CHAPTER IV

RESULTS AND DISCUSSION

1. PREPARATION OF TAMARIND PULP EXTRACT

Purified water was used as solvent to dissolve organic acids and other constituent in tamarind pulp. The physical properties of tamarind pulp extract were determined. Major composition of organics acid in tamarind pulp such as tartaric acid was determined. In this method tamarind pulp extract had density 1.0254-1.0255 g/mL, viscosity of extract was in range 3.64-4.68 mPas, pH was in range 2.61-2.84, titratability acidity 164.73-217.86 mL per tamarind pulp extract 100 grams, soluble solid was 5.2 °Brix, and tartaric acid was 0.99-1.34 %w/w, as show in table 6 (Appendix A, Table 23-33)

Table 6 Physical and chemical properties of tamarind pulp extract

	Physicochemical properties					
Lot	Density (g/mL)	Viscosity (mPas)	pH	Titratable acidity (mL/100g extract)	Soluble solid (° Brix)	Tartaric acid (% w/w)
R001	1.0255	4.46	2.65	181.12	5.2	1.19
R002	1.0254	4.68	2.61	217.86	5.2	1.34
R003	1.0255	3.64	2.84	164.73	5.2	0.99
Mean	1.0255	4.26	2.7	187.90	5.2	1.17
SD	0.0001	0.5481	0.1229	27.2068	0.0000	0.1756

The wide variation was met in titratable acidity. It was found that titratable acidity was related with quantitative amount of tartaric acid in tamarind pulp extract. Table 6, tamarind pulp extract Lot R003 show lower titratable acidity than R001 and R002, it refers to less quantitative amount of tartaric acid in tamarind pulp extract. The variation of titratable acidity and percent tartaric acid in tamarind pulp extract resulted from non homogeneity of tamarind pulp that might be due to the differece in contents of fiber and acid in their pulp.

Tartaric acid was found in tamarind pulp in range 8.32 – 12.35 %. Data shown in table 7 and Appendix A. Lewis and Neelakantan (1964) was reported that tamarind pulp had tartaric acid in range 8 – 18 percent. Thus, in this study the extraction gave tartaric acid in range similar to the previous study.

Table 7 Tartaric acid in tamarind pulp

Lot	Percent tartaric in tamarind pulp (% w/w)
R001	10.38
R002	12.35
R003	8.32
Mean	10.38
SD	1.99

2. DETERMINATION OF TARTARIC ACID IN TAMARIND PULP EXTRACT

The validation of analytical method is proceeded by which performance characteristics of method are established to meet the requirement for the intended analytical application. The performance characteristics of analytical method are expressed in term of analytical parameters. Parameters for validation of HPLC assay are specificity, linearity, accuracy and precision.

2.1 Specificity

The specificity of an analytical method is the ability to ensure of an analyst accurately and specificity in the presence of other components in the sample. The internal standard technique was performed by determining the peak area ratio of tartaric acid and acetic acid (internal standard) to give the complete separation, appropriate resolution and sharp peak of all components. Phosphate buffer, 0.05 mM potassium dihydrogen phosphate adjust pH to 2.50 with phosphoric acid, was developed and used as mobile phase. The chromatograms of mobile phase, standard solution, internal standard solution and other standard that might be in tamarind extract are presented in Figures 7 – 12. They showed peaks of tartaric acid and acetic acid which were not interfered and completely separated from peaks of mobile phase and other components. However, the chromatograms of mobile phase and tartaric acid showed peaks at retention time 3.8 min and 4.7 min, respectively (Figure 7 and 8).

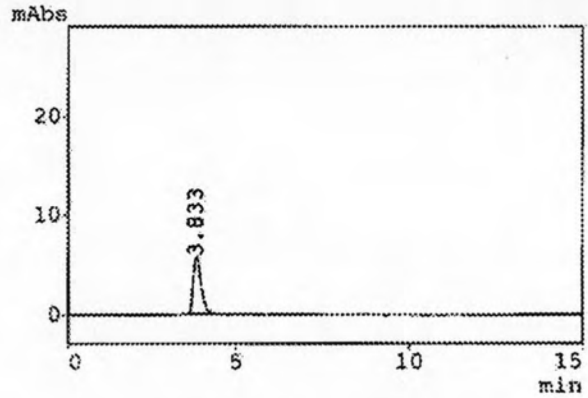


Figure 7 Chromatogram of 0.05 mM KH_2PO_4 pH2. 5

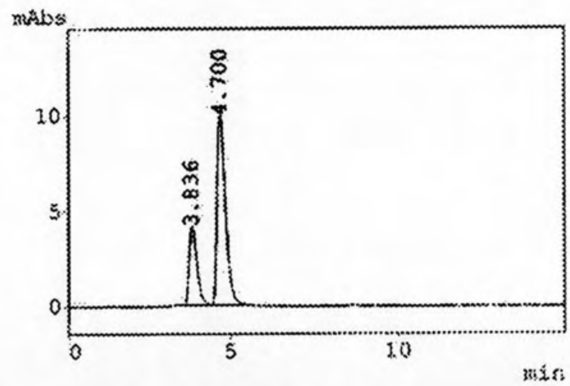


Figure 8 Chromatogram of tartaric acid solution

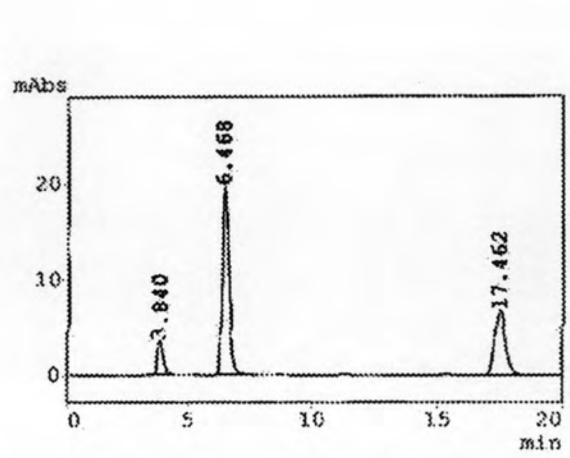


Figure 9 Chromatogram of malic acid solution

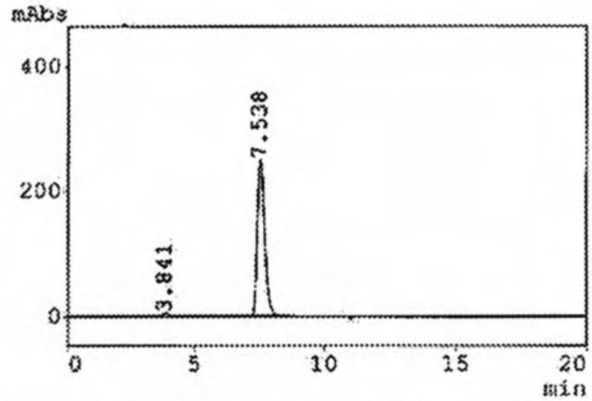


Figure 10 Chromatogram of vitamin C solution

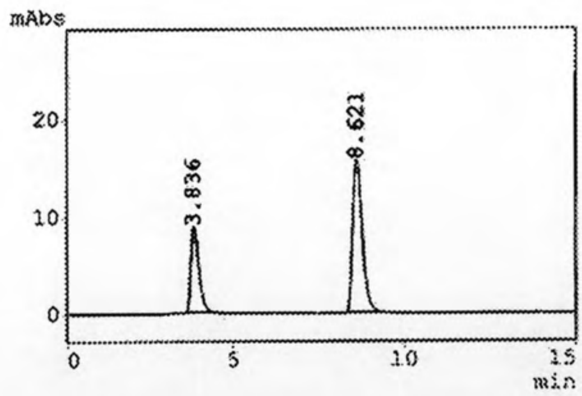


Figure 11 Chromatogram of acetic acid solution

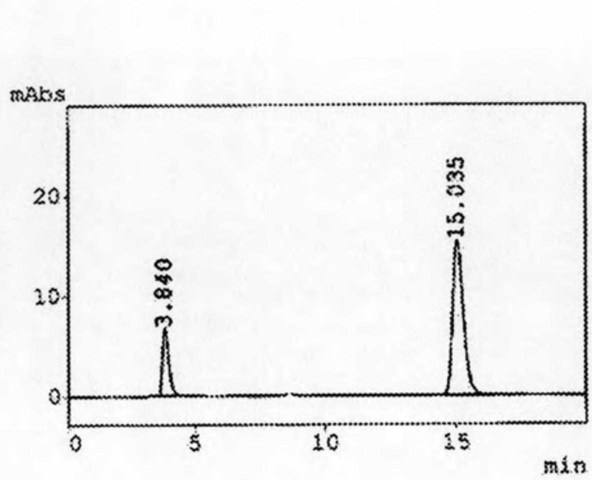


Figure 12 Chromatogram of citric acid solution

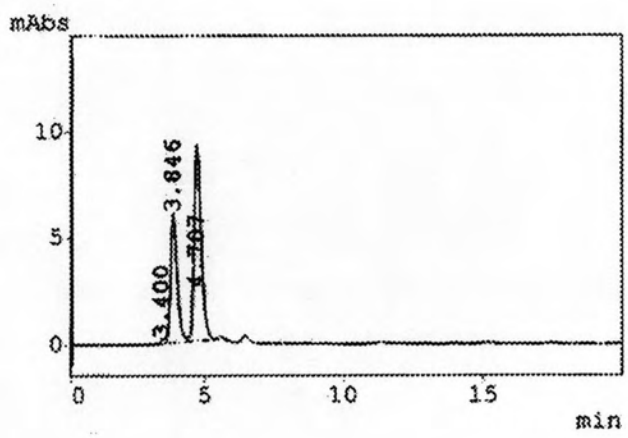


Figure 13 Chromatogram of tamarind pulp extract

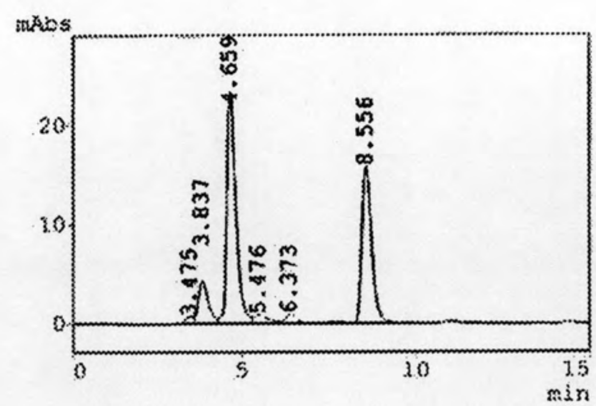


Figure 14 Chromatogram of tamarind pulp extract and acetic acid solution

The major composition of organic acids in tamarind pulp extract from this extraction is tartaric acid as shown in Figure 13, peak at retention time 4.7 min is tartaric acid when compare with Figure 8. Minor constituent was malic acid as shown at retention time nearly 6.4 min (Figure 9) and unknown interfere composition at retention time 5.4 min. From this study peak of tartaric acid do not interfere with other organic acids that might be found in tamarind pulp extract. Condition for determining tartaric acid content in tamarind pulp extract could be followed as this experiment.

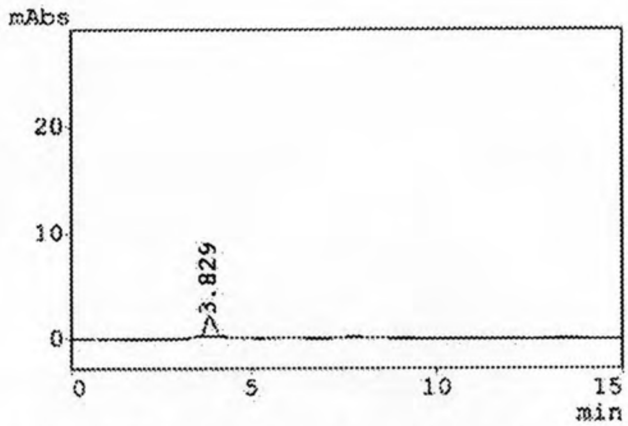


Figure 15 Chromatogram of acacia solution

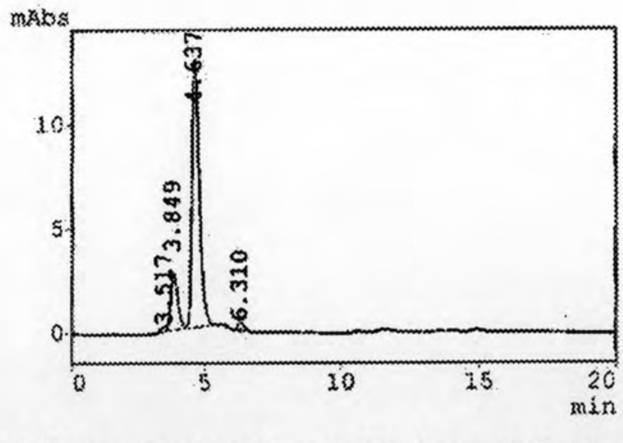


Figure 16 Chromatogram of spray-dried tamarind pulp extract

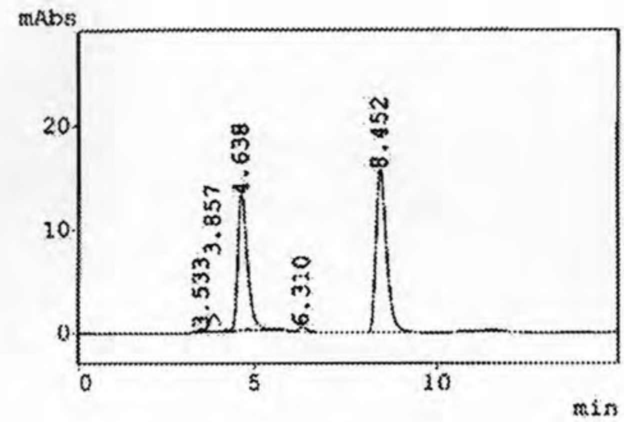


Figure 17 Chromatogram of spray-dried tamarind pulp extract and acetic acid (internal standard)

That chromatogram of acacia solution showed no peak of acacia solution in this HPLC condition, as demonstrated in Figure15. Peak at retention time 3.8 min is KH_2PO_4 pH 2.5 (mobile phase) when compared with Figure 7 and Figure 11, spray-dried tamarind pulp extract showed peak of tartaric acid at retention time 4.6 min and peak of minor composition of malic acid was shown at retention time 6.3 min. There was no appearance of the peak after retention time 6.3 min, thus, acetic acid could be used as internal standard for determining tartaric acid in spray-dried tamarind pulp extract. The retention time of acetic acid is 8.6 min (Figure 7 and 13)

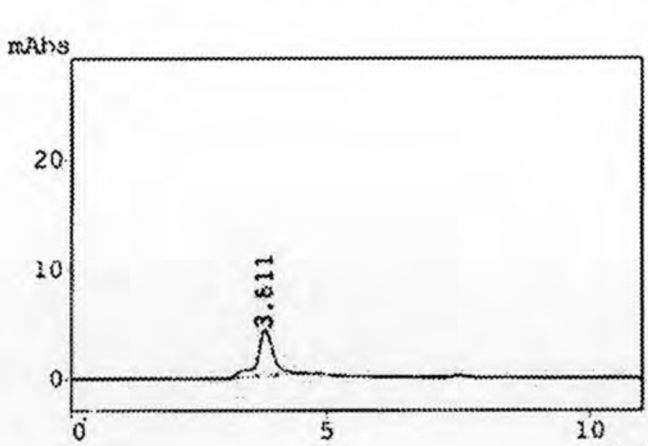


Figure 18 Chromatogram of oil-in-water emulsion

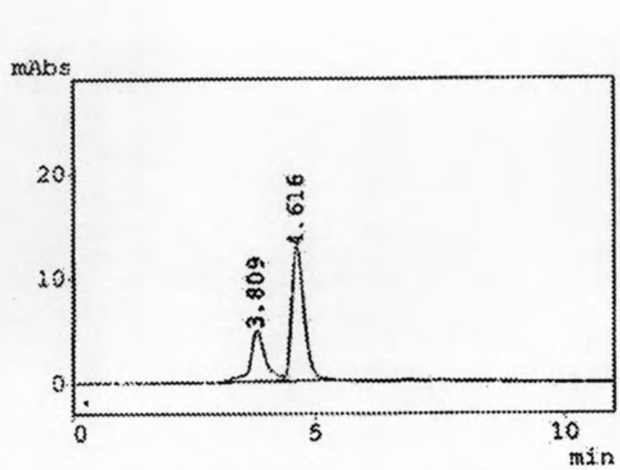


Figure 19 Chromatogram of oil-in-water emulsion with tartaric acid

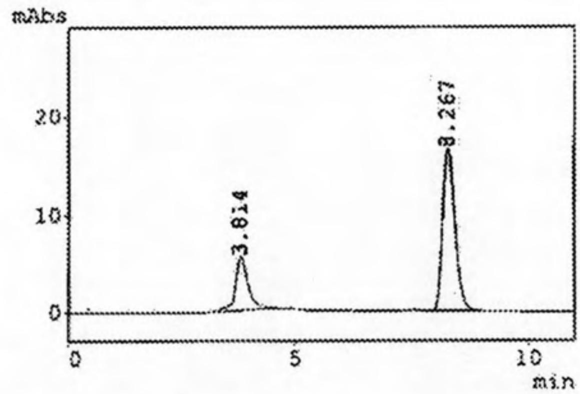


Figure 20 Chromatogram of oil-in-water emulsion with acetic acid
(internal standard)

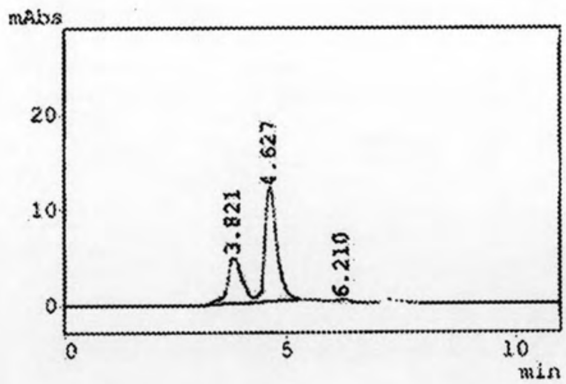


Figure 21 Chromatogram of oil-in-water emulsion containing
8 % w/w spray-dried tamarind pulp extract

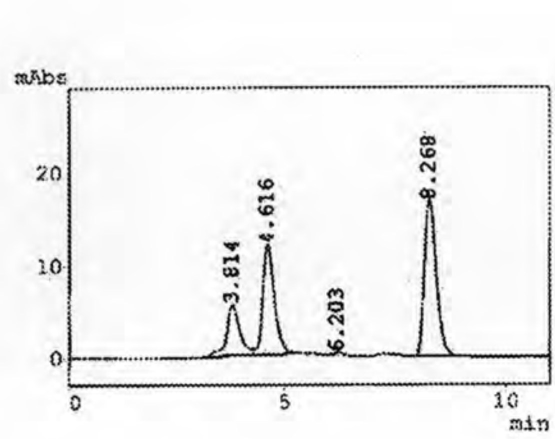


Figure 22 Chromatogram of oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract and acetic acid (internal standard)

The chromatogram of oil-in-water emulsion showed no peak of composition of oil-in-water emulsion in this HPLC condition, as demonstrated in Figure18. Peak at retention time 3.8 min is KH_2PO_4 pH 2.5 when compared peak at retention time with Figure 7. Chromatogram of oil-in-water emulsion with tartaric acid was shown in Figure 19. Peak of tartaric acid was separated from peak of KH_2PO_4 pH 2.5. Chromatogram of oil-in-water emulsion with acetic acid showed retention time 8.2 min (Figure 20) Chromatogram of oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract (Figure21) showed peak of tartaric acid at retention time 4.6 min and minor malic acid at retention time 6.2 min, there were not any peak after 4.6 min. So, acetic acid could be used as internal standard of this condition as shown in Figure 22.

Spray-dried tamarind pulp extract showed peak of tartaric acid at retention time 4.6 min and minor composition of malic acid showed peak at retention time 6.2 min. There was no peak appeared after retention time 6.2 min, thus, acetic acid was used as internal standard for determining tartaric acid in spray-dried tamarind pulp extract.

2.2 Linearity

The data of calibration curve of tartaric acid are shown in Table 8. The plot of concentration of tartaric acid versus the mean peak area ratio of tartaric acid and its internal standard illustrated linearity correlation in concentration range 50–300 µg/mL. The coefficient of determination (R^2) was 0.9999, as presents in Figure 23. This results indicated that this HPLC method was acceptable for qualitative analysis of tartaric acid in this range.

Table 8 Calibration curve data of tartaric acid by HPLC method

Concentration (µg/mL)	peak area ratio			mean	SD	%CV
	1	2	3			
50	0.5953	0.6160	0.5998	0.6037	0.011	1.81
100	1.1681	1.1426	1.1741	1.1616	0.017	1.44
150	1.7325	1.7597	1.7483	1.7468	0.014	0.78
200	2.2978	2.3000	2.3286	2.3088	0.017	0.74
250	2.9079	2.8911	2.9518	2.9169	0.031	1.07
300	3.4601	3.4861	3.5388	3.4950	0.040	1.15
R^2	0.9999	0.9996	0.9998	0.9999	-	-

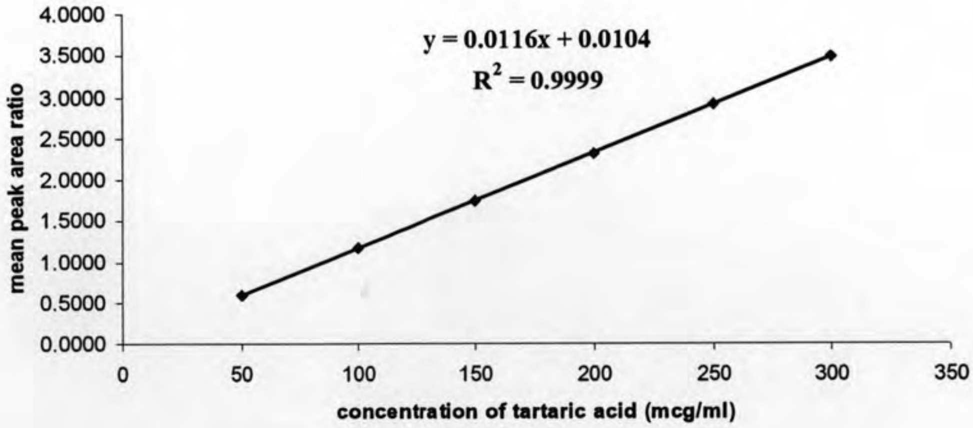


Figure 23 Calibration curve of tartaric acid by HPLC method

2.3 Accuracy

Accuracy of this experiment was estimated by using tartaric acid solution. They were prepared in five sets at concentrations 75, 175 and 275 μg/mL. The inversely estimated concentrations of tartaric acid and percentages recovery were calculated, as shown in Table 9. Percentage recovery of tartaric acid solution of each concentration was in range 99.33 – 99.72 % (Table10). This results indicated the high accuracy of this method.

Table 9.The inversely estimate concentration of tartaric acid in solution by HPLC method

Concentration	Inversely estimate concentration (μg/mL)					Mean ± SD
(μg/mL)	Set 1	Set 2	Set 3	Set 4	Set 5	
75	74.2957	74.3868	75.6699	75.3928	74.0429	74.7576 ± 0.72
175	172.1159	173.1453	175.3541	174.0973	174.4312	173.8288 ± 1.24
275	273.1050	277.5719	271.2953	275.3223	273.8542	274.2297 ± 2.37

Table 10 The percentage of estimate concentration of tartaric acid

Concentration	Percentage of estimate concentration (μg/mL)					Mean ± SD
(μg/mL)	Set 1	Set 2	Set 3	Set 4	Set 5	
75	99.0609	99.1823	100.8932	100.5238	98.7239	99.68 ± 0.97
175	98.3520	98.9402	100.2024	99.4842	99.6750	99.33 ± 0.71
275	99.3109	100.9352	98.6528	100.1172	99.5833	99.72 ± 0.86

Accuracy of formulation was investigated. Five sets of 3 concentrations, 75, 175 and 275 μg/mL, were used in this study. The inversely estimated concentration of tartaric acid emulsions and the percentage of estimated concentration of tartaric acid emulsions were determined as shown in Tables 11 and 12, respectively. The percentage of estimate concentration of tartaric acid emulsion was in range 99.97-100.14 %

Table 11 The inversely estimate concentration of tartaric acid in emulsion by HPLC method

Concentration (μg/mL)	Inversely estimate concentration (μg/mL)					Mean ± SD
	Set 1	Set 2	Set 3	Set 4	Set 5	
75	76.3629	73.7102	74.4783	75.5710	74.7229	74.97 ± 1.02
175	175.3857	175.6025	175.4678	175.2520	173.3173	175.01 ± 0.95
275	271.4206	276.1327	275.6675	279.9506	274.5439	275.54 ± 3.07

Table 12 The percentage of estimate concentration of tartaric acid in emulsion by HPLC method

Concentration (μg/mL)	Percentage of estimate concentration (μg/mL)					Mean ± SD
	Set 1	Set 2	Set 3	Set 4	Set 5	
75	101.8478	98.3098	99.2449	100.7916	99.6604	99.97 ± 1.38
175	100.1603	100.2841	100.2974	100.0839	99.0682	99.98 ± 0.52
275	98.6392	100.3517	100.1826	101.7392	99.7743	100.14 ± 1.12

2.4 Precision

The precision of tartaric acid determination by HPLC method was investigated. The tartaric acid solution was analyzed both within run and between run precision. The within run precision showed percent coefficient of variation of this series in range 0.72–0.98 %, as shown in Table 13. The between run precision was in range 1.25-1.85 %, as showed in Table14. Both of precision, within run and between run, their percent coefficient of variation were less than 2 %.

Table 13 Data within run precision of tartaric acid by HPLC method

Concentration (μg/mL)	Peak area ratio					Mean	SD	%CV
	Set 1	Set 2	Set 3	Set 4	Set 5			
75	0.859	0.860	0.875	0.871	0.856	0.8640	0.008	0.98
175	2.003	2.015	2.041	2.026	2.030	2.0231	0.015	0.72
275	3.185	3.237	3.163	3.211	3.193	3.1978	0.028	0.87

Table 14 Data between run precision of tartaric acid by HPLC method

Concentration (µg/mL)	Peak area ratio					Mean	SD	%CV
	set 1	set 2	set 3	set 4	set 5			
75	0.875	0.841	0.854	0.848	0.876	0.859	0.016	1.85
175	2.041	1.976	1.956	2.009	1.968	1.990	0.035	1.74
275	3.163	3.072	3.109	3.074	3.132	3.110	0.039	1.25

In conclusion, the analysis of tartaric acid by HPLC method was developed in this study. The results showed good specificity, linearity, accuracy and precision. So, this HPLC method was used for determining tartaric acid in tamarind pulp extract, spray-dried tamarind pulp extract and oil-in-water emulsion containing spray-dried tamarind pulp extract.

3. PREPARATION OF SPRAY-DRIED TAMARIND PULP EXTRACT

Spray-dried tamarind pulp extracts were prepared, conditions for spray drying were varied in 5 factors, type of carriers, concentration of carriers, concentration of silicon dioxide, inlet temperature and fan setting. Factors affected on properties of spray-dried tamarind pulp extract were evaluated. Percent yield and percent moisture content were observed. Products were collected from collecting chamber and cyclone. Amount of products from two sources were calculated as percent yield and percent moisture content was also determined.

3.1 Percent yield of tamarind pulp extract.

Percent yield of 32 experiments are shown in Figure 24.

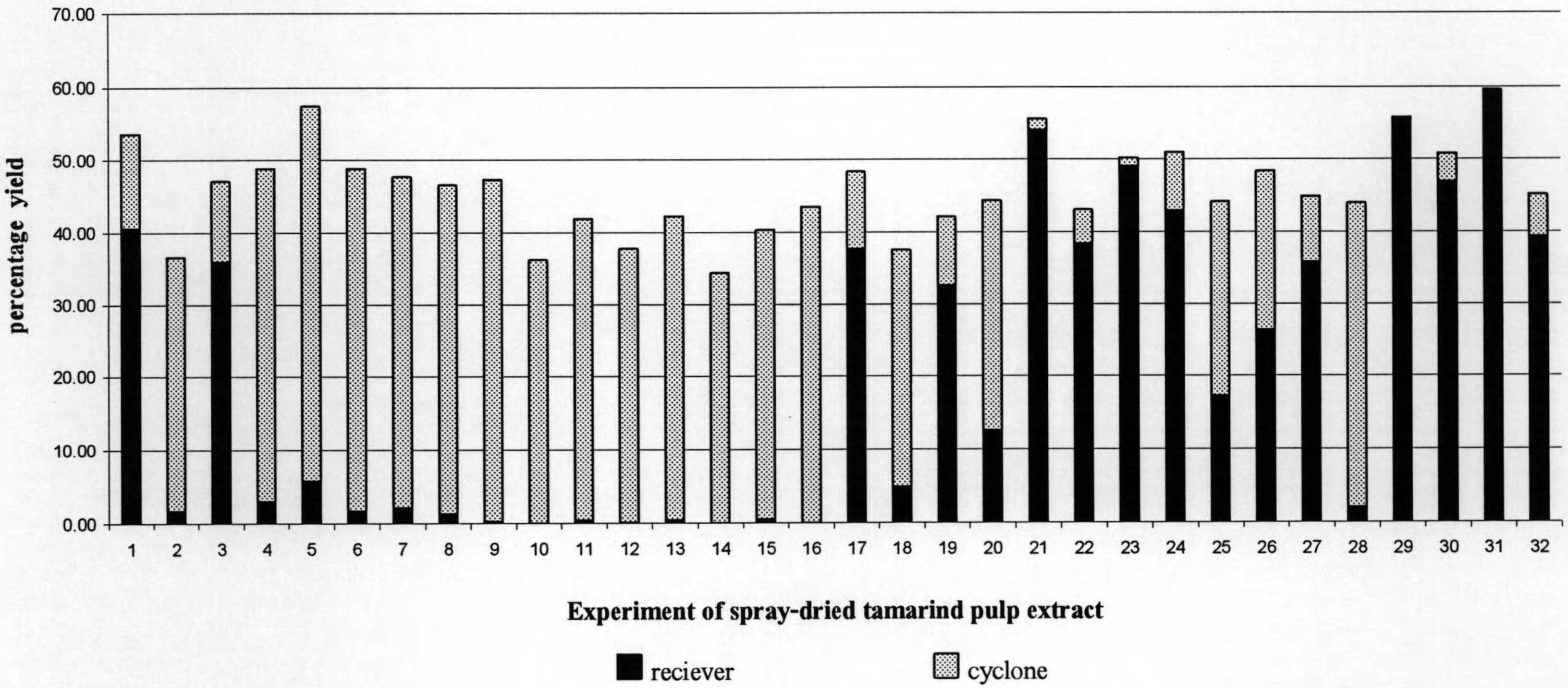


Figure 24 Percent yield of 32 experimental spray-dried

In study of the carriers effect, acacia was used as carrier for spray-dried tamarind pulp extract almost gave highest percent yield in collecting chamber as shown in Figure 24, (experiment 17-32). Condition of using acacia as carier gave higher percent yield in collecting chamber than maltodextrin (experiment 1-16), but maltodextrin gave percent yield in cyclone higher than in receiver.

In study of the effect of carriers concentration, condition of using maltodextrin, high concentration (10 % w/w, experiment 1-8) gave percent yield in collecting chamber higher than low concentration (5% w/w, experiment 9-16). But in condition of using acacia, it did not give the same results. Using acacia as spray-dried carrier in high concentration (10% w/w, experiment 17-24) and low concentration (5% w/w, experiment 25-32), the results showed variable of percent yield, both concentrations gave low and high percentage yield.

In study of he effect of concentration of silicon dioxide used as glidant together with carrier for spray-dried tamarind pulp extract. When maltodextrin was used as carrier, their results showed that silicon dioxide improved flow of the particles resulted free flowing of particle falling from cyclone into collecting chamber, as the results showed in Figure 24. Percent yield in collecting chamber of experiment 1-4, with 1% w/w silicon dioxide, gave higher percent yield than in experiment 5-8, without silicon dioxide. This results might be explained that the capability of silicon dioxide was rapid absorbed the excess of water. (Castro et al., 2005) Silicon dioxide improved flowability of particles from cyclone to collecting chamber. Moreover in other reason, silicon dioxide had anti-adherence property that reduced the adhesion of particles on the dryer wall (Pohlmann et al., 2002). But in the same conditions for spray drying of tamarind pulp extract that using acacia as carrier gave a contrast result with using maltodextrin as carrier. In the experiment that used acacia as carrier without silicon dioxide (experiment 21-24 and 29-32) gave higher percent yield in collecting chamber of spray drier than the experiment that used acacia as carrier with silicon dioxide (experiment 17-20 and 25-28). These results showed the failure of silicon dioxide addition in experiment 17-20 and 25-28, but in experiments without silicon dioxide drying aids have successful to obtain a nonsticky, free-flowing powder (Jaya and Das, 2004).

In study the effect of inlet temperature on the percent yield, inlet temperature did not improve percent yield of spray-dried tamarind pulp extract as showed in Figure 24. A decreasing inlet temperature could improve percent yield. The reason of this result was not clearly understood. This result is consistent with previous studying that decreasing inlet temperature could improve percent yield (Likitthanaset, 2006) The effect of inlet temperature might be explained in regarding to the moisture content of particles prepared from lower inlet temperature.

Fan setting were effected on percent yield of spray-dried tamarind pulp extract as showed in Figure 24 higher fan setting condition (fan setting 50, experiment 1 or 17) the more percent yield the more increased in collecting chamber than lower fan setting condition (fan setting 30, experiment 2 or 18). This finding might be explained that fan speed at high air flow rate might reduce adherent particles at cyclone and separate to collecting chamber. These results were related with lower moisture content in experiment with higher fan setting.

3.2 Percent moisture content of spray-dried tamarind pulp extract

The results of percent moisture content in spray-dried tamarind pulp extract were investigated.

In study the effect of spray-dried carrier type. The consideration of percent moisture content and percent yield, using maltodextrin as carrier gave lower percent moisture content than using acacia as spray dried carrier.

Both concentration and types of carriers have no influence on percent moisture of spray died powder. They showed tendency of higher percent moisture content in higher acacia concentration (10 % w/w, experiment 17 – 24) than lower acacia concentration (5 % w/w, experiment 25-32). In contrast to the previous study that increasing of the concentration of carrier could decrease the percent moisture content (Abadio, 2003). It might occur from the difference of preparation i.e. inlet temperature, outlet temperature and the humidity of the environment.

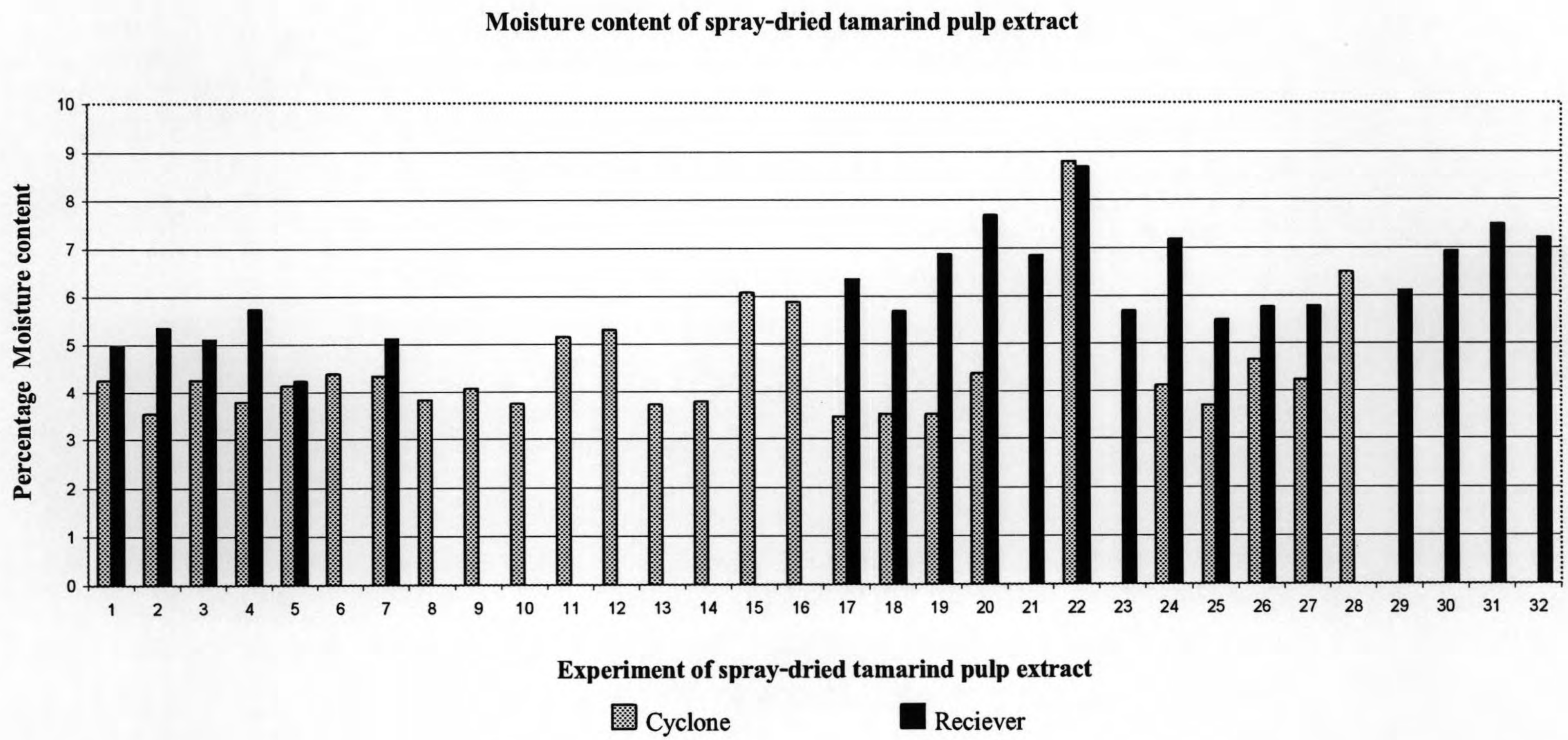


Figure 25 Percent moisture content of 32 experiment spray-dried tamarind pulp extract

The study effect of concentration of silicon dioxide, silicon dioxide was used as glidant to make lower percent moisture content of spray-dried tamarind pulp extract. The results displayed in Figure 25, experiment 25-28 (silicon dioxide 1 % w/w) showed less percent moisture content than in experiment 29-32 (no silicon dioxide). Due to silicon dioxide can absorb the moisture resulted in lower percent moisture content.

In study the effect of inlet temperature. Inlet temperatures were setted at 110°C and 130 °C. higher inlet temperature gave lower percent moisture content of spray-dried tamarind pulp extract. The results are shown in Figure 25. Percent moisture content in experiment 1 had lower moisture content than in experiment 3, or percentage moisture content in experiment 17 showed lower moisture content than in experiment 19. It could be explained that higher inlet temperature the more increasing energy in evaporation of water in spray-dried particles. The results were agreed with previous studied that an increase inlet temperature resulted in decrease percent moisture content (Likitthanaset, 2006)

In scope of studying fan setting, the results of fan setting were related to percent moisture content of spray-dried tamarind pulp extract. All spray dried experiments showed that experiment with higher air velocity had percent moisture content lower than experiment with lower air velocity. This result could explain that the increased air velocity influenced to the increased heat and mass transfer of moisture, which resulted in efficiency of drying (BÜCHI Labortechnik AG, 1997).

Particles obtained from spray drying experiment 1-32 were kept in tight, well closed protect from light bottle and placed in desiccator for 1 week. Characteristic of particles were observed. The results are shown in Table 15.

Table 15 Characteristic of spray-dried tamarind pulp extract keep in desicator

Ex	Type of carrier	Conc. of carrier	Conc. of adsorbents	Inlet temp	Fan setting	Free flowing of spray-dried tamarind pulp extract			
						After spray drying		1 week	
		(%w/w)	(% w/w)	(°C)		Cyclone	Reciever	Cyclone	Reciever
1	Maltodextrin	10%	1%	130	50	+	+	+	+
2	Maltodextrin	10%	1%	130	30	+	+	+	-
3	Maltodextrin	10%	1%	110	50	+	+	+	+
4	Maltodextrin	10%	1%	110	30	+	+	+	-
5	Maltodextrin	10%	0%	130	50	+	-	+	-
6	Maltodextrin	10%	0%	130	30	+	-	+	-
7	Maltodextrin	10%	0%	110	50	+	-	+	-
8	Maltodextrin	10%	0%	110	30	+	-	+	-
9	Maltodextrin	5%	1%	130	50	+	-	+	-
10	Maltodextrin	5%	1%	130	30	+	-	+	-
11	Maltodextrin	5%	1%	110	50	+	+	-	-
12	Maltodextrin	5%	1%	110	30	-	-	-	-
13	Maltodextrin	5%	0%	130	50	+	-	+	-
14	Maltodextrin	5%	0%	130	30	+	-	+	n/a
15	Maltodextrin	5%	0%	110	50	+	-	-	-
16	Maltodextrin	5%	0%	110	30	+	-	-	n/a
17	Acacia	10%	1%	130	50	+	+	+	+
18	Acacia	10%	1%	130	30	+	+	+	+
19	Acacia	10%	1%	110	50	+	+	+	+
20	Acacia	10%	1%	110	30	+	+	+	+
21	Acacia	10%	0%	130	50	+	+	+	-
22	Acacia	10%	0%	130	30	+	+	-	-
23	Acacia	10%	0%	110	50	+	+	+	+
24	Acacia	10%	0%	110	30	+	+	+	+
25	Acacia	5%	1%	130	50	+	+	+	+
26	Acacia	5%	1%	130	30	+	+	+	-
27	Acacia	5%	1%	110	50	+	+	+	-
28	Acacia	5%	1%	110	30	+	+	-	-
29	Acacia	5%	0%	130	50	+	-	-	-
30	Acacia	5%	0%	130	30	+	-	-	-
31	Acacia	5%	0%	110	50	-	-	-	-
32	Acacia	5%	0%	110	30	-	-	-	-

Remark : + is free flowing , - is Adherent, n/a is not analysis

Spray-dried tamarind pulp extract in experiment 1, 3, 17, 19, 20, 23 and 24 from collecting chamber did not show adherent of particles after storage in desiccator for 1 week. So that these six formulations of spray drying were repeated. The results were shown in Figure 26. The results from this study showed that experiment 23 had highest percent yield of spray-dried tamarind pulp extract. Moisture content of spray-dried particles was shown in Figure 27, experiment 23 had lower percent moisture content than experiment 24 but not less than experiment 1, 3 and 17.

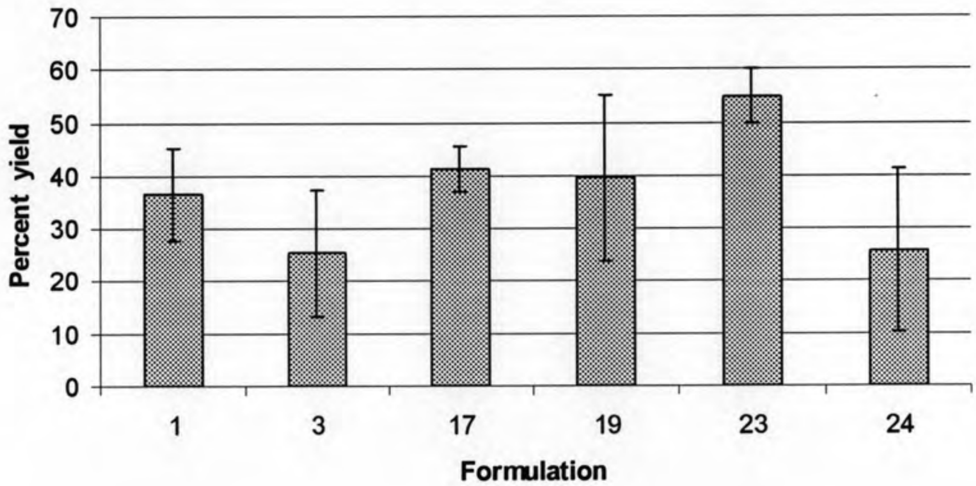


Figure 26 Percent yield of six formulation spray-dried tamarind pulp extract in collecting chamber

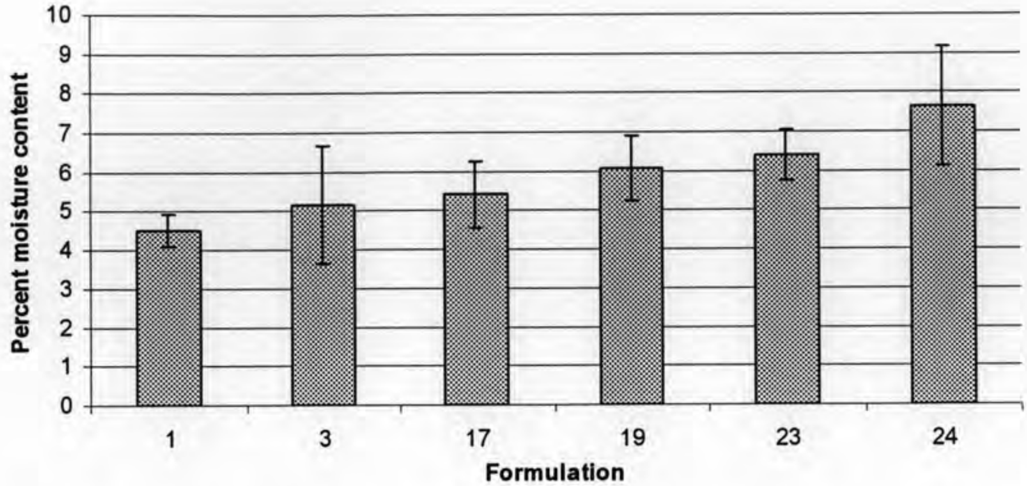
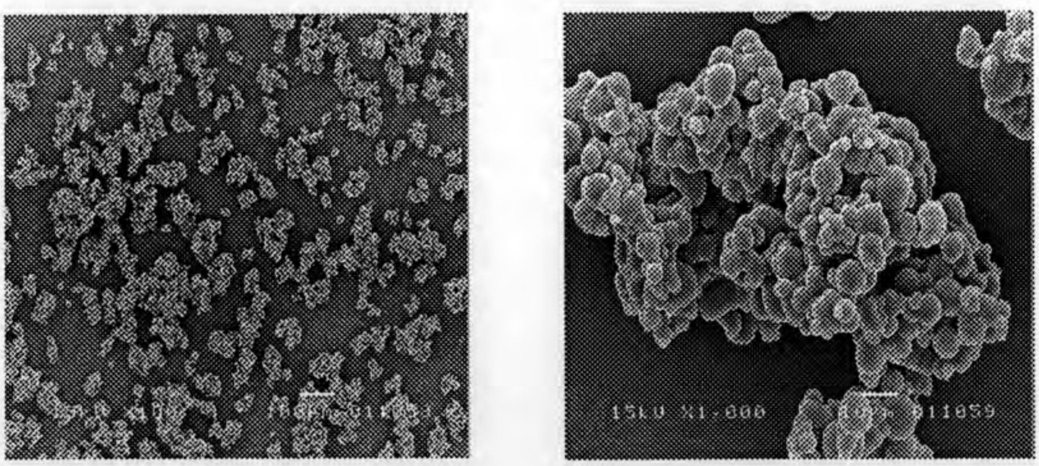


Figure 27 Percent moisture content of six formulation spray-dried tamarind pulp extract in collecting chamber

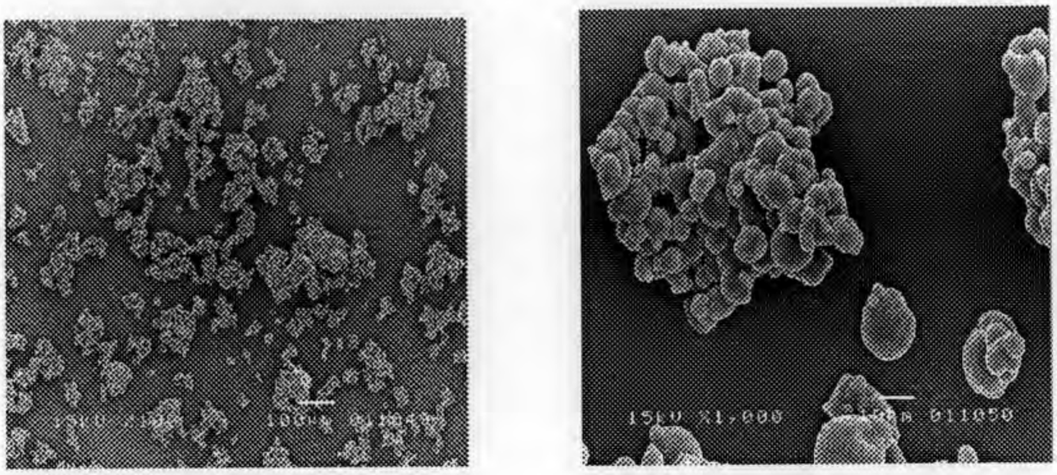
3.3 Morphology of spray-dried tamarind pulp extract

The shape and morphology of spray-dried tamarind pulp extract were observed by scanning electron microscope. The results of each experiment were shown below



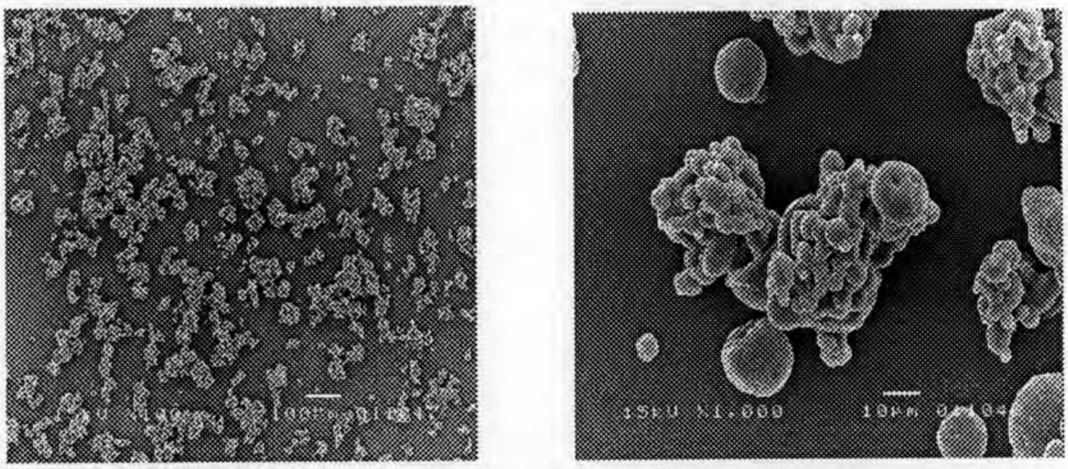
(a) (b)

Figure 28 Morphology of spray-dried tamarind pulp extract Experiment 1 Resolution x100 (a) and x 1000 (b)



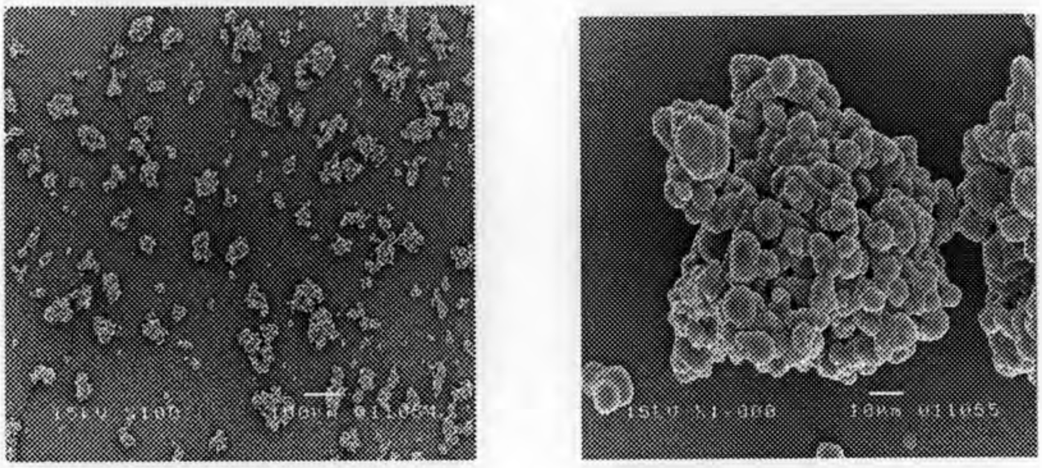
(a) (b)

Figure 29 Morphology of spray-dried tamarind pulp extract Experiment 3
Resolution x100 (a) and x 1000 (b)



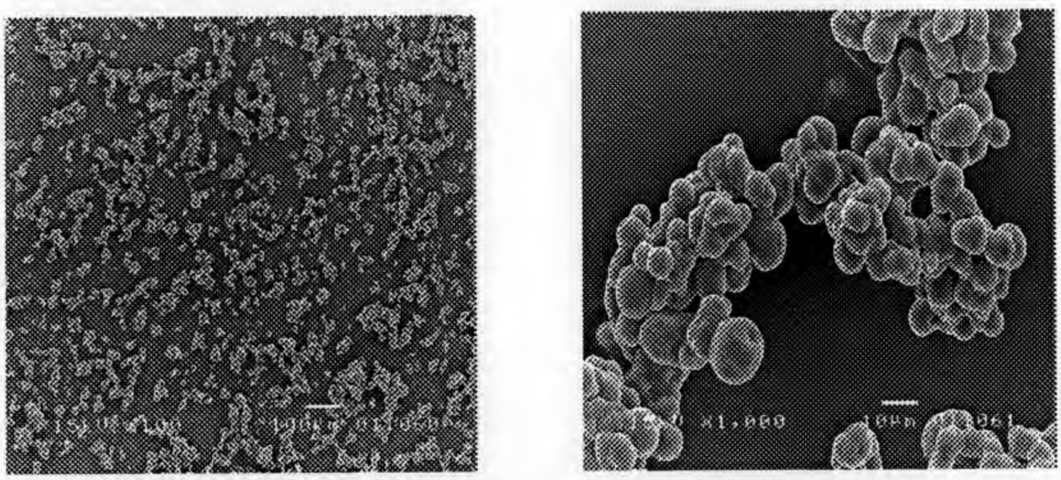
(a) (b)

Figure 30 Morphology of spray-dried tamarind pulp extract Experiment 17
Resolution x100 (a) and x 1000 (b)



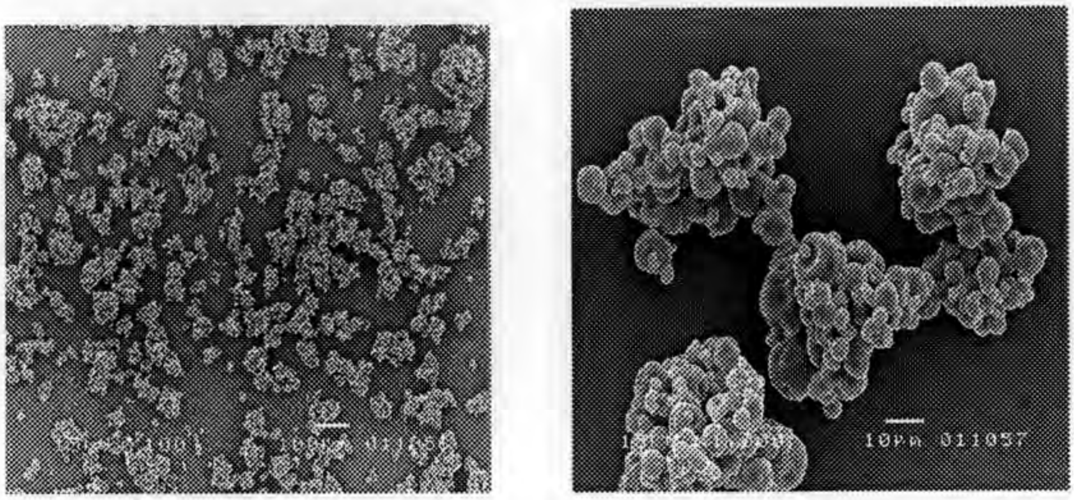
(a) (b)

Figure 31 Morphology of spray-dried tamarind pulp extract Experiment 19 Resolution x100 (a) and x 1000 (b)



(a) (b)

Figure 32 Morphology of spray-dried tamarind pulp extract Experiment 23 Resolution x100 (a) and x 1000 (b)



(a) (b)

Figure 33 Morphology of spray-dried tamarind pulp extract Experiment 24
Resolution x100 (a) and x 1000 (b)

Morphology of spray-dried tamarind pulp extract were investigated by SEM. Particles prepared from acacia as carrier were found to be almost spherical with smooth surface with aggregation as shown from experiment 17, 19, 23, 24 (Figure 30-33). The results demonstrated adherent of small particles in their surfaces and showed agglomerate of particles. These findings were similar to the previous investigations by Cano-chauca, (2005) and Shaikh et al., (2006).

Spray-dried tamarind pulp extract prepared from maltodextrin as carrier was found to be almost spherical with smooth surface with aggregation as showed in experiment 1 and 3 (Figure 28-29) This results also showed the adherent of small particle in their surface which agree with previous studied (Cano-chauca, (2005). The results demonstrated strong interaction among small particles on the surfaces and showed larger agglomerate of particle.

3.4 Particle sizes and size distribution

Generally, particles achievable from spray drying process had mean sizes of particles in range of 3-75 μm (Celik and Wendel, 2005). In this study, particle size [D 4,3] and size distribution (span) of spray-dried tamarind pulp extract were determined. Particle sizes were in range of 16.20 ± 0.09 to 91.45 ± 7.20 μm and the span value were in range 2.209 ± 0.013 to 4.414 ± 0.542 μm (Table 16).

Table 16 The particle sizes and size distribution of spray-dried tamarind pulp extract.

Experiment	Average particle size	
	D [4,3] ± SD (μm)	Span ± SD (μm)
1	36.67 ± 0.32	2.278 ± 0.022
3	91.45 ± 7.20	4.414 ± 0.542
17	23.34 ± 0.49	2.358 ± 0.028
19	22.36 ± 0.51	2.369 ± 0.014
23	16.20 ± 0.09	2.887 ± 0.006
24	36.60 ± 1.26	2.400 ± 0.028

Sizes determined by laser light scattering method were not correlated to size of particles determined by SEM method. Size of particles from SEM method was in range 3 to 15 μm, mostly size observe nearly to 10 μm. Size from laser light scattering method showed larger size than observed by SEM method. Since, it was aggregation of particles as shown in SEM method, thus, size of particles determined by laser light scattering method was not true size of particles but it related to clusters of particle size which were seen from SEM method.

In this study, suitable condition for spray drying tamarind pulp extract was found. Selection condition that gave good characteristic of particles from setting criteria, experiment 23, that gave particles with highest percent yield in collecting chamber of spray dryer, particles had low moisture content and still flow easily as previously kept in desiccator for 1 week. Experiment 23 acacia was used as carrier with concentration of 10 percent by weigh, instrument was setting at inlet temperature 110 °C, Fan setting 50 and feed rate level 3 (3 mL/min). The spray-dried tamarind pulp extract obtained from experiment 23 showed small aggregated particles with pale-yellow color as shown in figure 34.

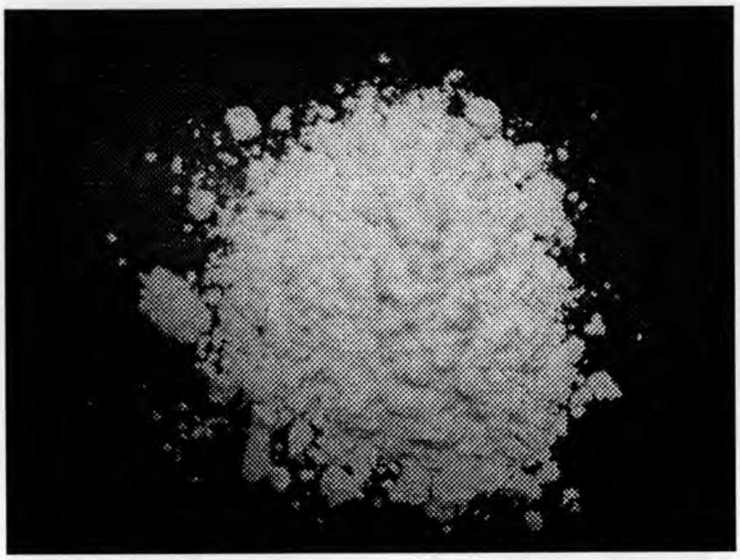


Figure 34 Spray-dried tamarind pulp extract powder from experiment 23

3.5 Determination of tartaric acid in spray-dried tamarind pulp extract

Content of tartaric acid in spray-dried tamarind pulp extract was determined by HPLC method. Content of tartaric acid in formulation 23 was 7.83 ± 0.13 % w/w.

3.6 Stability of spray-dried tamarind pulp extract

The stability of spray-dried tamarind pulp extract was determined by accelerated stability testing method. Formulation 23 was chosen for stability study. Spray-dried tamarind pulp extract was incubated at 40 ± 2 °C with relative humidity 75 ± 5 % for 6 months.. Concentrations of tartaric acid remaining in subsequent were calculated as percentage of initial concentration. The percent of tartaric acid remaining in spray-dried tamarind pulp extract after incubation for 0, 1, 2 3 and 6 months were presented in Figure 35.

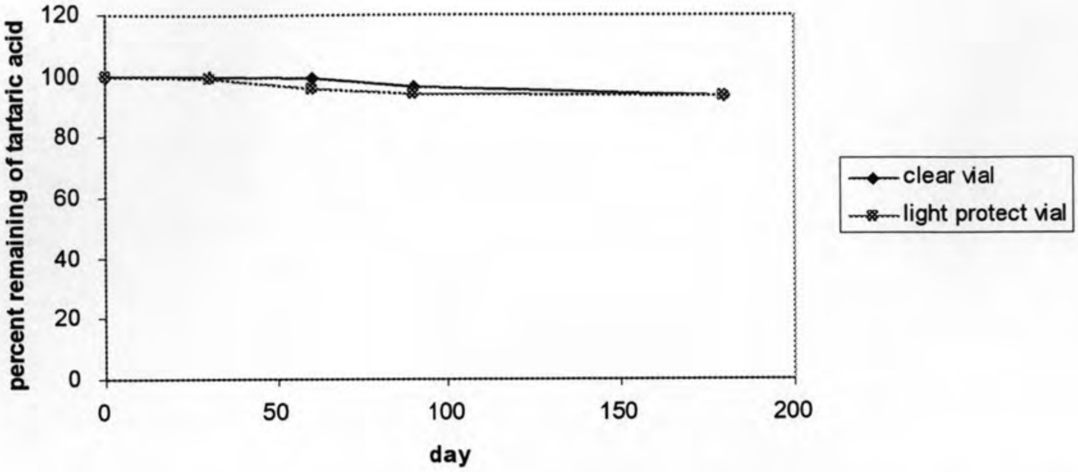


Figure 35 The percent remaining of tartaric acid in spray-dried tamarind pulp extract

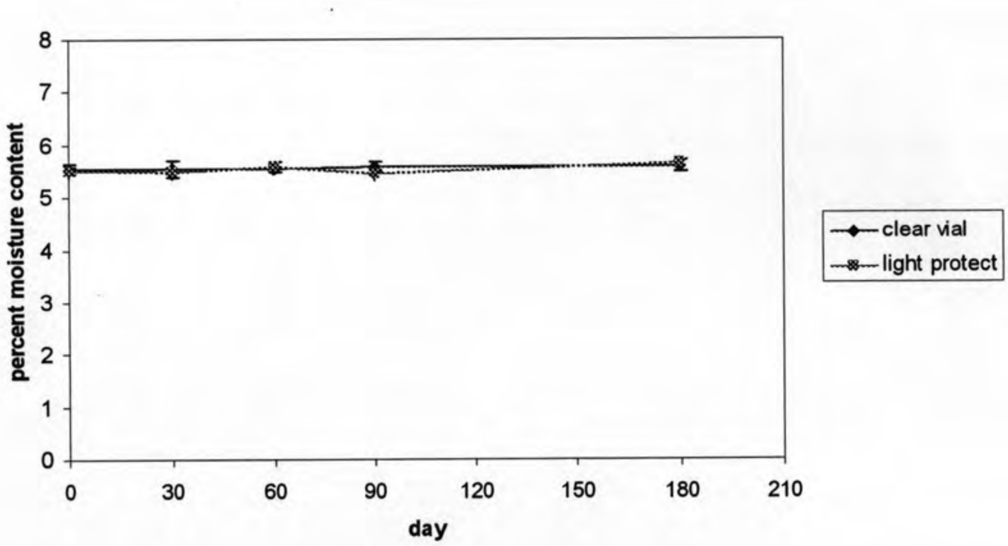


Figure 36 The percent moisture content in spray-dried tamarind pulp extract

Percent remaining of tartaric acid in spray-dried pulp extract which was kept in tightly closed vials and clear vials. It found that its percent remaining of tartaric acid slightly decreased comparing to light protected vial. But finally; at 6 months, the percent remaining of tartaric acid in both condition were decreased closely. The results were shown in figure 35. The percent remaining of tartaric acid appeared in spray-dried tamarind pulp extract were 93.16 from both clear and light protected vial.

Percentage moisture contents of spray-dried tamarind pulp extract after incubation at 40 ± 2 °C for 6 months, in both conditions of storage were slightly increased. The percent moisture contents of particles in clear vial increased from 5.54 to 5.59 and percentage moisture content of particle in light protect vial rising from 5.51 to 5.63, as shown in Figure 36.

Figure 37 Appearance of spray-dried tamarind pulp extract from clear vial.

Figure 38 Appearance of spray-dried tamarind pulp extract from light protected vial.

Spray-dried tamarind pulp extract obtained at initial day was pale yellow. Color of the particles from day 180 of stability testing became yellow, as shown in Figure37 and 38). The color of particles was not stable because tamarind pulp had reducing sugar and amino acid. Color of spray-dried tamarind pulp extract might change regarding to maillard reaction of reducing sugar and amino acid in tamarind pulp (Mottram, 2007).

In the study of the stability of spray-dried tamarind pulp extract, ICH guideline was followed (European medicines agency, 2006), stability testing of new drug substances and products. The significantly changing of product was found in spray-dried tamarind pulp extract, tartaric acid was changed more than 5 % in assay from its initial value, and discoloration of particles.

4. FORMULATION OF OIL-IN-WATER EMULSIONS CONTAINING SPRAY-DRIED TAMARIND PULP EXTRACT

4.1 Formulation of oil-in-water emulsion base

Formulations of oil-in-water emulsion base were developed. Appearances, skin feeling and physical properties of each formulation were investigated before and after 6 heating-cooling cycles. Formulations of oil-in-water emulsion are shown in table 5, the result as shown in tables 17-20.

In this study cyclomethicones was used for transient effects to give slightly lubricity, a slightly texture, fast sprading and good distribution of the product on application (Berthiaume, 1999; Blakely, 2001).

Steareth 2 and steareth 21 were selected, they were nonionic surfactants which they were widely used in topical pharmaceutical formulations. They were chemically stable in strongly acidic or alkaline conditions (Rowe, Sheskey and Weller, 2003). They got a great success with a pair of emulsifiers for a wide variety of lipids and mixtures of lipid (Courtney, 1997).

Formulation 1, steareth 2 and steareth 21 were used in concentration 4 and 2 % by weight, and 7 % by weight of cyclomethicone, this emulsions were stable after past accelerated test by temperature (heating cooling cycle for 6 cycles) but this formulation have sticky feeling (tackiness). So in formulation 2, steareth 2 was reduced to 2 % w/w, the results from steareth 2 reduction was unstable of oil-in-water emulsion. In formulation 3, steareth 2 was increased from 2 % w/w to 3 % w/w, at this concentration oil-in-water emulsion could be stable but skin feeling is not good. Stability of emulsion could be enhanced by increasing the viscosity of system. (Allen, 2002). In formulation 4-5, xantan gum was increased, xantan gum was used as viscosity enhancer in emulsion system but in this formulation it provided more sticky feeling in emulsion base and tamarind emulsion. In formulation 6, stiffening agent, cetly alcohol was increased from from 1 % w/w to 2 % w/w, it made more hardness of base and tamarind emulsion, but skin feeling of emulstion still not good. Formulation 7-9, polymethylmethacrylate, and cross-linked methylmethacrylate were used to help skin feeling problem, they reduced sticky feeling of base and tamarind emulsion. In this formulation, xantan gum was not used. Emulsion containing spray-dried tamarind pulp extract were not stable in heating cooling cycle test.

Cyclopentasiloxane and dimethicone crosspolymer were used for enhancing viscosity properties, it gave dry smoothness, non greasy skin feeling. It was used to improve thickening property of formulation. Formulation 10-13, Cyclopentasiloxane and dimethicone crosspolymer was added to formulation, it help stabilizing emulstion containing spray-dried tamarind pulp extract but it still gave sticky feeling. Formulation 14-17, only cyclopentasiloxane and dimethicone crosspolymer was used as thickening agent, it can stabilize emulsion containing spray-dried tamarind pulp extract in heating cooling cycle, and improved sticky feeling of emulsion. Formulation 18-19, steareth 2 was reduced from 3 % w/w to 2.6-2.8 % w/w it was shown unstable of emulsion containing spray-dried tamarind pulp extract. So, steareth 2 was used at 3 % w/w and steareth 21 was used at concentration 2 % w/w. Rice bran oil was medium chain triglyceride, it was used in formulation 20. Rice bran oil could improve skin feeling and smoothness of emulsion.

Table 17 Appearance of oil-in-water emulsion base before heating cooling cycle test

Formulation	1	2	3	4	5	6	7	8	9	10
Appearance										
Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Glossy	yes	yes	yes	no	no	no	no	no	no	no
Color	white	white	white	white	white	white	white	white	white	white
Odor	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly
Skin feeling										
Sticky	+++++	+++	+++	+++	++++	+++	+++	+++	++	+++
Spreadability	++++	+++	+++	+++	++++	++	++	++	++	++
Physical properties										
Viscosity (mPas ± SD)	1661.93 ± 80.61	570.14 ± 90.09	670.08 ± 24.52	3804.21 ± 344.59	3889.40 ±257.39	4009.00 ±131.18	4172.84 ± 184.36	1581.32 ± 55.09	1621.95 ± 68.02	521.97 ± 114.25
pH	3.20 ± 0.01	3.44 ± 0.21	3.45 ± 0.04	3.50 ± 0.04	4.32 ± 0.02	3.54 ± 0.02	4.62 ± 0.01	4.44 ± 0.01	4.58 ± 0.01	3.90 ± 0.06

Remark :

Sticky:
+ = little sticky
++ = good
+++ = normal
++++ = bad
+++++ = very stickly

Spreadability:
+ = very good
++ = good
+++ = normal
++++ = bad
+++++ = very bad

Table 17 Appearance of oil-in-water emulsion base before heating cooling cycle test (continue)

Formulation	11	12	13	14	15	16	17	18	19	20
Appearance										
Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Glossy	no	no	no	no	no	no	no	no	no	no
Color	white	white	white	white	white	white	white	white	white	white
Odor	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly
Skin feeling										
Sticky	+++	+++	+++	++	++	++	++	++	++	++
Spreadability	+++	+++	+++	++	+++	++	++	++	++	++
Physical properties										
Viscosity (mPas ± SD)	501.33 ± 106.59	677.78 ± 51.98	646.81 ± 54.01	613.39 ± 33.38	523.61 ± 13.63	483.31 ± 31.46	603.23 ± 85.70	567.19 ± 47.74	581.28 ± 53.12	497.07 ± 23.60
pH	3.84 ± 0.02	3.66 ± 0.06	3.95 ± 0.03	3.86 ± 0.03	3.37 ± 0.09	3.81 ± 0.27	4.27 ± 0.03	3.72 ± 0.08	4.10 ± 0.03	3.99 ± 0.02

Remark :

Sticky:
+ = little sticky
++ = good
+++ = normal
++++ = bad
+++++ = very stickly

Spreadability:
+ = very good
++ = good
+++ = normal
++++ = bad
+++++ = very bad

Table 18 Appearance of oil-in-water emulsion base after heating cooling cycle test

Formulation	1	2	3	4	5	6	7	8	9	10
Appearance										
Texture	smooth	-	smooth	smooth	smooth	smooth	smooth	smooth	-	smooth
Glossy	yes	-	yes	no	no	no	no	no	-	no
Color	white	-	white	white	white	white	white	white	-	white
Odor	slightly	-	slightly	slightly	slightly	slightly	slightly	slightly	-	slightly
Skin feeling										
Sticky	++++	-	+++	+++	++++	+++	+++	+++	-	+++
Spreadability	++++	-	+++	+++	++++	+++	+++	++	-	++
Physical properties										
Viscosity (mPas ± SD)	810.975 ± 49.67	-	262.789 ± 3.00	300.470 ± 12.45	433.175 ± 26.20	627.482 ± 25.86	790.987 ± 44.67	467.908 ± 22.99	-	357.812 ± 3.54
pH	3.36 ± 0.01	-	3.03 ± 0.01	3.17 ± 0.02	4.20 ± 0.01	3.26 ± 0.01	4.26 ± 0.01	4.14 ± 0.01	-	3.83 ± 0.02
Phase separation	no	yes	no	no	no	no	no	no	yes	no
Cycle of heating cooling test past	6	1	6	6	6	6	6	6	5	6

Remark :

Sticky:			Spreadability:		
+	=	little sticky	+	=	very good
++	=	good	++	=	good
+++	=	normal	+++	=	normal
++++	=	bad	++++	=	bad
+++++	=	very stickly	+++++	=	very bad

Table 18 Appearance of oil-in-water emulsion base after heating cooling cycle test (continue)

Formulation	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Appearance										
Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Glossy	yes	yes	yes	no	no	no	no	no	yes	no
Color	white	white	white	white	white	white	white	white	white	white
Odor	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly
Skin feeling										
Sticky	+++	++	++	++	++	++	++	++	++	++
Spreadability	+++	+++	+++	+++	+++	+++	++	+++	++	+++
Physical properties										
Viscosity (mPas ± SD)	380.421 ± 10.26	445.299 ± 17.69	515.420 ± 2.05	500.347 ± 27.24	428.916 ± 6.90	392.545 ± 21.68	336.186 ± 23.12	310.300 ± 38.79	399.426 ± 33.84	573.089 ± 76.67
pH	3.92 ± 0.08	3.39 ± 0.10	3.97 ± 0.06	3.80 ± 0.02	3.86 ± 0.03	3.94 ± 0.07	3.88 ± 0.03	4.16 ± 0.03	4.18 ± 0.06	4.08 ± 0.07
Phase separation	no	no	no	no	no	no	no	no	no	no
Cycle of heating cooling test past	6	6	6	6	6	6	6	6	6	6

Remark :

Sticky:
+ = little sticky
++ = good
+++ = normal
++++ = bad
+++++ = very stickly

Spreadability:
+ = very good
++ = good
+++ = normal
++++ = bad
+++++ = very bad

Table 19 Appearance of oil-in-water emulsion containing 8 % w/w tamarind pulp extract before heating cooling cycle test

Formulation	1 T	2 T	3 T	4 T	5 T	6 T	7 T	8 T	9 T	10 T
Appearance										
Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Glossy	yes	yes	yes	no	no	no	no	no	no	no
Color	cream	cream	cream	cream	cream	cream	cream	cream	cream	cream
Odor	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly
Skin feeling										
Sticky	+++++	++++	+++++	++++	++++	++++	++++	++++	+++	+++
Spreadability	+++++	+++	++++	+++	++++	+++	+++	+++	+++	++
Physical properties										
Viscosity (mPas ± SD)	848.657 ± 15.02	711.037 ± 24.74	737.250 ± 19.66	747.080 ± 26.21	815.890 ± 24.81	711.037 ± 34.52	797.541 ± 59.16	869.300 ± 11.05	821.133 ± 50.29	708.088 ± 76.04
pH	3.35 ± 0.05	3.49 ± 0.04	3.35 ± 0.04	3.37 ± 0.03	3.52 ± 0.02	3.23 ± 0.00	3.43 ± 0.02	3.35 ± 0.01	3.43 ± 0.01	3.74 ± 0.06

Remark :

Sticky:			Spreadability:		
+	=	little sticky	+	=	very good
++	=	good	++	=	good
+++	=	normal	+++	=	normal
++++	=	bad	++++	=	bad
+++++	=	very stickly	+++++	=	very bad

Table 19 Appearance of oil-in-water emulsion containing 8 % w/w tamarind pulp extract before heating cooling cycle test (continue)

Formulation	**11** **T**	**12** **T**	**13** **T**	**14** **T**	**15** **T**	**16** **T**	**17** **T**	**18** **T**	**19** **T**	**20** **T**
Appearance										
Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Glossy	no	no	no	no	no	no	no	no	no	no
Color	cream	cream	cream	cream	cream	cream	cream	cream	cream	cream
Odor	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly	slightly
Skin feeling										
Sticky	++++	+++	+++	+++	+++	++	++	++	++	++
Spreadability	+++	++	++	++	++	++	++	++	++	++
Physical properties										
Viscosity (mPas ± SD)	651.074 ± 30.73	771.655 ± 86.93	667.129 ± 11.35	556.050 ± 36.39	637.639 ± 19.52	672.700 ± 81.77	671.389 ± 56.32	442.350 ± 105.79	547.859 ± 21.86	487.240 ± 10.51
pH	3.51 ± 0.02	3.57 ± 0.03	3.48 ± 0.06	3.27 ± 0.04	3.31 ± 0.04	3.55 ± 0.12	3.35 ± 0.02	3.37 ± 0.01	3.35 ± 0.03	3.16 ± 0.02

Remark : Sticky:
+ = little sticky
++ = good
+++ = normal
++++ = bad
+++++ = very stickly

Spreadability:
+ = very good
++ = good
+++ = normal
++++ = bad
+++++ = very bad

Table 20 Appearance of oil-in-water emulsion containing 8 % w/w tamarind pulp extract after heating cooling cycle test

Formulation	**1 T**	**2 T**	**3 T**	**4 T**	**5 T**	**6 T**	**7 T**	**8 T**	**9 T**	**10 T**
Appearance										
Texture	smooth	-	smooth	smooth	smooth	smooth	-	-	-	smooth
Glossy	yes	-	yes	no	no	no	-	-	-	no
Color	cream	-	cream	cream	cream	cream	-	-	-	cream
Odor	slightly	-	slightly	slightly	slightly	slightly	-	-	-	slightly
Skin feeling										
Sticky	+++++	-	++++	++++	+++++	++++	-	-	-	++++
Spreadability	+++++	-	+++	++++	++++	+++	-	-	-	++++
Physical properties										
Viscosity (mPas ± SD)	570.146 ± 26.21	-	468.563 ± 24.74	498.053 ± 20.46	542.288 ± 54.11	540.650 ± 39.32	-	-	-	516.403 ± 10.09
pH	3.26 ± 0.05	-	3.35 ± 0.04	3.35 ± 0.03	3.37 ± 0.02	3.26 ± 0.04	-	-	-	3.39 ± 0.04
Phase separation	no	yes	no	no	no	no	yes	yes	yes	no
Cycle of heating cooling test past	6	1	6	6	6	6	5	5	5	6

Remark :

Sticky:			Spreadability:		
+	=	little sticky	+	=	very good
++	=	good	++	=	good
+++	=	normal	+++	=	normal
++++	=	bad	++++	=	bad
+++++	=	very stickly	+++++	=	very bad

Table 20 Appearance of oil-in-water emulsion containing 8 %w/w tamarind pulp extract after heating cooling cycle test (continue)

Formulation	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
	T	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**
Appearance										
Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	-	-	smooth
Glossy	no	no	no	no	no	no	no	-	-	no
Color	cream	cream	cream	cream	cream	cream	cream	-	-	cream
Odor	slightly	slightly	slightly	slightly	slightly	slightly	slightly	-	-	slightly
Skin feeling										
Sticky	++++	++++	++++	++	+++	+++	++	-	-	++
Spreadability	+++	+++	+++	++	++	++	++	-	-	++
Physical properties										
Viscosity (mPas ± SD)	384.025 ± 47.60	438.746 ± 54.40	501.330 ± 39.36	458.406 ± 52.96	491.828 ± 25.58	468.236 ± 26.40	539.667 ± 18.98	-	-	585.540 ± 39.78
pH	3.38 ± 0.06	3.30 ± 0.03	3.30 ± 0.02	3.24 ± 0.02	3.28 ± 0.02	3.34 ± 0.04	3.38 ± 0.02	-	-	3.35 ± 0.04
Phase separation	no	no	no	no	no	no	no	yes	yes	no
Cycle of heating cooling test past	6	6	6	6	6	6	6	3	3	6

Remark :

Sticky:				Spreadability:		
+	=	little sticky		+	=	very good
++	=	good		++	=	good
+++	=	normal		+++	=	normal
++++	=	bad		++++	=	bad
+++++	=	very stickly		+++++	=	very bad

4.2 Stability study of oil-in-water emulsions containing spray-dried tamarind pulp extract

4.2.1 Physical stability of emulsion

The formulation 20T, oil-in-water emulsions containing 8 % w/w spray-dried tamarind pulp extract, and formulation 20, oil-in-water emulsions base, were chosen to study stability at ambient temperature for 6 weeks. Appearances, physical properties and percent remaining of tartaric acid in emulsion were investigated. Viscosity of oil-in-water emulsions base was slightly decreased and pH of emulsion was slightly increased. Phase separation was not observed. The appearance and skin feeling were not changed. Results are demonstrated in Table 21 and 22.

Table 21 Physical stability of oil-in-water emulsion base formulation 20

Cream base Rx20	day 0	2 wk	4 wk	6wk
Appearance				
texture	smooth	smooth	smooth	smooth
Glossy	no	no	no	no
Color	white	white	white	white
Odor	slightly	slightly	slightly	slightly
Skin feeling				
Ssticky	++	++	++	++
Spreadability	++	++	++	++
Physical properties				
Viscosity (mPas ± SD)	3800.627 ± 66.31	3502.943 ± 39.36	3004.02 ± 263.06	2663.327 ± 160.04
pH	4.29 ± 0.02	4.43 ± 0.08	4.41 ± 0.19	4.65 ± 0.07
Phase separation	no	no	no	no

Remark : Sticky + = very sticky; ++ = good; +++ = normal; ++++ = bad; +++++ = very sticky

Spreadability + = very good; ++ = good; +++ = normal; ++++ = bad; +++++ = very bad

Oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract, formulation 20T, showed stable viscosity and slightly decreased in pH of emulsion. Phase separation was not occurred. The results were not changed in appearance. Color of emulsion was not changed from browning reaction of reducing sugar and amino acid that consisted in tamarind pulp, and skin feeling was slightly sticky. Results are demonstrated in table 21.

Table 22 Physical stability of oil-in-water emulsions containing 8 % w/w spray-dried tamarind pulp extract formulation 20T

Tamarind cream Rx 20T	day 0	2 wk	4 wk	6wk
Appearance				
texture	smooth	smooth	smooth	smooth
Glossy	no	no	no	no
Color	cream	cream	cream	cream
Odor	slightly	slightly	slightly	slightly
Skin feeling				
Sticky	++	++	++	++
Spreadability	++	++	++	++
Physical properties				
Viscosity (mPas ± SD)	2244.95 ± 56.87	2153.81 ± 25.58	2250.62 ± 44.13	2252.02 ± 12.37
pH	3.52 ±0.06	3.50 ± 0.06	3.45 ± 0.24	3.46 ± 0.22
Phase separation	no	no	no	no

Remark : Sticky + = very sticky; ++ = good; +++ = normal; ++++ = bad; +++++ = very sticky

Spreadability + = very good; ++ = good; +++ = normal; ++++ = bad; +++++ = very bad

4.2.2 Chemical stability of emulsions

Oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract, formulation 20T was kept in closed container and placed at ambient temperature for 6 weeks. Tartaric acid in product was determined by HPLC method. Percent remaining of tartaric acid was calculated. Percent remaining of tartaric acid was slightly decreased from 100 % w/w to 99.75% w/w as shown in Figure 39.

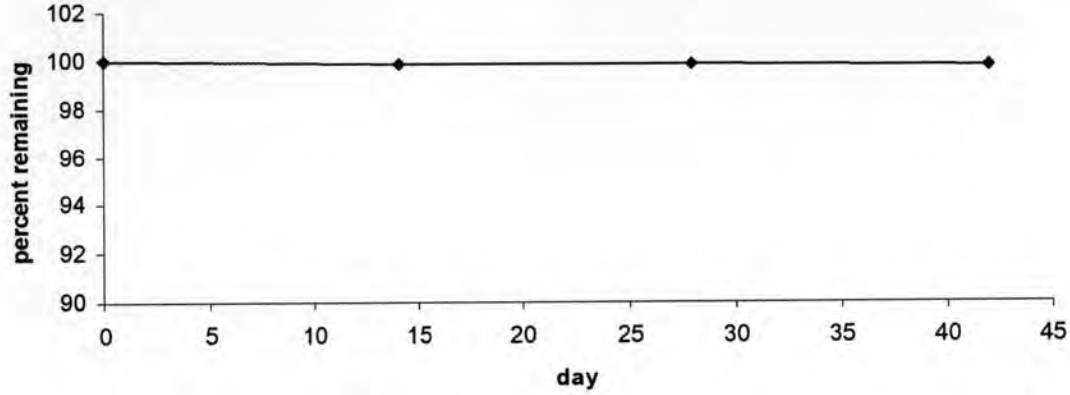


Figure 39 Percent remaining of tartaric acid in oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract at ambient temperature for 6 week

In this study, oil-in-water emulsion base formulation 20 and oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract formulation 20T were stable in physical and chemical properties at ambient temperature for 6 weeks.

5. STUDY OF WHITENING EFFICACY TEST IN VOLUNTEERS

Whitening and moisturizing efficacies of oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract were investigated. Thirty five healthy female volunteers, ages in range 20 – 45 years were enrolled in this study. Treatment emulsion was oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract, formulation 20T and control emulsion was oil-in-water emulsion, formulation 20. Volunteers received both formulation (20 and 20T) every week. Emulsions were applied on forehead of volunteers. This test is half forehead test, the amount of 0.5 g of each oil-in-water emulsion base or oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract was applied on each side of forehead twice daily after shower in the morning and evening everyday. The melanin value and moisture value at fixed area of forehead was measured once per weeks using probe of

Mexameter® MX18 and Corneometer®. Data were analysed using pair t-test statistic. Each group of data compared with their base line at beginning time (day 0) before starting use emulsions.

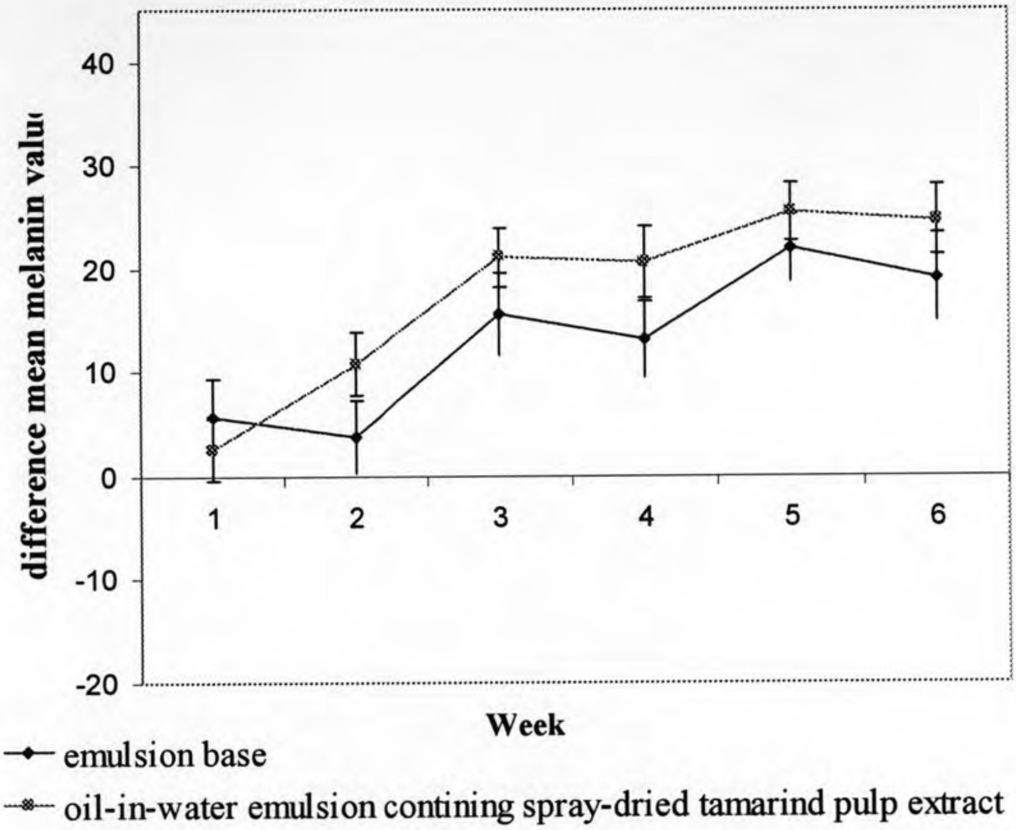


Figure 40 Difference mean melanin value during 6 weeks

Difference mean melanin values were shown in Figure 40. Mean melanin values measured from volunteers using oil-in-water base emulsion containing 8 % w/w spray-dried tamarind pulp extract were more significantly difference than oil-in-water emulsion base in lightening effect at 4 weeks (p=0.035) (Table 50, Appendix D). Lightening efficacy of oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract was not shown immediate effect. It might due to concentration of tartaric acid that contained in emulsion was lower than actual concentration of alpha hydroxyl acid when calculation from 8 % w/w spray-dried tamarind pulp extract.

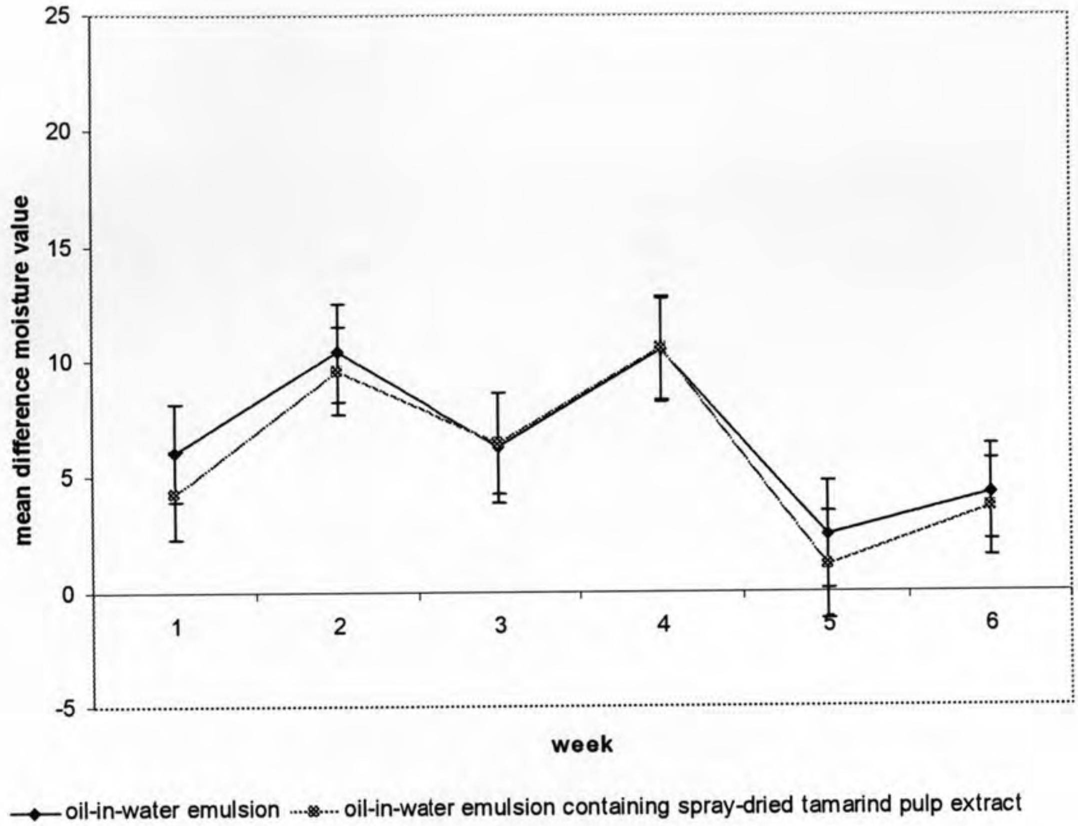


Figure 41 Difference mean moisture value during 6 weeks

Mean moisture values of oil-in-water emulsion base and oil-in-water emulsion containing 8 % w/w spray-dried tamarind pulp extract were increased from the first week and showed significantly difference after using emulsion containing 8 % w/w spray-dried tamarind pulp extract in 3 weeks (p=0.000) when compared with oil-in-water emulsion base. Difference mean moisture value during 6 weeks was shown in Figure 41.

In this study, five volunteers reported skin feeling after using product. Four volunteers were found that they had redness and itching in forehead area when using oil-in-water emulsion containing 8% w/w spray-dried tamarind pulp extract. This skin irritation effect appeared when applying products on skin and disappeared within half an hour. Nevertheless, effect from using oil-in-water emulsion containing 8% w/w spray-dried tamarind pulp extract happened only at first week after using the product. After that, volunteers have never found redness and itching at that area again. Skin itching described by appearing skin condition in which there was abnormal keratinization (Johnson, 2002) and burning itch sensed from disruption of skin pH which was far away from the physiological pH. To avoid irritation as much as possible, it was desired to formulate a cosmetic preparation at pH close to the normal range of pH of the skin. This might achieve by partial neutralization and by the addition of an effective buffer. However, data suggested that neutral pH of alpha hydroxyl acid products induced little effect on skin (Pierard et al., 2000). One of volunteers (2.86 %) was found redness and itching in forehead area that using oil-in-water emulsion containing 8% w/w spray-dried tamarind pulp extract. This effect was found after using product and disappeared within half an hour.