CHAPTER 2

Literature Reviews

2.1 Mango

Mango (*Mangifera indica* L.) comprising more than 70 genera is one of members of the *Anarcadiaceae* family. Nowadays, mango has become a major fruit crop of the tropical and subtropical countries, especially in Asia (Litz, 2009; Kamle *et al*., 2013; Sellamuthu *et al*., 2013; Chutichudet *et al*., 2016). The mango tree is an arborescent evergreen. Mature trees can attain more than a height of 40 meters, and can survive for hundreds of years. The trees that have been domesticated by selection from openly pollinated seedling populations show variation in shape and size of tree. The mango fruit is large and fleshy with an edible mesocarp of varying thickness. The mesocarp is resinous and highly fluctuating with size, shape, color, fiber content and flavor. The flesh of mango fruit has flavor ranging widely from turpentine to sweet. The exocarp of this fruit is thick and glandular. The shape of fruit has many types, including elongate, oblong and ovate or intermediate forms involving two of these shapes. Fruit length depending on the cultivar can range from 2.5 to more than 30 cm. The characteristics of endocarp are woody, thick and fibrous with the fibers arising from the endocarp (Litz, 2009).

The mango fruit is climacteric and during ripening, ethylene production occurs. Carotenes, anthocyanins, chlorophyll and xanthophylls can be found all in the fruit. The skin color is commonly a mixture of green, yellow and red pigments, however color of mature fruit is genotype dependent. The chloroplasts in the peel become chromoplasts, containing yellow and red pigments during ripening (Mitra and Baldwin, 1997). The red blush is caused by the presence of anthocyanins (Lizada, 1991). The pulp carotenoids in ripe fruit also change with respect to mango cultivar (Mitra and Baldwin, 1997). Mango fruit compositions include carbohydrates, proteins, amino acids, fatty acids, minerals, organic acids and vitamins. During the ripening process, the fruit is initially acidic and high in vitamin C content. Ripe mangoes have vitamin C quantity at moderate levels, but are fairly rich in provitamin A together with vitamins B_1 and B_2. The acidity of mango fruit is basically due to the presence of malic and citric acids. The function of carbohydrates, organic acids, lactones, monoterpene hydrocarbons and fatty acids form flavor of the mango mesocarp (Mitra and Badwin, 1997). During ripening, starch accumulating in the chloroplasts is hydrolyzed to sucrose, glucose and fructose (Kumar *et al*., 1994), concentration of sucrose is slightly higher concentrations than either fructose or glucose and it is also the principal sugar of ripe mangoes. Organic acid content decreases to 0.1-0.2 % during mango fruit maturation. Citric acid is the dominant organic acid however glycolic acid, malic acid, tartaric acid and oxalic acids are also present. The presence of lactones makes peach-like flavor of mangoes (Wilson *et al*., 1990; Litz, 2009).

Thailand is in the fourth rank of producing mango in the world (1.8 million tones production), with a 27 % share of world market and an emphasis in Japan and Europe (Wongkaew and Likittrakoolrung, 2009; Schulze *et al*., 2013). There are many popular types of mangoes grown for export in Thailand, including Chok Anan, Maha Chanok, Nang Klangwan, and Nam Dok Mai (Chutichudet *et al*., 2016). The Nam Dok Mai mango is one of the most famous cultivars for consumption at the ripe stage in Thailand and also for export (Thai Mango-Ma-Muang, 2008; Schulze *et al*., 2013). It is oval shape with a sharp-pointed tip. The ripe fruits are golden yellow with deep yellow flesh, and the taste is sweet, slightly sour and scented (Thai Mango-Ma-Muang, 2008). Many people like to eat the Nam Dok Mai because it is a fiber less mango with smooth flesh and a deliciously unique flavor (Kannika and Sanguansri, 2014). For nutritional value, the mango is rich in vitamin C, B_1, B_2, β-carotene, carbohydrates, protein, calcium, phosphorus and antioxidant potential (Vásquez-Caicedo *et al*., 2002).

Nam Dok Mai mangoes for exporting presented 9.7-16 ^{o}Brix according to the ripe stage of fruits at different harvest maturities (Watanawan *et al*., 2014). The fruit also possessed moisture and total phenolic content (TPC) about of 84 % wb (Laohaprasit *et al*., 2011) and 113 ± 5.5 mg GAE/100g FW (Patthamakanokporn *et al*., 2008), respectively. Jongsri *et al*. (2016) found that percentage of titratable acidity (TA) of this mango cultivar was in the range of 0.53-1.65 % following to storage time from 0-8 days, while the ascorbic content was from 90-175 μg/g FW. Nam Dok Mai mangoes were observed to contain 1658-11249 μg β-carotene/100 g DW as well (Vasquez-Caicedo *et al*., 2005).

2.2 Mango anthracnose (*Colletotrichum gloeosporioides*)

Anthracnose is one of the most prevalent postharvest diseases of mango in humid growing regions. This disease can affect almost 100 % on mango fruits produced under wet or very humid conditions (Litz, 2009). High relative humidity (95 %) and moderate temperature (20-30°C) for a period of 12 h are essential conditions for infection and growing of anthracnose disease on the fruits (Kamle *et al*., 2013). Mango anthracnose is caused mainly by the ubiquitous fungus *Colletotrichum gloeosporioides* Penz. and Sacc. which is the asexual stage of the pathogenic fungus (anamorph stage) (Nelson, 2008; Kamle *et al*., 2013). *C. gloeosporiodes* may attack many parts of the mango such as leaves, twigs, petioles, flower clusters and fruits, depicted in Figure 2.1 (Nelson, 2008; Kamle *et al*., 2013; Xu *et al*., 2017). On leaves, lesions are small, angular, brown or black spots that can enlarge to make huge dead areas. For flower clusters, the first symptoms on are small black or dark-brown spots, which can enlarge, coalesce, and the flowers will be killed before fruits are produced, enormously losing of yield. Petioles, twigs, and stems are also susceptible and easily become the typical black. Ripe fruits affected by anthracnose fungus develop sunken or prominent, dark brown to black decay spots before or after collecting. Fruits may drop from trees even before ripening. The spots on the infected fruit can and usually do coalesce and then penetrate deep into the flesh, forming considerable fruit rotting. Most immature fruit infections remain quiescent and commonly invisible until the postharvest time. According to Arauz (2000), infected mango fruits reaching approximately diameter of 4 cm possess the natural defense mechanisms that help to protect them from anthracnose by inducing the mold into a latent period. However, these natural defense mechanisms will be broken down when the fruits get ripe and soft during mature process. Therefore, mangoes appearing healthy at picking can grow significant anthracnose symptoms rapidly upon ripening and the whole fruits become rot in days.

Nelson (2008) explained that the cycle of anthracnose disease (Figure 2.2) is:

- Dissemination: splashing rain or irrigation water disperse spores (conidia) of the fungus pathogen.

- Inoculation: sites including panicles, leaves, branch terminals are infected fungus spores.
- Infection and pathogen development: on immature fruits and young tissues, spores germinate and penetrate through the cuticle and epidermis to enlarge through the tissues. On mature fruits, infections penetrate the cuticle, but remain latent until ripening of the climateric fruits begins.
- Symptom and disease development: black, sunken, rapidly expanding lesions develop on affected tissues.
- Pathogen reproduction: sticky masses of conidia are produced on symptomatic tissue, especially during moist conditions. Many cycles of disease can happen because the fungus continues to multiply during the season.
- Pathogen survival: on infected and defoliated branch terminals and mature leaves, the pathogen can survives.

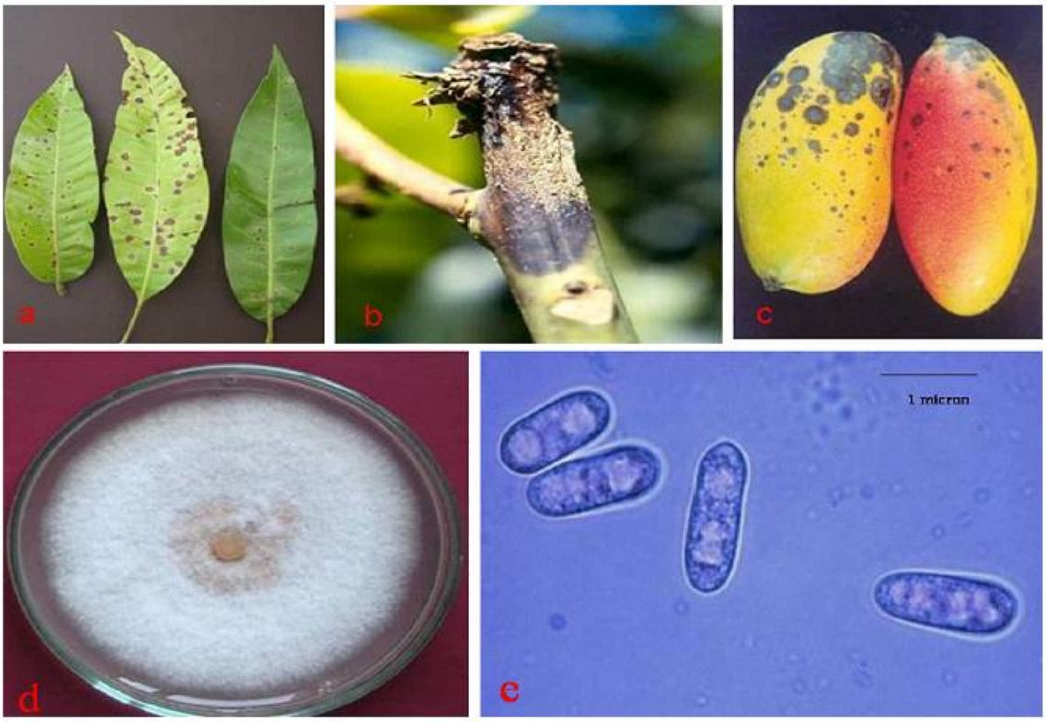

Figure 2.1 *Colletotricum gloeosporioides* caused anthracnose disease

(a) Black spot on leaf of mango, (b) on apical tip of mango, (c) symptoms of anthracnose on mango fruits, (d) pure culture of *C. gloeosporioides* and (e) spores of *C. gloeosporioides*

Source: Kamle *et al*. (2013)

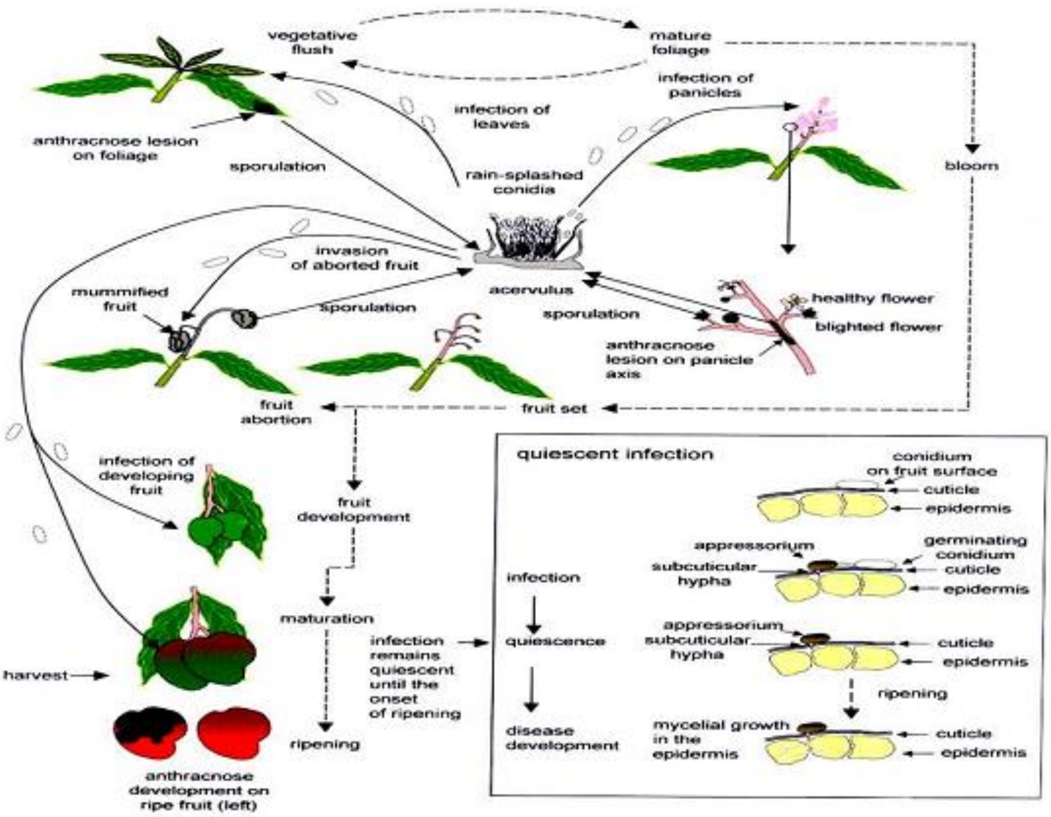

Figure 2.2 Disease cycle of anthracnose

Source: Arauze (2000)

C. gloeosporioides begins its life cycle by germination of spores on the surface of plant to form appressoria (melanized infection structure) which is followed by penetration of host tissue. At this point, the stage named biotrophic infection producing thick hyphaes in primary infected cells will happen, then the mold will switch suddenly to necrotrophic infection phase characterized by creation of thin secondary hyphases derived from the primary one which initiates colonizing the nearby cells and leads to visible lesions (black spots) at the infected point. Finally, the spores formed on the surface of infected tissue will be spread by insect, water or wind to begin another infection cycle (Sharma and Kulshrestha, 2015).

During colonization in host tissue, the fungus produces alkalinizes surroundings. The transcription factor, called pacC, is a regulator of pH-controlled genes and is important for the success of colonization. Pel-B gene is a virulent gene and encodes for pectate lyase which degrades the plant cell wall and can be observed easily in necrotrophic phase of infection. Pectate lyase expression is highly impacted by alkalinization occuring naturally during ripening of fruits, where the pH of the pericarp increases from 5.2 to 6.1 and the mold also helps in increase the amount of ammonia accumulated by the host. This increase pH may affect the pacC expression which is the important component of the pH-dependent genes known to regulate the expression of Pel-B gene (Sharma and Kulshrestha, 2015).

- **Conventional management of *C. gloeosporioides***

Generally, anthracnose disease of mango is controlled by applying suitable preharvest and postharvest management practices including hot temperature treatment (non-chemical control) and usage of fungicides (chemical control) (Sivakumar *et al*., 2011). In many countries, hot water treatment (HWT) is widely utilized for disease control in mango fruits. Reduction of anthracnose infection by more than 80 % was observed for the fruits treated with hot water (52-55ºC) for 10 min. The temperature of water and treatment time was decided by the size and maturity of mango (Jacobi *et al*., 2001). Applying HWT to mango is recommended within 24 h after harvest. However, improper HWT consisting of unfavorable higher temperature or increasing immersion time can cause scalds of skin, lenticels spotting and retention of immature starchy areas in flesh of fruit (Sivakumar *et al*., 2011). In addition, total soluble phenolics and antioxidant quality of mango treated HWT were found to be reduced after 4 days of storage. Thus, it is possible to lose some nutritious indexes of mango fruits subjected to HWT because of heat trigger oxidation process during long storage period and prolonged transportation (Kim *et al*., 2009).

The usage of fungicide as a chemical method in orchard having infected plants is also an effective control of this pathogen. There are diverse preharvest and postharvest types of fungicides against anthracnose mold applied at the interval of 2-4 weeks in the orchard in dry season. Postharvest fungicide usually is used for fruit crops which are already infected with the fungus, while the preharvest one are routinely used from flowering through to harvest (Sharma and Kulshrestha, 2015). Nevertheless, application of fungicide for extended durations may cause the formation of fungicide-resistant strains of the anthracnose mold. Besides, fungicide residues existing on the fresh produce are also harmful compounds to health of consumers (Ong and Ali, 2015).

2.3 Pesticides on mango

2.3.1 Pesticides and risk assessment

Pesticides are defined as any substances or substance mixtures that are applied for preventing, destroying or repelling of pests. The term of pests involves insects, bacteria, fungi, and viruses which mainly cause crop diseases. Categorization of pesticides can be based on their targeted pests or their chemical structures. For the former, pesticides are classified into insecticides, herbicides, fungicides, and many other substances, while there are three main chemical groups of them which are carbamates, organophosphates and organochlorinated pesticides (Hu *et al*., 2016). Pests and diseases may make up to one-third of loss of crop, therefore it is very important for using pesticides to increase agricultural production (Leo and Hamir, 2010).

However, pesticides are also toxic compounds, the rapidly increasing use of pesticides together with insufficient technical advice or research has caused many environmental and human health problems. A major concern regarding health hazards is primarily carcinogenicity, teratogenicity, allergic reactions, neurotoxicity, and effects on the immune and reproductive systems (Leo and Hamir, 2010). Direct exposure normally occurs during the application of pesticides while indirect exposure can happen through the environment or the ingestion of food (Tadeo, 2008). The problem is more considerable to fresh fruits and vegetables which are often consumed without washing or just with minimal processing (Misra, 2015). According to pesticide report of United State Food and Drug Administration (USDA), there are significantly higher number of domestic fruits and vegetables having detectable pesticide residue levels over the standard of Environmental Protection Agency (EPA) in comparison to other foods such as fish, dairy and grain products (FDA, 2012).

Maximum residue levels (MRLs) about pesticide residues in food stuff built from the assumption that good agriculture practices (GAPs) are applied to the usage of pesticides in plantations have been established in many countries for preventing the potential health hazards caused by these substances. When applying of pesticides follows to GAPs, MRLs will not be exceeded, however, the improperly application may leave toxic compounds involving possible health risks as well as environmental pollution (Basfar *et al*., 2012).

2.3.2 Common pesticides registered for mangoes

Among various pesticides, chlorpyrifos, belonging to organophosphorus (OP) group, is one of the most popular insecticides for many crops including mango (Srivastava *et al*., 2014). A systemic fungicide of carbamates group named carbendazim is also commonly used to control postharvest diseases of mango such as anthracnose (*C. gloeosporioides*) and stem end rot (*Lasiodiplodia thepbromae*) through both preharvest spray and postharvest dip or spray in hot fungicidal solution (Bhattacherjee and Pandey, 2010). Besides, the use of synthesis pyrethroid is approximately 5-7 % of the total quantity of pesticides largely applied in Thailand. Cypermethrin, one of pyrethroid pesticides, was observed to be the highest detection percentage in fruit samples at 95.3 % (Pakvilai *et al*., 2011).

2.3.2.1 Organophosphorus

Organophosphorus (OP) pesticides are hydrocarbon compounds containing one or more phosphorus atoms in their molecule (Tadeo, 2008). Among many types of pesticide, OP pesticide group is one of the most popular insecticides commonly applied to control noxious insects on farming for a wide range of fruits, vegetables and grains in the world (Bai *et al*., 2010). The diversity of organophosphorus insecticide types makes them to form the most versatile group. There are compounds with non-residual action and prolonged residual action, and compounds with a broad spectrum and very specific action (Tadeo, 2008).

Chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridinyl) phosphorothionate], an organophosphorous insecticide (Figure 2.3), is widely used for controlling pests of different agricultural products and applied as an alternative to oganochlorine compounds in various cases (Ling *et al*., 2011). However, chlorpyrifos can cause adversely environmental effects due to its considerable use, persistence nature and wide distribution (Saini and Kumar, 2016). In addition, the mode action of chlorpyrifos is binding and inhibition of enzyme acetyl cholinesterase disrupting nerve functioning and causing potential hazards in human beings (Ling *et al*., 2011). It is also reported that chlorpyrifos leads to the damage of lung and central nervous system, autoimmune disorders, and vomiting (Saini and Kumar, 2016). According to Codex Alimentarius Commission, FAO/WHO Food Standard Programme (2016), the

Maximum Residue Limits (MRLs) of chlorpyrifos on spices, fruits and berries is 1 ppm.

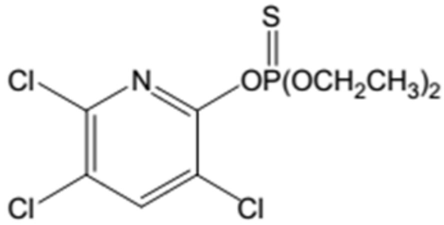

Figure 2.3 Chlorpyrifos

Source: Tadeo (2008)

2.3.2.2 Carbamates

Carbamates pesticides are the compounds derived from carbamic acid with a wide variety of chlorine, alkyl, akylthio, alkoxy and dialkylamino side chains. Carbamates pesticide group possesses useful insecticidal properties with high biocide activities (Tadeo, 2008; Bini Dhouib *et al*., 2016).

Carbamates are extensively used in modern agriculture all over the world as insecticides, fungicides, herbicides, nematicides, and sprout inhibitors. These compounds are part of a large group of synthetic pesticides which have been extensively developed, produced, and applied within the last 50 years. However, carbamates are also potentially toxicants to different kinds of organisms because they are easily absorbed and tend to accumulate in soil, plants, foodstuffs and ground or surface waters. They have the mode action which is quite same with organophosphate pesticides. It is the inhibition of cholinesterase enzymes affecting the nerve transmission function (Soloneski *et al*., 2015).

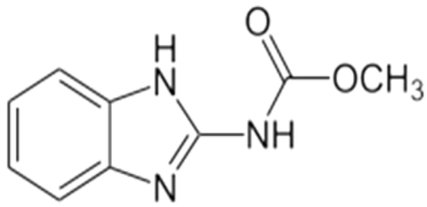

Figure 2.4 Carbendazim

Source: Tadeo (2008)

Belong to carbamate group, carbendazim (Methyl 1H-benzimidazol-2-ylcarbamate) is a kind of benzimidazone fungicide (Figure 2.4) used as, foliage spraying, seed dressing and soil treatment (Ma *et al*., 2015a). Carbendazim makes influence on the diffusion of cells as well as causing an effect in killing microorganisms by interfering the formation of pathogen during mitosis, which can prevent diseases caused by fungi on multiple kinds of crops (Chen *et al*., 2015). Carbendazim is a light toxicant to mammals, but with its higher dose, this compound may damages their reproduction and growth (Daundkar and Rampal, 2014). According to Codex Alimentarius Commission, FAO/WHO Food Standard Programme (2016), the Maximum Residue Limits (MRLs) of carbendazim on berries and other small fruits is 1 ppm, while MRLs of this pesticide on mango is 5 ppm.

2.3.2.3 Pyrethroids

Pyrethrins possessing insecticidal activity are natural chemicals obtained from pyrethrum, of the chrysanthemum species flowers (Barr, 2008; Tadeo, 2008). The insecticide properties are due to five esters that are mostly present in the flowers. These esters have asymmetric carbon atoms and double bonds in both alcohol and acid moieties (Tadeo, 2008). Natural pyrethrins have many isomeric forms and are usually classified as the pyrethrin I and II isomer (Barr, 2008). Synthetic pyrethrins, called pyrethroids and produced to mimic the action of natural ones, show more selective and better activity against a larger spectrum of pests than pyrethrins (Barr, 2008). Pyrethroids are considered as contact toxicants, affecting the nervous system of insects and depolarizing the neuronal membranes (Tadeo, 2008). However, pyrethroids can persist longer in the environment than their natural counterpart. The typical symptoms of pyrethroid poisoning in humans consist of nausea, vomiting, respiratory depression, mental change, acute kidney injury (Chen *et al*., 2016).

Cypermethrin ((RS)-alpha-cyano-3 phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate), a highly active synthetic pyrethroid insecticide (Figure 2.5), has been used to control pests in domestic, industrial and agricultural situations for more than 30 years. Formulations of cypermethrin consist of eight stereoisomers, however only (S)-(1R)-cis and (S)-(1R)-trans isomers have activity against insects (Liu *et al*., 2016). According to Codex Alimentarius

Commission, FAO/WHO Food Standard Programme (2016), MRLs of cypermethrin on mango is 0.7 ppm.

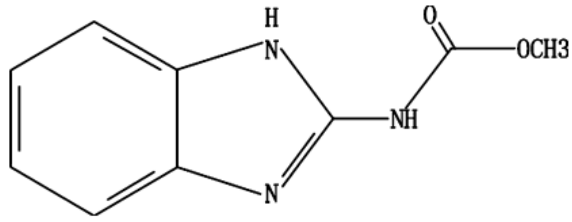

Figure 2.5 Cypermethrin
Source: Chen *et al*. (2015)

2.3.3 Analysis of pesticides residues in fruits and vegetables by using chromatographic methods

For determination pesticide residues in fruits and vegetables, various extraction and quantification methods are applied (Sharma *et al*., 2010). There are two mainly reasons for the difficulty of pesticide residue detection. The former is the wide varieties and complex composition of the pesticide, while another is the detection requirements of trace analysis (Chen *et al*., 2015). Recently, high performance liquid chromatography (HPLC) (Aschi *et al*., 2007), gas chromatography (GC) (Fenoll *et al*., 2007), gas chromatography–mass spectrometry GC-MS (Filho *et al*., 2010) and gas chromatography–tandem mass spectrometry (GC-MS/MS) (Arrebola *et al*., 2003) are common methods of pesticide residues detection due to their high separation power, selectivity and identification capabilities (Sharma *et al*., 2010; Chen *et al*., 2015).

Various sensitive detectors coupled with GC include electron capture detector (ECD) (Amvrazi and Tsiropoulos, 2009; Bhanti and Taneja, 2007), nitrogen phosphorus detector (NPD) (Amvrazi and Tsiropoulos, 2009; Hernández-Borges *et al*., 2009), flame ionization detector (FID), pulsed flame photometric detector (GC-PFPD), flame photometric detector (FPD) (Darko and Akoto, 2008) which help to improve the detection and quantification procedures of pesticide residues measuring in different matrices (Tadeo, 2008; Leo and Harmir, 2010; Sharma *et al*., 2010).

The most frequently used extraction solvents in pesticide residue studies are acetonitrile, acetone, dichloromethane, hexane, ethyl acetate and methanol which provide high recoveries of pesticides over a wide range of polarity (Hernandez *et al*., 2006; Knežević and Serdar, 2009; Chen *et al*., 2009).

2.4 Plasma and non-thermal plasma

Plasma or gas discharge plasma is a mixture of partly ionized gas (Niemira, 2012; Pankaj *et al*., 2014) that consists of active species (Surowsky *et al*., 2013; Mai-Prochnow *et al*., 2014). These include electrons, charged ions, free radicals, excited molecules, photons and atoms (Fernández *et al*., 2012; Stoica *et al*., 2013; Pankaj *et al*., 2014). Electron collision plays a key role to generate these reactive species (Mai-Prochnow *et al*., 2014). Artificial plasmas can be created when applying energy across a dielectric gas or fluid (Niemira, 2012; Shakila *et al*., 2012). Any kind of energy which can ionize a gas such as microwave, radio frequency, electric or electromagnetic field, thermal, optical, radioactive and X-rays can be employed for generation of plasma (Fridman, 2008; Bárdos and Baránková, 2010; Afshari and Hosseini, 2014; Pankaj *et al*., 2014; Thirumdas *et al*., 2014). Among them, electric or electromagnetic fields are the most useful ionization tools (Maricica and Liliana, 2014).

There are two types of plasma called thermal and non-thermal plasmas. This classification is based on the thermodynamic equilibrium between electrons and ions. The former are in a state of thermodynamic equilibrium having temperature as well as energy of electrons and ions are approximately equal. Therefore, these equilibrium plasmas have high overall temperature up to 10^4 K together with higher number of ions over total plasma particles (ionization degree). In contrast, non-thermal plasmas (NTP) are in a thermodynamic non-equilibrium state with low degree of ionization. In these plasmas, electrons have high temperatures in order of 10^4 K, whereas temperature of ions and neutrals is close to room temperature, thus the active gaseous medium remains at low temperature which is safety for applying of NTP on thermal sensitive materials (Surowsky *et al*., 2014).

2.4.1 Typical sources for generation of NTP

Typically, non-thermal or cold plasmas are obtained by means of electrical discharges in gases (Misra *et al*., 2016). The developments in plasma physics and engineering as well as innovative designs of various plasma sources have allowed the generation of non-thermal (cold) plasmas at atmospheric pressure conditions (Pal *et al*., 2016) with less power input requirements. This mild operation condition not only supports to the continuous food processing, but also prevents undesired phase transitions of applications at reduced pressure (<1013 mbar) or low pressure (<10 mbar) (Schluter *et al*., 2013). Plasma jets, dielectric barrier discharges (DBDs), corona discharges and microwave discharges depicted in Figure 2.6 are typical sources for the generation of NTP.

Plasma jets configured single electrode or two electrodes produce small flames of plasma typically in the range of radio frequency (Figure 2.6a). The electrode gap is commonly in the millimeter range and the utilized gas is ignited at voltages of 100V or higher. Atmospheric pressure plasma jets have the main advantages including narrow gap penetration ability, targeted applicability and their small dimensions (Surowsky *et al*., 2014).

Dielectric barrier discharges (DBDs) are applied to generate plasma in the space between their two electrodes which are separated by a dielectric. This setup (Figure 2.6b) can be operated in both diffuse mode and filamentary mode. Uniform discharge is commonly created by diffuse mode, while filamentary mode consists of a lot small discharged channel along the area of electrode. Type of process gas, distance of the electrodes and the discharge electrical operation are essential parameters to control for this atmospheric pressure plasma source. The major advantages of DBDs include the large variety of gas which can be applied for generation of plasma, the low gas flow needed, the homogeneous discharges ignited over comparably big area together with different geometries of electrode leading to good adaptability. However, certain precautions and significant isolations are required due to the high ignition voltage (>10kV) of these plasma systems (Surowsky *et al*., 2014).

Corona discharges are usually produced by sharp electrodes such as tips or thin wires imposed high voltage where intensity of electric field is large enough to form electrons with ionization energy level of surrounding gas molecules and generation of plasma happens (Scholtz *et al*., 2015). Point-to-plate (Figure 2.6c) and cylindrical setups are typical configurations (Surowsky *et al*., 2014). The advantages of corona discharges are the simple device required with rather low operating costs. However, non-uniform treatments together with rather small areas for treated samples are also their disadvantages (Scholtz *et al*., 2010).

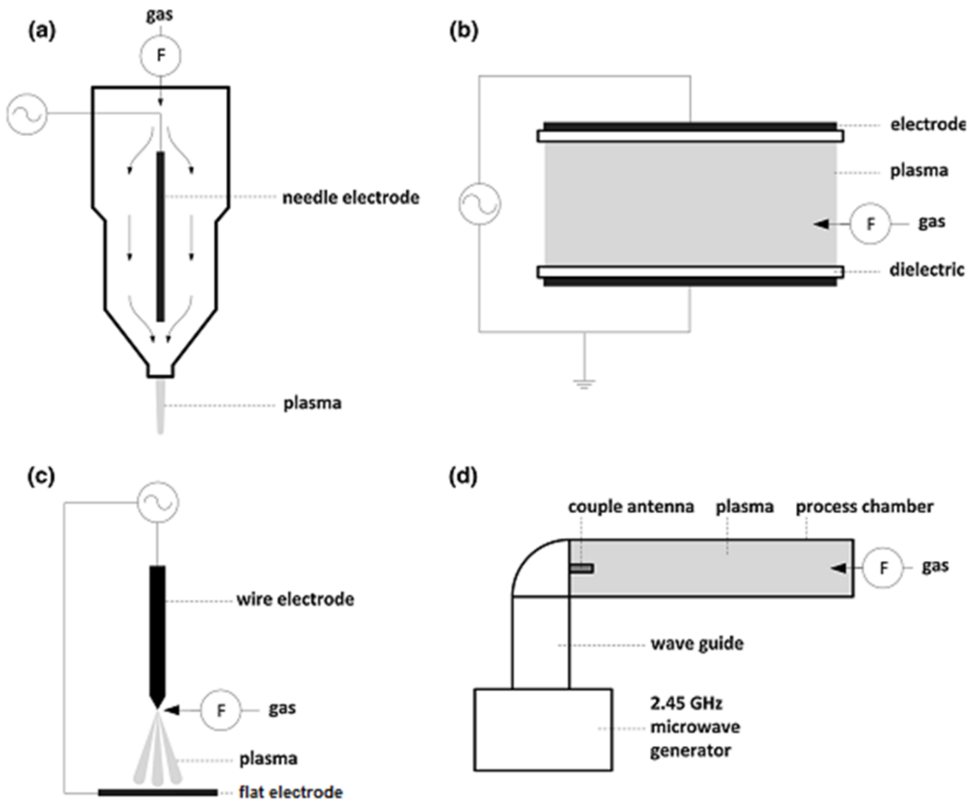

Figure 2.6 Schematic depiction of four common atmospheric plasma sources including (a) Plasma jet; (b) Dielectric barrier discharge (DBD); (c) Corona discharge (point-to-plate setup); (d) Microwave discharge

Source: Surowsky *et al*. (2014)

Microwave discharges (Figure 2.6d) are generated by electromagnetic waves at typically 2.45 GHz of frequency without electrodes. Existing electrons of process gas are increased in kinetic energy by absorbing the microwaves leading to the ionization reactions caused by inelastic collisions (Surowsky *et al*., 2014). Temperatures of neutral gas will be from room temperature to thousands of kelvin depending on the applied microwave power (Uhm *et al*., 2006). The no electrode setups and ignition in air environment are the main good points of microwave discharges, but the spatial limitation and ability of decontaminating large areas are the drawbacks of these atmospheric plasma sources (Surowsky *et al*., 2014).

2.4.2 Common gas used for forming NTP

Air, oxygen (O_2), nitrogen (N_2) or noble gases including helium (He), neon (Ne) and argon (Ar) are the most popular gases applied to generate NTP (Ma *et al*., 2017). The chemical composition of the feed gas is one of important factors determining reactions that the plasma can initiate. For any types of gas, the ionization voltage is identified by configured distance or the gap width between the electrodes and the gas pressure (Niemira, 2012). The impact of this relationship for various gases is showed in Figure 2.7. It can be seen that the lower pressure of gas is, the lower voltage for ionizing it is required, and at a given pd (pressure × distance), breakdown voltage (V_B) of noble gases is smaller than the others.

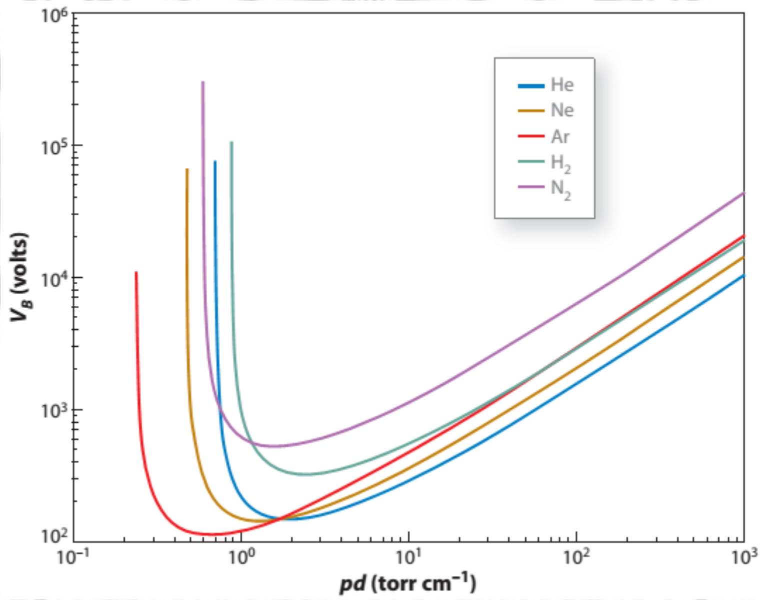

Figure 2.7 Ionization curve obtained for He, Ne, Ar, H_2 and N_2

V_B (breakdown voltage) as a function of pd (pressure × distance)

Assuming parallel plate electrodes

Source: Niemira (2012)

Reactive species like electrons, ions, radicals and UV radiation of gas plasma contributing to the effects against microorganisms or hazards may vary according to the type of feed gas and the energy sources applied to form plasma (Shintani *et al*., 2010). Further details about common gas types used for plasma generation are as follows:

- Oxygen: produces $O^{\bullet}$, $OH^{\bullet}$, $OOH^{\bullet}$ and other mixture of radicals for the sanitizing impact. Among them, $OH^{\bullet}$ radical often offers the most efficient for chemical and microbial decontamination, in spite of possessing extremely short lifetime. Etching effect is believed to be one of the main disinfect mechanisms of radicals derived from O_2 plasma. There was the more shrinkage of bacteria spores observed when applying the greater amount of plasma formed by O_2 gas. Nevertheless, the possible deterioration of sensitive materials due to etching phenomenon is also a mark drawback of O_2 gas plasma (Shintani *et al*., 2010).
- Nitrogen: requires high dissociation energy (approximately 9.91 eV) because of the triple bond in molecule leading to its resistance of ionization (Shintani *et al*., 2010).
- Noble gas (He, Ne or Ar): is quite more readily ionized than many other gases, thus they support to reduce the required ionization voltage as well as the consumed electricity and the cost of supplying power. However, cold plasma system processed with noble gas like He will be significantly more expensive than that applied air or other mixture of N_2 and O_2. Among noble gases, making plasma from Ar gas offers some advantages. Firstly, this gas is abundant in the atmosphere as a result of the radioactive decay of potassium and therefore cheaper than other noble gases. In addition, a pure Ar stream can only result in neutral, active, or ionized Ar species which will not react or combine with others to make molecules or substances (Niemira, 2012).
- Air: there is no expensive gas supply system needed for plasma sources operated with ambient air. However, plasmas driven in this type of gas will create a lot of different reactive agent composition, for instance active species, heat, radiation compared to pure argon plasma (Winter *et al*., 2013).

2.4.3 Measurement of active components produced by NTP

NTP which is generated at atmospheric pressure conditions normally comprises of different particles including excited or non-excited atoms and molecules, free electrons, charged ions, free radicals, coexisting with UV photons and visible light (quanta of electromagnetic radiation) (Fernandez *et al*., 2012; Misra *et al*., 2016). Most of plasma active species often possess high antimicrobial activity characteristically (Lacombe *et al*., 2015; Misra *et al*., 2016).

At atmospheric pressure conditions, generated NTP comprises of different active species such as UV photons, neutral or excited atoms and molecules, charged ions, free radicals and free electrons. However, the composition and density of these species alternate considerably according to the used plasma source (Scholtz *et al*., 2015). In general, the majority of reactive agents in commonly sources of NTP are as follows:

- excited oxygen O_2 and nitrogen N_2,
- reactive oxygen species (ROS) including atomic oxygen O, singlet oxygen $^1O_2$, superoxide anion O_2^- and ozone O_3,
- reactive nitrogen species (RNS) such as atomic nitrogen N, excited nitrogen N_2, nitric oxide NO•,
- if humidity is involved, H_2O^+, OH^- anion, OH• radical or hydrogen peroxide (H_2O_2) is also presented.

Plasma diagnostics can be carried out by optical emission spectroscopy (OES) and electric measurements. Additionally, image determined by an intensified CCD camera (ICCD) together with other visual records complete the description of the discharge creation and operation. Solution quality and formation of some chemical species is estimated by UV-VIS absorption spectroscopy, colorimetric methods or inductively coupled plasma (ICP) (Kozáková, 2011). Mass spectrometry also gives the possibility to measure the ion flux of electrodes directly and determine the ion composition of the plasma (Parvulescu *et al*., 2012).

2.4.4 Action of plasma on microorganisms and pesticides

2.4.4.1 Mechanism of degradation pesticide by NTP

The performance of NTP for pesticide degradation is mainly dependent on the average energy of electron and the production of reactive species (Bai *et al*., 2010; Misra *et al*., 2016). The average electron energy is between 0 and 10 eV while the bulk gas temperature is quite low and near room temperature. Therefore, all organic molecules which have similar ionization and dissociation energies from 3 to 6 eV can easily be destroyed when exposed to NTP by collision between high energy primary electrons and the molecules of the feed gas to form secondary electrons and highly reactive species (Bai *et al*., 2010).

Under selective non-thermal plasma treatment condition, pesticide molecules are firstly dissociated by the large energy from plasma and then this energy will also produce free radicals such as $OH^{\bullet}$ radicals (E^0=2.8 V_{NHE}), ozone (E^0=2.07 V_{NHE}) and hydrogen peroxide (E^0=1.77 V_{NHE}), which have a considerably high oxidation potential to initiate the degradation of pesticide (Bai *et al*., 2010; Jiang *et al*., 2014). Accordingly, the pesticide chemical bond can be broken effectively and different desired conversion of pesticides are formed which are less or not harmful compounds. Moreover, UV and irradiate light of plasma can also support the degradation of pesticide. Thus, physical conditions and chemical reactive species in NTP are combined and become a comprehensive process for rapid and efficient degradation of pesticide. This suggests a relatively lower consumption of energy used for operating the reaction system as well as not causing secondary pollution as the conventional processes for degradation pesticides (Bai *et al*., 2010).

There are many pesticides used as targets in the investigations applying non-thermal discharge plasma to remove the related potential health risks (Misra *et al*., 2016). Figure 2.8 shows the two pathways which possibly occur simultaneously for degrading dichlorvos (DDV), organophosphorous pesticide, of oxygen plasma treatment, NTP. The first one is based on free radicals generated in plasma which set the free radical reaction in motion. The other may be supported by electrons providing energy and affecting on the reaction involved in unsaturated bonds of DDV molecules (Bai *et al*., 2010). The degradation pathway of dimethoate using DBD plasma is also depicted in Figure 2.9. With the optimal levels of treatment parameters including applied power of 85 W, 5 mm air-gap distance, treatment time of 7 min, concentration of the pesticide was reduced by 96 % from the initial quantity of 20 mg/L. Generally, $OH^{\bullet}$ radicals played the key role in the decontamination of dimethoate. The degradation process was begun with the attack of $OH^{\bullet}$ at the double bond of P=S causing the formation of P=O of omethoate. After that, omethoate was further oxidized to C_2 and C_3 by $OH^{\bullet}$ through the break of P-S bonds and the release of N-methyl acetamide groups, respectively. The scission of S-C bonds by $OH^{\bullet}$ also led to the production of C_1 and C_4 compounds, and the successive attack of $^{\cdot}CH_3$ and $^{\cdot}OCH_3$. These intermediates can be finally converted into small nontoxic products such as PO_4^{3-}, H_2O, and CO_2 (Hu *et al*., 2013).

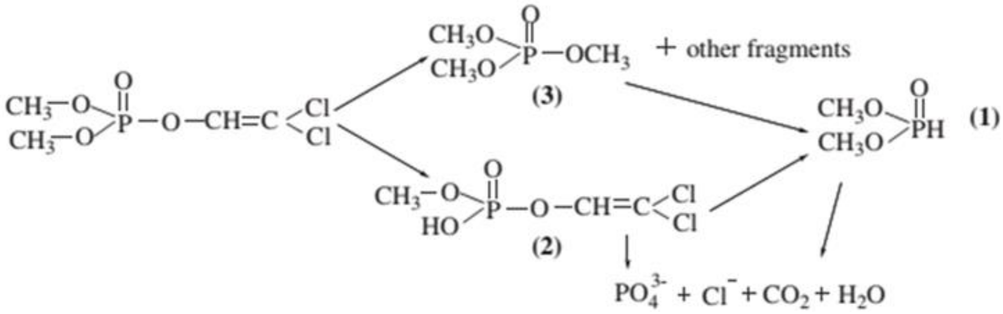

Figure 2.8 Degradation of dichlorvous (DDV) during oxygen plasma treatment (1) O,O-dimethyl phosphonic ester; (2) 2,2-dichlorovinyl O-methylphosphate; (3) O,O,O-trimethyl phosphoric ester

Source: Bai *et al*. (2010)

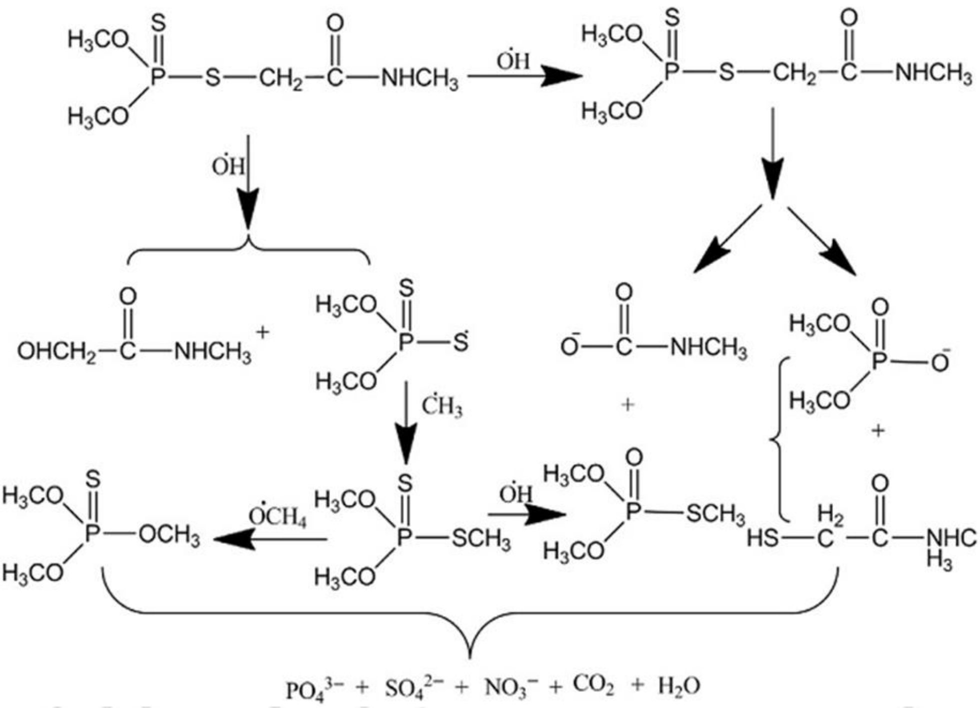

Figure 2.9 The degradation pathway of dimethoate in a dielectric barrier discharge plasma reactor

Source: Hu *et al*. (2013)

2.4.4.2 Microbial inactivation effect of NTP

Several synergistic mechanisms including 1) the direct chemical interaction of cell membrane with plasma reactive species such as O•, O_2, O_3, OH•, NO•, NO_2• and charged particles; 2) the damage of microbial cellular components, membranes, DNA and proteins by the generation of ultraviolet (UV) radiation and biocide active agents are currently attributed to explain the microbial inactivation effect of plasma treatment (Niemira, 2012; Bermúdez-Aguirre *et al*., 2013; Fernandez *et al*., 2013; Misra *et al*., 2016).

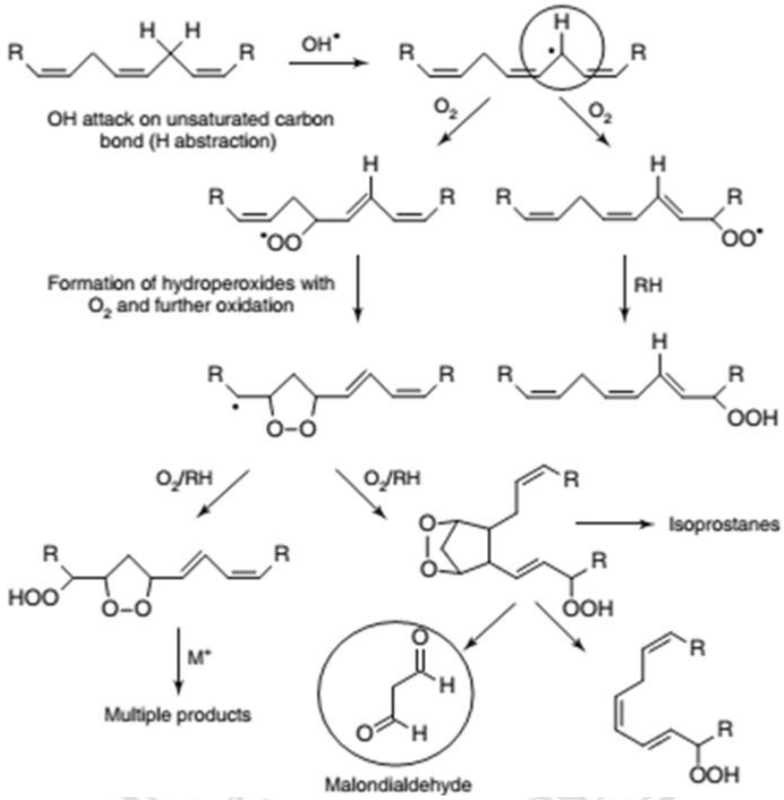

Figure 2.10 Scheme of OH radical attack on lipids

Source: Parvulescu *et al*. (2012)

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the major bactericidal agents (Misra *et al*., 2016), the inactivation process begins with the interaction between plasma active agents and microorganism cells at the surface of the cell where chemical destruction of the cell wall and membrane and related components can take place. These reactive species attack the membrane and degrade specific components such as the lipid part of phospholipids (Figure 2.10) and peptidoglycan due to the formation of peroxides and superoxide anions which could break the structurally important bonds (Song *et al*., 2009; Parvulescu *et al*., 2012; Misra *et al*., 2016). Accordingly, the cell membrane is weakened, and the inner fluid can leak out preventing the microorganisms from stimulating the anti-stress and repairing processes.

Subsequently, transport of active species into the cell where internal cell damage can occur through destruction of DNA, proteins, and other internal components of the cell (Parvulescu *et al*., 2012).

Non-thermal plasma is known to be highly effective in reducing pathogens and eliminating toxins in fresh fruits and vegetables while preserving the fresh taste, aroma, texture, wholesomeness, and nutritional content of food (Baier *et al*., 2013; Pankaj *et al*., 2014). However, antimicrobial effect of NTP is strongly dependent not only on the plasma forming gas applied and the specification of the apparatus producing plasma which mainly decide the composition and density of plasma reactive species, but also on the intrinsic factors such as water activity, texture, protein and fat content, pH and the type of produce being treated (Lacombe *et al*., 2015; Lee *et al*., 2015).

2.4.5 Effect of plasma on quality of fruits and vegetables

Although there is high promise for NTP industrial application as a new sanitizing method, the presence of reactive species as well as the residues of oxidation processes could probably promote the modification of physicochemical and sensory characteristics of the treated product (Pasquali *et al*., 2016). The pigments can be impacted by the plasma treatment and depended on time and NTP exposure conditions. Alterations in photosynthesis process and color parameters of fresh corn salad leaves (Baier *et al*., 2013; 2014) as well as slight color differences of bell peppers (Vleugels *et al*., 2005), blueberries (Lacombe *et al*., 2015) cucumber, carrot and pear slices (Wang *et al*., 2012) which were treated plasma have been observed. Wang *et al*. (2012) reported the reduction of antioxidant compounds in NTP treated cucumber. There were also significant reductions in anthocyanin content of blueberries (Lacombe *et al*., 2015), titratable acidity and total phenolic content (TPC) of mango after NTP treatment (Phan *et al*., 2017). The ascorbic acid content of strawberry has also been shown to be affected significantly by both applied voltage and treatment time of NTP. The changes induced with plasma chemistry, such as reactions of ascorbic acid to oxidative species could be used to explain the loss of fruit quality during processing (Misra *et al*., 2015). Recent research has indicated that the firmness of fresh cut apples decreased after treatment times of gas plasma ranging from 10 to 30 min. The prolonged exposure of fruit surface to gas plasma was one of the possible reasons causing cell leakage (Tappi *et al*., 2014). However, color of tomatoes treated using NTP showed insignificant changes (Misra *et al*., 2014a; Bermúdez-Aguirre *et al*., 2013). The cold plasma caused immediately slight decrease of fresh-cut kiwi pigments, but improved the color retention during storage time (Ramazzina *et al*., 2015). There was also an insignificant difference in the firmness values of treated cold plasma tomatoes (Misra *et al*., 2014a), mango (Phan *et al*., 2017), fresh-cut kiwi (Ramazzina *et al*., 2015) when compared to the non-plasma treated sample. Misra *et al*. (2015) found that the overall anthocyanin concentration of strawberry was insignificantly affected by either the applied voltage or treatment time of NTP. The higher values of TPC, crude protein, crude fat and crude fiber were also obtained in atmospheric radio-frequency plasma treated fresh-cut dragon fruit with green tea (Matan *et al*., 2015).

Overall, there are still limited studies conducting on physicochemical or nutritional qualities of food after plasma treatment. Nevertheless, the application of plasma is mainly on product surface, therefore any considerable modification caused by chemical reaction of NTP reactive species may only take place on the outer section of food (Critzer *et al*., 2007).