APPENDICES

APPENDIX A

Chemical analysis

Total acidity of Noni juice by a titrimetric method (an AOAC official method no 947.05, 2000)

Weight 10 ml of Noni juice into a 250 ml flask and dilute the fruit with distilled water for 2 times of its weight. Add 2 ml phenolphthalein indicator and titrate against 0.1 M NaOH until the first persistent pink appears. Calculate the total acidity by following an equation below;

$$\text{Citric acid}(\%) = \frac{\text{amount of 0.1 M NaOH (ml) x 100) x 0.00686}}{\text{Amount of Noni juice (ml)}}$$

Reducing sugar by the Lane and Eynon method (Rutjanakaikan, 2001)

Reagents

1. Fehling's solution no. 1. Dissolve copper sulfate ($CuSO_4 \cdot 5\ H_2O$) (Carlo Erba, Germany) 69.278 g in distilled water and then adjust to 1000 ml with distilled water in a volumetrick flask.
2. Fehling's solution no. 2. Dissolve 100 g sodium hydroxide and 346 g sodium potassium tartate (Univar, Australia) in distilled water and then adjust to 1000 ml with distilled water in a volumetric flask.
3. Carrez I solution. Dillolve 21.9 g zinc acetate dehydrate (Univar, Australia) in distilled water that has 3 ml gracial acetic acid and adjust to 100 ml with distilled water in a volumetric flask
4. Carrez II solution. Dissolve 10.6 g potassium ferrocyanide (Carlo Erba, Germany) in distilled water and then adjust to 100 ml with distilled water in a volumetric flask.

1. Preparation of Noni juice

Weight 100 g Noni juice and transfer into a 250 ml volumetric flask. Add 50 ml distilled water into the flask, followed by 5 ml Carrez I solution and 5 ml Carrez II solution. Adjust the volume of the volumetric flask to 250 ml by distilled water and leave the solution for 20 min. After that, filter the solution with a whatman filter paper no.4.

2. Analysis reducing sugar before inversion

2.1 Preliminary titration

Take the filtered solution from the preparation no.1 and transfer into a 50 ml burette, ready to be used for sugar determination. Pipette 10 ml of mixed Fehling reagents (each Fehling reagent is 5 ml) into a 250 ml flask and add glass beads (8-10 pieces). Boil the Fehling solution. When the solution has already boiled, titrate the Fehling solution with the filtered solution from the burette until the blue color of the Fehling solution almost disappear, and quickly add the Fehling solution with 1-2 drops of mehtylene blue indicator. After the addition, continue the titration until the blue color absolutely disappear. Take a note for the amount of the filtered solution from Noni juice sample that is used.

2.2 Accurate titration

Prepare the filtered solution from the preparation no.1 and the Fehling solution similar to the procedure of the preliminary titration (in 2.1). Add the Fehling solution with the filtered solution from a burette. The amount of the added filtered solution should be 1-2 ml less than the amount needed to reach the end point of titration in the 2.1. After the addition of the filtered solution, add the Fehling solution with 1-2 drops methylene blue indicator and quickly titrate the solution against the filtered solution from the burette until the blue color of the Fehling solution disappeared.

Calculate the amount of reducing sugar in Noni juice from the Table A1 : Invert Sugar for 10 ml Fehling's Solution (D_I)

3. Analysis reducing sugar after inversion

Transfer 70 ml of the filtered solution from preparation no. 1 into a 250 ml flask, and add with 10 ml of HCl 6.34 M. Warm the flask in a water bath that has been heated at 70^0C for 10 min and then cool it down immediately. After the solution in the flask is cool, adjust the pH of the solution to a pH value of 7.0 by adding NaOH 0.5 M. Transfer the whole solution to a 100 ml volumetric flask and adjust the volume of the solution to 100 ml by using distilled water. Mix the whole solution thoroughly. Transfer some of this solution into a 50 ml burette and use the solution to titrate the 10 ml mixed Fehling solution. Note the volume of the burette's solution that is needed to titrate the Fehling's solution. Calculate the amount of sugar in the Noni juice samples after inversion from the Invert Sugar Table for 10 ml Fehling's solution (D_2)

Reducing sugar = D_1

% sucrose = (D_2-D_1) x 0.95

Total Sugar = D_1 + %Sucrose

Table A1 Invert sugar Table for 10 ml Fehling's Solution

ml of sugar solution required	Solution containing besides Invert sugar : No sucrose		1 g sucrose per 100 ml		5 g sucrose per 100 ml		10 g sucrose per 100 ml		25 g sucrose per 100 ml	
	Invert sugar factor*	mg invert sugar per 100 ml	Invert Sugar Factor*	mg invert sugar Per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml
15	50.5	336	49.9	333	47.6	317	46.1	307	43.4	289
16	50.6	316	50.0	312	47.6	297	46.1	288	43.4	271
17	50.7	298	50.1	295	47.6	280	46.1	271	43.4	255
18	50.8	282	50.1	278	47.6	264	46.1	256	43.3	240
19	50.8	267	50.2	264	47.6	250	46.1	243	43.3	227
20	50.9	254.5	50.2	251.0	47.6	238.0	46.1	230.5	43.2	216
21	51.0	242.9	50.2	239.0	47.6	226.7	46.1	219.5	43.2	206
22	51.0	231.8	50.3	228.2	47.6	216.4	46.1	209.5	43.1	196
23	51.1	222.2	50.3	218.7	47.6	207.0	46.1	200.4	43.0	187
24	51.2	213.3	50.3	209.8	47.6	198.3	46.1	192.1	42.9	179
25	51.2	204.9	50.4	201.6	47.6	190.4	46.0	184.0	42.8	171
26	51.3	197.4	50.4	193.8	47.6	183.1	46.0	176.9	42.8	164
27	51.4	190.4	50.4	186.7	47.6	176.4	46.0	170.4	42.7	158
28	51.4	183.7	50.5	180.2	47.7	170.3	46.0	164.3	42.7	152
29	51.5	177.6	50.5	174.1	47.7	164.5	46.0	158.6	42.6	147
30	51.5	171.7	50.5	168.3	47.7	159.0	46.0	153.3	42.5	142
31	51.6	166.3	50.6	163.1	47.7	153.9	45.9	148.1	42.5	137
32	51.6	161.2	50.6	158.1	47.7	149.1	45.9	143.4	42.4	132
33	51.7	156.6	50.6	153.3	47.7	144.5	45.9	139.1	42.3	128
34	51.7	152.2	50.6	148.9	47.7	140.3	45.8	134.9	42.2	124
35	51.8	147.9	50.7	144.7	47.7	136.3	45.8	130.9	42.2	121
36	51.8	143.9	50.7	140.7	47.7	132.5	45.8	127.1	42.1	117
37	51.9	140.2	50.7	137.0	47.7	128.9	45.7	123.5	42.0	114
38	51.9	136.6	50.7	133.5	47.7	125.5	45.7	120.3	42.0	111
39	52.0	133.3	50.8	130.2	47.7	122.3	45.7	117.1	41.9	107
40	52.0	130.1	50.8	127.0	47.7	119.2	45.6	114.1	41.8	104
41	52.1	127.1	50.8	123.9	47.7	116.3	45.6	111.2	41.8	102
42	52.1	124.2	50.8	121.0	47.7	113.5	45.6	108.5	41.7	99
43	52.2	121.4	50.8	118.2	47.7	110.9	45.5	105.8	41.6	97
44	52.2	118.7	50.9	115.6	47.7	108.4	45.5	103.4	41.5	94
45	52.3	116.1	50.9	113.1	47.7	106.0	45.4	101.0	41.4	92
46	52.3	113.7	50.9	110.6	47.7	103.7	45.4	98.7	41.4	90
47	52.4	111.4	50.9	108.2	47.7	101.5	45.3	96.4	41.3	88
48	52.4	109.2	50.9	106.0	47.7	99.4	45.3	94.3	41.2	86
49	52.5	107.1	51.0	104.0	47.7	97.4	45.2	92.3	41.1	84
50	52.5	105.1	51.0	102.0	47.7	95.4	45.2	90.4	41.0	82

*mg of invert sugar corresponding to 10 ml of Fehling's solution.

Source : Rutjanakaikan (2001)

APPENDIX B

Proximate analysis

Moisture content (a method from an AOAC official Method no. 925.45, 2000)

Weight 3 g of Noni juice samples into a moisture can with a tight-fit cover, which was known accurately the weight. Place loosely the covered can in a hot air oven at 100^0C. Dry the samples until the weight is constant (about 4 h). Press the cover tightly into the moisture can, then removed them from the oven, cool in an active dessicator and weight. Express the loss weight as a moisture content of the Noni juice samples.

$$\%\text{Moisture content} = \frac{(a-b)}{c} \times 100$$

a = weight of can and sample before drying in an oven (g)

b = weight of can and sample after drying in an oven (g)

c = weight of sample (g)

Fiber (a modified method from an AOAC official method no. 978.10 (2000) and Rutjanakaikan (2001)

Procedure

One g of Noni juice is transfered into a 1000 ml flask. Add 200 ml of 0.12 M sulfuric acid solution into the flask and glass beads. Boil the solution and leave the solution boils exactly for 30 s. Prepare a funnel for filtration of fiber (using a whatman paper no. 41 that is known accurately its weight) by flowing boiling distilled water through the funnel to warm it. Then pour the acid solution that was boiled through the funnel and wash the solid that is collected on the funnel with boiled distilled water using vacuum. Wash the residue from the funnel in the flask that is used before with 200 ml of 0.31 M sodium hydroxide solution. Place the flask on a heater and boil for 30 min. Afterwards, pour the base solution that was boiled through a funnel using a whatman paper no.41 that is known accurately for its weight and wash the solid on the funnel with boiled distilled water using vacuum. Wash the residues from the funnel into the flask that is used before with 30 ml 1% of HCl. Next, wash the residues first with boiling distilled water and then 30 ml of 95% ethyl alcohol 2 times and 30 ml diethyl ether 3 times. Transfer the residues into a crucible that is known accurately for its weight. Dry the crucible in a boiling-water bath and then take the crucible into a hot air oven at 100 ^{0}C until the weight is constant. Cool the crucible in an active desiccator and weight again. After that, take the crucible into an oven at 500 ^{0}C for 3 h. Cool the crucible in an active desiccator and weight. Calculate the percentage of fiber in Noni juice samples.

Crude fiber in sample = weight of fiber – weight of ash

$$\%\text{Crude fiber} = \frac{\text{weight of fiber in sample (g)}}{\text{weight of sample (g)}} \times 100$$

Fat in Noni juice by the Rose-Gottlieb method (a modified method from an AOAC official methodno.905.02, 2000)

Determination

Weight 1 g Noni juice into a separatory funnel. Add 1 ml NH_4OH and mix thoroughly. Add with 10 ml ethyl alcohol 95% and mix well. Next, add with 25 ml diethyl ether that must be peroxide-free, and then close with a stopper, and shake very vigorously for 1 min. After that, add with 25 ml petroleum ether which has a boiling point range of 40-60^{0}C and repeat vigorous shaking for 30 s. Let the funnel stand until the liquid is separated into two layers. Upper liquid is separated into a flask that is dried and known accurately its weight. Repeat the extraction of the remaining liquid in the separatory funnel twice, using 15 ml diethyl ether and 15 ml petroleum ether. The upper liquid that is extracted for 3 times is added together into the dried flask. Then, the flask is taken into a hot air oven at 100°C, and dried to a constant weight.

$$\%\text{Fat} = \frac{\text{weight of fat (g)}}{\text{weight of sample (g)}} \times 100$$

Protein in Noni juice by the Kjeldahl method (a modified method from an AOAC official method no.991.20, 2000)

Determination

Weight 2 g of noni juice into a Kjeldahl digestion flask. Eight g of catalyst mixture and 20 ml sulfuric acid are added into the digestion flask. Place the flask in an incline position in a digestion machine. Next, increase the burner setting and boil until the noni juice is looked clear and then cool it to room temperature. Distilled water was added into the cooled flask. Then, transfer the noni juice that has been digested into a distilling flask. Distilled water of 400 ml is added into the distilling flask and swirl to mix. Three or four boiling chips are added into the flask too and a methyl red/bromocresol green indicator is also added. Connect up the distillation with a deliver tube dipping below a boric solution. Make a diluted digest alkaline with 50% sodium hydroxide solution in amount of 75 ml. Close the tap and distilled the ammonia into the boric acid solution. After about 30 ml the distillation is over, open the tap and wash down a condenser and the delivery tube into the receiver. Titrate the distillate with 0.05 M sulphuric acid.

Calculate the percentage of nitrogen in the sample (1 ml 0.05 M sulphuric acid equal to 0.014 g nitrogen). The crude protein can be calculated using an appropriate factor of 6.25

$$\%N = \frac{[(\text{ml of } H_2SO_4 - \text{ml of } H_2SO_4 \text{ blank}) \times 0.05 \times 0.014] \times 100}{\text{weight of sample (g)}}$$

$$\%\text{Protein} = \%N \times 6.25$$

Ash (AOAC official Method no. 945.46, 2000)

Weight 3 g of Noni juice and place on a ceramic dish that is known accurately for its weight. Heat the dish on a steam bath. Afterwards, transfer the dish into a hot air oven at 500 ^{0}C until the sample become an ash. Cool the dish in an active desiccator and weight it again. Calculate the percentage of ash in the noni juice.

$$\%\text{Ash} = \frac{\text{weight of ash (g)} \times 100}{\text{weight of sample (g)}}$$

Carbohydrate (Rutjanakaikan, 2001)

$$\%\text{Carbohydrate} = 100 - (\%\text{Moisture} + \%\text{Fat} + \%\text{Protein} + \%\text{Ash})$$

APPENDIX C

Carotenoid in noni juice (a modified method from an AOAC official method no. 941.5, 2000)

Procedure

1. Prepare a stock solution by dissolving 0.005 g β-carotene standard with 2.5 ml chloroform in a 50 ml beaker glass.
2. Adjust the volume of the solution with hexane in a 50 ml volumetric flask.
3. Dilute the stock solution by pipetting 5 ml of the solution into another 50 ml volumetric flask and then adjust the volume of the solution with hexane.
4. Pipette 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of the diluted solution into 10 ml volumetric flasks and adjust the volume with 10 % acetone in hexane.
5. Optimize the maximum wavelength by measuring the absorbance of the highest concentration of the standard solution.
6. Adjust λ max at the spectrophotometer from the previous step and measure all the standard concentrations. The blank was 10 % acetone in hexane solution. Record all the result measurements.
7. A linear equation was drawn from all the standard solution results and a graph between β-carotene concentration (ppm) and absorbance values was produced. The method for preparing 10% acetone in hexane solution was done by pipetting 10 ml acetone into a 100 ml volumetric flask and then adjust the volume with hexane.

The calculation to find out the amount of carotenoid in noni juice samples.

A correlation between the absorbance values and the β-carotene standard solution at λ = 450 nm was figured out in the standard curve and expressed in a linear equation which was:

$y = 0.1535x-0.0025$

Where y = absorbance values of carotenoid from noni juice samples.

x = the amounts of carotenoid in Noni juice samples.

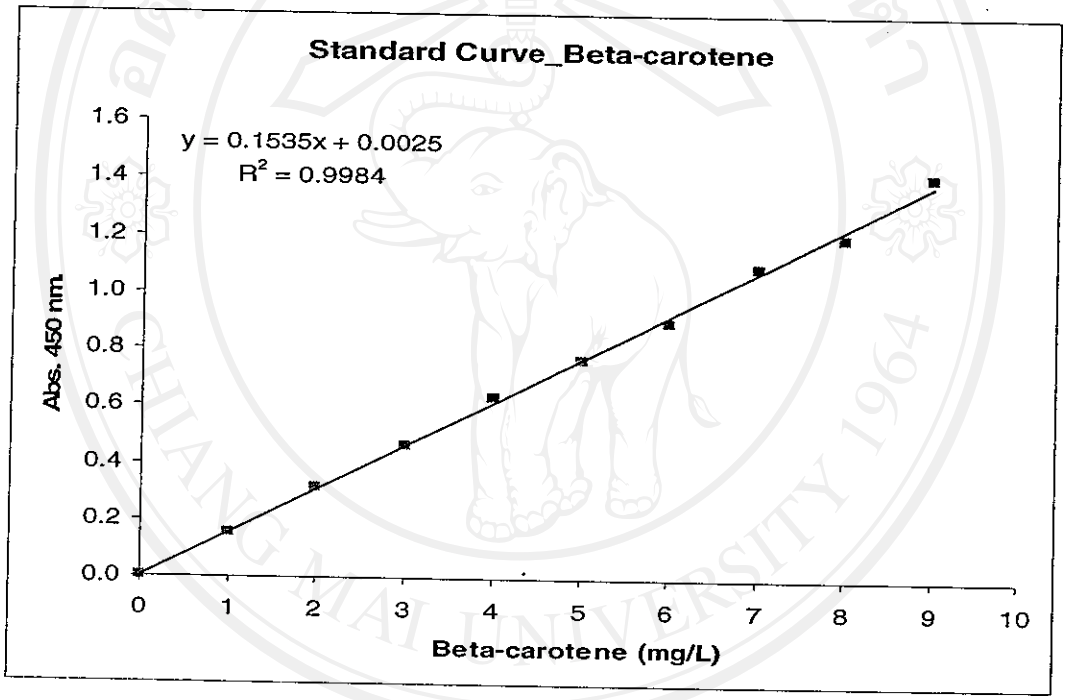


Figure C 1 Standard curve for carotenoid

An extraction method of noni juice samples.

Five g of noni juice was put into a 250 volumetric flask. A 100 ml of 40 % acetone in hexane was poured into the flask which was then stirred with a magnetic stirrer for 10 min. The supernatant of the solution was separated from the noni juice by doing a filtration with a whatman filter paper no. 4 and collected in a 250 ml separated funnel. Whereas, the Noni juice was washed for 2 times with 25 ml hexane. The solution from the washing steps was added into the separated funnel. The acetone in the separated funnel was removed by washing 5 times with 100 ml distilled water. Due to different solubility, the acetone which

was dissolved in distilled water could be separated from the carotenoid which was dissolved in hexane by using the separated funnel. The carotenoid which was dissolved in hexane was further filtered through a whatman filter paper no. 2 into a 250 ml beaker. The filtered solution was left in a hood until it was dried. After that the dried carotenoid was dissolved in 10% acetone in hexane in a 50 ml volumetric flask. The mixed solution was then measured for its absorbance at a wavelength of 450 nm and the absorbance value was recorded.

For the blank which was 10% acetone in hexane was measured 3 times for it absorbance.

Calculation the amount of carotenoid.

1000 ml diluted solution would have carotenoid = X mg.

50 ml diluted solution would have carotenoid = (X/1000) * 50 mg

Calculation for carotenoid in 5 g Noni juice.

5 g Noni juice would have carotenoid = Z mg.

1 g Noni juice would have carotenoid = Z/5 mg.

The unit was changed from mg to μg by multiplying the result with 1000 to be used in report.

APPENDIX D

Vitamin C (a modified method from an AOAC official method no. 967.21, 2000 and Rattanapanon, 2001)

An extraction solution (metaphosphoric acid-acetic acid solution)

Dissolve 15 g HPO_3 in 40 ml CH_3COOH, add with 200 ml distilled water and further dilute to 500 ml with distilled water in a volumetric flask.

An ascorbic acid standard solution

Accurately weight 0.05 g ascorbic acid reference standard that has been stored away direct sunlight. Transfer the acid to a 50 ml volumetric flask. Dilute the acid to the volume of volumetric flask with distilled water immediately before use with HPO_3-CH_3COOH solution.

Indophenol standard solution

Dissolve 0.05 g 2,6-dichoroindophenol in 50 ml distilled water to which has been added with 42 mg $NaHCO_3$ and then dilute to 200 ml with distilled water. Filter the solution through a fluted paper into an amber glass-stoppered bottle. Keep the bottle stoppered, out of direct sunlight and store in a refrigerator.

Vitamin C standard curve

Transfer 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml aliquots of ascorbic acid standard solution to each of 50 ml Erlenmeyer flask and then dilute the solution to 7.0 ml with HPO_3-CH_3COOH solution. Titrate the mixed solution rapidly with indophenol solution from a 50 ml buret until light but distinct rose pink persists for $\geq$ 5 s. Each value represented a mean value of triplicate determinations on each sample.

Similarly titrate 3 blanks composed of 7.0 ml HPO_3-CH_3COOH solution plus volume of distilled water equal to ascorbic acid standard solution and titrated with indopenol solution in direct titration. Calculate the amount of vitamin C in noni juice from the vitamin C standard curve in Figure C2

Preparation of noni juice samples for vitamin C determination

Weight 20 g noni juice samples and mix with 100 ml HPO_3-CH_3COOH solution. Shake the mixed solution thoroughly to ensure the uniformity of the solution and filter through a whatman filter paper no.4. Pipette 10 ml of the filtered solution into a 125 ml flask and then immediately titrate against indophenol

standard solution. The end point of titration caused by an excess unreduced dye in acid solution should appear as a pink color that persists for ≥ 5 s.

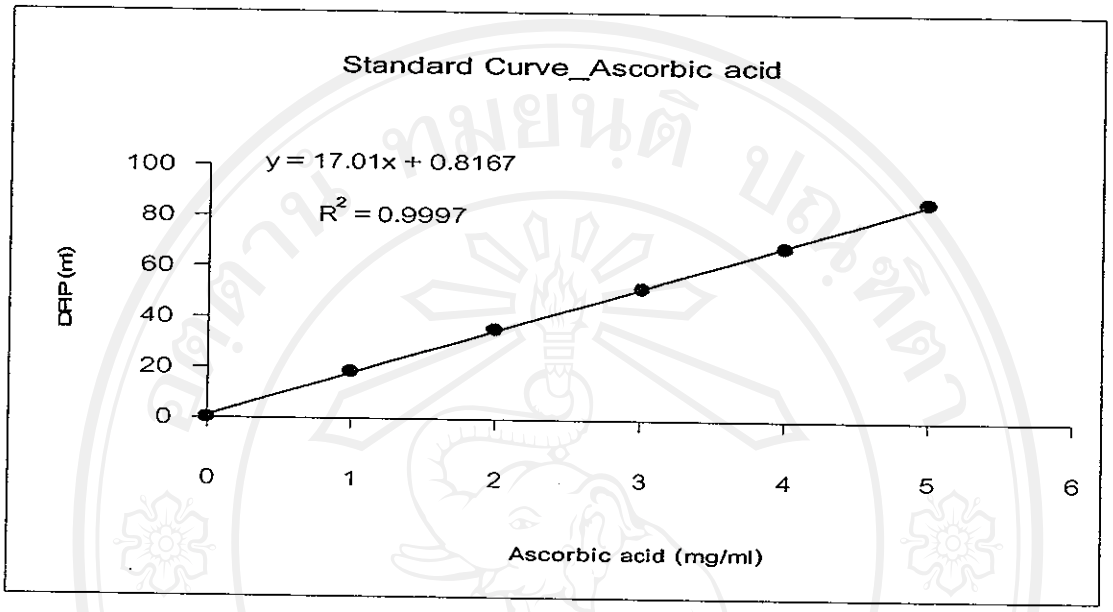


Figure D 1 Standard curve for ascorbic acid

Calculation the amount of ascorbic acid

For example : Unripen fruit 20.0050 g used 34.00 ml of 2,6-dichoroindophenol to titrate

$y = 17.01x + 0.8167$

where, y = amount of indophenol standard solution

x = amount of vitamin C

$34.00 = 17.01 (x) + 0.8167$

x = amount of vitamin C = 1.95 mg

10 ml sample solution would have ascorbic acid = 1.95 mg.

100 ml diluted sample solution would have ascorbic acid = (100x1.95)/10

= 19.5 mg

100 ml of sample solution from 20.0050 g of sample

20.0050 g of sample have ascorbic acid = 19.5 mg

sample 100 g would have ascorbic acid = 19.5 x 100 /20.0050

= 97.5 mg

sample 100 g have ascorbic acid = 97.5 mg

Unripen noni have ascorbic acid 97.5 mg/100g

APPENDIX E

Determination of Total Phenolic (a modified method from Kim *et al.*, 2003)

Total phenolics were evaluated using the spectrophotometric analysis with Folin-Ciocalteu's phenol reagent. In brief, a 20 g of sample was mixed with 20 ml of methanol/HCl 2% (95:5 v/v) in beaker (250 ml) and hold for 60 minutes. The solution was centrifuged at 3,000 rpm for 15 minutes. The supernatant was filter through a whatman filter paper no.4. Diluted to volume 50 ml with methanol/HCl 2% in 50 ml volumetric flask. An aliquot (1 ml) of appropriately diluted extracts was added to a 100 ml volumetric flask containing 9 ml of distilled water. A reagent blank using distilled water was prepared. Five milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and then shaken. After 5 minutes, 10 ml of a 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (100 ml) with distilled water and mixed thoroughly. After 90 minutes at room temperature, the absorbance was read against the prepared blank at 750 nm. The standard curve for total phenolics was made using gallic acid standard solution (100-800mg/l) under the same procedure as above. Total phenolics in noni were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh sample. All samples were analyzed in 3 replications.

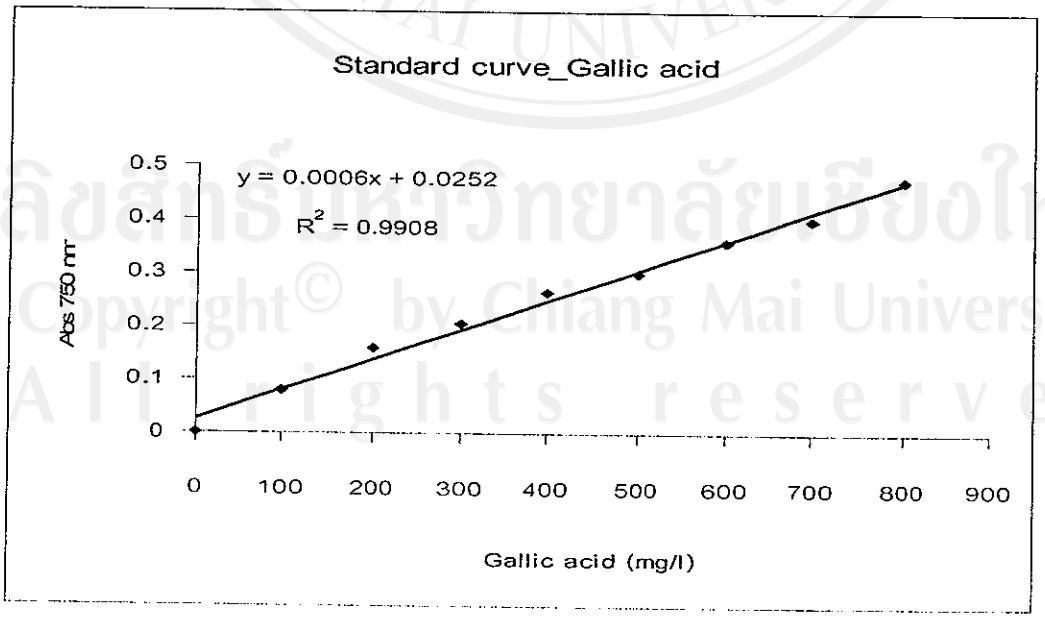


Figure E 1 Standard curve for total phenolic

Calculation the amount of total phenolic

For example

Unripen fruit 20.0936 g have absorbance at 750 nm = 0.473

$y = 0.0006x + 0.0252$

where, y = absorbance at 750 nm

x = amount gallic acid

$0.473 = 0.0006 (x) + 0.0252$

x = amount of gallic acid = 746.34 mg

1000 ml diluted solution would have total phenolic = 746.34 mg.

100 ml diluted solution would have total phenolic = (746.34/1000) * 100 mg

= 74.36 mg

Calculation for total phenolic in 20.0936 g noni.

20.0936 g noni would have total phenolic = 74.634 mg.

1 g noni would have total phenolic = 74.634/20.0936 mg.

= 3.714 mg

The unit was changed from mg/g to mg/100g by multiplying the result with 100 to be used in report.

The unripen noni fruit have 371.4 mg/100g.

APPENDIX F

Determination of Total Flavonoids (a modified method from Kim *et al.*, 2003)

Total flavonoids were measured according to a colorimeteric assay. A 1 ml aliquot of appropriately diluted sample was added to a 10 ml volumetric flask containing 4 ml of distilled water. At zero time, 0.3 ml of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the contents of the reaction flask were diluted to volume with the addition of 2.4 ml of distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm versus prepared water blank.

The standard curve for total phenolics was made using catechin standard solution (100-500mg/l) under the same procedure as above. The flavonoid in noni were expressed as milligrams of catechin equivalents per 100 g of fresh sample. All samples were analyzed in 3 replications.

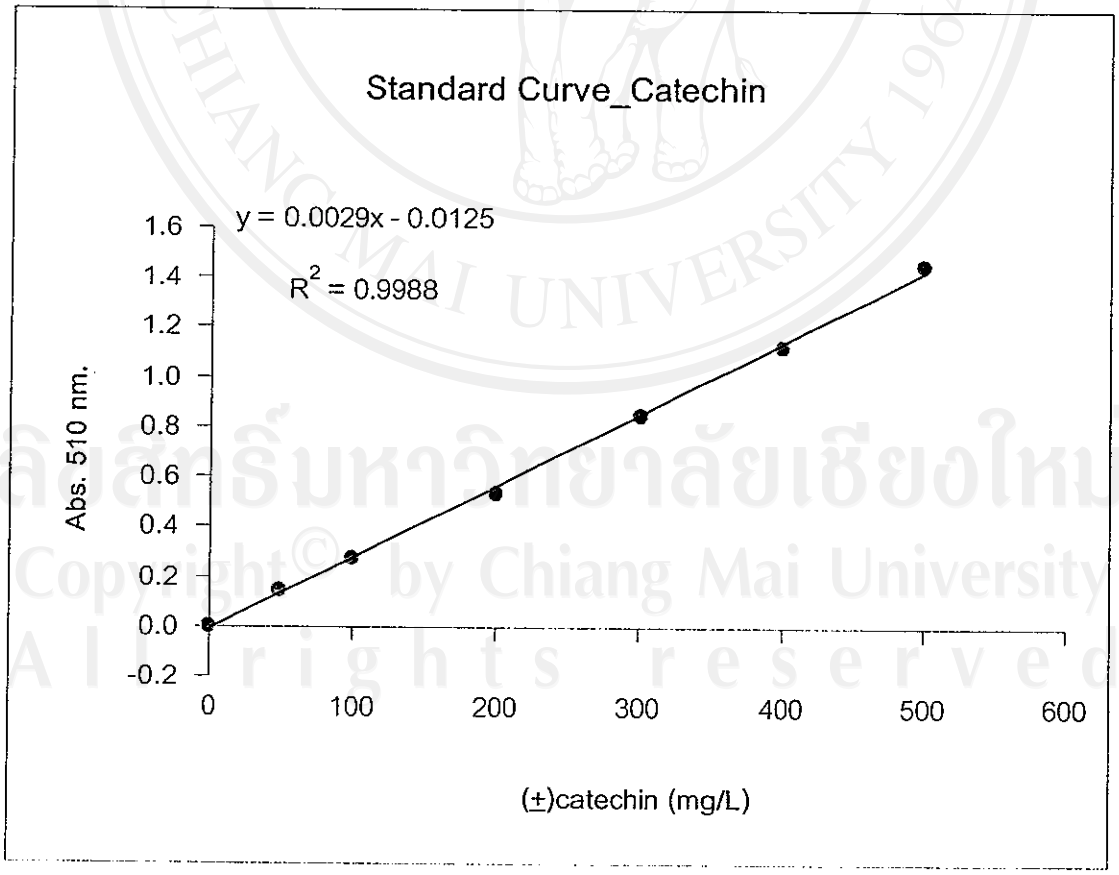


Figure F 1 Standard curve for flavonoid

Calculation the amount of flavonoid

For example

Unripen fruit 5.0057 g have absorbance at 510 nm = 0.305

$y = 0.0029x - 0.0125$

where, y = absorbance at 750 nm

x = amount of cetechin

$0.305 = 0.0029 (x) - 0.0125$

$x = 109.48$ mg

1000 ml diluted solution would have total phenolic = 109.48 mg.

100 ml diluted solution would have total phenolic = (109.48/1000) * 100 mg

= 10.948 mg

Calculation for total phenolic in 5.0057 g noni.

5.0057 g noni would have total phenolic = 10.948 mg.

1 g noni would have total phenolic = 10.948 /5.0057 mg.

= 2.187 mg

The unit was changed from mg/g to mg/100g by multiplying the result with 100 to be used in report.

The unripen noni fruit have 218.7 mg/100g.

APPENDIX G

Antioxidant activity assay (a modified method from Argolo *et al*, 2004 and Benvenuti *et al.*, 2004)

Spectrophotometric analysis was used in order to determine the inhibition concentration (IC_{50}) of the sasmples, which are widely used parameters for determining radical scavenging activity of pure samples and plant extracts. IC_{50} is the amount of antioxidant nessessary to decrease the initial concentration of DPPH radical by 50%. The experiments were performed using a spectrophotometer according to the methods of Moure *et al.* (2001). 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging activity of natural antioxidants (Brand-Williams *et al.*, 1995). In this study, increasing aliquots of each methanol/HCl 2% extract (3 replicates) were mixed with a methanolic solution of DPPH (1 mM, 0.5 ml) in 4-ml cuvettes and brought to 3.0 ml with methanol. To eliminate the interference of the extract pigments on the DPPH reaction, blanks of the fruit extracts were performed using 0.5 ml methanol instead of the DPPH solution. After incuation in the dark at room temperature for 30 minutes, the the spectrophotometric determination was assayed at 515 nm. A DPPH blank sample (containg 2.5 ml of methanol and 0.5 ml of DPPH solution) was prepared and measured daily. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4°C between measurements. The percent decrease in absorbance was recorded for each concentration, and percent quenching of DPPH radical was calculated on the basis of the observed decrease in absorbance of the radical. Percent inhibition of extract change curves were used to find the concentration at which 50% radical scavenging occurred. Percent inhibition was calculated according to the formula:

$$\%\text{inhibition} = [(A_{DPPH}) - A_{extr})/A_{DPPH}] \times 100$$

Where A_{DPPH} is the absorbance value of the DPPH blank sample and A_{extr} is the absorbance value of the test solution. A_{extr} was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank. IC_{50} was calculated by plotting the %inhibition at the steady state (30 min) against various concentrations of each extract (500, 250, 125, 62.5, 31.25 mg/l). The results were expressed as mg antioxidant/g DPPH $\pm$ standard deviation.

Table G1 Percent inhibition of gallic acid

IC_{50}_Std. Gallic acid		
conc. (mg/l) mg/l	abs. 515 nm	%inhibition
500	0.104	94.7074
250	0.106	94.6056
125	0.113	94.2494
62.5	0.115	94.1476
31.25	0.432	78.0153
16.625	0.781	60.2545
DPPH	1.965	0

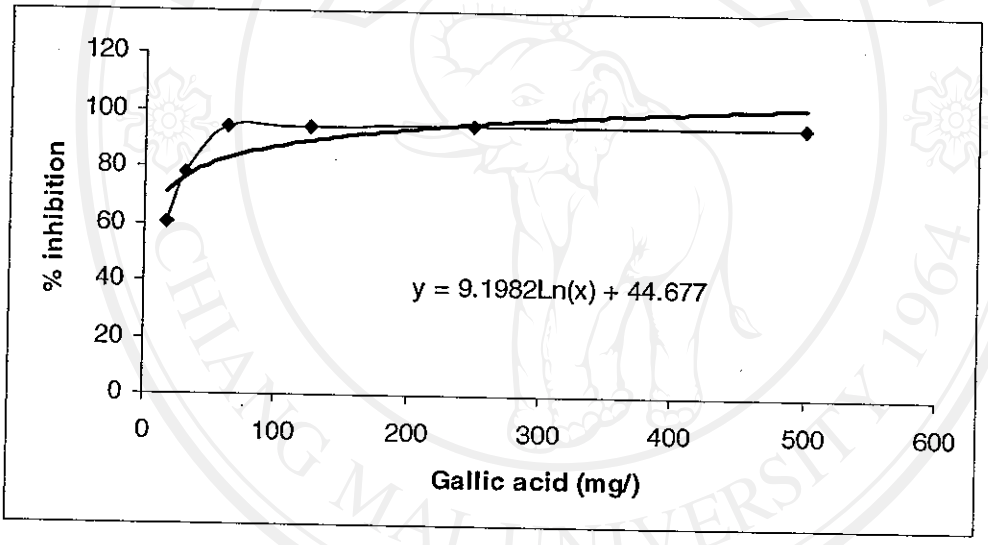


Figure G 1 Curve for gallic acid

$y = 9.1982 \text{ Ln } (x) + 44.677$

$50 = 9.1982 \text{ Ln } (x) + 44.677$

$\text{Ln } (x) = 0.58$

$(x) = e^{0.58}$

$IC_{50} = 1.78$ mg/g DPPH

APPENDIX H

Pasteurization apparatus

(1) Pump: Power 0.25 hp. to feed sample.

(2) Sample tank.

(3) Preheat unit: stainless tube (0.9525 cm inside diameter, 24 m length) coil in water bath that was controlled at a temperature of 60°C.

(4) Pasteurizing unit: stainless tube (inside diameter 0.9525 cm, length 24 m) coil in water bath that was controlled at temperature at 63°C., 71°C or 88°C.

(5) Cooling unit: cool water with ice in a tank that had a temperature between 2 - 6°C.

The temperature of the coil tube was checked using 4 thermocouples (6) (Gallenkamp, England)

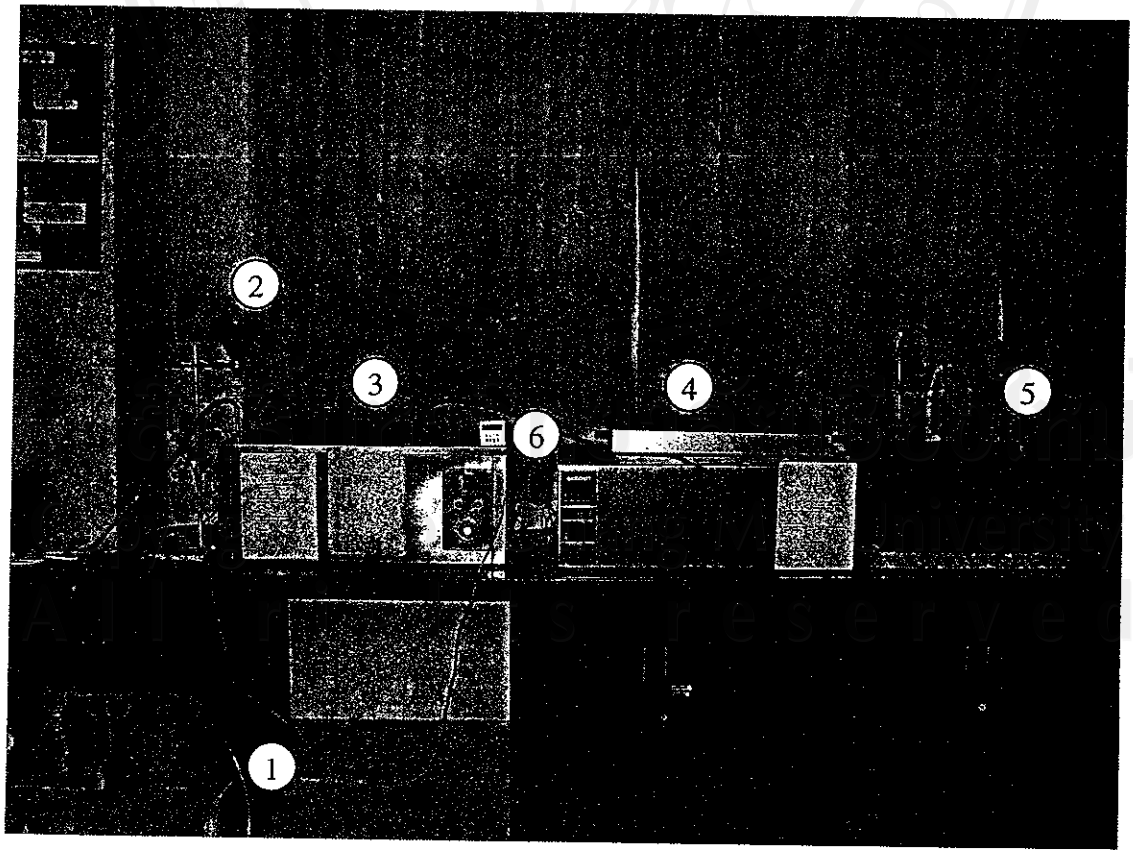
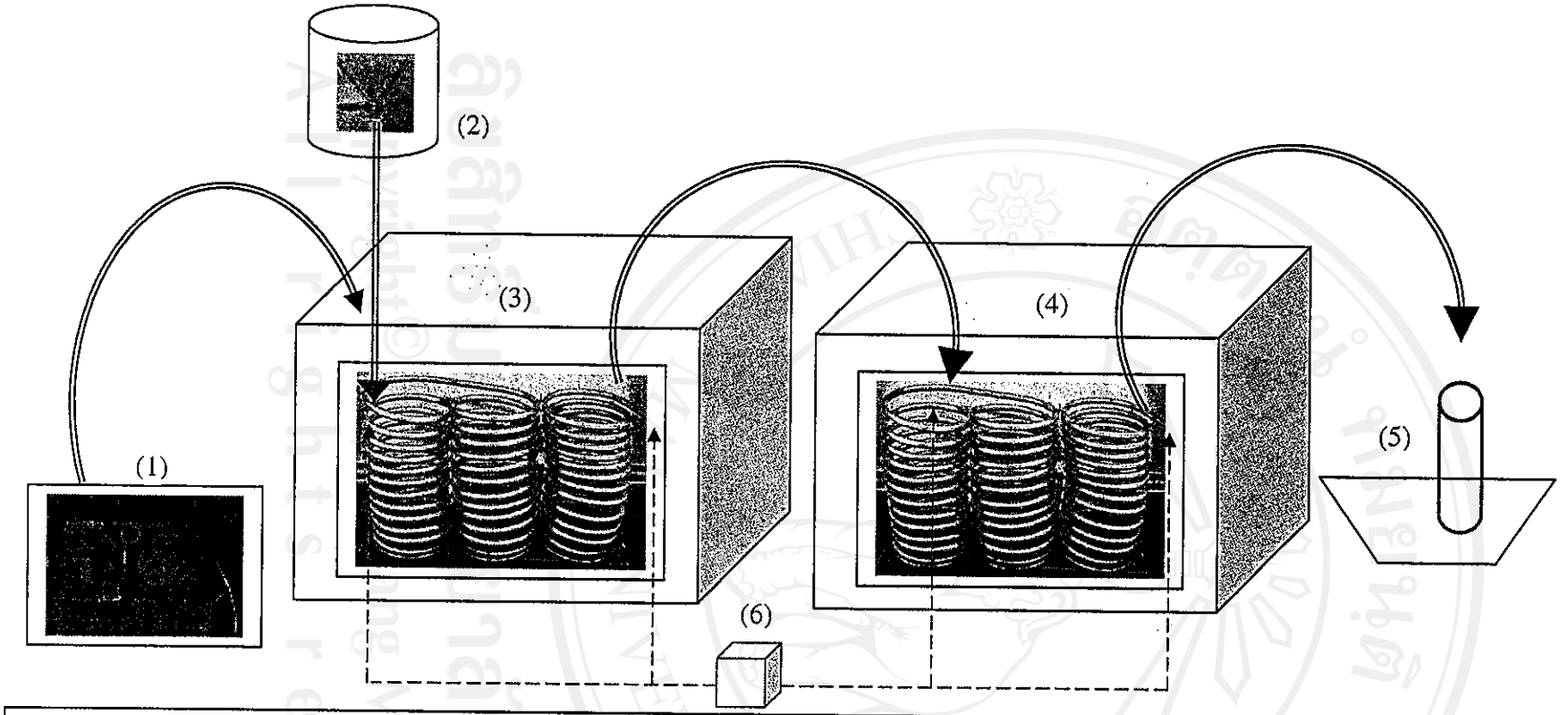


Figure H 1 Pasteurization apparatus



Procedure

Setting the temperature and checked by using thermocouple (6). Feed the sample (500 ml) from sample tank (2) to preheat unit (3). Link up the air pump (1) to preheat unit (3) and then open pump for air flow to feed sample to pasteurizing unit (4).

- Hold the sample in pasteurizing unit at 63°C for 30 minutes and then feed to cooling unit (5).
- Hold the sample in pasteurizing unit at 71°C for 1 minute and then feed to cooling unit (5).
- Feed the sample to flow through the pasteurizing unit within 16 seconds at 88°C (flow speed 1.5 m /seconds) to cooling unit (5)

Figure H 2 Schematic diagram of the pasteurization apparatus

CURRICULUM VITAE

Name	Mr. Phanlop Boonputh
Date of Birth	4 May 1978
Academic background	High school from Benchama maharat School. Ubonratchatani B.S. (Food Technology and Nutrition) Mahasarakham University 2002