CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Intelligent Packaging

Intelligent packaging is defined as packaging systems that monitor the condition of the food to provide information on the quality of the packaged food during transport and storage. A variety of indicators that belong to the group of intelligent systems are of interest to the food-packaging chain, such as indicators of temperature, time-temperature, pack integrity, microbial growth, product authenticity and physical shock. An indicator must be easily activated, exhibit an easily measurable, reproducible time/temperature-dependent change, be irreversible, and ideally correspond or easily be correlated to food quality. Commercially available indicators of interest for monitoring the food quality include indicators of time-temperature, leakage and freshness (Ahvenainen *et al.*, 2003).

These types of devices can be divided into three groups. The first type is the external indicators. These indicators are attached outside the package, and include time temperature indicators and physical shock indicators. The second type is the internal indicators, which are placed inside the package. They are either placed in the headspace of the package or attached to the lid - for example, oxygen leak indicators, carbon dioxide, microbial, and pathogen indicators (Ahvenainen *et al.*, 2003). The third type of devices is the indicators that increase the efficiency of information flow and effective communication between the product and the consumer. These products include special bar codes that store food product information such as use, and consumption date expiration. Product traceability, anti-theft, anti-counterfeiting, and tamperproof devices are also included in this category (Coles *et al.*, 2003).

For these packaging systems to be practical, they should be easy to use, cost effective, and capable of handling multiple tasks. Color indicating labels, for example, should have color changes that are irreversible, easy to read and easily understood by consumers.

In packaging, "smartness" can have many meanings, and covers a number of functionalities, depending on the product being packaged-food, beverage, pharma-

ceutical, household products etc. Examples of current and future functions that are considered to have "smartness" would be packages that:

1. Retain the integrity and actively prevent food spoilage (extend shelf life)

2. Enhance product attributes (look, taste, flavour, aroma, etc.)

3. Respond actively to changes in the product or in the package environment

4. Communicate product information, product history or other conditions to the user

5. Assist with opening and indicating seal integrity

6. Confirm product authenticity and act to counter theft.

Indicators are called smart or interactive because they interact with compounds in the food. Microwave heating enhancers, such as susceptors and other temperature regulation methods, are sometimes regarded as intelligent methods as well (Ahvenainen *et al.*, 2003). Tables 2.1 depict some currently available color indicators.

Table 2.1 Examples of external and internal indicators and their working principle or reacting compounds to be used in intelligent packaging for quality control of packed food (adapted from Ahvenainen *et al.*, 2003)

Indicator	Principle/reagents	Give information about	Application
Time-temperature indicators (external)	Mechanical, Chemical, Enzymatic	Storage conditions	Foods stored under chilled and frozen conditions
Oxygen indicators (internal)	Redox dyes, pH dyes, Enzymes	Storage conditions, Package leak	Foods stored in packages with reduce oxygen concentration
Carbon dioxide indicators (internal) packaging	Chemical	Storage conditions Package leak	Modified or controlled atmosphere food
Microbial growth indicators (internal/external) i.e. freshness indicators	pH dyes, all dyes reacting with certain metabolites (volatiles or non volatiles)	Microbial quality of food (i.e. spoilage)	Perishable foods such as meat, fish and poultry
Pathogen indicators (internal)	Various chemical and immunochemical methods reacting with toxins	Specific pathogenic bacteria such as *Escherichia coli* 0157	Perishable foods such as meat, fish and poultry

2.2 Intelligent Packaging for Improving Product Quality and Product Value

2.2.1 Time-Temperature Indicators

The temperature variations in a food product can lead to changes in product safety and quality. There are two types of temperature indicators; the simple temperature indicators and the time-temperature integrators (TTIs). Temperature indicators show whether products have been heated above or cooled below a reference (critical) temperature, warning consumers about the potential survival of pathogenic

micro-organisms and protein denaturation during, for example, freezing or defrosting processes. To determine the changes in the shelf life of the food product, the second type of indicator, a TTI, should be employed. These indicators display a continuous temperature-dependent response of the food product. The response is made to chemical, enzymatic or microbiological changes that should be visible and irreversible, and is temperature dependent. TTIs provide an overall temperature history of the product during distribution. The changes shown by TTI can easily be measured, and have been proven to give consistent responses under the same temperature conditions and when not under the influence of light, humidity, and contaminants. Ideal TTIs are able to adjust the residual shelf life of the food product by assessing the already possible quality deterioration. However, one drawback is that these integrators measure the surface temperature and not the actual temperatures of the product (Ahvenainen *et al.*, 1997).

2.2.2 Gas Concentration Indicators

Internal gas-level indicators are placed into the package to monitor the inside atmosphere. Most of these indicators induce a color change as a result of gas generated due to enzymatic and chemical reactions. Damage to individual packages can be determined by a fast visual check without opening the package. In addition, the rapid label check can allow consumers to view the quality of the food inside the package by examining common redox dyes (e.g. methylene blue, which is used as a leak indicator).

Oxygen indicators interact with oxygen penetrating the package through leakages to ensure that oxygen absorbers are functioning properly. Carbon dioxide indicators are also used in modified atmosphere packages (MAP) in which high carbon dioxide levels are desired. The indicators display the desired concentrations of carbon dioxide inside the package. This allows incorrectly packaged product to be immediately repacked, and eliminates the need for destructive, labor-intensive and time-consuming quality control procedures. The disadvantage of these oxygen and carbon dioxide indicators is that the color changes are reversible, which may cause possible false readings. For example, if the food is contaminated by micro-organisms, they will consume the oxygen inside the package and produce carbon dioxide, which will maintain the carbon dioxide levels in the headspace high even

though the package has been compromised. Therefore, the food is no longer safe to be consumed as a result of microbial contamination; however, the indicator still displays a "normal" status color, resulting in a false reading (Ahvenainen *et al.*, 1997).

2.2.3 Freshness Indicators

Freshness indicators are used to indicate if the product's quality has been impaired due to exposure to unfavorable conditions during storage and transportation. The package is usually equipped with a reversible color-changing device that tells the consumers if the package has undergone deterioration, along with a partial or complete history of the product.

Hydrogen sulfide indicators can be used to determine the quality of modified atmosphere packaged poultry products. It is based on detecting the color change of myoglobin caused by the production of hydrogen sulfide. During the aging process of packaged poultry meats, hydrogen sulfide is released from the meats. The indicator correlates with the color of myoglobin, which correlates with the quality deterioration of the poultry product (Ahvenainen *et al.*, 1997). In addition to hydrogen sulfide indicators, there are also indicators sensitive to microbial metabolites.

Smolander *et al.* (2002) used H_2S-sensitive indicators based on a visually detectable color change of agarose immobilized myoglobin. The color change of the indicators attached into the packages containing unmarinated broiler correlated with the microbiological and sensory quality of broiler samples and the onset of the color change took place concurrently with the sensory rejection of the odor.

In 2002, color indicators on nylon/polyethylene films were newly developed for kimchi (fermented vegetable products in Korea) packaging to examine the degree of fermentation of such products non-destructively during distribution and sale. The films are composed of calcium hydroxide as a carbon dioxide absorbent, bromocresol purple or methyl red as a chemical dye, and a mixture of polyurethane and polyester dissolved in organic solvents as a binding medium (Hong *et al.*, 2002). This is applied on a nylon film and then laminated with a polyethylene film to form the printed indicator. During the distribution process, the kimchi products undergo natural fermentation. Carbon dioxide, a by-product of fermentation, becomes the marker of kimchi ripeness, since the carbon dioxide concentration is correlated with the pH and the titratable acidity of the product. The absorption of carbon dioxide in calcium

hydroxide changes the pH of the indicator components and, accordingly, the color of the indicating chemical dye.

The use of pH dyes (e.g. bromothymol blue) as indicators to monitor the formation of carbon dioxide due to microbial growth is one of the most frequent applications in the food packaging industry. The increase in carbon dioxide levels can be used to detect microbial contamination in some products due to pH dyes that can react to the presence of this by-product. Other pH dye reagents include xylenol blue, bromocresol purple, cresol red, phenol red, and alizarin. Besides carbon dioxide, other metabolites (such as SO_2, NH_4, volatile amines, and organic acids) have been used as target monitoring molecules for pH-sensitive indicators (Ahvenainen *et al.*, 2003).

An inexpensive chemical bar-code was developed in the form of on-package sensor spots that monitored spoilage in fish and seafood products. When fish spoils, it releases a variety of basic volatile amines which are detectable with appropriate sensors. The sensor contains pH-sensitive dye, bromocresol green, that responds through visible color change (yellow to blue) to the basic volatile spoilage compounds such as trimethylamine (TMA), ammonia (NH_3) and dimethylamine (DMA) collectively known as Total Volatile Basic Nitrogen (TVB-N) (Pacquit *et al.*, 2004).

Two years later, Pacquit *et al.* developed volatile amine sensor for real-time monitoring of fish freshness. The sensor characteristics were studied as well as its response with standard ammonia gas (shown in Fig. 2.1). Trials on cod and under-utilised species have verified that the sensor response correlates with bacterial growth patterns in fish samples thus enabling the "real-time" monitoring of spoilage in various fish species (Pacquit *et al.*, 2006).

A year later, Pacquit *et al.* developed smart packaging to evaluate real-time freshness of fish and seafood products. These are prepared by entrapping within a polymer matrix a pH sensitive dye that responds, through visible color changes to the spoilage volatile compounds that contribute to a quantity known as total volatile basic nitrogen (TVB-N). Laboratory trials on fresh fish filets showed that the sensor accurately tracks the increase in amines concentration in the package headspace. The response was also found to correlate to changing microbial populations (total viable count or TVC and Pseudomonas spp.) (in Fig. 2.2) (Pacquit *et al.*, 2007).

Other spoilage detection methods include the incorporation of small analytical tools into the package, such as biosensors for the detection of biogenic amines. The increase in diamines in poultry meat can be detected with a putrescine oxidase reactor combined with an amperometric hydrogen peroxide electrode (Ahvenainen, 2003). The system can also be applied in the detection of histamine from rainbow trout meat and biogenic amines from fish muscles.

The "electronic nose" is an analytical tool composed of an array of sensors which respond to volatile compounds by changing their electrical properties (Blixt and Borche, 1999). The samples can then be classified as acceptable or unacceptable, referencing a sensory evaluation or microbiological analysis catalog. It has also been proved to be successfully in the quantitative determination of the degree of spoilage of vacuum-packaged beef (Blixt *et al.*, 1999).

"Doneness" indicators are convenience-, quality- and temperature-indicating packaging systems. They detect and indicate the state of readiness of heated foods. The "ready" button indicators are commonly placed in poultry products. When a certain temperature has been reached, the material expands and the button pops out, telling the consumer that the poultry product is frilly cooked (Ahvenainen *et al.*, 2003). There are also labels that change color when the desired temperature is reached. The drawback of "doneness" indicators is the difficulty of observing a color change without opening the oven.
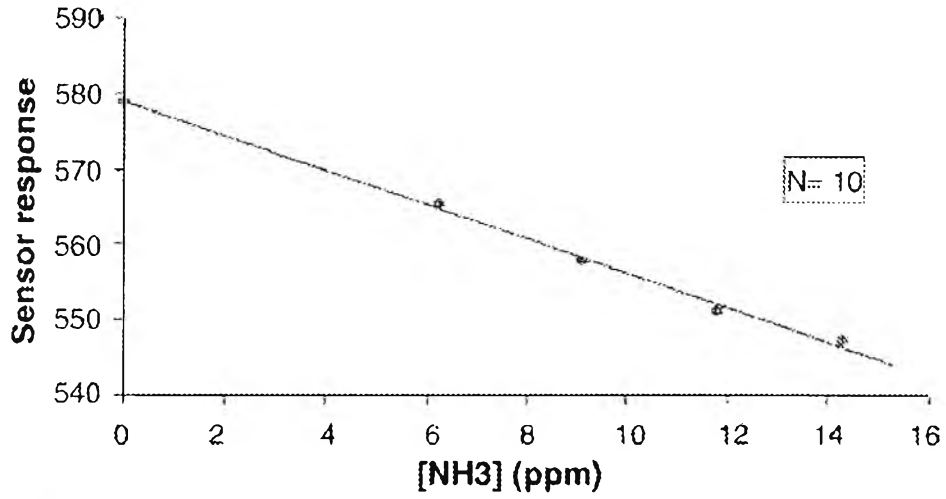
Figure 2.1 Sensor responses to increasing ammonia concentration monitored by the optical scanner.
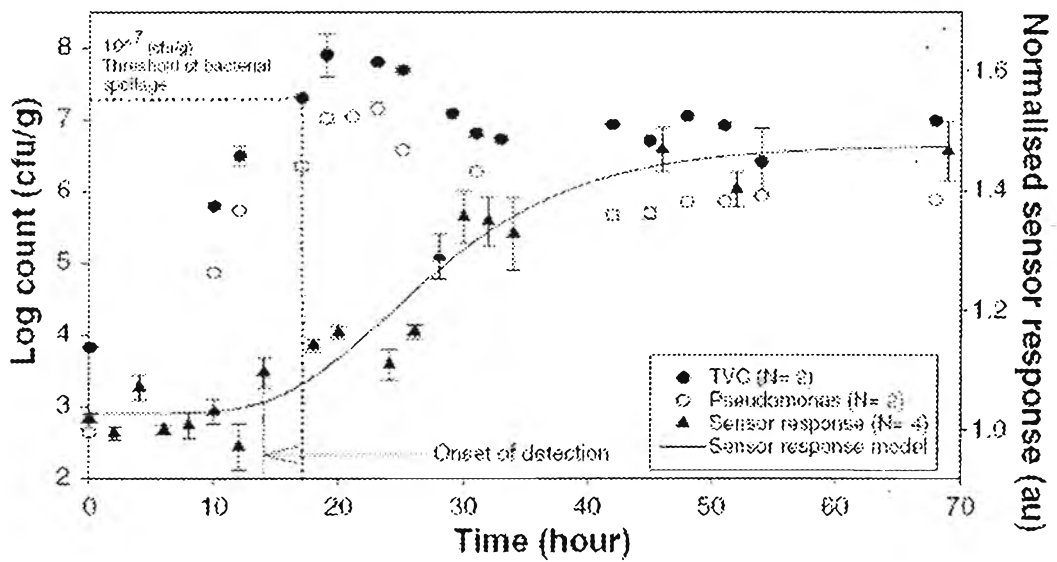

Figure 2.2 Correlation between sensor response and bacterial population in whiting filet samples at 21 °C.

2.3 Meat Spoilage

Food spoilage has been a continuing problem since humans first discovered they could produce more food than could be consumed in a single meal. Micro-organisms are capable of producing a wide range of chemicals associated with their metabolic activities (metabolic by-products) giving off-odours and off-flavours that are unacceptable or highly objectionable to the consumer. Off-odours and off-flavours are a common cause of spoilage in all branches of the food industry and the economic consequences can be serious. Consumption of microbially contaminated food can also cause serious infections or poisoning (Mielmann, 2006).

Growth of microorganisms in foods can cause spoilage by producing an un-acceptable change in taste, odor, appearance, texture, and a combination of the above. Spoilage is not only due to the visible growth of microorganisms, but also to the production of end-metabolites which result in off-odors, gas and slime production (Mielmann, 2006).

Spoilage of poultry is generally restricted to the outer surfaces of the skin and cuts and has been characterized by off-odors, which appears at a bacterial load between 10^6 and 10^8 /cm^2, sliminess generally occurs shortly after the appearance of off-odors, with log counts/cm^2 of about 8, and various types of discolorations. The inner portions of poultry tissue are generally sterile, or contain relatively few organisms, which generally do not grow at low temperatures. Poultry legs are more perishable. This is due to the slightly higher pH (6.2 to 6.4) of leg muscle compared to the 5.7 to 5.8 of breast meat (Mielmann, 2006).

Meat spoilage essentially, can be attributed to three main causative factors namely microbial, enzymatic or autolytic and chemical spoilage (oxidation) which occur in sequence as shown in Fig. 2.3.
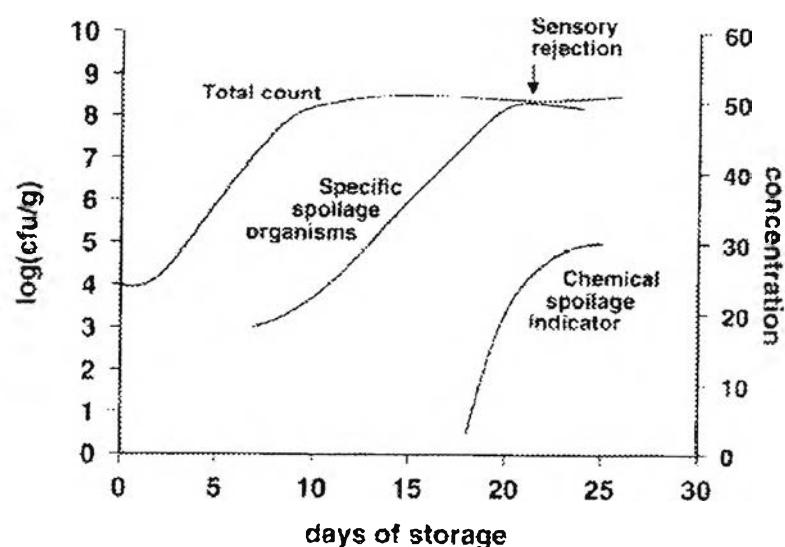
Figure 2.3 Model of changes in total count (TVC), specific spoilage organisms (SSO) and chemical spoilage indices during chill storage of a fish product.

2.3.1 Microbial Spoilage

Microbial spoilage of foods may take diverse forms, but all of them are the consequence of microbial growth, which are manifested as changes in the sensorial characteristics as shown in Table 2.2.

Important factors contribute to the microbiological complexity of fish and fish products (Milanes, 2004). They are:

- Contamination of the environment where the animal lives and of the products during processing

- Growth conditions for microorganisms due to specific intrinsic and extrinsic factors (temperature, Aw, pH, oxygen, light and microbial interactions).

Table 2.2 Microbiological spoilage of foods (Gram and Huss 1996).

Microbiological activity	Sensory manifestation
Breakdown of food components	Production of off-odors and flavor
Production of extra cellular Polysaccharide material	Slime formation
Growth of molds, bacteria, yeasts	Large visible pigmented or non-pigmented colonies
CO_2 – form carbohydrate or amino acids	Production of gas
Production of diffusible pigments	Discolouration

2.3.2 Autolytic Spoilage

The autolytic changes are responsible for the initial loss of quality in fresh meat, but contribute very little to spoilage of chilled meat and meat products. However, in frozen meat, the autolytic changes have great importance. Bacterial action is inhibited and TMAO is broken down by autolytic enzymes to dimethylamine (DMA) and formaldehyde (FA). The FA formed causes increased on denaturation of meat tissue, changes in texture and loss of water retention capacity (Milanes, 2004).

2.3.3 Chemical Spoilage

The most important chemical spoilage processes are changes taking place in the lipid fraction of the meat. The auto-oxidation process is a reaction involving only oxygen and unsaturated lipids. The first step of the oxidation process leads to formation of hydro-peroxides, which are tasteless but can cause brown and yellow discoloration of the meat tissue. The degradation of hydro-peroxides gives rise to formation of aldehydes and ketones. These compounds have a strong rancid flavor (Milanes, 2004).

2.4 Method to Determine Meat Spoilage

The various methods for assessing spoilage in meat can be placed in four broad categories namely, sensorial, microbiological, chemical and physical. Each of these methods measures different spoiling indicators or freshness in meat and/or meat products. Sensory evaluation gives direct measurements while the other three give indirect measurement, though the measurements are closely related.

2.4.1 Sensory Methods

Characteristic sensory changes occur in appearance, odor, taste and texture. Sensory methods are the most satisfactory way of assessing freshness of meat and meat products. This is because they can be applied to all meat, do not require elaborate laboratory facilities, they are quick non-destructive unless sample is being cooked, and they are closely allied to the criteria the consumer uses in evaluating acceptability. However, they are difficult to standardise and the results are subject to personal whims and bias of a taster (Masette, 1999).

2.4.2 Chemical Methods

Chemical methods, considered to be more objective than sensory methods, involve chemically analysing a sample to determine the concentration of a specific chemical(s) within a sample (Masette, 1999). Odor is one of the most important parameters to evaluate meat freshness. During storage of meat, the odor undergoes changes, from fresh odor, sweet and stale odors until the final phase of spoiled or putrid odors. Volatile compounds contributing to odor changes can be measured to evaluate the freshness and spoilage of meat. During the deterioration of fish amines are formed. One of the chemical methods makes use of the total volatile basic compounds (TVB) in meat, which contains mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA), the levels of which increase with spoilage by either bacterial or enzymic degradation (Milanes, 2004).

2.4.3 Microbiological Methods

The aim of microbiological examination of meat is to evaluate the possible presence of bacteria of public health significance. The number of specific spoilage bacteria will give information on the remaining shelf life which can be predicted from such numbers (Milanes, 2004). Bacterial activity is the prime cause of spoilage in fresh fish since they give rise to undesirable flavours and odours. The measurement of bacterial numbers therefore, should provide a direct index of freshness (Masette, 1999).

2.4.4 Physical Methods

Several physical measurements have been used to evaluate meat freshness. These include measurements of mechanical, microstructure, electrical properties, color measurements, spectroscopy and pH.

2.5 Polymer/Clay Nanocomposites

In recent year polymer/clay nanocomposites have attracted great interest, both in industry and in academia, because they often exhibit remarkable improvement in materials properties when compared with virgin polymer or conventional micro and macro-composites. These improvements can include high moduli, in-

creased strength and heat resistance, decreased gas permeability and flammability, and increased biodegradability of biodegradable polymers.

2.5.1 Clay Minerals

The commonly used clay minerals for the preparation of polymer/clay nano-composites belong to the same general family of 2:1 layered or phyllosilicates. Their crystal structure consists of layers made up of two tetrahedrally coordinated silicon atoms fused to an edge-shared octahedral sheet of either aluminum or magnesium hydroxide. The layer thickness is around 1 nm, and the lateral dimensions of these layers may vary from 30 nm to several microns or larger, depending on the particular layered silicate. Stacking of the layers leads to a regular Van der Waals gap between the layers called the interlayer or gallery. Isomorphic substitution within the layers (for example, Al^{3+} replaced by Mg^{2+} or Fe^{2+}, or Mg^{2+} replaced by Li^{+}) generates negative charges that are counterbalanced by alkali and alkaline earth cations situated inside the galleries. This type of clay is characterized by a moderate surface charge known as the cation exchange capacity (CEC), and generally expressed as me-quiv/100 gm. This charge is not locally constant, but varies from layer to layer, and must be considered as an average value over the whole crystal.

Montmorillonite (MMT), hectorite, and saponite are the most commonly used clays. clays have two types of structure: tetrahedral-substituted and octahedral substituted. In the case of tetrahedrally substituted layered silicates the negative charge is located on the surface of silicate layers, and hence, the polymer matrices can interact more readily with these than with octahedrally-substituted material. Details regarding the structure and chemistry for these layered silicates are provided in Fig. 2.4 and Table 2.3, respectively.
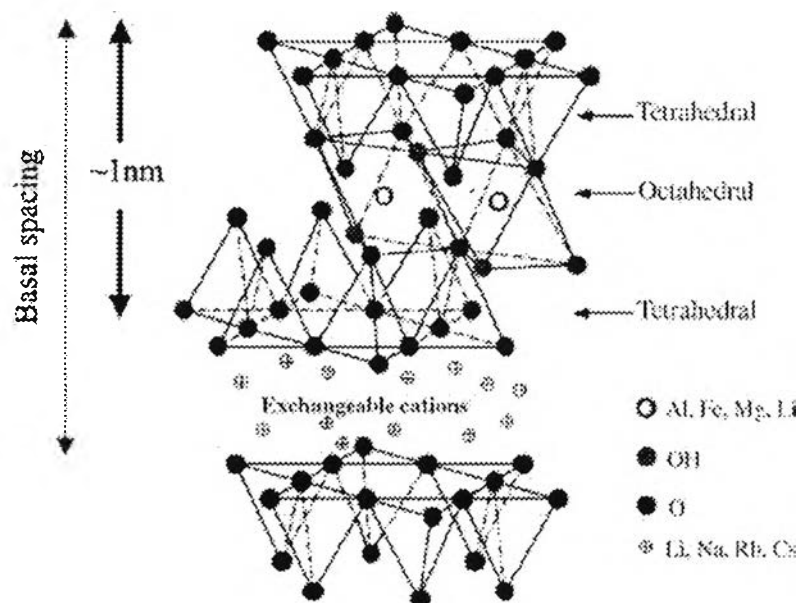
Figure 2.4 Structure of 2:1 phyllosilicates.

Table 2.3 Chemical formula and characteristic parameter of commonly used 2:1 phyllosilicates

2:1 phyllosilicates	Chemical formula	CEC (mequiv/100 g)	Particle length (nm)
Montmorillonite	$M_x(Al_{4-x}Mg_x)Si_8O_{20}(OH)_4$	110	100–150
Hectorite	$M_x(Mg_{6-x}Li_x)Si_8O_{20}(OH)_4$	120	200–300
Saponite	$M_xMg_6(Si_{8-x}Al_x)Si_8O_{20}(OH)_4$	86.6	50–60

M = monovalent cation; x = degree of isomorphous substitution (between 0.5 and 1.3).

Two particular characteristics of clay minerals that are generally considered for polymer/clay nanocomposites are: firstly, the ability of the clay particles to disperse into individual layers and secondly, the ability to fine-tune their surface chemistry through ion exchange reactions with organic and inorganic cations. These two characteristics are interrelated since the degree of dispersion of clay particles in a particular polymer matrix depends on the interlayer cation (Ray et al., 2003).

In the present work, bentonite is a clay mineral used for the preparation of polymer-clay nanocomposites. Bentonite is a clay mineral derived from the alteration of volcanic ash, comprising predominantly of montmorillonite. Depending on their sources, bentonite contains a small portion of accessory minerals and metal oxides in addition to montmorillonite.

2.5.2 Organically Modified Clay Minerals

Pristine clays usually contain hydrated Na^+ or K^+ ions. This pristine state, clay minerals are only miscible with hydrophilic polymers, such as poly(ethylene oxide) (PEO), or poly(vinyl alcohol) (PVA). To render clay minerals miscible with other polymer matrices, the normally hydrophilic silicate surface must be converted to an organophilic one, making the intercalation of many engineering polymers possible. Generally, this can be done by ion-exchange reactions with cationic surfactants including primary, secondary, tertiary, and quaternary alkylammonium or alkylphosphonium cations. Alkylammonium or alkylphosphonium cations in the organosilicates lower the surface energy of the inorganic host and improve the wetting characteristics of the polymer matrix, and result in a larger interlayer spacing. Additionally, the alkylammonium or alkylphosphonium cations can provide functional groups that can react with the polymer matrix, or in some cases initiate the polymerization of monomers to improve the strength of the interface between the inorganic and the polymer matrix (Ray *et al.*, 2003).

2.5.3 Types of Nanocomposites

Depending on the strength of interfacial interactions between the polymer matrix and clay (modified or not), three different types of polymer/clay nanocomposites are thermodynamically achievable (in Fig. 2.5):

a. *Intercalated nanocomposites*: in intercalated nanocomposites, the insertion of a polymer matrix into the clay structure occurs in a crystallographically regular fashion, regardless of the clay to polymer ratio. Intercalated nanocomposites are normally interlayer by a few molecular layers of polymer. Properties of the composites typically resemble those of ceramic materials.

b. *Flocculated nanocomposites*: conceptually this is same as intercalated nanocomposites. However, silicate layers are some times flocculated due to hydroxylated edge-edge interaction of the silicate layers.

c. *Exfoliated nanocomposites*: in an exfoliated nanocomposite, the individual clay layers are separated in a continuous polymer matrix by an average distances that depends on clay loading. Usually, the clay content of an exfoliated nanocomposite is much lower than that of an intercalated nanocomposite (Ray *et al.*, 2003).
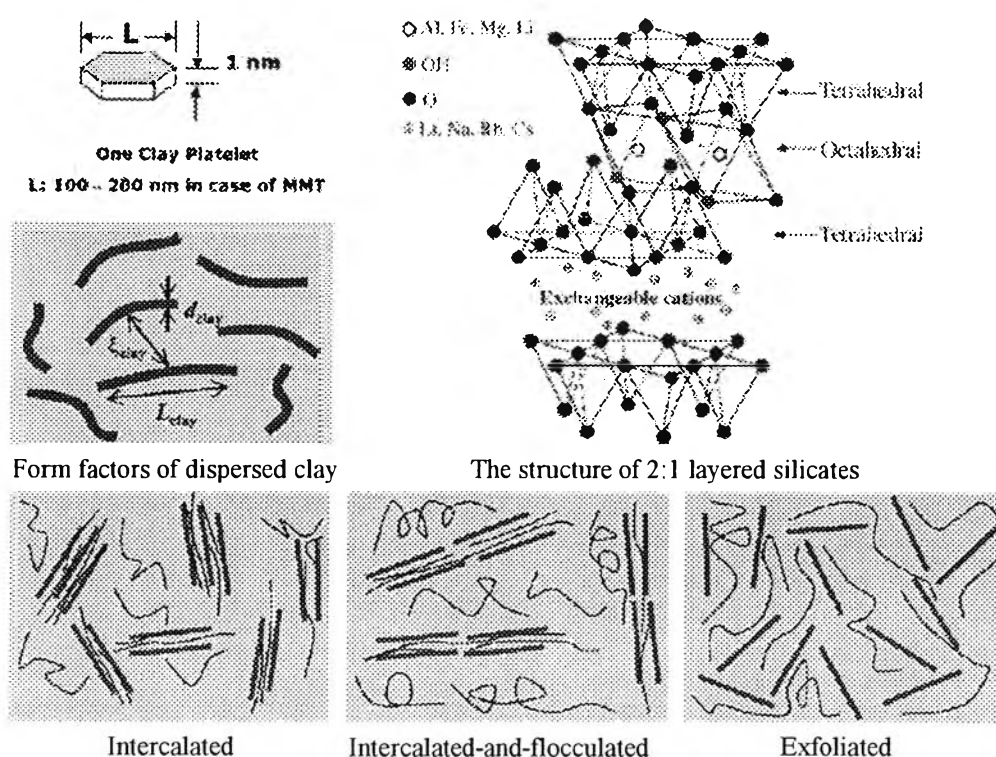

Form factors of dispersed clay

The structure of 2:1 layered silicates

Intercalated   Intercalated-and-flocculated   Exfoliated

Figure 2.5 Schematically illustration of three different types of thermodynamically achievable polymer layered silicate nanocomposites.

2.5.4 Preparative techniques

The preparative methods are divided into three main groups according to the starting materials and processing techniques.

a. *Intercalation of polymer or pre-polymer from solution*: This is based on a solvent system in which the polymer or pre-polymer is soluble and the clay minerals are swellable. The clay is first swollen in a solvent, such as water, chloroform, or toluene. When the polymer and clay minerals solutions are mixed, the polymer chains intercalate and displace the solvent within the interlayer of the clay. Upon

solvent removal, the intercalated structure remains, resulting in polymer/clay nano-composite.

b. *In situ intercalative polymerization method*: In this method, the layered clay is swollen within the liquid monomer or a monomer solution so the polymer-formation can occur between the intercalated sheets. Polymerization can be initiated either by heat or radiation, by the diffusion of a suitable initiator, or by an organic initiator or catalyst fixed through cation exchange inside the interlayer before the s-welling step.

c. *Melt intercalation method*: This method involves annealing, statically or under shear, a mixture of the polymer and organically modified clay above the sof-tening point of the polymer. This method has great advantages over either in situ intercalative polymerization or polymer solution intercalation. First, this method is environmentally benign due to the absence of organic solvents. Second, it is com-patible with current industrial process, such as extrusion and injection molding. The melt intercalation method allows the use of polymers which were previously not suitable for in situ polymerization or solution intercalation (Ray *et al.*, 2003).

2.5.5 Polypropylene/Clay Nanocomposites

The dispersal of clay minerals into the nonpolar polypropylene (PP) sys-tems proves to be a challenge since the polarity of organoclay does not match well with such polymers. Initial attempts to create PP/clay nanocomposites were based on the introduction a modified PP with polar groups to mediate the polarity between the clay surface and bulk PP. However, an organic solvent has to be used in order to fa-cilitate the formation of a modified PP intercalate. The mixture of stearylammonium-exchanged montmorillonite, maleic anhydride modified PP oligomer and homopoly-propylene was melt-processed to obtain successful PP/clay nanocomposites wherein a larger fraction of the clay minerals were found to be exfoliated. The hydrolyzed maleic anhydride PP is intercalated into the organoclay, expanding the galleries, and facilitated the incorporation of PP (LeBaron *et al.*, 1999).

Kawasumi et al. (1997) prepared PP/clay hybrids by simple melt-mixing of three components, i.e., PP, maleic anhydride modified PP oligomers (PP-MA), and clays intercalated with stearylammonium. The dispersibility of 10-Å-thick silicate layers of the clays in the hybrids was investigated by using a transmission electron

microscope and X-ray diffractometer. It was found that there are two important factors to achieve the exfoliated and homogeneous dispersion of the silicate clay layers in the hybrids. Firstly, the oligomers should include a certain amount of polar groups to be intercalated between silicate layers through hydrogen bonding. Secondly, the oligomers should be well miscible with PP. Since the content of polar functional groups in the oligomers should affect the miscibility of it with PP.

The driving force of the intercalation originates from the strong hydrogen bonding between the maleic anhydride group (or COOH group generated from the hydrolysis of the maleic anhydride group) and the oxygen groups of the silicates. The interlayer spacing of the clay increases and the interaction of the layers should be weakened. The intercalated clays with the oligomers contact PP under a strong shear field. If the miscibility of PP-MA with PP is good enough to disperse at the molecular level, the exfoliation of the intercalated clay should take place smoothly.

Surlyn is a commercial thermoplastic ionomer resin used in the packaging industry. It is excellent for packaging applications due to its sealing performance, formability, clarity, oil-grease resistance and high hot draw strength. Good hot draw strength allows faster packaging line speeds and reduces packaging failures. Shah *et al.* (2005) studied the structure-property relationships for nanocomposites by using melt processing techniques from poly(ethylene-co-methacrylic acid) ionomers, Surlyn® ionomers resins The chemical structure of the alkyl ammonium surfactants was systematically varied to determine how specific groups might affect the mechanical properties and morphology of these composites. Four distinct surfactant structural effects have been identified that lead to improved levels of exfoliation and higher stiffness for these nanocomposites: (1) higher number of alkyl tails on the amine rather than one, (2) longer alkyl tails instead of shorter ones, (3) 2-hydroxy-ethyl groups as opposed to methyl groups on the ammonium ion, and (4) excess amount of the amine surfactant on the clay instead of an equivalent amount.

2.6 Poly(3,4-ethylenedioxythiophene) (PEDOT)

Poly(3,4-ethylenedioxythiophene) (PEDOT) was used as sensor to determine meat freshness in this research work. PEDOT which is also known under the trade name Baytron[®] was initially developed to give a soluble conducting polymer that lacked the presence of undesired α,β- and β,β-couplings within polymer backbone. Prepared using standard oxidative chemical or electrochemical polymerization methods, PEDOT was initially found to be an insoluble polymer, yet exhibited some very interesting properties. In addition to a very high conductivity (ca. 300 S/cm), PEDOT was found to be almost transparent in thin, oxidized films and showed a very high stability in the oxidized state. The solubility problem was subsequently circumvented by using a water-soluble polyelectrolyte, poly(stylene sulfonic acid) (PSS), as a charge-balancing dopant during polymerization to yield PEDOT/PSS. This combination resulted in a water-soluble polyelectrolyte system with good film-forming properties, high conductivity (ca. 10 S/cm), high visible light transmissivity, and excellent stability (Kirchmeyer *et al.*, 2005).
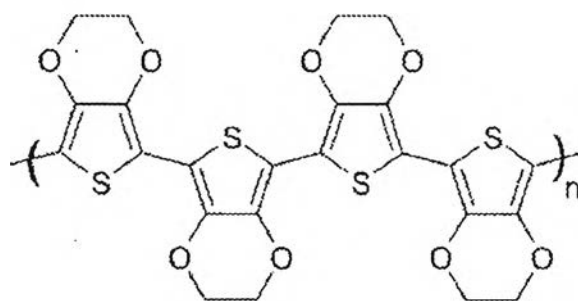

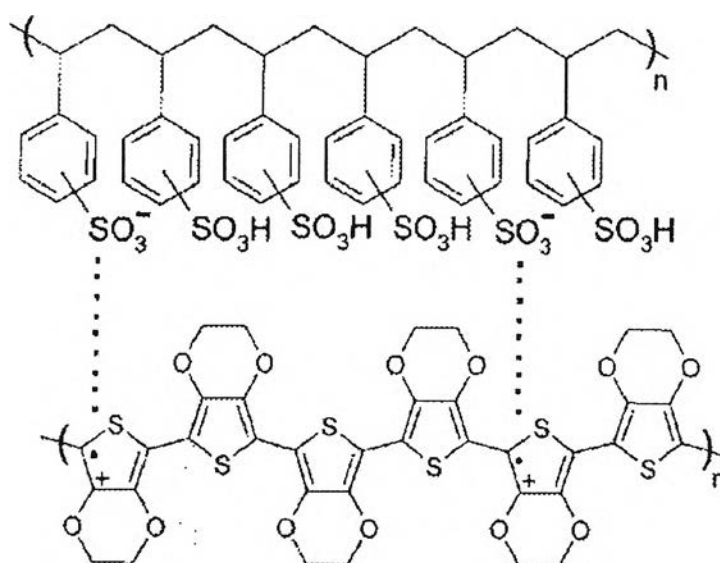**Figure 2.6** The backbone structure of PEDOT.

Figure 2.7 PEDOT:PSS blend (Baytron, P).

2.6.1 Optical Properties of PEDOT

PEDOT is conjugated and electroactive polymer that exhibits electrochromic properties. Electrochromic properties are properties that present reversible change in optical properties when material is electrochemically oxidized or reduced. This has led to studies of their use in smart windows and electrochromic displays. If polymers are to be used as electrochromic material in real displays and windows they need to have good stability and useful optical properties. They should be tuneable between one transparent and one opaque state (Gustafsson *et al.,* 1994). To achieve this, there are two possibilities:

1. A polymer with a band gap in the UV range, being transparent in its neutral state. When the polymer is doped, new electronic states in the band gap are induced and absorption of light in the visible range occurs. This means that the polymer becomes opaque, or heavily colored, upon doping (Gustafsson *et al.,* 1994).

2. A polymer with the onset of absorption at the low energy end of the visible range. Upon doping, new electronic states are produced in the bandgap, hence introducing new absorption peaks at energies in the near-IR region outside the visible range, and the material becomes transparent (Gustafsson *et al.,* 1994).

PEDOT is electrochromic polymer (of type 2 stated above) has an onset of optical absorption at about 1.5 eV and a maximum of optical absorption at 2.2 eV. In

its neutral state, it is blue and upon doping, it becomes highly transparent with a faint sky-blue color (Carlberg *et al.*, 1996).
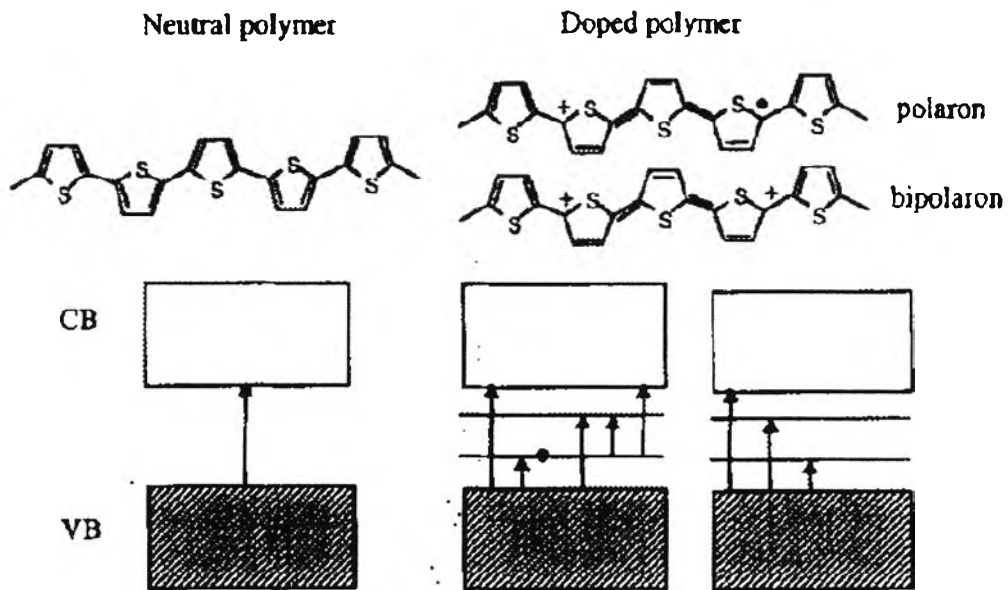

Figure 2.8 Chemical and electronic structure of neutral and doped polythiophene. These arrows indicate allowed electronic transitions.
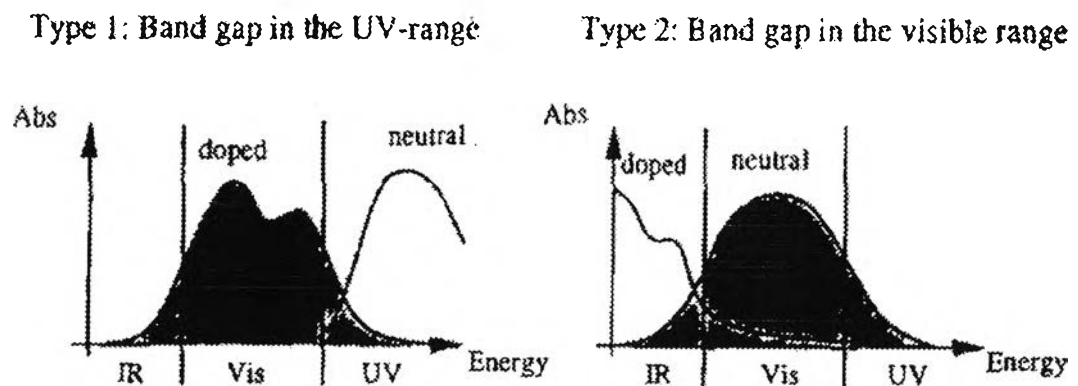

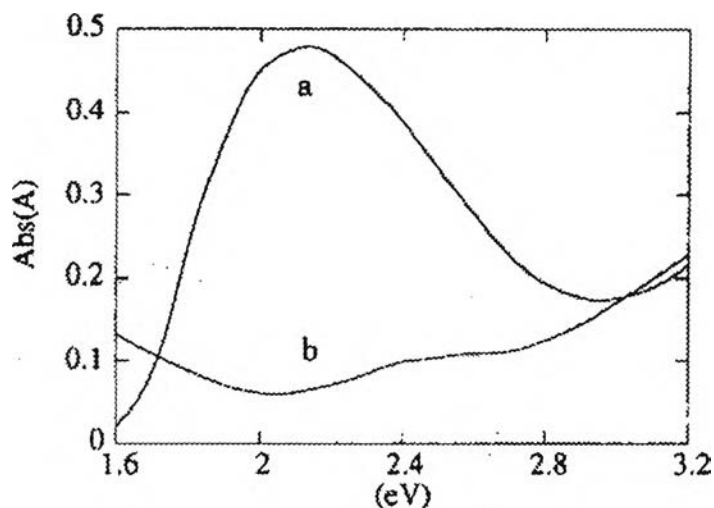**Figure 2.9** Two types of conjugated polymer: (1) high band gap polymer (2) low band gap polymer.

Figure 2.10 Optical absorption spectra of PEDOT in visible region: (a) U = -0.8 V (undoped polymer), (b) U = +1.5 V (doped polymer).

2.6.2 Utilising Redox Behavior of PEDOT

The PEDOT/PSS as a cathodically coloring polymer can be changed gradually by electrochemical means at different voltages. PEDOT/PSS therefore exhibits electrochromic properties, which can be utilized in appropriate devices when an electrical voltage is applied. The electrochromic is due to an electron transfer reaction that takes place during the electrochemical oxidation and reduction of the polymer.

Starting from the oxidized state, PEDOT/PSS accepts electrons from the cathode at a voltage of 2.5 V and is thereby switched to the reduced state. In accordance with this electrochemical reduction step, the color turns from slightly blue and transparent to deep blue. The reverse biasing with a voltage of -2.5 V leads to the back reaction, where the polymer in the reduced state will be oxidized on the electrode, which is now the anode. The original transparency, which depends on the layer thickness, is reversibly restored. The switching time from fully colored to almost transparent is in the range of seconds with liquid electrolyte systems, and in the range of minute with solid (gel) electrolyte systems. The beaching process is faster than the coloring process, which is accordance with the time needed for the oxidation and reduction of the polymer (Kirchmeyer *et al.*, 2005).

In 2007, Yang *et al.* prepared PEDOT nanoparticles by reverse micelle method, which could be well dispersed in water or alcoholic solvent with ultrasonic

treatment. Compare to electrical conductivity of conventional PEDOT particles (ca. 0.5 S/cm), the as-prepared palletized nanoparticles have higher conductivity (ca. 10.2 S/cm) and the conductivity varies with the doped and de-doped states of PEDOT. As for sensing property, it has been found that quartz crystal microbalance device coated with nanoparticles shows faster response and recovery to 20 ppm ammonia gas (ca. 50 s) than that of conventional PEDOT particles. The PEDOT nanoparticle covered device exhibits almost linear relation to lower NH_3 gas concentration and shows a saturate tendency of gas sensitivity with the increase of gas concentration over 500 ppm.

2.7 Copper

In the present work, copper was also used as sensor to evaluate meat fresh-ness. Copper is a transition metal (as shown in Fig. 2.11) which forms one or more stable ions which have incompletely filled d orbitals. Fig. 2.12 shows the electronic configurations starting from Argon. Fig. 2.13 shows the possible oxidation states of the first row of transition metals. It doesn't take a great deal of energy to convert from one oxidation state to another because the 4s and 3d orbitals are close together in energy. Transition metals are therefore easily oxidised and reduced and are useful as redox catalysts.

Figure 2.11 Shortened version of the Periodic Table which shows the first row of the d block.

3d Sub-shell · 4s Sub-shell

[Ar]
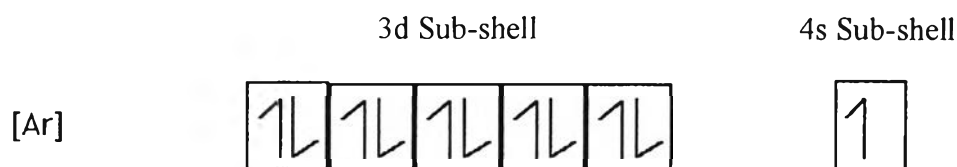
Figure 2.12 Electronic configuration of copper.

Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
+3	+4	+5	+6	+7	+6	+5	+4	+3	+2
	+3	+4	+5	+6	+5	+4	+3	+2	
	+2	+3	+4	+5	+4	+3	+2	+1	
	+1	+2	+3	+4	+3	+2	+1		
		+1	+2	+3	+2	+1			
			+1	+2	+1				
				+1					

Figure 2.13 Possible oxidation states of the first row of transition metals.

In 2002, Hammond *et al.* quantitatively measured the freshness of fish by using an array of semi-conducting metal-oxide (SMO). A variety of SMO films were tested, including films containing oxides of copper and tin, and commercially available tin-based SMO films. Sensory analysis, trimethylamine (TMA) content, pH analysis, and bacterial aerobic and anaerobic plate counts provided results that correlated well with each other and SMO sensor results.

Duchateau *et al.* (2004) described a colorimetric assay method for the screening of the activity of amino amidases that is based solely on the use of a metal sensor (Cu^{II}). The method is based on the difference in optical spectral properties of the copper complexes of an α-amino acid and an α-amino acid amide at alkaline pH. At pH < 8, α-amino acid amides form bidentate complexes with Cu^{II}, whereby two Cu–O and two Cu–N bonds are formed, resulting in a blue complex. At pH > 8, a violetred complex is formed. It was assumed that the color change originates from the displacement of the amide oxygen in the copper complex by the deprotonated amide nitrogen.