CHAPTER III
RESULTS

3.1 Determination of naringin in Thai tangerine juice by HPLC

Naringin and limonin were major bitter compounds generally found in citrus fruits but the naringin was not a major bitter compound in tangerine (Nagy *et al.*,1977). To confirm, the fresh Thai tangerine juice was passed through Extra Sep C-18 column to separate naringin and then determined for the naringin content by means of HPLC using the condition described in section 2.4.2 (Chapter II).

The % recovery and % C.V. represented the accuracy and precision of the method for naringin determination (Figure 19 and Table A 1). Using standard naringin, it can be seen that the accuracy was good (81% recovery) with the reasonable precision (%C.V.=6.29).

The HPLC chromatograms of standard naringin, naringin extract from fresh juice and spiked sample (standard naringin + naringin extract) were shown in Figures 20, 21, and 22 respectively. From the standard naringin chromatogram, it could be stated that, the retention time of the naringin peak was around 14 min. Figure 22 showed that naringin gave a distinct peak and was well differentiated from the rest in the condition used. When HPLC profile from the naringin extract was compared with that of the spiked sample, it was found that Thai tangerine juice did not contain the naringin peak (Figure 21)

Therefore, further work of this thesis will be concentrated on limonin, another major bitter compound in citrus.

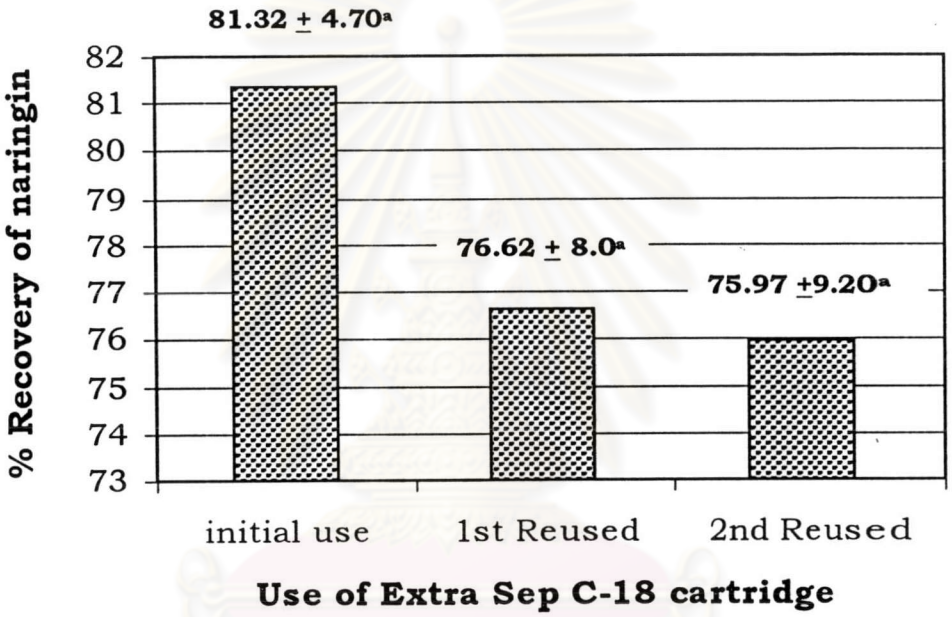


Figure 19 **%Recovery of naringin of initial and reused ExtraSep C-18 cartridge (Means with the same letter are not significantly different at P≤0.05, n=3)**

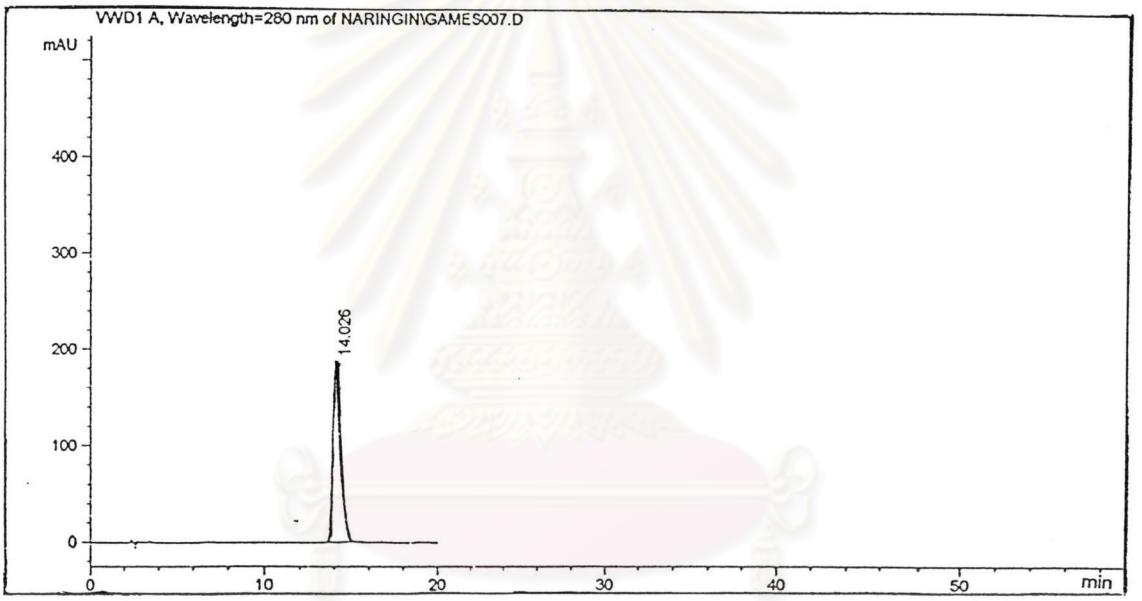


Figure 20 Chromatogram of standard naringin (20 µl sample injection containing 2 µg standard, C-18 column, acetonitrile:water (20:80 v/v), flow rate 1.0 ml/min, 280 nm)

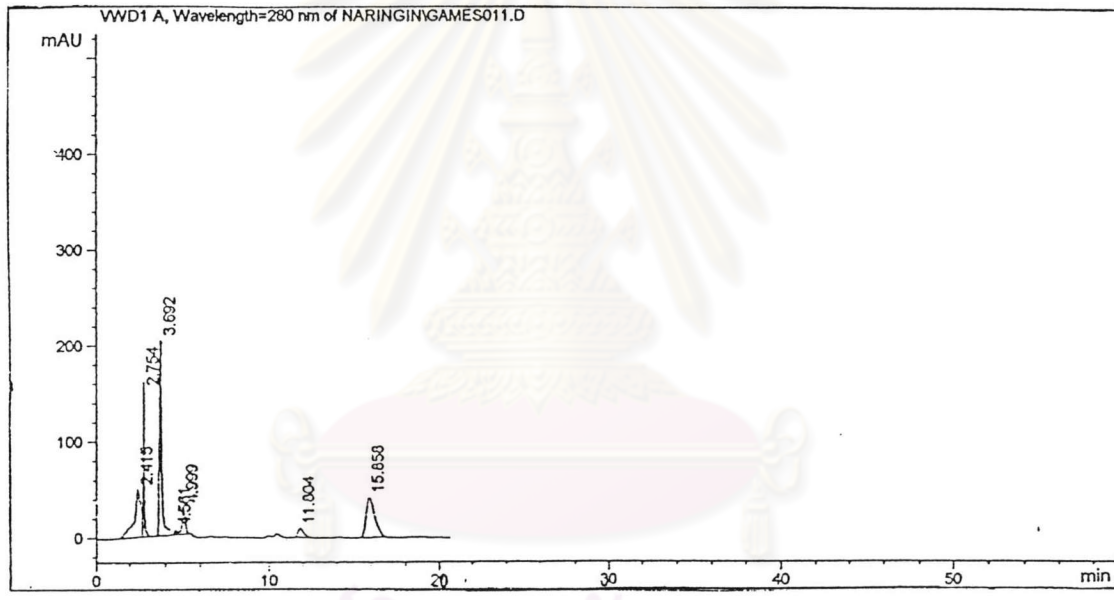


Figure 21 **Chromatogram of naringin extract (20 µl sample injection, C-18 column, acetonitrile:water (20:80 v/v), flow rate 1.0 ml/min, 280 nm)**

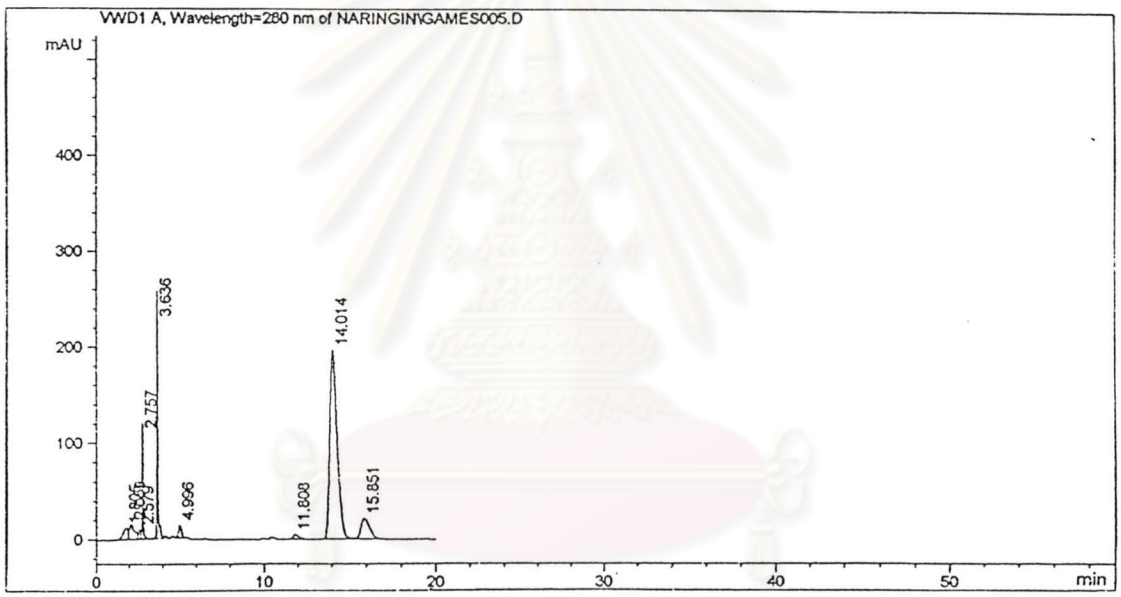


Figure 22 **Chromatogram of naringin sample spiked with standard naringin (20 µl sample injection containing 1 µg standard naringin, C-18 column, acetonitrile:water (20:80 v/v), flow rate 1.0 ml/min, 280 nm)**

3.2 Determination of limonin in Thai tangerine juice by HPLC

Two methods for limonin separation from tangerine juice, recommended by Shaw and Wilson (1984) and Mozaffar *et al.* (2000) as described in section of 2.4.2.2.1 and 2.4.2.2.2, Chapter II, were studied.

Limonin was extracted from tangerine juice and determined by HPLC using the condition described in section of 2.4.2 (Chapter II). The standard limonin chromatogram was shown in Figure 23. It was found that the retention time of limonin was around 22 min. Figures 24 and 25 show the chromatograms of limonin sample prepared by the method of Shaw and Wilson (1984) and Mozaffar *et al* (2000) respectively. It could be noted that the limonin sample prepared by using Extra Sep C-18 column gave much clearer and better resolution than the SE method.

The SPE method was further confirmed by comparing the chromatograms of limonin sample and the spiked limonin sample (Figures 26 and 27). The results strongly indicated that it was better to use Extra Sep C-18 column for limonin fractionation due to greater resolution of the peaks. Thereafter, extraction of limonin will be carried out by the SPE method.

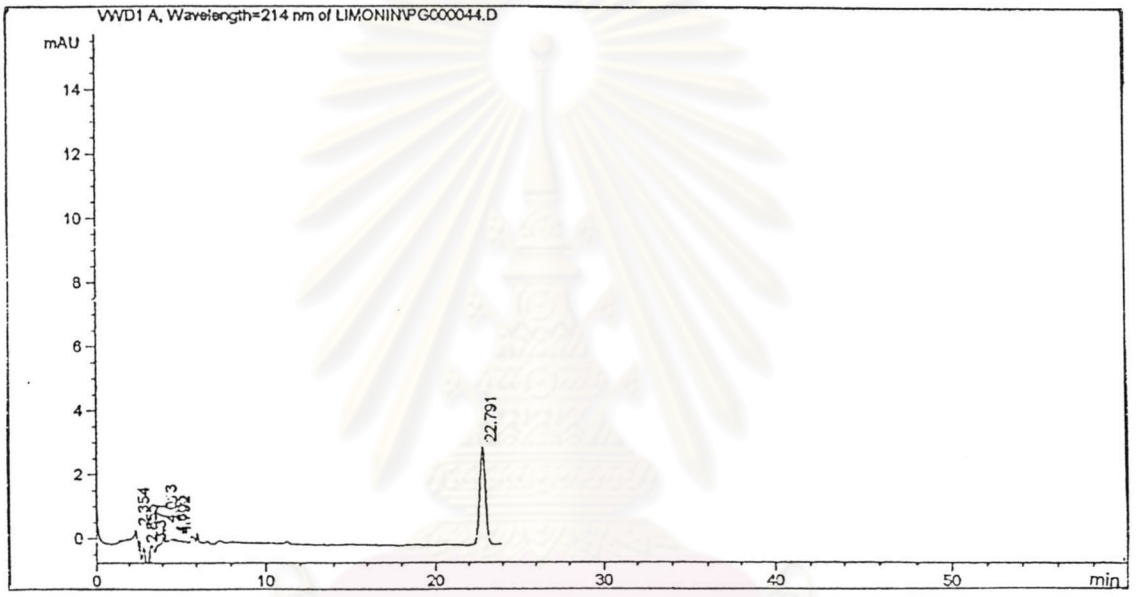


Figure 23 Chromatogram of standard limonin (20 µl sample injection containing 0.15 µg standard, C-18 column, acetonitrile:water (37:63 v/v), flow rate 1.0 ml/min, 214 nm)

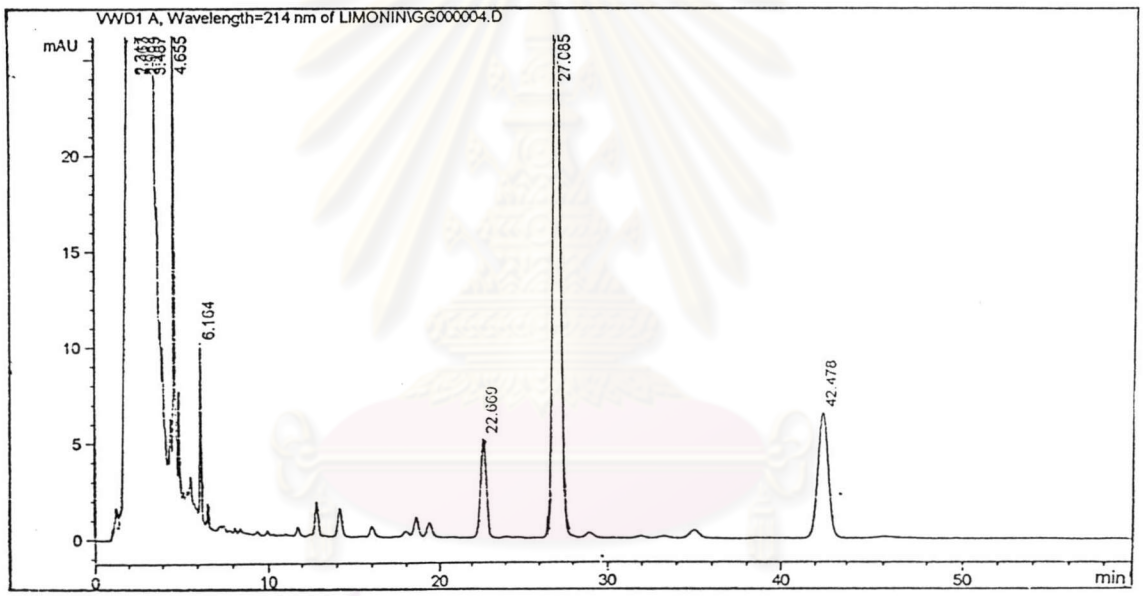


Figure 24 **Chromatogram of limonin sample extracted by SPE method (20 µl sample injection, C-18 column, acetonitrile:water (37:63 v/v), flow rate 1.0 ml/min, 214 nm)**

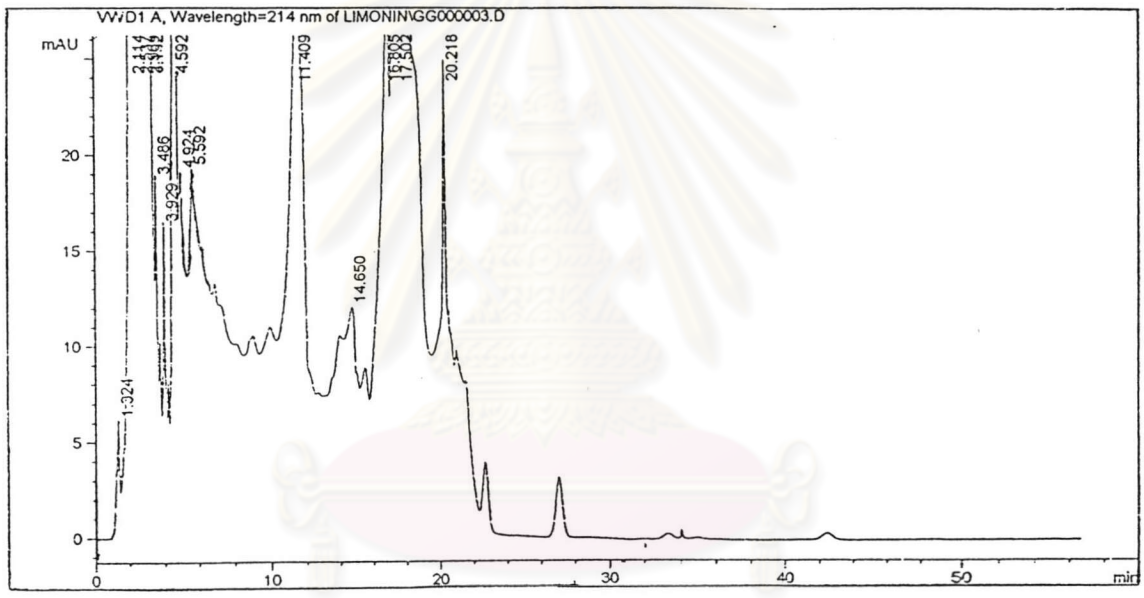


Figure 25 Chromatogram of limonin sample extracted by SE method (20 µl sample injection, C-18 column, acetonitrile:water (37:63 v/v), flow rate 1.0 ml/min, 214 nm)

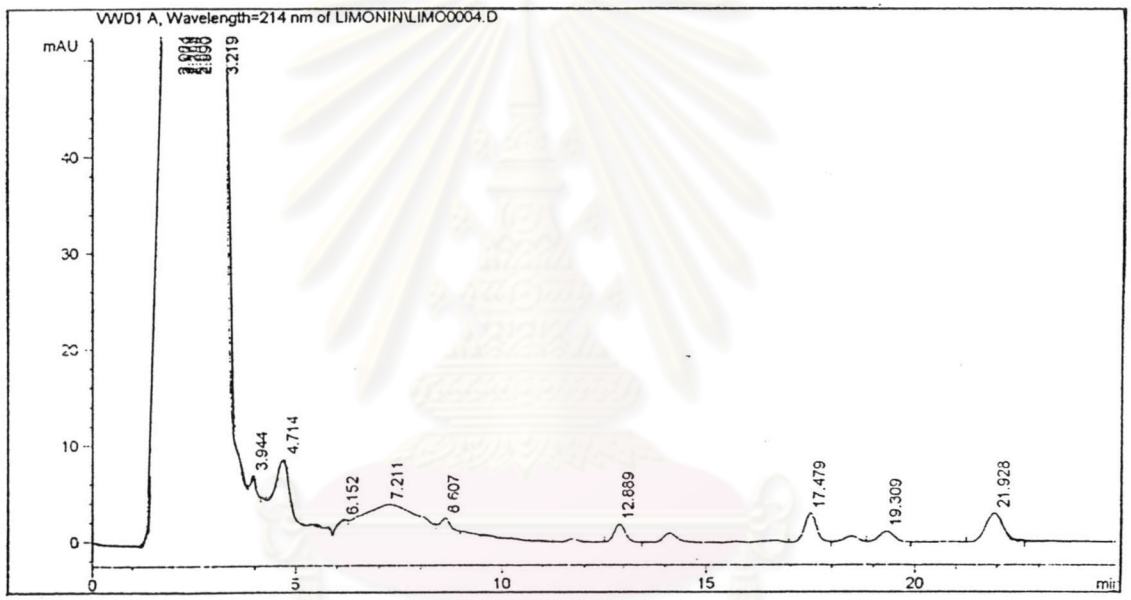


Figure 26 **Chromatogram of limonin sample prepared by SPE method (20 µl sample injection, C-18 column, acetonitrile:water (37:63 v/v), flow rate 1.0 ml/min, 214 nm)**

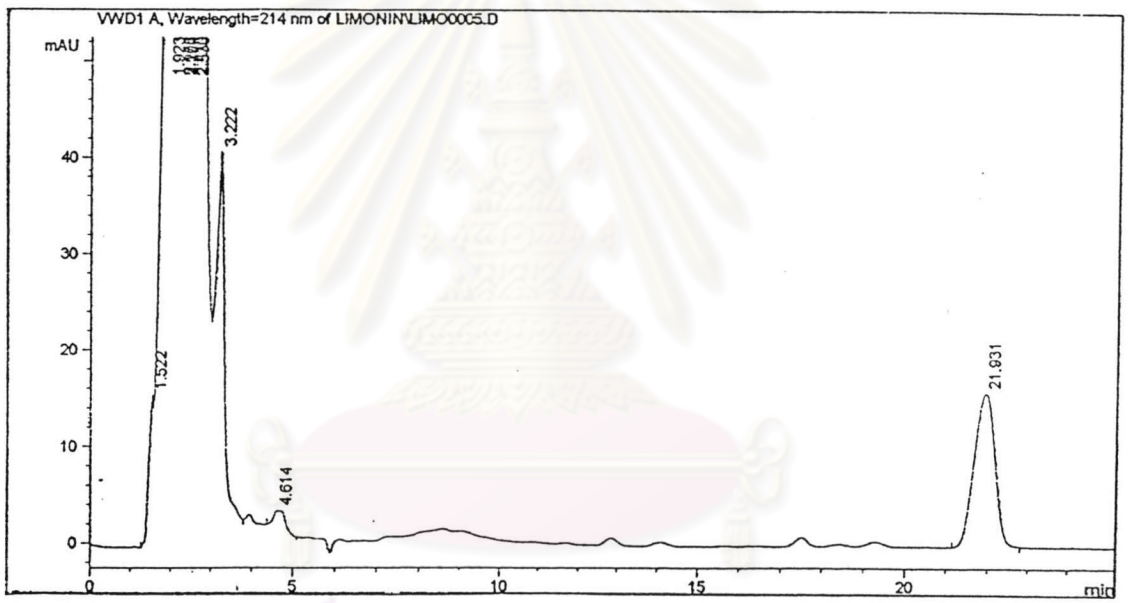


Figure 27 Chromatogram of spiked limonin sample prepared by SPE method (20 µl sample injection containing 1 µg standard limonin , C-18 column, acetonitrile:water (37:63 v/v), flow rate 1.0 ml/min, 214 nm)

3.3 Assessment of the efficiency of Extra Sep C-18 column for limonin separation

3.3.1 Efficiency of initial and reused Extra Sep C-18 column

To estimate the efficiency of Extra Sep C-18 column for limonin separation, 250 µl of 100 ppm standard limonin was loaded onto the Extra Sep C-18 column as the procedure described previously in section 2.5.1, Chapter II. Then the limonin content in samples eluted from the Extra Sep C-18 column was determined by HPLC as described in section 2.4.2.

Figure 28 shows the % recovery of limonin using Extra Sep C-18 column at each cycle. It was found that the accuracy of the method for limonin determination was 83%. However, the accuracy decreased gradually from 83 to 66 (~20% reduction) for third time of reuse and there was significant difference at the confidence level of 0.05 between each of reuse. Therefore, for effective determination, the Extra Sep C-18 column was recommended to be used only once (83% limonin recovery). The precision of the data was confirmed by % C.V. which was 0.94 as shown in Table A 1. In term of % C.V., it could be stated that the data in the experiment were reliable.

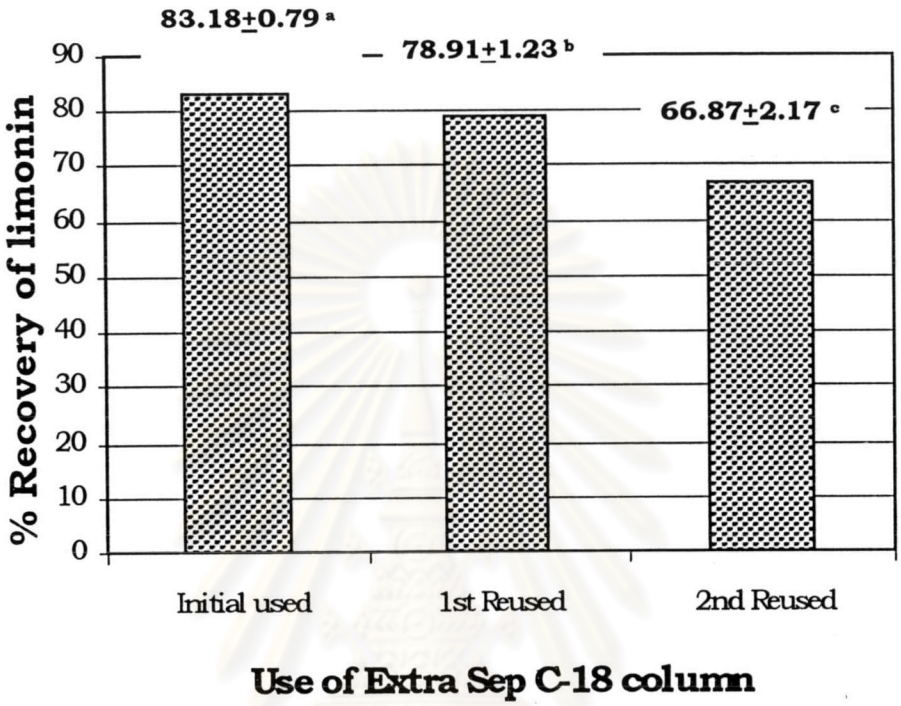


Figure 28 **% Recovery of limonin of initial and reused Extra Sep C-18 column (Means with the same letter are not significantly different at $P \leq 0.05$, n=3)**

3.3.2 Sensitivity of the method for limonin determination

The sensitivity of the technique using Extra Sep C-18 column and HPLC was also investigated (2.5.2).

Peak area of each concentration of standard limonin before and after passing through the Extra Sep C-18 column and % recoveries of limonin were shown in Table 3. It can be seen that the lowest concentration of standard limonin which could be detected by this method was at 0.3 ppm.

In addition, from the consideration of % recovery of limonin at each concentration in this experiment, lost of limonin occurred (~63% recovery) and they were lower than when the higher amount of limonin was loaded (83% recovery in Figure 28).

To evaluate the reliability of the method, five replications of 0.3 ppm standard limonin were assayed as usual. The result was shown in Table 4. The precision of the method was acceptable with error of $\pm$ 0.07 and %C.V. less than 10 even at 0.3 ppm.

Table 3 Limitation of limonin determination using Extra Sep C-18 column and HPLC

Standard limonin (ppm)	Peak area (mAUs)		% Recovery
	Before passing through Extra Sep C-18 column	After passing through Extra Sep C-18 column	
1	8.70	5.94	68.29
0.5	4.86	3.09	63.60
0.4	3.83	2.27	59.38
0.3	2.89	1.94	67.34
0.2	UD	UD	-
0.1	UD	UD	-

Note : UD = Undetectable

Table 4 Peak areas of 0.3 ppm standard limonin for 5 replications

Replications	Peak area (mAUs)	%Recovery
1	1.94	67.34
2	1.80	62.28
3	1.90	65.74
4	1.83	63.32
5	1.96	67.82
Average±SE	1.89±0.07	65.30±2.44
%C.V.	3.70	3.74

n=5

3.4 Optimization of juice preparation for study of limonin reduction

3.4.1 Limonin content in juice kept at different conditions

The limonin contents in fresh tangerine juice, pasteurized juice (70^{0}C for 15 min) and chilled juice (kept at 6 ^{0}C for 24 hrs) which are the general condition for consumption were explored. The three samples were derived from the same pool of tangerine extract. Limonin was separated by Extra Sep C-18 column and then analyzed by HPLC using the procedures described in 2.4.2.2.1 and 2.4.2 . The result was illustrated in Figure 29.

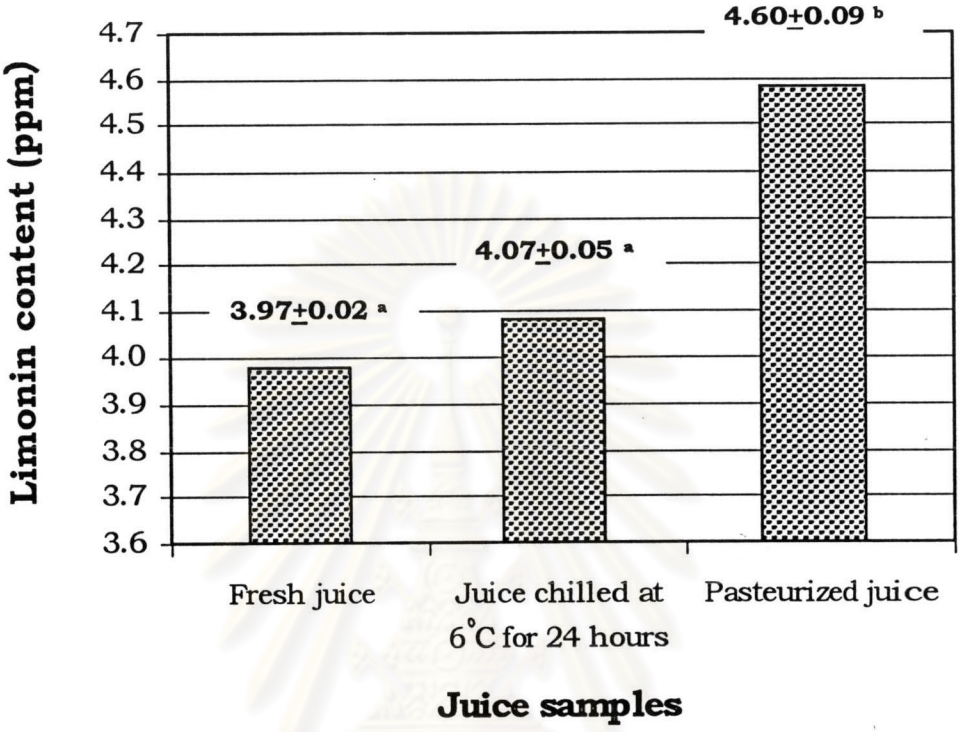


Figure 29 **Limonin content in sample juice at various conditions (Means with the same letter are not significantly different at $P \leq 0.05$, n=3)**

Figure 29 indicated that limonin content in fresh juice was low and slightly increased after 24 hours even when kept at low temperature. The limonin content in pasteurized juice was the highest (4.60±0.09 ppm) and significantly different from others at the confidence level of 0.05 with %C.V. 1.49 (Table A 3 and C 3). From the above data, it could be stated that temperature and storage time might affect limonin content in juice.

3.4.2 Effect of temperature on limonin content in tangerine juice

Since the limonin in untreated juice are too small for debittering study, juice were preheated to help increase the limonin content because there are several reports supported that increasing temperature will elevate limonin level (Shaw and Willson, 1983, 1988; Shaw *et al.*, 1984; Shaw and Buslig, 1986).

To find the suitable temperature for preheating juice sample, the juice was heated at 60, 70 and 80^{0}C for 15 minutes. Then limonin in each sample was separated by Extra Sep C-18 column as the method described in section 2.4.2.2.1 (Chapter II) and analyzed by HPLC.

Figure 30 shows the limonin content of each treatment with three replications. It was found that the limonin content slightly increased with temperature increased (7.90±0.03, 8.24±0.06 and 8.26±0.07 ppm respectively). However, there was no significant difference of limonin contents between 70 and 80 ^{0}C at the confidence level of 0.05. % C.V. of each treatment

was shown in Table A 3. Base on this experiment, the heating temperature for debittering study was selected at 70 ^{o}C.

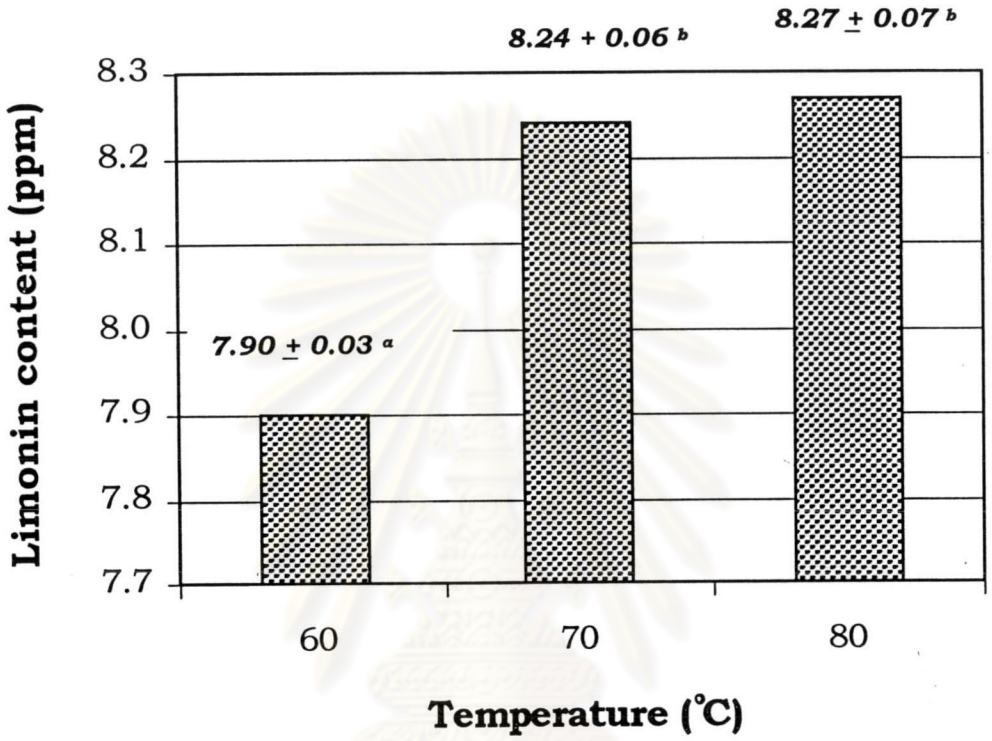


Figure 30 **Effect of heating temperature on limonin contents in Thai tangerine juice (Means with the same letter are not significantly different at $P \leq 0.05$, n=3)**

3.4.3 Effect of heating time on limonin level

To determine the suitable heating time for treating juice sample, the tangerine juice was heated at 70 ^{0}C for 5,10 and 15 min. Then the juice was suddenly cooled down at 2 ^{0}C and centrifuged at 10,000 rpm for 10 min. Limonin was then separated and determined.

From Table A 4, it was found that the limonin content in the tangerine juice gradually increased when the heating time was extended (2 - 6 % increase with %C.V. less than 2.5). There was no significant difference between limonin content heating for 10 and 15 minutes at the confidence level of 0.05. The effect of heating time on limonin content was displayed in Figure 31.

The preheating treatment of tangerine juice, based on the obtained results, would be at 70 ^{0}C for 10 minutes. The juice was used for following debittering processes.

In conclusion the optimum condition of juice preparation for debittering study was used as heating at 70 ^{0}C for 10 minutes.

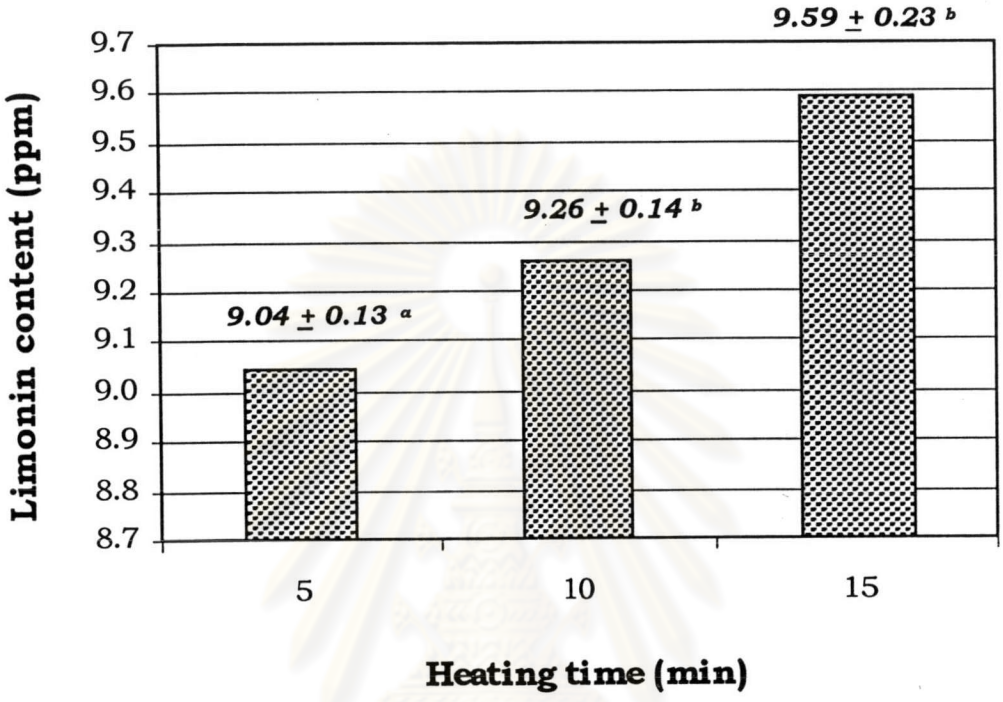


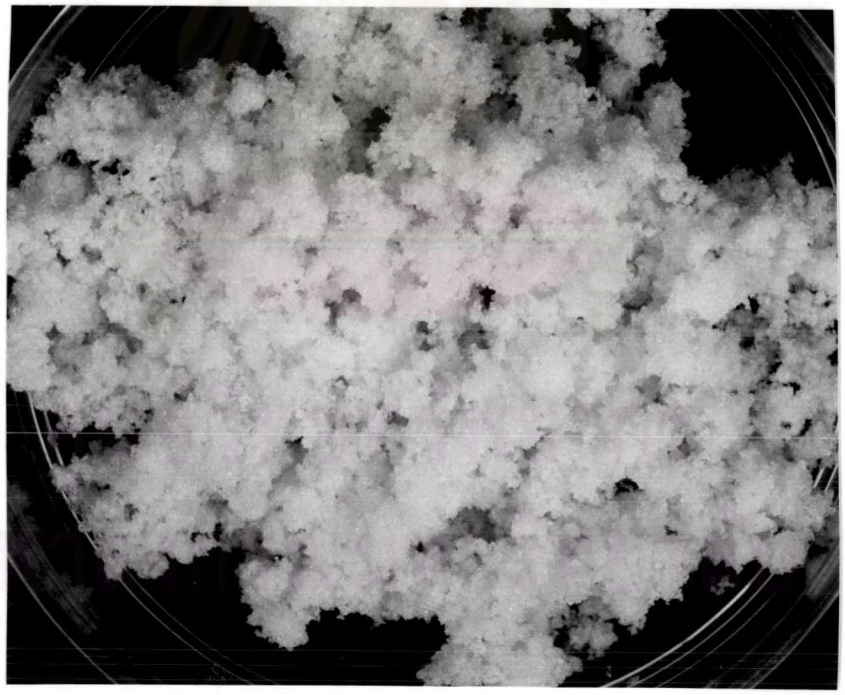
Figure 31 **Effect of heating time on limonin content (Means with the same letter are not significantly different at $P \leq 0.05$, n=3)**

3.5 Reduction of limonin by β-CD polymer

3.5.1 Preparation of β-CD polymer beads

Prior to use, the β–CD polymer beads were washed with acetone, water and ethanol as mentioned in 2.7.1. Figure 32 presents the color difference between pre-washed and washed β-CD polymer. It can be seen that, the fluffy polymer was cleaner, brighter and more uniform after washing with organic solvent followed by water until the pH was neutral. The treated polymer was ready to be used after washing and air-drying.

(A) Pre-washed β-CD polymer



(B) Washed β-CD polymer

Figure 32 Pre-washed and washed β-CD polymer

3.5.2 Batch process

3.5.2.1 Effects of temperature, amount of β-CD polymer used and processing time on % limonin reduction

The best condition for limonin reduction in batch process was explored. Temperature, time of process and the amount of β-CD polymer used were varied as 6, and 30^{0}C for temperature; 30, 60, and 90 min for processing time; and 1, 3, and 5g% for β-CD polymer used. The mixing speed of the rotor was set at No.3 (magnetic stirrer, Heidolph MR3003). The experiment was designed by using Factorial design 3x3x2x3 and analyzed by the SAS V 6.12 program.

Statistical analysis were sumerized in Appendix C (Table C 6). It showed that the testing model was good with % C.V. around 8. The interaction between temperature and β-CD polymer concentration was observed. It can be seen that both temperature and amount of β-CD polymer affect the % limonin reduction for batch process at the confidence level of 0.05.

Table 5 summarized the debittering observations in batch process. It can be proposed that % limonin reduction increased with the increasing amount of β-CD polymer at both temperatures tested. Marked increased of debittering was observed between 1g% and 3g% β-CD polymer. Only 10-15 % increased in debittered juice occurred for increment of 3g% to 5g% β-CD polymer.

Table 5 % Limonin reduction for batch process at each processing time, temperature and β-CD polymer concentration (Mean±SE)

Process time (min)	%Limonin reduction					
	Room Temperature (~ 30 °C)			Cold Room Temperature(6°C)		
	1g% CD/juice	3g% CD/juice	5g% CD/juice	1g% CD/juice	3g% CD/juice	5g% CD/juice
30	42.73±0.65 [a]	68.06±0.88 [b]	78.49±0.93 [e]	24.88±0.31 [e]	63.82±0.05 [h]	80.60±0.07 [d]
60	43.85±0.98 [a]	71.80±1.48 [c]	80.71±0.48 [d]	27.25±1.39 [f]	66.69±0.79 [i]	80.96±0.24 [d]
90	43.56±0.59 [a]	70.44±0.59 [c]	80.41±0.40 [d]	34.39±1.78 [g]	66.50±1.08 [i]	80.15±1.53 [d]

Note: Means with the same letter in the column and row are not significantly different at $P \leq 0.05$, n=3

It was also shown that, except for 5 g% β-CD polymer, debittering at normal room temperature (~30^{0}C) resulted in higher limonin reduction than at cold room temperature (6 ^{0}C). The effect of processing time on % limonin reduction was also observed. Although % limonin reduction moderately increased when debittering processing time was longer, the % limonin reduction became stable after 60 minutes of processing time. In this case, it was supported with the statistical analysis that there was no significantly different between 60 and 90 minutes at the confidence level of 0.05.

From Duncan's multiple range test (DMRT), the best condition which gave the highest % limonin reduction (80.96) for batch process was to mix tangerine juice with 5g% β-CD polymer at cold room temperature (~6 ^{0}C) for 60 minutes. Nevertheless, in practice, not only limonin reduction efficiency was considered but also other factors such as production cost, processing time, nutrition loss during process and limonin content in acceptable level. Employing the standard of limonin level in acceptable citrus products issued by the State of Florida, Department of Citrus, the final limonin content in debittered tangerine juice should not exceed 5 ppm.

In the above condition, the researcher decided to operate the batch debittering process at the condition of: room temperature (~30 ^{0}C), 3g% of β-CD polymer and 30 minutes, which gave around 68 % limonin reduction with ~ 2.80 ppm

Table 6 Limonin content (ppm) in debittered juice for batch process at each processing time, temperature and β-CD polymer concentration (Mean±SE)

Process time (min)	Limonin (ppm)					
	Room Temp (30 °C)			Cold Room (6°C)		
	1g%CD/juice	3g%CD/juice	5g%CD/juice	1g%CD/juice	3g%CD/juice	5g%CD/juice
30	4.41±0.12	2.80±0.04	1.97±0.03	4.23±0.07	2.35±0.08	1.67±0.06
60	4.16±0.10	2.77±0.32	1.70±0.13	4.15±0.09	2.07±0.11	1.50±0.04
90	4.34±0.03	2.52±0.20	1.86±0.07	4.17±0.08	2.17±0.028	1.52±0.04

Note: n= 3

limonin left in final juice product (Table 6). The limonin absorption capacity of this batch process was at 0.17 mg limonin/g β-CD polymer

3.5.2.2 Effect of mixing speed on % limonin reduction

The effect of mixing speed on % limonin reduction at each processing time was also investigated. At the chosen condition of the batch debittering process, the mixing speed of stirrer was varied into two levels - at speed No.3 and No.5 as mentioned in section 2.7.2.1 (Chapter II). The % limonin reduction of each mixing speed were displayed in Table 7.

Table 7 Effect of stirrer speed on %limonin reduction (Mean±SE)

Processing time (min)	% limonin reduction	
	Speed No3 [a]	Speed No 5 [b]
0	0	0
30	68.06±1.12	70.80±1.11
60	71.83±1.50	71.04±1.64
90	70.44±0.38	69.21±0.51

Note: a and b are not significantly different at P≤ 0.05, n=3

From ANOVA analysis, it also confirmed that processing time affected on % limonin reduction especially at 60 minutes, which gave the highest % limonin reduction and was significantly different from the results obtained at 30 and 90 minutes (Table C 7).

In case of the effect of mixing speed on % limonin reduction, it can be seen that the % limonin reduction between 2 levels of stirrer speed remained unchanged (at confidence level of 0.05). However it was observed that some polymer bead's deterioration occurred when rotor speed No.5 was used.

In conclusion, with economical consideration, the optimum condition for the batch debittering process should be as follows: room temperature (~30 ^{0}C), 3g% β-CD polymer, 30 minutes and mixing speed No.3.

3.5.3 Column process

The condition of the column was calibrated to have similar sample contact time with β-CD polymer as that of the batch process.

Three grams of washed β-CD polymer was swelled in water and packed into 12 cm x1.2cm i.d. glass column by the procedure described in section 2.7.2.2. The sample juice was passed through the column at the flow rate of 0.35 ml/min (30 minutes contact time). Five ml fraction was collected and determined for limonin content. The initial limonin content in the juice before

debittering was 8.70 ppm. The total amount of limonin in 100 ml of whole juice sample was 0.87 mg.

Table 8 shows the limonin content of each debitterred fraction. The average % limonin reduction of all fraction was around 94% and the capacity of β-CD polymer in limonin absorption at this condition was 0.27 mg limonin/g β-CD polymer. At all fractions eluted, the limonin content was well below 1 ppm. Total residual limonin in the debittered juice was about 0.05 mg.

Table 8 **Limonin content in debittered tangerine juice fractions (3g% β-CD polymer, flow rate 0.35 ml/min)**

Fraction no.	Average limonin content (ppm)	limonin in juice 5 ml (μg)
1	0.47	2.36
2	0.24	1.20
3	0.29	1.43
4	0.41	2.06
5	0.48	2.39
6	0.50	2.50
7	0.48	2.42
8	0.47	2.34
9	0.44	2.18
10	0.41	2.05
11	0.28	1.38
12	0.66	3.29
13	0.42	2.08
14	0.45	2.26
15	0.55	2.76
16	0.36	1.78
17	0.45	2.27
18	0.27	1.34
19	0.38	1.92
20	0.36	1.81
21	0.53	2.64
22	0.33	1.63
23	0.47	2.34
24	0.23	1.14
25	0.26	1.28
26	UD	UD
Total limonin in debittered juice (μg)		50.82

Note: UD=Undetectable, The initial limonin content 8.7 ppm (870 μg).

3.5.3.1 Practical maximum load of β-CD polymer column for debittering process

The practical maximum load of the β-CD polymer column was determined as maximum volume of juice by which the β-CD polymer column could still remove limonin from the juice to ≤ 5 ppm limonin.

This result could be used to indicate when the column should be regenerated. To investigate the practical maximum load of the β-CD polymer column, up to 350 ml of sample juice was loaded. Twenty-five ml fraction of debittered juice was collected from the β-CD polymer column. The limonin content in each fraction was determined as described in 2.4.2.2.1 and 2.4.2. Figures 31 and 32 show respectively the result from loading 100, 200 and 350 ml of clarified tangerine juice onto the β-CD polymer packed column. The running conditions of these experiments were similar.

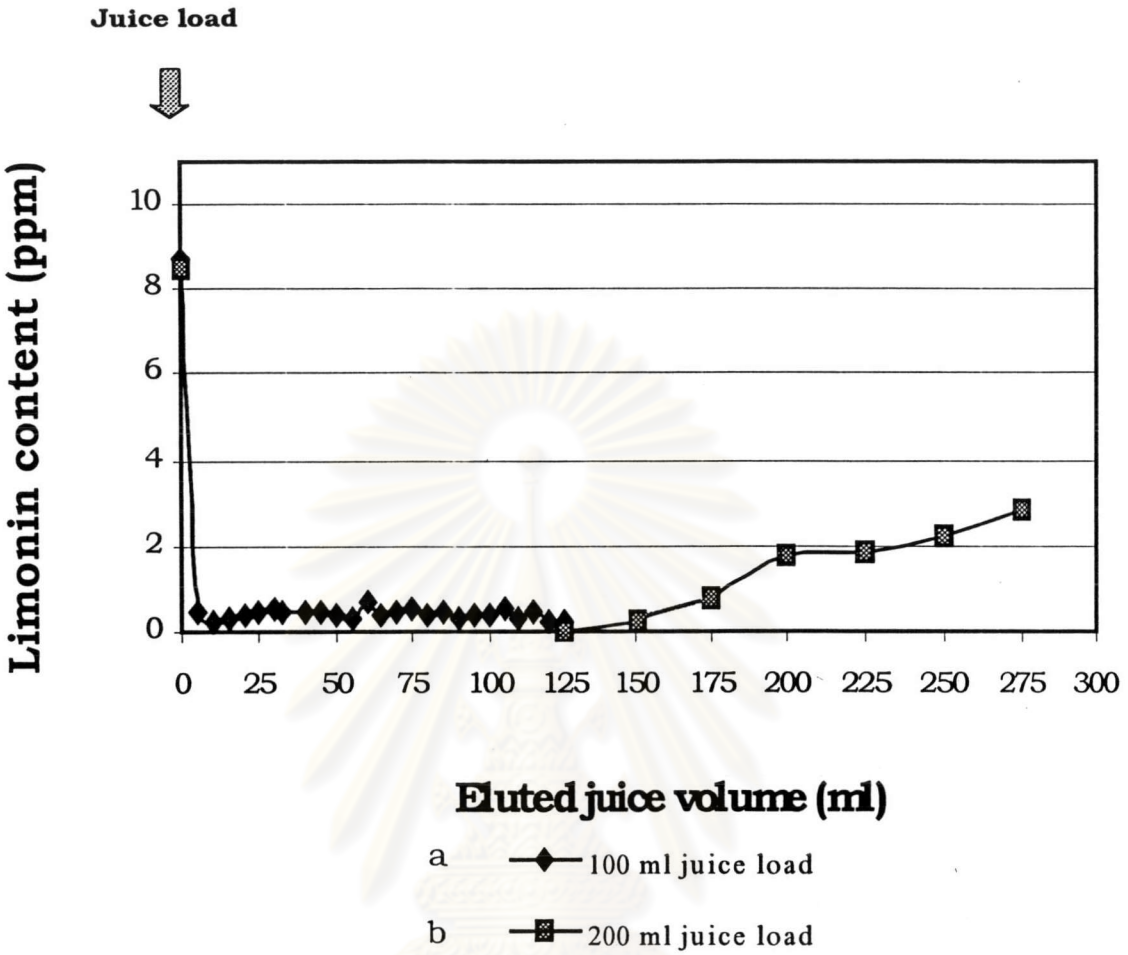


Figure 33 **Limonin content in debittered juice with 100-200 ml load a and b are separate experiments with initial limonin content of 8.70 and 8.43 ppm respectively. Five or 25 ml fractions were collected for limonin determination. In experiment b, the first collection was at 125 ml fraction.**

From Figure 33, it can be seen that the limonin content in debittered juice was marginal (around 0.2 ppm limonint) at the first of 160 ml of eluted juice, after that the value slowly increased. Even so, with 200 ml juice loading, the limonin content in the final fraction debittered juice was still less than 5 ppm. That means β-CD polymer column could be loaded with more than 200 ml volume. Then 350 ml juice was loaded for the next experiment.

When 350 ml of sample juice containing 10.11 ppm of limonin was loaded, the limonin content remaining in eluted juice was sharply increased after 200 ml, reaching non-absorbable level at 350 ml volume (Figure 34 and Table 8). Basing on the limonin taste threshold level (5 ppm), this result also suggests that the practical maximum load for this column was about 240 ml under the condition used. In term of absorption capacity, it was 0.81 mg limonin/g β-CD polymer at the practical maximum load (Appendix D).

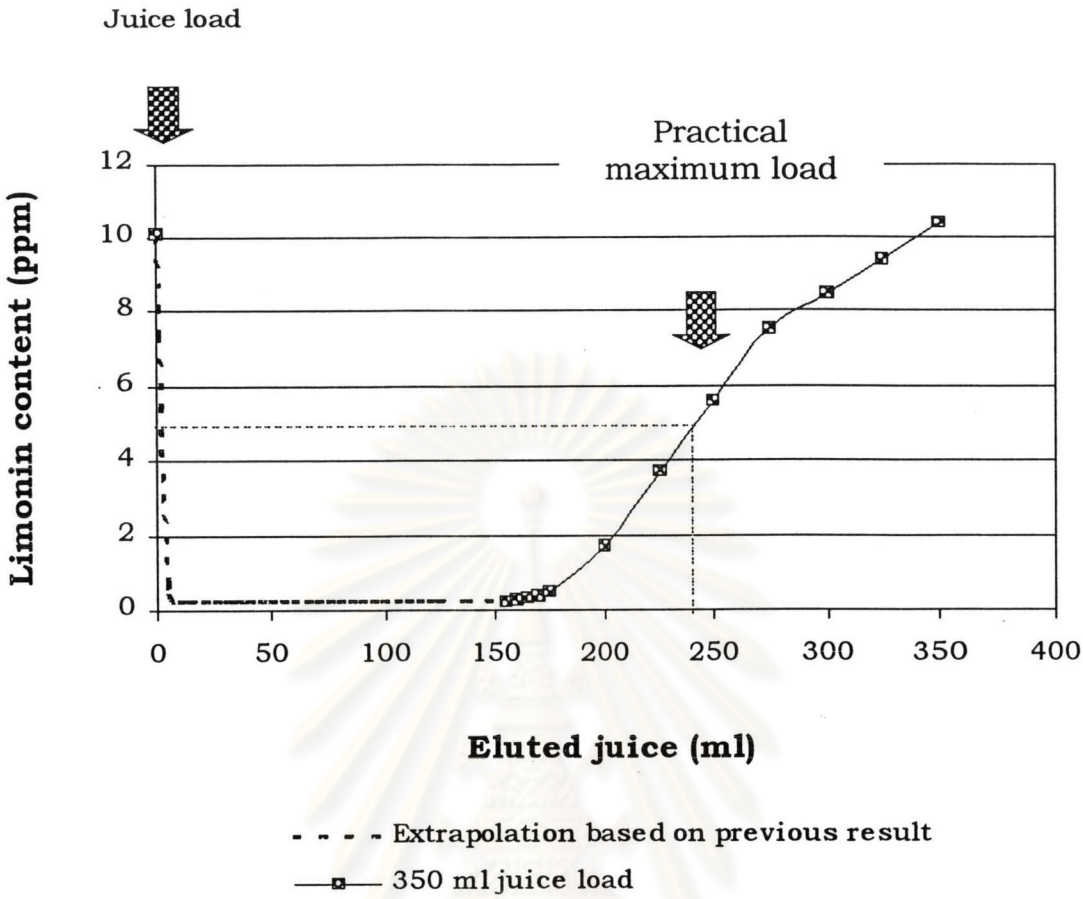


Figure 34 **Debittering of 350 ml juice load by β-CD polymer column The initial limonin was 10.11 ppm. Twenty-five ml fractions were collected and the first collection was at 155 ml.**

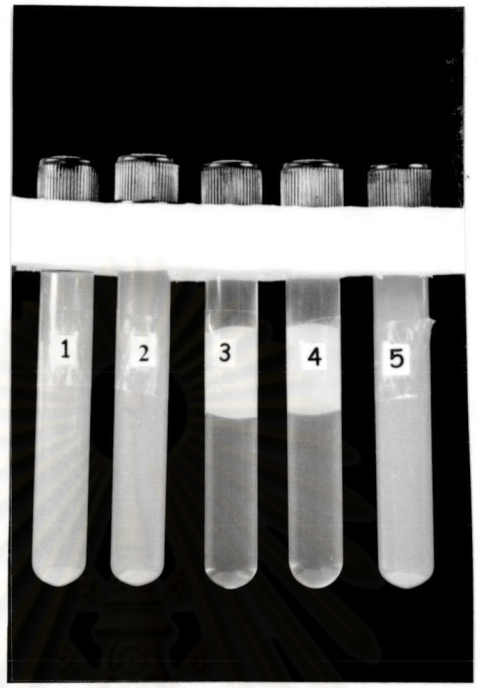
Table 9 Limonin content and % limonin reduction of 350 ml load by β-CD polymer column

Eluted juice (ml)	Limonin (ppm)	%limonin reduction
Before debittering	10.11	100
100	UD	≃ 100
125	UD	≃ 100
150	UD	≃ 100
155	0.22	97.82
160	0.27	97.26
165	0.36	96.47
170	0.40	96.07
175	0.52	94.86
200	1.70	83.21
225	3.72	63.19
250	5.60	44.58
275	7.50	25.79
300	8.46	16.28
325	9.38	7.19
350	10.38	≃ 0

Note: UD = Undetectable

3.5.3.2 Visual comparison of juice color

The objective of this experiment was to evaluate the debittered juice color which is the important consideration in citrus juice products affecting consumers' acceptance. The pulp was separated from the clarified juice and kept in the refrigerator. After the clarified juice was passed through the debittering column, the chilled pulp was blended into the debittered juice. The color of debittered juice before and after adding pulp was compared to each other and also compared with the fresh and pasteurized juice. The experiment was estimated by visual observation as shown in Figure 35. It can be seen here that the debittering process did not caused any visual color change on all occasions tested.



Tube 1 = Fresh juice

Tube 2 = Pasteurized juice

Tube 3 = Clarified juice before debittering

Tube 4 = Clarified juice after debittering

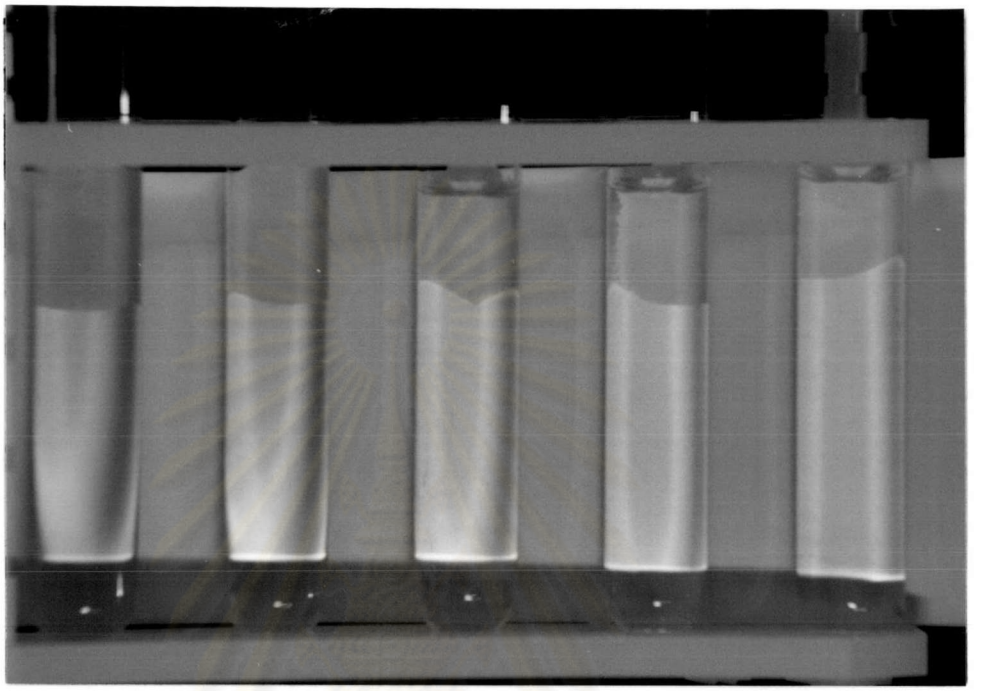
Tube 5 = Debittered juice after adding pulp

Figure 35 Color comparison between debittered and fresh juice

3.5.3.3 Regeneration of β-CD polymer

Regeneration of the polymer for reused was done by means of Shaw and Wilson (1985) who proposed that resulting with the reaction of flavonoid and alkali solution, the resulting mixture was yellow and easy to be assayed. Due to the ratio of Flavonoid and limonin was consistent this method was used to observe in limonin removal for regeneration.

The column was washed with excessive amount of water and 2% NaOH and finally by absolute ethanol as mentioned in section 2.7.4 (ChapterII). The color of the NaOH fraction was initially yellow and became paler on the prolonged wash (Figure 36). To follow the color spectrophotometrically, the absorption spectra of the NaOH washed was determined. Figure 37 shows that there were three peaks at 220, 275 and 360 nm respectively. The researcher chose to follow the regeneration process at 275 and 360 nm because the sodium peak was at 220 nm.



F10 **F20** **F30** **F40** **F50**

Figure 36 **Color comparison of each NaOH washed fractions (F=Fraction number)**

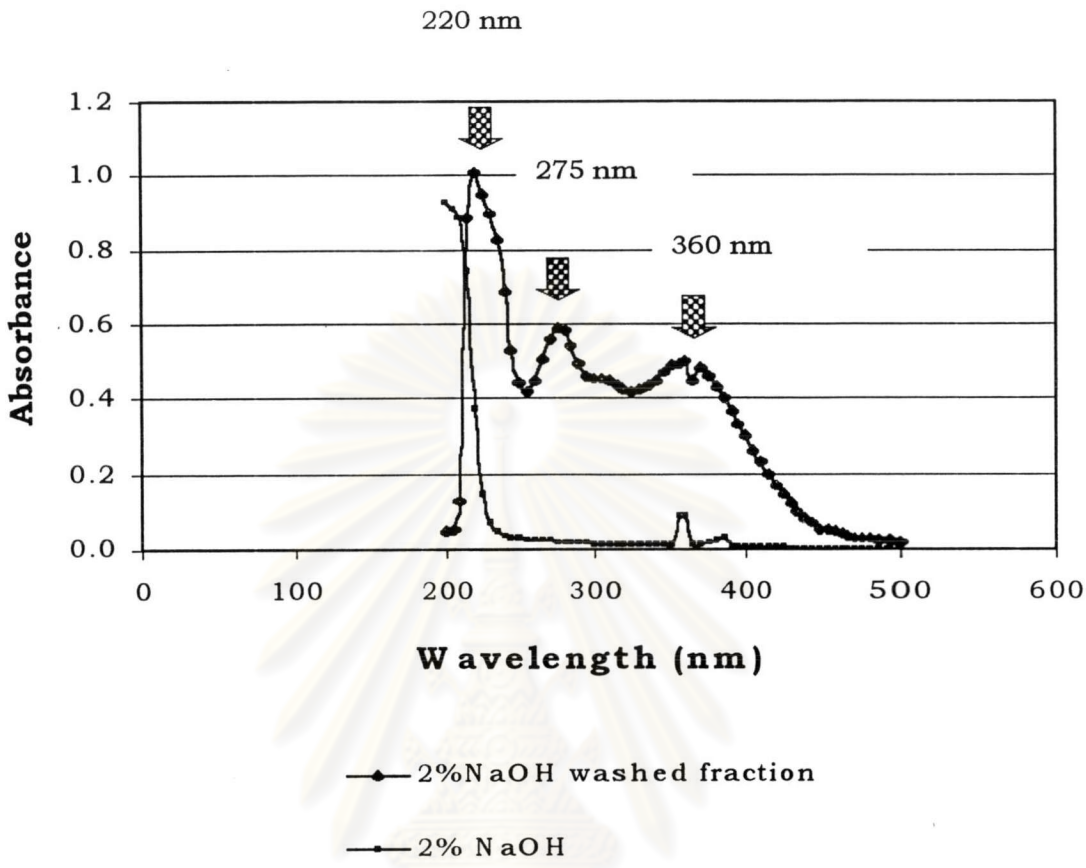


Figure 37 Absorption spectra of 2% NaOH washed fraction (F10)

During washing process, each 5 ml fraction of the NaOH washed was collected and measured by spectrophotometer at 275 and 360 nm as mentioned in 3.5.3.2. Figure 38 shows the absorbancy profile of the NaOH washed fraction from the β-CD polymer column. It could be seen that the absorbency gradually decreased and approached zero at the 40^{th} fraction (200ml). In other words, the regeneration process could be done by washing with 200 ml 2%NaOH for this column.

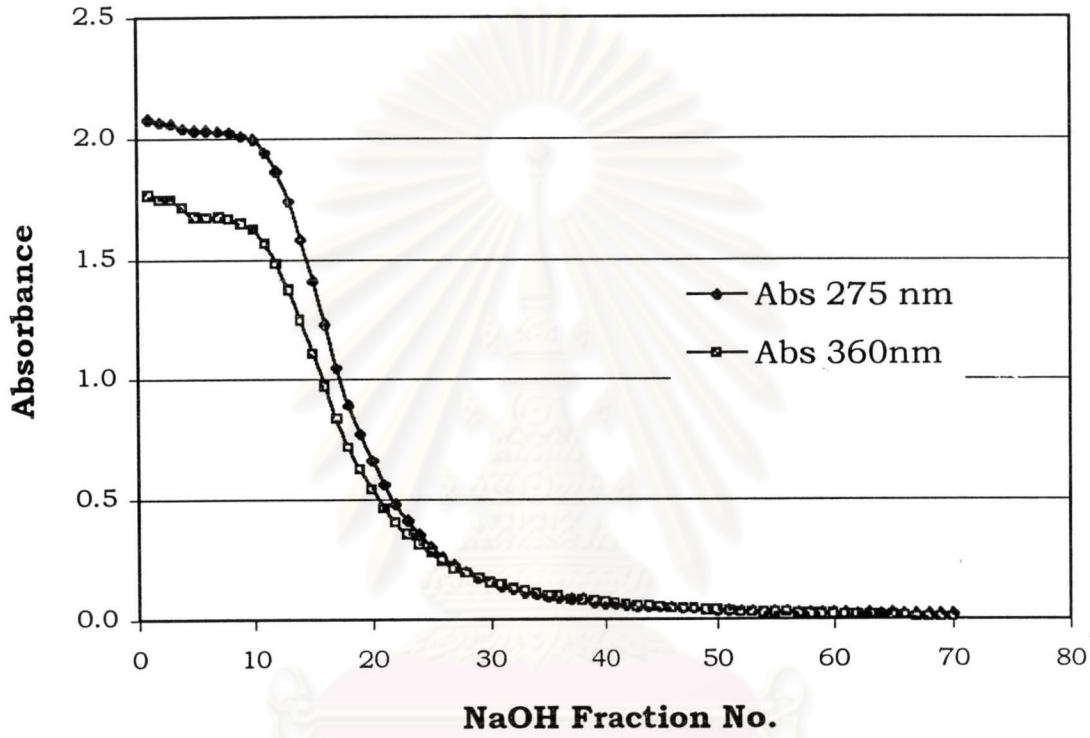


Figure 38 **Absorption profile of NaOH washed fraction at 275 and 360 nm**

3.6 Comparison of limonin reduction in Thai tangerine juice between batch and column process

Comparison of batch and column debittering processes was demonstrated in Table 10. It clearly indicated that the capacity of limonin absorption in column process was approximately 1.6 times higher than that obtained from the batch process.

Table 10 Comparison of limonin reduction efficiency between batch and column processes

Items	Batch process	Column process
% g β-CD polymer (g/100ml juice)	3	3
Process Temperature (^{o}C)	30	30
Contact time (minutes)	30	30 : BV
Initial limonin content in sample juice (ppm)	7.34±0.07	8.70±0.003
% Limonin reduction	68	94
Capacity of β-CD polymer for limonin absorption (mg limonin/g β-CD polymer)	0.17	0.27
Polymer destruction	LITTLE AT HIGH SPEED	NO

Note: BV = Bed Volume 10 ml

3.7 Column debittering process using XAD-16 resin

The XAD-16 resin was tested for column debittering process using the same bed volume (10 ml) and flow rate condition of the β-CD polymer column (section 2.8. Chapter II). Since XAD-16 resin has less sweeling property, 5.6 g% XAD-16 was used to provide 10 ml bed volume in the column. In this experiment, the initial limonin content in tangerine juice was 0.38 mg per 100 ml. It was found that the limonin content in every debittered fraction was undetectable (Table 11). In another word, the XAD-16 resin was effective for limonin adsorption even at room temperature. The capacity in limonin reduction of this XAD-16 resin column at the chosen condition was 0.15 mg limonin / g XAD-16 resin.

Table 11 Limonin reduction efficiency using XAD-16 column (5.6 g% XAD-16, flow rate 0.35 ml/min)

Eluted juice (ml)	Limonin content (ppm)	%Limonin reduction
Start	8.385	0
5	UD	≃ 100
10	UD	≃ 100
15	UD	≃ 100
20	UD	≃ 100
25	UD	≃ 100
30	UD	≃ 100
35	UD	≃ 100
40	UD	≃ 100
45	UD	≃ 100
50	UD	≃ 100
55	UD	≃ 100
60	UD	≃ 100
65	UD	≃ 100
70	UD	≃ 100
75	UD	≃ 100
80	UD	≃ 100
85	UD	≃ 100
90	UD	≃ 100
95	UD	≃ 100
100	UD	≃ 100
105	UD	≃ 100
110	UD	≃ 100
115	UD	≃ 100
120	UD	≃ 100
125	UD	≃ 100

Note: UD = Undetectable

3.8 Evaluation of debittering cost

Evaluation of debittering cost using β-CD polymer was described in Table A 8. The debittering cost was around 1,200 bahts/column and gave the productivity of 21 ml juice/column/hour. It was noticed that the 99 % of the operating cost was due to the β-CD polymer.