CHAPTER II
LITERATURE REVIEW

TANGERINE

The genus *Citrus* includes sweet citrus fruits (oranges, grapefruits) and sour citrus fruits (limes, lemons). Tangerine is a group name for a class of oranges with thin loose peel, which clearly dominate the citrus industry.

Tangerine (*Citrus reticulata* Blanco) belongs to the family *Rutaceae* and the subfamily *Aurantioideae* (Considine and Considine, 1982). Tangerine or Som Kheaw Wan in Thai, is oblate to pear-shaped of medium size (5.4-7.5 cm. wide and 4-5.4 cm. high), bright-orange or red orange peel when ripe, smooth, glossy, loose skinned, segments easily separable. Seeds are small, pointed at one end and green inside (Morton, Julia, and Miami, 1987). The flavor of tangerine is unique and richer than that of most citrus species. In Thailand, tangerines is separated into 6 sizes by diameter at 5.0, 5.5, 6.0, 6.5, 7.0, and more than 7.0 centimeter with the number of 3, 2, 1, 0, 00, and 000 respectively. The tangerine number 3 and number 2 are normally processed to tangerine juice.

The production of tangerine is increasing in response to strong demand from domestic and exports markets. Total production of tangerines in Thailand was around 600,000 tons in 2003 (DOAE, 2003). Although a high percentage of produce is consumed in its fresh form, an increasing proportion is being processed into juice. With

this growing potential, processing and packaging technologies to enhance the quality of the beverage for consumer acceptance are essential. Tangerine juice is preferable by consumers not only for desirable flavor but also for high nutritive value. Table 2.1 shows the quantity and value of citrus production in Thailand.

Table 2.1 Quantity and value of citrus fruits production in Thailand

Items	Quantity (tons)/ Value (million bahts)		
	1997	2000	2003
Tangerine	574,000/6.8	580,650/7.9	585,167/12.6
Neck orange	1,200/0.03	1,450/0.04	1,646/0.05
Sweet orange	4,400/0.7	6,380/0.13	7,775/0.16
Acidless orange	5,282/0.11	5,970/0.12	7,123/0.15
Pummelo	324,700/4.44	285,400/3.56	238,440/3.28

Available from: Department of Agriculture Extension (DOAE, 2003)

PHYSICAL STRUCTURE OF CITRUS FRUIT

Citrus fruit is composed of three distinctly different morphological parts. The cross-section diagram of typical citrus fruit is shown in Figure 2.1. The epicarp consists of the colored portion of the peel and is known as the flavedo. In the flavedo, there are cells containing the carotenoids that give the characteristic color to different citrus fruits, i.e., orange, tangerine, grapefruit, lemon, etc. The oil glands, also found in the flavedo,

are the raised structures in the skin of citrus fruits that contain the essential oils (Izquierdo and Sendra, 1993). Immediately under the epicarp is the mesocarp or albedo. This is typically a thick, white, spongy layer. The albedo consists of large parenchymatous cells that are rich in pectic substances and hemicelluloses. The combined albedo and flavedo are called the pericarp, commonly known as the rich or peel.

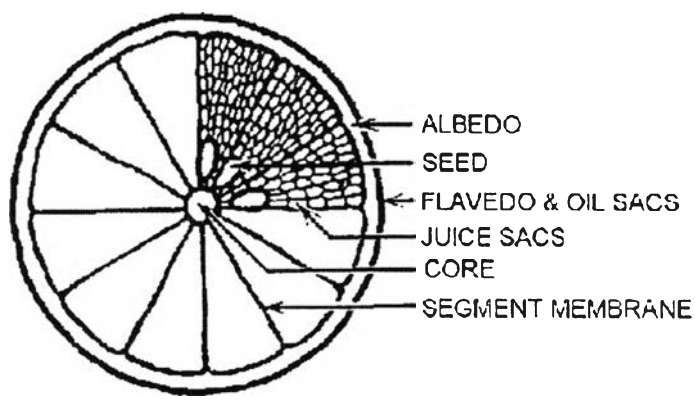


Figure 2.1 Cross-section of citrus fruit (Considine and Considine, 1982)

The edible portion of the citrus fruit, or the endocarp, is composed of many carpels or segments. Inside each segment there are juice vesicles, which are attached to the segment membrane by the vesicle stalk. Many chemical constituents are distributed among the various tissues. Some are more concentrated in one tissue than the other (Izquierdo and Sendra, 1993).

CHEMICAL COMPOSITION OF CITRUS FRUIT

The composition of citrus fruits varies with cultivar, climate, rootstock and cultural practices. During the fruit development, the major changes in chemical constituents include those in fruit color (carotenoids), total nitrogen, carbohydrates, organic acids, lipids, volatile compounds, waxes, steroid, terpenoids, vitamins, inorganic mineral constituents, and flavonoids and limonoids (Kale and Adsule, 1995).

1. Pigments

The color of orange and tangerine juices is due to carotenes and xantophylls. As chlorophyll in citrus peel decreases, carotenoids increase. In mature green fruit, xantophylls predominate (Ting and Attaway, 1971a cited Miller *et al.*, 1940b).

2. Nitrogenous compounds

The nitrogenous constituents of citrus fruit include alanine, asparagine, aspartic acid, glutamic acid, proline and serine, amines, peptides and proteins (Ting and Attaway, 1971b cited Underwood and Rocklands, 1953). These nitrogenous compounds distribute in various parts of the fruit. Bain (1958) observed that in the early stage of development of the fruit, protein nitrogen is predominant (Ting and Attaway, 1971c cited Bain, 1958). As the fruits mature, soluble nitrogen that is mostly found in the juice increases and is almost equal to protein nitrogen at the early stage of development.

3. Carbohydrate

The principal carbohydrates in both citrus fruit and juice are sugars and polysaccharides, 75-85% of the total soluble solids of orange juice are sugars, (Ting and

Attaway, 1971d cited Bartholomew and Scinclair, 1943). Curl and Veldhuis (1948) concluded that the main sugars in Florida valencia oranges were sucrose, glucose and fructose at the ratio of 2:1:1. As the early and mid season oranges and tangerines ripped on the tree, the total sugars in the juice increase rapidly due to the accumulation of sucrose (Ting and Attaway, 1971e cited Curl and Veldhuis, 1948).

During the course of development of fruits, starch is found in all components of the fruit including the juice vesicles (Webber and Bachelor, 1943). It is especially abundant in the albedo, but is also found in flavedo. The other principal polysaccharide found in citrus fruits is pectic substances which are mostly distributed in albedo and citrus tissue. The pectic substances are important to the processing industry because of their function as a cloud stabilizer in the juice.

4. Organic acids

Citrus fruits are classified as acid fruits, as their soluble solids are composed mainly of organic acids and sugars. The acidity of citrus is due primarily to citric and malic acid (Gorini and Testoni, 1988). Traces of tartaric, benzoic, oxalic, and succinic acids have also been reported (Sinclair, Bartholowen and Ramsey, 1945). The titratable acidity of oranges and grapefruits plays an important part in determining the maturity of these fruits.

5. Lipids and oil

Oleic, linoleic, linolenic, palmitic and stearic acids, glycerol, and a phytosterol in the pulp and locular tissues of California Valencias, have been reported (Cameroon and

Frost, 1968) The off-flavors in canned orange juice might be related to change in fatty acid constituents. The changes in lipid composition of orange juice due to pasteurization are very slight and are not related to changes in flavor. The unsaturated fatty acids occupy a large percentage of the total fatty acid of the citrus seed oils. This fact makes citrus oil a desirable substitute for other unsaturated fats food.

In the extraction of the juice, different proportions of oil may be extracted from peel. The main constituent of oil is *d*-limonene. A small amount of peel oil in fresh orange juice gives a pleasant aroma and flavor. Under certain conditions, even the small amounts of oil in canned juice may give rise to objectionable flavors upon storage. Efforts to increase the yield of the juice may result in the possible contamination of the juice with peel juice constituent, which may contribute bitterness or other off-flavor.

6. Volatile compounds

The most important volatile materials of citrus fruit are those associated with flavor and aroma. The volatile constituents of citrus which can be removed from the juice by distillation consist of water-insoluble and water–soluble fractions (Izquierdo and Sendra, 1993). The predominant off-flavor in stored canned orange appears to come from nonvolatile precusors.

7. Vitamins

Vitamin C, or ascorbic acid, is by far the most abundant vitamin in citrus fruits, which are important sources of this vitamin. On a whole fruit basis, the juice contained about 25 % of the total vitamin C of the oranges (Ting and Attaway, 1971f cited Atkins,

1945). Tangerines generally contain from 20 to 50 mg of ascorbic acid /100ml of juice. Harding reported that ascorbic acid was usually high in immature oranges, but as fruit ripen and increase in size, the concentration generally decreased. Besides vitamin C, citrus juice also consists of others important vitamins such as inositol, tocopherol, vitamin A, thiamin, niacin and riboflavin (Ting and Attaway, 1971 g; h cited Harding *et al.*, 1940; Harding and Fisher, 1945).

8. Inorganic constituents

In common with other fruits, citrus fruits have a high content of potassium (100-350 mg/100 g edible portion) and low content of sodium (1-10 mg/100 g). Potassium accounts for 60-70% of the total ash content of juice. The major portion of the calcium and magnesium is in the water-insoluble forms that combine with pectin. The juice of citrus fruit contains about 0.4% ash. The ash content of orange juice was generally the highest in immature fruit and gradually decreased as fruit maturity progressed (Harding, Wilston and Fisher, 1940).

9. Miscellaneous compounds

These compounds (waxes, coumarins, triterpenoids and steroids) are found in the residue after distillation of citrus oil and they are all solute in lipid solvents.

10. Flavonoids and Limonoids

The two principal compounds that impart the bitterness in citrus fruits are flavonoids and limonoids (Table 2.2). Citrus fruits contain complex mixture of flavonoid compounds, which include flavanone and flavone glycosides and also highly

methoxylated flavanones and flavones. The principal citrus flavonoid in sweet oranges, tangerines, and lemons is hesperidin, while in grapefruit, pomelo and sour orange, naringin predominates. These two flavonoids are important as quality indicators for citrus industry. For example, the cloudiness of marmalade made from sweet oranges is due to precipitation of hesperidin, which is less soluble than naringin (Ting and Attaway, 1971i cited Smith, 1953). Moreover, high concentration of naringin also reduces juice quality because of its bitter taste, which can be detected at a dilution of 1:50,000 (Ting and Attaway, 1971j cited Zoller, 1918). Its concentration is least amount in ripened fruit and its taste threshold in orange juice is approximately 600 ppm (Kimball, 1991a).

Limonoids, the other biiter compound, are found in all citrus species. It is generally known in the processing industry that the juice of some varieties of oranges, such as Washington Navel, sometimes becomes unpalatably bitter a few hours after extraction. Limonin are widely distributed throughout the Rutaceae species (Macintoch and Rouseff, 1982). Consequently, the major bitter compound of Thai tangerine (*Citrus reticulata* Blanco) is also limonin (Savitree Jungsakulrujirek, 1997; Piriya Rodart, 2001). Limonin is exceedingly bitter component with taste threshold of 6 ppm in orange juice (Guadagni *et al.*, 1973).

Table 2.2 Chemical compounds of flavonoids and limonoids

Flavonoids	Limonoids
Naringin	Limonin
Neohesperidin	Nomilin
Poricidin	Obacunone
Hesperidin	Ichangin
Rutinoside of naringenin	Deoxylimonin
Tangeritia	Etc.
Nobiletin	
Etc.	

NARINGIN IN CITRUS FRUITS

Naringin ($C_{27}H_{32}O_{14}$) is a flavanone glucoside with molecular weight of approximately 580 and approximate volume of 465 cubic angstroms. The melting point of naringin is 81^{0}C. Naringin is composed of one flavonoid group attached to a disaccharide (glucose-rhamnose). The structure of naringin is shown in Figure 2.2. If the rhamnose attached to the C-2 position of the flavonoid, the compound is non-bitter however if it attached at C-7 position of the flavonoid, the compound is bitter. Naringin is slightly water soluble component found in the fruit membrane and albedo and could be extracted in to fruit juices (Fisher and Wheaton, 1976). The taste threshold level of naringin in citrus juice issued by the State of Florida, Department of citrus (1982) was around 600 ppm.

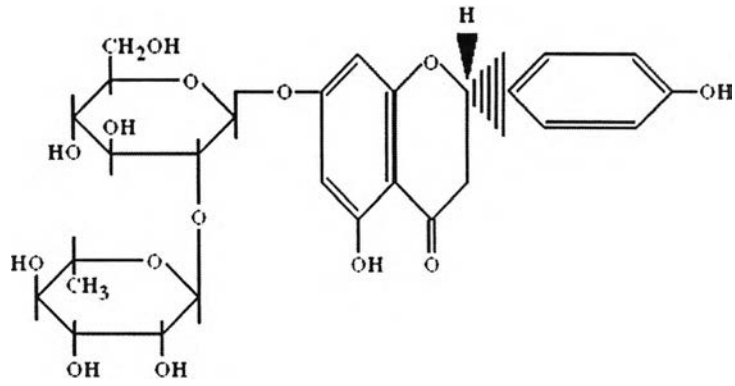


Figure2.2 Chemical structure of naringin (Mozaffar, Miranda and Saxena, 2000)

LIMONIN IN CITRUS FRUITS

Limonin ($C_{26}H_{30}O_8$) is a highly oxygenated tetracyclic triterpene dilactone. The molecular weight is approximately 470 and a volume of approximately 402 cubic angstorms. The melting point of limonin is 298^{0}C and the size is 13.3 x 7.6 x 3.4 ^{o}A (Norman, 1989). Limonin includes an epoxidic, two lactone rings, a five-membered ether ring, and a furan ring as shown in Figure 2.3. Limonin is only slightly soluble in water and alcohol. It is soluble in acetonitrile, chloroform and glacial acetic acid (Windholz, Budavari and Stroumtsos, 1976).

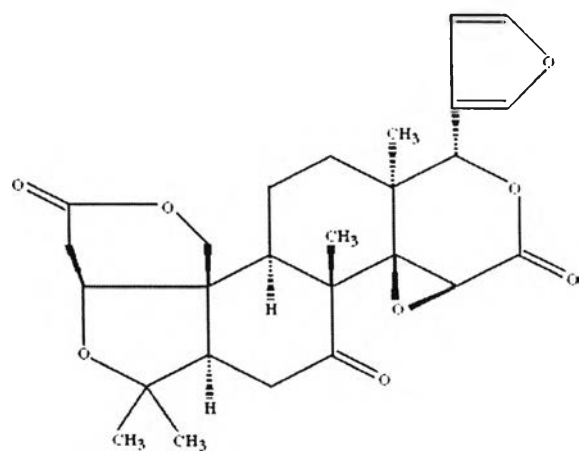


Figure 2.3 Chemical structure of limonin (Windholz *et al.*, 1976)

Limonin appears to be ubiquitous in all citrus species (Maier, Bennett and Hasegawa,1977). The observed phenomenon that a variety of citrus juice are not bitter-tasting when eaten fresh or freshly prepared juice, but turn quite bitter a few hours after extraction even kept at low temperature, is known as "delayed bitterness". In term of delayed bitterness in which a non-bitter precursor was converted to bitter compound due to the process of juice extraction or heat treatment and upon prolonged standing. The non-bitter precursor, limonoate-A-ring lactone (LARL), is found to be endogenously present in membrane sacs which is probably at a neutral to slightly alkali pH. When these sacs are ruptured during juice processing, the LARL encounters the net acidic pH of the juice, which limonin D-ring lactone hydrolase gradually catalyzes closure of the ring to form limonin (Kimball, 1991b). The conversion of LARL to limonin was illustrated in Figure 2.4.

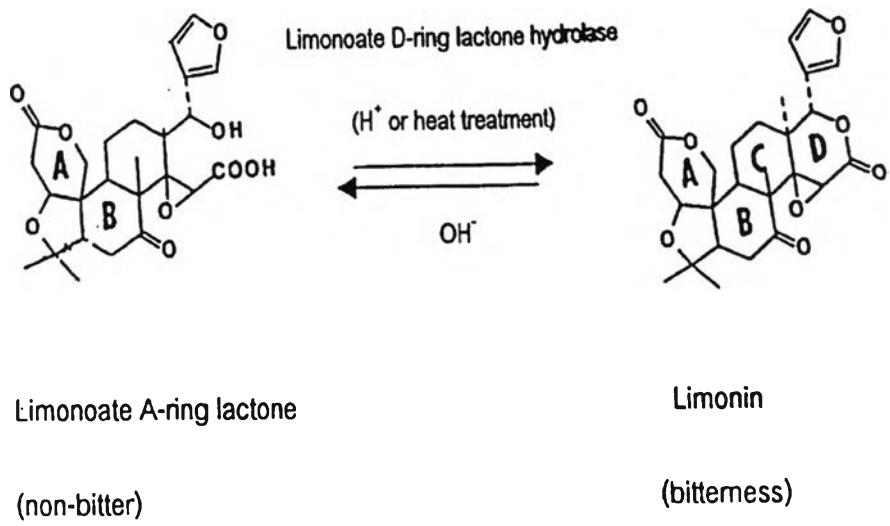


Figure 2.4 Conversion of LARL (a non-bitter precursor) to limonin (a bitter end product) (Puri *et al.*, 1996)

The catabolic pathway for the conversion of limonin to its nonbitter metabolites is shown in Figure 2.5. An isolate from soil, *Arthrobacter globiformis* and *Corynebacterium fascians* can produce limonoate dehydrogenase and limonol dehydrogenase which convert LARL and limonin to nonbitter 17-dehydroxy-limonoate A ring lactone and limonol respectively (Hasegawa *et al.*, 1972; Hasegawa *et al.*, 1983; Hasegawa *et al.*, 1985).

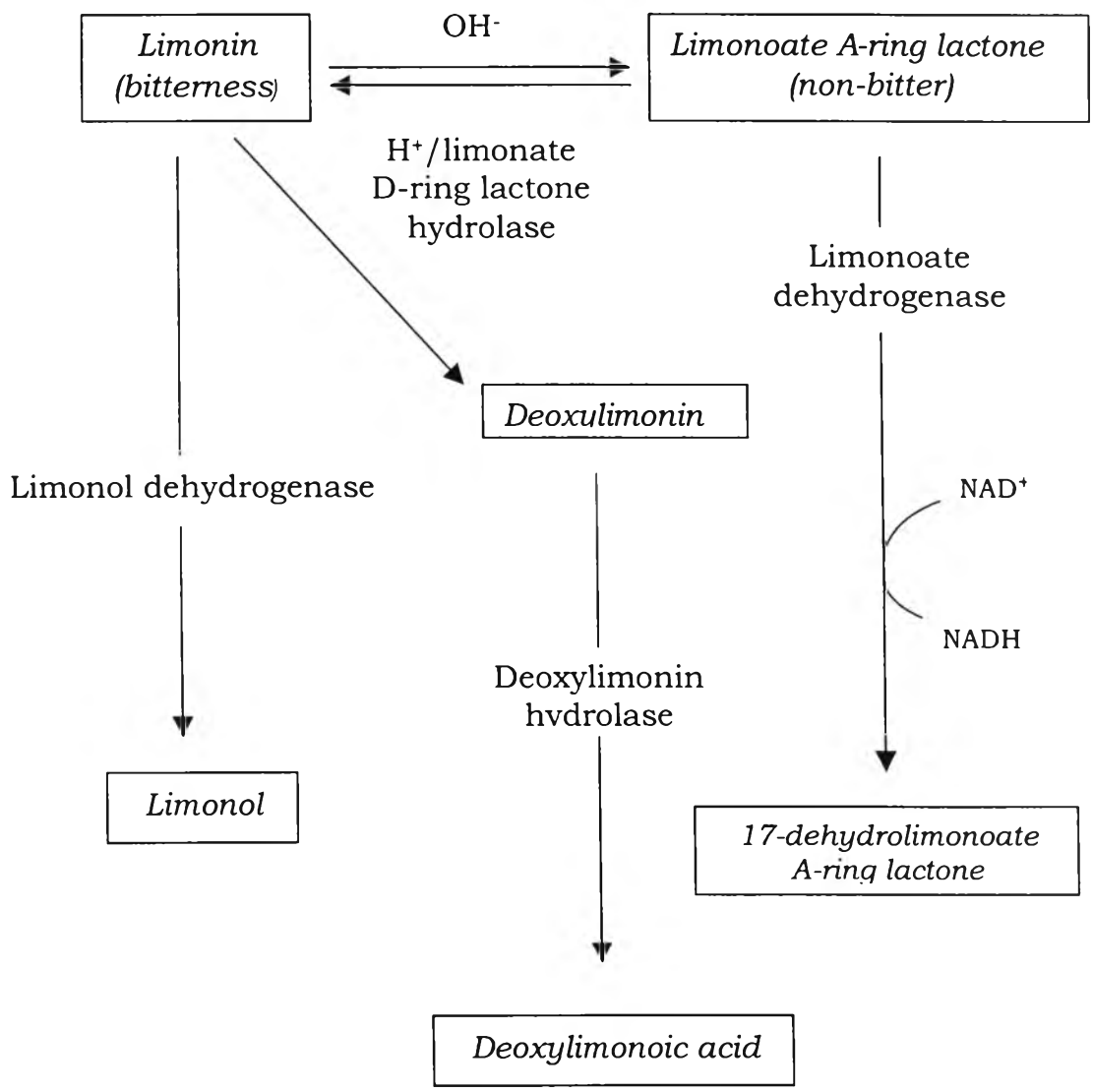


Figure 2.5 Catabolic pathways for the conversion of limonin to its nonbitter metabolites (Puri *et al.*, 1996)

ANALYSIS OF LIMONIN

A variety of methods have been proposed for the detection and quantification of bitter limonoids in citrus. Most methods were developed specifically for the quantification of citrus juice limonin. The early techniques included colorimetry, fluorometry and gas chromatography.

The colorimetric method (Wilson and Crutchfield, 1968) and fluorometric method (Fisher, 1973) involve the chemical derivative of the limonin. However, the reactions are not specific for limonin, and these techniques depend on isolating limonin from interfering compounds. Kruger and Colter (1972) developed a derivitization procedure to allow for the non-volatile limonin in citrus juices to be analyzed by gas chromatography. Since the detection system was nonspecific, any compound which overlapped the limonin peak would interfere.

TLC is the easiest and most economical technique of detecting limonoid. Unlike other methods, TLC can be used for a variety of tissue samples. TLC solvent systems are well developed and an appropriate system can be found for particular type of tissue sample with just a few trials. The disadvantage is the use of subjective visual comparison with standards for qualitation and quantitation (Tatum and Berry, 1973).

Most of the high-performance liquid chromatography (HPLC) methods developed are for the analysis of limonin in citrus juices. Rouseff and Fisher (1980) developed the normal-phase HPLC, this method is accurate but requires lengthy chloroform extraction. Several reverse-phase HPLC methods were also developed (FMC

Corporation, 1983; Shaw and Wilson, 1984; Shaw and Buslig, 1986; Van Beek and Blaakmeer, 1989). At present, this method is considered to be the best method for limonin determination. Either a C-8 or C-18 column was used for analysis with rapid solid phase extraction (SPE) to separate limonin from interfering components. Many solvent combinations used as a mobile phase were investigated.

A rapid method with enzyme-linked immunoassay has also been developed and made into a commercially available kit (Jourdan, Mansell and Oliver, 1984). The method has the advantage of short analyzing time and the equipment cost is less than HPLC. However, a collaborative study done by Widmer and Rouseff (1991) using the commercial kit indicated some problems with reproducibility of results.

In the food industry, high-performance liquid chromatography (HPLC) is the most widely used technique for the determination of limonin levels in citrus juices, since it is accurate and reliable.

TASTE THRESHOLD LEVELS OF LIMONIN

There are many reports concerning the threshold level of limonin in citrus juice. For example, Barmore *et al.* (1986) reported that the threshold point for sensory detection of limonin in distilled water was at 1 ppm. While Guadagni *et al.* (1973) stated a threshold of 6.5 ppm in orange juice at a pH of 3.8 suggesting that natural orange components are effective in neutralizing or masking limonin bitterness for a certain degree. In addition, only 17 % of panels could detect limonin in orange juice at 1 ppm

and 75 % of tasters at 5-6 ppm. The permissible limits of limonin and naringin in grapefruit juice issued by the State of Florida, Department of Citrus (1982) were 6 ppm and 600 ppm respectively.

FACTORS AFFECTING BITTERNESS IN CITRUS JUICE

The level of bitter compound in citrus fruit varies with choice of rootstock, climatic condition, degree of ripeness at harvesting time, horticulture practices. In addition, many processes in juice production such as extraction pasteurization and storage conditions also affect its quality.

DEBITTERING OF CITRUS JUICE

The negative impact created by bitter taste in citrus juice has made debittering a generally incorporated step in industrial juice processing technology. Several methods to reduce or control the bitter compounds in citrus juices below a threshold level for consumer acceptability had been developed through the years.

The methods are described below.

1. Controlling harvesting time and processing parameters

Both harvesting time and processing methods have been investigated for reducing the bitterness of citrus fruit, as well as methods for debittering harvested juice (Rangana, Govindarajan and Ramana, 1983). Because of higher level of limonin in early season fruits, only mid to late season fruits is advised to

be processed. Furthermore, the processing parameters are regulated, for example, the use of low pressures during juice extraction to prevent albedo disruption and the immediate separation of pulp from the juice after extraction.

2. Chemical methods

Pritchet (1957) used lengthy selective extraction techniques, and Swisher (1958), process involved adjustment of the juice pH both after extraction and immediately prior to consumption.

Maier, Brewster and Hsu (1973) used brief post-harvest fruit treatment with ethylene (20μg/ml) for 3 hrs to accelerate limonoid metabolism in navel orange, lemon, and grapefruit with a concomitant reduction in bitterness. Using carbon dioxide at pressures of 21 to 41 MPA at 30^0C to 60^0C for 1 hr resulted in average removal of 25% of the limonin from navel orange juice. By extending the treatment to 4 hrs, 60% of the limonin was removed (Kimball, 1987).

Although using chemical treatment seems to be effective in bitterness reduction, either through removal of nutrients, flavor, color, etc., the chemicals used in certain case cannot be disposed and might cause environmental problem (Puri *et al.*, 1996).

3. Enzyme techniques

3.1 Immobilized microbial mass for debittering

Limonoid catabolic pathways in microorgamisms have been studied. An isolate of *Anthrobacter globiformis* secreted intracellular

limonoate dehydrogenase which catalyzed reversible conversion of LARL to 17-dehydrolimonoate A-ring lactone in the presence of NAD as shown in Figure 2.5 (Hasegawa *et al.*, 1972).

The application of two other bacterial isolates, *Pseudomonas* 321-18 and *Bacterium* 342-152-1 was extended successfully to reduce limonin in grapefruit juice by Hasagawa *et al.* (1972) and Hasegawa and Kim (1975) respectively.

Vaks and Lifshitz (1981) purified *Acinetobacter* sp. from soil and entrapped the bacteria in a dialysis sac. This bacterium could convert limonin to deoxylimonic acid, a nonbitter compound as also shown in Figure 2.5.

Hasegawa *et al.* (1983) reported that packing *Pseudomonas* sp. and *Arthrobacter globiformis* II isolated from soil in column could reduce limonin in citrus juice by metabolizing the limonin to nonbitter metabolites named 17-dehydroxy-limonoate A ring lactone and limonol respectively. Another bacterium, *Corynebacterium fascians* was immobilized in acrylamide gel, which produce limonol dehydrogenase for the metabolism of limonin to limonol (Hasegawa and King, 1983; Hasegawa *et al*, 1985).

3.2 Immobilized enzyme for debittering

Immobilization of naringinase from *Aspergillus niger* and *Penicillium* sp. to hydrolyze naringin in citrus juice have been reported (Goldstein *et al.*, 1971; Ono *et al.*, 1978; Tsen, 1984; Tsen *et al*, 1989). The operational stability of naringinase from *Penicillium* sp. was better than that from *Aspergillus niger* for debittering process in citrus juice. The immobilized enzyme column could remove bitter compounds simultaneously without affecting to levels of total organic acids and pulp contents (Tsen and Yu, 1991).

However, techniques of immobilization are difficult to control optimum conditions for enzyme activity. Moreover, it is very difficult to separate the enzymes because they are dissolved in citrus juice and this may affect juice flavor. Hasegawa *et al* (1985) cautioned that the use of immobilized bacteria or enzymes for removing limonin and naringin was disqualified for scale-up purposes.

4. Adsorptive debittering

Chandler and Kefford (1968) successfully used polyamides to absorb significant quantities of limonin from Washington navel orange juice. Reduction of limonin in orange juice has also been achieved by adsorption on cellulose acetate gels (Chandler and Johnson, 1977). In 1988, Johnson and Chandler reported that the variety of adsorbents such as cellulose acetate, nylon-based

matrices, porous polymers, and ion exchangers could reduce bitterness and acidity in grapefruit juice.

Treatment of grapefruit juice with Florisil (activated magnesium silicate) in a batch mode significantly reduced limonin, naringin, narirutin, and total acid without reducing vitamin C, sugars or flavor. In fact, the flavor of the Florisil-treated juice was improved significantly (Barmore *et al.*, 1986).

Fernandez *et al* (1992) proposed that the application of ultrafiltration in tandem with adsorption effectively removed limonin from grapefruit juice at a pilot plant scale.

In batch operations, Puri (1984) used cross-linked divinylbenzene-styrene adsorbent resin to reduce limonin and naringin in grapefruit juice by 90 and 80 %, respectively. Laboratory studies on the effect of mean pore diameter, percent cross-linkage and specific area of polystyrene- divinylbenzene resins for the adsorption of limonin and naringin from grapefruit juice have also been reported (Manlan *et al.*, 1990).

Other debittering methods for citrus juice include the debittering with neutral XAD-16 resin, the polystyrene-divinylbenzene, and deacidifying with weak base anionic exchange (IRA-93) for sour orange juice in a series of four glass columns (30cm x 15 mm i.d.) were studied by Couter and Rouseff (1992). Limonin reduction by XAD-16 and IRA93 were about 100% and 24-30% respectively.

However, despite the large number of methods studied to remove bitterness from citrus juice, most of the previously reported methods have serious limitations. For example, carbon adsorbents are non-specific and consequently remove other components present in the juice (Kimball, 1987). The use of polyamide has a major drawback in that it results in the substantial loss of ascorbic acid from orange juice. Thus, this method is not commercially viable.

5. Debittering by cyclodextrin

Konno *et at.* (1981) effectively used soluble β-CD to reduce the bitter taste of limonin and naringin from grapefruit juice, Iyo orange, and *Citrus natsudaidai* due to its ability to form inclusion complexes with bitter substances.

This information led to utilization of insoluble β-CD polymer in a batch/continuous column mode to remove limonin and naringin from their aqueous solutions in citrus juices. But, it has no effect on the amount of total solid, acidity and ascorbic acid as well. The ability to regenerate of the β-CD polymer by extraction with ethanol enhanced its use for scale-up trials.

Shaw, Wilson and James (1984) then stated that the significant quantities of limonin, nomilin, and naringin from grapefruit juice as well as limonin and nomilin from navel orange juice were removed in a continuous flow fluid column. Flavor results indicated a preference for such debittered juice when compared with control juice. This led to a scale-up application of the β-CD polymer on a

pilot scale fluidized bed column, enhancing the possibilities of using such a system for commercial operations (Wagner *et al.*, 1988). Recently, Fontananova *et al.* (2003) immobilized O-octyloxycarbonyl-β-cyclodextrin onto polymeric membrane, which was able to form a complex with naringin in grapefruits.

CURRENT COMMERCIAL DEBITTERING PROCESS

The first commercial debittering unit was installed in a plant in Australia in the late 1970's, based on the research of Johnson and Chandler (1982). To begin with, cellulose acetate was packed in 12 long cylindrical column however there was a continuing problem of column plugging. In early 1980's, the several kinds of neutral and ion exchange resins used commercially. Using styrene-divinylbenzene and acrylonitrile-divinylbenzene are the most effective resins for reduction of naringin (60%) and limonin (75-90%) (Shaw, 1999a cited Maeda *et al.*, 1984).

In 1988 the first commercial debittering unit used in the United States was installed in California, The using a styrene-divinylbenzene polymer is still in use in 1999 (Shaw, 1999b cited Kimball and Norman, 1990). That unit consists of two stages, centrifugation of the juice and passage of the clarified juice through column packed with a hydrophilic styrene-divinylbenzene adsorbent. The second unit was designed to use ultrafiltration for pulp removal in conjunction with the use of neutral styrene-divinylbenzene as adsorbent. The schematic processes involving combined technology of ultrafiltration and adsorption are illustrated in Figures 2.6. In current debittering unit,

the styrene-divinylbenzene resin is popularly used and they remain to be a suitable adsorbent for debittering unit as long as they satisfied the U.S. Food and Drug Administration requirements for food contact use.

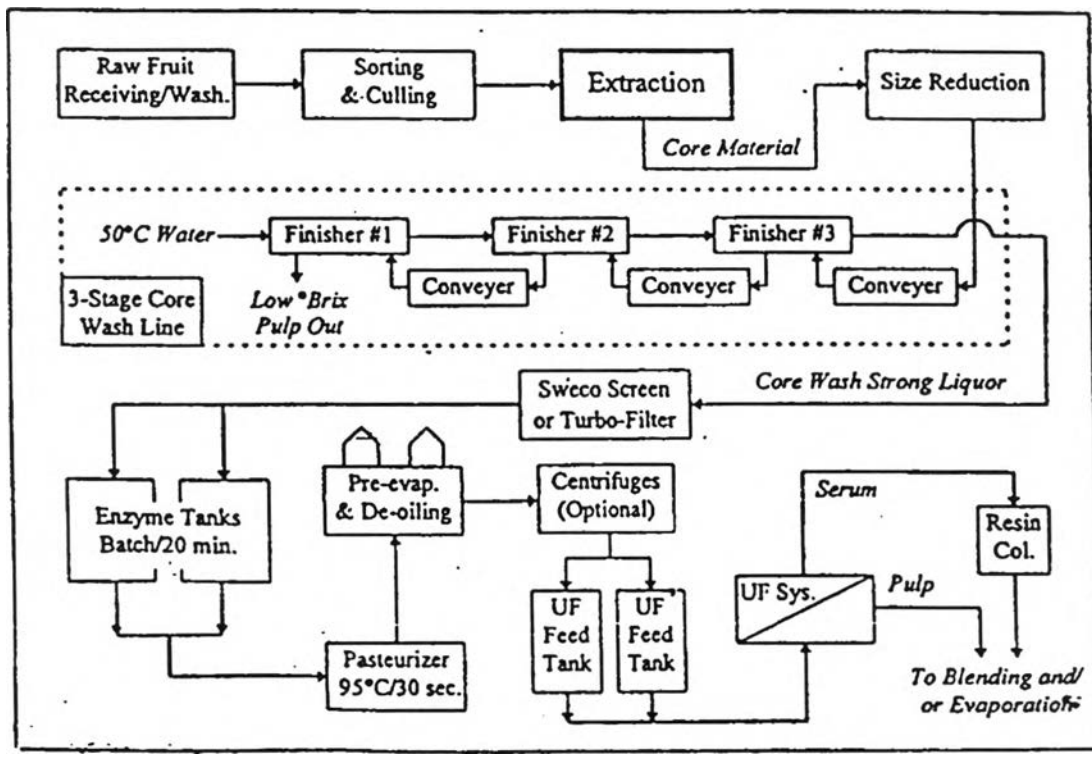


Figure 2.6 Schematic flow of commercial debittering process (Milnes, 1995)

CYCLODEXTRINS

Cyclodextrin (CDs) are cyclic oligosaccharides that are composed of α-1,4-linked glucopyranose subunits. They are produced from starch by enzyme degradation. Currently α, β and γ -cyclodextrin, which are built up from 6, 7 and 8 α-D-glucose units respectively as shown in Figure 2.7 and 2.8, are available in relatively large amounts. The physical properties of α, β and γ -CD are shown in Table 2.3. Primary hydroxy groups are located on the narrower rim of the cyclodextrin cavity, while the secondary hydroxy groups are on the wider rim of the cyclodextrin cavity. The numbers of the primary and secondary hydroxy groups are one and two, respectively, per a single glucose unit in cyclodextrin. Because of the presence of the hydrophilic hydroxy groups existing at the two ends of the cavity, cyclodextrins are soluble in water. On account of their relative hydrophobic interiors, CDs have the ability to form inclusion complex with a suitable wide range molecules by hydrophobic interactions, van der waals forces, and, in some cases, also hydrogen bridge bonds (Szejtli, 1998).

Cyclodextrins could form inclusion complexes with bitterness producing component such as naringin and limonin. Moreover, in term of the molecular study, NMR spectra have been recognized as direct methods of verification of the formation of inclusion complexes. The formation of inclusion complex between naringin and β-CD has been reported by Konno *et al.*, (1981). From NMR spectra, the chemical shifts of the β-CD protons definitively established that the phenyl ring of naringin not with the rhamnose moiety is positioned within the β-CD cavity. In case of limonin, examination of

^{1}H NMR spectra was impossible because of its low solubility. From solubility measurement and chemical structure of limonin, Konno and co-workers (1981) also assumed that β-CD could from a similar inclusion complex with limonin at the position of its furan ring.

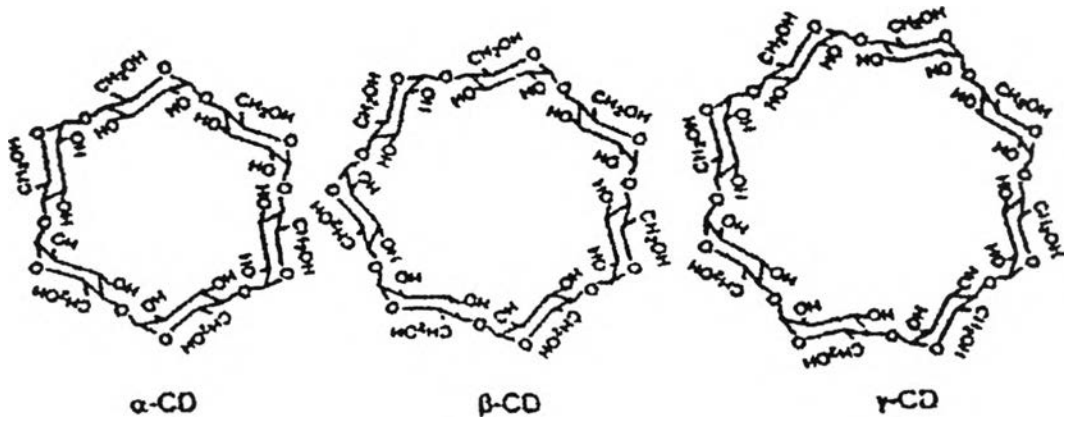


Figure 2.7 Chemical structure of α, β, and γ- CD (Szejtli, 1998)

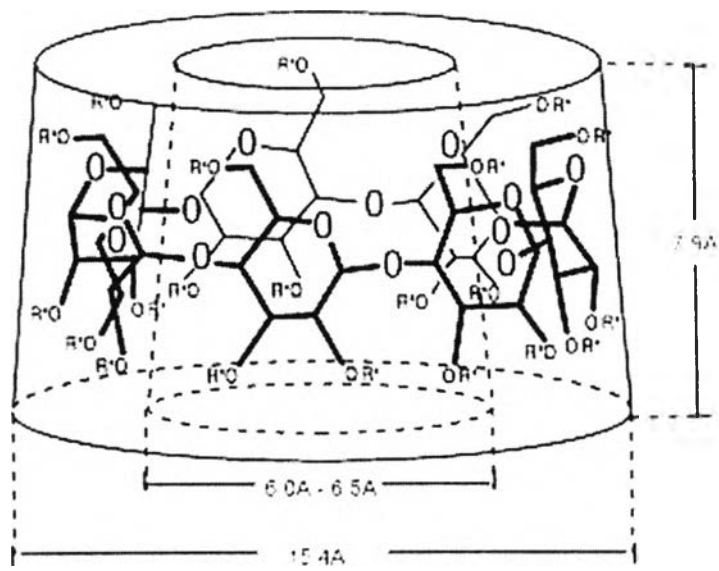


Figure 2.8 Structure of β-CD (Szejtli, 1998)

Table 2.3 Physical properties of α, β, and γ- CD (Szejtli, 1998)

Physical properties	α	β	γ
Molecular weight	972	1135	1297
Glucose monomers	6	7	8
Internal cavity diameter (angstroms)	5	6	8
Water solubility (g/100mL: 25 ^{0}C)	14.2	1.85	23.2
Surface tension (mN/m)	71	71	71
Melting range (^{0}C)	255-260	255-265	240-245
Water of crystallization	10.2	13-15	8-18
Water molecules in cavity	6	11	17

CYCLODEXTRINS AND INCLUSION COMPLEX

The cyclodextrin is "host molecule", and the driving force of the complex formation is the substitution of the high enthalpy molecules by an appropriate "guest molecule". One, two or three cyclodextrin molecules contain one or more entrapped guest molecules. Most frequently the host:guest ratio 1:1. This is the essence of molecular encapsulation. This is the simplest and most frequent case. However, host:guest in ratio 2:1, 1:2, 2:2, or even more complicated associations, and higher order equilibrium exist, almost always simultaneously. The inclusion complex formation of β-CD is shown in Figure 2.9. The circles represent water molecules. The outer surface of the β-CD is hydrated, but the water molecules in the ring cavity are in the energetically unfavorable position due to its non-polar surface cavity. The potential guest molecule repulses the water molecules. The result of the complex formation is that the non-polar the guest molecule penetrates into the β-CD cavity (Pagington, 1985).

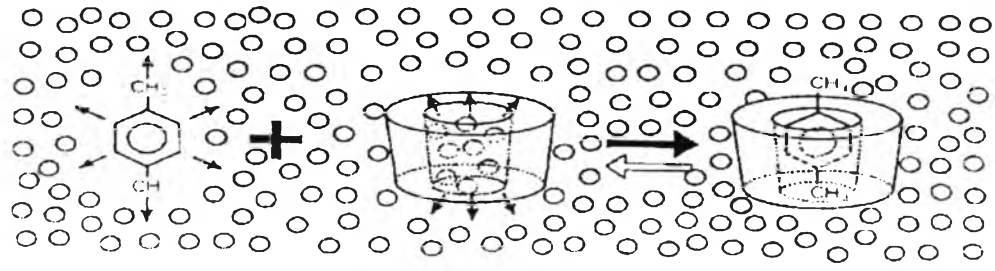


Figure 2.9 The inclusion complex formation of β-CD (Pagington, 1985)

APPLICATIONS OF CYCLODEXTRINS

Cyclodextrins are used in separation science because they have been shown to discriminate between positional isomers, functional groups, homologues and enantiomers. This property makes them a useful agent for a wide variety of separations. The utility of these water-soluble cyclic glucans in a variety of foods, cosmetics and toiletries, pharmaceuticals, agriculture, chemical technology and packaging is also well known (Table 2.4). Application of cyclodextrins in various sections could be represented by Figure 2.10.

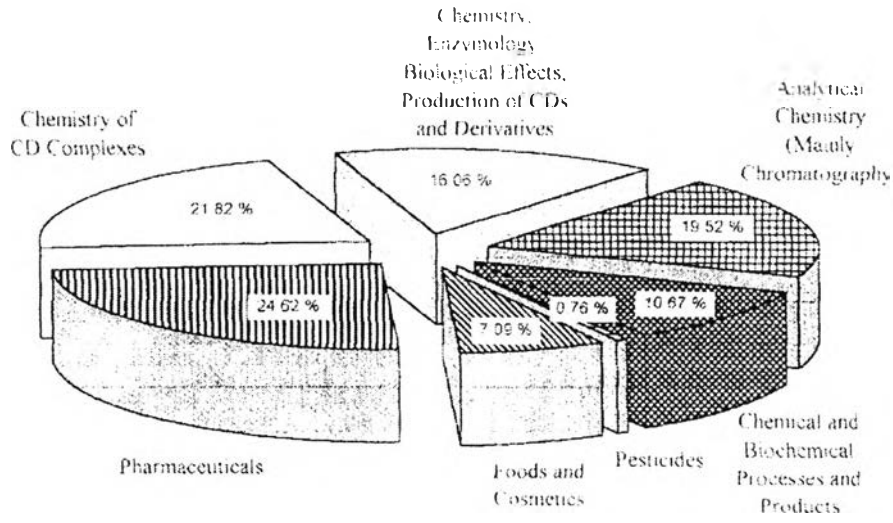


Figure 2.10 Distribution of the 1706 CD relevant abstract published in 1996 by Cyclodextrin News (Szeijtli, 1998)

Table 2.4 Industrial applications of cyclodextrin (Horokoshi, 1982; Bender, 1986; Schmid, 1989;Szejtli and Pagington, 1991)

Use	Guest compounds and end products
Foods	
1) Emulsification	Eggless mayonnaise, seasoning oil, Whipping cream, etc.
2) Increase of foaming power	Egg white (freeze-dry), hotcake-mix, Cake-mix, etc.
3) Stabilization of flavors and seasonings	Chewing gum flavor, biscuit flavor, powdered seasoning, instant noodles, seasoning paste, etc.
4) Taste masking	Meat paste
5) Reduction of hygroscopicity	Powder flavour products
6) Elimination of unpleasant tastes	Juice, milk, casein, ginseng, propyleneglycol
7) Elimination of cholesterol	Egg yolk, milk, butter
8) Reduction of odor	Mutton, fish, soybean
Cosmetics and toiletries	
1) Color masking and control	Fluorescein, bath agents
2) Stabilization of fragrances	Menthol
3) Stabilization	Chalcone, dihydrochalcone (tooth paste), Perfume
4) Preventing inflammation of skin	Skin lotion, sun block cream
5) Deodorant	Mouth wash, in refrigerator
6) Reduction irritation	Shampoo, cream, skin powder
7) Enhancement of attained concentration	Skin moisturizing lotion
8) Defoaming effect	laundry liquid

Table 2.4 Industrial applications of cyclodextrins (continued)

Use	Guest compounds and end products
Pharmaceuticals	
1) Increase of solubility	Prostaglandin, Phenobarbital, chloromphenicol
2) Taste making	Prostaglandin
3) Powdering (nonvolatile)	Nitroglycerin, clofibrate
4) Stabilization (UV, thermal)	Prostaglandin, vitamins
5) Decrease of irritation	Cu-alcanolamine complex
6) Enhancement bioavailability	Barbiturate, flufenamic acid, digoxin
7) Reduction of systemic toxicity	2-amino, 4-methyl-phosphonobutyric acid
Agriculture	
1) Stabilization of volatility	Tobacco aroma
2) Stabilization of nutrient	Animal-feed
3) Improvement of palatability	Bone-powder, microbial cell-mass
Chemical technology	
Catalyzation for reaction	Products of hydrolysis, substitution, Diels-Alder reaction, stereospecific reaction, etc
Plastics	
Stabilization of colors and flavors	Colors, flavors
Others	Adhesives

β-CYCLODEXTRIN SAFETY STATUS

It has taken many years to dispel the notion of β-cyclodextrin toxicity created by some earlier work (1956) using adulterated material. Major pharmaceutical companies are routinely approaching the FDA to get approval for the use of derivatives of β-cyclodextrin and food companies have confirmed the GRAS status of the natural CD's as food additives. Recently, used of β-CD as a food additive is approved in United States by Generally Recognized as Safe (GRAS) in many food categories with different limitations, for use as a flavor carrier or protectant as described in the table 2.5 below.

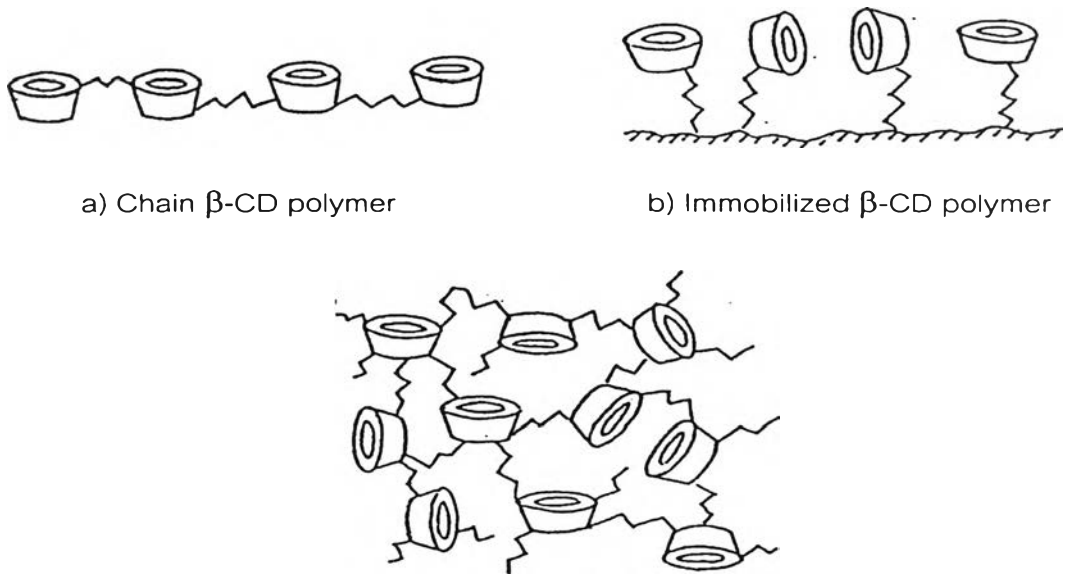
Table 2.5 Limitations of using β-CD as a food additive

Food Category	Maximum Level of Use
Baked goods prepared from dry mixes Breakfast cereal Chewing gum Compressed candies	2 percent
Gelatins and puddings Flavored coffee and tea Processed cheese products Dry mix for beverages	1 percent
Flavored savory snacks and crackers	0.5 percent
Dry mixes for soups	0.2 percent

Available from: http://www.cyclodex.com/about.asp?page_id=8&n=8

β-CYCLODEXTRIN EPICHLOROHYDRIN POLYMER (β-CD POLYMER)

The β-cyclodextrin epichlorohydrin polymers (β-CD polymer) have a large number of potential applications such as analytical chemistry, thermochemistry, catalysis, wastewater treatment, pharmaceutical and food industries (Szeijtli, 1998). The usefulness of these polymers is mainly in their ability to form an inclusion with hydrophilic guests. In food industries, β-CD polymers are used in food formulations for flavor protection or flavor delivery by means of encapsulation technology. They are used as process aids for example, to remove cholesterol from products such as milk, butter and eggs. The use of β-CD polymer in food industries is also interesting because it is natural material which can form inclusion complex with suitable guest molecules. In addition, in form of insoluble materials, the β-CD polymer is easily removed from the food processes. Figure 2.11 shows the structure of many kinds of β-CD polymer.



a) Chain β-CD polymer

b) Immobilized β-CD polymer

c) Network β-CD polymer

Figure 2.11 The predicted structure of many kinds of β-CD polymer (Szejtli and Pagington, 1987)

β-CD polymer is prepared by cross-linking β-CD with epichlorohydrin (Renard *et al.*, 1997). Reacting one or more of 21 hydroxyl groups with epichlorohydrin can polymerize the β-CD generally. This cross-linking agent, which cointains two reactive functional groups can react with β-CD molecules in cross- linking step and/ or itself in polymerization step. Therefore, the polymeric form can increase the stability and complication. All the possible reactions are presented in Figure 2.12. Hydroxyl groups can react with one reactive group of the bifunctional agent (Reaction 1). The side chain obtained can further react in two different ways: the epoxide ring can react with another hydroxyl group of a second β-CD molecule (Reaction 2), resulting in a glyceryl bridge connecting two CD cavities or the epoxyde ring is hydrolysed (Reaction 3). Moreover, epichlorohydrin has the capability to react on itself (Reaction 4) to form homopolymers of epichlorohydrin. Consequently, the glyceryl bridges and the glycerol tails can have different lengths.

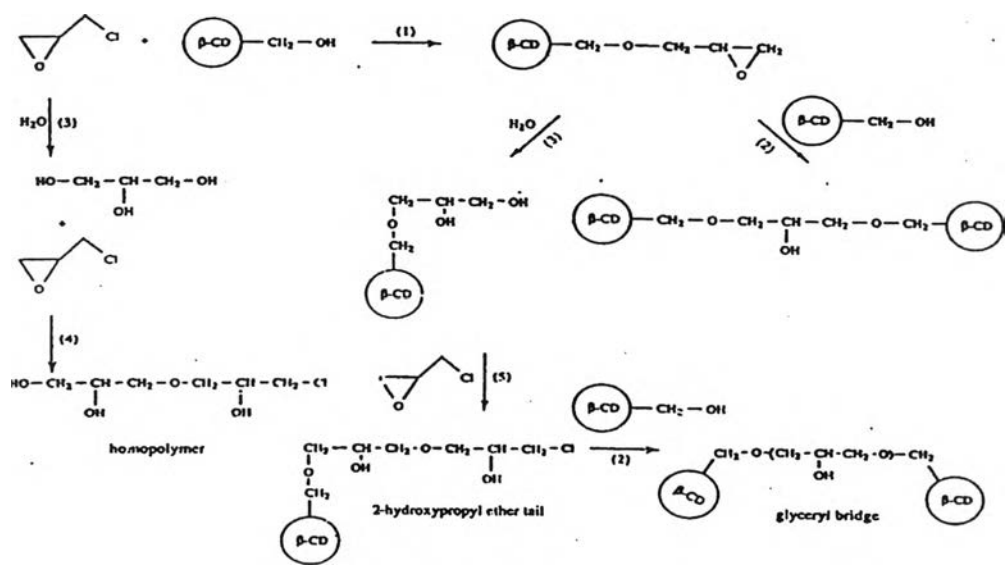


Figure 2.12 Reaction scheme of the polycondensation of β-CD with epichlorohydrin (Renard *et al.*, 1997)

FLUIDIZATION

Fluidization is the operation be which fine solids are transformed in to a fluidlike state through contaction with a gas or liquid. This method of contacting has a number of unusual characteristics, and fluidization application in concerned with efforts to take advantage of this behavior and put it in good use (Kunii and Levenspiel, 1969).

Phenomenon of fluidization

A fluidized bed is considered a layer of solid particles, displayed in a vertical column, the bottom of which is closely by a distributor that supports the solids. If a rising fluid flow is established through the bed of particles, different phenomena as shown in Figure 2.13 may happen:

1. For low fluid flows, the bed of particles remains fixed. This is a fixed bed.

2. With an increment of flow rate, particles move apart and a few are seen to vibrate and move about in restricted regions. This is the expanded bed.

3. At a still higher velocity, a point is reached when the particles are all just suspended in the upward flowing gas or liquid. At this point the frictional force between a particle and fluid counterbalances the weight of the particle, the vertical component of the compressive force between adjacent particles disappears, and the pressure drop through any section of the bed about equals the weight of fluid and particles in that section. The bed is considered to be just fluidized and is referred to as an incipiently fluidized bed or a bed at minimum fluidization velocity.

4. For an increment of flow rate above minimum fluidization velocity, both gas and liquid fluidized beds are considered to be dense phase fluidized beds. However, at a sufficiently high fluid flow rate the terminal velocity of the solids is reached, the upper surface of the bed disappears. It is the phenomena of pneumatic or hydraulic transport at higher fluid flow rate.

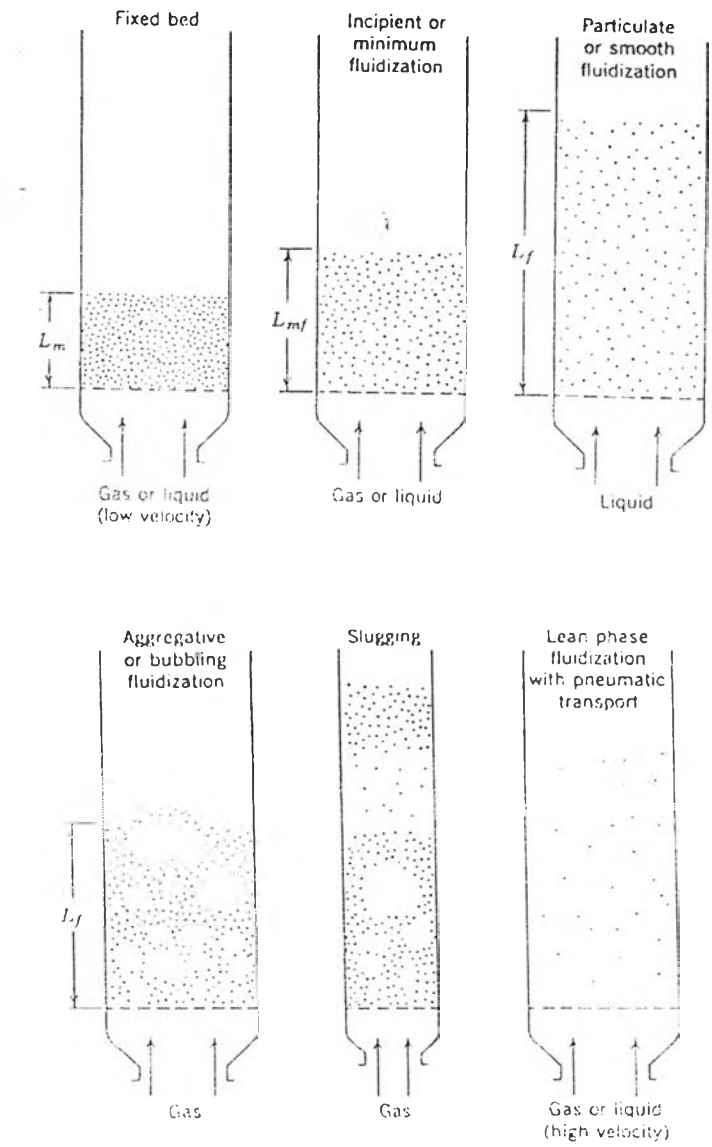


Figure 2.13 Flow pattern in fluidized bed

Pressure drop

The simplest fact about a fluidized system is that the drag exerted by the gas flow supports the weight of the bed. Many reports (Leva *et al.*, 1951; Lewis *et al.*, 1949; Toomey and Johnstone, 1952) have demonstrated that the pressure drop (ΔP) across the bed of area (A_b) is essentially equal to the weight per unit area, mg, of particles in the bed. Thus:

$$\Delta P\, A_b = m(\rho_s - \rho_f)g / \rho_s \qquad (2.1)$$

where ρ_s is the density of the solid (kg/m^3) , ρ_f is the density of fluid (kg/m^3), m is mass of particls in bed (kg) and g is acceleration due to gravity (m/s^2). The effective ΔP excludes the hydrostatic head pressure drop across the bed which can neglected in gas-fluidized systems and, indeed, gas densities are so low, except in ver high pressure systems, that the equation may be simplified to:

$$\Delta P\, A_b = mg \qquad (2.2)$$

Or, when no channeling occurs through the bed, this may be expressed in terms of the bed height (H) as:

$$\Delta P = (1-\varepsilon)\, g\, \rho_s\, H \qquad (2.3)$$

where ε is the bed voidage. These simple relationships imply that the pressure drop across the bed is independent of the gas velocity and this has been confirmed experimentally at moderate gas flow rate for uniformly fluidized systems.

MINIMUM FLUIDIZATION VELOCITY

The bed behaves as a static packed bed up to the point of fluidization although there will be some rearrangement of particles as the state of fluidization is approached. Minimum fluidization velocity have been estimated by relating the pressure drop through a packed bed when the bed is at the voidage for minimum fluidization to the weight of the bed per unit area (Wen and Yu 1966). The equation in term of minimum fluidization is:

$$\Delta P = (\rho_s - \rho_f)(1-\varepsilon_{mf}) L_{mf} g \qquad (2.4)$$

For, turbulent flow, the correlation is Ergun equation must be used for the pressure drop through packed bed. Geldart (1970) suggested combination of Kozeny-carman's and Burke-Plummer's correlation, as shown in Equation 2.5

$$\Delta P = \frac{150 (1-\varepsilon_{mf})^2 \mu U_{mf}}{\varepsilon_{mf}^3 d_p^2} + \frac{1.75 (1-\varepsilon_{mf}) \rho_f U_{mf}^2}{\varepsilon_{mf}^3 d_p} \qquad (2.5)$$

In the bed at onset of fluidization the voidage is larger than in a packed bed, and it actually corresponds to the loosest state of a packed bed of hardly any weight. Thus ε_{mf} can be estimate from random packing data, by pouring a known weight of particles very gently into measuring cylinder. Then:

$$\varepsilon_{mf} = 1 - \frac{m}{L_{mf} A_b \rho_s} \quad (2.6)$$

where m is weight of solid in bed and A_b = cross-sectional area of bed.

In case of U_{mf}, minimum fluidization velocity is found by combinding Equation 2.5 with Equation 2.6. Gledart (1970) recommended the following equations in designing a fluidize bed;

for small particles which Reynold number < 50, the equation is:

$$U_{mf} = \frac{688\, d_p^{1.82} (\rho_s - \rho_f)^{0.94}}{\mu^{0.88}} \quad (2.7)$$

and for large particles which Reynold number of fluid > 1000 the equation is:

$$U_{mf} = \frac{d_p (\rho_s - \rho_f) g \varepsilon_{mf}^3}{1.75 \rho_f} \quad (2.8)$$

FLUIDIZED BED AND FIXED BED COMPARISON

Despite the many interesting aspects of fluidization, experience so far indicated that a wholesale substitution of fixed beds by fluidized beds is by no means in sight. As with most processes and operations there are advantages and disadvantages on both sides (Richardson, 1971).

Advantages

1. Owing to the intense agitation in a well-fluidizing dense-phase bed, local temperatures and solids distributions are much more uniform than in fixed bed.

2. Since in a fluidized bed particle size is smaller than in the fixed bed, the resistance to diffusion through the particle is smaller in the fluidized bed.

3. Fluidization will permit the ready additions of solids to or the withdrawals of solids from the bed. This is an important advantage over the fixed bed, especially where rapid-activity losses are involved. This property of the fluidized system is responsible for the ease with which continuous operation is achieved.

4. In many instances fluidization will cause a smaller pressure drop than will fixed-bed operation.

5. Fluidization will eliminate catalyst pelleting, an important cost item in many catalytic processes.

6. The product can rapidly transport from the fluidized bed.

Disadvantages

1. The average flow of the solids in the single-bed fluidized reactor is concurrent. This has an unfavorable effect on the driving force. In order to approach countercurrent flow, a multicompartment reactor is required. This is much more expensive than a fixed bed.

2. From low height-diameter ratios there may result appreciable longitudinal mixing of fluid and solids in the fluidized reactor. This may lead to lower conversion rates and a reduction in selectivity.

3. During a fluidized operation, a catalyst may undergo attrition, or size reduction. Thus the fluidization properties of the material may become different and require adjustment of fluid rates.

4. In the fluidized reactor the fluid velocity must be closely coordinated with the properties of the solids so that adequate fluidization results. Thus the fluidized reactor is in this respect restricted, and the fixed bed offers a great degree of freedom and adjustment of space velocity.

5. In fluidized-operation equipment, erosion may be serious. Special and generally expensive designs may be required to eliminate or minimize wear in reactors and transfer lines.

7. Attrition and formation of fines leads to catalyst losses. These must be replaced in the fluidized unit. The cost involved may be appreciable.

ADSORPTION ISOTHERMS

The simplest theoretical model used to describe monolayer adsorption is the Langmuir isotherm (Langmuir, 1918). It assumes a uniform adsorbent surface with energetically identical sorption sites. The Langmuir equation is

$$q_s = \frac{K_L\ C_s}{1 + a_L C_s}$$

where q_s (mg/g) is the adsorbate concentration per unit of weight of adsorbent (solid phase), C_s (mg/L) is the concentration of adsorbate in solution (fluid phase) at equilibrium, and K_L (L/g) and a_L (L/mg) are the Langmuir constants. The Langmuir isotherm well describes the adsorption data of gases on solid surfaces, but it is less

accurate for the equilibria involving the solid-liquid interfaces where adsorption continues beyond a monolayer.

The Freundlich isotherm is an empirical equation for nonideal adsorptions (Freundlich, 1907). It describes the equilibrium conditions on heterogeneous surfaces and therefore does not assume the monolayer capacity. It is expressed by the formula below:

$$q_s = K_F C_s^{bF}$$

where q_s and C_s are as defined above and K_F (L/g) and b_F (dimensionless) are the empirical constants dependent on several environmental factors; K_F provides an indication of the adsorption capacity of the adsorbent, and b_F represents the adsorption intensity. A larger value for b_F indicates a larger change in effectiveness over different equilibrium concentrations; when b_F is > 1, the change in adsorbed concentration is greater than the change in the solute concentration.

FACTORS AFFECTING ADSORPTION IN FLUIDIZED COLUMN

It had been found that the serious problem of clogging in packed beds operation led by fine adsorbent could be solved by fluidized-solid contacting technique. There are several factors, which influence the mass transfer of adsorption in fluidized column, for example concentration, feed rate and Reynolds number of flufid. Besides, the surface area and particle size also affected its efficiency (Weber and Keinath, 1967; Ganho, 1974; Pol Sagetong, 1975).

THE OBJECTIVES OF THIS STUDY

This study is continuously from the previous work of Piriya Rodart (2001), the insoluble β-CD polymer was used to reduce limonin in Thai tangerine juice by batch and column process. In batch process, acceptable level of limonin was observed with 3 g β-CD polymer/100 ml juice for 30 min at room temperature. Greater limonin reduction (94%) was observed in the packed bed column process with 3 g β-CD polymer, the flow rate of 0.35 ml/min and room temperature. From preliminary information, the purpose of this further study is to investigate the commercial possibilities of using insoluble β-cyclodextrin polymer to debitter Thai tangerine *Citrus reticulata* Blanco juice in a laboratory-scale fluidized bed column. A large amount of tangerine juice, fast feed rate and simple process with low cost is aimed for small scale orange juice producers. Therefore, the objectives of this study are

1. To develop the method of determination of limonin from tangerine juice using HyperSEP C-18 cartridge and reverse phase HPLC.
2. To investigate the optimum condition for a scale-up debittering by fluidization process using β-CD polymer as an adsorbent.
3. To compare the debittering efficiency of β-CD polymer and regenerated β-CD polymer.
4. To compare the % limonin reduction efficiency of β-CD polymer, XAD-16 resin fluidized column and prepared β-CD polymer.
5. To evaluate quality of the debittered juice by β-CD polymer fluidized column.