

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Chemical Reagents

3.1.1 Chemical reagents

Names of chemical reagents	Production company
Aluminium chloride hydrated	Ajax Finchem, New Zealand
Acetic acid glacial	Merck, Germany
Acetone	Fisher Scientific, U.K.
Ascorbic acid	Sigma-Aldrich, Switzerland
Boric acid	Merck, Germany
(±) Catechin hydrate	Sigma-Aldrich, Switzerland
Copper sulphate	J.T. Baker, U.S.A.
Chloroform	Merck, Germany
2,6-Dichlorophenolindophenol	Merck, Germany
2,2-Diphenyl-1-picrylhydrazyl	Fluka, Switzerland
Folin-Ciocalteu's phenol reagent	Merck, Germany
Gallic acid	Fluka, Switzerland
Hexane	J.T. Baker, U.S.A.
Hydrochloric acid	J.T. Baker, U.S.A.
Methanol	J.T. Baker, U.S.A.
Methylene blue	Merck, Germany
Oxalic acid	Carlo Erba Reagenti, Germany
Potassium ferrocyanide	J.T. Baker, U.S.A.
Sodium carbonate	Merck, Germany
Sodium hydroxide	Merck, Germany
Sodium hydrogen carbonate	Merck, Germany
Sodium nitrate	Merck, Germany

Sodium potassium tartrate	J.T. Baker, U.S.A
Standard β -carotene	Fluka, Sigma-Aldrich, USA
Zinc acetate dihydrate	J.T. Baker, U.S.A

3.1.2 Equipment

Blender	Imarflex, France
Centrifuge	Z 200A HERMLE, Germany
ColorQuest II Hunterlab	Minolta Camera, Japan
Electronic analytical balance	Sartorius CP224S, Germany
Filter papers	Whatman No.1 and No.4 ; 12cm diameter, USA
Hot air oven	Memmert, Germany
Hot plate and Magnetic stirrer	Whatman; HPMS, USA
Juicer	Imarflex, France
pH meter	LabincowTW pH 537, USA
Muffle furnace	Gallenkamp, England
Semi micro Kjeldahl apparatus	Tecator, Germany
Spectrophotometer	Biomate 5, Thermo spectronic, England
Water bath	Gallenkamp, England

3.1.3 Fresh noni fruits.

Noni fruit (unripen, half-ripen and fully ripen) used in this study was harvested from Tumbon Maehea and Tumbon Suthep, Muang district, Chiang Mai province.

3.1.4 Commercial Noni Products

Siam noni cool, 20% noni juice honey flavor (noni juice 20%, sugar 8%, honey 2% and citric acid 0.4%) was purchased from Health food creation Co. Ltd., Bangkok. Concentrate noni juice was purchased from Ban Arokaya, Bangkok. Fermented noni juice was purchased from Kaset Somphong noni plantation, Sa-kaew province.

3.1.5 Pasteurization apparatus

Pump: Power 0.25 hp to feed sample.

Sample tank.

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.

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.

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 (Gallenkamp, England)

The photograph of the pasteurization apparatus was shown in Appendix H.

3.2 Methods

3.2.1 Nutritional value and composition of fresh noni fruits.

Analysis of fresh noni fruits.

This study used three harvesting stages of fresh noni fruits including unripen fruits (green noni fruit), half-ripen fruits (light yellow noni fruit), and fully ripen (light brown fruit). These different ripening stages of noni fruits were analyzed as followed:

a) Chemical and physical properties including pH measurement by using pH meter, color by using a colorimeter (Minolta, Japan), weight of individual fruit by using an analytical balance, total acidity, reducing sugar, and total sugar. The procedures for the last 3 analyses could be seen in Appendix A.

b) Proximate analysis, including moisture content, ash, fat, protein, fiber, and carbohydrate as described in Appendix B.

c) Antioxidant component including ascorbic acid content, carotenoid content, flavonoid content, total phenolic content as described in Appendix C, D, E, and F, respectively.

d) Antioxidant activity as described in Appendix G.

3.2.2 Nutritional value and composition of fresh noni juices.

Analysis of noni juice.

Noni fruit juice was produced using half-ripen of noni fruit. The production steps were inspection of the fruit, washing the fruit, slicing the fruit into 8 section, blending the fruit with juicer and extraction the noni juices using a mesh that had a size of 20 mesh (Subcharoen, 2002). The fresh noni juice was analyzed as followed:

a) Chemical and physical properties including pH measurement, color, total acidity, reducing sugar, and total sugar. The procedures for the last 3 analyses could be seen in Appendix A.

b) Proximate analysis, including moisture content, ash, fat, protein, fiber, and carbohydrate as described in Appendix B.

c) Antioxidant component including ascorbic acid content, carotenoid content, flavonoid content, total phenolic content as described in Appendix C, D, E, and F, respectively.

d) Antioxidant activity as described in Appendix G.

3.2.3 The effect of different pasteurization conditions and boiling on the antioxidant contents of noni juices

Noni juices (section 3.2.2) were pasteurized or boiled using different time and temperature conditions as followed:

3.2.3.1 An amount of 0.5 l of noni juice was pasteurized at 63°C for 30 minutes using a pasteurization apparatus (section 3.1.5)

3.2.3.2 An amount of 0.5 l of noni juice was pasteurized at 71°C for 1 minute using a pasteurization apparatus (section 3.1.5)

3.2.3.3 An amount of 0.5 l of noni juice was pasteurized at 88°C for 16 seconds using a pasteurization apparatus (section 3.1.5)

3.2.3.4 An amount of 0.5 l of noni juice was boiled for 10 minutes using 1,000 ml beaker and a magnetic stirrer on a hot plate

The heat-treated noni juices and 2 brands of commercial noni juices were analyzed for

a) Antioxidant component including ascorbic acid content, carotenoid content, flavonoid content, total phenolic content as described in Appendix C, D, E, and F, respectively.

b) An antioxidant activity as described in Appendix G.

3.2.4 The effect of fermentation periods on the antioxidant contents of noni fruit

Study the effect of fermentation on the antioxidant stability.

Noni fruit was fermented by doing an inspection for 1 kg half-ripen noni fruit, washing the fruit, slicing the fruit into 8 sections, filling to a bottle, adding 3 kg of cane sugar and 10 liter of drinking water and mixing properly (Subcharoen, 2002). During a fermentation period of 120 days to be analyzed as followed:

a) Antioxidant component including ascorbic acid content, carotenoid content, flavonoid content, total phenolic content as described in Appendix C, D, E, and F, respectively.

b) An antioxidant activity as described in Appendix G.

At the same time, a commercial brand of fermented noni juice was also analyzed.

3.2.5 The effect of storage temperature on the antioxidant contents of noni juices

One heating condition to pasteurize noni juice from the section 3.2.3 that had the highest antioxidant activity was used in this section. Noni juice was pasteurized

and stored at 2 different temperatures of 4°C in a refrigerator and at ambient temperature for 21 days. During the storage period, samples of noni juices were taken on 1, 7, 14 and 21 days to be analyzed for:

a) Antioxidant component including ascorbic acid content, carotenoid content, flavonoid content, total phenolic content as described in Appendix C, D, E, and F, respectively.

b) An antioxidant activity as described in Appendix G.

3.3 Statistical analysis

Experimental results were expressed as mean \pm standard deviation. Collected data was analyzed statistically using one – way analysis of variance for three replications by applying a complete randomized experimental design. A Duncan's New Multiple Comparison Test was used to test the differences among treatments. All the statistical analysis was conducted in a statistical program SPSS for windows version 10.0.7 (Microsoft, USA). The level of confidence required for significance was selected at $p \leq 0.05$ (Phongsirigul, 2002).