

Chapter 2

Literature Review

2.1 Lime fruit (*Citrus aurantifolia* (Christm.) Swingle)

2.1.1 Scientific classification (Liogier, 1988)

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Sapindales

Family : Rutaceae

Genus : *Citrus*

Species : *C. aurantifolia*

Binomial name : *Citrus aurantifolia* (Christm.) Swingle

Common name : Lime, Key lime (Mexican) and Ma-nao (Thailand)



(a)



(b)

Figure 2.1 Lime fruits (a) and the half fruits of lime juice (b)
(Source: http://en.wikipedia.org/wiki/Lime_%28fruit%29)

2.1.2 General description

Limes (*C. aurantifolia*) (Figure 2.1) are the fruit of tropical citrus tree closely related to lemons because of their distinctive flavor. This evergreen tree is in the Rue family, Rutaceae, which also includes citrus fruits such as oranges, lemons and kumquats. Limes are native to Southeast Asia and probably originated in Indonesia or Malaysia (Little and Wadsworth, 1964).

Limes are a small citrus fruit, which have an oval or round shape with a diameter between 1-2 inches. The fruits have juicy, very acidic, greenish-yellow flesh, usually picked green and are yellow at maturity. It is smaller, seedier, has a higher acidity, a stronger aroma and a thinner rind than that of the more common Persian lime. It is valued for its unique flavor compared to other limes, with Ma-nao usually having a more tart and bitter flavor (Liogier, 1988; Little and Wadsworth, 1964). Flowers and fruit appear throughout the year but are most abundant from May to September. The only two acid fruits of the lime group that have attained commercial importance are the key lime (Mexican or West Indian) and Ma-nao (Thailand) (Chaisawadi, 2003).

2.1.3 Constituents and composition

Lime exocarp contains an essential oil (7%) or typical volatile, whose main components are citral, limonene, β -pinene and fenchone (up to 15%). Further aroma compounds are terpineol, bisabolene and other terpenoids. Limonene was regarded as the major volatile component. Major fractions of peel oil include: monoterpenes (76%), sesquiterpenes (3.8%) and oxygen containing compounds (18.1%) such as aldehydes,

esters and alcohols. Citrus essential oils are made up mainly of hydrocarbons designated as terpenes and of a smaller amount of sesquiterpenes; these two components serve as carrier for more important class of oxygenated compounds which are usually the bearers of the characteristic odor of the oil in which they are contained (Nagy *et al.*, 1977).

The composition of lime juice varies considerably with the variety of fruit and with the location where the fruit is grown which is based on analyses of juice from different varieties and from different production areas (Braverman, 1949).

2.1.4 Botanical

The fruit of citrus structure is called a hesperidium. The exocarp colored yellow, orange, or green and is called flavedo. Located in the exocarp are spherical oil glands containing, e.g., lemon oils. The white, spongy portion of the fruit wall is the mesocarp and is called albedo. The locule is an air-filled chamber in which the future seeds, the ovules, are found. In the hesperidium, the layer of endocarp lining the outside of the locule grow as hairs that project into the locule. Cells of these hairs store water, organic acids and sugars, and each hair therefore becomes juice sacs. These juice sacs eventually fill the locule. The locule, when mature, becomes the section of a tangerine containing the juice sacs (hairs) within (Nagy *et al.*, 1977).

2.1.5 Benefits

Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. Within these flavonoids, d-limonene has been shown to inhibit mammary

tumours in rats. The interesting property of flavonoids in lime juice is perhaps their antibiotic effects, for example against *Vibrio cholera* (Mata *et al.*, 1994). Limes are an excellent source of vitamin C, one of the most important antioxidants in nature (Wood, 1988).

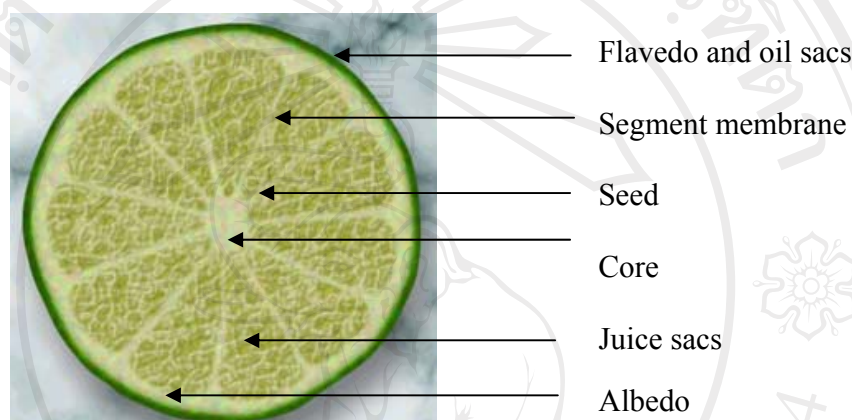


Figure 2.2 Cross section diagram of lime fruit
(Source:

<http://www.botgard.ucla.edu/html/botanytextbooks/economicbotany/Citrus/b1241tx.html>)

Lime is sold commercially and is the basis for a juice and lime oil industry (Morton, 1987). The principal use is still for food, refreshing drinks, tasty desserts and for seasoning meats, vegetables, salads, sauces and casseroles (Ehler, 2002 and Katzer, 2002). The wild type is superior in flavor to the improved varieties because it has a stronger flavor and a higher acid content (Ehler, 2002). The fresh fruits and bottled juice are an excellent source of vitamin C and were once relied upon to prevent scurvy (Bruneton, 1999).

2.2 Citrus limonoids

Citrus fruits are one of the most popular foods in the world with worldwide agricultural production over 100 million metric tons per year. While a significant quantity of citrus is consumed as fresh fruit, more and more of the crop is being consumed in the form of processed products such as juices, concentrates, flavored, citrus beverages and other food products. However, one of the long-standing problems in processed citrus products has been bitter. The level of bitterness varies among different cultivars. Bitterness in citrus juices is caused by two groups of chemicals: the flavanone neohesperidosides, such as naringin and limonin, found in species relates to the pummelo and lemon, respectively, and the limonoids (Maier *et al.*, 1980 and Nagy *et al.*, 1977).

2.2.1 Limonin and other limonoids

A group of chemically related triterpene derivatives found in the *Rutaceae* and *Meliaceae* families has been named limonoids, after the first known compound of this type, limonin. The latter has received considerable attention due to the bitter taste of most limonoids; *Rutaceae* includes the citrus species of commerce (Nagy *et al.*, 1977).

Limonoids have recently attracted attention because compounds belonging to this group have exhibited a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, anticancer, antiviral and a number of other pharmacological activities on humans. Interest in limonoids research has become greater than before also because some of them are responsible for producing bitterness in citrus fruits, which has negative impact on citrus

fruit and juice industry worldwide. The major limonoid in this family is limonin, accountable for the bitterness of poor quality juices (Mark *et al.*, 2000).

2.2.2 Structure of limonin

Characteristic structural features of limonoids are seen in the formula of limonin (Figure 2.3). All naturally occurring citrus limonoids contain a furan ring attached to the D-ring, at C-17, as well as oxygen containing functional groups at C-3, C-7, C-16 and C-17 (Somrutai *et al.*, 2005). The structural variations of limonoids found in *Rutaceae* are less than in *Meliaceae* and are generally limited to the modification of A and B rings, the limonoids of *Meliaceae* are more complex with very high degree of oxidation and rearrangement exhibited in the parent limonoid structure (Suarez *et al.*, 2002).

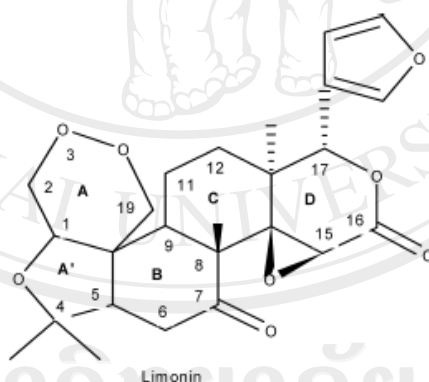


Figure 2.3 Structure of limonin
(Source: Maier *et al.*, 1980)

2.2.3 Properties of limonin

Limonin's chemical composition is $C_{26}H_{30}O_8$ with a molecular weight of 470 which contains two lactones groups. Limonoids are heavily oxygenated, modified terpenoids dominant in the plant family *Meliaceae*, and to a lesser extent in *Rutaceae*.

They may be the most extreme examples of oxidation of triterpenes in nature. They have moderate polarity and are insoluble in water and hexane but soluble in hydrocarbons, alcohol and ketone. The limonoids are mostly bitter in taste and account for the scent of fresh peels of citrus fruits. Limonoids are present in neutral (noncarboxylated/aglycon) as well as acidic (carboxylated / glucoside) forms, the former are insoluble and bitter while the latter are soluble and tasteless (Mark *et al.*, 2000; Kale and Adsule, 1995 and Nagy *et al.*, 1977).

2.2.4 Biosynthesis of limonoids

There are five groups of enzymes involved in the biosynthesis and biodegradation of limonoids in *Citrus* (Hasegawa and Miyake, 1996). One group is present only in the phloem region of citrus stem tissues dedicated to the production of nomilin. A second group which converts nomilin to the other limonoid aglycones occurs in all citrus tissues, including leaves, stems, fruit juice sac segments, fruit peel and seed regardless of maturity. The biosynthetic pathways of limonoids postulated thus far have been based upon reasonable sequences of known limonoids. The generally accepted sequence of

limonin synthesis in citrus is (Figure 4): deacetylnomilin \longrightarrow nomilin \longrightarrow obacunone \longrightarrow obacunoic acid \longrightarrow isoobacunoic acid \longrightarrow limonin (Mark *et al.*, 2000).

An alternative to the previous pathway is a pathway via ichangin (Dreyer, 1966). Kefford and Chandler (1970) suggested that this pathway may be a minor one, since ichangin had only been isolated from the rare Ichang lemon, while nomilin and

obacunone are present in most citrus species. However, ichangin had been isolated also from both lemon and grapefruit seeds by other researchers (Nagy *et al.*, 1977).

All limonoids (Figure 2.5) involved in the pathways may be present in the open D-ring form in citrus tissues. It has been shown that limonoate A-ring lactone (the salt of limonoic acid A-ring lactone) is the form of limonin in citrus tissues (Nagy *et al.*, 1977), except in seeds where limonin is also present. Maier and Margileth (1969) showed the presence of a limonoid enzyme, limonin D-ring lactone hydrolase, in citrus.

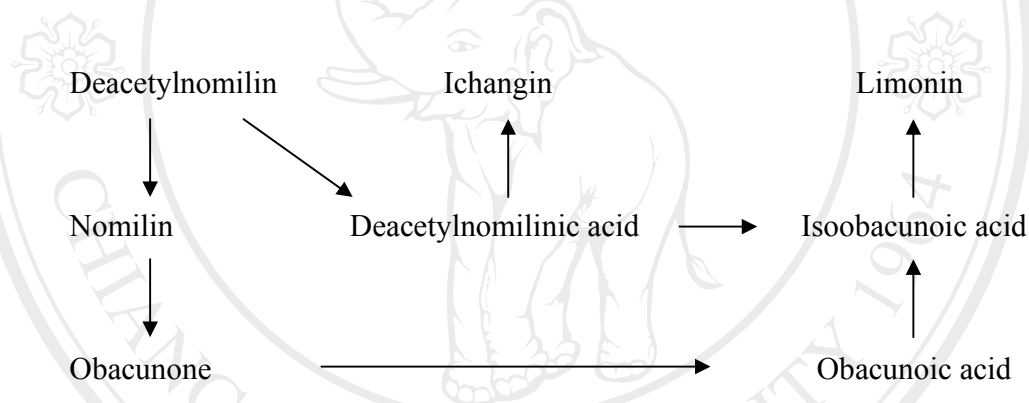


Figure 2.4 The proposed biosynthetic pathways of limonin in citrus
(Source: Mark *et al.*, 2000)

The enzyme catalyzes reversibly the hydrolysis of D-ring of limonin: limonin \leftrightarrow limonoate A-ring lactone. It does not attack the open or closed A-ring lactone of the compounds. Since the hydrolase has fairly broad substrate specificity for limonoids containing a D-ring lactone, its role in the biosynthetic scheme may be to keep the limonoids in their more soluble salt forms (D-ring lactone open) (Nagy *et al.*, 1977).

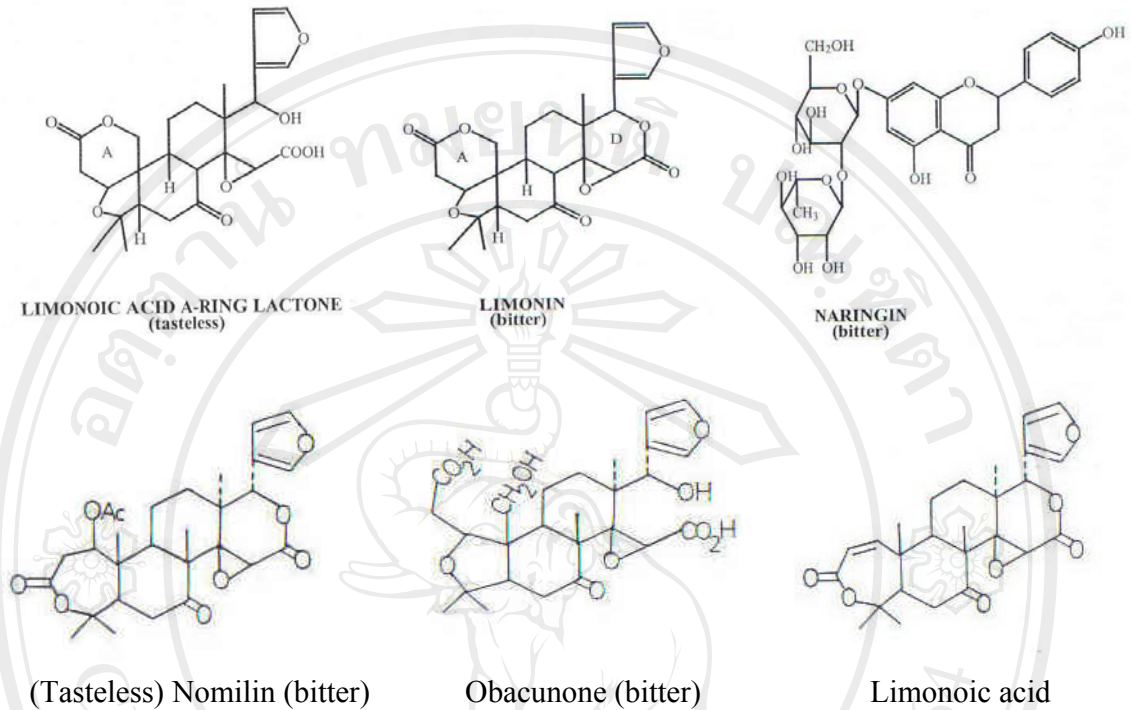


Figure 2.5 Citrus limonoids
(Source: Mark *et al.*, 2000)

In mature fruit tissue, glucosides, predominantly limonin glucoside, accumulate while in seeds both aglycones and glucosides, predominantly nomilin glucoside, are found (Figure 2.7). The limonin glucosides stored in the fruit tissue are very stable. Hence fresh tissue and freshly squeezed juice do not taste bitter. However, tissues and seeds were crushed during commercial juice processing release bitter aglycones and glucosidases hydrolyzing limonoid glucosides to bitter aglycones or crashing may release this β -glucosidase activity in the juice (Mark *et al.*, 2000). These may increase the level of the bitter aglycones, such as limonin, by hydrolyzing the tasteless limonoid glucosidase. Citrus juice turns bitter during storage. This delayed bitterness is caused by limonin and nomilin forming from monolactones (Ashurst, 2005).

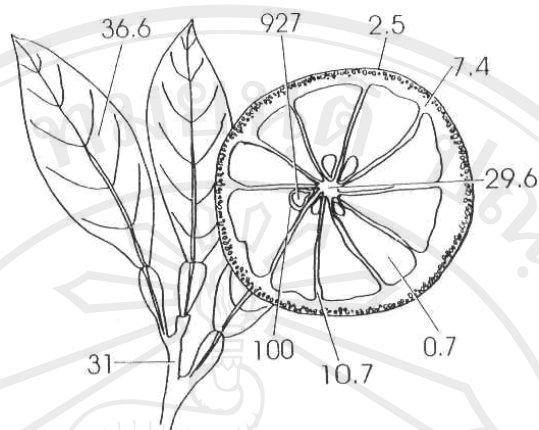


Figure 2.7 Distribution of limonin in a fruit and vegetable part (as $\mu\text{g}/100\text{ mg}$)
(Source: Nagy *et al.*, 1977)

2.2.5 Delayed bitterness in citrus juice

Most citrus fruits do not taste bitter if eaten fresh or if freshly squeezed juice is consumed. However, within a few hours after juicing at room temperature, or overnight if stored in a refrigerator, the juice extracted become bitter (Hasegawa and Maier, 1990). This gradual development of bitterness in these citrus juices, sometimes referred to as delayed bitterness, is caused by the formation of d-limonin, from a tasteless precursor (Nagy *et al.*, 1977). The precursor was limonate A-ring lactone, which is present in the capillary membrane and albedo tissue of citrus fruits (Ashurst, 2005).

When the juice is extracted, this precursor comes into contact with the acid environment of the juice and undergoes a slow conversion to the bitter d-limonin (Figure 2.8). A bitter taste can be detected in the juice of immature fruit within a half-hour at room temperature, but these periods increase to several hours as the fruit becomes more mature (Braverman, 1949).

The reaction proceeds under acidic conditions below pH 6.5 and is accelerated by the enzyme limonin D-ring lactone hydrolase. This enzyme has been isolated from citrus and shown to be an extremely heat stable enzyme (Maier *et al.*, 1980).

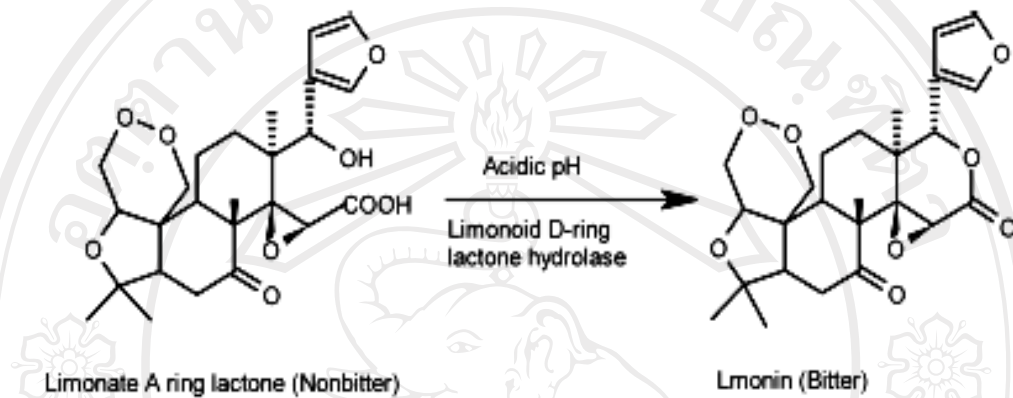


Figure 2.8 Mechanism of delayed bitterness
(Source: Mark *et al.*, 2000)

Thermal processing accelerates this reaction (Maier *et al.*, 1980). Hard extraction of fruit with a thick albedo layer may place more albedo into the juice than soft extraction and is one of the reasons hard extracted juices are of lower quality due to higher quantities of undesirable compounds (Nagy *et al.*, 1977 and Maier *et al.*, 1980). Other bitter tasting limonoids are nomilin and ichangin, although they are found in much lower concentrations than limonin in the citrus fruits (Hasegawa *et al.*, 1995).

The delayed bitterness lowers the quality and value of commercial juices and has significant negative economic impact to the citrus industry. Unusual weather and harvesting conditions which cause disruption of fruit tissues, such as freezing or mechanical damage, can promote the acidic pH and enzyme activity in the fruit tissues and the conversion of the precursor to limonin (Fong *et al.*, 1993).

2.3 Hydrocolloids

The term 'hydrocolloids' refers to a range of polysaccharides and proteins. Hydrocolloids are hydrophilic polymers of vegetable, animal, microbial or synthetic origin that generally contain many hydroxyl groups and may be polyelectrolytes. Nowadays they are widely used in a variety of industrial sectors. They are naturally present or added to control the functional properties of aqueous foodstuffs. They have many properties including emulsion stabilization, prevention of ice-recrystallization, organoleptic properties, etc (Phillips and Williams, 2000). The commercially important hydrocolloids and their origin are given in Table 2.1.

The food industry, in particular, has seen a large increase in the use of hydrocolloids in recent years. Eventhough they are often present only at concentrations of less than 1%, they can have a significant influence on the textural and organoleptic properties of food products (Phillips and Williams, 2000).

2.3.1 Gum acasia

2.3.1.1 Source

A natural gum also called gum arabic (Figure 2.9), is prepared from an exudate of the stems and branches of sub-Saharan *Acacia senegal* and *Acacia seyal* trees (Colloïdes Naturels International, 2007). It is produced naturally as large nodules exudate from their stems and branches to seal wounds in the bark of the tree, in the form of droplets of sap approximately five centimeters in diameter. It is a less consistent material than other hydrocolloids (Verbeken *et al.*, 2003).

Table 2.1 Source of commercially important hydrocolloids

Sources		Hydrocolloids
Botanical :	Tree	Cellulose
	Tree gum exudates	Gum acasia, Gum karaya, Gum ghatti and Gum tragacanth
	Plants	Starch, pectin and cellulose
	Seeds	Guar gum, locust bean gum, tara gum and tamarind gum
	Tubers	Konjac mannan
Algal :	Red seaweeds	Agar, carrageenan
	Brown seaweeds	Alginate
Microbial		Xanthan gum, curdlan, dextran, gellan gum and cellulose
Animals		Gelatin, caseinate, whey protein and chitosan

Source: Phillips and Williams (2000)

2.3.1.2 Properties

Gum arabic readily dissolves in water to give clear solutions ranging in color from very pale yellow to orange-brown and with a pH of ~ 4.5. The highly branched structure of *A. senegal* gum (Figure 2.10) gives rise to compact molecules with a relatively small hydrodynamic volume and as a consequence gum solutions become viscous only at high concentrations (Phillips and Williams, 2000).

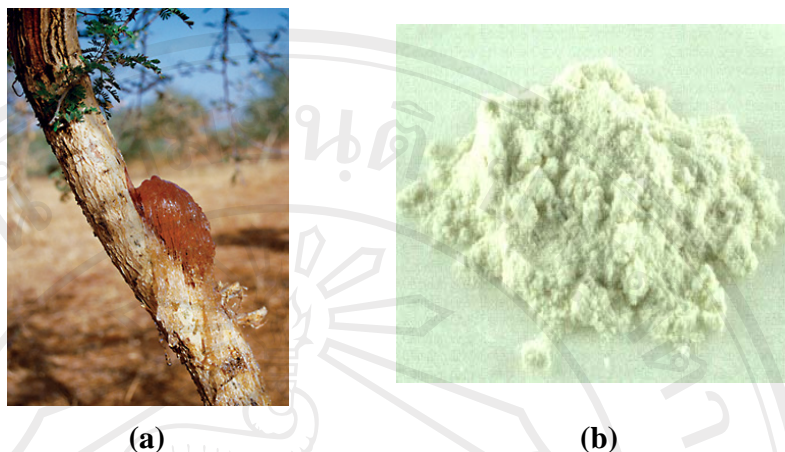


Figure 2.9 The nodules exudates of gum acasia (a) and gum acasia powder (b)
 (Sources: http://www.saudiaramcoworld.com/issue/200502/gum_arabic.htm and
http://www.gasid.it/prodotti/inglese/gommaarabica_1.html)

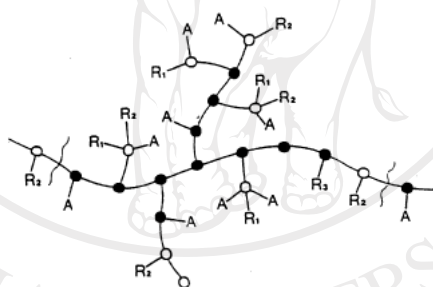


Figure 2.10 Possible structure of the carbohydrate component of gum from *A. senegal*
 (Source: Phillips and Williams, 2000)

2.3.1.3 Functionality

As with many other hydrocolloids, gum arabic is a useful as a prebiotic promoting beneficial physiological effect (Phillips *et al.*, 2007). It has a very high solubility in water (Fennema, 1996) and is used primarily in the food industry as a stabilizer. Gum acasia is a complex mixture of saccharides and glycoproteins, which has several properties, including very low viscosity, absence of taste and odor and perfectly edible (Verbeken, 2003).

Gum acasia can be incorporated in large amount in foodstuffs without disturbance of their organoleptic properties. As a food additive, it is a useful, but slightly expensive hydrocolloid emulsifier, texturizer and film-former. Gum arabic is stable in acid conditions and is widely used as a stabilizer and emulsifier in the production of concentrated citrus and cola flavour oils for application in soft drinks. The gum is able to inhibit flocculation and coalescence of the oil droplets over several months and the emulsions remain stable for up to a year (Phillips and Williams, 2000).

The simultaneous presence of hydrophilic carbohydrate and hydrophobic protein enables its emulsification and stabilization properties. Emulsification is particularly enhanced due to molecular flexibility which allows greater surface interaction with the oil droplets (Chaplin, 2007). Gum arabic is used in confectionery such as traditional hard (wine) gums and pastilles and as a foam stabilizer in marshmallows (Sanchez *et al.*, 2002).

2.3.2 Pectin

2.3.2.1 Source

Pectins (Figure 2.11) are heterogeneous grouping of acidic structural polysaccharides and are present in the primary cell walls and middle lamella of many plants, where they are frequently associated with other cell wall components, such as cellulose, hemicellulose and lignin. Pectin is found in fruit and vegetables and mainly prepared from 'waste' citrus peel and apple pomace (Kertesz, 1951).

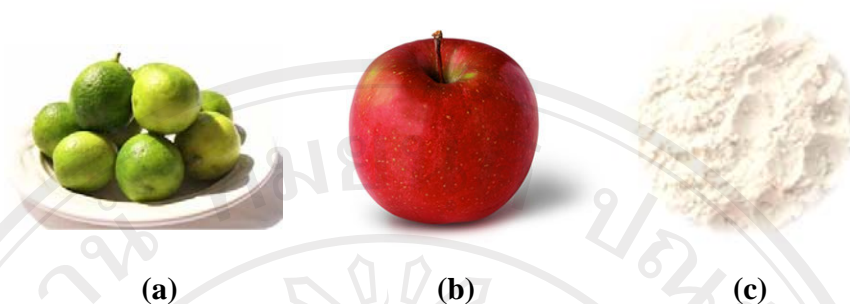


Figure 2.11 Pectin from citrus (a), from apple (b) and pectin powder (c)
(Source: <http://en.wikipedia.org/wiki/Pectin>)

2.3.2.2 Properties

Pectin is a complex polysaccharide consisting mainly of esterified D-galacturonic acid residues in an α -(1-4) chain (Figure 2.12). The acid groups along the chain are largely esterified with methoxy groups in the natural product. There can also be acetyl groups present on the free hydroxy groups. The galacturonic acid main chain also has the occasional rhamnose group present which disrupts the chain helix formation (Croak and Corredig, 2006).

Pectin is also known to contain other neutral sugars which are present in side chains. The most common side chain sugars are xylose, galactose and arabinose. The side chains tend to occur in groups and have led to the description of the pectin molecule as having hairy and smooth regions. The commercial pectin molecules which contain largely galacturonic acid units consist of a mixture of methyl ester, free acid and salt derivatives of the carboxyl group of the acid (Phillips and Williams, 2000).

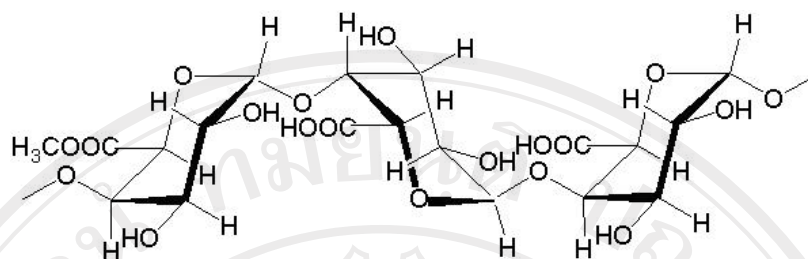


Figure 2.12 Structure of pectins
(Source: <http://www.kjemi.uio.no/Polymerkjemi/Research/pectin.jpg>)

2.3.2.3 Functionality

Pectins are mainly used as gelling agents, but can also act as a thickener, water binder and stabilizer. Low methoxyl pectins (< 50% esterified) form thermoreversible gels in the presence of calcium ions and at low pH (3 - 4.5) (Dickinson, 2003). Applications in food industry are diversified, including jams and jellies of low-sugar content; dairy desserts, where usually addition of calcium salt is not needed; fruit gels for use in ice cream; as food coatings and as thickening agents of syrups for fruit and vegetable canning (Kertesz, 1951).

Whereas high methoxyl pectins rapidly form thermally irreversible gels in the presence of sufficient sugars such as sucrose and at low pH (< 3.5). The lower the methoxyl content, the slower the set will form. The high methoxyl pectins are mainly used as gelling agents in fruit-based products, especially in the manufacture of jams and fruit preservatives. Other food applications include milk desserts, fruit preparations for yoghurt, confectionery jellies and stabilization of acidified milk products, fruit juices or soft drinks (Kertesz, 1951).

2.3.3 Carboxymethylcellulose (CMC)

2.3.3.1 Source

Carboxymethylcellulose is a derivative of cellulose formed by its reaction with alkali and chloroacetic acid (Nussinovitch, 2003).

2.3.3.2 Properties

The CMC structure is based on the β -(1 \rightarrow 4)-D-glucopyranose polymer of cellulose (Figure 2.13). Different preparations may have different degrees of substitution, but it is generally in the range of 0.6 - 0.95 derivatives per monomer unit (Nussinovitch, 1997).

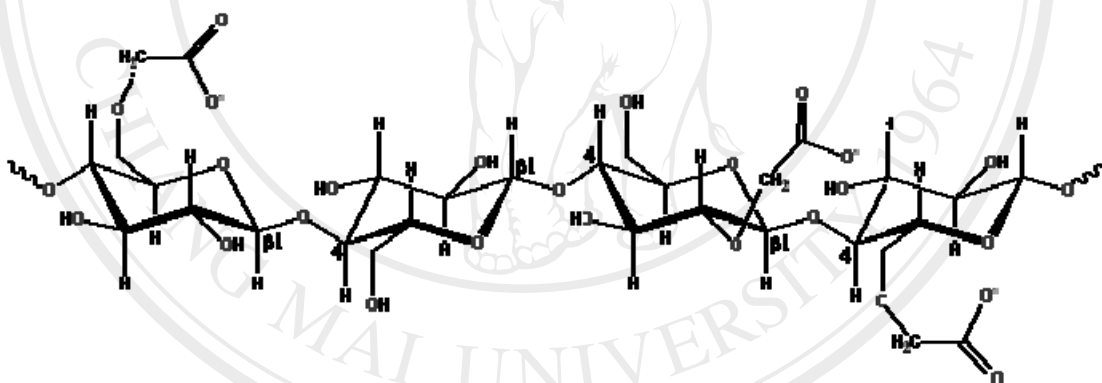


Figure 2.13 Structure of Carboxymethylcellulose (CMC)
(Source : <http://www.lsbu.ac.uk/water/hycmc.html>)

2.3.3.3 Functionality

CMC is used in food science as a viscosity modifier or thickener and to stabilize emulsions, for instance in ice cream. They have high viscosity, are not toxic and are generally non-allergenic. CMC dissolves rapidly in cold water and is mainly used for controlling viscosity without gelling. The CMC, at typical concentrations, does not form gel even in the presence of calcium ions (Phillips and Williams, 2000).

2.3.4 Carrageenan

2.3.4.1 Source

Carrageenan is a collective term for polysaccharides prepared by alkaline extraction (and modification) from red seaweed (*Rhodophyceae*), mostly of genus *Chondrus*, *Eucheuma*, *Gigartina* and *Iridaea* (Ceamsa Compañía Española de Algas Marinas S.A, 2002). Different seaweeds produce different carrageenans. There are basically three main types of commercial carrageenan that have been identified. These are known as kappa, iota and lambda which are idealized molecules assigned a definitive structure. Hardly any seaweed yields this ideal carrageenan, but rather a range of intermediate structures (Nussinovitch, 2003).

2.3.4.2 Properties

Carrageenan is a high molecular weight linear polysaccharide comprising from repeating galactose units and 3,6-anhydrogalactose (3,6 AG), both sulfated and non-sulfated, joined by alternating α -(1,3) and β -(1,4) glycosidic links (Figure 2.14). The thickening and gelling properties of different types of carrageenan are quite different. For example, kappa carrageenan forms a firm gel with potassium ions while iota and lambda are only slightly affected (Nussinovitch, 1997).

Iota carrageenan interacts with calcium ions to give soft elastic gels, but salts have no effect on the properties of lambda carrageenan. All carrageenans are soluble in hot water. However, only lambda and the sodium salts of kappa and iota are soluble in cold water. Carrageenan solutions will lose viscosity and gel strength in systems below pH values of about 4.3 (Phillips and Williams, 2000).

2.3.4.3 Functionality

Carrageenans are versatile as food additives as they are capable of binding water, promoting gel formation and acting as thickeners and stabilizing agents. Further advantages lie in the improvement of palatability and appearance.

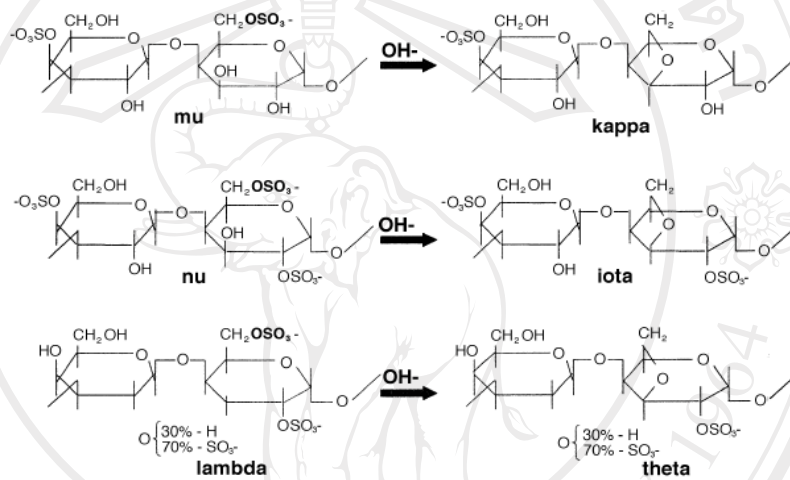


Figure 2.14 Carrageenan structures
(Source: Phillips and Williams, 2000)

Traditional classification of food applications depends on whether carrageenan is added to a milk system (Drohan *et al.*, 1997) or to an aqueous system lacking the casein that interacts so strongly. The widespread uses of κ -carrageenan in food preparations include the important range of dairy desserts (milk gels flans, custards, chocolate milks and ice creams) and meat preserves including pet foods (Kampf and Nussinovitch, 1997).

2.4 High Pressure Processing (HPP)

Traditional methods of food preservative are being used for many years with the principle objective of preservation and extension of shelf-life. In general, the nutritional attributes, color, flavor and sensory qualities are often lost to some extent in order to achieve these objectives. In this century, customer awareness and expectations for food with higher organoleptic quality and safety has been increased (Riahi, 2003).

Despite the effectiveness achieved by traditional thermal technologies from a microbial safety standpoint, thermal technologies also cause nutritional and sensory food deterioration. In traditional thermal processing, heat is mainly transferred to food by conduction or convection. This energy not only inactivates microorganisms by disrupting the chemical bonds of cellular components such as nucleic acids, structural proteins and enzymes (Farkas and Hoover, 2000), but also affects desirable food components that are responsible for flavor, aroma, texture, some nutritional aspects and appearance. Although food fortification can overcome certain nutritional degradation attributes, sensory attributes are difficult to retain in foods treated with traditional heat treatments.

In addition, consumers are looking for food products with superior nutritional value, freshness, no addition of food preservatives and assurance that there are no pathogenic or contaminant microorganisms present in food products. Novel technologies been focused and recognized in the user community are microwave heating, ohmic heating, pulsed electric field, pulsed light irradiation and high-pressure treatments. These methods are generally recognized as “non-thermal or novel/enhanced thermal” methods (Houska *et al.*, 2006; Riahi, 2003 and Thumthanaruk, 2002).

High pressure processing (HPP) is not a novel technology in the food industry, although interest has been renewed in the last decade (Deliza *et al.*, 2005). HPP (100-1000 MPa) treatment concept originated from applications in material science such as ceramics and artificial diamond, is currently being expanded to biological and food systems due to its potential for achieving microbial destruction and enzyme inactivation at low or moderate temperatures. HPP is less destructive to low molecular weight food compounds such as flavoring agents, pigments and vitamins (Houska *et al.*, 2006; Riahi, 2003 and Tausher, 1995).

2.4.1 Principles of High Pressure Processing

High pressure processing relies on Le Chatellier's and isostatic principles. Based on the Le Chatellier principle, pressure can enhance or retard chemical reactions if a volume decrease or increase accompanies them, respectively. The isostatic principle indicates that pressure is transmitted in a uniform and quasi-instantaneous manner throughout the biological sample. Therefore, in contrast to thermal processing the pressurization process time is independent of sample volume. Keeping the sample for extended period does not require any additional energy. The low energy levels involved in pressure processing may explain why covalent bonds of food constituents are usually less affected than weak interactions (Cheftel and Culioli, 1997).

High Pressure satisfies the demand from consumers in developed countries for minimally processed, higher quality and safe foods with extended shelf life and without any chemical additive. HP does not affect covalent bonds; therefore, flavor and

appearance are similar to fresh food, usually superior to heat treated foods. HP has been used effectively for sterilizing and preserving citrus juice without changing the original flavor and taste. Unlike thermal processing, HP treatment is independent of product size and geometry, and all food particles receive the same pressure and the process pressure is achieved virtually instantaneously (Houska *et al.*, 2006; Deliza *et al.*, 2005 and Bull *et al.*, 2004).

HP is effective at ambient temperature reducing thermal effects on food quality. HP is effective with mild heating and even at ambient temperature reducing thermal energy consumption compare to conventional sterilization processes (Houska *et al.*, 2006 and Farr, 1990).

2.4.2 Engineering Aspects

High Pressure Processing technology is based on the use of pressure to compress food to 300-700 MPa inside a pressure vessel for a short period, typically ranging from a few seconds to several minutes. The pressure vessel is the main component of a HHP system. Pre-stressed vessels were designed with residual compressive stress to lower the maximum stress level in the vessel wall during pressurization, hence reducing the cost of producing the equipment (Mertens and Deplace, 1993). The pressure vessel is pressurized by means of a pressure medium which usually is water mixed with a small percentage of oil for pump lubrication purposes (Van der Berg *et al.*, 2002).

In the equipment utilized for food applications, pressure is transmitted by two methods (Figure 2.15). In a direct method, a piston is pushed by pressure transmitting liquid at one end, directly pressurizing the food contained in the pressure chamber at its other end. This method allows very fast compression but requires a pressure resistant dynamic seal between the piston and the internal vessel surface. In an indirect method, high pressure intensifiers are used to pump the pressure medium from a reservoir into the closed vessel until the selected pressure is achieved (Farr, 1990 and Mertens and Deplace, 1993).

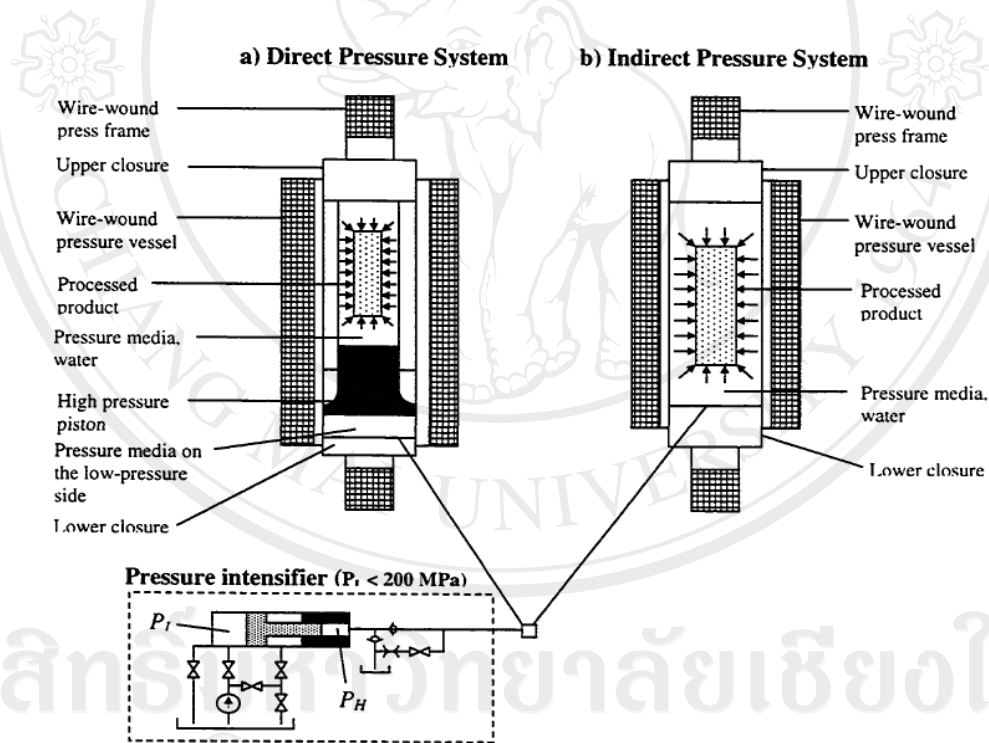


Figure 2.15 High pressure machines with wire-wound vessels and two pressure transmission systems

(Source: Barbosa-Canovas and Rodriguez, 2002)

In both methods, pressure is transmitted isostatically by a pressure transmitting fluid, according to Pascal's law (Earnshaw, 1996). In the case of liquid foods that are pressurized in bulk (i.e. without an intermediate package) the pressure is transmitted by a piston (or isolator) to the pressure chamber, where the food is contained. In this way, an uniform pressure from all directions compresses the food, which then returns to its original shape when the pressure is released (Olsson, 1995).

HHP has been proposed and reported for processing of citrus and other juices (Basak and Ramaswamy, 1996 and Morild, 1981), processing milk and pork with minimal changes in color, microorganisms and enzymes (Mussa, 1999); processing fruits with minimal effect on its texture (Basak and Ramaswamy, 1998); formation of gel from starch (Cheftel and Culioli, 1997); production of cheese (Pandey *et al*, 2000); digestion of starch (Hayashi, 1989) and processing of fish enzymes (Ashie, 1995).

The processing cost of Ultra-High Pressure (UHP) has been reported to be lower than thermal processing (Cheftel and Culioli, 1997). HHP processing is an alternative non-thermal food processing method that is considered as emerging technology in the new millennium. Application of HP in food processing provides unique opportunities for production of food with high safety, high quality and shelf life.

2.4.3 The advantage of High Pressure Processing

In recent year, HPP of food has received attention due to its potential benefits compared to thermal treatments. The advantages of UHP processing include (Heremans, 1995 and Galazka *et al.*, 1995) :

- 1) Retention of flavor, color, vitamin and taste because covalent bonds (rupture of peptide bonds and disulfide bonds) are not affected by the pressures while they may be broken by temperature.
- 2) The independent effect of the treatment towards the size and geometry of the sample since it is an isostatic process, thus pressure is applied uniformly throughout products, while heat generates gradients and may thus over-process for some part of the food.
- 3) Improvement of food texture.
- 4) Producing additive free foods.
- 5) No environmental pollution.
- 6) Involving low energy and running cost.

Pressure treatment is still costly, mainly because of the initial capital expenditure, and this limits its applications to high-value products. However, it can be expected that those costs will go down as a consequence of further progress in technology (UK High pressure club for food processing, 2002).