CHAPTER V
DISCUSSION

The trend toward greater consumer demand for healthy foods and beverages will provide the citrus industry with a significant opportunity to improve profitability during the remainder of this decade. The production of tangerines or Som Khaew Wan in Thailand is well in excess of 600,000 tons/year (DOAE, 2003). Because of the excessive production, tangerines are processed to many products including jam, canned single strength juice and frozen concentrated juice. However, a major problem in the case of mass production is the formation of bitterness in citrus juice since it reduces the quality and commercial value of the product. Bitterness in citrus juice is caused by limonin and naringin (Rouseff and Fisher, 1980). There have been many attempts to eliminate or control levels of bitterness in citrus juice products. Among these, using of the β-CD and XAD- 16 resin are available for debittering process (Ribeiro, 2002; Manlan *et al.*, 1990; Konno *et al.*, 1981). Reports for debittering citrus juices with β-CD are quite limited because it was still present in the finished product and could result in rejection by some countries (Konno *et al.*, 1981). However, β-CD is allowed to be used as processing aids and is generally recognized as safe (GRAS). For more consumer acceptance, the insoluble β-CD polymer was prepared to be used for debittering because it could reduce the bitter compounds without significantly affect the

soluble solids, total acid, or ascorbic content of the juice and it could be removed by filtration (Wagner *et al.*, 1988).

The current study reports scale-up of β-CD polymer to remove limonin from Thai tangerine juice by fluidization process. There are two main parts of studies reported in this experiment. The first one is the development of the method for limonin extraction, determination and the optimization of β-CD polymer fluidized column debittering process, including effects of repetitive regeneration on effectiveness of the fluidized column and comparison of the use of XAD-16 resin and prepared β-CD polymer fluidized column for debittering. The second part is the analysis of tangerine juice composition comprising color, total soluble solids and vitamin C content before and after debittering process. An assessment of the production cost and potential of β-CD polymer for the scale-up application were also reported.

Naringin and limonin are two major bitter compounds generally found in citrus fruits. Naringin is a bitter flavonoid common in citrus species such as pomelo, grapefruit, sour orange. This flavonoid is an indigeneous component in the membrane and albedo of the fruit and contributes to the bitterness of fresh fruit and juice (Mozaffar *et al.*, 2000). Limonin, the other bitter compound, is found in all citrus species and formed from a non-bitter precursor (limonoate A-ring lactone) present in juice sacs when the juice is extracted and allowed to stand or are heated during processing (Kimball, 1991b).

The study of Nagy and co-workers (1977) revealed that naringin did not occur in some citrus species such as sweet orange (*Citrus sinensis*), lemon (*Citrus limon)* and tangerine *(Citrus reticulata)*. To confirm this finding, Piriya Rodart (2001) extracted naringin from Thai tangerine juice by Extra Sep C-18 column and determined naringin content by reverse phase HPLC. It has been reported that naringin was not detectable in Thai tangerine juice. Moreover, it could be stated that limonin is the only major bitter compound in Thai tangerine. The compound exists in the range of 1-4 ppm in fresh juice from fruits harvested during 8-12 months of age (Savitree Jungsakulrujirek, 1997). Therefore, the debittering process of Thai tangerine juice in this experiment was mainly the reduction of the limonin content.

Since the level limonin in bitter tangerine juice (Tipco Foods Thailand Public Co., Ltd.) was still low for debittering study, it was necessary to increase the level by heating the juice (Piriya Rodart, 2001). Therefore tangerine juice was preheated at 70 ^{0}C for 15 minutes to adequately convert the non-bitter precursor to limonin. 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 gradually catalyzes closure of the ring to form limonin (Kimball, 1991b). In addition, this conversion is accelerated by the action of an endogenous enzyme named

limonin D-ring lactone hydrolase which works well under heat treatment and in the acidic condition (Maier *et al.*, 1977).

There are several methods for quantitative determining limonin content in citrus juice. GC (gas chromatography), TLC (thin layer chromatography), RIA (radioimmunoassay), EIA (enzyme-linked-immunoadsorbent assay) and HPLC techniques were reported by Kruger and Colter (1972); Tatum and Berry (1973); Weiler and Mansell (1980); Jourdan *et al* (1984), and Rouseff and Fisher (1980) respectively. At present, reverse phase HPLC is considered to be the best method for limonin determination because analyses can be carried out with good precision. Shaw and Wilson (1984) recommended using solid phase extraction (SPE) to separate limonin from interfering components before limonin determination by HPLC. They also reported that the rapid simple the preliminary separation by SPE could be used on all samples to protect the column and guard column from possible contamination by solid juice particles. In addition, the State of Florida, Department of Citrus (1982) used rapid and simple HPLC method for routine analysis to determine limonin content in grapefruit juice processed in Florida.

In this study, HyperSEP C-18 cartridge (C-18 octadecyl silane chemically bond to< 5 μm microsilica packing) was used for limonin separation. The efficiency of SPE using HyperSEP C-18 cartridge can be explained in term of reverse phase adsorption chromatography (Shaw and Wilson, 1984). The limonin in sample juice was passed through the HyperSEP C-18 cartridge with gravity flow (~1.0 ml/min) and retained in the

cartridge before washed with water to remove sugar and collected limonin by acetonitrile. It was demonstrated here that water could not extract limonin. However, extraction of limonin took place when the initial 0.3 ml of acetonitrile was passed through the cartridge and limonin could be collected in 1.5 ml acetonitrile fraction (Figure 4.1). The acetonirile collected volume in this study is lower than the method reported by Piriya Rodart (2001) which was in 3 ml acetonitrile fraction. Therefore, the limonin content in this study was more concentrated.

In this experiment, the retention time of limonin peak was at 16 minutes. The limonin standard curve was established by using six standard concentrations at levels of 2.5 to 25 ppm in 5 ppm increments at 20 μl injection volume. The standard curve showed a reliable linear response (R^2 = 0.9983).

The % recovery of limonin by initial use of HyperSEP C-18 cartridge was around 84 % (at the started limonin concentration of 25 ppm) with very good precision (% C.V. of 2.77) (Figure 4.5). It can be seen that the % limonin recoveries decreased when the HyperSEP C-18 was reused (83.95±2.6%, 80.29±0.26%, 76.89±1.07%, 63.37±0.19% and 63.99±0.43% respectively). It might be because the HyperSEP C-18 was contaminated from the other hydrophobic substance. The limonin which tightly binding with octadecyl silane by hydrophobic interaction could not be eluted by the regeneration step so the HyperSEP C-18 could lost its limonin adsorption ability (Shaw and Wilson, 1984). Although, there was significant difference of % limonin recovery between each reused at the confidence level of 95%. The HyperSEP C18 cartridge was recommended

to be used 3 times (~80% limonin recovery) because the difference of % recovery in 3 times is still in acceptable level (less than 7%) when considered with the high cost of HyperSEP C-18 cartridge. Besides rapid limonin extraction, this separation technique by SPE using HyperSEP C-18 cartridge could be reused 3 times. Piriya Rodart (2001) recommended to use the Extra Sep C-18 only once for the best separation and reported that the limonin extraction was done at 0.35 ml/min of flow rate which is lower than the flow rate (gravity flow) of this experiment. However, she stated that the flow rate also affected the accuracy of this method resulted in lower accuracy a higher flow rate. In addition, the vacuum manifold (12 ports) which could adjust the flow rate was used to extracted limonin (Piriya Rodart, 2001). Nevertheless, the lower flow rate gave a similar result with using the gravity flow for extraction limonin in preliminary studies of this research. Because of equipment limitation, the flow rate that is lower than the gravity flow was not done. Therefore, the gravity flow was the highest flow rate for limonin extraction. The advantage of the method for limonin extraction in this experiment was more rapid than the 0.35 ml/min of flow rate that reported by Piriya Rodart (2001).

The sensitivity of the method for limonin determination was observed at the lower limit of 2.4 ppm (Table 4.1). The lower limit of the method in this study is almost equal to 2 ppm level reported by Shaw and Wilson (1984), on the 4.6 mm i.d. x 100 mm analytical C-18 column. That means the method used in this study is adequately sensitive. However, the condition during SPE extraction such as flow rate, composition of solvent or type of HPLC column may be different. In addition, the % recoveries of

HyperSEP C-18 cartridge at the concentration 2.4 ppm were average 83.69±2.20. The precision of the method was represent by % C.V. at 3.24.

For debittering process, the β-CD polymer was preferred over β-CD monomer because of its specific characteristics. Since β-CD monomer is slightly soluble and difficult to be separated from the juice and some monomers may still be present in the finished product which could result in rejection by the regulation of some countries (Konno *et al.*, 1981). On the other hand, β-CD polymer has larger size than the monomer and can be prepared as insoluble form, it can be easily filtered from the juice after debittering process. Besides, the adsorption ability of the β-CD polymer was more effective than monomer because of its porosity, particle size and surface area (Su and Yang, 1991).

The β-CD polymer in this study was obtained as a gift from Cerestar Inc., U.S.A. It is a β-CD crosslinked with epichlorohydrin. It is insoluble, bright yellow puffy bead that can swell in water. However, it needed to be activated by acetone, water and ethanol before applying to the debittering process. The ethanol wash gave a polymer that dried and handled easier than the last wash with water (Shaw *et al.*, 1984).

To scale up the debittering process, a fluidized bed was performed. Fluidized process was developed from the data of the batch and column process by Piriya Rodart (2001). In batch process, the highest limonin reduction was 81% at the condition of 5 g β-CD polymer/100 ml juice (5g%) for 60 min at 6°C. Considering the production cost and energy consumption cost, the appropriate batch processing with acceptable level

of limonin was observed with 3 g β-CD polymer/100 ml juice (3g%) for 30 min at room temperature. Moreover, she reported that the use of 3 g β-CD polymer in a packed bed column (12 cm x 1.2 cm i.d. glass column; 10 ml bed volume) provided the sample with greater debittering result (94% limonin reduction). The maximum volume of sample juice that could be debittered through the column while still maintain the limonin level to less than 6 ppm (limonin taste threshold level) was 240 ml. However, the flow rate (0.35 ml/min) for the column process was used to the requirement of resident time which equal with the batch process in order to compare the difference between the batch and column process. In addition, this flow rate (0.35 ml/min) may not yet optimized for the column process. Therefore, it was extremely low and impractical.

In this work, two parts of fluidized column study were reported. Firstly, the optimization of β-CD polymer fluidized column including effects of repetitive regeneration on effectiveness of the fluidized column were studied. Secondly, the use of XAD-16 resin and prepared β-CD polymer fluidized column for debittering were compared.

The first part of optimization of fluidized process involves the determination of two important variables in fluidized column design i.e. the size of fluidized column and minimum fluidization velocity of juice fluid. Leva's equation (Equation 2.7 Chapter II) was used to evaluate the size of fluidized column since it covers the diameter of β-CD polymer, density of β-CD polymer and tangerine juice, and juice viscosity which were found to be 0.015 in, 67.8 lbm/ft^3, 64.74 lbm/ft^3 and 1.2 centipoise respectively. The

sized of fluidized column was calculated (Appendix B), there are two sizes of fluidized column used in this work. The height of column was 50 cm, but diameter of fluidized column was varied as 5 and 3 cm i.d. which had 950 and 350 ml bed volume respectively.

The appropriate size of fluidized column for this experiment was chosen from the determination of minimum fluidization velocity. The expansion of bed increased when the velocity of fluid increased. With further increase in the upward velocity, the expansion continues and a stage will be reached where the drag forces exerted on the particles was sufficient to support the weight of the particle. In this stage, the fluid/particle system begins to behave like a fluid, this is a point of fluidization (Kunii and Levenspiel, 1969). Among these, β-CD polymer as a fluidized bed seems to be an efficient process because fluidization has many advantages such as the temperature and solid distribution were much more uniform than in fixed bed and has a large surface area for mass transfer (Leva, 1951). The minimum velocity is estimated by relating the pressure drop, the velocity at the intersectional point of fixed bed region line and fluidized bed region line is minimum fluidization velocity (Figure 3.4, Chapter III).

In the case of the 50 cm x 5 cm i.d. fluidized column, the fluidizing point could not be determined even when the velocity almost increased to 40 cm/min or 500 ml/min of flow rate (Figure 4.6A). This means that a large number of tangerine juice was required for debittering. Therefore, the 50 cm x 3 cm i.d. fluidized column was selected as the more appropriate fluidized column in this experiment. The minimum velocity

of fluidization of this column was about 13 cm^2/min or 90 ml/min in the flow rate (Figure 4.6B).

The use of β-CD polymer as an adsorbent in the debittering by fluidization process was studied. First of all, it was observed that limonin reduction varied with flow rate of clarified juice. The minimum fluidization flow rate of the 50 cm x 3 cm i.d. fluidized column was 90 ml/min.

The appropriate flow rate to reduce the limonin content in tangerine juice by fluidized β-CD polymer process was investigated. The flow rate of clarified juice was varied into three levels - at 75, 100 and 120 ml/min (Figure 4.7). At 75 ml/min, the fluidization may not be obtained completely since the level of residual limonin still fluctuated. At too high flow rate (120 ml/min), the contact time between limonin in the juice and the polymer decreased, thus reducing the debittering efficiency. The effective debittering process was observed at the 100 ml/min flow rate.

At the flow rate of 100 ml/min, room temperature (27°C), the optimum amount of β-CD polymer was explored by varying the amount of bed (15, 20 and 25 g β-CD polymer). Konno *et al.* (1981) reported that increasing concentration of β-CD resulted in a decreasing intensity of bitterness. All level of the tested bed showed rapid limonin reduction (~80%) in the first 25 ml of debittered juice (Figure 4.8B). However, the polymer gradually lost their capacities to remove limonin (Shaw and Wilson, 1983). In the adsorption of biological compounds, Freundlich isotherm was an empirical equation for nonideal adsorption. It described the adsorption data of liquid on solid surfaces

(Freundlich, 1907). This isotherm model has been widely used since it is a mathematically simple model adequate to describe (i) non-linear adsorption in a narrow range of solute concentration and also (ii) adsorption processes on surface adsorption sites that are energetically heterogeneous (Reibeiro, 2002). The typical adsorption graph in term of contact time on adsorption kinetics explained that the adsorption gradually increased until approach the steady state (Reibeiro, 2002). In earliest stage of this study, the exterior β-CD polymer could immediately adsorbed limonin in their hydrophobic cavities. When the cavities of these polymers were occupied, the limonin could be adsorbed by the interior β-CD polymer later on. The possibility of adsorption and adsorption rate might be lower. Considerable deviations from the typical adsorption graph were observed when the β-CD polymer was used because of the characteristics of β-CD polymer that was soft, puffy and porous bead like sponge. In addition, the structure of many kinds of β-CD polymer were chain, network and immobilized β-CD polymer (Figure 2.11, Chapter II). There was a several types of the orientation of adsorption.

From Figure 4.8A, it can be seen that the use of 25 g of β-CD polymer was the worst in both efficiency and maximum practical load and 20 g of β-CD polymer gave the same result as with 15 g of β-CD polymer. Considering the production cost, the use of 15 g polymer should be appropriate and amount of the acceptable debittered juice (limonin below 6 ppm) was about 900 ml for these columns (50-80 % reduction). In this study, increasing the amount of β-CD polymer did not increase the capacity and

efficiency of the process. It might be because the maximum complexation of limonin in the sample juice was already attained at this bed concentration and lower amount of β-CD polymer could be used.

Piriya Rodart (2001) reported that the use of 5 g β-CD polymer gave the highest limonin reduction (81%) in 100 ml tangerine juice. However, she decided to operate the batch debittering process with 3 g β-CD polymer (68% limonin reduction) because the final limonin in debittered in tangerine juice was still maintain under acceptable limonin level (< 5 ppm). In the reduction of limonin by β-CD polymer, not only limonin reduction efficiency was considered but also other factors such as production cost, processing time, nutrition loss during process, initial limonin concentration and limonin content in acceptable level. Determining the adequate β-CD polymer from Rodart (2001) experiment, the maximum volume of tangerine juice that could be debittered through 3 g β-CD polymer (1.25 g%, w/v) packed bed column and still maintain the limonin level below 6 ppm (llimonin taste threshold level) was 240 ml (24 times bed volume). In this experiment, 15 g β-CD polymer (1.67 g%, w/v) could reduce limonin in the maximum juice load (900 ml). It can be seen that the use of β-CD polymer in this study (1.67 g%, w/v) was higher than that reported by Piriya Rodart (2001) (1.25 g%, w/v). Therefore, the lower of amount of β-CD polymer in the further work was varied at 8, 11 and 15 g of β-CD polymer which was 0.89 g%, 1.25 g% and 1.67 g% respectively.

Since the researcher had limit amount of β-CD polymer, the regenerated β-CD polymer was used to optimize the appropriate amount of β-CD polymer for debittering

by fluidized column. It was observed that limonin reduction varied with amount of debittering agent (8, 11 and 15 g of regenerated β-CD polymer). From Figure 4.9, regenerated β-CD polymer was highly effective initially in removing limonin from tangerine juice. However, regenerated β-CD polymer lost its capacity to remove limonin later on. The use of 11 g of regenerated β-CD polymer was the most appropriate because it gave the same result with 15 g. Moreover, the lowest amount at 8 g of regenerated β-CD polymer gave the least desirable result in both efficiency and maximum practical load.

In conclusion, the optimum condition for fluidized debittering process (initial limonin~12 ppm) was; 50 cm x 3 cm i.d. fluidized column, 11 g of β-CD polymer, 100 ml/min of juice flow rate and room temperature (27 °C).

The β-CD polymer fluidized column was investigated for the practical maximum load. It was found that the maximum load for sample juice of the debittering by 15 g of β-CD polymer (1.67g%, w/v) was 900 ml. The limonin absorption capacity of the fluidized column was 0.35 mg limonin/g β-CD polymer which was similar with the result of Piriya Rodart (2001), the limonin absorption capacity of the packed bed column at the practical maximum load was 0.36 mg limonin/g β-CD polymer. This method gave around 50-80% limonin reduction.

A laboratory scale of fluidized process was reported by Shaw *et al.* (1984), the use of 1 g β-CD polymer/50 ml of juice, fluidized bed by bubbling nitrogen gas at 200 ml/min reduced the limonin in grapefruit juice and navel orange juice about 50%. Shaw

and Wilson (1985) proposed that the level of limonin were reduced at an average of 50% in clarified juice by treatment with 69 g β-CD polymer packed in a 30 cm x 3.2 cm i.d., at 15 ml/min of juice. Beside this, a pilot-scale fluidized bed column (76.2 cm x 7.6 cm i.d.) containing 200 g β-CD polymer, 160 ml/min of grapefruit juice flow rate reduced limonin level as concentrations range from 30-59% (Shaw and Buslig, 1986). In commercial debittering unit, more than one adsorption resin columns were installed to provide high productivity and to insure that the process could be carried on continuously while the other columns were regenerated.

In some previous works, Shaw and Wilson (1985) and Wagner *et al.* (1988) reported that the β-CD polymer would effectively be used for batch and fluidized column for 19 and 21 times respectively. Shaw *et al.* (1989) proposed that β-CD polymer retained their capacity for bitterness reduction for over 30 regeneration with 2% sodium hydroxide solution. Su and Yang (1991) reported that, the β-CD polymer could be regenerated 7 cycles without apparent loss in capacity. This is advantage for reducing the operating cost. However, for the examination of the efficiency of regenerated β-CD polymer in this experiment, the adsorption capacity of regeneration of β-CD polymer was decreased around 60 %. The practical maximum load for 15 g regenerated β-CD polymer fluidized column under the condition used was about 350 ml. The adsorption capacity of the column was 0.14 mg limonin/g regenerated β-CD polymer. It can be seen that the characteristic observed from scanning electron micrograph of the regenerated β-CD polymer bead revealed more ruptured surface. Therefore, the

polymer tremendously lost its capacity to remove limonin. It might be because of severely regeneration treatment. The physical nature of the β-CD polymer was changed by this treatment, probably due to agglomeration of the polymer beads (Shaw et al., 1989). In the regeneration step, Shaw and Wilson (1985) suggested that the use of magnetic stirring caused mechanical breakdown of some polymer beads. In addition, the small particles could pass through the screen and end up in the debittered juice.

The commercial adsorbent XAD-16, the neutral resin cross-linked polystyrene adsorbent was tested for fluidized column debittering process using the same condition of regenerated β-CD polymer. It was found that the limonin reduction obtained from the XAD-16 resin fluidized column was nearly complete. In addition, the limonin adsorption capacity was 1.58 mg limonin/g XAD-16. High efficiency of XAD-16 resin was due to abundant surface area and durability. Shaw *et al.* (1989) reported that using β-CD polymer and XAD-16 resin in pilot-scale fluidized bed column gave the limonin reduction range from 28-67% and 90-97% respectively.

In commercial debittering process, the establishment of XAD-16 debittering unit is very costly because it is patented. Moreover, the process for XAD-16 resin preparation was more complicated than that of the β-CD polymer (section 3.4.1) (Shaw *et al.*, (1989). On the other hand, the use of β-CD polymer as food processing aids was generally recognized as safe (GRAS) in most countries and the market price of this kind of polymer is gradually decreasing. In addition, the β-CD polymer could be prepared by cross-linking β-CD with epichlorohydrin in the laboratory. Therefore, β-CD polymer

has a good potential for being the absorbent for debittering process in citrus industry if further development both in equipment design and process are carried out.

Due to the high cost of commercial β-CD polymer the researcher decided to prepare the β-CD polymer by the method of Shaw *et al* (1984). The β-CD polymer was prepared with the molar ratio of epichlorohydrin:β-CD = 15:1. Su and Yang (1991) reported that using insoluble polymeric gel (epichlorohydrin:β-CD >15) gave higher capacity to form complex with naringin than using soluble polymeric gel (epichlorohydrin:β-CD <15). They describe that it might be because of the higher degree of crosslinking or the greater ability for interaction of the epichlorohydrin structure in an insoluble polymeric gel. And, they belief that the inclusion of naringin is due not only to the cavities in the β-CD structure itself but also to some form of interaction between naringin and the cross-linked ECH structure of the gel.

Scanning electron micrograph was used to evaluate the surface area of prepared β-CD polymer (Figure 4.15). The surface area and porosity of prepared β-CD polymer was not apparently and the product apparence was different from the commercial β-CD polymer. Shaw and Buslig (1986) stated that the failure of controlling β-CD polymer preparation could occurs very often because the reaction was difficult to control and the washing and activation treatment affected the cross-linked of β-CD polymer.

In case of epichlorohydrin contamination, the prepared β-CD polymer was softly washed with ethanol until the epichlorohydrin is removed completely. The final ethanol

wash was checked for residual epichlorohydrin by gas chromatography (Figure 4.16-4.18). It was observed that the third cycle of ethanol wash solution was no epichlorohydrin peak. In conclusion, the prepared β-CD polymer should be washed for three times to decrease offensive in taste when added to tangerine juice. Shaw *et al.* (1984) proposed that the final ethanol wash was checked for removal of epichlorohydrin before the polymer was added to juice to be used in sensory evaluation.

The prepared β-CD polymer fluidized column seemed to lost some ability to remove limonin. The adsorption capacity was very low (0.028mg limonin/g prepared β-CD polymer). In further preparation, to increase the effective of prepared β-CD polymer, the preparation and activation process should be done gently, considering stirring movement and washing technique.

In term of consumer acceptance, the evaluation of the debittering process was investigated. The fresh juice, clarified bitter juice and debittered juice by β-CD polymer, XAD- 16 resin and prepared β-CD polymer fluidized column was evaluated in color, total soluble solids and vitamin C content.

Color values (CIE *L*, *a*, *b*) of sample juice were measured in transmittance mode by a Minolta chromameter CR-300. From Table 4.4, it was found that there was no significant different in color values between clarified juice and debittered juice at the confidence level of 0.05. Thus, the debittering process did not cause any color values change on all conditioned tested. In addition, Piriya Rodart (2001) stated that after adding the pulp into the clarified debittered juice, the color of the juice was similar to

fresh juice by visual observation. Therefore, the process did not cause apparent change in the debittered juice.

Since 75-85% of the total soluble solid of tangerine juice is sugars, the sweetness of tangerine was referred to the total soluble solids (Ting and Attaway, 1971d cited Bartholomew and Scinclair, 1943). Total soluble solids as degree brix of sample juice were measured by a Hand refractometor MNL-1125. It was shown that there was no significant different of color values between each sample juice at the confidence level of 0.05 (Table 4.5). Likewise, total soluble solids of tangerine juice were not affected by the debittering process.

Analysis of vitamin C in clarified and debittered juice was carried out according to the titration procedure by AOAC method (AOAC, 1995). This method was applied to determine the reduction in vitamin C that is not suitable for highly colored juice. Because vitamin C loss can probably be attributed to juice handling during processing, it was immediately determined after finish debittering process (Lee and Chen, 1998). The result indicated that the vitamin C content in tangerine juice before and after debittering process were not significantly different ($P<0.05$) (Table 4.6). This implies that the quality of juice in considering of color, flavor and vitamin C remain nearly the same after the debittering process

As regards to the quality of debittered juice, there are a number of reports concerning the nutritional value of the juice in terms of both quantitatively and qualitatively. Shaw and Wilson (1983) and Shaw *et al* (1984) showed that both the

vitamin C content and total soluble solids of debittered juice were unchanged whereas the reduction of essential oil level was about 40 %. In case of sensory evaluations, there are a number of studies. Konno *et al* (1982); Shaw *et al* (1984); and Wagner *et al* (1988), reported that the flavor evaluations on debittered juice sample and their original samples showed a significant preference for the debittered juice at a confidence level of 95 % or greater.

The debittering cost of β-CD polymer column was estimated at the practical maximum load. The estimation cost of this process included the expense on tangerine, β-CD polymer for debittering column, energy consumption and chemicals for regeneration process was around 4,500 bahts/column (details in Table A5). The majority of total debittering cost was due to the price of β-CD polymer (~98%). The productivity of the β-CD polymer column was 6 L/hour.

For possibility in citrus industry, the fluidized column process can be developed by increasing the size of fluidized column and the flow rate to increase production rate of debittered juice or decrease contact time. The limonin was decreased to the point where limonin content in the fruit juice is just below 6 ppm. To reduce the operating cost, the regeneration technique and the β-CD polymer preparation must be improved.

There are some recommendations from researcher for the further study in term of process development as described below. For enhancing knowledge of the debittering of Thai tangerine juice by β-CD, additional studies on the following topics should be achieved.

1. Development of the column design and scale-up the process to pilot-scale.
2. Simultaneous installation of the adsorption column and regenerated column to provide high productivity and carry on continuously debittering process.
3. Improvement of the β-CD polymer regeneration in order to maintain the ability to adsorb bitter components.
4. Investigation of the method for the β-CD polymer preparation in order to overcome the highest efficiency for debittering.
5. Development of the production of β-CD and β-CD polymer using the local raw materials to reduce cost.
6. Sensory evaluation of the debittered juice is needed for evaluating consumer acceptance.