# CHAPTER II
# THEORY AND LITERATURE REVIEW

## 2.1 Bacterial cellulose

Cellulose is the major biopolymer of earth and tremendous economic importance globally. It forms the basic structure matrix of cell walls of all plants, many fungi and some algae.

Apart from plants, certain bacteria, algae and fungi produce cellulose as well. Among the cellulose-forming bacteria, *Acetobacter* strains (reclassified as the genus *Gluconacetobacter*) are especially suitable for the formation of cellulose. They are not pathogenic and can procure glucose, sugar, glycerol, or other organic substrates to convert them into pure cellulose.

*Acetobacter xylinum* (*A. xylinum*) is a simple Gram-negative bacterium which has an ability to synthesize a large amount of excellent quality of cellulose formed as twisting ribbons of microfibrillar bundles (Czaja et al., 2006). A single cell of *Acetobacter* has a linear row of pores from which glucan chain polymer aggregates are spun as shown in Figure 2.1.

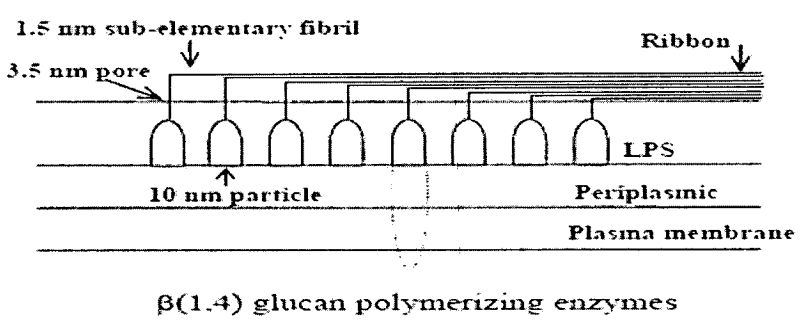



**Figure 2.1** Scheme for the formation of bacterial cellulose (Jonas and Farah, 1998).

Bacterial cellulose is an organic compound with the formula $(C_6 H_{10}O_5)_n$ as shown in Figure 2.2, which is the repeating unit of D-glucose joined by β-1,4-glycosidic linkages (glucan) as polysaccharides as carbohydrate. Bacterial cellulose is of particular importance owing to its unique structure which is quite different from the common synthetic polymers. Formed by repeated connection of glucose building blocks, the highly functionalized, linear stiff-chain homopolymer is characterized by its hydrophilicity, chirality, biodegradability, and broad chemical-modifying capacity (Klemm et al., 2005). Therefore, it is an insoluble structure and most organic solvents.

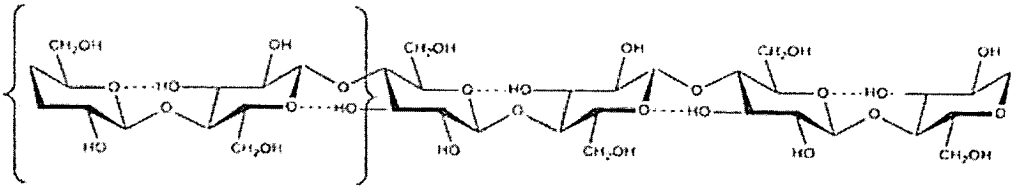


**Figure 2.2** Structure of bacterial cellulose (Brown, 1996).

When cellulose molecule is completely extended, its chain resembles a flat ribbon with hydroxyl groups extending laterally from the edges. This molecular structure is also the basis for extensive inter-and intra-molecular hydrogen bond networks forming semi-crystalline fiber morphologies. The hydrogen atoms oriented above and below the plane of the ribbon and are thus hydrophilic. This structure allows for Van der Waals interactions between the hydrogen atoms as shown in Figure 2.3. The properties of cellulose are determined by the supra-molecular order and specific assembling, these again being controlled by the origin and treatment of the cellulose.

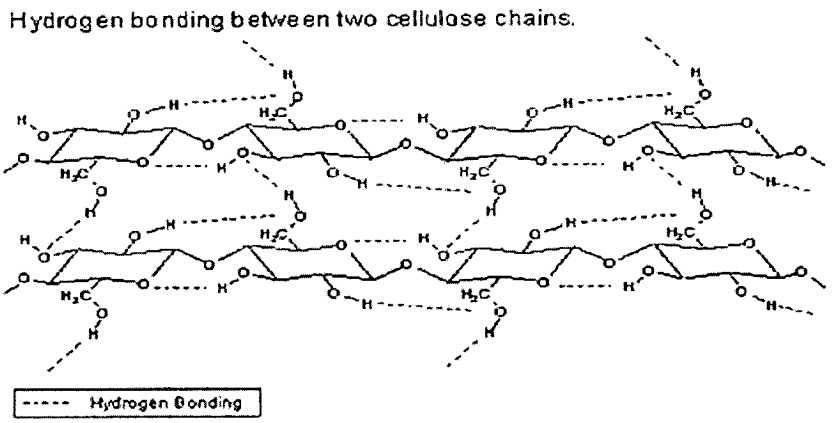



**Figure 2.3** Hydrogen bond system of the cellulose

Moreover, macroscopic morphology of BC strictly depends on culture conditions. In static conditions, bacteria accumulate cellulose mats on the surface of nutrient broth at the oxygen-rich air-liquid interface. The subfibrils of cellulose are continuously extruded from linearly ordered pores at the surface of the bacterial cell, crystallized into microfibrils and forced bons to forming parallel but disorganized planes.

### 2.1.1 Properties of cellulose nanofibrils produced by bacteria

The cellulose nanofibrils produced by bacteria are 3-8 nm in diameters and the entangled mesh of these fibrils produces a white gelatinous membrane known as a pellicle. This membrane of pure cellulose and cells entrapped within it could be cleaned and dried and the product used for many exciting new applications.

In terms of the molecular formula, BC is identical to cellulose of plant origin apart from alien groups such as carbonyl and carboxyl units in the latter as a result of the plant cellulose processing. But important structural features and properties significant for practical application of BC are quite different from wood cellulose:

- BC is high purity
- BC is high degree of polymerization (up to 8000)
- BC is high crystallinity (of 70 to 80%)

- BC is high water content (to 99% )
- BC is high mechanical stability

Due to these reasons, BC provides significant advantages over plant cellulose including:

- BC has finer and more complex structure.
- BC is composed of pure cellulose, no need to remove hemicellulose or lignin.
- BC has longer fiber length so it is much stronger.
- BC can be grown to virtually any shape.
- BC can be produced on a variety of substrates.

One of the unique features of this pure cellulose membrane is that it is very strong in the never dried state and it could hold hundreds of times its weight in water. This great absorptivity and strength constitute two of the many novel features of microbial cellulose.

In addition, the size of BC fibrils is about 100 times smaller than that of plant cellulose as shown in Figure 2.4.

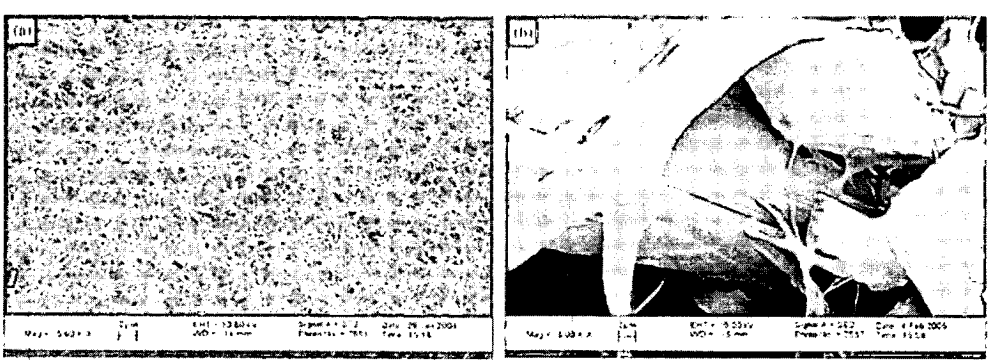


**Figure 2.4** A comparison of microfibrillar organization between BC (a) and wood pulp (b) (Czaja et al., 2006).

### 2.1.2 Application of BC as film packaging

Among available packaging materials, cellulose-based products have attracted increasing interest due to their edibility, biodegradability and potential. Moreover, bacterial cellulose can be altered to suit many potential commercial applications due to physical properties such as hydrophilicity, tensile strength and high purity.

The specific application of bacterial cellulose as a dialysis membrane was examined by Shibazaki et al. (1993). Bacterial cellulose film showed a significantly higher permeation rate and a greater molecular weight cut-off when compared to a commercial dialysis membrane (regenerated cellulose membrane, RBC). Similar observations were reported by Shanshan et al. (2012) prepared cellulose films from solution of bacterial cellulose in NMMO (regenerated BC films). It found that RBC films had better mechanical and barrier properties, and the thermal stability was similar to that of the native BC

Therefore, the additional benefit of the bacterial cellulose film compared to the regenerated cellulose membrane was that the added mechanical property allowed the use of a thinner material.

Currently, BC based materials have been modified for applications as antimicrobial film. For example, BC films were developed to control *L. monocytogenes* and other bacteria on the surface of frankfurters as models for higher value meat products by containing nisin (Nguyen et al., 2008) and to control release of sorbic acid (Jipa et al., 2012a), vanillin (Stroescu et al., 2013) and potassium sorbate (Jipa et al., 2012b) as antimicrobial agent for antimicrobial food packaging material. Biodegradable packaging and edible films also gain much more interesting worldwide. Biodegradable food packaging materials from poly (vinyl alcohol) and BC was investigated in order to use them as food packaging materials and also irradiated to avoid microbial recontamination (Stoica-Guzun et al., 2013).

## 2.2 Sodium alginate

Sodium alginate is water soluble sodium salt of alginic acid. It is a linear polysaccharide copolymer of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers with the formula $(C_6H_7O_6Na)_n$ as shown in Figure 2.5. It can be isolated from the cell wall of brown algae and brown seaweed, and its form as gum. Sodium alginate is widely used in food and pharmaceutical industries because of non-toxic polysaccharide.

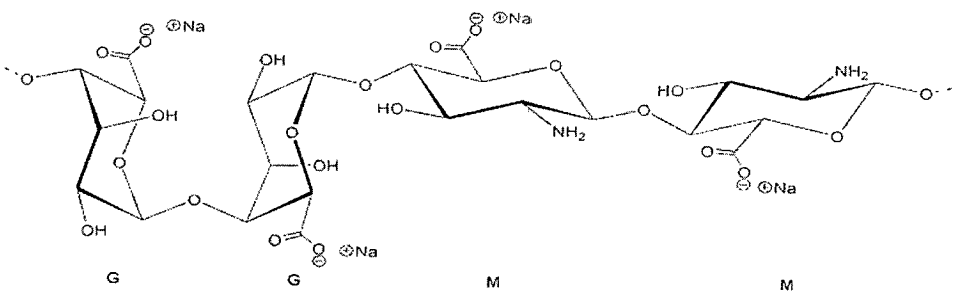



**Figure 2.5** Structure of sodium alginate

### 2.2.1 Properties of sodium alginate

Sodium alginate forms well-characterized hydrogel with water resistance. It is recognized as a cold gelling agent that needs no heat to gel by adding divalent cations as crosslinked agents. The gelation and crosslinking of the polymers are mainly achieved by the exchange of sodium ions from the guluronic acids with the divalent cations, and the stacking of these guluronic groups to form the characteristic 'egg-box structure' (Gombotz and Wee, 1998).

Generally, alginate that coordinated to sodium is a very flexible chain. When sodium is replaced by calcium ion which is circle dots in the image below coordinates to two alginate chains, linking them together. The flexible chains become less flexible and form a huge network–a gel. Mechanism of sodium alginate and calcium ions (which holds a charge of +2) knocks away two sodium ions (each holding a charge of

+1). The alginate molecule contains loads of hydroxyl groups (-OH) that can be coordinated to cations-that is ions with a positive charge such as sodium and calcium (Draget et al., 2005) as shown in Figure 2.6.

Alginate gel is of interest since alginate is able to use for viscosity tuning. The gel can be formed without any heating and the gel network still remains through freeze and thaw cycles. Its gel is thermally stable and therefore continues to provide functionality even when food is heated. It is also effective at both highly acidic and neutral pH levels and it is no thermo-reversible as gelatin.

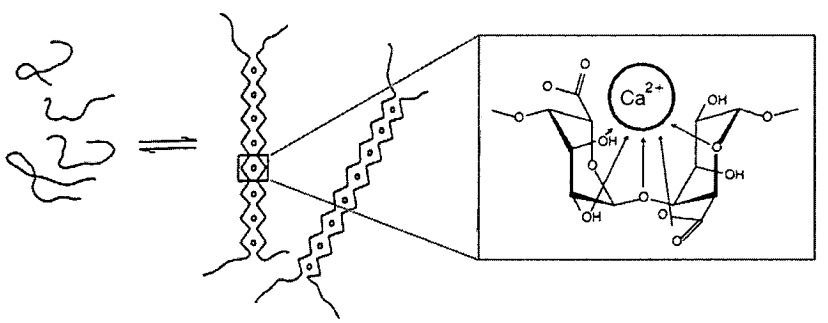



**Figure 2.6** Schematic representation of the egg-box association of the poly-L-guluronate sequences of alginate crosslinked by calcium ions. The figure shows conversion of random coils to buckled ribbon like structures which contain arrays of $Ca^{2+}$ ions. The magnitude figure shows the proposed stereochemistry of $Ca^{2+}$ ion complexation. The oxygen atoms involved in the coordination sphere are shown as filled circles (Rees, 1981, modified).

Sodium alginate crosslinking will reduce significantly its swelling in the presence of the solvent, resulting generally in a reduction of the permeability of different solutes. Moreover, alginate films exhibit poor moisture barriers due to hydrophilic property, but incorporation of calcium can reduce the water vapor permeability of these films, making them water insoluble (Rhim, 2004).

As with other edible films, behavior of alginate films will depend upon the surrounding relative humidity (RH) as well as the plasticizer. Therefore, analyzing the properties of alginate films under different RH conditions would use different

plasticizers. It is important in determining whether these films are suitable for a specific food.

### 2.2.2 Application of sodium alginate as film packaging

Alginate is of interest as a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing. Though edible films prepared from hydrocolloids like alginate form strong films, they exhibit poor water resistance because of their hydrophilic nature. The ability of alginate to make strong and insoluble gels with calcium ions can be utilized to improve such properties of alginate films. However, gel formation of alginate with calcium ions is so instantaneous that it might prevent casting to make films in some cases (Rhim, 2004).

Sodium alginate was used for edible films, biodegradable, antimicrobial film or combined them by blending with other compound such as pectin (Galus and Lenart, 2013; Da Silva et al., 2009) and natamycin as antimicrobial agent (Bierhalz et al., 2012).

Carbohydrate based films occurred strong cohesive films in step dehydration. The addition of plasticizer leads to a decrease in intermolecular forces along polymer chains which improves the flexibility and chain mobility. The plasticizers commonly used are polyols, mainly glycerol and sorbitol (Yang and Paulson, 2000).

## 2.3 Gelatin

Gelatin is a water soluble protein with formula $C_{102}H_{151}N_{31}O_{39}$ as shown in Figure 2.7, which is produced by a controlled hydrolysis of the fibrous insoluble collagen, which is a protein widely found in nature and is the major constituent of skin, bones and connective tissue. The main of fibrous protein gelatins consist of cartilages and skins. Therefore, the source, age of the animal, and type of collagen, are all intrinsic factors influencing the properties of the gelatins.

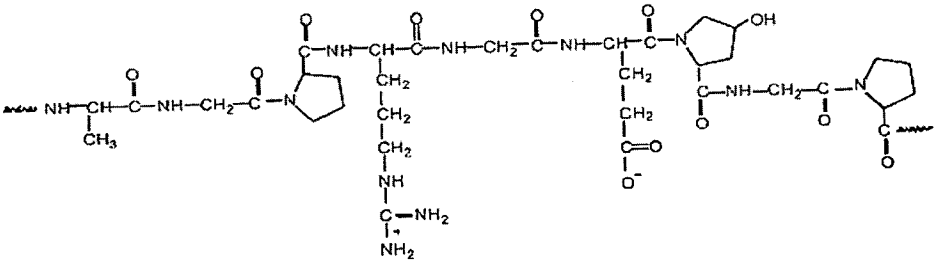


**Figure 2.7** Structure of gelatin (Peña et al., 2010)

Gelatin could be divided two type, depending on the pre-treatment procedure and are known commercially as type-A gelatin (isoelectric point at pH 7-9) and type-B gelatin (isoelectric point at pH 4.8-5.2) obtained under acid and alkaline pre-treatment conditions, respectively. It was generally agreed that alkaline processing is more effective than the acid-extraction method.

According to the total gelatin production in 2007, the most abundant sources of gelatin are pig skin (46%), bovine hide (29.4%), pork and cattle bones (23.1%) and fish gelatin was accounted for less than 1.5% (Gómez-Guillén et al., 2009). Most commercial gelatins are made from pork or non-religiously slaughtered beef. It was generally agreed that beef sources carry more of a risk than those from pork and bones carry a higher risk than skins. The main drawback of fish gelatins is that gels based on them tend to be less stable, lower gelling and poorer mechanical properties, and have worse rheological properties than gelatins from land mammals (Fernandez-Diaz et al., 2001).

All of gelatin is unique among hydrocolloids in forming thermo-reversible with a melting point close to body temperature and it is excellent biodegradability and biocompatibility, which is particularly significant in edible, pharmaceutical applications. Moreover, it also could be added to provide the necessary workability to composite packaging film.

Gelatin is particularly attractive for forming hydrogel packaging because it is inexpensive and biodegradable. In addition, its structure facilitates multiple combinations of molecular interactions. Extensive covalent cross-linking during the cooling of set gelation may cause an almost complete loss of thermo-reversibility in the resultant gelatin gel, depending on whether covalent crosslinking occurs predominantly before or after formation of the hydrogen-bonded triple-helix junction zones (Babin and Dickinson, 2001).

Normally, gelatin gel became stiffer when left at room temperature for a few minutes. This phenomenon of gelation can be explained by chemical crosslinking of gelatin molecules which when cooled to lower temperatures will reinforce the physical crosslinks such as ionic and hydrogen bonding interactions in particular. At the optimum pH conditions used for the crosslinking reaction, it could be increased number of carboxylate ions from both the protein and phenolic.

### 2.3.1 Properties of gelatin

Besides their basic hydration properties, such as swelling and solubility, the most important properties of gelatin can be divided into two groups (Schrieber and Gareis, 2007):

i) Properties associated with their gelling behavior, i.e. gel formation, texturizing, thickening and water binding capacity

ii) Properties related to their surface behavior, which include emulsion and foam formation and stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity

The major properties involving packaging films are below:

#### 2.3.1.1 Gelling properties

Gel formation, viscosity and texture are closely related properties, which vary mainly by the structure, molecular size and temperature of the system. The basic mechanism of gelatin is related to the reverse coil-to-helix transition triggered by cooling solutions below 30 °C. The gelation process for gelatin is thermo-reversible; gelatin gels melt by raising the temperature.

#### 2.3.1.2 Water binding properties

Gelatin chains in solution may be covalently crosslinked to form matrix capable of swelling in the aqueous forming solutions, which are commonly known as gelatin hydrogels. Hydrogels are characterized by hydrophilicity and insolubility in water and swelling to an equilibrium volume while preserving their shape. The chemical cross-linkers are small bifunctional molecules or polyfunctional macromolecules, for instance polyphenolic compound such as tannic acid (Cao et al., 2007; Zhang et al., 2010; Peña et al., 2010; Frazier et al., 2010).

#### 2.3.1.3 Surface properties

Gelatin surface properties are based on the presence of charged groups in the protein side chains and on certain parts of the collagen sequence containing either hydrophilic or hydrophobic amino acids. Both hydrophobic and hydrophilic parts tend to migrate towards surfaces. Hence, reducing the surface tension of aqueous systems and forming the required identically charged film around the components of the dispersed phase which could be additionally strengthened by gel formation.

#### 2.3.1.4 Film-forming properties

Gelatin-based biodegradable materials for food packaging or biomedical applications is focused on developing films with improved mechanical and water resistance properties by combining gelatin with biopolymers with different characteristics, such as polysaccharides as bacterial cellulose (Nakayama et al., 2004; Lin et al., 2009), chitosan (Arvanitoyannis et al., 1998), new hydrophobic or hydrophilic plasticizers (Andreuccetti et al., 2009; Cao et al., 2009), as well as cross-linking agents such as phenolic compound as tannic acid (Zhang et al., 2010; Peña et al., 2010; Deaville et al., 2007).

The conformational state of dehydrated gelatin films obtained by casting method differs when the solvent is evaporated at room temperature or lower or at temperatures above 35 °C (cold- and hot-cast films, respectively). At room temperature, a helical structure is obtained. At temperatures above 35 °C, the conformation of a statistical coil is obtained and films are typically more brittle than cold-cast films and do not shows the helix-coil transition temperature (Fakirov and Bhattacharyya, 2007). The tightly bounds (hydrogen bonds and hydrophobic interactions) presented in gelatin structure and the polar groups of amino acids result in brittle materials in dry state with high moisture absorption (Karnnet et al., 2005).

### 2.3.2 Application of gelatin as film packaging

Gelatin films or Protein-based films were reported to have better oxygen barrier properties but poor water barrier due to their hydrophilic nature with other types of films. Gelatin films may serve as gas and solute barriers, thereby improving the quality and shelf life of muscle foods. Despite these successes, gelatin lacks strength and requires a drying step to form more durable films. Moreover, gelatin was widely used to prepare edible and biodegradable films for food packaging with plasticizer addition. For improved properties, gelatin was combined with polysaccharide compounds such as sodium alginate (Dong et al., 2006) and starch (Veiga-Santos et al., 2007).

In drying processes, it was found that plasticizers affected the quality of the formed films because dehydration may produce brittle films. Thus, plasticizers must be added to reduce inter-chain interactions improving film flexibility.

Currently, Meat industry uses collagen films during the processing of meat products. When heated, intact collagen films can form a "skin" or edible film that becomes an integral part of the meat product (Cutter, 2006). These commercially available collagen films have been purported to reduce shrink loss, increase permeability of smoke to the meat product, increase juiciness, allow for easy removal of nets after cooking or smoking, and absorb fluid exudates. Protein coatings derived from collagen also have been used to reduce transport of gas and moisture in meats (Baker et al., 1994; Gennadios, 2002).

Additional studies have demonstrated that gelatin could be used to carry antioxidants to reduce oxidation, enhance color stability, to retain flavor, taste and aroma of foods during refrigerated or frozen storage.

## 2.4 Plasticizers

Polyols are the most used of plasticizers that are widely used in hydrocolloid-based films or protein-based materials due to their ability to reduce intermolecular hydrogen bonding while increasing intermolecular spacing (Audic and Chaufer, 2005). The composition, size and shape of a plasticizer as well as its compatibility could affect the interactions between the plasticizer and the polymer, including its ability to attract water to the plasticized protein films (Sothornvit and Krochta, 2001). Moreover, plasticizers with characteristics such as small size, high polarity, more polar groups per molecule, and greater distance between polar groups within a molecule generally impart greater plasticizing effects on a polymeric system.

The selection of a plasticizer for a specified system is normally based on the compatibility between plasticizer and protein, permanence of the plasticizer in the film, the amount necessary for plasticization, and the desired physical properties of the films (Cheng et al., 2006). Sorbitol, glycerol or the combination of sorbitol and glycerol are generally used as plasticizer for gelatin-based films (Thomazine et al., 2005; Jongjareonrak et al., 2006).

### 2.4.1 Glycerol

Glycerol (*syn.* glycerine or glycerin) is a simple polyol compound with formula $C_3H_8O_3$ as shown in Figure 2.8. It can be obtained by the saponification of natural fats and oils or by the fermentation of glucose. Glycerol consists of a propane molecule attached to three hydroxyl (OH) groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol backbone is central to all lipids known as triglycerides. Glycerol is a colorless, odorless and viscous liquid with sweet-tasting and low toxicity. It is widely used in food industry and pharmaceutical formulations.

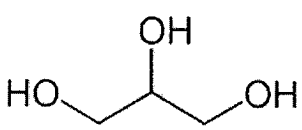



**Figure 2.8** Structure of glycerol

### 2.4.2 Sorbitol

Sorbitol is a polyol (sugar alcohol) with formula $C_6H_{14}O_6$ as shown in Figure 2.9. It is found naturally in a number of fruits, including apples, pears, peaches, and prunes. It can be obtained by reduction of glucose, changing the aldehyde group to a hydroxyl group or synthesized by sorbitol-6-phosphate dehydrogenase, and converted to fructose by succinate dehydrogenase and sorbitol dehydrogenase. Sorbitol can be metabolized slowly in human body.

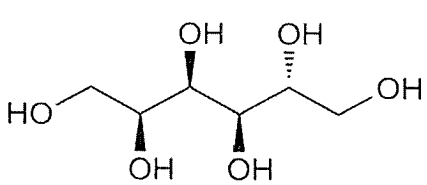



**Figure 2.9** Structure of sorbitol

## 2.5 Tannic acid

Tannic acid (*syn.* Tannin) is a phenolic compound with the formula $(C_{76}H_{52}O_{46})_n$ as shown in Figure 2.10. Tannic acid extracted from nutgall (chestnut) or bark contains a glucose linking through ester bonds to an average of nine to ten molecules of gallic acid.

Tannic acid is a part of hydrolysable tannin, which is a diverse group of polyphenols that are formed as secondary metabolites in plants. It includes a wide range of oligomeric and polymeric polyphenols. It is usually classified in hydrolysable tannin (*syn.* gallotannin) and condensed tannin (*syn.* proanthocyanidins) (Hagerman and Butler, 1991). Moreover, tannic acid is high water-soluble phenolic compounds, relatively rigid and spherical, and high molecular weights between 500 and 3000 Da (Taguri et al., 2004). Thermal processing could breakdown the ester bonds of a polygalloyls and could enhance hydrolysis of tannic acid.

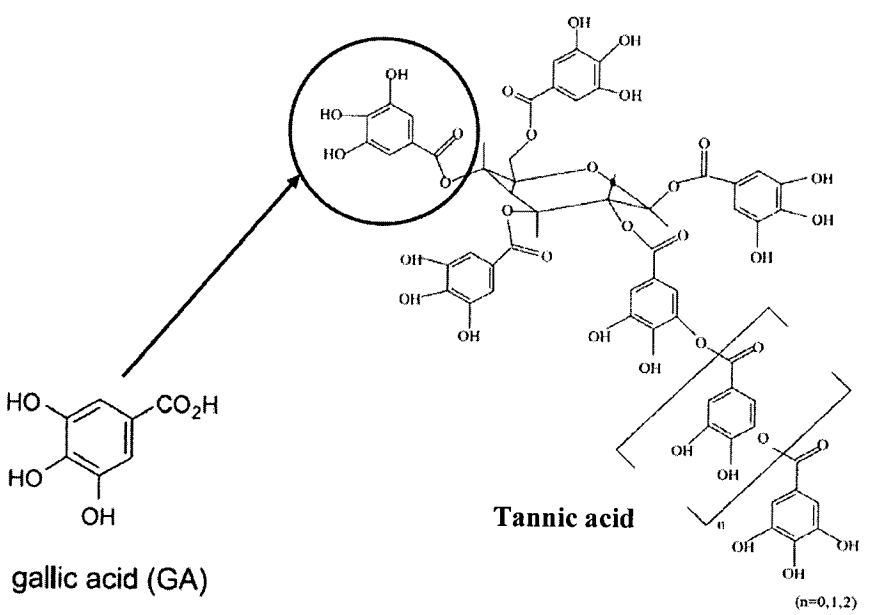



**Figure 2.10** The chemical structures of tannic acid and gallic acid

Tannic acid acts like mild acid on the basis of many phenolic-OH groups. High quality tannin contains 65-76% tannic acid. It is present in a variety of plants and fruits and considered as a 'generally recognized as safe' (GRAS) food additive (Akiyama et al., 2001). In addition, tannic acid has several feature properties including antioxidant capacity, astringency properties and anti-allergenic, antiinflammatory, antimicrobial, cardioprotective and anti-thrombotic activities (Balasaundram et al., 2006). Hence, it is a very interesting raw material for the development of green polymeric materials for food packaging and medical applications.

### 2.5.1 Properties of tannic acid

#### 2.5.1.1 The physical properties of tannic acid

Gelatin-tannic acid films turned from light yellow to brownish as tannic content increased. The transparency of these films was reduced with increasing tannic acid content. Sodium hydroxide (NaOH) also has effect on the transparency of these films, since NaOH can oxidize tannic acid, causing intense yellow colour (Peña et al., 2010).

#### 2.5.1.2 Tannic acid as protein-binding agents

Tannic acid tends to have a strong affinity for proline-rich protein as gelatin (Taylor et al., 2007). Moreover, the conformational flexibility of tannic acid is necessary for strong binding, which is unlikely to be bound to specific ligand binding sites on the protein (Deaville et al., 2007). Tannic acid-protein interactions were suggested as a surface phenomenon in which tannic molecules effectively coat the surface of the protein (Spencer et al., 1988).

The combination of polyphenol-protein was the duple functions of hydrogen bonds and hydrophobic interaction. The mechanism of tannic acid-gelatin (protien) precipitation or tannic acid combining with protein was suggested as following:

In the first stage, tannic acid which contained hydrophobic groups such as galloyl group entered into hydrophobic district of gelatin by hydrophobic reaction to form stable soluble tannic acid-gelatin complexes (Yi et al., 2006; Hagerman, 1992). These were confirmed by absorption peak of tannic at 280 nm shifts toward longer wavelength from the UV-visible absorption spectra (Cao et al., 2007). Moreover, the phenolic hydroxyl groups of the tannic acid molecules were able to enter the hydrophobic areas of the gelatin resulting in a strong hydrophobic interaction.

In the second stage, phenolic hydroxyl groups of tannic acid combined with polar groups of gelatin (amide carbonyl of the peptide backbone) under alkaline conditions to form covalent C-N bonds and generated cross-linked networks by hydrogen bonds. When the degree of combination was suffice, the complex precipitated from the solution. This stage was dominated by less specific binding and aggregation (Frazier *et al.*, 2003).

The mechanism could be expressed as follows:

Tannin acid solution + protein solution

↔ [Tannin acid m · protein] solution ↔ [Tannin acid n · protein] precipitate

The reaction was reversible and alkali could make the complex reversed to polyphenol and protein. Continuously increased protein could result in more cross-linking between tannic acid and protein molecules (CaO et al., 2007).

Hydrophobic interaction was the driving influence in the reaction between tannic acid and gelatin. Hydrophobic interactions between tannic acid and protein contribute to the formation of complexes but are considered far weaker than hydrogen bonding. Moreover, larger molecular weight tannic acid is better protein precipitants than smaller tannic acid and the ratio of tannic acid to protein also affects precipitation in vitro (Hagerman and Klucher, 1986).

In the other hand, the polyphenolic structure of tannic acid enabled it to interact by hydrogen bonding with the polar groups of the gelatin, such as peptide, carbonyl, and guanidine groups. Hydrophobic areas were formed by tannic acid combining with many sites of the protein molecules by hydrogen bonding and then

the complexes of tannic and gelatin began to precipitate (Baxter *et al.*, 1997). The formation of covalent cross-links between gelatin and tannic acid is also possible but only under oxidizing conditions in which quinones react with side chain amino groups of peptides (Strauss and Gibson, 2004).

### 2.5.1.3 Effect of pH to tannic acid binding protein

Tannic acid binds proteins in acidic to mildly basic aqueous solutions, so the pH played an important role in tannic acid-gelatin systems. When the pH varied from 5 to 7, the system was stable and the diameters of the nanospheres were distributed evenly. When the pH was below 4, the solution appeared turbid and began to show flocculent deposits. If the pH was low enough, the tannic acid was easy to condense, leading to an increased molecular weight of the tannic acid, resulting in a stronger ability of gelatin to be precipitated (Yi et al., 2006). When the pH was above 8, tannic would be subject to oxidative hydrolysis.

Thus, hydrogen bonding is greatly decreased and protein precipitation does not readily occur at high pH. Besides molecular size and shape of tannic acid, factors that affect tannic acid-protein interactions have been studied in vitro (Martin et al., 1985).

### 2.5.1.4 Antimicrobial properties

Numerous studies have suggested that tannic acid has antimicrobial activity against foodborne pathogens. Tannic acid inhibited growth of *Escherichia coli* (Pyla et al., 2010), *Listeria monocytogenes* (Rhode et al., 2006), *Staphylococcus aureus* (Akiyama et al., 2001) and *Clostridium perfringens* (Fernandez-Miyakawa, 2010) etc. The antimicrobial activity of the water fraction was further enhanced when this fraction was thermally processed and it was shown that the thermal treatment of pure tannic acid enhanced the antimicrobial activity by inducing the partial hydrolysis of this compound at an ester linkage between two gallic acids or between gallic acid and polyol (Kim et al., 2010).

## 2.6 Mangosteen ethanolic extracts

Mangosteen is very famous fruit and has been known as the "Queen of fruits" or "super fruits" in Thailand. It has been interested to be used as active constituents in functional products.

Mangosteen extract is isolated from all parts of mangosteen (syn. *Garcinia mangostana* Linn. or *G. mangostana*). The fruit rind of *G. mangostana*, especially contains many complex phenolic compounds such as xanthones, flavonoids, tannins, and other bioactive substances (Phothitirat et al., 2009).

Mangostins are one of derivative of xanthone obtained by boiling the rind in water. After tannic is removed by exhausting by boiling in alcohol and evaporating, the obtained product is mangostin and resin. The resin is precipitated by re-dissolving it in alcohol and water, and then evaporating the water. It occurs in small yellow scales, tasteless neutral, insoluble in water, but readily soluble in alcohol and ether. Mangostins included $\alpha$-, $\beta$- and $\gamma$-mangostins. Mangostin in $\alpha$-form is a major compound in mangosteen, so $\alpha$-mangostin could specify quality of mangosteen extract.

The fruit shell contains 7-13% tannin and the seeds contain 3% oil. The rind of the fruit contains tannin, a resin and a bitter principle called mangostins as shown in Figure 2.11. The rind contains 5.5% of tannin, and a resin as well as a yellow crystalline bitter principle. Mangostins contain three compounds including α-mangostin, β-mangostin and γ-mangostin that α-mangostin is a major compound in xanthone and is usual specified for the quality of mangosteen extract.

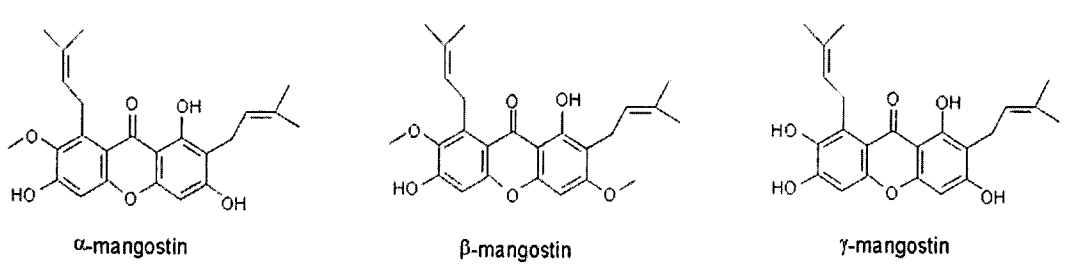



**Figure 2.11** Mangostins structure (Chaverri et al., 2008)

### 2.6.1 Xanthones

The chemical structure of xanthones consists of two benzene rings connected by carbonyl group (C=O) and oxygen. Each ring is conjugated in a fused formation not allowing free rotation on the carbon-carbon bonds. The xanthones backbone is attached to distinct functional groups at benzene ring in various positions. The difference in functional groups and positions affect to specific functionalities or properties of xanthones (Jantaravinid, 2009) as shown in Figure 2.12.

### 2.6.2 Alpha-Mangostin (α-mangostin)

α-Mangostin, yellow-colored, is the major extracted derivative of xanthones which has demonstrated active antimicrobial activities against gram-positive bacteria including *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* (MRSA) (Sundaram et al., 1983; Mahabusarakam et al., 1986).

The chemical IUPAC name of α-mangostin is 1, 3, 6-Trihydroxy-7-methoxy-2, 8-diprenylxanthone with formula $(C_{24}H_{26}O_6)_n$ and molecular weight of 410.47 g/mole as shown in Figure 2.11. Its boiling point is about 180-181 °C. The solubility of α-mangostin in water is very poor, whereas it solutes clearly in organic solvent, for example, ethanol, chloroform and methanol, etc (Phadungkarn et al., 2009).

In addition, Kaomongkolgit et al. have reported that α-mangostin had no cytotoxic effects on human gingival fibroblasts up to 4000 μg/mL (2009). Antibacterial action of α-mangostin was first studied by Nguyen and Marquis. They reported that the antimicrobial action of α-mangostin was from targeting cytoplasmic enzymes (2011). However, the action of enzymetargeted antimicrobials would be expected to require considerable time (Grohs et al., 2003), which seems in contrast to the reported rapid bactericidal action of α-mangostin. The rapid antimicrobial action is suggestive of the antimicrobial action of the natural antimicrobial peptides or peptidomimetics which act on bacterial membrane (Bai et al., 2009; Isaksson et al., 2011).

The stability of α-mangostin from dichloromethanic extraction was studied by Yodhnu et al. (2009). It was found that α-mangostin was stable in these condition:

storage at 80 °C for 3 h, storage under UV-light at 254 or 366 nm of wavelength for 6 h and under 3 N NaOH solution supplementation followed by heating at 80 °C for 3 h.

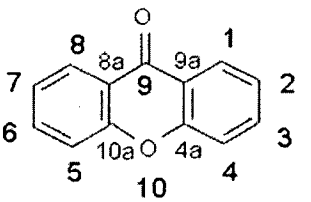
### 2.6.3 Properties of xanthone involving with antimicrobial including antifungal and antibacterial in food and medicine

#### 2.6.3.1 Antifungal activity

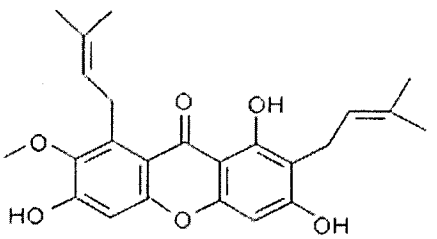
The antifungal activity of several xanthones isolated from fruit hulls of *G. mangostana* and α-mangostin-derivatives could against three phytopathogenic fungi including *Fusarium oxysporum vasinfectum*, *Alternaria tenuis* (*A. alternate*) and *Drechslera oryzae* (*Cochliobolus miyabeanus*) (Gopalakrishnan et al., 1997).

*Epidermophyton floccosum, Alternaria solani, Mucor* sp., *Rhizupus* sp. and *Cunninghamella echinulata* were highly susceptible to xanthones, whereas *Trichophyton mentagrophytes, Microsporum canis, Aspergillus niger, Aspergillus flavus*, *Penicillium* sp., *Fusarium roseum* and *Curvularia lunata* were only moderately susceptible to them (Sundaram et al., 1983).

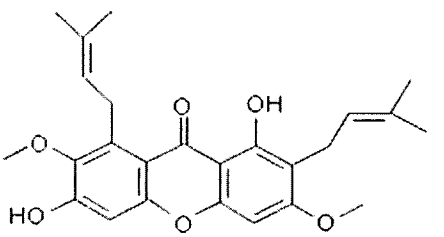
In addition, the activities of mangostin, gartanin and γ-mangostin against *Candida albicans, Cryptococcus neoformans, T. mentagrophytes* and *Microsporum gypseum* were tested. All of the components showed moderate activities against *T. mentagrophytes* and *M. gypseum* but exhibited no activity against *C. albicans* and *C. neoformans* (Mahabusarakam et al., 1986).



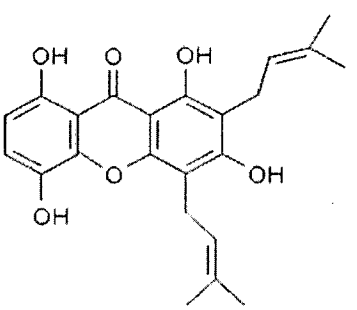

Xanthone nucleus



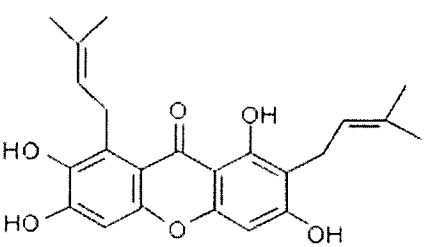

$\alpha$-mangostin



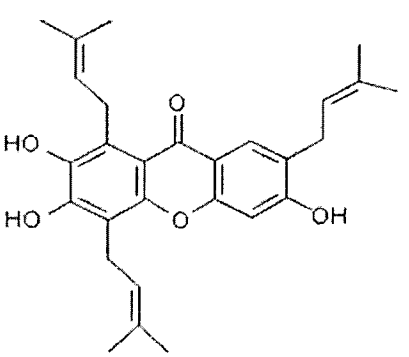

$\beta$-mangostin



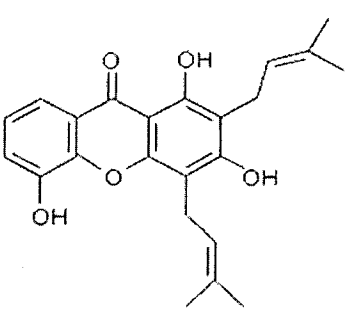

Gartanine




$\gamma$-mangostin




Garsinone E




8-deoxygartanine

**Figure 2.12** Xanthone nucleuses with IUPAC numbers of carbons and chemical structure of the most studied xanthones (Chaverri et al., 2008)

#### 2.6.3.2 Antibacterial activity

α-Mangostin was reported to have activity against normal, methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA) (Mahabusarakam et al., 1986; Iinuma et al., 1996) and *S. aureus, P. aeruginosa, Salmonella typhimurium* and *Bacillus subtilis* were highly susceptible to xanthones, whereas *Proteus sp., Klebsiella sp.* and *Escherichia coli* were only moderately susceptible to them (Sundaram et al., 1983).

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC is important to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. Another, the minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

The order of the efficacy determined by the MIC (mg/ml) showed that α-mangostin and most of xanthone derivatives could have activities against bacterial strain.

## 2.7 Gelatin-Bacterial cellulose Blends

Gelatin is rarely used alone because of its low intensity and high brittleness and being often used after modification through several methods. Blending of polymer is one of the simplest methods to obtain a variety of physical and chemical properties from the constituent polymer at a molecular level. The gain in newer properties depends on the degree of compatibility or miscibility of the polymer.

The efficacy of cross-linked gelatin-based sponges composed of gelatin and BC (polysaccharides) for food material and food packaging was reported. BC nano-composites films by using gelatin and its enzymatically modified form (EMG) could enhance the rehydration abilities properties of BC. Due to the polar functional groups

of gelatin and EMG as well as BC's porous networks with lower level of crystallinity contributed to the rehydration ability of composites (Lin et al., 2009) and gelatin crosslinking disrupted the crystallization formed from the hydrogen bonds between cellulose molecules. Crosslinking can also enhance the rehydration ratio (Nakayama et al., 2004).

Moreover, alkaline treated BC/gelatin composites (ATBC/G) crosslinked with EDC (ATBC/G/E) could improve the mechanical strength and hydrophilic property of BC composites. While increased gelatin concentrations in addition to the EDC treatment decreased crystallinity in the composites. The FT-IR spectrograph of the ATBC/G composites revealed that the OH groups of the composites tended to increase (Chang et al., 2012).

## 2.8 Gelatin cross-linked by tannic acid

The crosslinking methods can be classified as physical crosslinking and chemical crosslinking. Physical treatments such as UV-and -radiation could induce crosslinked gelatin gel with weak interaction, whereas chemical agents such as aldehydes could cause crosslinking between the amino acid chains of gelatin with strong interaction. However, these agents have high toxicity and might contaminate the product. Therefore, the natural crosslinking agent such as genipin, ferulic and tannic acid has been used as crosslinking agent for gelatin (Bigi et al., 2002; Cao et al., 2007).

Tannic acid could be a suitable crosslinking agent for gelatin to be used in packing as edible films. Gelatin-tannic acid (GT) film has decreased swelling ratio and solubility, since the presence of tannic reduced the water affinity of gelatin (Peña et al., 2010). However, no obvious effect was detected on water vapor permeability of the film. Mechanical and thermal behaviors varied as a function of the tannic acid content. Moreover, it was found that the properties of the films treated by tannic acid became better after being stored for more than 90 days (Cao et al., 2007). In addition, hydrogen and/or hydrophobic interactions between gelatin and tannic molecules induce changes in the formation of triple helix in gelatin and surely reduced the

mobility of the side chains, as reflected in an increment of the glass transition temperature of GT films.

Cross-linking reactions between gelatin and tannic acid also involved with tannic acid (TA) content. At a low TA content, the crosslinking effect was predominant and the cross-linked structure was stable even under boiling. Both the rigidity of the protein matrix increased and the mechanical properties of the GT films were enhanced. At higher TA content, grafting and branching reactions between gelatin and TA were enhanced, whereas some amount of TA molecules not involved in cross-linking (Zhang et al., 2010). Therefore, the small amount of TA is sufficient to crosslink gelatin in GT film. Addition of TA in powder form is better than TA solution.

## 2.9 Tween-80 (Polysorbate 80)

Tween-80 is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. It is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers, also known as polyoxyethylene groups which are polymers of ethylene oxide.

Tween-80 is often used in food and other products as an emulsifier, and it was added to prevention of phase separation (Brandelero et al., 2012).

## 2.10 The ethanol extracts

Many solvents were used to extract compound from plants such as DMSO, ethanol, methanol and water. In this work, mangosteen that was extracted by ethanol was applied as natural antimicrobial. Crude mangosteens from the ethanol extracts have better antimicrobial activity than those from water extract and were safety to apply for food packaging. Moreover, mangosteens from ethanol extract was potent in inhibiting bacterial growth of both gram-negative and gram positive bacteria (Mishra et al., 2009).

## 2.11 The pH and microbial growth

The most of foodborne microorganism suitably grows at approximate pH 7 (6.6-7.5) but some microorganism can grow below pH 4. Generally, bacteria, yeast and fungi widely grow at pH range of 6.0-8.0, 4.5-6.0 and 3.5-4.0, respectively. When pH in a food is reduced below the lower limit for growth of microbial species, the cells not only stop growing but also lose viability. The minimum pH value for the growth of some foodborne bacteria is shown in Table 2.1 and 2.2.

**Table 2.1** Reported minimum pH values for the growth of some foodborne bacteria (James et al., 2000, modified)

| Foodborne Bacteria | pH |
|---|---|
| *Escherichia coli* 0157:H7 | 4.5 |
| *Listeria monocytogenes* | 4.1 |
| *Salmonella* spp. | 4.5 |
| *Staphylococcus aureus* | 4.0 |

**Table 2.2** Characteristic and survival/growth parameters of pathogenic microorganisms commonly associated and processed meat products (Tarté, 2009, modified)

| Organism | pH range | Associated meat products |
|---|---|---|
| **Gram positive** | | |
| *Listeria monocytogenes* | 4.4-9.0 | Delicatessen meats, frankfurters, seafood |
| *Staphylococcus aureus* | 4.4-10.0 | Delicatessen meats, meat salads |
| **Gram negative** | | |
| *Escherichia coli*<br>*Salmonella typhimurium* | 4.4-9.0 | Fresh meat |
| **Molds** | | |
| *Aspergillus niger* | 2.0-8.5 | Low pH meat |

The pH in food can vary to a great extent, depending on types. Foods can be divided into 2 groups as shown in Table 2.3 and 2.4:

1) high-acid foods (pH below 4.6) such as fruits, fruit juices, fermented foods and salad dressings

2) low-acid foods (pH 4.6 and above) such as vegetable, meat, fish, milk and soups

**Table 2.3** Approximate pH values of some fresh fruits and vegetables (James et al., 2000, modified)

| Product | pH | Product | pH |
|---|---|---|---|
| **Vegetables** | | **Fruits** | |
| Asparagus (buds and stalks) | 5.7–6.1 | Apples | 2.9–3.3 |
| Beans (string and Lima) | 4.6–6.5 | Apple cider | |
| Beets (sugar) | 4.2–4.4 | Apple juice | 3.6–3.8 |
| Broccoli | 6.5 | Bananas | 3.3–4.1 |
| Cabbage (green) | 5.4–6.0 | Figs | 4.5–4.7 |
| Carrots | 4.9–5.2; 6.0 | Grapefruit (juice) | 4.6 |
| Cauliflower | 5.6 | Grapes | 3.0 |
| Celery | 5.7–6.0 | Limes | 3.4–4.5 |
| Corn (sweet) | 7.3 | Melons (honeydew) | 1.8–2.0 |
| Cucumbers | 3.8 | Oranges (juice) | 6.3–6.7 |
| Eggplant | 4.5 | Plums | 3.6–4.3 |
| Lettuce | 6.0 | Watermelons | 2.8–4.6 |
| Onions (red) | 5.3–5.8 | | 5.2–5.6 |
| Parsley | 5.7–6.0 | | |
| Potatoes (tubers and sweet) | 5.3–5.6 | | |
| Pumpkin | 4.8–5.2 | | |
| Spinach | 5.5–6.0 | | |
| Squash | 5.0–5.4 | | |
| Tomatoes (whole) | 4.2–4.3 | | |

**Table 2.4** Approximate pH values of dairy, meat, poultry, and fish products (James et al., 2000, modified)

| Product | pH | Product | pH |
|---|---|---|---|
| **Dairy products** | | **Fish and shellfish** | |
| Butter | 6.1–6.4 | Fish (most species)* | 6.6–6.8 |
| Buttermilk | 4.5 | Clams | 6.5 |
| Milk | 6.3–6.5 | Crabs | 7.0 |
| Cream | 6.5 | Oysters | 4.8–6.3 |
| Cheese (American mild and | 4.9; 5.9 | Tuna fish | 5.2–6.1 |
| cheddar) | | Shrimp | 6.8–7.0 |
| **Meat and poultry** | | Salmon | 6.1–6.3 |
| Beef (ground) | 5.1–6.2 | White fish | 5.5 |
| Ham | 5.9–6.1 | | |
| Veal | 6.0 | | |
| Chicken | 6.2–6.4 | | |
| Liver | 6.0–6.4 | | |
| *Just after death. | | | |

From Table 2.1, 2.2, 2.3 and 2.4, it demonstrates that pH of vegetables is more than pH of fruits. Therefore, typically, fresh fruits are subjects to spoilage by yeast and fungi, whereas fresh vegetables are subject to infection by bacterial soft rots. Fish rot faster than meat, because the pH of meat after rigor mortis is approximately 5.6 (pork: 5.3-6.9, beef: 5.1-6.2 and mutton: 5.4-6.7), which are below the pH of fish (6.2-6.5).