CHAPTER 2

Literature Reviews

2.1 Mango Fruit and Ethylene Role during Ripening

Mango (*Mangifera indica* Linn.) is an economically important fruit, which widely cultivated in the tropics and subtropics (Sivakumar, Jiang, & Yahia, 2011). Mangoes belong to the family Anacardiaceae and they have 150 cultivars produced around the world (Medina & García, 2002). In the international market, the popularity of mangoes is due to its excellent flavor, attractive fragrance, beautiful color, delicious taste, and high nutritional properties (Table 2.1). These reasons making mango are usually referred to be as the king of fruits (Tharanathan et al., 2006). For the world mango production, it is reported more than 87 countries in which the prominent mango producing countries are India, China, Thailand, Indonesia, Philippines, Pakistan, and Mexico (Tharanathan et al., 2006). In addition, mango fruits are good sources of antioxidants such as carotenoids and phenolic compounds (Talcott, Moore, Lounds-Singleton, & Percival, 2005). Thus the consumption of mangoes can get significant amounts of antioxidants.

In Thailand, mangoes are considered as one of the most popular fruit and here is the third country with the largest mango production in the world as it produces around 2,985,530 tons a year (FAOSTAT, 2012). All regions of the country have widely cultivated the mangoes in several cultivars. Among all of the mango's cultivars, "Nam Dok Mai" mango (Figure 2.1) is the most popular exporting cultivar, because its beautiful color, attractive fragrance, and delicious taste is match to the consumer's demand (Laohaprasit, Kukreja, & Arunrat, 2012). Total production ranks the first among commercial mango cultivars. Moreover, the physical properties of Nam Dok Mai mango fruit are suitable for transportation (Suwapanich, 2006). As each fruit consists of 271.9 g weight, 144.4 mm length, 62.4 mm width, and 54.1 mm thick, and it has 69.8 % flesh, 13.1 % seed, and 17.2 % peel (Vásquez-Caicedo et al., 2002).

Table 2.1 Compositions of the edible portion of mango fruits

Compositions		Value per 100 g edible portion
Proximate	Water	83.46 g
	Energy	60 kcal
	Protein	0.82 g
	Total lipid	0.38 g
	Carbohydrate	14.98 g
	Total dietary fiber	1.6 g
	Total sugar	13.66 g
Minerals	Calcium	11 mg
	Iron	0.16 mg
	Magnesium	10 mg
	Phosphorus	14 mg
	Potassium	168 mg
	Sodium	1 mg
	Zinc	0.09 mg
Vitamin	Vitamin C	36.4 mg
	Thiamin	0.028 mg
	Riboflavin	0.038 mg
	Niacin	0.669 mg
	Vitamin B-6	0.119 mg
	Folate	43 µg
	Vitamin A	54 µg
	Vitamin E (alpha-tocopherol)	0.90 mg
	Vitamin K (phylloquinone)	4.2 µg

Source: USDA National Nutrient Database for Standard Reference (2016)

Figure 2.1 Characteristic of mango fruits cv. Nam Dok Mai

However, the amount of mangoes exported from Thailand to the world market is limited, due to highly perishable, short shelf life, and susceptible to postharvest decay (Suwapanich, 2006). As the mango fruit is a climacteric having a short shelf life and continue to ripen after harvest. During the ripening process, the climacteric fruit emits ethylene (C_2H_4), a naturally occurring plant growth hormone, along with increases rate of respiration (Alexander & Grierson, 2002). Fruit ripening is accompanied by an auto-catalytic increase in ethylene production during ripening (Mattoo & Modi, 1969; Litz, 2009). Ethylene has various effects on the growth, development, and storage life of many climacteric fruits (Saltveit, 1999). This plant hormone is synthesized from the methionine cycle through S-adenosyl-L-methionine (AdoMet) and the cyclic non-protein amino acid 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams & Yang, 1979). The enzymes catalyzing the conversion of AdoMet to ACC and of ACC to ethylene are ACC synthase and ACC oxidase, respectively (Figure 2.2) (Kende, 1993). For an economically important role, ethylene acts as an inducer of climacteric fruit ripening (Bleecker & Kende, 2000). It is predominantly postharvest applied in term of gas phase that diluted in the air. Ethylene concentrations commercially used are 10 to 1000 µL/L to trigger the ripening of climacteric fruits, *i.e.*, avocados, bananas, honeydew melons, mangos, kiwifruit, and tomatoes (Saltveit, 1999). Whereas, some situations the ethylene is considered as detrimental for the quality of fruits, for example, enhances excessive softening of fruits, promotes discoloration (browning), and induces germination of fungi spore causing postharvest diseases (Flaishman & Kolattukudy, 1994; Saltveit, 1999).

The accumulation of ethylene in storage areas increases susceptibility to senescence, fruit pathogen, and physiological disorders (Kader, 1985; Flaishman & Kolattukudy, 1994). Moreover, almost stress conditions can also increase ethylene biosynthesis resulting in over ripening and eventual reduces of postharvest life in fruits (Hyodo, 1991; Bleecker & Kende, 2000). These detrimental effects depend on a number of variables, the most important being tissue sensitivity to ethylene, temperature, duration of exposure, ethylene concentration, and atmospheric composition (Saltveit, 1999).

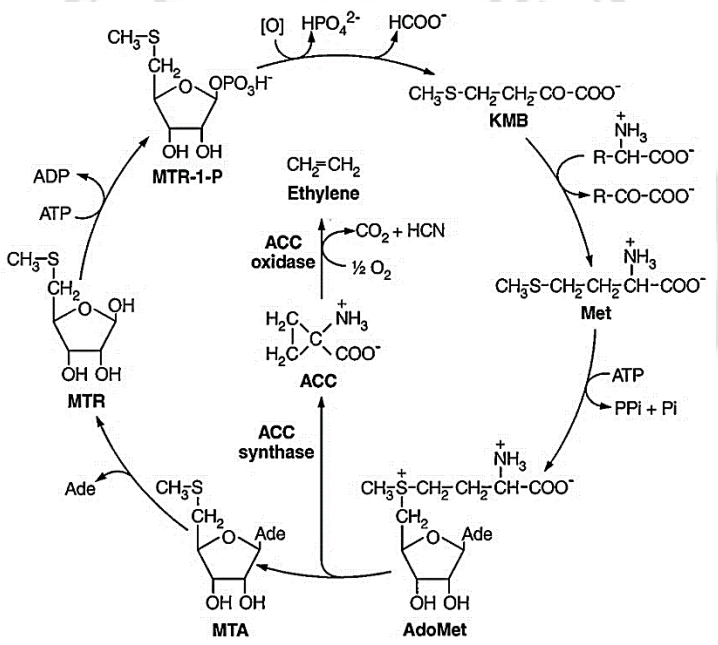


Figure 2.2 The ethylene biosynthesis pathway in plants (Bleecker & Kende, 2000)

ACC, 1-aminocyclopropane-1-carboxylic acid

Ade, adenine

AdoMet, S-adenosyl-L-methionine

KMB, 2-keto-4-methylthiobutyric acid

Met, L-methionine

MTA, 50-methylthioadenosine

MTR, 50-methylthioribose

MTR-1-P, 50-methylthioribose-1-phosphate.

The excessive ethylene exposure of mango fruit reduces its shelf life. Because the ethylene can induce the adverse physiological responses, *i.e.*, color changes, increase respiration, fruit softening, and aroma volatile production, and led to the physiological deterioration of fruits which related to over ripening and by pathogen infection (Watada, 1986; Wills et al., 1998; Tharanathan et al., 2006). The postharvest life of mangoes stored at 10 – 15 °C usually does not exceed 2 – 3 weeks (Yahia, 1998; Tharanathan et al., 2006). Furthermore, the excessive ethylene exposure led to increased severity of anthracnose. Brown and Barmore (1976) demonstrated that increasing the ethylene concentration in increment of 10 ppm from 0 to 50 ppm enhanced the occurrence of anthracnose in inoculated tangerines until a maximum was reached with 40 ppm, percent of anthracnose increased from 0 % to maximum at 86.7 %. This indicates that the severity of anthracnose is ethylene dependent.

2.2 Anthracnose Disease in Mango Fruit

Postharvest diseases of mangoes are symptoms of infected mango fruits including Botryodiplodia fruit rot, Dothiorella fruit rot, Phomopsis fruit rot, Aspergillus rot, and anthracnose rot, which caused by *Botryodiplodia theobromae*, *Dothiorella dominicana*, *Phomopsis mangiferae*, *Aspergillus niger*, and *Colletotrichum gloeosporioides*, respectively (Sangchote, 1987). These symptoms cause postharvest loss during transportation and storage (Sharma, Singh, & Singh, 2009). Among all postharvest diseases, anthracnose caused by *Colletotrichum* spp. is the most severe disease causing the postharvest loss to mango fruits (Sangchote, 1987). Rivera-Vargas, Lugo-Noel, McGovern, Seijo, and Davis (2006) studied the anthracnose disease caused by *Colletotrichum* spp. in mango fruits, which was conducted in different locations of Puerto Rico and Florida, USA. The authors found that 93 % of the isolates were identified as *Colletotrichum gloeosporioides* (*C. gloeosporioides*), while only 5 % as *Colletotrichum acutatum* (*C. acutatum*). Typically, symptoms on infected mango fruits appear as rounded brown to black lesions with an indefinite border on the fruit surface (Figure 2.3). After the initial infection, the fungus usually remains dormant until fruit begins to ripen. During ripening, dark depressed circular lesions develop and increase rapidly in size to cover almost the entire fruit in extremely severe cases (Siddiqui & Ali, 2014).

Figure 2.3 Anthracnose rot of mango fruits

Conidia are formed abundantly in the mango canopy, which is considered to be the primary source of inoculum (Fitzell & Peak, 1984; Arauz, 2000). As seen in Figure 2.4, *C. gloeosporioides* produces conidia on lesions on leaves, twigs, panicles, and mummified fruit in the field and conidia produced can be rain-splashed to other flowers or leaves causing the secondary infections (Fitzell & Peak, 1984; Arauz & González-Lobo, 1986; Arauz, 2000). Thus the disease is polycyclic in these organs (Arauz, 2000). In the some cases, developing fruit can be infected by some isolates and leading to preharvest fruit loss (Gantotti & Davis, 1993). For postharvest anthracnose, developing fruit are infected in the field, but infections remain quiescent until the onset of ripening, which occurs after harvest (Figure 2.4). Once the climacteric period of the fruit starts, lesions begin to develop. There is no fruit-to-fruit infection. Thus, postharvest anthracnose is a monocyclic disease (Arauz, 2000). Colony characteristics and spore colony of *C. gloeosporioides* in a laboratory media are shown at Figure 2.5 (A) and (B) respectively.

The fungus develops abundant orange to pink masses of conidia, the microscopic images of conidia are shown at Figure 2.6 (A) and (B). The conidia is frequently occur in concentric rings (Arauz, 2000). Difference of conidia between *C. gloeosporioides* and *C. acutatum* has been reported by Rivera-Vargas et al. (2006). Conidia of the isolates were hyaline, one-celled, ovoid to oblong, slightly curved, and ranged from 12 – 20 × 3.5 – 6.0 µm for *C. gloeosporioides* and 8 – 13 × 2 – 5 µm for *C. acutatum*. Additionally, results of ELISA and PCR assays and the result of morphological observation confirmed that

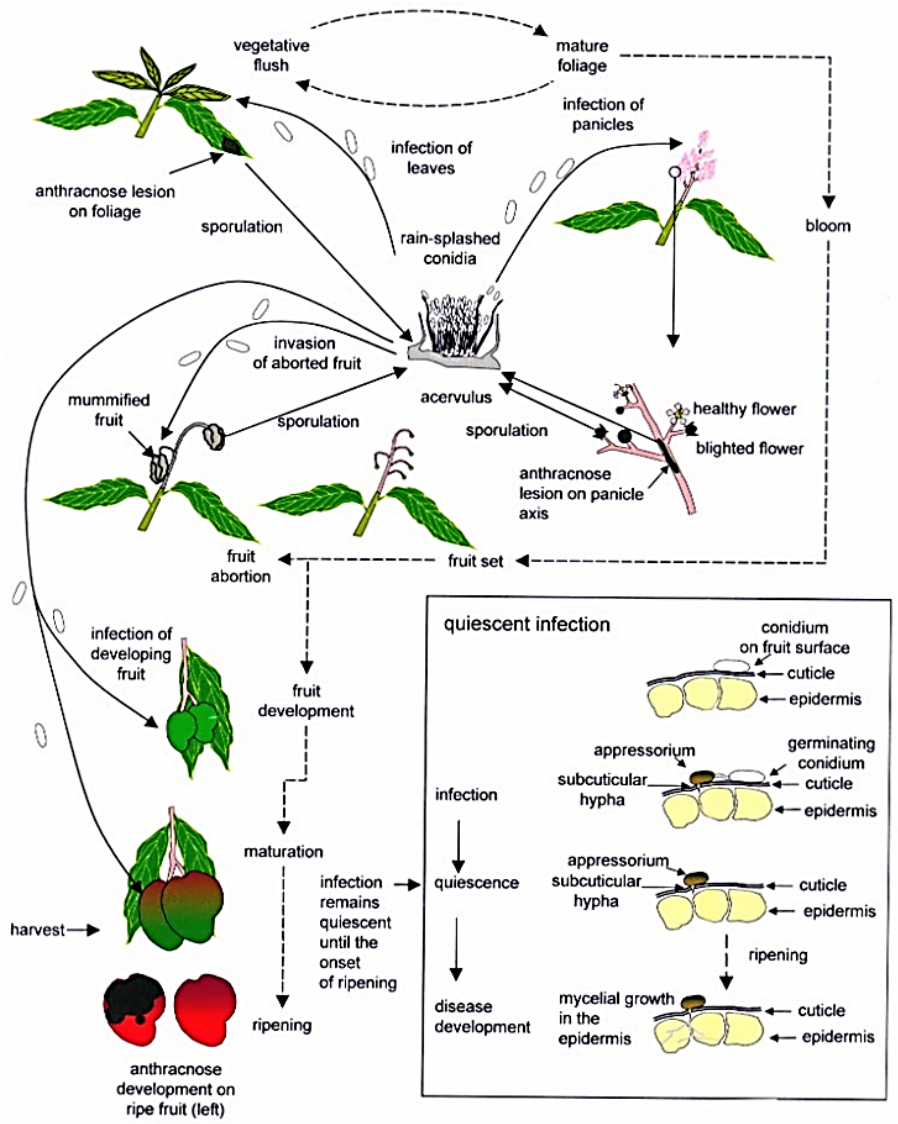
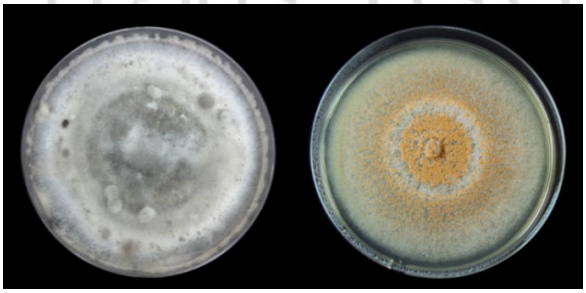


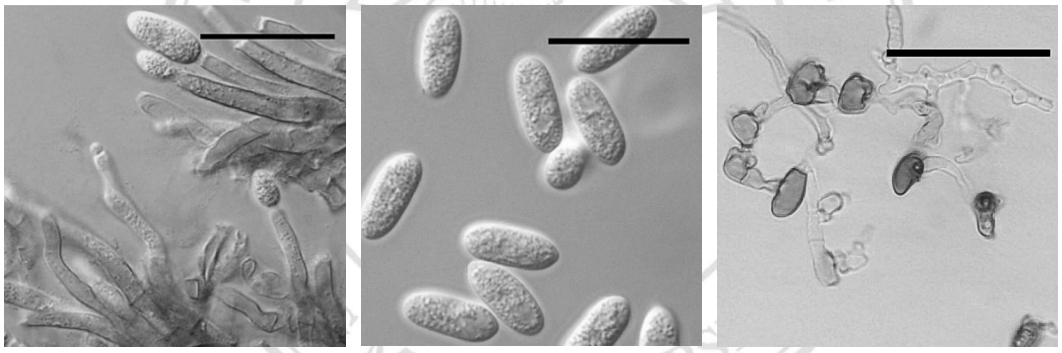
Figure 2.4 Anthracnose cycle on mango (Arauz, 2000). Solid lines represent disease cycle. Dotted lines represent mango phenology.



(A) (B)

Figure 2.5 Colony characteristic (A) and spore colony of *C. gloeosporioides* (B)

C. acutatum is a cause of anthracnose in mango's flower, peduncle, and immature fruit (Rivera-Vargas et al., 2006). For the pathogen infection of fruits, conidia are locally dispersed from infected plants by rain splashes prior to being disseminated by the wind (Siddiqui & Ali, 2014). Initially, the conidia infects immature green fruits in the field, followed by conidiospore germination and appressoria formation (Figure 2.6 (C)). The germinated spore of fungus would produces appressoria to enzymatically penetrate and degrade the cuticle of plant cell wall resulting in quiescent infection (Siddiqui & Ali, 2014). Furthermore, most mango fruits can be cross-infected with conidia from isolates of *Colletotrichum* spp. regardless of the original host plant. For example, the isolates of *Colletotrichum* spp. from other fruits (such as avocado, papaya, and citrus) can also cause infection in mango fruits (Freeman & Shabi, 1996).



(A) (B) (C)

Figure 2.6 Characteristics of conidiogenous cell (A), conidia (B), and appressoria (C) of *C. gloeosporioides* (Rojas et al., 2010). Bar = 20 µm.

Conditions that promote the development of anthracnose pathogens in postharvest period including the presence of high relative humidity (RH), warm temperature, and nutrients from fruit surfaces, mainly sugars (Flaishman & Kolattukudy, 1994; Gadgile et al., 2009; Rathod, 2011). Recent researches, Gadgile et al. (2009) studied the influence of environmental factors (temperature and RH) on the development of anthracnose rot in mango fruits caused by *C. gloesporioides*, they found that development of anthracnose was found to be very less at low temperature (10 °C) and low RH (30 %), whereas it was highest at 25 °C and at 100 % RH. The similar result is confirmed by Rathod (2011), the author also reported that anthracnose is maximum developed at conditions of 25 °C and

100 % RH. In addition, the quiescent infections in climacteric fruit are terminated with reduction of antifungal compounds, production of ethylene, and changes in nutritional and texture (Flaishman & Kolattukudy, 1994). Flaishman and Kolattukudy (1994) indicated that timing of fungi infection at host ripening is a significant cause of postharvest loss. Ethylene level produced at ripening would stimulate latent fungi to form branched mycelia and multiple appressoria, and induce any ungerminated spore to germinate and form appressoria. Multiple appressoria amplify the capability of the fungus to enter into the host by allowing penetration at multiple points (Flaishman & Kolattukudy, 1994). This is a reason that climacteric fruit at ripening is more susceptible to infection when its surface is soften. Thus, during handling, packing, and transportation operations, small openings in the skin or wounded areas on fruit surface become the excellent sites for the pathogens to entry into the fruit tissue.

In order to extend the shelf life of mango fruit and reduce postharvest loss, there are several techniques (such as cold storage, hot water treatment, controlled and modified atmospheric storages, irradiation, film packaging, coating, and the use of chemicals) that can be applied (Johnson et al., 1990; Baldwin et al., 1999; Srinivasa, Baskaran, Ramesh, Prashanth, & Tharanathan, 2002; Anwar & Malik, 2007; Kim, Brecht, & Talcott, 2007). However, most techniques for shelf life extension of mango fruit focus on the inhibitions of anthracnose pathogen and ethylene production, which are the major causes of the mango fruit deterioration during storage and export.

2.3 Active Packaging and Application on Climacteric Fruits

The climacteric fruit is highly perishable and susceptible to several postharvest diseases. Anthracnose is the most devastating disease of climacteric fruit which infects immature fruit in the field while symptoms appear only after ripening (González-Aguilar et al., 2003). Moreover, they demonstrate more dramatic changes in their physiological activity after ripening. Storage techniques have been developed to extend the storage life of fruits. Some extension of shelf-life has been demonstrated using controlled atmosphere (CA) storage (relatively low O_2 and high CO_2) (Pariasca, Miyazaki, Hisaka, Nakagawa, & Sato, 2000). The prevailing low O_2 and high CO_2 concentration levels also depress the biosynthesis of ethylene which can trigger the activity of ripening genes that affect color

changes, aroma and degradation of cell walls that results in softening. However, Lakshminarayana and Subramanyam (1970) reported that CO_2 injury, increased ethanol production and flavor problems due to anaerobic respiration. Edible coatings appears to be an alternative approach to extend the shelf life of climacteric fruit due to its acting as controlled atmospheres (Park, 1999). Additionally, edible coatings also provide more advantages than synthetic materials in terms of edibility, biocompatibility, being non-toxic and low cost. Edible coatings can be formulated from different materials including polysaccharides, proteins, lipids or combination of these materials (Lieberman & Gilbert, 1973). Some advantages to using coatings include reduction of water loss, retardation of ripening, reduction of chilling and mechanical injury. The coatings can prevent quality changes in foods by acting as barriers to control moisture transfer, oxygen uptake, lipid oxidation and loss of volatile flavors and aromas (Stuchell & Krochta, 1994). Moreover, coatings can also be used as carriers of useful ingredients such as antimicrobial compounds, color or aroma additives, antioxidants or anti-ripening compounds (McGuire & Hallman, 1995). The benefits of applying coatings to fresh fruits include control of ripening and browning, and delayed color, flavor, moisture and firmness loss (Li & Barth, 1998). In edible film or coated material containing antimicrobial, the gradual release of an antimicrobial from edible film or active coated material to the food surface may have an advantage over dipping and spraying. In the latter processes, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix (Appendini & Hotchkiss, 2002). However, the atmosphere created by skin coatings can change in response to environmental conditions, such as temperature and humidity, due to combined effects on fruit respiration and coating permeability. So, it causes changes in flavor as result of anaerobic respiration and thus increasing ethanol concentrations (Baldwin et al., 1999).

An active packaging is defined as packaging which changes the condition of the package system included in the packaging material or the package headspace to extend shelf-life or improve sensory properties while maintaining the food quality (Robertson, 2006; Day, 2008). Active packaging systems can be classified into two systems including active-releasing systems and active-scavenging systems (Barros-Velázquez, 2016). For the active-releasing systems, active compounds including antimicrobial agents and anti-

oxidant agents were incorporated into a package to improve its functionality or give it new functions. In this system, active compounds are released to react with specific targets through suitable release mechanisms. While the active-scavenging systems, this system removes undesired compounds, *i.e.*, water, oxygen, ethylene, carbon dioxide, and other compounds. Among these, antimicrobial packaging and ethylene-scavenging systems are interested in this study.

2.4 Antimicrobial Packaging

The antimicrobial packaging is a system, which aims to reduce, inhibit or retard the growth of microorganisms and thus extend the shelf-life of perishable products (Han, 2000). It can be constructed by using antimicrobial packaging materials or antimicrobial agents inside the package headspace or inside foods. It can take several forms including: (Appendini & Hotchkiss, 2002).

2.4.1 Type of Antimicrobial Packaging

1) Insertion of sachets containing volatile antimicrobial agent into packages

Oxygen and moisture absorbers in sachets are commercial used in bakery, pasta and meat packaging to prevent oxidation and water condensation. A reduction in oxygen and a_w inhibit the growth of aerobic microorganism, especially molds. Other examples include ethanol vapor generators which ethanol is released into the headspace of packaging via the selective barrier to retard molds in bakery and dried fish products (Smith, Hoshino, & Abe, 1995).

2) Incorporation of volatile and non-volatile antimicrobial agents directly into polymers.

The antimicrobial is incorporated into the packaging material, particularly films. Antimicrobial enzymes including lactoperoxidase and lactoferrin, antimicrobial peptides including magainins, cecropins, defensins, natural phenols like hydroquinones and catechins, fatty acid esters, antioxidant phenolics, antibiotics, metals like copper and silver

substituted zeolites and others may be useful (Hotchkiss, 1997; Brody, Strupinsky, & Kline, 2001). They are taken up by microbial cells disrupting the cells' enzymatic activity. Antimicrobial packaging materials must contact the surface of the food if they are non-volatile, so the antimicrobial agents can diffuse to the surface, therefore, characteristics of surface and diffusion kinetics become crucial. Han (2000) demonstrated that antimicrobial release from the polymer must be maintained at a minimum rate so that the surface concentration is above a critical inhibitory concentration. To achieve appropriate controlled release to the food surface, the use of multilayer films control layer, matrix layer barrier layer has been proposed (Floros, Nielsen, & Farkas, 2000). The inner layer controls the rate of diffusion of the active substance while the matrix layer contains the active substance and the barrier layer prevents the migration of agent towards the outside of a package. For volatile antimicrobials, the advantage is that they can penetrate the bulk matrix of food and that the polymer need not necessarily directly contact the product (Appendini & Hotchkiss, 2002).

3) Coating or adsorbing antimicrobials onto polymer surfaces.

Some antimicrobial agents are sensitive to polymer forming temperatures. Thus, coating onto the material after forming is an alternative way. Cast edible films, have been used as carriers for antimicrobials and applied as coatings onto packaging materials and foods. Examples include nisin-methylcellulose coatings for polyethylene films (Cooksey, 2001), nisin-zein coatings for poultry (Food Safety Consortium Newsletter, 2000), nisin coating on PE, EVA, PP, polyamide, PET, acrylics and PVC (Daeschel & McGuire, 1995; Wilhoit, 1996) and nisin-EDTA-citric solutions coated onto PVC, nylon and LLDPE films (Natrajan & Sheldon, 2000).

4) Immobilization of antimicrobials to polymers by ion/covalent linkages.

Immobilization requires the presence of functional groups on both of antimicrobial and polymer. Examples of antimicrobials with functional groups are peptides, enzymes, polyamines and organic acids. Immobilized peptides have shown antimicrobial activity against microorganisms in foods due to peptides can be covalently immobilized through amino and carboxylic groups, they may be suitable for attachment to functionalized polymer surfaces. Ionic bonding of antimicrobials onto polymers allows slow

release into the food. However, diffusion to the product is less of a concern when the antimicrobial is covalently bonded to the polymer unless conditions within the product promote reactions such as hydrolysis. The potential reduction in antimicrobial activity due to immobilization must be considered. For proteins and peptides, changes in conformation and denaturization by solvents may result in low activity per unit area. Approaches to increasing activity per unit area include the protection of active sites during film formation and incorporation of dendrites to increase the surface area of the supports (Appendini & Hotchkiss, 2002).

5) Use of polymers that are inherently antimicrobial.

Some polymers are inherently antimicrobial and they have been used in coatings and films. Cationic polymers such as chitosan and poly-L-lysine promote cell adhesion (Goldberg, Doyle & Rosenberg, 1990). Since charged amines interact with negative charges on the cell membrane, causing leakage of intracellular constituents. Chitosan has been applied as a coating and appears to protect fresh vegetables and fruits from the fungal degradation. Although the antimicrobial effect is attributed to antifungal properties of chitosan, it may be that the chitosan acts as a barrier between the nutrients contained in the produce and microorganisms (Cuq, Gontard, & Guilbert, 1995). In addition, chitosan-based antimicrobial films have been used to carry organic acids and spices (Ouattara, Simard, Piette, Bégin, & Holley, 2000). Calcium alginate films reduced the growth of the natural flora and coliform inoculate on beef, possibly due to the presence of calcium chloride (Cuq et al., 1995). Bactericidal acrylic polymers made by co-polymerizing acrylic protonated amine co-monomer have been proposed as packaging materials for increased fruit and vegetable shelf life (Pardini, 1987).

2.4.2 Chitosan

Chitosan, a linear copolymer of glucosamine and N-acetyl glucosamine connected through β-(1-4) glycosidic linkages (Figure 2.7), is a polysaccharide, which has received a considerable attention due to its potential in a broad range of applications (Tharanathan & Kittur, 2003). Chitosan is an edible and biodegradable material that has attracted notable interest in the food packaging area (Despond, Espuche, & Domard, 2001). It has been docu-

mented to possess film forming properties for use as edible films or coatings and also bio-active properties either in its polymeric or oligomeric form (Coma, Deschamps, & Martial-Gros, 2003). Chitosan is inherently antimicrobial and has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of foodborne filamentous fungi, yeasts, and bacteria by disrupts the barrier properties of the outer membranes of Gram-negative bacteria, which leads to the leakage of intracellular constituents (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001). The chitosan films are tough, long-lasting, flexible, and very difficult to tear. Most of their mechanical properties are comparable to many medium-strength commercial polymers. It has been reported that chitosan films have moderate water vapor permeability (WVP) values and could be used to increase the storage life of fresh produce and foodstuffs with higher water activity values. Chitosan exhibits excellent oxygen-barrier properties due its high crystallinity and the hydrogen bonds between the molecular chains (Kittur, Kumar, & Tharanathan, 1998; Gallstedt, 2001). Moreover, chitosan is a good barrier against grease (Kittur et al., 1998). Due to its positive charge on the amino group under acidic conditions, chitosan binds to negatively charged molecules such as fats and lipids (Shu, Zhu, & Song, 2001). These properties make chitosan an attractive polymer for the barrier coating of cellulose-based materials for food packaging purposes.

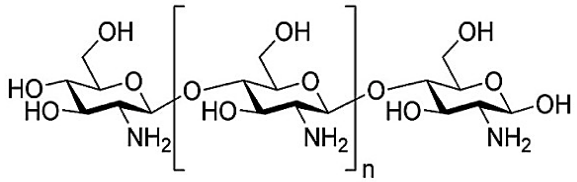


Figure 2.7 Chemical structure of chitosan (Mahapatro & Singh, 2011)

2.4.3 Carboxymethyl Cellulose

Carboxymethyl cellulose (CMC) is a copolymer of two units: ß–D–glucose and ß–D–glucopyranose 2–O (carboxymethyl)–monosodium salt which are connected via ß–1,4–glycosidic bonds (Figure 2.8). It contains a hydrophobic polysaccharide backbone and many hydrophilic carboxyl groups, showing amphiphilic characteristic (Zhang, Bacik, Roberts, & Zhao, 2013).

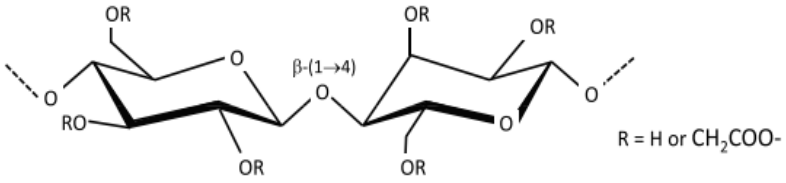


Figure 2.8 Chemical structure of carboxymethyl cellulose (Tong, Xiao, & Lim, 2008)

CMC is prepared by an alkali-catalyzed reaction with chloroacetic acid, in which some of the hydroxyl groups of the glucopyranose units in cellulose are replaced by carboxymethyl groups. CMC absorbs moisture, dissolves easily in cold water, shows thermal gelatinization, and forms films. CMC finds its uses as a viscosity modifier, thickener, water retention agent, or a structural or adhesive component in various applications. Edible film is a common packaging-related application. CMC is often used as a structural polymer or reinforcement in a film. The film-forming capabilities are due to the long chain length of the CMC molecules. As for other cellulose-based materials, the mechanical properties of CMC-containing films are lessened with increasing moisture content (Feng, Pelton, & Leduc, 2006). The concentration of alkali during functionalization of cellulose has a direct effect on tensile properties of CMC films (Rachtanapun, Luankamin, Tanprasert, & Suriyaterm, 2012; Rachtanapun & Rattanaponone, 2011). The alkali concentration affects the degree of substitution of the CMC molecules. The more carboxymethyl groups there are in the cellulose molecule, the stronger the CMC film is, due to strong intermolecular forces. At high alkali concentrations, sodium glycolate is formed, which reduces the strength properties. In addition to using CMC in blends, CMC was filled with clay, microcrystalline cellulose, and chitosan. If the filler is well dispersed in the polymer matrix, an improvement in the mechanical properties is usually observed. Typically, the tensile strength and the E-modulus are increased, and the strain at break is reduced. Filling has other effects as well, *i.e.*, clay filler reduced the water solubility as well as the moisture sensitivity of CMC-starch films. Glycerol is a regularly used plasticizer in CMC-based films. Increasing the amount of glycerol typically increases the ductility of the film significantly, but also leads to a decrease in the tensile strength and the modulus of elasticity. CMC may reduce the water vapor permeability (WVP) of the composite, depending on other polymers included in it, such as a starch/CMC material (Ghanbarzadeh, Almasi, & Entezami, 2010). However, CMC films are generally perme-

able to water vapor, and several approaches have been tested to reduce their permeability. Lipids are among the potential additives that increase barrier to water vapor in hydrophilic films. Increasing the mass fraction of lipids reduces the WVP of CMC film, but it also decrease mechanical properties of the films. The behavior was observed, for example, with oleic in CMC films and palm oleic in CMC/glucomannan blend films (Cheng, Karima, & Seow, 2008; Ghanbarzadeh & Almasi, 2011). The preparation method, type, and amount of the CMC component in the blend can slightly reduce the WVP value, but greater reductions are achieved using inorganic or organic fillers. The degree of substitution (DS) of CMC affects the hydrophilicity of the film, which in turn is directly proportional to the WVP of CMC films (Rachtanapun et al., 2012). The added compounds affect the barrier and mechanical properties of the packaging film due to chemical interactions between polymer and active compound. Potassium sorbate has been studied as an antimicrobial agent in CMC films (Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011). It provides the desired antimicrobial effect, but weakens the polymer structure. Water extracts from different types of murta leaves are antioxidant solutions from nature (Bifani et al., 2007; Gutiérrez, Echeverría, Ihl, Bifani, & Mauri, 2012; Ramírez, Gallegos, Ihl, & Bifani, 2012). The extracts give CMC films a yellowish color. In addition to the antioxidative effect, the solids present in the extract may slightly reduce the WVP of films, but significant reductions in the WVP are obtained by fillers. Murta extract plasticizers the CMC-matrix and thus reduces oxygen and CO_2 permeability (Paunonen, 2013).

2.4.4 Vanillin

Vanillin or 4-hydroxy-3-methoxybenzaldehyde (Figure 2.9) is the major components of vanilla (*Vanilla planifolia* A.), which is an economically important flavoring plant. Most contents of vanillin contain in the vanilla pod (Walton, Mayer, & Narbad, 2003). Vanillin has generally recognized as safe (GRAS) status and is used as a flavouring compound in several industries, *i.e.*, foods, beverages, pharmaceutics, and cosmetics (Medina et al., 2009). Recent reports have shown that vanillin can play a role as antioxidant, antibacterial, and antifungal (Burri, Graf, Lambelet, & Loliger, 1989; Lopez-Malo, Alzamora, & Argaiz, 1995; Moon, Delaquis, Toivonen, & Stanich, 2006). Moreover, vanillin also have positive effects to human health that involved with its function can act as antimutagenic

agent, inhibit chemical carcinogenesis, and suppress invasion and migration of cancer cells (Gustafson et al., 2000; Lirdprapamongkol et al., 2005; King et al., 2007).

The antimicrobial properties of vanillin is involved with its aldehyde and hydroxyl groups. The aldehyde group of vanillin play a key role in its antimicrobial activity, while side-group position on the benzene ring also influences this activity (Fitzgerald et al., 2005). It is well known that aldehyde group is very reactive and can form covalent bonds with DNA and proteins, thereby potentially interfering with their normal functions (Feron et al., 1991; Fitzgerald et al., 2005). Likewise, recent researches have been suggested that antimicrobial action of phenolic compounds is due to the presence of hydroxyl group (Wendakoon & Sakaguchi, 1995; Aziz, Farag, Mousa, & Abo-Zaid, 1998; Dorman & Deans, 2000; Ultee, Bennik, & Moezelaar, 2002), which can reacts with enzyme active sites via formation of hydrogen bonds (Wendakoon & Sakaguchi, 1995; Aziz et al., 1998) and acts as a transmembrane carrier for monovalent cations (Dorman & Deans, 2000). On the same way, Fitzgerald et al. (2004) exhibited that the mode of antimicrobial action of vanillin against *Escherichia coli*, *Listeria innocua*, and *Lactobacillus plantarum* is primarily a membrane-active compound, resulting in the dissipation of ion gradients and the inhibition of respiration.

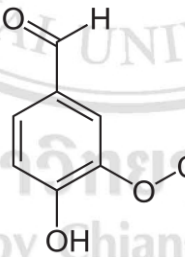


Figure 2.9 Structure of vanillin (Walton et al., 2003)

Several researches have reported the antimicrobial activity of vanillin (Table 2.2), depending on several factors such as target microorganism, concentration, temperature, and pH (Lopez-Malo et al., 1995; Cerrutti & Alzamora, 1996; Lopez-Malo, Alzamora, & Argaiz, 1997). Jay and Rivers (1984) reported that the inhibitory activity of vanillin was more effective against Gram-positive than Gram-negative bacteria. However, Fitzgerald et al. (2004) reported that Gram-negative *Escherichia coli* had the lowest minimum inhi-

bitory concentration (MIC) compared with Gram-positive bacteria of *Lactobacillus plantarum* and *Listeria innocua*, indicating that Gram-negative bacteria can be equally susceptible to the antimicrobial activity of vanillin. For the food spoilage yeasts, Fitzgerald, Stratford, and Narbad (2003) also demonstrated the antimicrobial potential of vanillin against the growth of three yeasts of food spoilage including *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, and *Zygosaccharomyces bailii*. The authors determined that the MIC values were 21, 20, and 13 mM vanillin for the three yeast strains, respectively.

For inhibitory effect against molds, Lopez-Malo et al. (1995) studied the effect of vanillin concentrations (0 – 2000 ppm) on the growth of four *Aspergillus* spp. molds, including *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, and *Aspergillus parasiticus*, in potato dextrose agar (PDA) and five fruit-based agar (apple, pineapple, mango, banana, and papaya). The authors reported that the increase in vanillin concentration and the type of fruit used in agar preparation affected the radial growth rate. In general, the vanillin inhibition concentrations were lower than 2000 ppm. They also found that the lower inhibitory effect of vanillin was observed in mango and banana, due to their fat and/or protein contents. Additionally, Lopez-Malo et al. (1997) demonstrated the effects of pH (3.0 – 4.0), incubation temperatures (10 – 30 °C), and vanillin concentrations (350 – 1200 ppm) on the fungal growth including *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, and *Aspergillus niger* on PDA. The authors found that the increase in both pH and temperature enhanced the growth of all molds, and the increased concentrations of vanillin decreased the growth of all molds.

There are few studies of vanillin with regard to its antifungal effect in practical applications. Recent research, the application of vanillin as active substance incorporating with the wrapping film to control *Saccharomyces cerevisiae* and *Escherichia coli* in the fresh-cut cantaloupe and pineapple have been reported by Sangsuwan, Rattanapanone, and Rachtanapun (2008). Likewise, Rakchoy et al. (2009) have applied the vanillin solution for coating paperboard intend for packaging bakery product. Paperboard coated with vanillin can inhibited food spoilage bacteria including *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* and greatly extended the shelf-life of that bakery product. However, the application of vanillin as antifungal substance incorporated in active packaging for controlling micro-biological quality of climacteric fruits during exportation is an interest issue.

Table 2.2 Inhibitory effects of vanillin on spoilage microorganisms

Microorganisms	Food systems	MIC	References
Bacterias			
- *Escherichia coli*	Laboratory media	15 mM	Fitzgerald et al. (2004)
- *Lactobacillus plantarum*	Laboratory media	75 mM	Fitzgerald et al. (2004)
- *Listeria innocua*	Laboratory media	35 mM	Fitzgerald et al. (2004)
- *Pseudomonas aeruginosa*	Laboratory media	18 mM	Rupasinghe et al. (2006)
Yeasts			
- *Saccharomyces cerevisiae*	Laboratory media	21 mM	Fitzgerald et al. (2003)
- *Saccharomyces cerevisiae*	Apple juice and peach-flavored soft drink	17 mM	Fitzgerald et al. (2004)
- *Zygosaccharomyces bailii*	Laboratory media	20 mM	Fitzgerald et al. (2003)
- *Zygosaccharomyces rouxii*	Laboratory media	13 mM	Fitzgerald et al. (2003)
- *Candida parapsilosis*	Apple juice and peach-flavored soft drink	9 mM	Fitzgerald et al. (2004)
- *Debaryomyces hansenii*	Apple puree	2000 ppm	Cerrutti & Alzamora (1996)
Moulds			
- *Aspergillus flavus*	Mango and banana-based agars	1000 ppm	Lopez-Malo et al. (1995)
- *Aspergillus niger*	Mango and banana-based agars	1500 ppm	Lopez-Malo et al. (1995)
- *Aspergillus ochraceus*	Mango and banana-based agars	1000 ppm	Lopez-Malo et al. (1995)
- *Aspergillus parasiticus*	Mango and banana-based agars	1000 ppm	Lopez-Malo et al. (1995)

MIC: Minimum inhibitory concentration

2.5 Ethylene-Scavenging Systems

Most substances designed to remove ethylene from packages are delivered either as sachets that place or stick inside the package or are integrated into the packaging material, usually a plastic polymer film or the ink used to print on the package. Many vendors offer ethylene adsorbers based on $KMnO_4$ immobilized on any of several minerals. The performance and useful lives of these scavengers depends on the substrate surface area and the content of reagent ($KMnO_4$). Formulations differ in density and surface area of substrate and the loading of reagent. Potassium permanganate is not integrated into food-contact packaging because of its toxicity. However, sachets could be used inside produce packages and have been shown to effectively scavenge ethylene from packages of bananas, persimmons, kiwi fruit, avocados (Ben-Arie & Sonego, 1985; Fuchs & Temkin-Gorodeiski, 1971; Hatton & Reeder, 1972; Krishnamurthy & Kushalappa, 1985; Liu, 1970; Maotani et al., 1982; Scott, McGlasson, & Roberts, 1970). An alternative ethylene-scavenging system had been proposed. Activated carbon, bentonite, Kieselguhr, and crystalline alumino-silicates, *i.e.*, zeolite, had been reported the capable of adsorbing ethylene. Film incorporating the Orega compound is claimed to have an ability to scavenge ethylene at a rate of at least 0.005 ppm per hour per square meter. The ethylene adsorptive activity of this film results from adding a fine porous into the film, inorganic material containing a large number of fine pores, 2 to 2,800 Aº in size, such as pumice, zeolite, activated carbon, cristobalite, and clinoptilolite. The finely divided porous materials are sintered with a small amount of a metal oxide before being added to the film. The particle size of the fine powder should be at least 200 mesh, and at least 1% by weight should be contained in the film. Treating the porous mineral with oxygen enhances the ethylene absorbing activity. The film containing the fine porous material not only has ethylene scavenging activity, but reportedly also has excellent permeability to gases such as oxygen, carbon dioxide, nitrogen, ethylene, and water vapor. Consequently, ethylene gas is discharged outside the film wrapping vegetables and fruit, and the inside of the film is maintained at a suitable relative humidity (Choi, 1991).

2.5.1 Mechanism of Adsorption at the Solid/Gas Interface

Gas adsorption is the phenomenon in which a gas is accumulated on the solid surface only (Bahl, Bahl, & Tuli, 1977). Mechanisms of gas adsorption mainly occur from a spontaneous accumulation of a gas (adsorbate) at the surface of solid (adsorbents) through intermolecular forces or called as van der Waal's attractive forces that referred to as physical adsorption and/or the formation of chemical bonds, which may be covalent or ionic in nature, this is referred to as chemical adsorption (IUPAC Compendium of Chemical Terminology, 2014).

2.5.2 Factors Affecting Adsorption of Gas on Solid Adsorbent

1) Nature of adsorbents

The adsorption of the gas depends on the nature of the adsorbents that differed in their size, surface area, and functional groups on the surface (Pan & Xing, 2008). Increase in the surface area of the adsorbent increased in the adsorption of gases (Bahl et al., 1977) due to more number of adsorbing sites.

2) Nature of gas

A gas, which has higher in its critical temperature, will be more easily to be adsorbed. For example, 1 g of activated carbon can adsorbs 380 mL of sulphur dioxide (critical temperature 157 °C), 16 mL of methane (critical temperature –83 °C) and 4.5 mL of hydrogen (critical temperature –20 °C) (Bahl et al., 1977). Likewise, the activated carbon (2.5 to 7.5 μL/L) can adsorb over 70 % of the exogenous ethylene gas within 24 h (Martínez-Romero et al., 2007).

3) Temperature

Physical adsorption occurs rapidly at low temperature and decreases with increasing temperature, which is in accordance to Le Chatelier's principle. On the other hand, an increase in the temperature can cause physical adsorption to change to chemical adsorption. For example, nitrogen is physically adsorbed on iron at 190 °C but chemically adsorbed to form a nitride at 500 °C (Bahl et al., 1977).

4) Pressure

Increase of pressure leads to increase of gas adsorption and decrease of pressure causes desorption (Bahl et al., 1977).

2.5.3 Ethylene Adsorbents

Exposure to ethylene accelerates the ripening and senescence of climacteric fruit (Kanellis, Chang, Kende, & Grierson, 1997). This gas is increasingly produced in its ripening period of fruit. To extend the shelf life of climacteric fruit during storage, the ethylene absorbents can be successfully applied to remove exogenous ethylene existing in the storage atmosphere (Kanellis et al., 1997). Scott et al. (1970) demonstrated that potassium permanganate can used as an ethylene adsorbent in polyethylene bags to delay ripening of bananas during storage. Bananas in polyethylene bags containing potassium permanganate was firmer than the one without the adsorbent. There are various kinds of ethylene absorbents such as silica gel, potassium permanganate, activated carbon, zeolite, montmorillonite, natural clay, etc. Among these ethylene absorbents, two commercial ethylene absorbents, activated carbon and zeolite, are often used in active packaging because they are available, non-toxic, low cost, and wide usability.

1) Activated carbon

Activated carbon is the most widely used adsorbent, which can be typically produced from a variety of heterogeneous carbonaceous materials, such as coconut shells, wood, coal, fruit nuts, etc., which are materials with high carbon content and low inorganic components (Yang, 2003; Dabrowski, Podkościelny, Hubicki, & Barczak, 2005; Kose, 2010; Sivakumar, Kannan, & Karthikeyan, 2012; Du et al., 2014). In general, this adsorbent can be found in the forms of granular activated carbon (GAC) and powdered activated carbon (PAC). The particles of GAC have irregular shapes with commercially available sizes ranging from 0.5 – 2.5 mm, while PAC is a pulverized form of GAC that its size is predominantly less than 0.15 mm (Karanfil, 2006). Bailen et al. (2007) reported that both GAC and PAC with concentration of 1.25 g/L effectively absorbed exogenous ethylene. GAC was the most effective. To increase the ethylene removal, a system containing GAC and 1 % of palladium as a catalyst was developed and applied on tomatoes. It was found

that ethylene removal led to a delay in tomato ripening. For the unique adsorption properties of activated carbon, they are results from high surface area, high adsorption capacity, and broad range of surface functional groups (Yang, 2003; Sivakumar et al., 2012). The activated carbon contains a high porous with an internal surface area about 500 – 2500 m^2/g (Derbyshine et al., 2001). Li, Quinlivan, and Knappe (2002) exhibited that increases in BET surface area of activated carbon fibers were primarily attributable to increases in micropore volume. Likewise, the adsorbent pore size can also be influenced the adsorption capacity. The sizes of adsorbent pore's width are classified into four groups according to the International Union of Pure and Applied Chemistry recommendations: (1) macropores (larger than 500 Å), (2) mesopores (20 – 500 Å), (3) secondary micropores (8 – 20 Å), and (4) primary micropores (smaller than 8 Å), as shown in Figure 2.10 (Sing et al., 1985; Kose, 2010). Karanfil and Dastgheib (2004) demonstrated that the increase in the pore volume in the region of micropore less than 10 Å enhanced trichloro-ethylene by GAC. While the optimum pore size region for TCE adsorption was in between 5 – 8 Å region. On the other hand, Guo, Yadav, and Karanfil (2007) reported that optimum pore size region for the adsorption of atrazine by GAC was 10 – 20 Å. The optimum pore size for the adsorption of ethylene gas (molecular diameter approximately 0.358 nm) by a commercial activated carbon was reported by Suzuki and Sakoda (1982), ranging of 0.95 – 1.10 nm. In addition, it is known that activated carbon has heterogeneous surfaces, which are associated with different functional groups. Several techniques can be modified the chemical surface of activated carbon such as heat treatment, oxidation, amination, and impregnation with various inorganic compounds (Karanfil & Kilduff, 1999; Kose, 2010). Increasing the oxygen-containing functional groups that's carboxylic acid, phenolic hydroxyl, and quinone carbonyl groups on the activated carbon surface also increased its acidity and polarity (Karanfil & Kilduff, 1999). On the other side, acidity and polarity reduced if the activated carbon surfaces contained more nitrogen functional groups (Mangun, Benak, Economy, & Foster, 2001). Above predominant features of activated carbon resulted to it is an excellent and versatile adsorbent, and widely applied in several industries including the adsorptive removal of color, odor, taste, and other undesirable organic and inorganic pollutants from drinking water, the treatment of industrial waste-water, solvent recovery, air purification and purification of chemical, food, and pharmaceutical products (Levine & LaCourse, 1967).

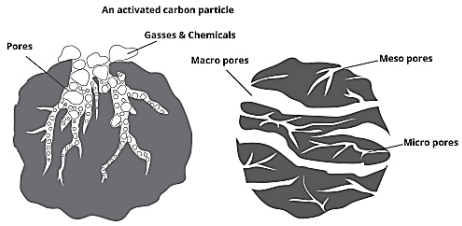


Figure 2.10 Characteristic of activated carbon
(http://www.keywordsking.com/emVvbGl0ZSBhIHN1cmZhY2UgYXJlYQ [1.14.2017])

2) Zeolite

Zeolites are environmentally compatible crystalline alumino-silicates, which have well defined micropore dimensions and composition in a rigid crystal lattice. Zeolite frameworks consist in tetrahedral units of silicate (SiO_4) and aluminate (AlO_4), and their silica alumina ratio (SAR) determines zeolite polarity. Its three-dimensional frame-work, generating nanometer sized channels and cages, imparts high porosity and a large surface area on this material. One of its defining features is the shape of internal pore structure that can strongly affect its adsorption selectivity toward host molecules (Cejka, van Bekkum, Corma, & Schueth, 2007). Utilization of natural zeolite as an adsorbent has gained interest among researchers, mainly because its adsorption properties provide a combination of ion exchange and molecular sieve properties which can also be easily modified (Cincotti et al., 2006). The isomorphic substitution of Si by Al, leads to a negative charge density in the zeolite lattice. This charge is neutralized by introducing monovalent, divalent or trivalent cations in structural sites of zeolite (Sue-aok, Srithanratana, Rangsriwatananon, & Hengrasmee 2010). Ion exchange of the balanced cations can result in zeolites with specific properties such as selective adsorption. Alver and Sakızcı (2012) reported that natural zeolite (clinoptilolites) had considerable good ethylene removal properties. They found that zeolites containing K^+ ions showed greater capacity for ethylene adsorption than zeolite exchanged with Na^+ and Ca^{2+}. Sue-aok et al. (2010) reported the enhancement of ethylene absorptivity upon modification of NaY zeolite by group I metal cations. Patdhanagul, Srithanratana, Rangsriwatananon, and Hengrasmee (2010) demonstrated that zeolite NaY modified with phenyl trimethyl ammonium bromide (PTAB) as a cationic surfactant, is capable to modified only the external part of zeolite and not capable to enter the micropores of zeolite due to its large long chain, showing an appreciable

increase in ethylene adsorption. On the other hand, the zeolite modified with palladium presented a significant ethylene adsorption capacity (4162 μLg^{-1} material), far superior to $KMnO_4$-based scavengers when used in low amounts (10 mg modified zeolite/L) and in conditions of high relative humidity (Terry et al., 2007). Moreover, it is not only to provide several advantages similar to other typical packing materials including high porosity for easy air flow, extensive surface area for biofilm attachment, but also has high moisture holding capacity, diverse nutrient content, very slow degradation rate as well as more economic advantages (Stoeckinger, 2004).

2.6 Active Agent Release Mechanism

2.6.1 Type of Active Agent Release Mechanism

1) Diffusion limited release: reservoir systems

A reservoir system consists of an active agent contained within a rate-controlling barrier (Figure 2.11). Barriers may be microporous, macroporous or non-porous; these last systems barriers are the most commonly used. The release rate from a reservoir system depends on the thickness, area and permeability of the barrier. In a reservoir containing an excess of active agent, the release rate follows zero-order kinetics (Pothakamury & Barbosa-Canovas, 1995). According to Pothakamury and Barbosa-Canovas (1995), the principal steps in the release of an active ingredient from a reservoir system are: (1) diffusion of the active agent within the reservoir, (2) dissolution or partitioning of the active agent between the reservoir carrier fluid and the barrier, (3) diffusion through the barrier and partitioning between the barrier and the elution medium (*i.e.*, the surrounding food), and (4) Transport away from the barrier surface into the food.

2) Swelling induced release

In swelling-controlled systems, the active agent, dissolved or dispersed in a polymeric matrix, is unable to diffuse to any significant extent within the matrix because of its low diffusion coefficient. When the polymer matrix is placed in a thermodynamically compatible medium, the polymer swells owing to absorption of fluid (penetrant) from the medium. The active agent diffusion coefficient in the swollen part of

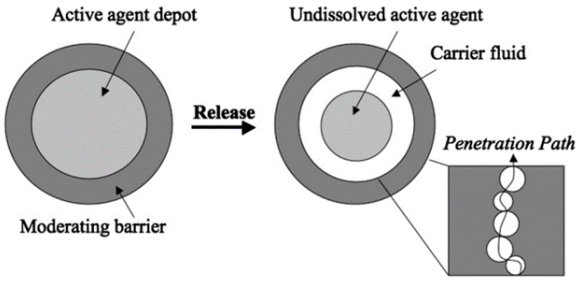


Figure 2.11 Reservoir system (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010)

the matrix increases then it diffuses out. In a diffusion controlled system, the matrix is assumed to be ineffective during the process of release, whereas in swelling-controlled systems, the membrane undergoes a transition from glassy to rubbery state, upon interaction with the penetrant. The polymer chains in the rubbery state, being more mobile than in the glassy state, allow the active agent to diffuse out of the matrix more rapidly. The release rate is determined by the process of glassy to rubbery transition. Sanchez-Gonzalez, Cháfer, González-Martínez, Chiralt, and Desobry (2011) studied the release kinetics of limonene, the major oil component of bergamot oil, from chitosan film to five liquid food stimulants (aqueous solutions with 0, 10, 50, and 95 % of ethanol and isooctane). It was found that kinetic constants for all films increased exponentially when the ethanol concentration increased in the aqueous system and were slightly greater when the film thickness was lower. They mentioned that the hydration of film to promote the molecular mobility was essential to ensure the compound release.

The release rate is determined by the transition of glass-to-rubbery (Mastromatteo et al., 2010). In the case of plane geometry, the quantity of active substance, M_t, released at time t is given by Equation 2.1 (Crank, 1975; Peppas, 1985).

$$\frac{M_t}{M_\infty} = kt^n \tag{2.1}$$

Where M_∞ is the amount of active substance released at equilibrium, k is a constant which characterizes the polymer network system (s^{-1}), t is time (s), and n is the diffusion exponent.

The parameter n can take a range of values that indicate the type of transport. When $n \leq 0.5$, the active substance is released by simple Fickian diffusion (Stochastic phenomenon) in which the rate of diffusion is much less than that of the film relaxation. A diffusion exponent in the range of 0.5 and 1 is a combination of Fickian and non-Fickian, and is known as anomalous diffusion which occurs when the diffusion and relaxation are comparable. The last case in which $n > 1$ shows that diffusion is very rapid compared with the relaxation processes. Values of n and k can be obtained from the slope and intercept of the ln M_t/M_∞ and ln t plot. The diffusion coefficients (D) ($m^2.s^{-1}$) of active substance was later calculated using the half-time method equation (Lim & Tung, 1997).

$$D = \frac{0.049h^2}{t_{0.5}} \tag{2.2}$$

Where h is the film thickness (m), and $t_{0.5}$ is the time (s) at which $M_t = 0.5\, M_\infty$. The diffusion coefficients, obtained from Equation (2.3), was substituted in Crank's equation (Crank, 1975).

$$\frac{M_t}{M_\infty} = 1 - \sum\left[\frac{8}{(2n+1)^2\pi^2}\right]\exp[-(2n+1)^2\pi^2\frac{Dt}{h^2}] \tag{2.3}$$

In order to assess the temperature dependence of diffusion, an Arrhenius activation energy calculated by Equation (2.4) was used.

$$D = D_0\exp(-\frac{Ea}{RT}) \tag{2.4}$$

In which D_0 is a constant ($m^2.s^{-1}$), Ea is the activation energy ($J.mole^{-1}$), R is the universal gas constant (8.314 $J.mole^{-1}.K^{-1}$), and T is the absolute temperature (K).

3) Biodegradation induced release

Two different erosion mechanisms have been proposed: (1) surface or heterogeneous erosion, and (2) bulk or homogeneous erosion (Langer & Peppas, 1983). Firstly, polymer degradation is much faster than water intrusion into polymer bulk. The degradation occurs mainly in the outermost polymer layers, thus affecting only the surface and not the inner parts of the matrix (heterogeneous process). Bulk eroded polymers in contrast degrade slowly and water uptake by the system is much faster than polymer degradation. Thus, the entire system is rapidly hydrated and polymer chains are cleaved throughout the device. Consequently, erosion is not restricted to the polymer surface (homogeneous process). As a basic rule, polymers that are built from reactive functional groups tend to degrade fast and to be surface eroded, whereas polymers containing less reactive functional groups tend to be bulk eroded. Polyanhydrides are examples for predominantly surface eroded polymers, while polylactide and polylactide-co-glycolide are examples for predominantly bulk eroded materials.

2.6.2 Factors Affecting the Release of Active Substance

1) Temperature

The glass transition temperature (T_g) of a polymeric matrix is one of the important physicochemical properties of the matrix. Polymeric matrix exists in a soft rubbery state above T_g and changes to a hard or brittle structure of the glassy state below T_g. The permeability properties of polymer materials are higher in the rubbery state (above T_g) where polymer chains are more mobile than in the glassy state (Gontard & Ring, 1996). Therefore, above the glass transition temperature the molecular mobility in a system increases with temperature, which leads to an increment in the ability of the material to transport substances through its network. For example, increasing the temperature from 4 – 24 °C resulted in a faster rate of diffusion for acetic and propionic acids from chitosan-based films (Ouattara et al., 2000). These can be explained by the change of free volume in films which response to temperature. The transport of solutes is presumed to permeate through the free-water region in the swollen film (Fang, Cheng, & Lu, 1998). As the total volume of pores or channels in the surface of film at higher temperature, resulting in the increase in an effective diffusion area, the amount of solute transport increases (Yoshizawa,

Shin-ya, Hong, & Kajiuchi, 2005). Hence, the diffusion coefficient of the film increased as temperature increased (Sangsuwan, Rattanapanone, Auras, Harte, & Rachtanapun, 2009; Bastarrachea et al., 2010).

2) Relative humidity

Relative humidity can affect the release of active agent from biopolymer matrix in relation to glass transition change (Whorton, 1995; Busso et al., 2007). The glass transition phenomenon is not only related to the molecular mobility of the matrix but also that of components entrapped in the matrix such as active compounds. Changes in the glass transition temperature can be explained behavior of release from a biopolymer matrix. As the relative humidity increases, the water concentration and the transition occurs leading to a high release of the active compound (Chalier, Ben Arfa, Guillard, & Gontard, 2009). For example, the diffusion of sugars in the model food with a_w of 0.85 is slower than the model food with a_w of 0.95 (Petersson, Thomsen, Hauggaard-Nielsen, & Thomsen, 2007). The diffusion might have been slower due to less free water that can act as diffusion medium of the sugars and a higher viscosity in the low a_w model food. Moreover, the combined effect of temperature and relative humidity can be strongly dependent on diffusion coefficient. Increasing storage temperature from 5 – 30 ºC and relative humidity from 60 – 100 % led to an increase in carvacrol diffusivities from SPI-coated paper (Chalier et al., 2009). The influence of temperature and relative humidity on release was related to the glass transition phenomenon and its effect on chain polymer mobility and substances diffusivity. Therefore, the diffusion coefficient of substance increased as temperature and relative humidity increased (Chalier et al., 2009).

3) pH value

The charge density of the polymers depends on pH and ionic composition of the solution that the polymer is exposed. Altering the pH of the solution will cause swelling or de-swelling of the polymer. Thus, active substance release from these polymers will display release rates that are pH sensitive. There are two kinds of pH sensitive materials: one which have acidic group (-COOH, $-SO_3H$) and swell in basic pH because the acidic groups will be protonated and unionized are known as polyacidic polymers such as poly-

acrylic acid, and others which have basic groups ($-NH_2$) and swell in acidic pH because the ionization of the basic groups will increase with decreasing pH are known as polybasic polymers such as chitosan (Balamuralidhara et al., 2011). For the example, the diffusion coefficient of vanillin from chitosan/methyl cellulose films to citrate buffer increased with decreasing pH from 6.5 – 3.5 (Sangsuwan et al., 2009). In agreement with these finding, Shu et al. (2001) and Yoshizawa et al. (2005) reported that the decrease in pH facilitated the amino groups in chitosan film were in the form of NH_3^+ which caused film swelling and led to the higher leaching of migrant from the chitosan network.