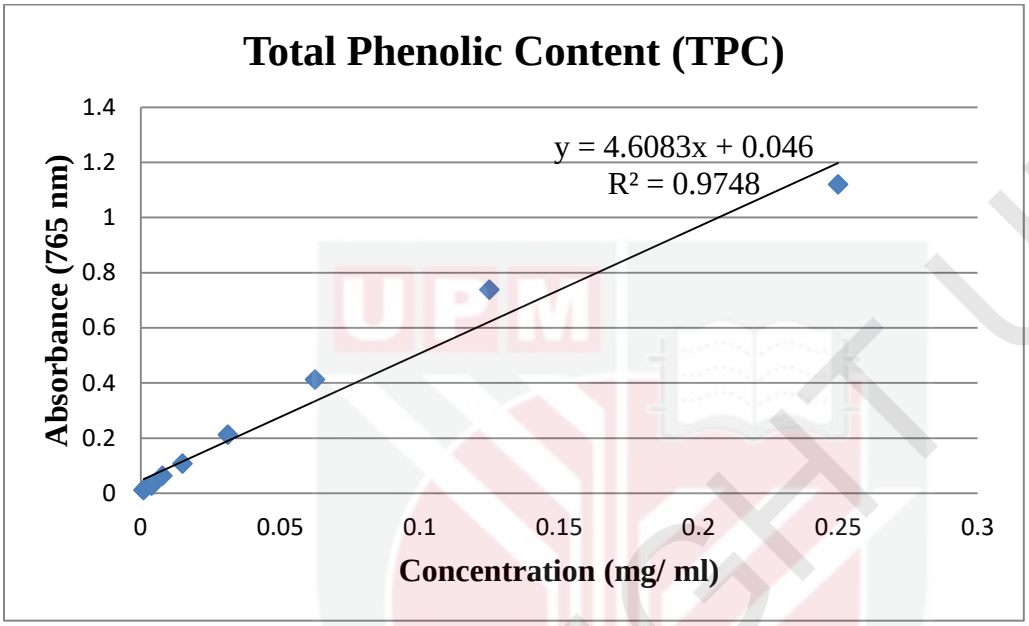
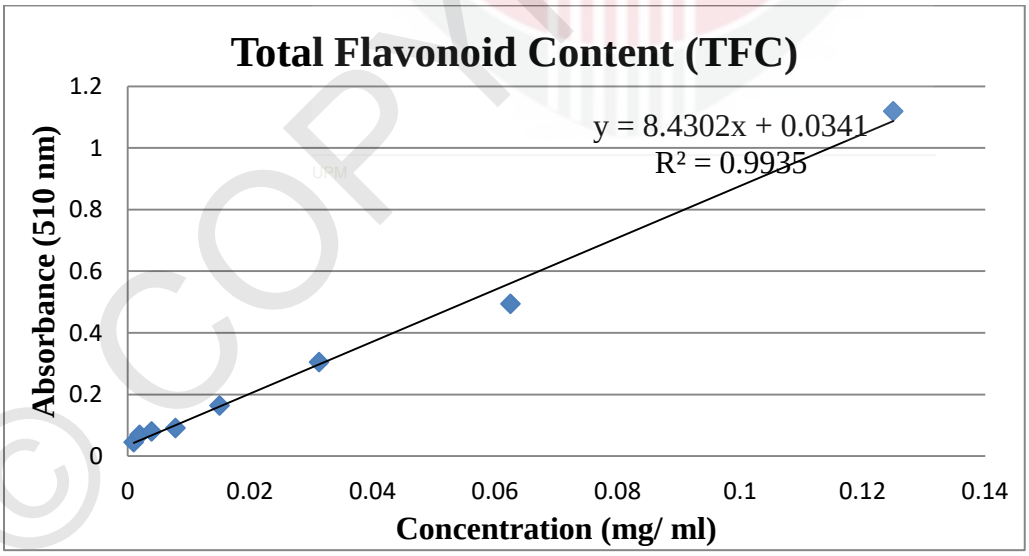
APPENDICES

Appendix A: Gallic acid standard curve for total phenolic content (TPC)



Appendix B: Catechin standard curve for total flavonoid content (TFC)



Appendix C: Ferrous sulphate standard curve for FRAP assay