CHAPTER 3

RESULTS AND DISCUSSION

3.1 Relationship among Carbon Dioxide Concentration with Other Parameters for Standard Curve

The parameters of wine fermentation without nitrogen source supplementation were investigated. The relationships among carbon dioxide concentration and alcohol, reducing sugar, biomass and total soluble solid (^{o}Brix) were plotted.

Table 3.1 Parameters of Wine Fermentation without the Addition of Ammonium Salts.

Time (h)	TSS (^{o}Brix)	pH	RS (g/L)	Biomass (g/L)	Alcohol (g/L)	CO_2 (g/L)	CO_2 (g/L-h)
0	17.8	3.90	193.81	0.13	0.00	0.0000	0.0000
3	17.6	3.89	186.90	0.20	0.40	0.0000	0.0000
6	17.5	3.84	181.14	0.23	0.40	0.0980	0.0163
9	17.5	3.82	175.57	0.31	1.20	0.2380	0.0264
12	17.4	3.85	172.49	0.62	2.80	1.1700	0.0975
13	17.4	3.74	169.72	0.57	3.84	1.3850	0.1065
14	17.3	3.71	166.73	0.37	5.04	1.6920	0.1209
15	17.2	3.70	158.86	0.47	5.52	2.0500	0.1367
16	17.1	3.69	155.20	0.57	6.24	2.5300	0.1581
17	17.1	3.66	153.67	0.62	6.40	3.3380	0.1964
18	16.7	3.63	150.98	0.49	6.56	3.6180	0.2000
19	16.6	3.65	147.52	0.49	6.72	4.1130	0.2165

Table 3.1 (continued).

Time (h)	TSS (°Brix)	pH	RS (g/L)	Biomass (g/L)	Alcohol (g/L)	CO_2 (g/L)	CO_2 (g/L-h)
20	16.4	3.69	144.64	0.37	7.20	5.0120	0.2506
21	16.2	3.62	140.61	0.40	8.00	5.5640	0.2650
22	16.0	3.58	137.92	0.45	8.96	6.4170	0.2917
23	15.6	3.56	134.84	0.48	9.84	7.6380	0.3321
24	15.5	3.56	132.25	0.52	10.56	8.6430	0.3601
26	15.2	3.54	128.44	0.57	13.20	10.6650	0.4102
28	15.0	3.50	125.43	0.64	15.04	12.3880	0.4424
30	14.5	3.51	121.97	0.71	18.48	15.6570	0.5219
33	14.1	3.49	119.48	0.73	22.56	18.6080	0.5639
36	13.5	3.56	109.49	0.75	25.92	22.6800	0.6300
39	12.3	3.54	96.81	0.81	30.40	26.7420	0.6857
42	11.6	3.43	94.12	0.89	35.12	31.3780	0.7471
45	11.3	3.45	77.99	0.95	39.52	35.9840	0.7996
48	11.0	3.50	72.61	1.06	43.52	39.9160	0.8316
54	9.5	3.44	64.73	1.20	49.44	47.5370	0.8803
60	9.2	3.60	54.17	1.41	55.84	51.8600	0.8643
66	8.4	3.55	39.95	2.11	62.64	59.4500	0.9008
72	7.5	3.62	24.39	1.63	64.72	64.9770	0.9025
78	7.1	3.54	21.90	1.83	65.68	68.0960	0.8730
84	6.7	3.47	18.35	1.90	68.00	69.9050	0.8322

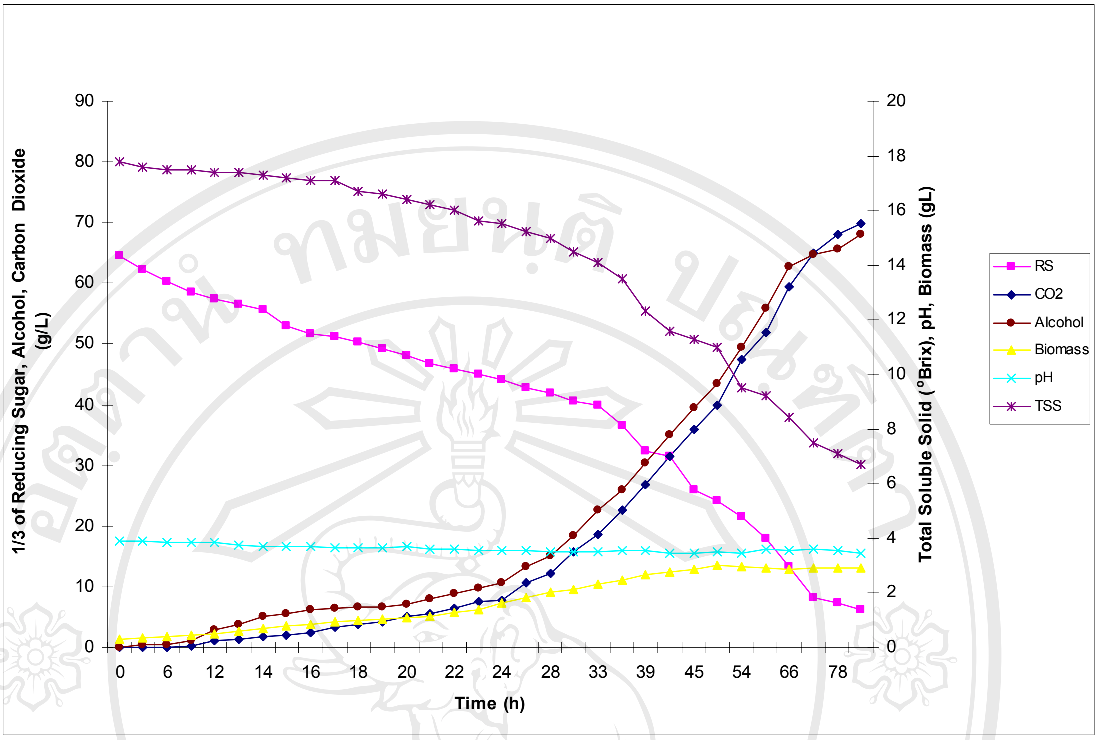

Figure 3.1 Changes of pH, Reducing Sugar, Biomass, Alcohol, Carbon Dioxide Concentration and Total Soluble Solid along with Time during Wine Fermentation without the Addition of Ammonium Salts.

Table 3.1 and figure 3.1 showed pH, reducing sugar, biomass, alcohol, carbon dioxide concentration, and total soluble solid during wine fermentation without supplementation of ammonium salt. Reducing sugar decreased sharply from 193.81 to 24.39 g/L in 72 h and, then, flattened to 18.35 g/L at the end of fermentation. The pH decreased from 3.90 to 3.49 in 39 h of fermentation and, then, remained more or less constant at the end of fermentation. The total soluble solid decreased from 17.8 to 6.7 °Brix at the end of fermentation. Biomass concentration increased from 0.13 to 2.11 g/L after 66 h of fermentation, then, decreased to 1.63 g/L at 72 h of fermentation and further increased to 1.90 g/L at the end of fermentation. Alcohol content increased from 0 to 10.56 g/L after 24 h of fermentation. Then, it increased sharply to 64.72 g/L at 72 h of fermentation. After that, it increased to 68.00 g/L at the end of fermentation. Carbon dioxide concentration increased from 0 to 8.6430 g/L after 24 h of fermentation. After that, it increased sharply to 68.0960 g/L at 78 h of fermentation and then flattened to the end of fermentation.

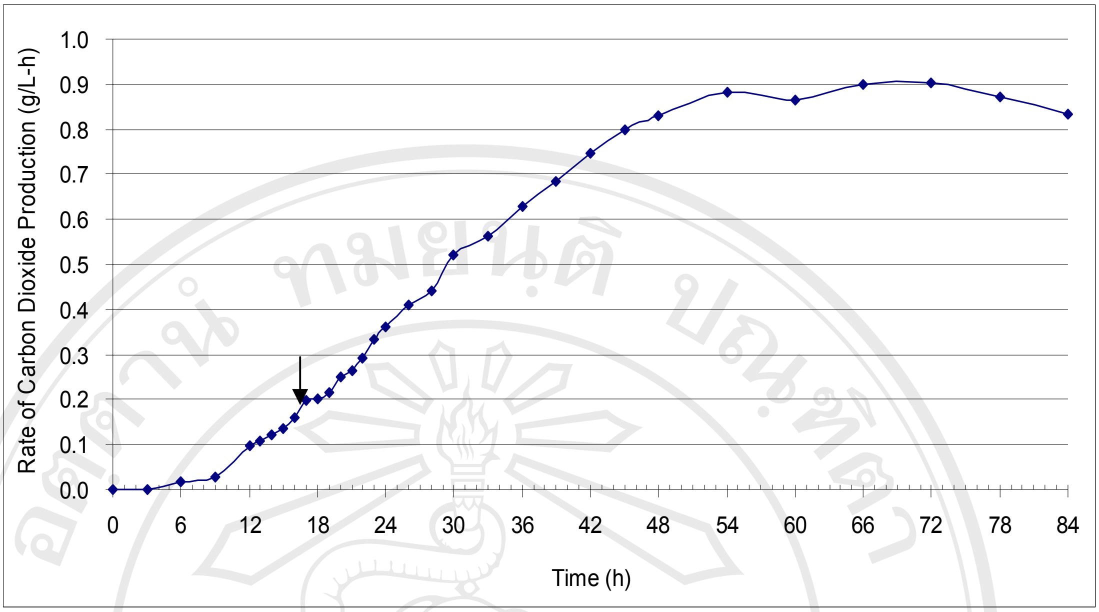

Figure 3.2 Relationship between Time and Rate of Carbon Dioxide Production.

Figure 3.2 showed the rate of carbon dioxide production. It was found that the rate of carbon dioxide production increased during 17 h of fermentation. Then, it dropped very slowly until 19 hrs which is caused by nitrogen deficiency in wine fermentation, then continuous increased sharply until 54 h of fermentation. Then, the rate of carbon dioxide production was constant until the end of fermentation.

Figure 3.3 to figure 3.6 showed the relationship among carbon dioxide concentration (CO_2) with alcohol (alc), reducing sugar (RS), biomass (X) and total soluble solid (TSS). Their equations and R-squares are as follows:

$y_{co_2/alc} = - 0.0053X^2 + 1.5148X$ ($R^2 = 0.9961$)

$y_{co_2/RS} = - 2x10^{-6}X^5 + 0.0003X^4 – 0.0217X^3 + 0.6587X^2 – 10.255X + 182.48$ ($R^2 = 0.9937$)

$y_{co_2/X} = - 6x10^{-9}X^5 + 6x10^{7}X^4 + 3x10^{-6}X^3 - 0.0011X^2 + 0.0376X + 0.3323$ ($R^2 = 0.941$)

$y_{co_2/TSS} = 0.0008X^2 – 0.2043X + 17.503$ ($R^2 = 0.9963$)

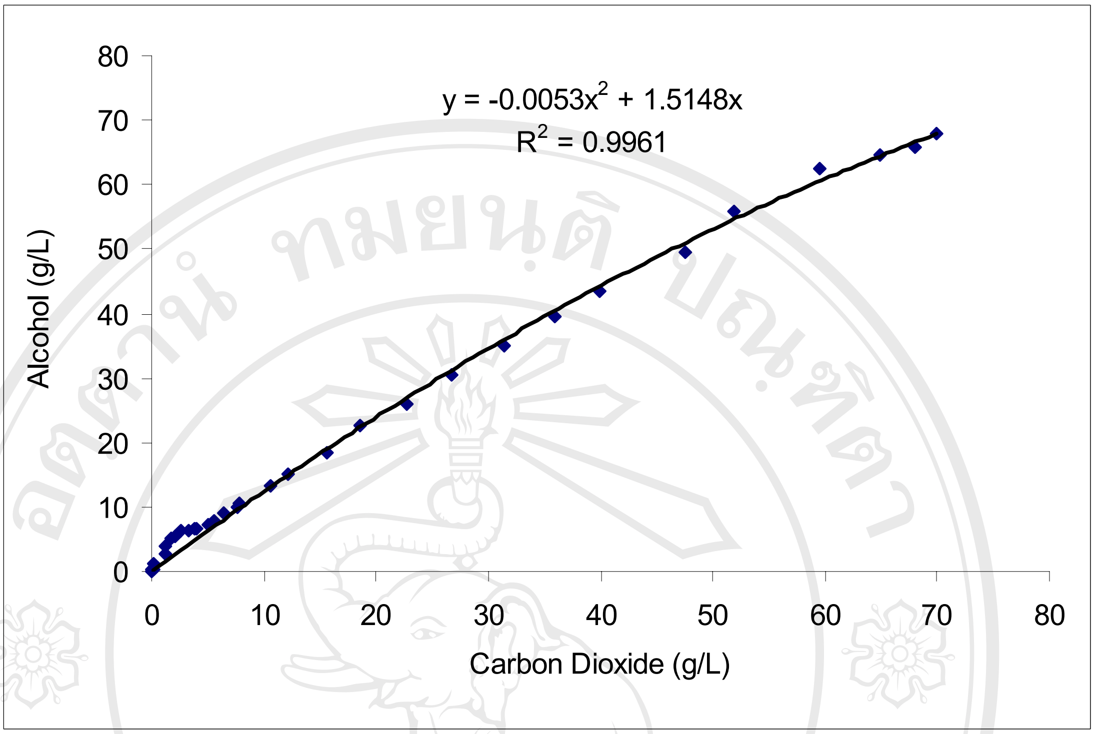

Figure 3.3 Relationship between Alcohol and Carbon Dioxide Concentrations.

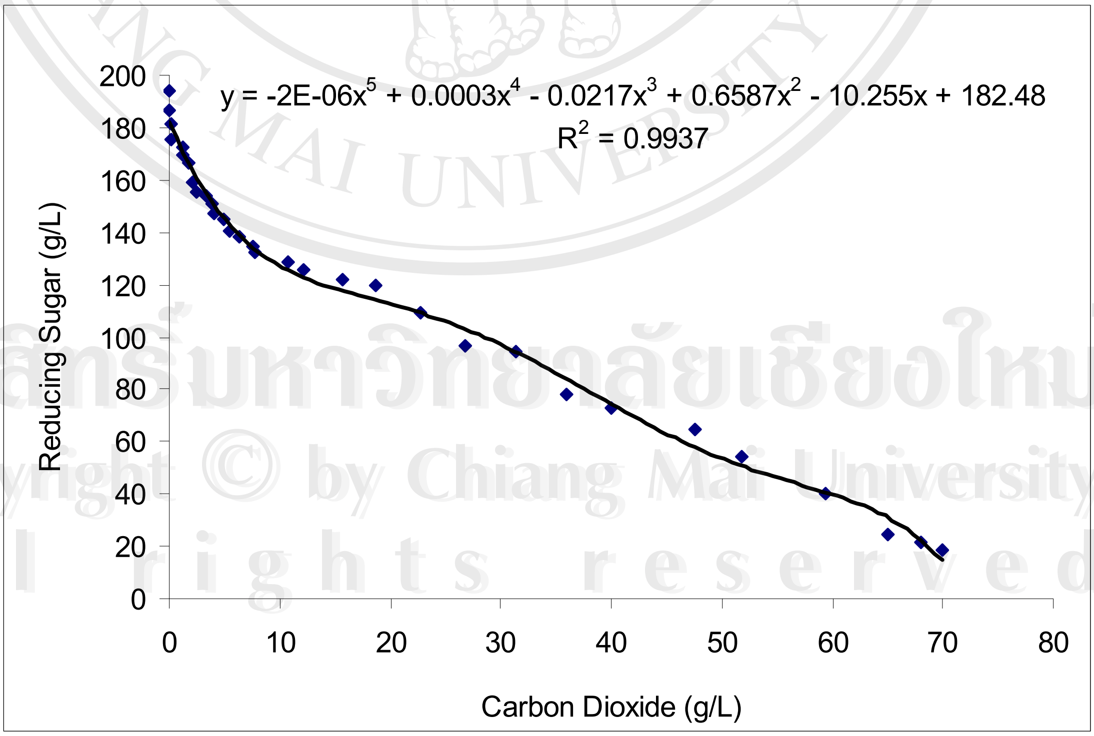

Figure 3.4 Relationship between Reducing Sugar and Carbon Dioxide Concentrations.

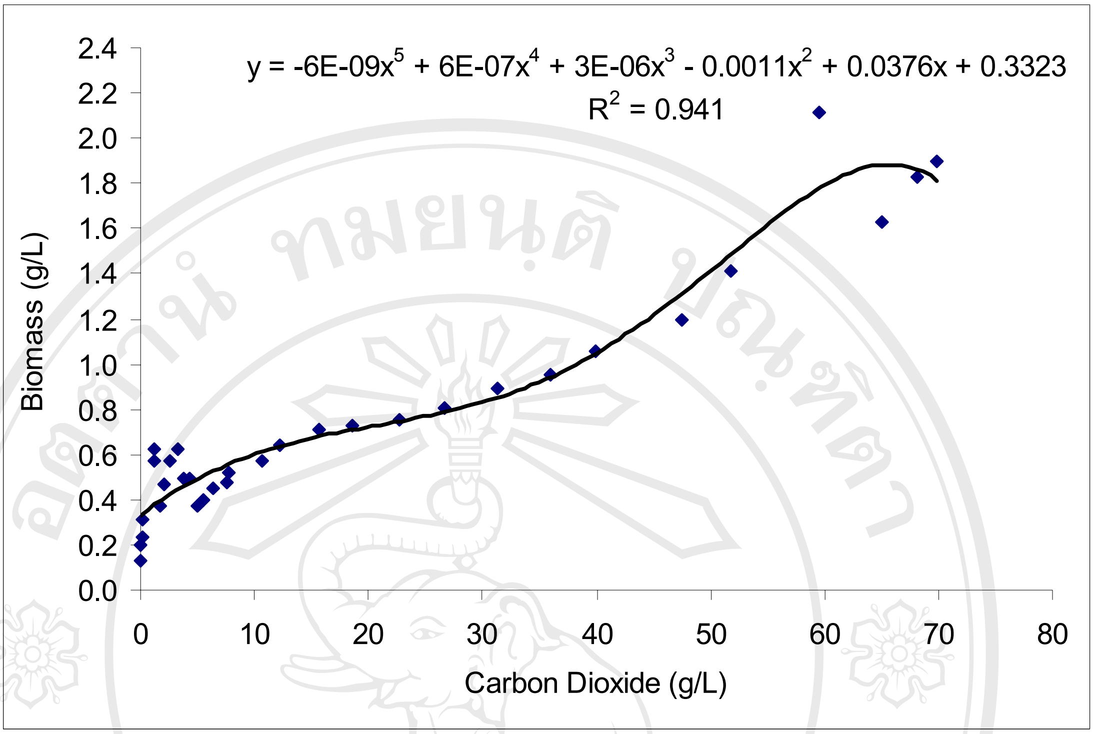

Figure 3.5 Relationship between Biomass and Carbon Dioxide Concentrations.

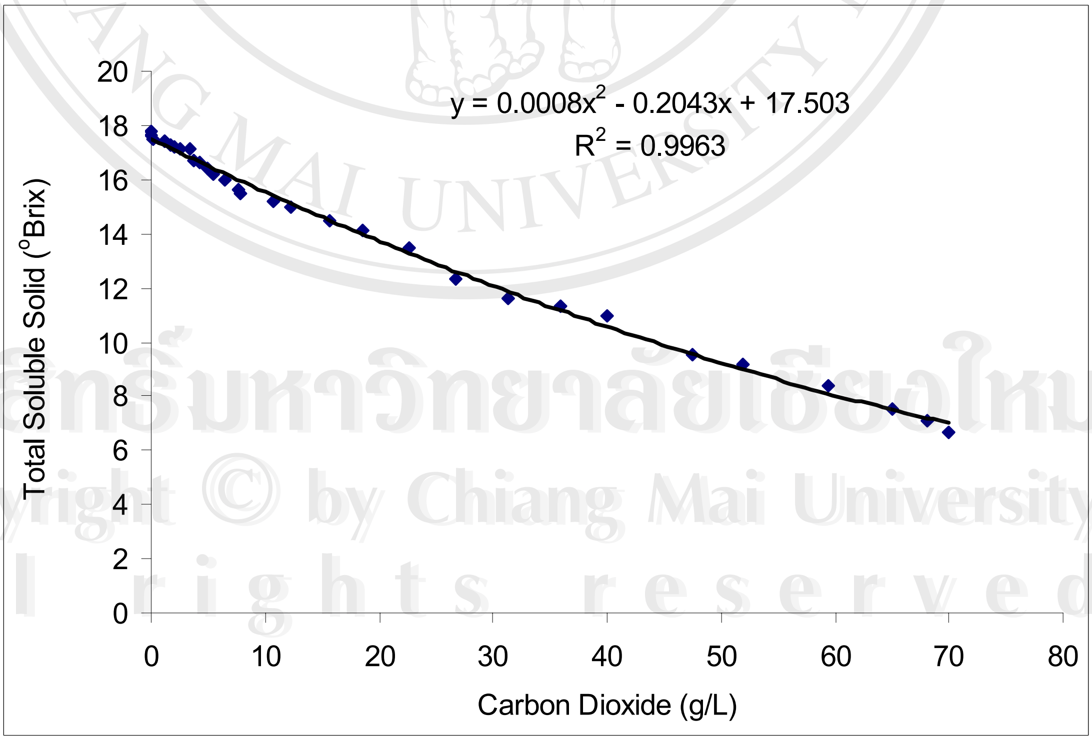

Figure 3.6 Relationship between Total Soluble Solid and Carbon Dioxide Concentration.

3.2 The Suitable Time for Ammonium Salts Supplementation in Wine Fermentation

Slow fermentation is caused by nitrogen deficiency which limits yeast growth and its metabolism. So, the timing of nitrogen addition is very important in the development of wine fermentation. From figure 3.2 shown nitrogen deficiency after 17 hrs of fermentation. In this step, the influences of wine fermentation without and with supplementation of ammonium phosphate at the beginning and after 17 hrs of fermentation were investigated.

Table 3.2 Carbon Dioxide Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Time (h)	Carbon dioxide (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
0	0.00	0.00	0.00
3	0.00	0.00	0.00
6	0.19	0.17	0.19
9	0.39	0.39	0.39
10	0.67	0.65	0.67
11	0.89	0.87	0.89
12	1.19	1.15	1.19
13	1.49	1.44	1.49
14	1.81	1.72	1.81
15	2.24	2.09	2.24
16	2.82	2.64	2.82
17	2.88	3.16	2.88
18	3.59	4.14	3.96
19	4.12	4.76	5.15
20	4.68	5.44	6.20
21	5.44	6.16	6.96
22	6.19	6.97	7.77
23	7.02	7.81	8.61

Table 3.2 (continued).

Time (h)	Carbon Dioxide (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
24	7.96	8.83	9.63
26	9.75	10.82	11.62
28	11.25	12.89	13.71
30	13.00	14.93	16.08
33	16.32	18.48	19.44
36	19.99	22.36	23.21
39	23.36	26.10	27.24
42	27.38	30.18	31.41
45	31.12	34.25	36.10
48	34.62	38.01	40.63
54	40.81	44.54	48.22
60	46.90	50.98	56.88
66	52.73	57.06	63.90
72	57.67	62.20	68.40
78	62.68	67.35	71.47
84	67.08	71.47	75.08

The profiles of wine fermentation without and with supplementation 700 ppm of ammonium dihydrogen phosphate at the beginning of the fermentation and after 17 h of fermentation were as follows :

Table 3.2 and figure 3.7 showed carbon dioxide concentrations during wine fermentation. It was found that carbon dioxide concentration increased gradually from 0 to 67.08, 71.47 and 75.08 g/L, respectively.

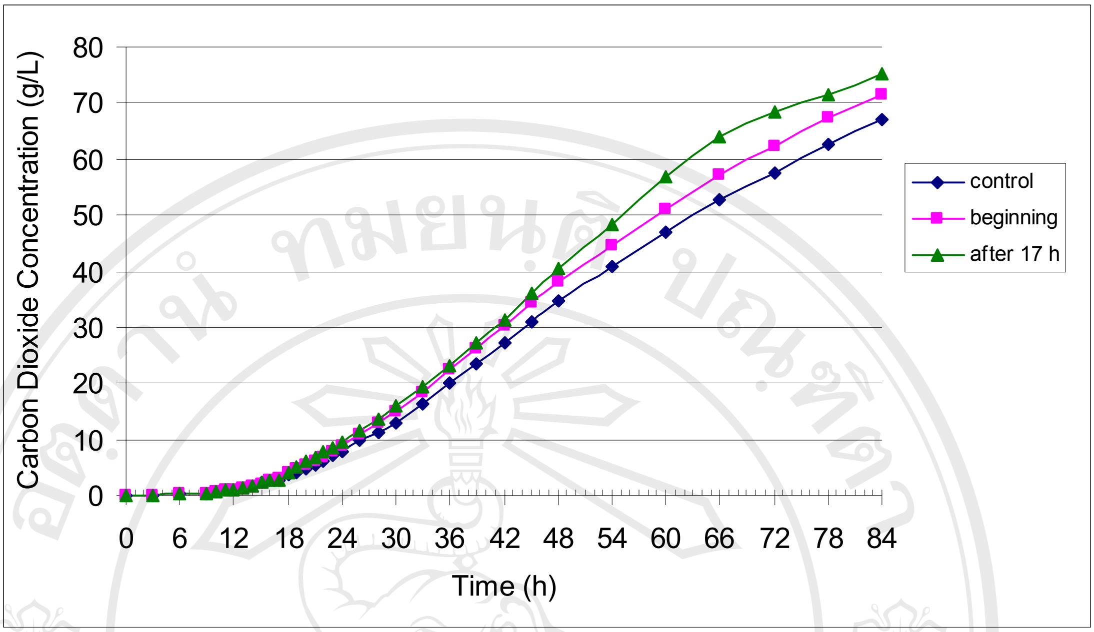

Figure 3.7 Carbon Dioxide Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

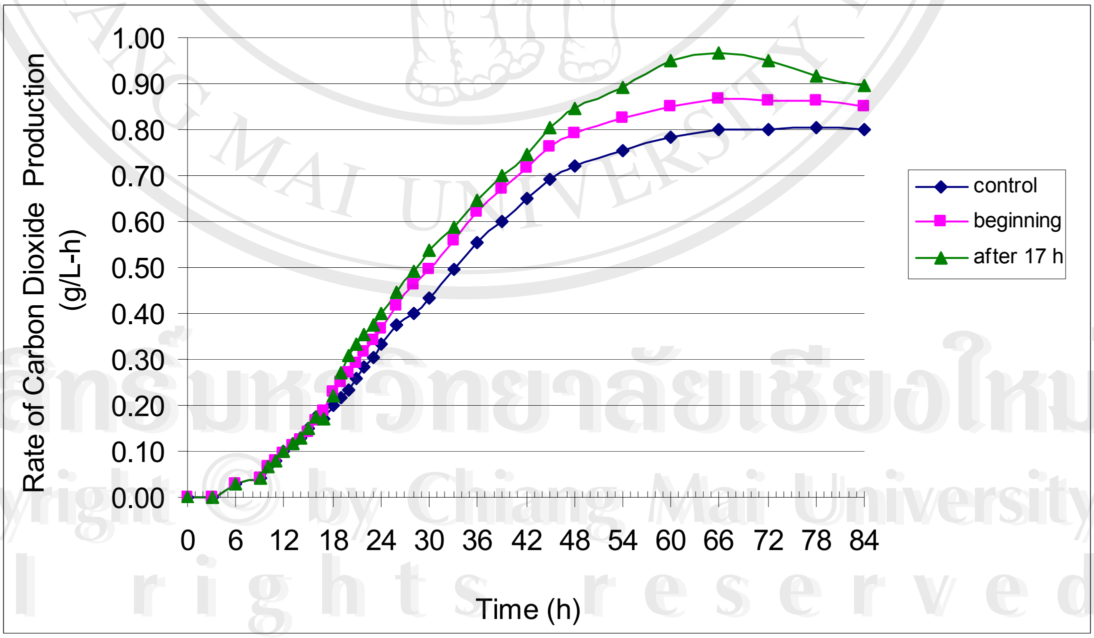

Figure 3.8 Rates of Carbon Dioxide Production during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Table 3.3 Rates of Carbon Dioxide Production during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Time (h)	Rates of Carbon Dioxide (g/L-h)		
	Control	Time of Supplementation (h)	
		0 h	17 h
0	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000
6	0.0308	0.0289	0.0308
9	0.0437	0.0435	0.0437
10	0.0668	0.0652	0.0668
11	0.0808	0.0792	0.0808
12	0.0988	0.0957	0.0988
13	0.1147	0.1109	0.1147
14	0.1290	0.1231	0.1290
15	0.1493	0.1396	0.1493
16	0.1764	0.1651	0.1764
17	0.1692	0.1859	0.1692
18	0.1996	0.2298	0.2198
19	0.2168	0.2504	0.2712
20	0.2338	0.2720	0.3101
21	0.2588	0.2935	0.3316
22	0.2813	0.3168	0.3531
23	0.3051	0.3396	0.3743
24	0.3316	0.3679	0.4012
26	0.3750	0.4162	0.4470
28	0.4017	0.4605	0.4896
30	0.4334	0.4977	0.5359
33	0.4945	0.5601	0.5891
36	0.5554	0.6210	0.6446
39	0.5991	0.6693	0.6984
42	0.6519	0.7186	0.7478

Table 3.3 (continued).

Time (h)	Rates of Carbon Dioxide (g/L-h)		
	Control	Time of Supplementation (h)	
		0 h	17 h
45	0.6914	0.7611	0.8023
48	0.7212	0.7919	0.8464
54	0.7557	0.8248	0.8929
60	0.7817	0.8497	0.9481
66	0.7990	0.8646	0.9682
72	0.8010	0.8639	0.9500
78	0.8036	0.8634	0.9163
84	0.7986	0.8509	0.8938

Table 3.3 and figure 3.8 showed rates of carbon dioxide production during wine fermentation. It was found that the maximum rates of carbon dioxide productions of wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning and after 17 h of fermentation increased gradually from 0 to 0.8036, 0.8646 and 0.9682 g/L-h after 78, 66 and 66 h of fermentation, respectively.

Table 3.4 Alcohol Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Time (h)	Alcohol (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
0	0.00	0.00	0.00
3	0.00	0.00	0.00
6	0.27	0.26	0.27
9	0.57	0.59	0.57
10	0.98	0.99	0.98
11	1.30	1.32	1.30
12	1.73	1.73	1.73
13	2.17	2.17	2.17
14	2.63	2.60	2.63
15	3.25	3.15	3.25
16	4.09	3.96	4.09
17	4.17	4.73	4.17
18	4.86	6.18	5.91
19	5.45	7.09	7.66
20	6.17	8.08	9.19
21	7.15	9.13	10.29
22	8.12	10.30	11.45
23	9.18	11.51	12.65
24	10.38	12.96	14.10
26	12.63	15.77	16.89
28	14.49	18.65	19.77
30	16.65	21.44	22.98
33	20.64	26.19	27.45
36	24.94	31.22	32.30
39	28.78	35.93	37.33
42	33.21	40.89	42.35

Table 3.4 (continued)

Time (h)	Alcohol (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
		700 ppm	700 ppm
45	37.19	45.67	47.79
48	40.81	49.93	52.80
54	46.93	56.97	60.73
60	52.59	63.47	69.04
66	57.69	69.20	75.18
72	61.76	73.74	78.84
78	65.65	78.01	81.22
84	68.88	81.23	83.89

Table 3.4 and figure 3.9 showed alcohol concentrations during wine fermentation. It was found that alcohol concentrations of wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning and after 17 h of fermentation increased gradually from 0 to 68.88, 81.23 and 83.89 g/L, respectively. The specific rates of substrate formation (q_p) and yield coefficients of product formation from substrate ($Y_{p/s}$) were 0.013, 0.016, 0.021 g/L-h and 0.397, 0.444, 0.443 g/g, respectively.

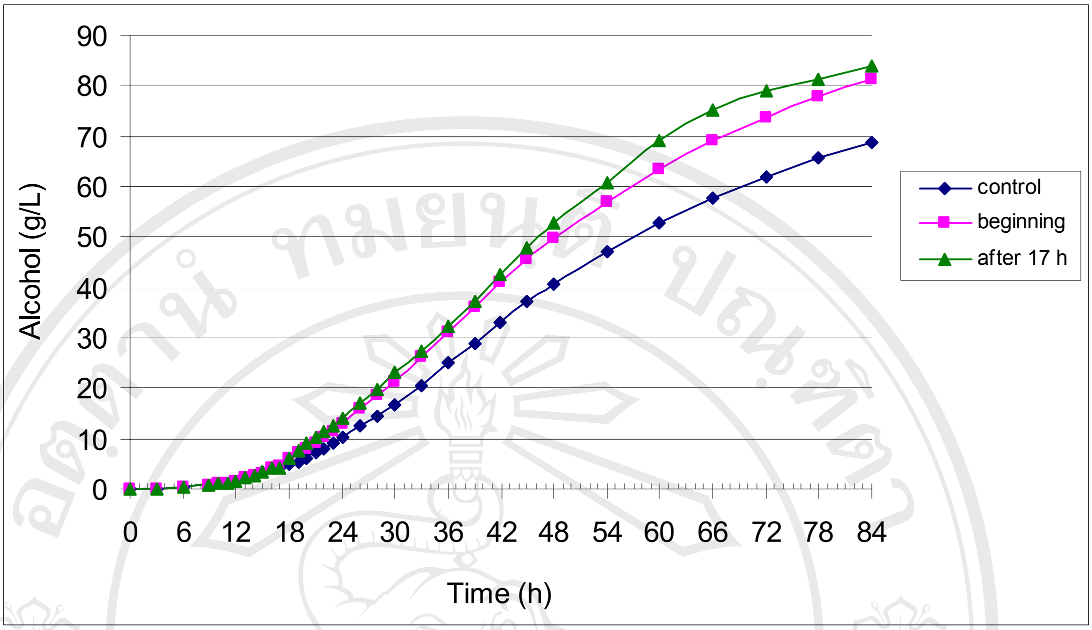

Figure 3.9 Alcohol Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

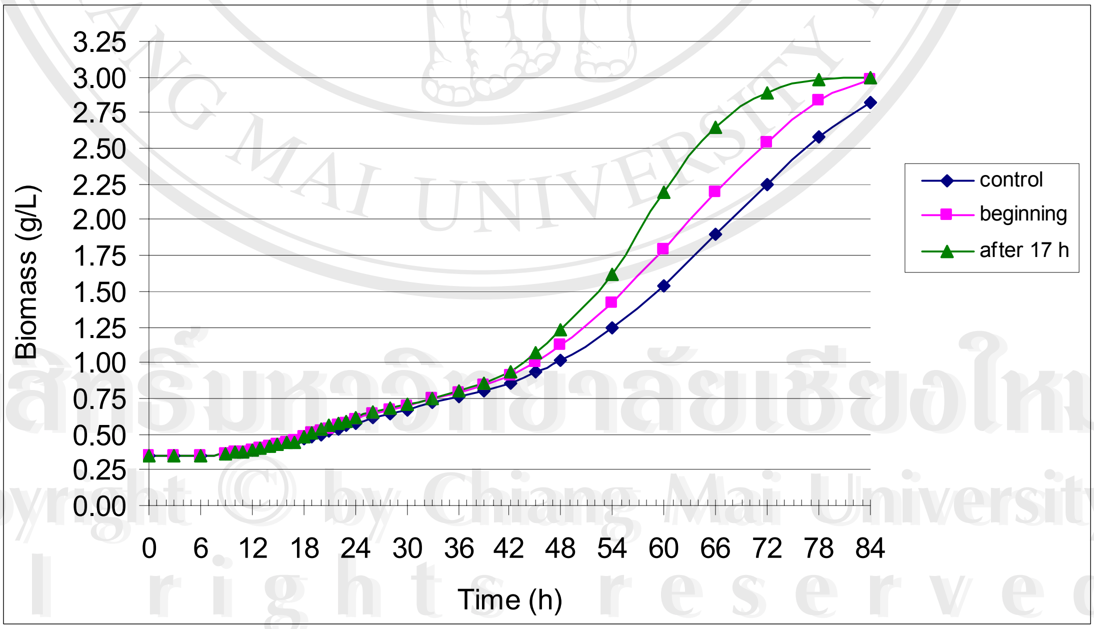

Figure 3.10 Biomass Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Table 3.5 Biomass Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Time (h)	Biomass (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
0	0.3475	0.3475	0.3475
3	0.3475	0.3475	0.3475
6	0.3544	0.3540	0.3544
9	0.3621	0.3621	0.3621
10	0.3721	0.3716	0.3721
11	0.3801	0.3794	0.3801
12	0.3905	0.3892	0.3905
13	0.4011	0.3995	0.4011
14	0.4118	0.4091	0.4118
15	0.4263	0.4215	0.4263
16	0.4450	0.4392	0.4450
17	0.4467	0.4555	0.4467
18	0.4686	0.4846	0.4794
19	0.4841	0.5021	0.5128
20	0.4998	0.5205	0.5399
21	0.5203	0.5390	0.5583
22	0.5396	0.5584	0.5766
23	0.5596	0.5775	0.5946
24	0.5808	0.5991	0.6149
26	0.6172	0.6367	0.6503
28	0.6440	0.6703	0.6822
30	0.6719	0.6990	0.7138
33	0.7168	0.7427	0.7538
36	0.7602	0.7882	0.7988
39	0.8008	0.8387	0.8564
42	0.8588	0.9103	0.9367
45	0.9302	1.0083	1.0637

Table 3.5 (continued).

Time (h)	Biomass (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
48	1.0188	1.1286	1.2311
54	1.2388	1.4146	1.6187
60	1.5424	1.7898	2.1878
66	1.9043	2.2000	2.6526
72	2.2425	2.5473	2.8830
78	2.5775	2.8374	2.9775
84	2.8248	2.9776	2.9906

Table 3.5 and figure 3.10 showed biomass concentrations during wine fermentation. It was found that biomass concentrations of wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning and after 17 h of fermentation increased gradually from 0.3475 to 2.8248, 2.9776 and 2.9906 g/L, respectively. The specific growth rates (μ) and yield coefficients of biomass formation from substrate ($Y_{x/s}$) were 0.034, 0.037, 0.047 h^{-1}, and 0.0143, 0.0144, 0.0140 g/g, respectively.

Table 3.6 Reducing Sugar Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Time (h)	Reducing Sugar (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
0	192.96	192.96	192.96
3	192.96	192.96	192.96
6	191.81	191.88	191.81
9	190.54	190.55	190.54
10	188.90	188.99	188.90
11	187.62	187.72	187.62
12	185.95	186.16	185.95
13	184.28	184.54	184.28
14	182.61	183.04	182.61
15	180.40	181.13	180.40
16	177.56	178.43	177.56
17	177.31	175.99	177.31
18	174.05	171.70	172.47
19	171.77	169.14	167.58
20	169.48	166.47	163.64
21	166.49	163.78	160.93
22	163.68	160.91	158.20
23	160.75	158.06	155.46
24	157.57	154.76	152.26
26	151.88	148.65	146.29
28	147.39	142.61	140.29
30	142.30	136.83	133.61
33	132.93	126.87	124.18
36	122.63	116.00	113.62
39	113.17	105.54	102.42
42	102.02	94.45	91.22
45	91.98	83.95	79.42

Table 3.6 (continued).

Time (h)	Reducing Sugar (g/L)		
	Control	Time of Supplementation (h)	
		0 h	17 h
48	83.04	74.93	69.08
54	68.69	60.98	53.96
60	56.41	48.97	38.69
66	45.90	38.38	25.93
72	37.31	29.15	16.75
78	28.26	18.99	9.83
84	19.55	9.82	3.58

Table 3.6 and figure 3.11 showed the decrease of reducing sugar concentrations during wine fermentation. It was found that reducing sugar concentrations of wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning and after 17 h of fermentation decreased gradually from 192.96 to 19.55, 9.82 and 3.58 g/L, respectively. The specific rates of substrate formation (q_s) were 2.38, 2.57 and 3.36 g/g-h, respectively.

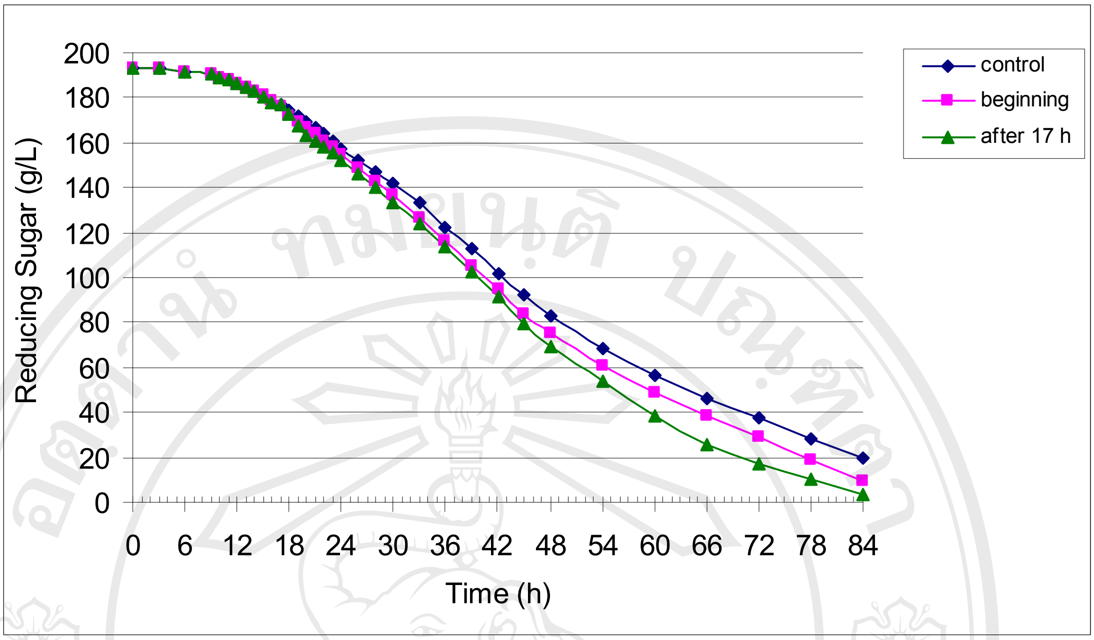

Figure 3.11 The Decrease of Reducing Sugar Concentrations during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

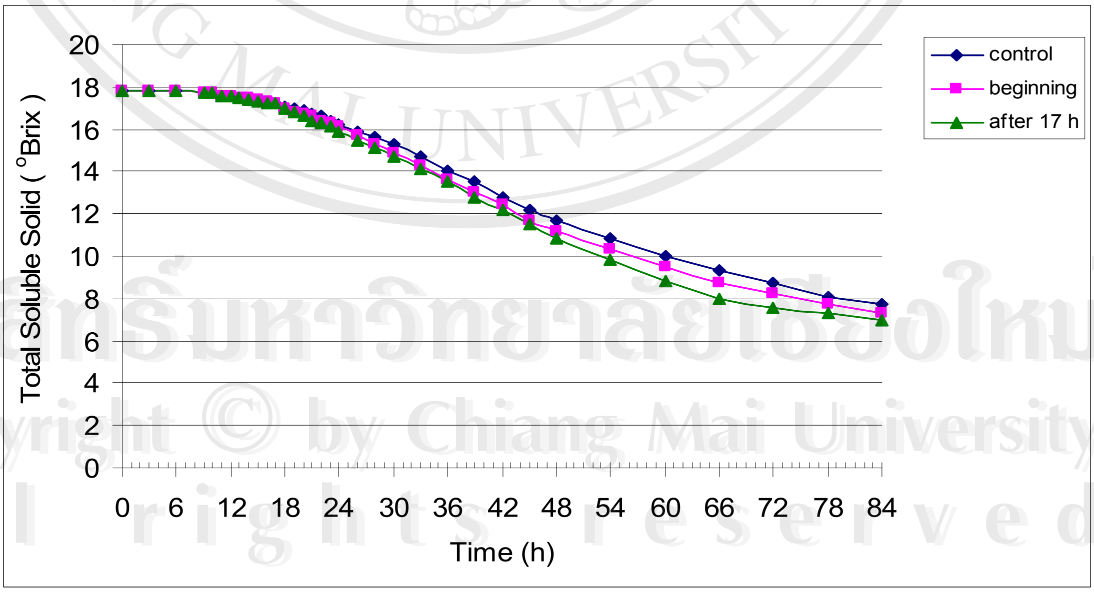

Figure 3.12 The Decrease of Total Soluble Solid during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Table 3.7 Total Soluble Solid during Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Time (h)	Total Soluble Solid (°Brix)		
	Control	Time of Supplementation (h)	
		0 h	17 h
0	17.8	17.8	17.8
3	17.8	17.8	17.8
6	17.8	17.8	17.8
9	17.7	17.7	17.7
10	17.7	17.7	17.7
11	17.6	17.6	17.6
12	17.6	17.6	17.6
13	17.5	17.5	17.5
14	17.4	17.5	17.4
15	17.3	17.4	17.3
16	17.2	17.3	17.2
17	17.2	17.2	17.2
18	17.1	17.0	17.0
19	17.0	16.8	16.8
20	16.9	16.7	16.6
21	16.7	16.6	16.4
22	16.6	16.4	16.3
23	16.4	16.3	16.1
24	16.2	16.1	15.9
26	15.9	15.7	15.5
28	15.6	15.3	15.1
30	15.3	14.9	14.7
33	14.7	14.3	14.1
36	14.0	13.6	13.5
39	13.5	13.0	12.8
42	12.8	12.4	12.2
45	12.2	11.7	11.5

Table 3.7 (continued).

Time (h)	Total Soluble Solid (^{o}Brix)		
	Control	Time of Supplementation (h)	
		0 h	17 h
48	11.7	11.2	10.8
54	10.8	10.3	9.8
60	10.0	9.5	8.8
66	9.3	8.7	8.0
72	8.7	8.2	7.6
78	8.1	7.7	7.3
84	7.7	7.3	7.0

Table 3.7 and figure 3.12 showed the decrease of total soluble solid (^{o}Brix) during wine fermentation. It was found that total soluble solid of wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning and after 17 h of fermentation decreased gradually from 17.8 to 7.7, 7.3 and 7.0 ^{o}Brix, respectively.

Table 3.8 Comparison of Kinetic Parameters in Wine Fermentation without and with 700 ppm of Ammonium Dihydrogen Phosphate Supplementation at the beginning and after 17 h of Fermentation.

Kinetic Parameters	Time of Supplementation (h)		
	control	0 h	17 h
The specific growth rate(μ),h^{-1}	0.034	0.037	0.047
The specific rate of substrate consumption (q_s), g/g-h	2.38	2.57	3.36
The specific rate of product formation (q_p), g/g-h	0.013	0.016	0.021
Yield coefficient of biomass formation from substrate ($Y_{x/s}$), g/g	0.0143	0.0144	0.0140
Yield coefficient of product formation from substrate ($Y_{p/s}$), g/g	0.397	0.444	0.443
Alcohol (g/L)	68.88 c	81.23 b	83.89 a

Mean separation within row by Duncan's multiple range test, $p < 0.05$.

Table 3.8 showed kinetic parameters of wine fermentation. It was found that wine fermentation supplemented with 700 ppm of ammonium dihydrogen phosphate after 17 h of fermentation was superior to wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning, respectively. Fermentation kinetics of wine fermentation supplemented with 700 ppm of ammonium phosphate after 17 h of fermentation were calculated out. It was revealed that the specific growth rate (μ) was 0.047 h^{-1}, the specific rate of substrate consumption (q_s) was 3.36 g/g-h, the specific rate of product formation (q_p) was 0.021 g/g-h, yield coefficient of biomass from substrate ($Y_{x/s}$) was 0.0140 g/g and yield coefficient of production from substrate ($Y_{p/s}$) was 0.443 g/g. Alcohol content of 83.89 g/L and the final total soluble solid of 7.0 ^{o}Brix in wine were obtained.

The timing of the addition of ammonium salts appears to be important. Ribereau-Gayon *et. al.,* (1975) had suggested their addition in must before the initiation of fermentation. Yeasts react best to stimuli during the growth phase in a medium containing little ethanol. They witnessed an assimilation of ammoniacal nitrogen (100 mg/L) varying between 100 and 50 per cent when the addition was made before the initiation of fermentation or on the forth day. The addition of nitrogen is not significantly effective for accelerating a slow fermentation in its final stage and even less so for restarting a stuck fermentation. Sablayrolles *et. al.*, (1996b) demonstrated that nitrogen added at mid-fermentation at the same time as an aeration gave the best results. This dual operation had more effect on fermentation kinetics than aeration alone.

Nitrogen and oxygen deficiencies are of major problems. Oxygen is required by yeasts for synthesis of cellular compounds, particularly sterols and unsaturated fatty acids while assimilable nitrogen, which is usually considered to include ammonium salt and α-amino acids are necessary for protein synthesis and yeast growth. The additions of these two nutrients are very effective if the amount added and the timing of addition is controlled. The best time for oxygen addition is at the end of the cell growth phase (Sablayrolles *et. al.*, 1996b). While nitrogen is most effective when added at the halfway of fermentation process (Bely *et. al.,* 1990a). Sablayrolles *et. al.*, (1996b) showed that the combined addition 5 mg/L oxygen at the end of the cell growth phase and 300 mg/L diammonium phosphate at the halfway fermentation process was very effective in preventing stuck and sluggish fermentations.

Ammonium ion is consumed preferentially by yeasts to FAN amino acids. Therefore, timing of nutrient addition is important. One large addition of diammonium phosphate at the beginning of fermentation may delay/inhibit uptake of amino acids. Multiple addition sources are preferred. First addition should be a nutrient mix, such as Super Food or Fermaid K, followed by diammonium phosphate. Adding nutrient supplements all at once can lead to too fast a fermentation rate, and an imbalance in uptake and usage of nitrogen compounds. Supplement added to late (after mid-fermentation) may not be used by the yeasts, in part because the alcohol prevents their development. For the same reason, adding nutrients to a stuck fermentation seldom does any good at all (http:// www.vtwines.info, 2001).

Watunyoo (2003) studied wine and brandy productions from dried longan. It was found that longan wine was supplemented with triammonium phosphate of 0.15 per cent (w/v) was the best condition for longan wine production. Wine sample showed that it contained alcohol of 84.0 g/L, the specific growth rate (μ) was 0.019 h^{-1}, the specific rate of substrate consumption (q_s) was 0.38 g/g-h, the specific rate of product formation (q_p) was 0.17 g/g-h, yield coefficient of biomass from substrate ($Y_{x/s}$) was 0.05 g/g and yield coefficient of production from substrate ($Y_{p/s}$) was 0.44 g/g.

3.3 The Effects of Various Concentrations of Ammonium Dihydrogen Phosphate Supplementation in Wine Fermentation

The concentration of nitrogen salt is as very important as the timing of nitrogen salt addition in the development of wine fermentation. Previous results (topic 3.2) revealed that kinetic parameters of wine fermentation supplemented with 700 ppm of ammonium dihydrogen phosphate after 17 h of fermentation was superior to wine fermentation without and with 700 ppm of ammonium dihydrogen phosphate supplementation at the beginning of fermentation. In this study, the influence of wine fermentation supplemented with various concentrations of ammonium dihydrogen phosphate after 17 h of fermentation were investigated.

Table 3.9 Carbon Dioxide Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Dihydrogen Phosphate after 17 h of Fermentation.

Time (h)	Carbon Dioxide (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
6	0.18	0.18	0.18	0.18	0.18	0.18
9	0.38	0.38	0.38	0.38	0.38	0.38
10	0.65	0.65	0.65	0.65	0.65	0.65
11	0.87	0.87	0.87	0.87	0.87	0.87
12	1.17	1.17	1.17	1.17	1.17	1.17
13	1.47	1.47	1.47	1.47	1.47	1.47
14	1.78	1.78	1.78	1.78	1.78	1.78
15	2.21	2.21	2.21	2.21	2.21	2.21
16	2.78	2.78	2.78	2.78	2.78	2.78
17	2.84	2.84	2.84	2.84	2.84	2.84
18	3.35	3.80	3.84	4.05	3.97	3.99
19	3.87	4.12	4.37	4.63	4.49	4.57
20	4.42	4.61	4.97	5.27	5.10	5.18
21	5.17	5.18	5.68	6.01	5.83	5.91
22	5.92	6.05	6.38	6.74	6.63	6.77
23	6.74	6.83	7.21	7.60	7.27	7.65
24	7.67	7.69	8.14	8.54	8.23	9.24
26	9.44	9.14	9.86	10.31	9.87	10.69
28	10.92	10.89	11.77	12.36	11.82	12.70
30	12.66	12.85	13.81	14.46	14.01	15.03
33	15.93	15.78	17.08	17.67	17.02	18.36

Table 3.9 (continued).

Time (h)	Carbon Dioxide (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
36	19.56	19.45	20.83	21.42	20.65	22.29
39	22.90	22.96	24.52	25.29	24.56	26.35
42	26.87	26.90	28.50	29.44	28.64	30.40
45	30.56	30.93	32.97	34.03	33.22	35.08
48	34.02	34.79	37.08	38.39	37.85	39.33
54	40.14	41.25	44.48	46.28	45.06	47.39
60	46.16	47.95	51.61	54.29	53.50	55.97
66	51.93	54.31	58.10	61.54	61.15	64.42
72	56.81	59.67	63.39	66.74	66.87	70.55
78	61.76	65.31	68.12	71.01	72.30	75.14
84	66.11	70.18	71.45	73.79	74.60	77.49

The profiles of wine fermentation without (control) and with supplementation 100, 300, 500, 700, and 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation were as follows :

Table 3.9 and figure 3.13 showed carbon dioxide concentrations during wine fermentation. It was found that carbon dioxide concentrations increased gradually from 0 to 66.11, 70.18, 71.45, 73.79, 74.60, and 77.49 g/L, respectively.

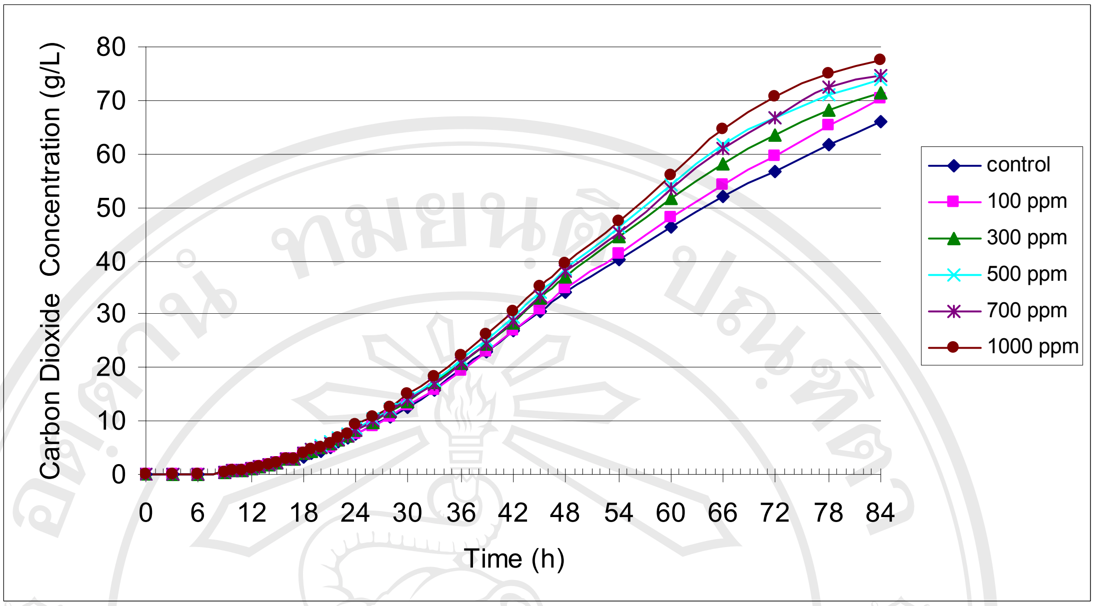

Figure 3.13 Carbon Dioxide Concentrations during Wine Fermentation Supplemented with Ammonium Dihydrogen Phosphate of 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

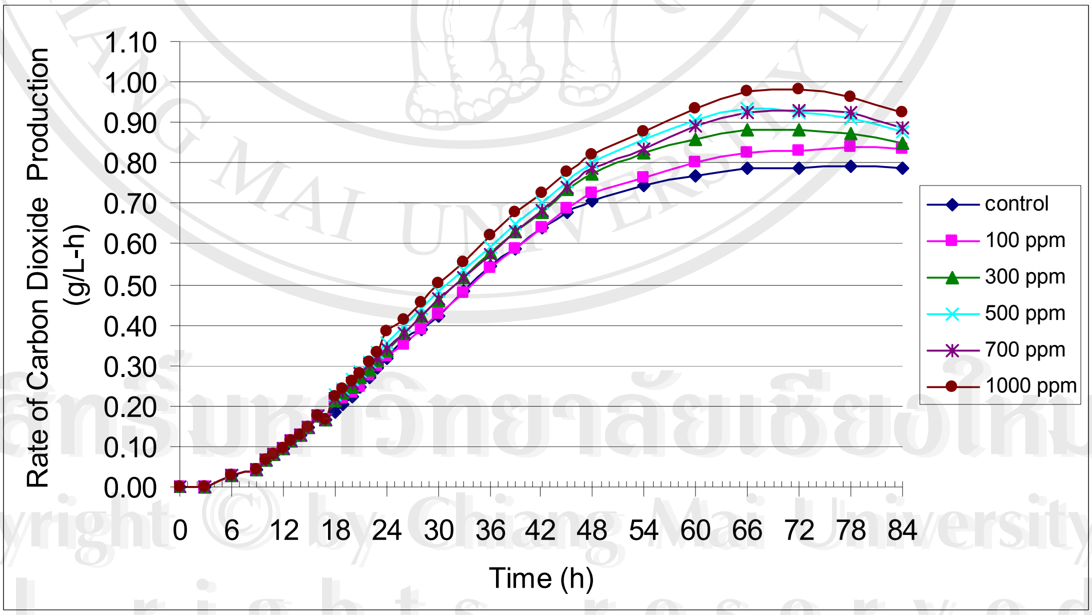

Figure 3.14 Rate of Carbon Dioxide Production during Wine Fermentation Supplemented with Ammonium Dihydrogen Phosphate of 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

Table 3.10 Rates of Carbon Dioxide Production during Wine Fermentation Supplemented with Various Concentrations of Ammonium Dihydrogen Phosphate after 17 h of Fermentation.

Time (h)	Rates of Carbon Dioxide (g/L-h)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.0295	0.0295	0.0295	0.0295	0.0295	0.0295
9	0.0425	0.0425	0.0425	0.0425	0.0425	0.0425
10	0.0654	0.0654	0.0654	0.0654	0.0654	0.0654
11	0.0793	0.0793	0.0793	0.0793	0.0793	0.0793
12	0.0971	0.0971	0.0971	0.0971	0.0971	0.0971
13	0.1129	0.1129	0.1129	0.1129	0.1129	0.1129
14	0.1271	0.1271	0.1271	0.1271	0.1271	0.1271
15	0.1472	0.1472	0.1472	0.1472	0.1472	0.1472
16	0.1740	0.1740	0.1740	0.1740	0.1740	0.1740
17	0.1669	0.1669	0.1669	0.1669	0.1669	0.1669
18	0.1863	0.2111	0.2135	0.2253	0.2203	0.2216
19	0.2039	0.2168	0.2301	0.2437	0.2365	0.2403
20	0.2212	0.2303	0.2484	0.2634	0.2552	0.2592
21	0.2464	0.2467	0.2703	0.2861	0.2775	0.2815
22	0.2691	0.2749	0.2900	0.3063	0.3015	0.3077
23	0.2930	0.2968	0.3136	0.3305	0.3163	0.3324
24	0.3196	0.3203	0.3390	0.3557	0.3427	0.3849
26	0.3631	0.3515	0.3793	0.3965	0.3797	0.4110
28	0.3900	0.3889	0.4205	0.4416	0.4223	0.4537
30	0.4218	0.4282	0.4603	0.4820	0.4669	0.5008
33	0.4828	0.4782	0.5175	0.5353	0.5157	0.5563
36	0.5435	0.5401	0.5785	0.5949	0.5737	0.6192

Table 3.10 (continued).

Time (h)	Rates of Carbon Dioxide (g/L-h)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
39	0.5871	0.5886	0.6286	0.6484	0.6299	0.6757
42	0.6397	0.6405	0.6786	0.7010	0.6820	0.7239
45	0.6791	0.6874	0.7326	0.7562	0.7383	0.7796
48	0.7088	0.7248	0.7725	0.7999	0.7886	0.8194
54	0.7433	0.7639	0.8237	0.8571	0.8345	0.8775
60	0.7694	0.7992	0.8602	0.9048	0.8916	0.9329
66	0.7868	0.8228	0.8803	0.9324	0.9265	0.9761
72	0.7890	0.8287	0.8804	0.9269	0.9287	0.9798
78	0.7918	0.8373	0.8734	0.9103	0.9269	0.9634
84	0.7870	0.8355	0.8506	0.8784	0.8881	0.9225

Table 3.10 and figure 3.14 showed rates of carbon dioxide production during wine fermentation. It was found that the maximum rates of carbon dioxide production of wine fermentation without (control) and with supplementation 100, 300, 500, 700, and 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation increased gradually from 0 to 0.7918, 0.8373, 0.8804, 0.9324, 0.9287, and 0.9798 g/L-h after 78, 78, 72, 66, 72, and 72 h of fermentation, respectively.

Table 3.11 Alcohol Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Dihydrogen Phosphate after 17 h of Fermentation.

Time (h)	Alcohol (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
6	0.26	0.26	0.26	0.26	0.26	0.26
9	0.56	0.56	0.56	0.56	0.56	0.56
10	0.96	0.96	0.96	0.96	0.96	0.96
11	1.27	1.27	1.27	1.27	1.27	1.27
12	1.70	1.70	1.70	1.70	1.70	1.70
13	2.14	2.14	2.14	2.14	2.14	2.14
14	2.59	2.59	2.59	2.59	2.59	2.59
15	3.21	3.21	3.21	3.21	3.21	3.21
16	4.03	4.03	4.03	4.03	4.03	4.03
17	4.11	4.11	4.11	4.11	4.11	4.11
18	4.54	5.68	5.74	6.05	5.92	5.96
19	5.13	6.15	6.52	6.90	6.70	6.81
20	5.84	6.86	7.40	7.83	7.59	7.71
21	6.81	7.70	8.43	8.91	8.65	8.77
22	7.78	8.97	9.45	9.97	9.81	10.01
23	8.83	10.09	10.65	11.21	10.74	11.27
24	10.01	11.33	11.98	12.55	12.10	13.54
26	12.24	13.40	14.42	15.05	14.44	15.58
28	14.09	15.87	17.10	17.92	17.17	18.39
30	16.22	18.59	19.91	20.80	20.18	21.57
33	20.18	22.58	24.32	25.11	24.25	26.02
36	24.45	27.45	29.25	30.01	29.03	31.14
39	28.25	31.99	33.96	34.92	34.02	36.24
42	32.65	36.92	38.87	40.01	39.05	41.16

Table 3.11 (continued).

Time (h)	Alcohol (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
45	36.61	41.79	44.18	45.42	44.48	46.63
48	40.21	46.29	48.89	50.36	49.75	51.39
54	46.28	53.48	56.90	58.77	57.51	59.90
60	51.93	60.47	64.08	66.63	65.89	68.20
66	57.01	66.65	70.14	73.17	72.83	75.61
72	61.07	71.54	74.75	77.52	77.62	80.52
78	64.95	76.35	78.63	80.87	81.85	83.93
84	68.18	80.24	81.21	82.95	83.54	85.60

Table 3.11 and figure 3.15 showed alcohol concentrations during wine fermentation. It was found that alcohol concentrations of wine fermentation without (control) and with supplementation 100, 300, 500, 700, and 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation increased gradually from 0 to 68.18, 80.24, 81.21, 82.95, 83.54, and 85.60 g/L, respectively. The specific rates of substrate formation (q_p) and yield coefficients of product formation from substrate ($Y_{p/s}$) were 0.014, 0.016, 0.018, 0.019, 0.019, 0.021 g/L-h, and 0.398, 0.445, 0.444, 0.439, 0.438, 0.445 g/g, respectively.

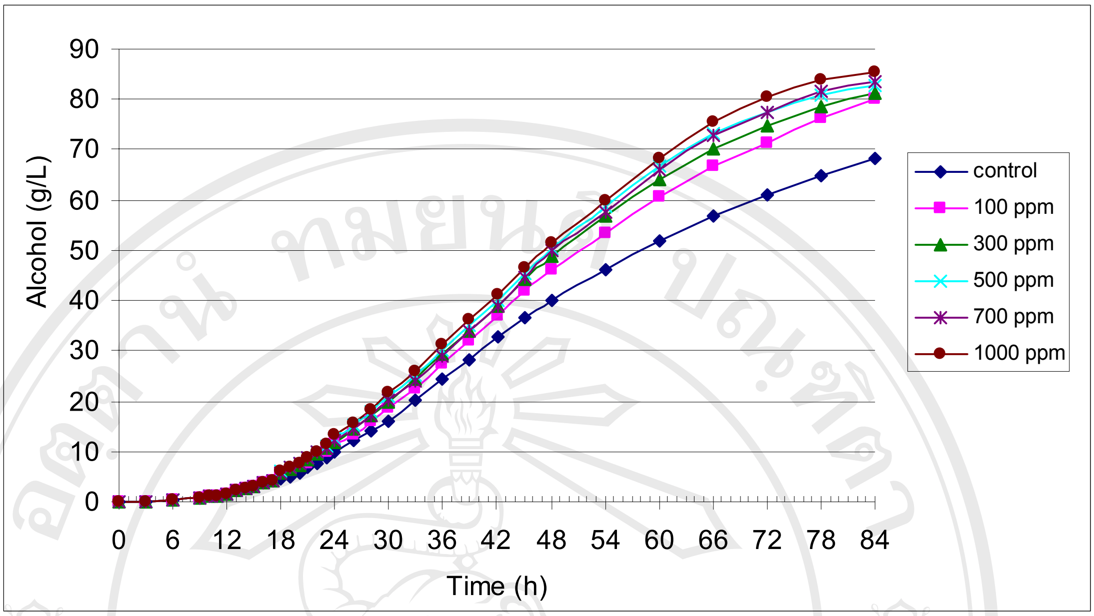

Figure 3.15 Alcohol Concentrations during Wine Fermentation Supplemented with Ammonium Dihydrogen Phosphate of 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

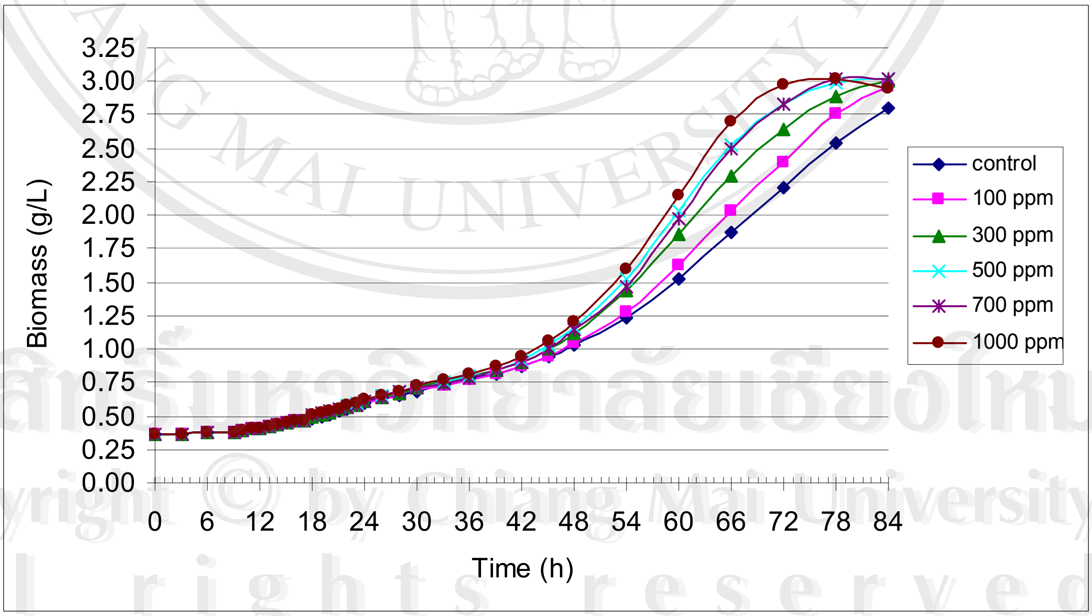

Figure 3.16 Biomass Concentrations during Wine Fermentation Supplemented with Ammonium Dihydrogen Phosphate of 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

Table 3.12 Biomass Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Dihydrogen Phosphate after 17 h of Fermentation.

Time (h)	Biomass (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.3687	0.3687	0.3687	0.3687	0.3687	0.3687
3	0.3687	0.3687	0.3687	0.3687	0.3687	0.3687
6	0.3753	0.3753	0.3753	0.3753	0.3753	0.3753
9	0.3829	0.3829	0.3829	0.3829	0.3829	0.3829
10	0.3928	0.3928	0.3928	0.3928	0.3928	0.3928
11	0.4007	0.4007	0.4007	0.4007	0.4007	0.4007
12	0.4110	0.4110	0.4110	0.4110	0.4110	0.4110
13	0.4215	0.4215	0.4215	0.4215	0.4215	0.4215
14	0.4321	0.4321	0.4321	0.4321	0.4321	0.4321
15	0.4464	0.4464	0.4464	0.4464	0.4464	0.4464
16	0.4650	0.4650	0.4650	0.4650	0.4650	0.4650
17	0.4666	0.4666	0.4666	0.4666	0.4666	0.4666
18	0.4826	0.4960	0.4972	0.5034	0.5008	0.5015
19	0.4982	0.5053	0.5125	0.5198	0.5160	0.5180
20	0.5140	0.5191	0.5291	0.5371	0.5328	0.5349
21	0.5346	0.5348	0.5478	0.5563	0.5517	0.5539
22	0.5541	0.5573	0.5655	0.5742	0.5717	0.5749
23	0.5742	0.5763	0.5853	0.5941	0.5867	0.5951
24	0.5956	0.5960	0.6059	0.6143	0.6077	0.6285
26	0.6325	0.6266	0.6405	0.6488	0.6407	0.6555
28	0.6596	0.6591	0.6740	0.6834	0.6748	0.6886
30	0.6879	0.6908	0.7049	0.7139	0.7076	0.7215
33	0.7332	0.7312	0.7473	0.7543	0.7465	0.7624
36	0.7765	0.7751	0.7911	0.7981	0.7891	0.8086
39	0.8161	0.8168	0.8372	0.8479	0.8378	0.8636
42	0.8717	0.8723	0.8993	0.9169	0.9019	0.9362

Table 3.12 (continued).

Time (h)	Biomass (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
45	0.9394	0.9474	0.9953	1.0234	1.0019	1.0535
48	1.0232	1.0449	1.1171	1.1638	1.1441	1.1996
54	1.2320	1.2792	1.4326	1.5289	1.4630	1.5914
60	1.5223	1.6244	1.8516	2.0302	1.9766	2.1459
66	1.8723	2.0316	2.2930	2.5257	2.4997	2.7045
72	2.2040	2.4005	2.6430	2.8296	2.8358	2.9772
78	2.5397	2.7551	2.8928	2.9887	3.0123	3.0109
84	2.7977	2.9669	2.9984	3.0215	3.0174	2.9427

Table 3.12 and figure 3.16 showed biomass concentrations during wine fermentation. It was found that biomass concentrations of wine fermentation without (control) and with supplementation 100, 300, 500, 700, and 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation increased gradually from 0.3687 to maximum amounts of 2.7977, 2.9669, 2.9984, 3.0215, 3.0174, and 3.0109 g/L after 84, 84, 84, 84, 84, and 78 h of fermentation, respectively. The specific growth rates (μ) and yield coefficients of biomass formation from substrate ($Y_{x/s}$) were 0.034, 0.037, 0.040, 0.044, 0.044, 0.047 h^{-1}, and 0.0142, 0.0144, 0.0144, 0.0141, 0.0139, 0.0134 g/g, respectively.

Table 3.13 Reducing Sugar Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Dihydrogen Phosphate after 17 h of Fermentation.

Time (h)	Reducing Sugar (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	193.81	193.81	193.81	193.81	193.81	193.81
3	193.81	193.81	193.81	193.81	193.81	193.81
6	192.71	192.71	192.71	192.71	192.71	192.71
9	191.45	191.45	191.45	191.45	191.45	191.45
10	189.83	189.83	189.83	189.83	189.83	189.83
11	188.56	188.56	188.56	188.56	188.56	188.56
12	186.91	186.91	186.91	186.91	186.91	186.91
13	185.26	185.26	185.26	185.26	185.26	185.26
14	183.60	183.60	183.60	183.60	183.60	183.60
15	181.41	181.41	181.41	181.41	181.41	181.41
16	178.60	178.60	178.60	178.60	178.60	178.60
17	178.35	178.35	178.35	178.35	178.35	178.35
18	175.96	173.99	173.81	172.90	173.27	173.17
19	173.67	172.63	171.56	170.51	171.06	170.77
20	171.35	170.61	169.15	167.98	168.62	168.31
21	168.34	168.32	166.42	165.19	165.86	165.55
22	165.52	165.04	163.84	162.57	162.94	162.46
23	162.57	162.26	160.92	159.60	160.71	159.46
24	159.38	159.32	157.83	156.54	157.55	154.33
26	153.69	154.63	152.39	151.04	152.37	149.90
28	149.21	149.30	146.70	144.99	146.55	144.01
30	144.15	143.60	140.85	139.01	140.29	137.42
33	134.87	135.30	131.66	130.01	131.82	128.07
36	124.68	125.02	121.14	119.49	121.63	117.03

Table 3.13 (continued).

Time (h)	Reducing Sugar (g/L)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
39	115.33	115.16	110.80	108.65	110.67	105.71
42	104.28	104.18	99.82	97.28	99.43	94.71
45	94.30	93.31	88.04	85.35	87.39	82.74
48	85.37	83.47	77.95	74.90	76.15	72.78
54	70.99	68.59	61.95	58.44	60.80	56.35
60	58.67	55.30	48.71	44.04	45.42	41.12
66	48.16	44.01	37.41	31.23	31.95	25.77
72	39.66	34.63	27.76	21.10	20.84	12.82
78	30.83	24.00	18.19	11.76	8.71	6.31
84	22.40	13.66	10.72	5.07	3.01	1.66

Table 3.13 and figure 3.17 showed the decrease of reducing sugar concentrations during wine fermentation. It was found that reducing sugar concentrations of wine fermentation without (control) and with supplementation 100, 300, 500, 700, and 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation decreased gradually from 193.81 to 22.40, 13.66, 10.72, 5.07, 3.01, and 1.66 g/L, respectively. The specific rates of substrate formation (q_s) were 2.39, 2.57, 2.78, 3.12, 3.17, and 3.51 g/g-h, respectively.

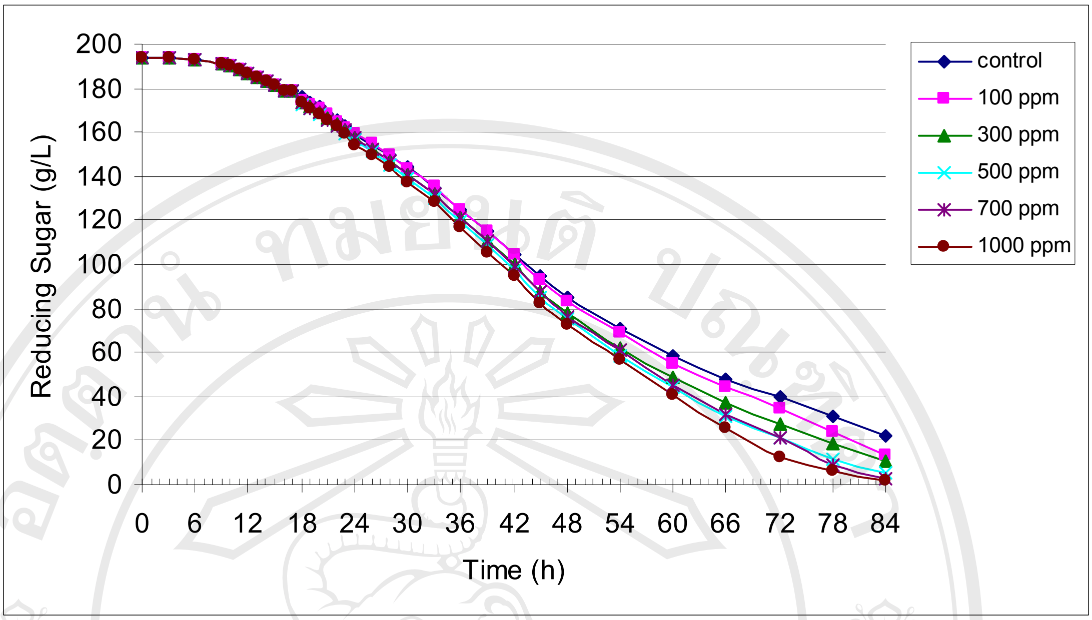

Figure 3.17 The Decrease of Reducing Sugar Concentrations during Wine Fermentation Supplemented with Ammonium Dihydrogen Phosphate of 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

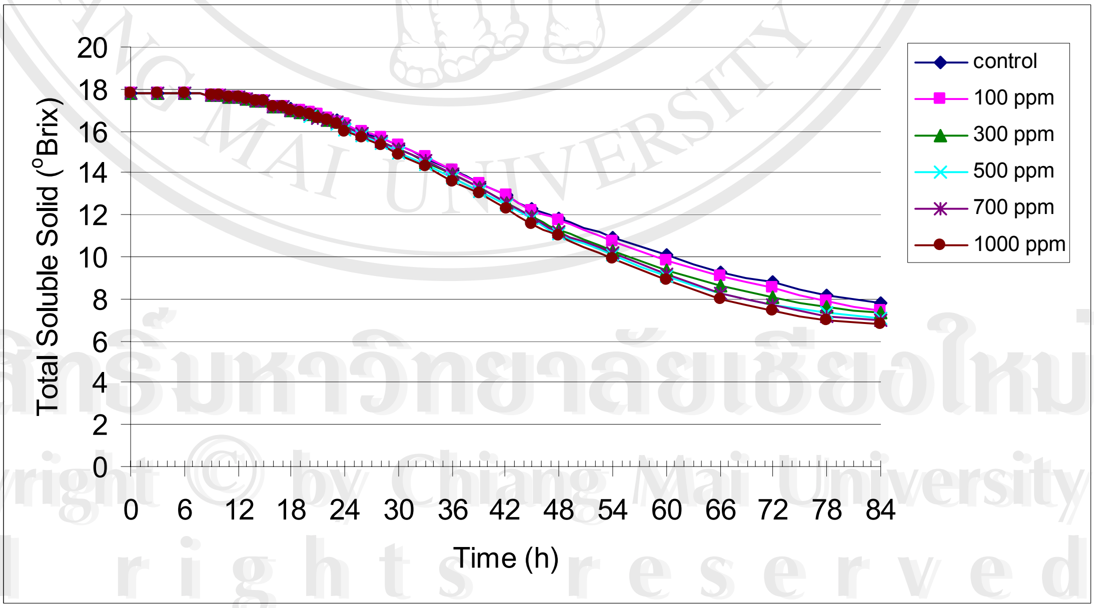

Figure 3.18 The Decrease of Total Soluble Solid during Wine Fermentation Supplemented with Ammonium Dihydrogen Phosphate of 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

Table 3.14 Total Soluble Solid during Wine Fermentation Supplemented with Various Concentrations of Ammonium Dihydrogen Phosphate after 17 h of Fermentation.

Time (h)	Total Soluble Solid (^oBrix)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	17.8	17.8	17.8	17.8	17.8	17.8
3	17.8	17.8	17.8	17.8	17.8	17.8
6	17.8	17.8	17.8	17.8	17.8	17.8
9	17.7	17.7	17.7	17.7	17.7	17.7
10	17.7	17.7	17.7	17.7	17.7	17.7
11	17.6	17.6	17.6	17.6	17.6	17.6
12	17.6	17.6	17.6	17.6	17.6	17.6
13	17.5	17.5	17.5	17.5	17.5	17.5
14	17.4	17.4	17.4	17.4	17.4	17.4
15	17.4	17.4	17.4	17.4	17.4	17.4
16	17.2	17.2	17.2	17.2	17.2	17.2
17	17.2	17.2	17.2	17.2	17.2	17.2
18	17.1	17.0	17.0	17.0	17.0	17.0
19	17.0	17.0	16.9	16.9	16.9	16.9
20	16.9	16.9	16.8	16.7	16.8	16.8
21	16.8	16.8	16.7	16.6	16.6	16.6
22	16.6	16.6	16.5	16.5	16.5	16.5
23	16.5	16.4	16.4	16.3	16.4	16.3
24	16.3	16.3	16.2	16.1	16.2	16.0
26	15.9	16.0	15.9	15.8	15.9	15.7
28	15.7	15.7	15.5	15.4	15.5	15.3
30	15.3	15.3	15.1	15.0	15.1	14.9
33	14.7	14.8	14.5	14.4	14.6	14.3
36	14.1	14.1	13.9	13.8	13.9	13.6
39	13.5	13.5	13.3	13.1	13.3	13.0
42	12.9	12.9	12.6	12.5	12.6	12.3

Table 3.14 (continued).

Time (h)	Total Soluble Solid (^{o}Brix)					
	Control	Ammonium Dihydrogen Phosphate (ppm)				
		100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
45	12.3	12.2	11.9	11.8	11.9	11.6
48	11.8	11.7	11.3	11.1	11.2	11.0
54	10.9	10.7	10.3	10.1	10.2	9.9
60	10.1	9.8	9.4	9.1	9.2	8.9
66	9.3	9.1	8.6	8.3	8.3	8.0
72	8.8	8.5	8.1	7.7	7.7	7.4
78	8.2	7.9	7.6	7.3	7.2	7.0
84	7.8	7.4	7.3	7.1	7.0	6.8

Table 3.14 and figure 3.18 showed the decrease of total soluble solid (^{o}Brix) during wine fermentation. It was found that total soluble solid of wine fermentation without (control) and with supplementation 100, 300, 500, 700, and 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation decreased gradually from 17.8 to 7.8, 7.4, 7.3, 7.1, 7.0, and 6.8 ^{o}Brix, respectively.

Table 3.15 Comparison of Kinetic Parameters in Wine Fermentation Supplemented with Various Ammonium Dihydrogen Phosphate Concentrations after 17 h of Fermentation.

Kinetic Parameters	Ammonium Dihydrogen Phosphate (ppm)					
	control	100	300	500	700	1000
The specific growth rate (μ), h^{-1}	0.034	0.037	0.040	0.044	0.044	0.047
The specific rate of substrate consumption (q_s), g/g-h	2.39	2.57	2.78	3.12	3.17	3.51
The specific rate of product formation (q_p), g/g-h	0.014	0.016	0.018	0.019	0.019	0.021
Yield coefficient of biomass formation from substrate ($Y_{x/s}$), g/g	0.0142	0.0144	0.0144	0.0141	0.0139	0.0134
Yield coefficient of product formation from substrate ($Y_{p/s}$), g/g	0.398	0.445	0.444	0.439	0.438	0.445
Alcohol (g/L)	68.18 e	80.24 d	81.21 c	82.95 b	83.54 b	85.60 a

Mean separation within row by Duncan's multiple range test, $p < 0.05$.

Table 3.15 showed kinetic parameters of wine fermentation. It was found that wine fermentation supplemented with 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation was superior to wine fermentation with 700, 500, 300, and 100 ppm of ammonium dihydrogen phosphate supplementation after 17 h of fermentation and control, respectively. Fermentation kinetics of wine fermentation supplemented with 1000 ppm of ammonium dihydrogen phosphate after 17 h of fermentation were calculated out. It was revealed that the specific growth rate (μ) was

0.047 h^{-1}, the specific rate of substrate consumption (q_s) was 3.51g/g-h, the specific rate of product formation (q_p) was 0.021 g/g-h, yield coefficient of biomass formation from substrate ($Y_{x/s}$) was 0.0134 g/g and yield coefficient of product formation from substrate ($Y_{p/s}$) was 0.445 g/g. Alcohol concentration of 85.60 g/L and the final total soluble solid of 6.8 ^{o}Brix in wine were obtained.

Insufficient levels of yeast nutrient have been considered to lead to a reduced yeast tolerance of ethanol, and of these nutrients, assimilable nitrogen levels can be critical. Additives of assimilable nitrogen have been shown to increase sugar usage and ethanol formation (Allen and Auld, 1988)

Yeast hulls, or yeast ghosts, have been reported to enhance fermentation rates and prevent stuck or sluggish fermentations. These act as adsorbing agents lowering the concentration of toxic substances in fermentation medium. Yeast hull additions (0.2 g/L) can stimulate fermentation not simply by detoxification as was previously believed, but by supplying unsaturated fatty acid (C-16, C-18) as an oxygen substitute (Munoz and Ingledew, 1990).

3.4 The Effects of Various Concentrations of Ammonium Sulfate in Wine Fermentation

The species of nitrogen were as important as the timing addition and concentration of nitrogen in the development of wine fermentation. Timing of nitrogen addition in wine fermentation in topic 3.2 revealed that kinetic parameter of wine fermentation supplemented with 700 ppm of ammonium phosphate after 17 h of fermentation was superior to wine fermentation without and with 700 ppm of ammonium phosphate supplementation at the beginning of fermentation. In addition, the result in topic 3.3 revealed that kinetic parameter of wine fermentation supplemented with 1000 ppm of ammonium phosphate after 17 h of fermentation was superior to wine fermentation supplemented with other concentrations of ammonium phosphate after 17 h of fermentation. In this study, the influences of ammonium sulfate of 700 ppm at the beginning of fermentation and 100, 300, 500, 700, and 1000 ppm after 17 h of fermentation were investigated.

Table 3.16 Carbon Dioxide Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Sulfate.

Time (h)	Carbon Dioxide (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.18	0.17	0.18	0.18	0.18	0.18	0.18
9	0.39	0.41	0.39	0.39	0.39	0.39	0.39
10	0.66	0.63	0.66	0.66	0.66	0.66	0.66
11	0.88	0.85	0.88	0.88	0.88	0.88	0.88
12	1.18	1.15	1.18	1.18	1.18	1.18	1.18
13	1.49	1.49	1.49	1.49	1.49	1.49	1.49
14	1.80	1.80	1.80	1.80	1.80	1.80	1.80
15	2.23	2.20	2.23	2.23	2.23	2.23	2.23
16	2.82	2.73	2.82	2.82	2.82	2.82	2.82
17	2.87	3.28	2.87	2.87	2.87	2.87	2.87
18	3.39	4.04	3.79	4.44	4.65	4.68	4.60
19	3.92	4.98	4.33	5.38	5.64	5.75	5.76
20	4.48	5.70	4.91	6.09	6.35	6.47	6.51
21	5.24	6.54	5.57	6.93	7.25	7.43	7.41
22	5.99	7.34	6.48	7.86	8.13	8.33	8.30
23	6.82	8.18	7.37	8.82	9.18	9.34	9.30
24	7.76	9.23	8.32	9.96	10.37	10.56	10.45
26	9.55	11.09	9.87	12.01	12.49	12.72	12.49
28	11.05	13.09	11.71	14.15	14.71	14.88	14.69
30	12.80	15.08	13.66	16.53	17.14	17.32	16.91
33	16.12	18.67	17.05	20.08	20.94	21.17	20.60

Table 3.16 (continued).

Time (h)	Carbon Dioxide (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
36	19.79	22.72	20.76	24.14	25.02	25.31	24.55
39	23.16	26.60	24.34	28.11	29.11	29.30	28.66
42	27.18	30.98	28.47	32.55	33.71	33.86	33.19
45	30.92	35.70	32.54	37.24	38.69	38.91	38.33
48	34.42	40.35	36.35	41.61	43.58	43.76	43.13
54	40.61	48.74	43.01	49.06	51.80	52.62	51.71
60	46.70	57.43	49.76	56.45	59.67	61.35	60.63
66	52.53	64.72	55.97	62.97	66.42	68.01	68.69
72	57.47	69.50	61.26	68.14	71.09	72.41	74.18
78	62.48	72.91	66.72	72.34	74.63	75.58	76.96
84	66.88	74.95	71.31	75.27	77.33	77.60	78.90

The profiles of wine fermentation without the addition of ammonium sulfate (control) and wine fermentation supplemented with 700 ppm at the beginning of fermentation and 100, 300, 500, 700, and 1000 ppm of ammonium sulfate after 17 h of fermentation were showed in table 3.16 and figure 3.19. It was found that carbon dioxide concentrations increased gradually from 0 to 66.88, 74.95 71.31 75.27, 77.33, 77.60, and 78.90 g/L, respectively.

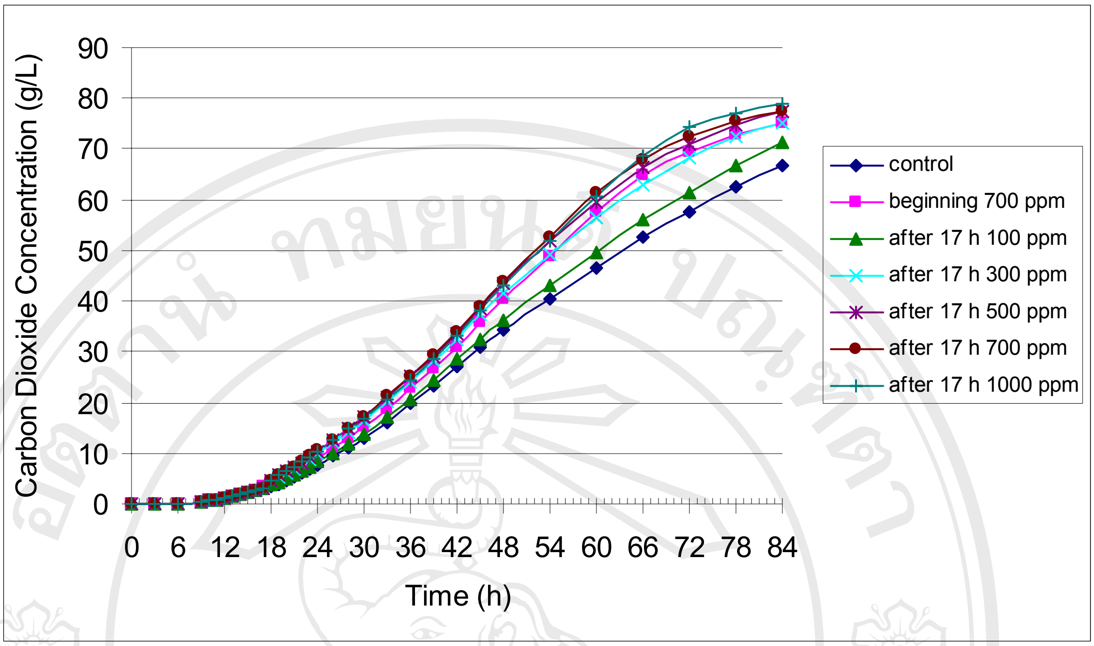

Figure 3.19 Carbon Dioxide Concentrations during Wine Fermentation Supplemented with Ammonium Sulfate of 700 ppm at the beginning and 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation**.**

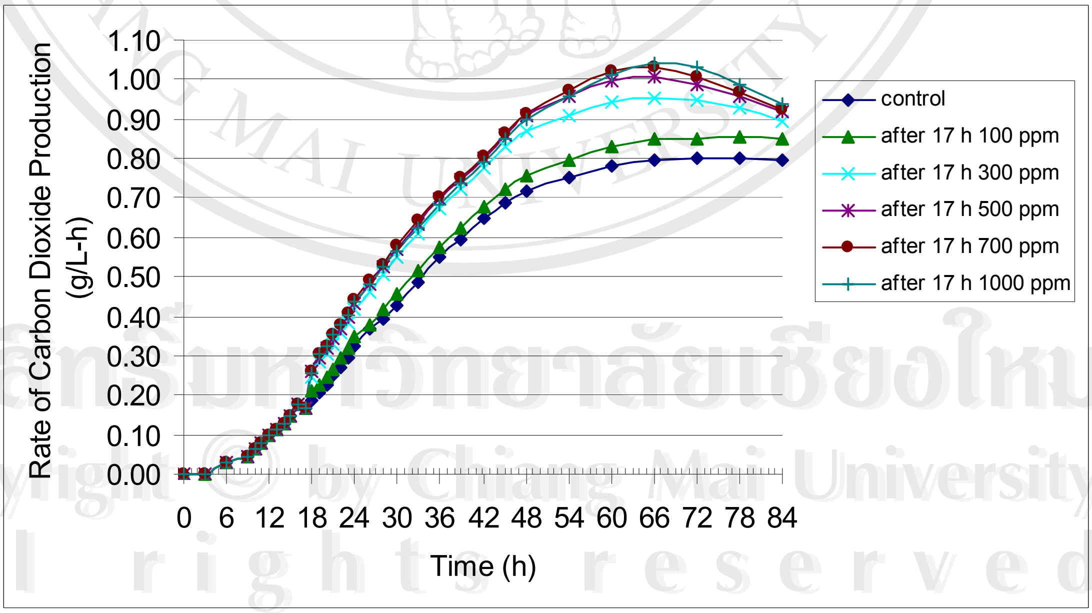

Figure 3.20 Rates of Carbon Dioxide Production during Wine Fermentation Supplemented with Ammonium Sulfate of 700 ppm at the beginning and 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

Table 3.17 Rates of Carbon Dioxide Production during Wine Fermentation Supplemented with Various Concentrations of Ammonium Sulfate.

Time (h)	Rates of Carbon Dioxide (g/L-h)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.0298	0.0282	0.0298	0.0298	0.0298	0.0298	0.0298
9	0.0430	0.0454	0.0430	0.0430	0.0430	0.0430	0.0430
10	0.0662	0.0631	0.0662	0.0662	0.0662	0.0662	0.0662
11	0.0803	0.0770	0.0803	0.0803	0.0803	0.0803	0.0803
12	0.0983	0.0958	0.0983	0.0983	0.0983	0.0983	0.0983
13	0.1142	0.1150	0.1142	0.1142	0.1142	0.1142	0.1142
14	0.1286	0.1286	0.1286	0.1286	0.1286	0.1286	0.1286
15	0.1489	0.1468	0.1489	0.1489	0.1489	0.1489	0.1489
16	0.1761	0.1706	0.1761	0.1761	0.1761	0.1761	0.1761
17	0.1688	0.1930	0.1688	0.1688	0.1688	0.1688	0.1688
18	0.1885	0.2246	0.2107	0.2468	0.2581	0.2599	0.2554
19	0.2063	0.2619	0.2281	0.2830	0.2969	0.3025	0.3030
20	0.2238	0.2849	0.2457	0.3043	0.3173	0.3237	0.3256
21	0.2493	0.3113	0.2652	0.3302	0.3450	0.3539	0.3528
22	0.2722	0.3334	0.2947	0.3575	0.3696	0.3786	0.3771
23	0.2964	0.3556	0.3204	0.3834	0.3989	0.4063	0.4045
24	0.3233	0.3845	0.3466	0.4150	0.4323	0.4398	0.4354
26	0.3673	0.4264	0.3798	0.4621	0.4803	0.4891	0.4802
28	0.3946	0.4676	0.4184	0.5053	0.5254	0.5313	0.5246
30	0.4268	0.5025	0.4554	0.5510	0.5714	0.5774	0.5638
33	0.4884	0.5657	0.5165	0.6084	0.6347	0.6416	0.6242
36	0.5498	0.6311	0.5768	0.6704	0.6949	0.7031	0.6818
39	0.5939	0.6821	0.6240	0.7208	0.7463	0.7513	0.7349
42	0.6471	0.7376	0.6780	0.7750	0.8025	0.8061	0.7903

Table 3.17 (continued).

Time (h)	Rates of Carbon Dioxide (g/L-h)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
45	0.6870	0.7933	0.7231	0.8276	0.8597	0.8648	0.8517
48	0.7170	0.8406	0.7573	0.8668	0.9078	0.9116	0.8985
54	0.7520	0.9026	0.7965	0.9085	0.9592	0.9745	0.9577
60	0.7784	0.9572	0.8293	0.9408	0.9945	1.0225	1.0104
66	0.7960	0.9806	0.8481	0.9542	1.0063	1.0305	1.0408
72	0.7983	0.9653	0.8509	0.9464	0.9873	1.0057	1.0303
78	0.8010	0.9347	0.8554	0.9274	0.9568	0.9689	0.9867
84	0.7962	0.8922	0.8489	0.8960	0.9206	0.9238	0.9393

Table 3.17 and figure 3.20 showed rates of carbon dioxide production during wine fermentation. It was found that the maximum rates of carbon dioxide production of wine fermentation without the addition of ammonium salt (control) and wine fermentation supplemented with 700 ppm at the beginning of fermentation and 100, 300, 500, 700 and 1000 ppm of ammonium sulfate after 17 h of fermentation increased gradually from 0 to 0.8010, 0.9806, 0.8554, 0.9542, 1.0063, 1.0408, and 1.0408 g/L-h after 78, 66, 78, 66, 66, 66, and 66 h of fermentation, respectively.

Table 3.18 Alcohol Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Sulfate.

Time (h)	Alcohol (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.26	0.26	0.26	0.26	0.26	0.26	0.26
9	0.57	0.62	0.57	0.57	0.57	0.57	0.57
10	0.97	0.95	0.97	0.97	0.97	0.97	0.97
11	1.29	1.28	1.29	1.29	1.29	1.29	1.29
12	1.72	1.73	1.72	1.72	1.72	1.72	1.72
13	2.16	2.25	2.16	2.16	2.16	2.16	2.16
14	2.62	2.71	2.62	2.62	2.62	2.62	2.62
15	3.24	3.31	3.24	3.24	3.24	3.24	3.24
16	4.08	4.09	4.08	4.08	4.08	4.08	4.08
17	4.16	4.91	4.16	4.16	4.16	4.16	4.16
18	4.59	6.04	5.67	6.63	6.92	6.97	6.85
19	5.18	7.41	6.47	7.99	8.38	8.53	8.55
20	5.91	8.46	7.31	9.02	9.40	9.58	9.64
21	6.89	9.68	8.27	10.25	10.70	10.97	10.93
22	7.86	10.83	9.60	11.59	11.97	12.25	12.20
23	8.93	12.04	10.87	12.95	13.45	13.69	13.63
24	10.12	13.53	12.24	14.56	15.15	15.40	15.25
26	12.38	16.14	14.44	17.43	18.09	18.41	18.09
28	14.25	18.93	17.02	20.37	21.14	21.36	21.11
30	16.40	21.63	19.71	23.59	24.41	24.65	24.11
33	20.40	26.44	24.28	28.28	29.40	29.70	28.96
36	24.71	31.68	29.17	33.48	34.58	34.95	33.99
39	28.55	36.55	33.73	38.40	39.61	39.84	39.07
42	32.99	41.85	38.84	43.70	45.04	45.22	44.45

Table 3.18 (continued).

Time (h)	Alcohol (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
45	36.98	47.33	43.68	49.07	50.68	50.93	50.28
48	40.61	52.50	48.07	53.86	55.96	56.15	55.48
54	46.74	61.26	55.36	61.57	64.26	65.05	64.18
60	52.41	69.54	62.26	68.64	71.54	73.01	72.38
66	57.52	75.86	68.20	74.40	77.26	78.54	79.07
72	61.60	79.71	72.93	78.64	80.93	81.93	83.24
78	65.50	82.30	77.50	81.88	83.57	84.25	85.22
84	68.74	83.79	81.10	84.02	85.48	85.67	86.56

Table 3.18 and figure 3.21 showed alcohol concentrations during wine fermentation. It was found that alcohol concentrations of wine fermentation without the addition of ammonium salt (control) and wine fermentation supplemented with 700 ppm at the beginning of fermentation and 100, 300, 500, 700, and 1000 ppm of ammonium sulfate after 17 h of fermentation increased gradually from 0 to 68.74, 83.79, 81.10, 84.02, 85.48, 85.67, and 86.56 g/L, respectively. The specific rates of substrate formation (q_p) and yield coefficients of product formation from substrate ($Y_{p/s}$) of them were 0.013, 0.021, 0.017, 0.019, 0.023, 0.022, 0.023 g/L-h, and 0.397, 0.439, 0.444, 0.441, 0.448, 0.448, 0.452 g/g, respectively.

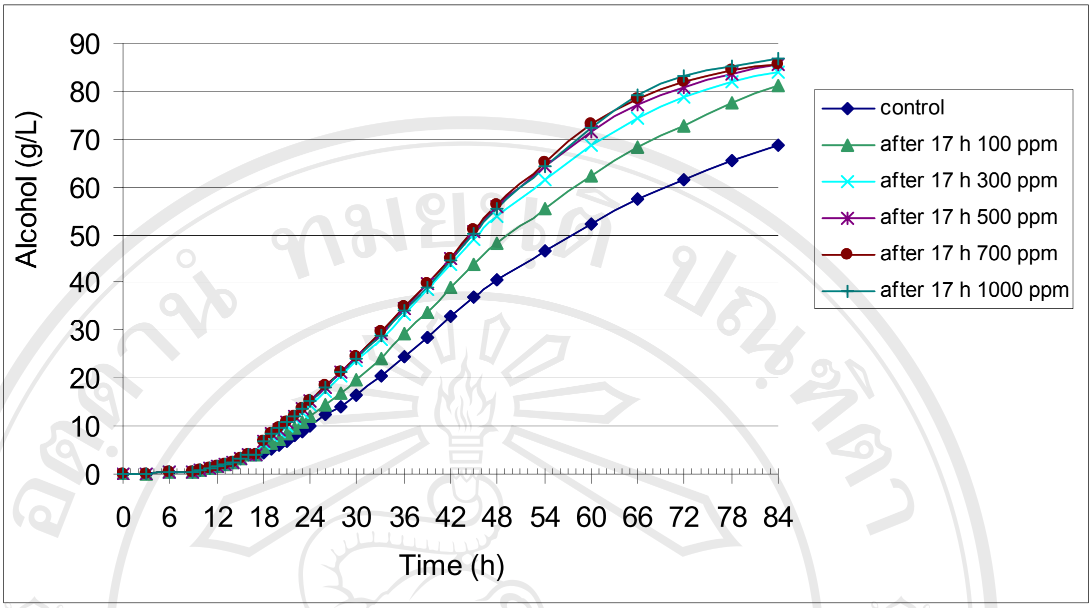

Figure 3.21 Alcohol Concentrations during Wine Fermentation Supplemented with Ammonium Sulfate of 700 ppm at the beginning and 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

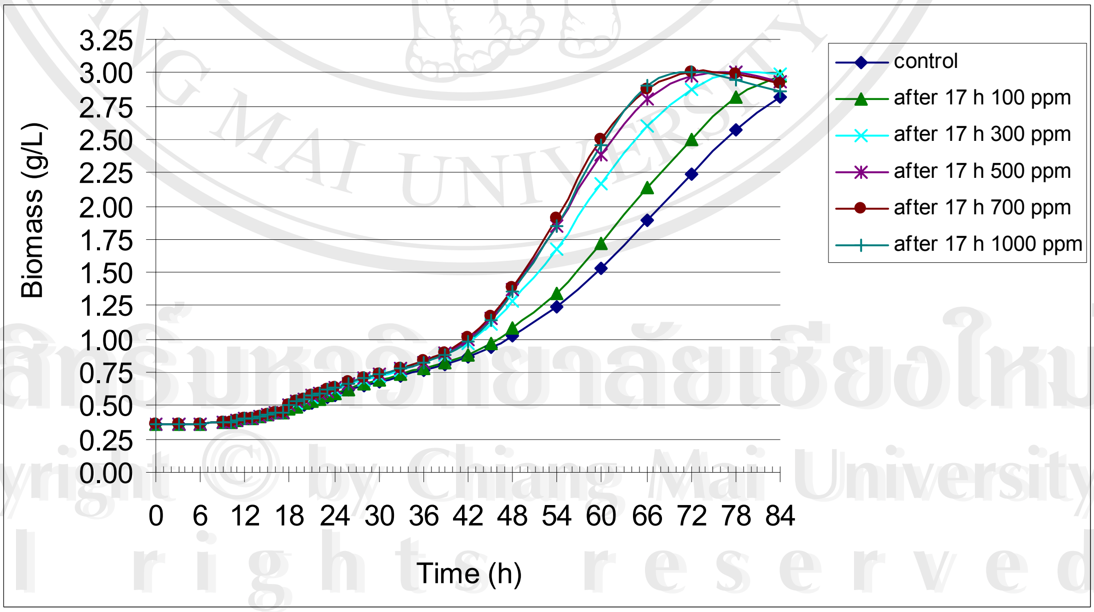

Figure 3.22 Biomass Concentrations during Wine Fermentation Supplemented with Ammonium Sulfate of 700 ppm at the beginning and 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

Table 3.19 Biomass Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Sulfate.

Time (h)	Biomass (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	0.3546	0.3546	0.3546	0.3546	0.3546	0.3546	0.3546
3	0.3546	0.3546	0.3546	0.3546	0.3546	0.3546	0.3546
6	0.3613	0.3609	0.3613	0.3613	0.3613	0.3613	0.3613
9	0.3690	0.3698	0.3690	0.3690	0.3690	0.3690	0.3690
10	0.3790	0.3779	0.3790	0.3790	0.3790	0.3790	0.3790
11	0.3869	0.3857	0.3869	0.3869	0.3869	0.3869	0.3869
12	0.3974	0.3964	0.3974	0.3974	0.3974	0.3974	0.3974
13	0.4080	0.4084	0.4080	0.4080	0.4080	0.4080	0.4080
14	0.4187	0.4187	0.4187	0.4187	0.4187	0.4187	0.4187
15	0.4332	0.4321	0.4332	0.4332	0.4332	0.4332	0.4332
16	0.4519	0.4491	0.4519	0.4519	0.4519	0.4519	0.4519
17	0.4536	0.4663	0.4536	0.4536	0.4536	0.4536	0.4536
18	0.4697	0.4890	0.4817	0.5004	0.5061	0.5070	0.5047
19	0.4854	0.5152	0.4974	0.5259	0.5328	0.5355	0.5358
20	0.5013	0.5343	0.5135	0.5441	0.5506	0.5537	0.5546
21	0.5221	0.5553	0.5309	0.5647	0.5720	0.5762	0.5757
22	0.5417	0.5740	0.5540	0.5858	0.5916	0.5958	0.5951
23	0.5620	0.5926	0.5748	0.6060	0.6132	0.6165	0.6157
24	0.5835	0.6142	0.5956	0.6283	0.6359	0.6391	0.6372
26	0.6205	0.6484	0.6266	0.6638	0.6712	0.6747	0.6712
28	0.6477	0.6804	0.6589	0.6955	0.7032	0.7054	0.7029
30	0.6760	0.7080	0.6887	0.7265	0.7339	0.7361	0.7312
33	0.7214	0.7520	0.7328	0.7683	0.7784	0.7811	0.7744
36	0.7650	0.7998	0.7763	0.8180	0.8300	0.8342	0.8235

Table 3.19 (continued).

Time (h)	Biomass (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
39	0.8054	0.8534	0.8207	0.8784	0.8964	0.9000	0.8881
42	0.8626	0.9343	0.8848	0.9708	1.0005	1.0045	0.9870
45	0.9330	1.0582	0.9705	1.1085	1.1607	1.1693	1.1472
48	1.0201	1.2267	1.0787	1.2808	1.3732	1.3821	1.3513
54	1.2374	1.6572	1.3458	1.6764	1.8498	1.9041	1.8442
60	1.5382	2.2330	1.7193	2.1647	2.3868	2.4992	2.4510
66	1.8981	2.7076	2.1318	2.6032	2.7993	2.8741	2.9018
72	2.2357	2.9313	2.4934	2.8796	2.9764	2.9996	3.0063
78	2.5719	3.0044	2.8146	2.9987	3.0031	2.9892	2.9501
84	2.8223	2.9995	2.9813	2.9948	2.9356	2.9239	2.8532

Table 3.19 and figure 3.22 showed biomass concentrations during wine fermentation. It was found that biomass concentrations of wine fermentation without the addition of ammonium salt (control) and wine fermentation supplemented with 700 ppm at the beginning of fermentation and 100, 300, 500, 700, and 1000 ppm of ammonium sulfate after 17 h of fermentation increased gradually from 0.3546 to maximum amounts of 2.8223, 3.0044, 2.9813, 2.9987, 3.0031, 2.9996, and 3.0063 g/L after 84, 78, 84, 78, 78, 72, and 72 h of fermentation, respectively. The specific growth rates (μ) and yield coefficients of biomass formation from substrate ($Y_{x/s}$) were 0.034, 0.048, 0.038, 0.044, 0.052, 0.049, 0.051 h^{-1}, and 0.0143, 0.0139, 0.0144, 0.0139, 0.0135, 0.0134, 0.0131 g/g, respectively.

Table 3.20 Reducing Sugar Concentrations during Wine Fermentation Supplemented with Various Concentrations of Ammonium Sulfate.

Time (h)	Reducing Sugar (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	193.19	193.19	193.19	193.19	193.19	193.19	193.19
3	193.19	193.19	193.19	193.19	193.19	193.19	193.19
6	192.07	192.13	192.07	192.07	192.07	192.07	192.07
9	190.80	190.68	190.80	190.80	190.80	190.80	190.80
10	189.17	189.35	189.17	189.17	189.17	189.17	189.17
11	187.89	188.09	187.89	187.89	187.89	187.89	187.89
12	186.22	186.38	186.22	186.22	186.22	186.22	186.22
13	184.54	184.49	184.54	184.54	184.54	184.54	184.54
14	182.87	182.88	182.87	182.87	182.87	182.87	182.87
15	180.66	180.82	180.66	180.66	180.66	180.66	180.66
16	177.82	178.24	177.82	177.82	177.82	177.82	177.82
17	177.57	175.67	177.57	177.57	177.57	177.57	177.57
18	175.17	172.32	173.40	170.65	169.82	169.69	170.02
19	172.86	168.50	171.10	166.94	165.94	165.54	165.50
20	170.52	165.73	168.75	164.29	163.35	162.89	162.75
21	167.49	162.66	166.21	161.27	160.19	159.56	159.64
22	164.64	159.88	162.85	158.11	157.23	156.59	156.70
23	161.67	157.08	159.77	155.03	153.90	153.37	153.50
24	158.46	153.73	156.62	151.47	150.22	149.67	150.00
26	152.73	148.10	151.74	145.38	144.01	143.35	144.02
28	148.21	142.27	146.25	139.27	137.68	137.21	137.74
30	143.10	136.66	140.65	132.57	130.85	130.35	131.49
33	133.73	126.57	131.13	122.62	120.19	119.55	121.16
36	123.42	115.21	120.69	111.24	108.78	107.97	110.10
39	113.96	104.39	110.68	100.25	97.56	97.04	98.76
42	102.80	92.57	99.27	88.48	85.54	85.17	86.84

Table 3.20 (continued).

Time (h)	Reducing Sugar (g/L)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
45	92.74	80.62	88.52	76.95	73.61	73.10	74.44
48	83.76	69.91	79.06	67.21	63.13	62.77	64.05
54	69.35	53.23	64.28	52.65	47.76	46.32	47.91
60	57.02	37.96	51.39	39.68	34.00	30.95	32.28
66	46.48	24.56	40.50	27.93	21.15	17.81	16.34
72	37.89	14.56	31.12	17.53	10.96	9.82	8.45
78	28.87	6.61	20.52	7.99	5.62	5.32	4.63
84	20.19	2.54	10.44	2.67	2.43	2.08	1.87

Table 3.20 and figure 3.23 showed the decrease of reducing sugar concentrations during wine fermentation. It was found that reducing sugar concentrations of wine fermentation without the addition of ammonium sulfate (control) and wine fermentation supplemented with 700 ppm at the beginning of fermentation and 100, 300, 500, 700, and 1000 ppm of ammonium sulfate after 17 h of fermentation decreased gradually from 193.19 to 20.19, 2.54, 10.44, 2.67, 2.43, 2.08, and 1.87 g/L, respectively. The specific rates of substrate consumption (q_s) were 2.38, 3.45, 2.64, 3.17, 3.85, 3.65, and 3.89 g/g-h, respectively.

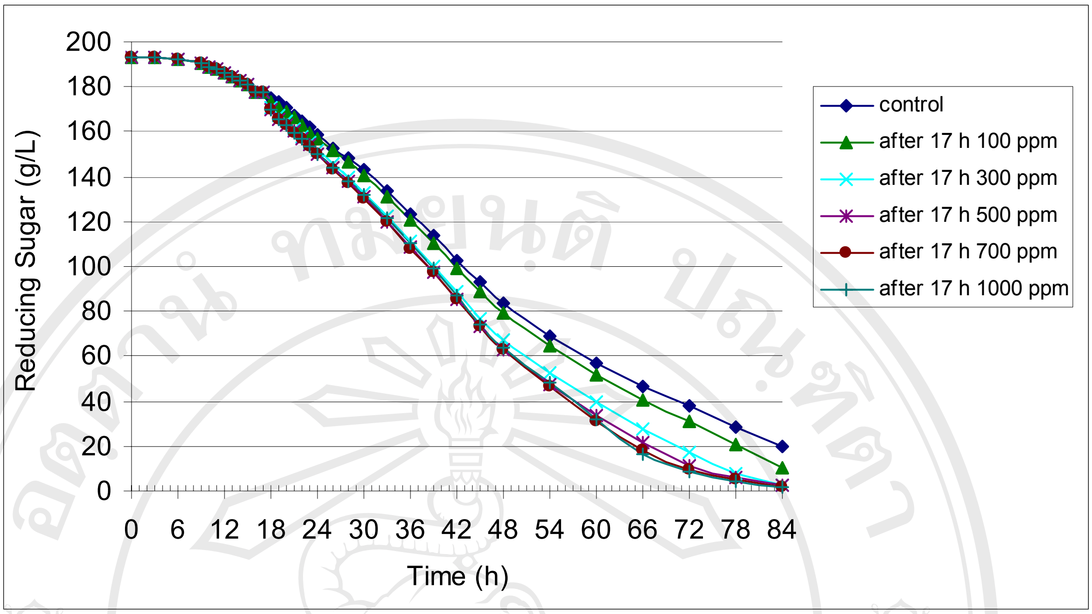

Figure 3.23 The Decrease of Reducing sugar Concentrations during Wine Fermentation Supplemented with Ammonium Sulfate of 700 ppm at the beginning and 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

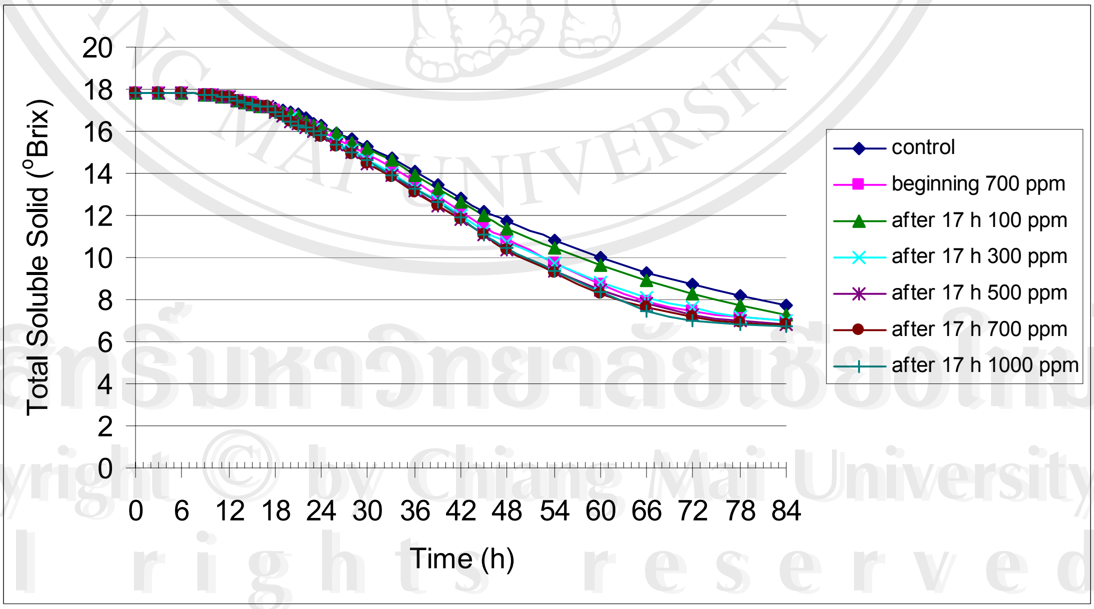

Figure 3.24 The Decrease of Total Soluble Solid during Wine Fermentation Supplemented with Ammonium Sulfate of 700 ppm at the beginning and 100, 300, 500, 700, and 1000 ppm after 17 h of Fermentation.

Table 3.21 Total Soluble Solid during Wine Fermentation Supplemented with Various Concentrations of Ammonium Sulfate.

Time (h)	Total Soluble Solid (^{o}Brix)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
0	17.8	17.8	17.8	17.8	17.8	17.8	17.8
3	17.8	17.8	17.8	17.8	17.8	17.8	17.8
6	17.8	17.8	17.8	17.8	17.8	17.8	17.8
9	17.7	17.7	17.7	17.7	17.7	17.7	17.7
10	17.7	17.7	17.7	17.7	17.7	17.7	17.7
11	17.6	17.6	17.6	17.6	17.6	17.6	17.6
12	17.6	17.6	17.6	17.6	17.6	17.6	17.6
13	17.5	17.5	17.5	17.5	17.5	17.5	17.5
14	17.4	17.4	17.4	17.4	17.4	17.4	17.4
15	17.3	17.4	17.3	17.3	17.3	17.3	17.3
16	17.2	17.2	17.2	17.2	17.2	17.2	17.2
17	17.2	17.1	17.2	17.2	17.2	17.2	17.2
18	17.1	17.0	17.0	16.9	16.9	16.9	16.9
19	17.0	16.8	16.9	16.7	16.7	16.7	16.7
20	16.9	16.7	16.8	16.6	16.5	16.5	16.5
21	16.8	16.5	16.7	16.4	16.4	16.3	16.3
22	16.6	16.3	16.5	16.2	16.2	16.2	16.2
23	16.4	16.2	16.3	16.1	16.0	16.0	16.0
24	16.3	16.0	16.2	15.8	15.8	15.7	15.8
26	15.9	15.6	15.9	15.5	15.4	15.3	15.4
28	15.6	15.3	15.5	15.1	15.0	14.9	15.0
30	15.3	14.9	15.2	14.6	14.5	14.5	14.6
33	14.7	14.3	14.6	14.0	13.9	13.8	13.9
36	14.1	13.6	13.9	13.3	13.2	13.1	13.3

Table 3.21 (continued).

Time (h)	Total Soluble Solid (^oBrix)						
	Control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
39	13.5	12.9	13.3	12.7	12.5	12.5	12.6
42	12.8	12.2	12.6	12.0	11.8	11.8	11.9
45	12.2	11.5	12.0	11.3	11.1	11.1	11.1
48	11.7	10.9	11.4	10.7	10.4	10.4	10.5
54	10.8	9.7	10.5	9.7	9.4	9.3	9.4
60	10.0	8.7	9.6	8.8	8.5	8.3	8.4
66	9.3	7.9	8.9	8.1	7.8	7.6	7.5
72	8.7	7.5	8.3	7.6	7.3	7.2	7.0
78	8.2	7.2	7.7	7.2	7.0	6.9	6.8
84	7.7	7.0	7.3	7.0	6.8	6.8	6.7

Table 3.21 and figure 3.24 showed the decrease of total soluble solid (^oBrix) during wine fermentation. It was found that total soluble solid of wine fermentation without the addition of ammonium salt (control) and wine fermentation supplemented with 700 ppm at the begining of fermentation and 100, 300, 500, 700, and 1000 ppm of ammonium sulfate after 17 h of fermentation decreased gradually from 17.8 to 7.7, 7.0, 7.3, 7.0, 6.8, 6.8, and 6.7 ^oBrix, respectively.

Table 3.22 Comparison of Kinetic Parameters in Wine Fermentation Supplemented with Various Ammonium Sulfate Concentrations at the beginning and after 17 h of Fermentation.

Kinetic Parameters	control	Time of Supplementation (h)					
		0 h	17 h				
		700 ppm	100 ppm	300 ppm	500 ppm	700 ppm	1000 ppm
The specific growth rate(μ),h^{-1}	0.034	0.048	0.038	0.044	0.052	0.049	0.051
The specific rate of substrate consumption (q_s), g/g-h	2.38	3.45	2.64	3.17	3.85	3.65	3.89
The specific rate of product formation (q_p), g/g-h	0.013	0.021	0.017	0.019	0.023	0.022	0.023
Yield coefficient of biomass formation from substrate ($Y_{x/s}$), g/g	0.0143	0.0139	0.0144	0.0139	0.0135	0.0134	0.0131
Yield coefficient of product formatiom from substrate ($Y_{p/s}$), g/g	0.397	0.439	0.444	0.441	0.448	0.448	0.452
Alcohol (g/L)	68.74 e	83.79 c	81.10 d	84.02 c	85.48 b	85.67 b	86.56 a

Mean separation within row by Duncan's multiple range test, $p < 0.05$.

Table 3.22 showed kinetic parameters of wine fermentation. It was found that wine fermentation supplemented with 1000 ppm of ammonium sulfate after 17 h of fermentation was superior to wine fermentation supplemented with other concentrations of ammonium sulfate at the beginning of fermentation and after 17 h of fermentation. Fermentation kinetics of wine fermentation supplemented with 1000 ppm of ammonium sulfate after 17 h of fermentation were carried out. It was revealed that the specific growth rate (μ) was 0.051 h^{-1}, the specific rate of substrate consumption (q_s) was 3.89 g/g-h, the specific rate of product formation (q_p) was 0.023 g/g-h, yield coefficient of biomass formation from substrate ($Y_{x/s}$) was 0.0131 g/g and yield coefficient of product formation from substrate ($Y_{p/s}$) was 0.452 g/g. Alcohol content of 86.56 g/L, and the final total soluble solid of 6.7 ^{o}Brix in wine were obtained.

A deficiency of assimilable nitrogen in must has been linked to poor yeast growth, stuck fermentations and production of hydrogen sulfide. Pre-fermentation processing of musts may lower the concentration of essential yeast nutrients, thereby increasing the possibility of a sluggish or stuck fermentation and reduced yeast growth (Eglinton and Henschke, 1993). In brewing, stuck and sluggish batch fermentation in very high gravity worts seemed to be caused by insufficient level of yeast nutrients and the effect of these nutrients on tolerance of the culture to ethanol (Ingledew and Kunkee, 1985). Many high gravity worts are known to be nitrogen can increase fermentation rate and can reduce time required for fermentation completion (Bely *et. al.,* 1990b). It is generally accepted that 150 mg/L of free amino nitrogen (FAN) is required in a wort for a normal fermentation. Enologists have demonstrated that large amounts (over 500 mg/L) of assimilable nitrogen when present with oxygen (and/or substitutes), resulted in very rapid batch fermentations of musts and worts of very high initial sugar concentrations (Ingledew *et. al*., 1986).

Table 3.23 Comparison of Alcohol Concentrations in Wine Fermentation Supplemented with Various Concentrations of Ammonium Salts.

Ammonium Salts	Concentration (ppm)	Alcohol (g/L)
Ammonium dihydrogen phosphate	100	80.24 f
	300	81.21 e
	500	82.95 d
	700	83.54 cd
	1000	85.60 b
Ammonium sulfate	100	81.10 e
	300	84.02 c
	500	85.48 b
	700	85.67 b
	1000	86.56 a

Mean separation within column by Duncan's multiple range test, $p < 0.05$.

Table 3.23 showed alcohol concentrations in wine fermentation. It was found that alcohol concentrations of wine fermentation supplemented with 1000 ppm of ammonium dihydrogen phosphate, and 500 and 700 ppm of ammonium sulfate were not significantly different at $p< 0.05$. In addition, alcohol concentrations of wine fermentation supplemented with 100 ppm of ammonium dihydrogen phosphate and 1000 ppm of ammonium sulfate were the lowest and the highest at $p< 0.05$, respectively.

3.5 Conclusions

1. The equations and R-square values of carbon dioxide concentration (CO_2) with alcohol (alc), reducing sugar (RS), biomass (X) and total soluble solid (TSS) are as follows :

$y_{co_2/alc} = -0.0053X^2 + 1.5148X$ ($R^2 = 0.9961$)

$y_{co_2/RS} = -2x10^{-6}X^5 + 0.0003X^4 - 0.0217X^3 + 0.6587X^2 - 10.255X + 182.48$ ($R^2 = 0.9937$)

$y_{co_2/X} = -6x10^{-9}X^5 + 6x10^{7}X^4 + 3x10^{-6}X^3 - 0.0011X^2 + 0.0376X + 0.3323$ ($R^2 = 0.941$)

$y_{co_2/TSS} = 0.0008X^2 - 0.2043X + 17.503$ ($R^2 = 0.9963$)

2. The suitable time for supplementation of ammonium salt was after 17 h of fermentation. It was better than supplementation at the beginning of the fermentation. It was found that the specific growth rate (μ) was 0.047 h^{-1}, the specific rate of substrate consumption (q_s) was 3.36 g/g-h, the specific rate of product formation (q_p) was 0.021 g/g-h, yield coefficient of biomass formation from substrate ($Y_{x/s}$) was 0.0140 g/g and yield coefficient of production formation from substrate ($Y_{p/s}$) was 0.443 g/g. The alcohol of 83.89 g/L and the final total soluble solid of 7.0 ^oBrix in wine were obtained in wine fermentation supplemented with 700 ppm of ammonium dihydrogen phosphate after 17 h of fermentation.

3. The optimum concentration of ammonium dihydrogen phosphate as nitrogen sources for *Saccharomyces cerevisiae* was 1000 ppm. It was better than those of 100, 300, 500, and 700 ppm. The fermentation kinetics were higher than that obtained from other concentrations. It was found that the specific growth rate (μ) was 0.047 h^{-1}, the specific rate of substrate consumption (q_s) was 3.51 g/g-h, the specific rate of product formation (q_p) was 0.021 g/g-h, yield coefficient of biomass formation from substrate ($Y_{x/s}$) was 0.0134 g/g and yield coefficient of product formation from substrate ($Y_{p/s}$) was 0.445 g/g. The alcohol concentration of 85.60 g/L and the final total soluble solid of 6.8 ^oBrix in wine were obtained.

4. The optimum concentration of ammonium sulfate as nitrogen source for *Saccharomyces cerevisiae* was 1000 ppm. The fermentation kinetics were higher than that obtained from other concentrations. It was found that the specific growth rate (μ) was 0.051 h^{-1}, the specific rate of substrate consumption (q_s) was 3.89 g/g-h, the specific rate of product formation (q_p) was 0.023 g/g-h, yield coefficient of biomass formation from substrate ($Y_{x/s}$) was 0.0131 g/g and yield coefficient of product formation from substrate ($Y_{p/s}$) was 0.452 g/g. The alcohol concentration of 86.56 g/L and the final total soluble solid of 6.7 ^{o}Brix in wine were obtained.

5. Comparison of wine fermentation kinetics supplemented with ammonium sulfate and ammonium dihydrogen phosphate at the same concentration, it was found that the kinetics of wine fermentation supplemented with ammonium sulfate was superior to wine fermentation supplemented with ammonium dihydrogen phosphate.

6. Alcohol concentrations of wine fermentation supplemented with 1000 ppm of ammonium dihydrogen phosphate, 500 and 700 ppm of ammonium sulfate were not significantly different at $p< 0.05$. Though, wine supplemented with ammonium sulfate of 1000 ppm after 17 h of fermentation as nitrogen source for *Saccharomyces cerevisiae*, the alcohol concentration was the highest at 86.56 g/L. In addition, wine supplemented with ammonium sulfate of 500 ppm after 17 h of fermentation, the alcohol of 85.48 g/L was obtained. For economic point of view and health concern, 500 ppm of ammonium sulfate supplementation after 17 h of fermentation as nitrogen sources are recommended.