

3. RESULTS

The results were divided into two stages. First stage was the preliminary analysis of hydrolysis skimmed milk with bromelain enzyme, consists of degree of hydrolysis, pH, and antioxidant activity analysis. These analysis determined the effective hydrolysis time, that used for the next stage. Second stage was debittered skimmed milk hydrolysis with bromelain enzyme, followed by several analysis such as degree of hydrolysis, pH, antioxidant activity, and sensory analysis.

3.1. Preliminary Analysis of Hydrolysis Skimmed Milk

Preliminary analysis of hydrolysis skimmed milk with bromelain enzyme, consists of degree of hydrolysis, pH, and antioxidant activity analysis.

3.1.1. Skimmed Milk Degree of Hydrolysis (DH)

The hydrolysis time and percentage of degree hydrolysis for each hour(s) is shown in Table 1.

Table 1 Skimmed Milk Degree of Hydrolysis Time

Hydrolysis Time (hour(s))	Degree of Hydrolysis (%)
Control (without enzymatic hydrolysis)	12.74±0.01
Skimmed milk with enzymatic hydrolysis	
0	13.08±0.03
1	13.77±0.05
2	14.69±0.03
3	16.35±0.01
4	15.19±0.05
5	15.17±0.02
6	13.90±0.05
7	13.28±0.02
8	12.08±0.04
9	12.30±0.01
10	10.87±0.01

*Numbers show the average ± standard deviation (n=6)

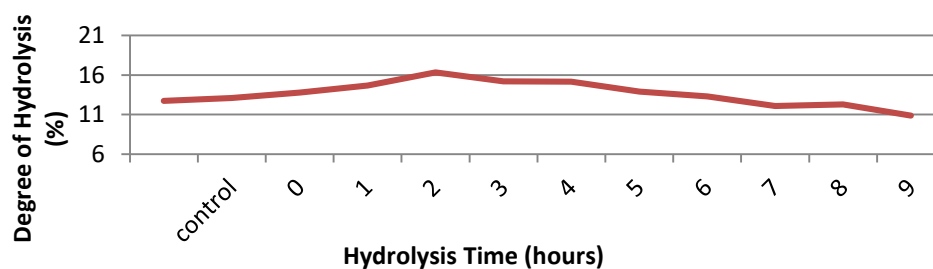


Figure 2 Degree of Hydrolysis of Skimmed Milk Hydrolysate

Table 1. and Figure 2. showed the rate and values of degree hydrolysis control skimmed milk and several hours of hydrolysis (0 to 10 hours hydrolysis time). The lowest DH value presented at 10 hours of hydrolysis, which is $10.87 \pm 0.01\%$. The highest DH value presented at 3 hours of hydrolysis, which is $16.35 \pm 0.01\%$. The graphic also showed that in the first 3 hours of hydrolysis, from control to 0 until 3 hours, the higher rate of degree of hydrolysis achieved. After that, the reactions tended to decrease and have lower degree of hydrolysis than the first 3 hours hydrolysis.

3.1.2. pH of Hydrolysis Skimmed Milk

The pH values of control skimmed milk and effect hydrolysis time on pH are shown in Table 2.

Table 2 pH Values of Hydrolysate Milk with Bromelain Enzyme

Time (hour(s))	pH Value
Control (without enzymatic hydrolysis)	6.37
Skimmed milk with enzymatic hydrolysis	
0	6.38
1	6.45
2	6.51
3	6.64
4	6.62
5	6.63
6	6.61
7	6.68
8	6.68
9	6.68
10	6.7

*Numbers show the average \pm standard deviation (n=6)

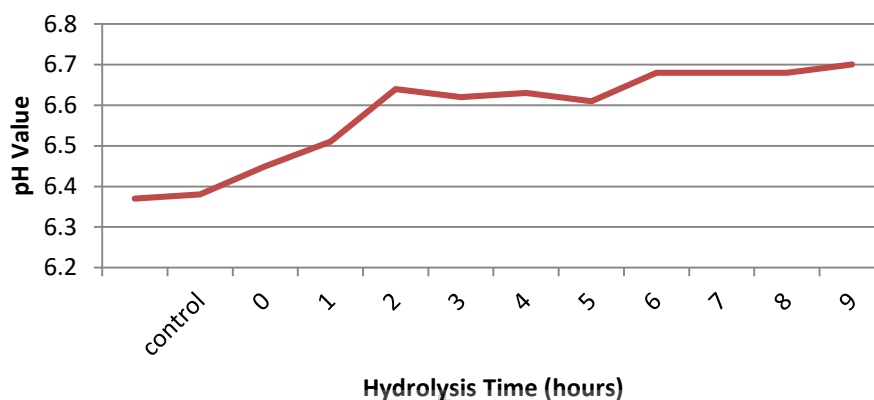


Figure 3 pH Values of Hydrolysate Milk with Bromelain Enzyme

Table 2. and Figure 3. showed that the highest pH value is 6.68 which belongs to 7, 8, and 9 hours of hydrolysis time. The lowest pH value is 6.37, which belongs to control skimmed milk. After incubated with several hydrolysis times, all samples has higher pH value than the control skimmed milk pH value.

3.1.3. Antioxidant Activity of Hydrolysis Skimmed Milk

The antioxidant activity of hydrolysis skimmed milk is shown in Table 3.

Table 3 Antioxidant Activity of Hydrolysis Skimmed Milk

Hydrolysis Time	Antioxidant Activity (%)
Control (without enzymatic hydrolysis)	0.57 \pm 0.006
Skimmed milk with enzymatic hydrolysis	
0	2.06 \pm 0.006
1	18.64 \pm 0.001
3	15.63 \pm 0.006
5	14.28 \pm 0.001
7	19.10 \pm 0.006
9	19.29 \pm 0.006

*Numbers show the average \pm standard deviation (n=6)

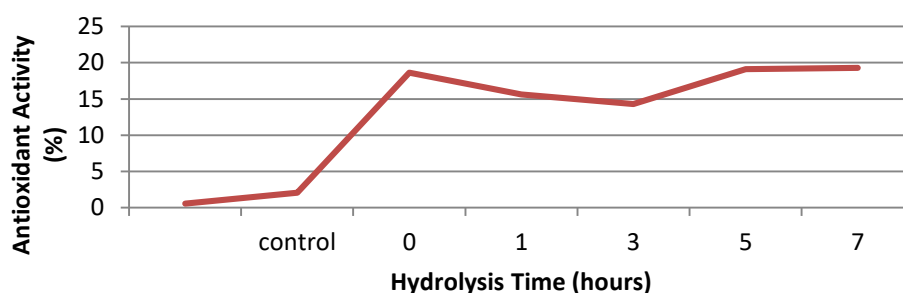


Figure 4 Antioxidant Activity of Hydrolysis Skimmed Milk

Based on Table 3. And Figure 4., showed that the antioxidant activity values from control skimmed milk, 0 hours to 9 hours of hydrolysis. Based on the table above, the highest antioxidant activity is 9 hours of hydrolysis time, which is 19.29%. The lowest antioxidant activity is control, which is 0.57%.

3.2. Debittered Hydrolyze Skimmed Milk

3.2.1. Chemical Analysis of Debittered Skimmed Milk

The chemical analysis of 3 hours incubated hydrolysis skimmed milk and debittered treatments is shown in Table 4.

Table 4 Chemical Analysis Debittered Hydrolyze Skimmed Milk

Treatment	Degree of Hydrolysis (%)	Antioxidant Activity (%)	pH
Control	13.98±0.02 ^b	9.39±0.61 ^a	6.92±0.01 ^a
Masking 2%	14.31±0.03 ^b	63.37±0.89 ^b	7.26±0.01 ^b
Masking 4%	17.98±0.03 ^c	69.02±0.95 ^c	7.4±0.01 ^b
Spray Dry 60:40	11.94±0.03 ^a	14.27±1.06 ^d	6.56±0.01 ^a
Spray Dry 80:20	10.45±0.01 ^a	16.40±0.64 ^e	6.74±0.003 ^a

*Numbers show mean ± standard deviation (n=6)

*Different superscript showed there was a significant difference at 95% (p<0.05) confidence level based on Independent T-test and followed by Duncan's multiple range test as significantly different test

Table 4. showed that the degree hydrolysis percentage for 3 hours incubation with several treatments. Control treatment is 3 hours milk hydrolyze with bromelain enzyme.

Masking 2% is treatment with addition of cocoa powder 2 grams for every 100 ml. Masking 4% ml is treatment with addition of cocoa powder 4 grams for every 100ml. Spray dry 60:40 is treatment with spray drying method that combine the carrier between SPI (Soy Protein Isolate) dan gelatine, 60% is for the hydrolyze milk, and 40% is for the carriers. The highest degree of hydrolysis is $17.98 \pm 0.03\%$ with masking method addition of 4 grams/100ml cocoa powder. The lowest degree of hydrolysis is $10.45 \pm 0.01\%$ with spray dry method 80:20.

The highest antioxidant activity is masking method with 4 grams cocoa powder/100ml hydrolyze milk, with $69.02 \pm 0.95\%$ antioxidant activity. The lowest antioxidant activity is control hydrolyze skimmed milk, with $9.39 \pm 0.61\%$ antioxidant activity. Meanwhile, pH value is variance between the treatments. The highest pH value is owned by masking method with 4 grams cocoa powder/100 ml hydrolyze milk with 7.4 ± 0.01 . The lowest pH is owned by spray dry method 60:40 with 6.56 ± 0.008 .

3.2.2. Sensory Analysis of Debittered Skimmed Milk

The sensory analysis of 3 hours incubated hydrolysis skimmed milk and debittered treatments is shown in Table 5.

Table 5 Sensory Analysis of Debittered Hydrolyze Skimmed Milk

Treatment	Analysis				
	Color	Odor	Texture	Taste	Overall
F0	3.7 ± 1.18^a	3.275 ± 1.20^a	3.3 ± 1.30^a	3.275 ± 1.28^a	3.29 ± 1.26^a
F1	2.13 ± 1.02^b	2.02 ± 0.99^b	2.16 ± 1.11^b	2.08 ± 1.08^b	2.03 ± 1.06^b
F2	1.73 ± 0.94^c	1.81 ± 0.89^c	2.01 ± 0.99^c	2.05 ± 1.04^{bc}	2.03 ± 1.04^{bc}
F3	3.73 ± 1.19^a	3.98 ± 1.11^a	3.78 ± 1.24^a	3.77 ± 1.24^a	3.84 ± 1.19^a
F4	3.63 ± 1.24^a	3.88 ± 1.16^a	3.71 ± 1.25^a	3.75 ± 1.24^a	3.76 ± 1.20^a

Key:

*F0 = Hydrolyze milk with 3 hours incubation; F1 = 3 hours hydrolysis milk spray dried 60:40; F2 = 3 hours hydrolysis milk spray dried 80:20; F3 = 3 hours hydrolysis milk with masking method 2% addition of cocoa powder; F4 = 3 hours hydrolysis milk with masking method 4% addition of cocoa powder

* Numbers show mean \pm standard deviation (n=2)

*Sensory test was done by hedonic ranking test as acceptance product to panelist for 60 people.

*Different superscript showed there was a significant difference at 95% ($p < 0.05$) confidence level based on Kruskal Walls test and followed by Mann Whitney test as significantly different test

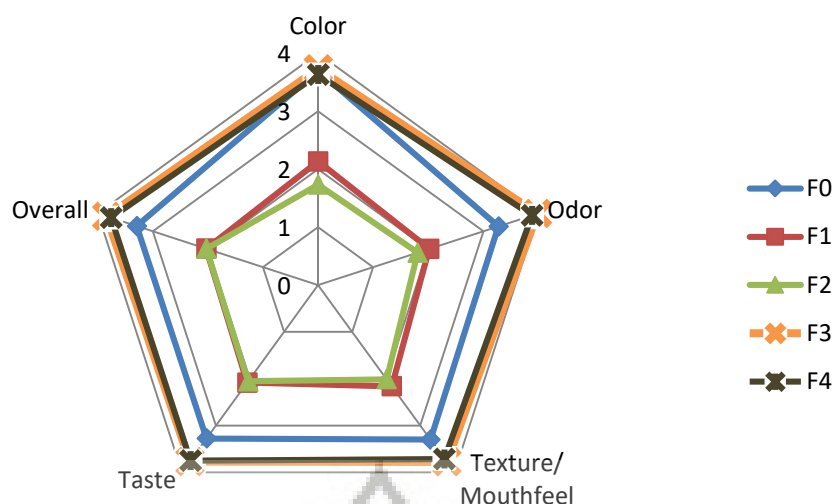


Figure 5 Sensory Analysis of Debittered Hydrolyze Skimmed Milk

Table 5. and Figure 5. showed that sensory test was runned based on color, odor, texture, taste, and overall to 5 different samples. F0, F3 and F4 samples showed the exact same significantly different results on all attributes. Meanwhile, F1 and F2 showed the same significantly different on all attributes. The range of radar showed from the number 0 to 4 on the figure. The wider range of radar showed more favourable type of samples and attributes to the panelists. The widest radar range is F4, and the smallest radar range is F2. The masking method with 4% cocoa powder is most liked among all of the samples, followed by F3, which is masking method with 2% cocoa powder, and the third place of most liked there is F0 or control hydrolyze skimmed milk, second place is F1 or debittered hydolyze skimmed milk with spray dry 60:40 method, and the most unfavourable sample is F2 or debittered hydolyze skimmed milk with spray dry 80:20 method.