CHAPTER 2

Literature Review

Application with high frequency fields to heat a product is a physical method of energy input to eliminate insect pest has been developed since 19th century (Martens *et al*., 2012). Not only electromagnetic radiation (Figure 2.1) but also microwave and radio frequency, have the potential for fast heating in solid and semisolid materials having dielectric property especially those with low moisture. Dielectric heat is formed and transferred directly between electromagnetic wave and insect infested products. In 1934, Davis investigated the process to control insects in grain by high frequency waves (cited by Nelson and Whitney, 1960). The use of RF was further investigated during 1940s and 1960s including mostly disinfestations of insects (Fleming, 1944; Webber *et al*., 1946; Frings, 1952; Baker *et al*. 1956; Nelson and Whitney, 1960; Nelson and Kantack, 1966)

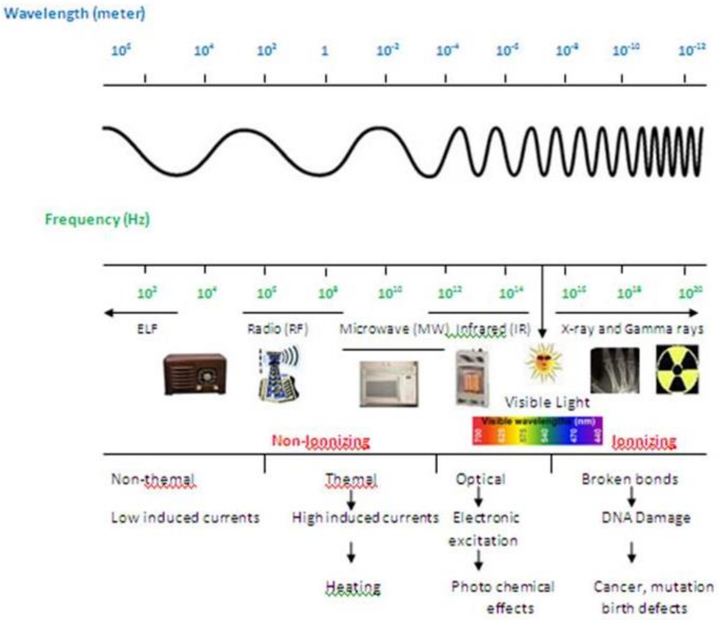

Figure 2.1 Electromagnetic radiations

2.1 Dielectric heating

Dielectric deals with poor electrical conductor between two parallel electrodes. Material, which containing no free ions and no unsymmetrical molecule (e.g. paper) is confirmed as no dielectric heating. This principle is carried a resemblance to exploit an energy loss to generate a thermal in the material filling the electrode-plates. Most of all of the matter containing polar molecule such as water which have conducting component and most readily heated and absorbed the energy. Dielectric heating is generated from electromagnetic energy at radiofrequency (13.56 27.12 and 40.68 MHz) or microwave (433, 915, 2,450 MHz). A high frequency is converted from oscillating electromagnetic field to RF power. As polar molecules in the material continually arrange in a line and reorient themselves to change electric field, then friction is formed and causes heat within conductive materials (Fig 2.2). Therefore, the water content of the matter is an important factor for these heating performances. In addition the heat is generated inside out and distributed throughout its mass within the peripheral of the container often hot rapidly. This is in contrast to conventional heating where heat enters the sample through its surface and transferred toward the center by thermal conduction and/or convection. The application of microwave and RF dielectric heating to agricultural products after post-harvest management is a new technology and has attracted great interest following many researches (Wang *et al*., 2003). In this technology, the radiation energy is dissipated within the sample afterward a great rate of heating can be obtained rapidly. In recent year, the radio frequency unit has been developed to large scale processing applications of materials by operating with belt conveyer system in big scale (Tang *et al*., 2000; Nijhuis *et al*., 1998).

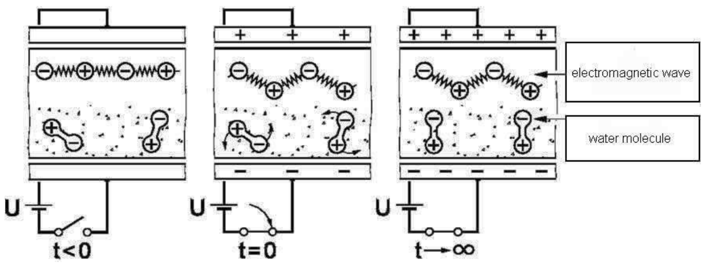

Figure 2.2 Molecules in the material continually arrange in a line and reorient themselves to changed electric field (Cwiklinski and von Hoersten, 2001)

2.2 Radio frequency heat treatment

Heating with radio frequency is operated by the wave generator, which made from vacuum circuit tube or semiconductor. High-energy radio waves are created through the electrode plates of which electric field are assigned to heat target material. The band of 13.56, 27.12 and 40.68 MHz are also differing in energy or powers, which proceed through the material depending on the material properties and frequency. For heating the large material, lower frequency waves can pass into material deeper than high frequency, which is an ideal for heating small or slight material.

2.2.1 Mechanisms of radio frequency heat treatment

Dielectric material delivers low power when passed high voltage energy as alternating current at a frequency of 27.12 MHz, or 27,120,000 times per second. Electromagnetic fields at low frequency are produced with long wavelengths resulting in a possibility to control the direction. Dielectric is an insulating power which has no independent capacity (or free electrons) that moves within a conductor. However, the dielectric molecules can also arrange position by the electric field arising from electric dipole properties. This is caused by electric dipolar properties of the material that molecules can be divided into two categories. Types of heat from electromagnetic absorption are as followed:

- **Ionic polarization**

Generally, the un-pole molecule in the absence of electric field, electron moves around nucleus showing a positively charged and the center of positive charge overlap exactly through the center of the negative ions. Nevertheless, when the electric field acting on the molecule, electron in an atom is induced until the center of positive charge and negative ions move away from each other slightly. Then, it becomes a molecular electric dipole and the moment is immerged by the inductance.

- **Orientation polarization**

Orientation polarization always occurs with a polar molecule, such as water molecules where the electrons is divided not equal or unsymmetrical on each atom of a molecule and shows electrical neutrality resulting from a 104° angle between the two OH- anions. However, polar molecules which randomly oriented usually confirm a stability of electric dipole moment value. When pass through the electric field, the positive and negative charges in material move to change direction in an orderly arrangement by moving back and forth rotation which occurs rapidly as the frequency of the waves. In the radio-frequency with ion mobility of 3-300 million times per second, resulting the speed of rotation and friction, causing the heat up rapidly within 2-3 seconds, or about 1 minute after receiving electromagnetic radiation, then the heat generated distribute to other parts of the matter.

2.2.2 Radio frequency systems

Since, pesticides, refrigeration, packaging and modified atmosphere storage are the technologies most often used today for disinfection and disinfestations. New, noninvasive, user-friendly and economically viable processing technologies are needed to meet involving consumer expectations and trade standards. A research project was established to study, evaluate and demonstrate new RF processing applications materials. The research respecting review laboratory-scale results for several RF processes with potential commercial applications, and provide preliminary economic estimates for their installation and operation. Traditionally, RF energy refers to non-ionizing electromagnetic radiation with frequencies ranging from approximately 30 megahertz (MHz) (wavelength=11 yards [10 meters] to 300 MHz (wavelength =1.1 yard [1meter]). However, the U.S. Federal Communications Commission (FCC) allows other frequencies to be utilized for industrial, scientific and medical applications (e.g. 13.56 and 27.12 MHz) (Kasevich, 1998). The studies focused on the use of lower frequencies outside the FCC domain, ranging from 300 kilohertz (kHz) (wavelength=1,094 yards [1,000 meters]) to 10 MHz (wavelength=36.1 yards [33 meters]). Early tests demonstrated the potential advantages of lower frequencies, in terms of the type and efficiency of RF interactions with different materials. Within the lower frequency range, very high (>80%) overall energy-use efficiencies are achievable with modern design and engineering systems. These novel RF systems can be manufactured and operated with significant savings, and increased ruggedness and reliability as compared with conventional RF systems.

RF power is produced when electricity is applied to an RF generator whose signal is amplified and delivered to a parallel electrode system (RF cavity), in which a selected material is placed. Within the RF cavity, an oscillating electric field is created, and energy is transferred to the treated material through electronic-field interactions with dipole or induced dipole molecules (those formed by the polarization of neutral molecules). These dipole molecules are forced to reorient within the changing electronic field, which results in movement or drifting that causes internal friction and creates thermal energy (heat). The process is known as "RF thermal processing" or simply "RF heating". At certain frequencies or frequency bands, some foods and nonfood materials can be heated preferentially and faster, creating rapid thermal effects on pests but minimal interactions with the host material. This is due to the difference in electrical conductivity between arthropod pests (high) and the host commodity (low). This process is called "selective or differential RF heating" and could provide an alternative disinfestations process for thermally sensitive fruit and vegetable products. In general, complex organisms such as arthropod pests are more severely and easily affected by heat. The higher response of pests and lesser sensitivity of host commodities offer a window of opportunity for disinfection with minimal or no impact on the commodity. The differential effect is generally less effective with microbial contaminants, since microbes are significantly smaller in mass and are usually well attached to a much larger volume (and mass) host, thus being rapidly and effectively cooled. For disinfection to occur, the microbe must reach lethal temperatures, which are usually also deleterious to the host commodity.

However, RF heating induces the thermal inactivation of biological organisms (such as fungi, bacteria, protozoa, parasites and nematodes), viruses and enzymes, as well as arthropod pests present in heat-tolerant commodities (such as dried fruits, grains, seeds, wastewater and soils). Unlike traditional surface heating, RF penetrates deeply into foods and agricultural materials. The thermal power induced by RF is given by the following formula:

$$P = 55.61 \times 10\text{-}14\ E^2\ \nu\ \acute{\varepsilon}$$

Where **P** is the thermal power generated (W/cm^3)

E is the RF frequency in (Hz)

$\acute{\varepsilon}$ is the dielectric loss factor of the material (intrinsic property)

The dielectric loss factor (**$\acute{\varepsilon}$**) largely depends on the material's chemical composition and is essentially the ease by which molecules can be heated by and RF field. The surfaces of the treated materials are slightly colder because of radiation losses, and the insides are heated homogenously and at controllable rates. In general, RF heating eliminates surface overheating, reducing thermal loads and allowing a food's quality and nutritional attributes to be maintained.

2.2.3 Application of radio frequency heat

Radio frequency wave does not cause disintegration of the charge and the energy is transferred without breakdown of molecules, which causes mutations. When the wave disperses and contacts with materials, the power is partly absorbed by the elements having electrical conductivity, then heat is generated within the material. Since the energy is transferred to the object without the need for medium to convey the heat, subsequently reaction arises very rapidly within electromagnetic field especially the polar molecule illustrating the most intense. Therefore, the water content of the materials is an important factor for the radio frequency heating performance.

Usually, a pulse or signal of any specified radio frequency generator can be constructed in any infinite length and duration. The velocity with constant-phase surface of waves is propagated depending on the propagation constant. The complex permittivity of the medium as ε´ relative to a vacuum has a value of 8.85×10^{-12} Fm^{-1}. If the medium is non-conducting and the applied field is independent on frequency, the signal is propagated without distortion. However, in absorptive medium the harmonic wave is displaced in phase in the direction of propagation since the signal is dispersed. A significant change in dielectric properties when misplaced or overlapped in frequency range is called a dielectric dispersion. The quantity ε″ is a measure of the polarization. The number of polarizing mechanism is took place in each matter or element will reflect a characteristic relaxation frequency. The relaxation frequency may be understood as the relation between the electrical conductivity and the relative dielectric constant of the sample. At the relaxation of frequency, the material will show a maximal absorption. Thus, this principle has been applied to an indicated object with high moisture content such as in the case of the beetle or rice weevil infested with wheat kernel (Figure 2.3). The curves illustrate observed dielectric loss factor in materials in the frequency range 10^2 to 10^{11}Hz. Energy from radio frequency and microwave distribute in biological dielectric as material and cause partial loss of the dielectric when wheat are exposed in electromagnetic field. The molecular structure of materials in polar form tries to sort the field in the direction of the passing wave, then induces molecular friction within the frequency range of amplitude showing the most intense molecules vibrations which present high performance of energy transfer known as dielectric relaxation frequency. However, the difference between the forms of water (free water, bound water and constituent water) in the matter also affects the curve of loss factor of dielectric by the influence of electromagnetic fields.

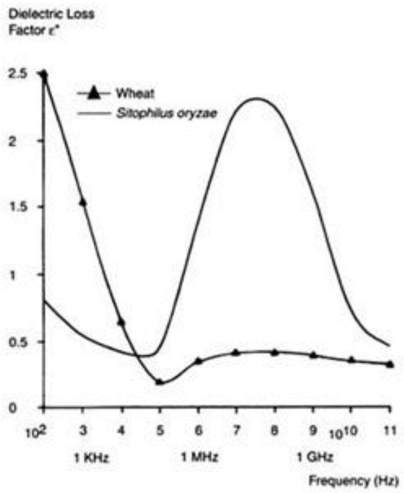

Figure 2.3 Change of dielectric properties of rice weevil (*Sitophilus oryzae* L.) in different spectrum of radio frequency (Nelson and Charity, 1972)

The permittivity of dielectric is performed by the electric field consisting of two parts: 1) ε′ is a dielectric constant and presents the capacity of a material at a certain frequency and 2) ε″ is dielectric loss factor which shows electric energy dissipates into heat. The average power (***P***) dissipating in material volume (***V***) which is related to electronic field (***E***) and transformed into heat energy by dielectric property are expressed in terms of equation (1) or (2)

$$\boldsymbol{P = 2.\text{¶}.f.\varepsilon_0.\varepsilon''.E^2.V} \qquad (1)$$

$$\boldsymbol{P = 55.63 \times 10^{-12} f.\varepsilon''.E^2} \qquad (2)$$

Amount of heat released into object depending on the specific heat itself and the relative amount of the electromagnetic field passes into the surface and is adsorbed (in some cased, thermal energy becomes 100%) however in theory, the heat is caused by all of absorbed power (no energy loss). A simplified model used to describe the power with the rate of rising temperature during heating can be expressed as equation (3).

$$\boldsymbol{Q= d\theta.M.c.1.1627 \times 10^{-3}} \qquad (3)$$

Where Q is absorbed power per hour (kWh^{-1})

$d\theta$ Temperature increase during exposure (°C)

M Mass of material (kg)

c Specific thermal value of product (Cal g^{-1} $°C^{-1}$)

However, the relation between equation (2) and (3) can be performed when the absorbed power is completely transferred 100% in theoretical given by the equation

$$\boldsymbol{dt = Q P_{th}^{-1}} \qquad (4)$$

Where dt is exposure duration in hour and P_{th} is all absorbed energy which is transferred to material in kW (if dt=1, then $P_{th}= Q$), then heat transfer into the matter can be calculated from the equation

$$\boldsymbol{P = 4.186 \times 10^6 .c. d\theta\, dt^{-1}} \qquad (5)$$

Where $d\theta\, dt^{-1}$ means temperature in °C per time (s^{-1}) and P is absorbed power in Wm^{-3}

According to theory, the power from electromagnetic wave passed through material P_{th} fluctuates more from the monitored heat that is conveyed to the matter when the energy deliver is fragile.

Many studies have confirmed the merit of radio frequency to control insect pests during storage whereas the specific heat delivering to material has been adjusted in the form of thermal to control insect by lacking of excessive heat to be damage the mass or reduce the food quality. Nelson and Charity (1972) showed that the dielectric property of insect is higher than cereal grain by investigating the dielectric constant of insect at the frequency of 9.4 GHz and dielectric loss factor of wheat grain. The result was that the dielectric loss factor wheat was 0.9-1.8 depending on moisture content and the rice weevil (*Sitophilus oryzae* L.) was 13. The loss factors derived from estimation shows that insect takes up more energy from the wave than wheat matter (Nelson, 2004). In addition, the low loss factor was also found in wheat indicating expressed the ability of microwave and radio frequency to be able to penetrate more intensely. The absorbed power and heat generated in insects differ from heat in material such as food products and are based on their moisture content. Generally, insects contain water or moisture between 50-65%, while the grain moisture is only 11-16%. The low frequency use accomplishes a divergence in heat dramatically accumulated in insect and material. Presently, only certain frequency bands are allowed by laws for industrial and scientific use in order to avoid interference with band used in telecommunications. Uses for high moisture solid foods have been less successful in extensive research conducted at 2450 MHz, but combined with convection heating method. The central wavelength is 12.2 and results in low penetrate depth then, the signal below 2450 MHz has been developed, such as 915 and 27.12MHz (Orsat and Ranhavan, 2005). Especially at 27.12 MHz, the wavelength is in the order of 10m and some research has been conducted in sterilization of food products. The investigation was found that using radio frequency at 27.12 MHz in process of a model food, macaroni and cheese produced better quality of them (Wang, 2003).

2.3 Radio frequency heating application for controlling insect

The ideal conditions for stored product insects normally range between 25-32°C and 65-75%RH (Robinson, 2005). Above and below these temperatures, insects pest grow growth and fitness will be affected and extreme conditions can be lethal to the insects.

Heat treatment has been used against stored product pests and most of the storage insect response to elevated temperature. As in generalized report by Field (1992) that at 35-42°C insect population decline and move to cooler environment and will be eliminated when exposed to 45-50°C in 24 hr, 50-62°C in 1hr, and above 62°C in 1 min. However, temperature effect may vary with individual tissue within the insects. Perez-Mendoza *et al*. (2004) used near-infrared spectroscopy to determine water content in adult rice weevil and the result confirmed that younger weevil (12 days old) tended to have higher water content (about 51%) than older weevil (95 days old, water content 47-49%) in both male and female.

In brief, radio frequency as an alternative heat treatment to disinfest food products and stored-products has been used for more than 50 years. Then these technique can be apply to the problem about stored grain insect pest disinfest mentioned before (Fig 3), insect shows high dielectric loss factors, heat can be transferred more rapidly under electromagnetic field. When the energy is absorbed, the heat is generated rapidly by dielectric material like insect. Nelson (1996) found that many kinds of insects, which destroy the agricultural products, could be controlled by radio frequency application with the short period of exposure time without demolishing the quality. In general, the successful temperatures to control the pests through radio frequency are 40-90°C depending on the properties of the product, insect characteristics and the wave's nature. The radio frequency heat treatment differently results in the individual insect species and each growth stage due to peculiarity of species, which perform phenotype as biological or physiological or body composition of each unusual insect. Many researches involved with the influence of heat in insect metabolism and they demonstrated concomitant increase in both metabolism and respiration up to a critical thermal limit as well as heat effect on nervous and endocrine system (Neven, 2000) and mortality ultimately. In addition, the radio frequency has effect on the insect with incomplete development of deformation such as the head and chest of the larva and pupa of flour moth infested with wheat grain when exposed to radio frequency of 39 MHz The treated insect had reproductive rate lower than 50% due to the heat from wave frequency damage to ovarian tissue then, the rate of hatching eggs dropped out and become small in size (Nelson, 1996). For tolerance in each stage of insects, Johnson *et al*. (2004) has identified growth stages of the red flour beetle infested in nut which is resistance to radio frequency. The larvae old age stage is most tolerance to radio frequency at the temperature of 48-50°C, and followed by pupae, adult, egg and larvae respectively. In addition, it was found that the temperature of 52°C for 2 minute completely removed old age worm infesting almond, pins and pistachio.

Among the beetles, *Lasiodermaserricorne* and *Rhyzopertha dominica* are highly tolerant to heat, while *Sitophilus* spp. and *Tribolium castaneum* are moderately tolerant (Robinson, 2005). The heat from radio frequency at 27 MHz is also used to eradicate codling moth (*Cydia pomonella* (L.)) infesting walnut. The experiment reported that the target temperature of 43 and 53°C for 2 and 3 minute to control the codling moth at 3th and 4^{th} old age stage resulted in worm mortality 78.6 and 100% without impact on the quality i.e. peroxide content, total fatty acid and color of walnut kernels (Wang *et al*., 2001). Moreover, the temperature of 55°C completely eliminated worm of fifth-in star navel orange worm (*Amyelois transitella*) whereas the moisture content of walnut slightly decreased with no effect on the quality (Wang *et al*. 2002). In addition, radio frequency 27.12 MHz at the lower temperature of 50°C with 3 minutes of exposure time has been developed and completely eradicated third stage of rice weevil worm filled with walnut (Wang *et al*. 2003). Therefore, radio frequency heat treatment is possible to apply in a mass walnut products in a short period of exposure time and replace current fumigated methyl bromide in processing plant. Nevertheless, not only in shell walnut but also in rice, the systemically fumigation is also used to eliminate the pests immediately before and after processing. The radio frequency also has been considered to replace chemical fumigation. Janhang *et al*. (2005) determined the effect of radio frequency 27.12 MHz at the temperature of 70, 75, 80, 85°C on *disease i.e Trichoconis padwickii, Fusarium* sp.and *Bipolaris oryzae and Curvularia lunata* with rice weevil infested in Kao Dok Mali 105 rice (KDML105). The result noted that the fungal contamination, viability of rice weevil and seed quality decreased with increasing temperature and the temperature of 75°C could completely control rice weevil and reduced fungal contamination by 41%. The same frequency can also be irradiated to eradicate codling moth, which is a major pest in rice. The radio frequency heat treatments at 60°C to various stages of codling moth infested in rice and review that it was most effective in survival stages i.e. egg and larvae by showing 98.9 and 98.35 percent mortality while the adult and worm were completely destroyed (Luechai *et al*., 2008).

2.4 Rice bran: An overview

2.4.1 General characteristic of rice bran

Rice (*Oryza sativa* L.) grain or paddy rice composes with 2 main parts (Figure 2.4)

1) **Outer protective covering** so called hull (husk) which consists of lemma, palea, pubescent, awn, sterile lemmas and rachilla.

2) **Rice caryopsis** or true fruit or brown rice comprise with the details by following:

   2.1) **Pericarp**: After husking, pericarp is an outer layer of brown rice and thick about 10 microns covering the true fruit inside the hull. The pericarp is a cell wall fibre 6 layers and restrains pigment resulting in color of brown rice as seen in light or deep brown, purple and brown almost black and etc. as well as its contains hemicellulose and major protein.

   2.2) **Seed coat**: Seed coat is next to a pericarp. Two layers of long cell size formed in transverse and separated by thin cells (thickness about 0.5 microns). Lipid and pigment also are within the cell as same as pericarp which made of color of seed coat.

   2.3) **Nucellus**: Nucellus is a cell layer adjacent to the seed coat (thickness about 0.8 -2.5 microns), however the bond between nucellus and seed coat is firmly attached which it can be separated from each other.

   2.4) **Aleurone layer**: Aleurone tissue is next to seed coat consisting of 7 cell layers. The thickness varies by rice genetic variety which aleurone layer of short-thick grain is thicker than long grain.

   2.5) **Endosperm**: Component of the grain kernel of endosperm is about 80% of total grain. The endosperm is divided into two parts: sub-aleurone layer is the 2 layers cell next to aleuroneand starchy endosperm.

2.6) **Embryo**: Embryo is a living part locating at the base of seed. The bottom part of seed is radical, plumule, coleorhizae, coleoptiles, epiblast and scutellum as a monocot. Embryo is function as a source of food for growth of the seedling as well as it is rich in protein and fat at various parts.

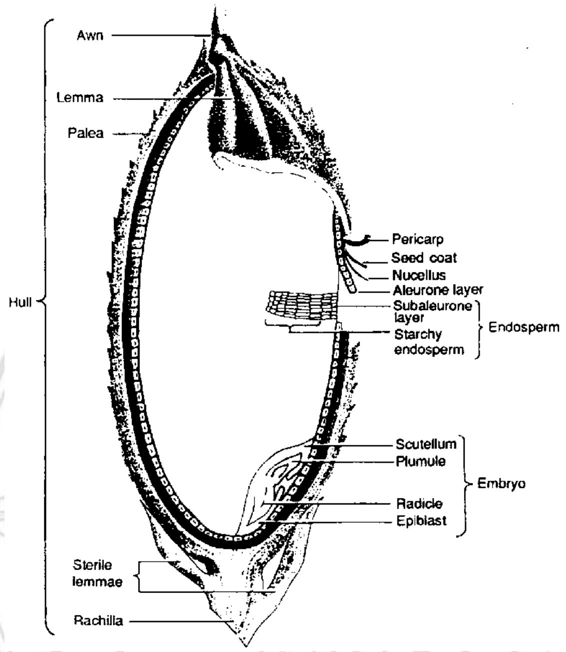

Figure 2.4 Composition of rice grain or paddy rice and brown rice (Juliano, 1993)

When comparing the fraction of grain from rough rice yield 100 percent, it includes with 20 percent of husk and the remaining is brown rice. When brown rice was compared to 100 percent, the ratio consists of pericarp and seed coat layer is 6.5 percent, and embryo 3 percent, starchy endosperm 90.5 percent. However, milling and polishing process play an important role on rice fraction yield (Juliano, 1993, Naivikul, 2007).

After rough rice of paddy rice is dehulled, the husk and brown rice were obtained. When brown rice is continuously milled with whitener machine, the bran and white rice is achieved. The bran is acquired from pericarp, seed coat, nucellus, aleurone layer and sub-aleurone layer. The amount of bran, structure and chemical composition vary with milling process, rice variety and environment during planting time until whitening process (Oranong, 2004). Juliano, (1993) reported that chemical composition of paddy rice is different from brown rice and whitened rice as shown is Table 1. Protein of paddy rice is about 5.8-7.7 gram, lipid 1.5-2.3 g, crude fibre 7.2-10.4 g and ash 2.9-5.2 g. When the rice is dehulled to be brown rice contains higher chemical composition which is protein (7.1-8.3 g), lipid (1.6-2.8 g), carbohydrate (73-87 g) and fibre content (0.6-1.0 g) and dietary fibre decrease to 2.9-3.9 g. Nutrition of white rice getting from whitening process is decreased when compared with brown rice. For a by-product of rice milling and polishing, rice bran is mixed between the aleurone and embryo piece results on consisting of high nutrient for human health which is protein 11.3-14.9 g, fat 15.0-19.7 g, crude fibre 7.0-11.4 g, ash 6.6-9.9 g),carbohydrate 34-62 g and dietary fibre 24-29 g. In addition, Tanaka *et al*., (1973) found that the lipid content of rice bran is approximately 18–22%, with the lipids existing as lipid bodies or spherosomes in the aleuronelayer. Additional constituents of the bran include protein, carbohydrates, ash, vitamins, and minerals (Qureshi *et al*., 2000; Saunders, 1985)

Table 2.1 Proximate chemical composition of rough rice and its milling fraction at moisture content of 14%

Rice fraction	Crude protein	Crude fat	Crude fibre	Ash	Available Carbohydrates	Neutral detergent fibre
				(%)		
Rough rice	5.8-7.7	1.5-2.3	7.2-10.4	2.9-5.2	64-73	16.4-19.2
Brown rice	7.1-8.3	1.6-2.8	0.6-1.0	1.0-1.5	73-87	2.9-3.9
Milled rice	6.3-7.1	0.3-0.5	0.2-0.5	0.3-0.8	77-89	0.7-2.3
Rice bran	11.3-14.9	15.0-19.7	7.0-11.4	6.6-9.9	34-62	24-29
Rice hull	2.0-2.8	0.3-0.8	34.5-45.9	13.2-21.0	22-34	66-74

2.4.2 Purple rice and their nutrients

Rice is a staple food of Thai people for a long time. Many report indicated rice contained various nutraceutical compounds that can prevent many disease. Tanaka *et al.*, (1973) found that the lipid content of rice bran is approximately 18–22%, with the lipids existing as lipid bodies or spherosomes in the aleurone layer. Additional constituents of the bran include protein, carbohydrates, ash, vitamins, and minerals i.e. vitamin B, E, beta-carotene, gamma-oryzanol and etc. (Qureshi *et al*., 2000; Saunders, 1985; Mori, 1999). The nutrients not only in the white rice, but also purple rice which is a genetic diversity of landrace Thai rice because of their accumulation of pigment colored on purple or deep red in husk and pericarp so called anthocyanin (Abdel-Aal *et al*., 2006) which also provided health benefits.

2.4.3 Anthocyanin

Anthocyanin, the biological active pigment compounds among flavonoids group which is classified as water soluble pigment accumulated in plant vacuole and its contribute to the high anti-oxidant potential. The base structures of anthocyanins are $C_{15}H_{10}O_2$ so called anthocyanidin or flavan nucleus or flavalium cation combined with glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium salts.

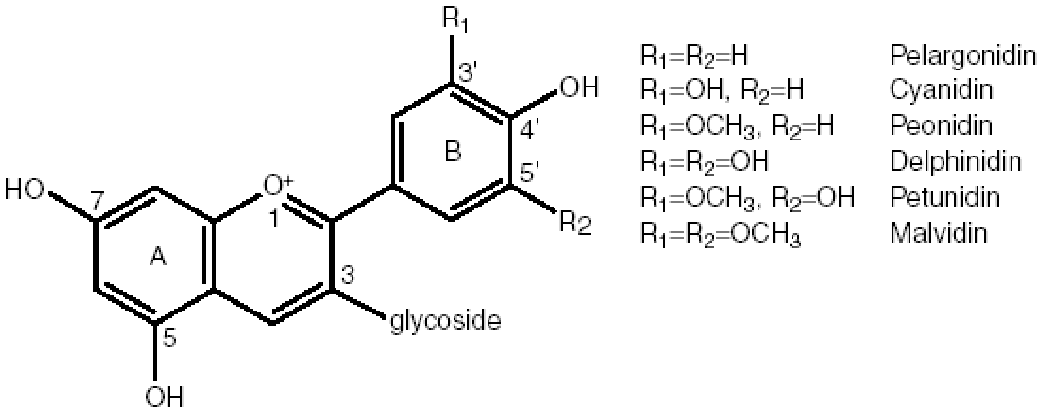

Figure 2.5 General structure of anthocyanidin (Clifford, 2000)

Clifford (2000) noted that several hundred anthocyanins are known varying in; the identity, number and positions at which carbohydrate in form of sugar (glucose, rhamnose, xylose, galactose, arabinose, rutinose) are attached to the skeleton; the extent of sugar acylation and the identity of the acylating agent; and the basic anthocyanidin skeleton i.e. the number and position of hydrogen ion (H), hydroxyl (OH) and methoxyl (–OCH_3) group substituent at position of 3′ and 5′, which are classified into six common variations (Figure 2.5). The substituent of the structure presents various glucosides which response for plant colors and effect on human color vision with differently. For the pigmented rice, the color is classified as followed:

- **Red** : Red color is the pigment color structure so called cyanidin which OH group substituent at 3′position while hydrogen (H) substituent at 5′ position.
- **Purple**: Purple color occurrence is the pigment color structure so called delphinidin which OH substituent at 3′and 5′ position.
- **Deep purple**: Deep purple incidence color is the pigment color structure so called peonidin which –OCH_3 substituent at 3′position while hydrogen (H) substituent at 5′ position.

Hayashi *et al*. (1952) indentified anthocyanins derivatives of pigmented rice seed. It was found the main component of cyanidin about 0-470 mg/100g, peonidin 0-40 mg/100g with small amount of mulvanidin and pelargonidin, and then it was called purple rice. Moreover, the isolated anthocyanins were differ in each variety of rice species especially *indica* type rice obviously showed diversity of pigment color and the pigment is accumulated at outer layer of rice seed and In addition, the anthocyanins of purple glutinous rice as black rice (*Oryza sativa* L. *indica*) was detected and the pigments accumulated at outer layer of the rice was cyanidin-3-glucoside 85% and others was peonidin-3-glucoside (Chun *et al*., 2003). Recently, Zhang *et al*. (2010) determine the phytochemical profiles and antioxidant activity of rice bran samples from 12 diverse varieties of black rice. It was found that the percentage contribution of free anthocyanins to the total amount ranged from 99.5 to 99.9%. Cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside were detected in black rice bran samples and ranged from 736.6 to 2557, from 22.70 to 96.62, and from 100.7 to 534.2 mg/100 g of DW, respectively.

The purple rice is a nutraceutical plant as medical uses and also a food colorance mixer. The natural products of anthocyanins were known for their beneficial effect on human health since ancient time. Presently, several researchers recognized anthocyanins for their antioxidant properties (Robards *et al*., 1999; Karalaya *et al.*, 2001; Rossi *et al*., 2003), help for blood circulation system and anti-aging. The research recently found that cyanidin 3-glucoside inhibited lung cancer in organisms (Chen *et al*., 2005; Okarter and Liu, 2010)

2.4.4 Chemical change of lipid

Juliano (1993) noted that rice bran contains 15.0-19.7%t oil which include 20% saturated fatty acids and approximately equal amounts of oleic (40-50%) and linoleic (30-35%) fatty acids (Rukmini and Raghuram, 1991). Rancidity can be happened when the bran attached with the air surround with reaction by following:

1) Lipolysis: Lipolysis is hydrolysis of the primary and secondary ester bonds between long chain fatty acids and the glycerol back bone in triacylglycerols. Important enzymes and regulatory protein factors involved in lypolysis have been identified. Three enzyme has been implicated which include an essential TAG hydrolase named adipose triglyceride lipase selectively performs the first and rate-limiting step hydrolyzing TAG to generate diacylglycerols, hormone sensitive lipase which is a multifunctional enzyme capable of hydrolyzing a variety of acylesters including TAG, diacylglycerols and monoacylglycerols (MAG) and monoglyceride lipase which efficiently cleaves MAG into glycerol and non-esterified fatty acids (Lass *et al*., 2011).

2) Oxidative rancidity: Oxidative rancidity is a result from more complex auto-oxidations involves the reaction between double bond of unsaturated fatty acid and oxygen molecule act with O_2 in surrounding air to produce peroxide linkage at α-methylene group. Auto oxidation can be accelerated by singlet oxygen, free radicals, metal ions (iron, copper, and cobalt), light and radiation (Barnes and Galliard, 1991). The mechanism of oxidation is considered to arise in three phase as followed:

2.1) Initiation phase or induction phase

The reaction between oxygen molecule and unsaturated fatty acids produce hydroperoxides and free radicals which these forms are highly reactive.

$$RH \longrightarrow R\bullet + H\bullet$$

```
   H   H                          H  H
   |   |                          |  |
R – C = C - R′ + O2  ———→  R - C – CH –R′
                                     |
                                     O – O•
```

• means unpaired electron

2.2) Propagation phase

The propagation of further oxidation by lipid oxidation products as peroxy radical, (ROO•), hydroperoxide (ROOH) and hydrocarbon radical (R•) increased to be auto-oxidation.

Propagation

$$R\bullet + O_2 \longrightarrow ROO\bullet$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet$$

$$2\ ROOH \longrightarrow RO\bullet + ROO\bullet + H_2O$$

2.3) Termination phases

Un-reactive compounds are formed in this termination phase of lipid oxidation including hydrocarbons aldehydes, and ketones which leads to production of various off-odor compounds.

Rice bran contains active enzymes. Germ and the outer layers of the caryopsis have higher enzyme activities. Rice bran contains several types of lipases as well as phospholipases, glycolipases, and esterases (Takano, 1993). In addition, Orthoefer (2005) also confirmed that some enzymes that are present include α-amylase, β-amylase, ascorbic acid oxidase, catalase, cytochrome oxidase, dehydrogenase, deoxyribonuclease, esterase, flavin oxidase, α and β -glycosidase, invertase, lecithinase, lipase, lipoxygenase, pectinase, peroxidase, phosphatase, phytase, proteinase, and succinate dehydrogenase. After the bran is re-moved from the rice, lipolysis activity continues during postharvest processes and storage resulting free fatty acids increase bran acidity and reduce pH; an off-flavor and soapy taste is produced, and functional properties change. Saunders (1986) reported that the rate of free fatty acid formation by lipase promotion is also depended on environmental condition which the formation is as high as 5–7% free fatty acids per day and up to 70% in a month. Therefore, the reduction of rice bran quality must be stabilized immediately after production.

2.4.5 Rice bran stabilization

Prevention of lipolysis caused by rice bran lipase primary means for rice bran stabilization which include deactivating the enzymes through a variety of methods like cold storage, sun drying, steaming and expelling as well as the chemical stabilizer like sodium meta-bisulphate was also used.

The stabilization of rice bran by dry and moist thermal is accomplished commercially *i.e.* hot air, drum drying, extrusion and microwave. Prabhakar, (1987) and Narisullah and Krishnamurthy (1989) found that an effective method of stabilization is hot air; however the heating uniformity of the sample in the tray is a limiting factor for the method. Fernando and Hewavitharana (1993) developed fluidized bed drying and the rice bran was stabilized at temperature of 90-130ºC. On the other hand, the fluidized bed method was required high air velocity which was less uses as uneconomical. As well as drum drying at temperature of 156ºC was also developed for rice bran stabilization (de Delahaye *et al.*, 2005).

In recent year, ohmic heating is a promising method of stabilizing rice bran (Lakkakula *et al*., 2004). The principle of the method regarding to the route of alternating current was passed through a food sample which results in ohmic or electrical heating by virtue of the sample's electrical resistance (Halden, *et al*., 1990).

To achieve proper stabilization, microwave energy is use an inexpensive source of thermal processing. In addition, the method has been considered to be one of the most energy-efficient types and a rapid method for heating food items (Yoshida *et al.,* 2003). Tao *et al*. (1993) showed that microwave heating is an effective method for the inactivation of lipase, the major enzyme responsible for hydrolytic rancidity for a storage period of 8 weeks.

The microwave process has been irradiated to rice bran and it was found that results in inactivating lipase, the major enzyme responsible for hydrolytic rancidity. Ramezanzadeh *et al*. (2000) also revealed that stabilizing rice bran by the microwave heating process did not result in any deleterious effect on major nutrients in the bran. Microwave heating at temperature over 100ºC is an effective method for stabilizing rice bran with the addition of moisture (Malekian *et al.*, 2000).

2.5 Mango qualities

The mango fruit (*Mangifera indica* L.) is one of the most important seasonal fruits in Thailand. Besides the local consumption, foreign exchange is a commodity earning. With an annual harvest of 1.25 million tons of fresh mangoes, Thailand is one of the main producers in Asia 2000 (De la Cruz Medina and Garcia, 2003) and the 3rd largest mango exporter in the world. According to FAO record, Thailand exported about 2.4 million metric tons of mangos (FAO, 2009) worth US$ 48 million during 2009 (Office of Agricultural Economics, 2009). Not only good quality mango is needed but also served for imposing restriction of importing countries like USA (irradiation) and China (hot water quarantine treatment; HWQT) and etc. However, a large number of disorder rights of Thai mango are also occurred from the plant in orchard to the fruit during transit and storage. During postharvest, pest especially mango fruit fly (*Bactrocera frauenfeldi*) (Diptera: Tephritidae) is a pest of fleshy fruits and plays important role in infestation in mango of Thailand.

2.5.1 Fruit fly life cycle

The mango fruit fly life cycle initiate from the adult of female fruit fly lays their eggs in batches under the skin of fruits with her needle-like ovipositor in average of at least 25 eggs in 24 hours. The egg is creamy-white, spindle-shaped and length about 1 mm (Figure 2.6). Meanwhile the fly punctures the fruit, bacteria on the fruit surface are pushed in the fruit of which cause fruit decay while also providing a medium for the larvae feed which egg hatch to become larvae in about two days. The larva grows in size to be three larval stages (instars) and fully grown in 10.5 days after egg was laid. The size of larva is 10 mm long and 2 mm wide.

A **B**

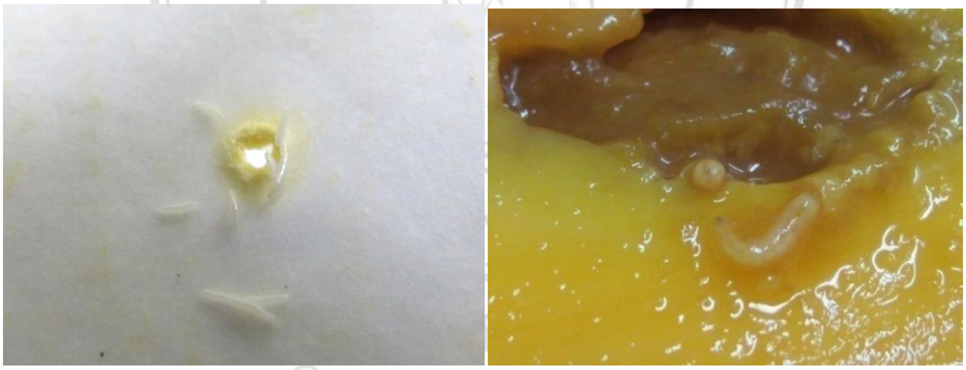

Figure 2.6 The egg (A) and 2nd star larvae (4-6 days old) stages (B) of oriental fruit fly

2.5.2 Mango fruit fly disinfestations during postharvest

Disinfestations of fruit fly during postharvest is aim to kill all eggs and larvae of fruit flies which present inside mango fruit before export to importing countries as a quarantine security. Fumigation with ethylene dibromide or methyl bromide is an official quarantine process used for treating fruit. Presently, it is becoming progressively out of use due the fumigant is banned in USA because of its evidence of the carcinogenic and mutagenic effects in test animals (Yadav and Parmar, 2014). Moreover, methyl bromide is ozone depletes, and then the imminent removal of methyl bromide from use is in 2010. Therefore, development of methyl bromide alternatives for quarantine applications on mango commodities has been developed and become a number of alternative thermo physical treatments in use for various perishables as followed:

1) Hot Water

Hot water treatments of perishable fruit are classical thermo physical methods of plant protection. Not only disease infection but also the insect pest is completely disinfestations by developing several methods against these pests. The pathogen and insect pest egg are eliminated as far as possible by dipping the fruit in hot water for certain duration without adverse effect in and outside fruit. On hot water treatment, an exact temperature has to be maintained throughout the application. Dipping during post-harvest to control decay was applied for only a few minutes at higher temperatures than water heating designed to kill insect pests where located at the interior of a commodity, (Lurie, 1998). Many procedures have been developed to disinfest a number of subtropical and tropical fruits from various species of fruit fly (Paul, 1994). The times of immersion can be 1 Hr. or more and temperatures are below 50°C, in contrast to many antifungal treatments which are for minutes at temperatures above 50°C (Lurie, 1998).As well as exporting to the United States also set the conditions for control fruit fly pests in mango of which mango fruit must be soaked in hot water temperature of 46°C for 90 minutes (USDA-APHIS-PPQ, 2002).

2) Vapor heat treatment

Vapor heat is a technique of heating fruit through air saturated with water vapor at temperatures of 40–50°C to kill insect eggs and larvae as a quarantine treatment before fresh market shipment (Animal and Plant Health Inspection Service, 1985). The technology developed from differential pressure treatment system to induce efficient circulation of vapor heat through all spaces in-between fruits which expanded the scope of vapor heat application to fresh fruit in widely range, therefore every individual fruit is always evenly and uniformly exposed to vapor heat atmosphere (JAFTA, 2009). However, Lurie (1998) reviewed that the high humidity in vapor heat can sometimes damage the fruit being treated. Nevertheless, the present mango export markets from Thailand to Japan has been required the steam process at a temperature of 47°C for 20 minutes and the air must be saturated with heat at the relative humidity over 95% (Chomchalow *et al*., 2008).

3) Hot air

Hot air is also physical treatment with thermotherapy which is an effective way to control pathogen by dry heat in a heated chamber with a ventilating fan, or by applying forced hot air where the speed of air circulation with precisely controlled (Lurie, 1998). The basic principle of thermotherapy is the application of heat, usually in conjunction with moisture, to kill harmful pathogen on the surface and interior of a fruit without impairing the functional capacity of the fruit.

The early literature also found that Mediterranean fruit fly was killed by high temperature forced air as using as a quarantine treatment (Armstrong *et al*., 1989) In addition, the method of the hot air has been developed for elimination of melon fly and oriental fruit fly on papayas (Hansen *et al*., 1990). However, Lurie (1998) also suggested that the slower heating time of forced hot air and lower humidity of the system can cause less damage that vapor water. Nonetheless, the heat also causes acceleration in fruit ripening process such as producing more ethylene, increase in breathing, color change and fruit injury. Many researchers have reported heat temperature treatment after harvest affected potential damages of mango (Shellie *et al*., 1993; Shellie and Mangan, 2000; Obenland *et al*. 1999a)

4) Combined heat with microwave

Varith and Kiatsiriroat (2004) investigated the microwave (2,450-MHz/800-W) heating on mangoes cv. Chok Anan. The inside mango was observed an increase of temperature up to 40ºC within 40 second. The heat distribution inside the fruit depended on position of orientation, microwave power and treatment time. The result noted that horizontal position of treated mango with 505 microwave power showed better heat distribution than vertical position. Continuously, the researcher team developed a microwave-vapor heat treatment for mangoes *cv*. Nam Dok Mai Si Thong to disinfest the oriental fruit fly (*Bactrocera dorsalis* (Hendel)). The resulted found that temperature between 46-55ºC with time ranging from 2 to 20 minutes are effectively disinfested oriental fruit fly eggs by using microwave for preheating followed by vapor for holding processes. Physiochemical properties of treated mango *i.e*. color, titrable acid, total soluble solid and firmness were not significantly different from non-treated mangoes. The result retained equivalent or better lethality of oriental fruit fly eggs and showed less heat damage of mangoes than conventional vapor heat treatment as well as its spent shortened process time more than 90% during preheating time (Varith *et al*., 2007). The microwave at frequency of 915 MHz was also investigated to control instars codling moth in cherry. Treatment temperature of 45, 50 and 55 can past through the fruit; however the fruit quality was most promising at temperatures less than 50ºC (Ikediala *et al*., 1999).

5) Application of radio frequency heat treatment in thermal sensitive fruit

Developing alternative treatment methods for control fruit flies has been increased since the use of chemical fumigation (methyl bromide) up to 2010. Radio frequency is a process which can achieve fast and effective thermal processing, however variations in radio frequency fields is a major obstacle in developing postharvest insect control treatments based on RF energy. Therefore, Birla *et al*. (2004) developed a laboratory scale fruit mover for improving uniformity of radio frequency heating suiting for movement and spinning of fruit while compare the method with conventional heating. The experiment found that fruit mover containing exactly volume of water can properly move a fruit as vortex formation while applied with radio frequency heating. The results showed that, with rotation and movement of fruit, temperature uniformity in oranges and apples was significantly improved after an average temperature rise of about 30˚C in 7.8 min. Based on the thermal death kinetics of the Mediterranean fruit fly (Medfly), Birla *et al*. (2005) continued to study the effect of Radio frequency heat treatments on the quality of treated 'Navel' and 'Valencia' oranges. The results indicated that the treatment that raises fruit temperature from 19 to 48 ºC by RF heating in saline water and held then for 15 min in 48º C hot water would meet the quarantine security without impairing the quality of the treated oranges. The potential of using radio frequency heating for pest control in 'Bing' sweet cherry (*Prunnus avium* L.) was also done by Monzon *et al.*(2006). The fruit were heated with radio frequency energy to four target temperatures (50, 52, 53 and 54 ºC) held in the RF-heated water for various holding times