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노니 과실에 함유된 항산화물의 추출 공정

곽미경* · 최희선** · Benya Manochai*** · 홍정화**†

*자혜식품(주) 연구개발부, **인제대학교 식품생명과학부, ***Department of Horticulture, Kasetsart University

Extraction Procedures for Free Radical Scavenging Activity from Noni Fruit (*Morinda citrifolia*)

Mi Kyung Gwak*, Hee Sun Choi, Benya Manochai*** and Jeong Hwa Hong**†**

**Jahye Food Co. Ltd., Jincheon-gun 365-843, Korea.*
***Biohealth Products Research Center, School of Food and Life Science, Inje University, Gimhae 621-749, Korea.*
****Faculty of Agriculture, Department of Horticulture, Kasetsart University, Bangkok 10900, Thailand*

ABSTRACT : In this study, we investigated maximum yield of radical scavenging activity (RSA) using each of various extraction solvents and procedure from noni friut (*Morinda citrifolia*). Noni fruits were either sun-dried (FS) or hot-air dried (FO) at 60℃ after steam blanching. For optimum aqueous extraction, noni fruits should be sun dried and autoclave extraction time should not be over 30 min to produce extraction with high RSA with low cost. In case of 50% ethanol extraction, reflux extracts of FS and FO resulted in IC_{50} of 1.92 ㎎/mL and 3.06 ㎎/mL at 8 hr. When IC_{50} values were lower than 5 ㎎/mL, coefficient of correlation was 0.71 indicating that 71% of the phenolic antioxidants in noni fruits were accounted for the activity by scavenging free DPPH. However, coefficient of correlation significantly decreased to 0.63 over IC_{50} values of 5 ㎎/mL. Autoclave extraction contained chlorogenic acid of 14.69 ㎍/mL and scopoletin of 3.86 ㎍/mL. Reflux extraction showed all three compounds, chlorogenic acid (26.19 ㎍/mL), quercetin (19.59 ㎍/mL), and scopoletin (17.4 ㎍/mL). Therefore, the result of this study indicated that the potential antioxidant activities and functional values were obtained significantly with reflux extraction from noni fruit.

Key Words : Noni (*Morinda citrifolia*), DPPH, Chlorogenic Acid, Scopoletin, Quercetin

INTRODUCTION

Many diseases are associated with free radicals because oxidative damage to DNA, proteins, and other macromolecules accumulates with age and has been postulated to constitute a major type of endogenous damage leading to aging (Fraga *et al*., 1990). Although almost all organisms are equipped with antioxidant defense and repair systems that have involved protecting them against oxidative damage, these systems are often inadequate to completely prevent the damage (Simic, 1988). However, antioxidant supplements or natural products containing antioxidants maybe used to help to reduce oxidative damage to human body.

*Morinda citrifolia*L. (Noni) is one of the traditional folk medicinal plants that have been used for over 2000 years in Polynesia (Whistler, 1985). Noni is native plant from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, and central and northern South America (Ross, 2001; Dixon *et al*., 1999). Noni is the Hawaiian name for the fruit of *Morinda citrifolia* L. (*Rubiaceae*). The noni plant is a small evergreen tree found growing in open coastal regions at sea level and in forest areas up to about 1300 ft above sea level (Morton, 1992). The plant is often found growing along lava flowers. It's identifiable by its straight trunk, large, bright green and elliptical leaves, white tubular flowers, and its distinctive, ovoid, "grenade-like" yellow fruit (Nelson, 2001; Wang *et al*., 2002). The noni fruit (3-10 ㎝ length, 3-6 ㎝ width) is oval and fleshy with an embossed appearance. It is slightly wrinkly, semi-translucent, and ranges in color from green to yellow, to almost white at the time of picking. It is covered with reddish-brown buds containing the

†Corresponding author: (Phone) +82-55-321-0691 (E-mail) fdsnhhon@inje.ac.kr
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seeds. The ripe fruit exhales a strong butyric acid-like rancid smell (Morton, 1992; Cardon, 2003). The pulp is juicy and bitter, light dull yellow or whitish, gelatinous when the fruit is ripe; numerous hard triangular reddish-brown pits are found, each containing four seeds (3.5 ㎜) (Dittmar, 1993).

Biological compounds such as glycosides, polysaccharides, iridoids, alkaloids, lignans, trisaccharide fatty acid esters, anthraquinones, scopoletin, morindin, vitamins, and minerals have been isolated from noni fruits, roots, and leaves (Furusawa *et al*., 2003; Hirazumi and Furusawa, 1999; Liu *et al*., 2001; Sang *et al*., 2001; Sang *et al*., 2003; Shotipruk *et al*., 2004; Su *et al*., 2005; Wang *et al*., 1999; Wang *et al*., 2000). *In vitro* and *in vivo* laboratory experiments on functions of noni juice, extract, or isolated biological compounds demonstrate that noni can confer health benefits in the form of scavenging of free radicals, antimutagenicity, anticarcinoma activity, anticlastogenic activity, inhibition of low density-lipoprotein oxidation, anti-inflammatory activity, blood purification, stimulation of the immune system, regulation of cell function, and regulation of cholesterols (Wang *et al*., 2002; Furusawa *et al*., 2003; Hirazumi and Furusawa, 1999; Hornick *et al*., 2003; Kamiya *et al*., 2004; Saludes *et al*., 2002; Yamaguchi *et al*., 2002; Zin *et al*., 2006).

However, only a few researches were reported about extraction process of noni fruit despite producing some noni fruit products (Mohd *et al*., 2002; Calzuola *et al*., 2006; Pongnaravane *et al*., 2006; Hemwimon *et al*., 2007). The objective of the study was to determine extraction process of noni fruit products in large scale and contribute to develop the functional products of noni fruit.

MATERIALS AND METHODS

1. Chemicals

2, 2-Diphenyl-1-picryl-hydrazyl (DPPH), Folin-Ciocalteu reagent, and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA.). Na_2CO_3 (7.5% w/v) solution was obtained from Katayama Chemical (Kansai, Japan). All other chemicals used in this study were of reagent grade and were obtained from commercial sources.

2. Plant Materials

The noni fruits were purchased from the market located in Singburi province, Thailand. Noni fruits were sliced at 5 ㎜ thickness and blanched with steam for 5 minutes. Sun-drying and hot-air drying methods were employed for this study. During sun drying average light intensity was 172.90 μmoles/m^2/s. Samples were moved to well ventilated warehouse just before sunset to prevent from moisture reabsorption. For hot-air drying, batch tray dryer equipped with 2 kw electric fan heater wired with external thermostat was used and drying temperature was set to 60℃. Total drying time for sun-drying and hot-air drying was 5 days and 24 hour, respectively. Dried noni fruits were ground using pin mill with 40 mesh sieve and stored at 4℃.

3. Extraction with various solvents and stability comparison test

To determine suitable solvent, noni fruits (*Morinda citrifolia)* powder was extracted with ethanol (EtOH), ethyl acetate (EtOAc), hexane, and water in a shaking incubator for 48 hr at room temperature (25℃). This extracted solution was filtered and the extraction process was repeated twice (Mohd *et al*., 2002).

To stability comparison test, noni fruit extracts were stored in shaded area at room temperature for three months. Storage stability was monitored by radical scavenging activity on each sampling day (Pia *et al*., 2008).

4. Aqueous extraction

To determine the appropriate aqueous extraction time, noni fruits (*Morinda citrifolia)* powder was extracted with water in a shaking incubator for 2, 4, 8, 12, 24, and 48 hr at room temperature and autoclave process for 15, 30, 60, 90, and 120 min at 121℃.

5. Ethanol extraction

To determine the appropriate ethanol concentration, noni fruits powder was extracted in a shaking incubator for 48 hr at room temperature using 25, 50, 70, and 95% EtOH. After optimal ethanol concentration was determined by this test, optimal extraction temperature and time were determined using same method as aqueous extraction. To achieve maximum extraction yield, reflux extraction was employed. Noni fruit powder was refluxed with 50% ethanol at 80℃. Optimal extraction time was also determined by measuring radical scavenging activity (RSA) at different extraction times (Zhu *et al*., 2006).

6. Radical scavenging activity assay

The antioxidant activity of the extract was evaluated by measuring the free radical DPPH *in vitro*. The assay method was modified from that described in the reported by Ollanketo *et al.,* 2002. For the purpose of comparing the antioxidant activity in various extracts, concentration (㎎ noni fruit powder/mL solvent)

of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index. To find this value, the extract was diluted in series with ethanol and 200 μL of each diluted extract was added to 800 μL of DPPH solution. The solutions were mixed and allowed to stand for 30 min in the dark at room temperature, after which the absorbance was measured at the wavelength of 517 ㎚ using ethanol as a reference. The IC_{50} values were determined using linear regression of RSA versus the concentration of noni fruit powder. The values of RSA were calculated using the following equation: RSA (%) = 1 − (Sample absorbance / Blank absorbance) × 100.

7. Determination of total phenolic content

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent assay with gallic acid as standard. Briefly, 500 μL of noni fruit extracts or a standard solution of gallic acid were added to a test tube containing 2.25 mL distilled water. After the addition of 250 μL of Folin-Ciocalteu reagent, the mixture was stirred for 1 min followed by the addition of Na_2CO_3 (7.5% w/v) solution and the mixture was incubated for 120 min at 25℃. The absorbance, relative to that of blank prepared using distilled water, was measured at 760 ㎚ using a spectrophotometer (HITACHI U-1800, Chryssaygi, 2008).

8. Biologically active substances

The noni fruit extract was concentrated using a rotary evaporator and the extract was transferred into a 10 mL volumetric flask to make up to volume with extraction solvent. After filtration of the sample through a 0.45-㎛ membrane, it was injected into the HPLC system. An Agilent 1100 liquid chromatograph system (Dionex HPLC, Ultramate 3000, USA) consisting of a quaternary pump, an auto sampler and a photodiode array detector coupled with Agilent Chemstation was used. Separations were carried out with a Gemini C_{18} reversed-phase column (250 ㎜ × 4.6 ㎜, 5 m) (Phenomenex Sciences Instrument Co., Ltd., USA). The mobile phase consisted of 0.04% phosphoric acid aqueous (A) and acetonitrile (B) with the gradient program (0-12 min, 10-12% B; 12-17 min, 12-16% B; 17-40 min, 16-25% B; 40-50 min, 25-38% B). Chromatography was performed at 35℃, the flow rate 1.0 mL/min, and injection volume 5 μL. The UV detection wavelength was set at 280, 325, 345, and 355 ㎚ and absorption spectra of compounds were recorded between 200 and 400 ㎚. The compounds were identified by comparing their retention times and UV spectra with those of the markers (Tan *et al*., 2008).

9. Statistical Analysis

All data were analyzed using Microsoft Excel (Microsoft Office 2003) to evaluate the values and were reported as the mean ± standard deviation (SD) of three replications. Mean differences between treatments were statistically evaluated via one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences, version 12.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

1. Antioxidative activity of various solvent extracts

The noni fruit powders were extracted with EtOH, EtOAc, n-hexane, and water. The antioxidative activities of these extracts were evaluated by a DPPH radical (Jeong *et al*., 2009). The result was expressed as $IC_{50.}$ The lowest IC_{50} values were obtained from water extract with FS of 10.85 ㎎/mL followed by FO of 29.85 ㎎/mL. Ethanol extracts also showed relatively low IC_{50}: 25.87 ㎎/mL (FS) and 48.03 ㎎/mL (FO). Hexane extract showed very high IC_{50} value (FS 49.3 ㎎/mL, FO; 57.61 ㎎/mL) in contrast, IC_{50} of ethyl acetate extract was unable to measure because of very low RSA value with DPPH method. This result indicated that compounds with high RSA in noni fruit were largely polar compounds. Only low and insignificant amount of non-polar and neutral compounds having low RSA were in noni fruit. Sun-drying resulted in noni fruit extract with higher RSA than that hot-air drying. In terms of RSA, the order of extract efficiency was water > ethanol > hexane > ethyl acetate. Therefore, water and ethanol were selected for extraction solvent for further study.

Ethanol extraction showed the second best result in terms of RSA yield. If ethanol concentrations are varied, the polarity of solvent will be changed; as a result, the solvent will have partly ethanol and water characteristics. Ethanol solvents were prepared at the concentrations of 50%, 70%, and 95%. As shown in Table 1, the lowest IC_{50} values were obtained by 50% ethanol followed 70% ethanol. With FS IC_{50} values were 5.68 and 6.19 ㎎/mL for 50% and 70% ethanol extraction, respectively; likewise, with FO IC_{50} values were 11.47 and 11.92 ㎎/mL for 50% and 70% ethanol extraction, respectively. With differing ethanol concentration, high RSA values were obtained in the order of 50% > 70% > 25% > 95% ethanol. In addition, IC_{50} values of FO were two times higher than those of FS, indicating sun-drying resulted in high RSA yield as was the case of aqueous extraction.

Table 1. Comparison of IC_{50} values as affected by ethanol concentration of noni fruit extracts.

	25%-EtOH	50%-EtOH	70%-EtOH	95%-EtOH
	IC_{50} (㎎/mL)			
FS[1)]	7.96±0.20[b]*	5.68±0.11[a]	6.19±0.21[a]	14.37±0.62[c]
FO[2)]	17.14±0.66[b]	11.47±0.19[a]	11.92±0.25[a]	30.35±1.49[c]

*Each value represents the mean ± S.D of triplicate determinations. Values followed by the same letter within a row are not significantly different ($p < 0.05$).
1) FS: Sun-dried, 2) FO: Hot-air dried

2. Effect of aqueous extraction time and temperature on the RSA yield

To find the time to obtain maximum yield of RSA, DPPH values were measured during aqueous extraction of noni fruit at predetermined time intervals. In case of FS, aqueous extraction resulted in IC_{50} of 9.22, 9.06, and 10.9 ㎎/mL at 2, 4, and 8 hr extraction time, respectively; in contrast, FO extract showed IC_{50} of 22.14 and 23.85 ㎎/mL at 8 and 12 hr extraction time. Maximum yield of RSA was obtained with FS with aqueous extraction time between 2 and 4 hr. In autoclave process extraction temperature, pressure, and time were 121℃, 1.5 atmosphere, and 15, 30, 60, 90, and 120 min. At extraction time of 60 and 90 min with FS, IC_{50} values were 5.64 and 5.82 ㎎/mL, respectively. In case of FO, IC_{50} values were 11.15 and 11.78 ㎎/mL for 90 and 120 min extraction, respectively (Table 2). It is concluded that sun-drying was better pretreatment for autoclave aqueous extraction of noni fruit. Optimum extraction time was 60 min for FS.

Based upon the above result, very high RSA yield could be obtained through autoclave process in case of aqueous extraction of noni fruit. The compounds having high RSA in noni fruit were heat stable; as a result, high RSA were not inactivated upon high heat treatment. In general extraction efficiency is a function of temperature; therefore, extraction yield is proportional to temperature employed. However if the target compounds are heat sensitive, the yield decreases considerably at high temperature extraction. High RSA yield of noni fruits can be obtained by high extraction temperature and prolonged extraction time. However, Madhujith and Shahidi showed different result from this study (Madhujith and Shahidi, 2006). In their study with six barley cultivars, extraction at a low temperature yielded lower antioxidant activity, which gradually increased up to about 60℃ and then exhibited a downward trend. They concluded that high temperatures might lead to destruction of antioxidative components. On the contrary, in case of noni fruit extraction, high temperature up to 121℃ achieved the best RSA yield. Antioxidant compounds of noni fruit must be far more stable than those of barley. It is safely confirmed by the result of prolonged extraction time with barley. Madhujith and Shahidi claimed that times of < 30 min did not yield high antioxidant activity due to insufficient time duration available for the extraction process, whereas prolonged extraction time might lead to destruction of antioxidative compounds (Madhujith and Shahidi, 2006). In contrast, prolonged extraction time up to 120 min at 121℃, the RSA yield remained relatively stable in case of noni fruit extract.

3. Effect of 50% ethanol extraction and reflux process on RSA yield

At room temperature, time course of 50% ethanol extraction of noni fruit was monitored (Table 3). With FS IC_{50} values of 7.34 and 6.93 ㎎/mL at 4 and 8 hr extraction, respectively; in contrast IC_{50} values of 17.88 and 17.36 mg/mL were obtained with FO at the extraction time of 12 and 24 hr, respectively. This result

Table 2. Comparison of IC_{50} values as affected by aqueous (25℃) and autoclave (121℃) extraction time of noni fruit extracts.

(unit: ㎎/mL)

Aqueous Extraction			Autoclave Process		
Time (hr)	FS[1)]	FO[2)]	Time (min)	FS[1)]	FO[2)]
2	9.22±0.02[a]*	26.39±1.24[c]	15	6.78±0.05[d]	15.34±0.54[c]
4	9.06±0.02[a]	25.71±1.18[bc]	30	6.05±0.10[c]	12.64±0.36[b]
8	10.90±0.01[b]	22.14±0.77[a]	60	5.64±0.03[a]	12.23±0.69[b]
12	12.90±0.41[c]	23.85±1.77[ab]	90	5.82±0.03[b]	11.15±0.22[a]
24	10.85±0.11[b]	29.85±1.16[d]	120	5.86±0.08[b]	11.78±0.63[ab]
48	12.81±0.06[c]	33.66±1.06[e]			

*Each value represents the mean±S.D of triplicate determinations. Values followed by the same letter within a column are not significantly different ($p < 0.05$).
1) FS: Sun-dried, 2) FO: Hot-air dried

Table 3. Comparison of IC_{50} values as affected by 50% ethanol (25℃) and reflux extraction (80℃) time of noni fruit extracts.

(unit: ㎎/mL)

50% EtOH Extraction			Reflux Process		
Time (hr)	FS[1)]	FO[2)]	Time (hr)	FS[1)]	FO[2)]
1	8.12±0.01[c*]	21.59±0.79[c]	1	4.70±0.38[d]	9.08±0.09[e]
2	7.84±0.14[bc]	20.02±0.87[b]	2	3.23±0.09[c]	8.76±0.13[d]
4	7.34±0.56[ab]	19.95±0.76[b]	4	3.21±0.11[c]	5.98±0.32[c]
8	6.93±0.21[a]	19.98±0.74[b]	6	1.95±0.02[a]	3.38±0.12[b]
12	7.49±0.38[b]	17.88±0.91[a]	8	1.92±0.03[a]	3.06±0.21[a]
24	7.66±0.26[bc]	17.36±0.64[a]	10	2.51±0.07[b]	3.56±0.04[b]
48	8.99±0.14[d]	20.01±0.69[b]	12	2.29±0.05[b]	3.38±0.11[b]

*Each value represents the mean±S.D of triplicate determinations. Values followed by the same letter within a column are not significantly different ($p < 0.05$).
1) FS: Sun-dried, 2) FO: Hot-air dried

indicated that maximum RSA yield was achieved with 50% ethanol extraction at 8 hr for FS and 24 hr for FO.

To maximize the RSA yield with 50% ethanol extraction, time course of reflux extraction of noni fruit was carried out at 80℃ (Table 3). The advantage of reflux system is that samples are extracted with fresh solvent all the time. Reflux extract of FS resulted in IC_{50} values of 1.95 and 1.92 ㎎/mL at 6 and 8 hr, respectively. In contrast, IC_{50} values of FO reflux extracts were 3.38 and 3.06 ㎎/mL at 6 and 8 hr. This result indicated that reflux extraction of noni fruit produced the highest RSA yield of all the extraction methods in this experiment. Many researches supported this result of reflux extraction, concluding reflux system was the best extraction method in case of solvent extraction (Pongnaravane *et al*., 2006; Li *et al*., 2004; Sharma *et al*., 2008; Kalia *et al*., 2008).

FS extract, in this case, resulted in the higher RSA yield than FO extract, which was the same as other extraction methods. The antioxidant capacity tended to increase significantly with the lower drying temperature. We can presume, therefore, that high temperature stabilization procedures may lead to the degradation of phenolic compounds with higher antioxidant activity. Three possible mechanisms can be proposed to explain the reduction of the phenolic content of samples dried at high temperature. Maillard and Berset proposed three hypotheses to explain the decrease of bound phenolic acids: release of bound phenolic compounds; partial degradation of lignin which could lead to the release of phenolic acid derivatives; and/or the beginning of thermal degradation of the phenolic compounds (Maillard and Berset, 1995). In this case, the fact that a significant reduction of the polyphenol content was observed from hot air dry suggests that thermal degradation is the main mechanism.

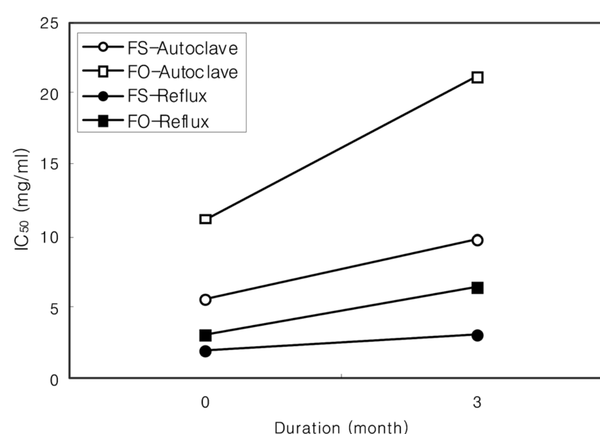

Fig. 1. Stability comparison of noni fruit extracts as affected by autoclave and reflux process.

4. Stability on the RSA yield

Aqueous extract with autoclave process and 50% ethanol extract with reflux process were selected for stability test because they showed the highest RSA yield in the various extraction methods using same solvent.

In case of autoclave extract of FS, IC_{50} value of 5.64 ㎎/mL was changed to 9.71 ㎎/mL after three months storage; likewise IC_{50} values of FO were increased from 11.15 to 21.14 ㎎/mL (Fig. 1). However, reflux extract with 50% ethanol showed significant loss of RSA; IC_{50} values changed from 1.92 to 3.00 ㎎/mL for FS and 3.06 to 6.36 ㎎/mL for FO. FS extract showed better stability in both aqueous and 50% ethanol reflux extraction; in addition, reflux extracts showed better stability than aqueous extract.

5. Total phenolics analysis

Noni fruit extracts with 50% ethanol reflux were selected for

Table 4. Total phenolic contents of noni fruits extracted by reflux process.

Time (hr)	Total Phenolic Contents (g/L) FS[1)]	FO[2)]
1	1.70±0.01*	1.13±0.02
2	2.62±0.03	1.61±0.03
4	3.08±0.05	1.81±0.03
6	3.98±0.11	1.82±0.04
8	3.16±0.07	2.18±0.06
10	3.30±0.06	2.18±0.04
12	3.48±0.10	2.53±0.09

* Each value represents the mean±S.D of triplicate determinations. Values are not significantly different ($p < 0.05$).
1) FS: Sun-dried, 2) FO: Hot-air dried

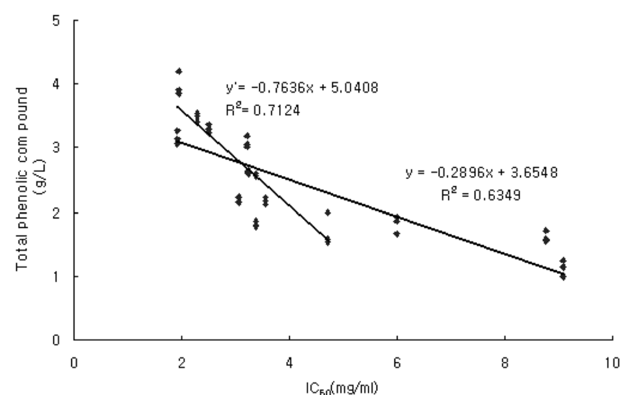

Fig. 2. Correlation between total phenolic compounds content and IC_{50} of noni fruit extracts.

total phenolic content analysis. In case of FS, total phenolic content was increased up to extraction time of 6 hr followed by significant decrease in total phenolic content; in contrast FO extract showed steady increase in total phenolic content during 12 hr extraction (Table 4). When total phenolics contents of FS and FO were compared, FS showed higher total phenolic content than FO at the identical extraction time. FS exhibited the highest total phenolic content (3.98 mg/mL) at the extraction time of 6 hr. In case of FO maximum total phenolic content (2.53 mg/mL) was obtained at 12 hr extraction. These results indicate that sun dry method degrades some phenolic compounds and partially converts phenolic glycosides into their corresponding aglycones. Thus the drying process is an important step for making and improving the quality characteristics of noni fruit products.

6. Correlation between radical-scavenging activity and total phenols

Fig. 2 shows the significant linear correlation of total phenolic compound to IC_{50} values. When IC_{50} values were lower than 5 mg/mL, coefficient of correlation was 0.71 indicating that 71% of the phenolic antioxidants in noni were accounted for the activity by scavenging free DPPH. However, coefficient of correlation significantly decreased to 0.63 over IC_{50} values of 5 mg/mL. As Yang *et al.* pointed out; several factors may contribute to this result (Yang *et al.*, 2007). First, the DPPH radical-scavenging assay determined free antioxidants in noni extract whereas the assay of total phenols with Folin-Ciocalteu reagent determined both free phenolics and bound phenolics in noni extract (Singleton *et al.*, 1999). Therefore, the bound antioxidants in noni fruit may not contribute RSA in the DPPH assay. Second, the reactions of antioxidants to the DPPH free radicals were different from their reactions to the Folin-Ciocalteu reagent in the total phenol assay. The Folin-Ciocalteu reagent is sensitive to a broad range of substrates, which are easily oxidized, but the DPPH free radicals exhibit different sensitivity to various antioxidants, which present fast, intermediate, or slow kinetic reactions to the DPPH free radicals; they reach steady state of scavenging free DPPH radicals within 1 min, 30 min, and 1-6 hr, respectively (Brand-William *et al.*, 1995). Nonphenolic antioxidants such as ascorbic acid exhibit rapid reactions to the DPPH radicals. Most phenolic antioxidants, such as gallic acid, tannic acid, rutin, ferulic acid, quercetin, and resveratrol exhibit intermediate of slow reactions (Sanchez-Moreno *et al.*, 1998). In this experiment, the observation time was 6 hr, so the phenolic antioxidants in noni fruit with slow kinetic reactions may not have responded well to the DPPH free radical in this work.

7. Biologically active substances

Chlorogenic acid, scopoletin, and quercetin were selected as biologically active substances in noni fruit based upon the various researches on antioxidant activity (Tan *et al.*, 2008; Kayano *et al.*, 2002; Adam *et al.*, 2009). Autoclave extract and reflux extract of FS were analyzed based upon the high RSA yield. Autoclave extract contained chlorogenic acid of 14.69 μg/mL and scopoletin of 3.86 μg/mL however, quercetin was not detected (Fig. 3). Reflux extract showed all three compounds in the chromatogram: chlorogenic acid (26.19 μg/mL), quercetin (19.59 μg/mL), and scopoletin (17.4 μg/mL). Aqueous extraction method did not extract quercetin, whereas 50% ethanol reflux extraction method successfully extracted quercetin.

This study demonstrated that noni contained substantial amounts of phenolic antioxidants that effectively scavenge free

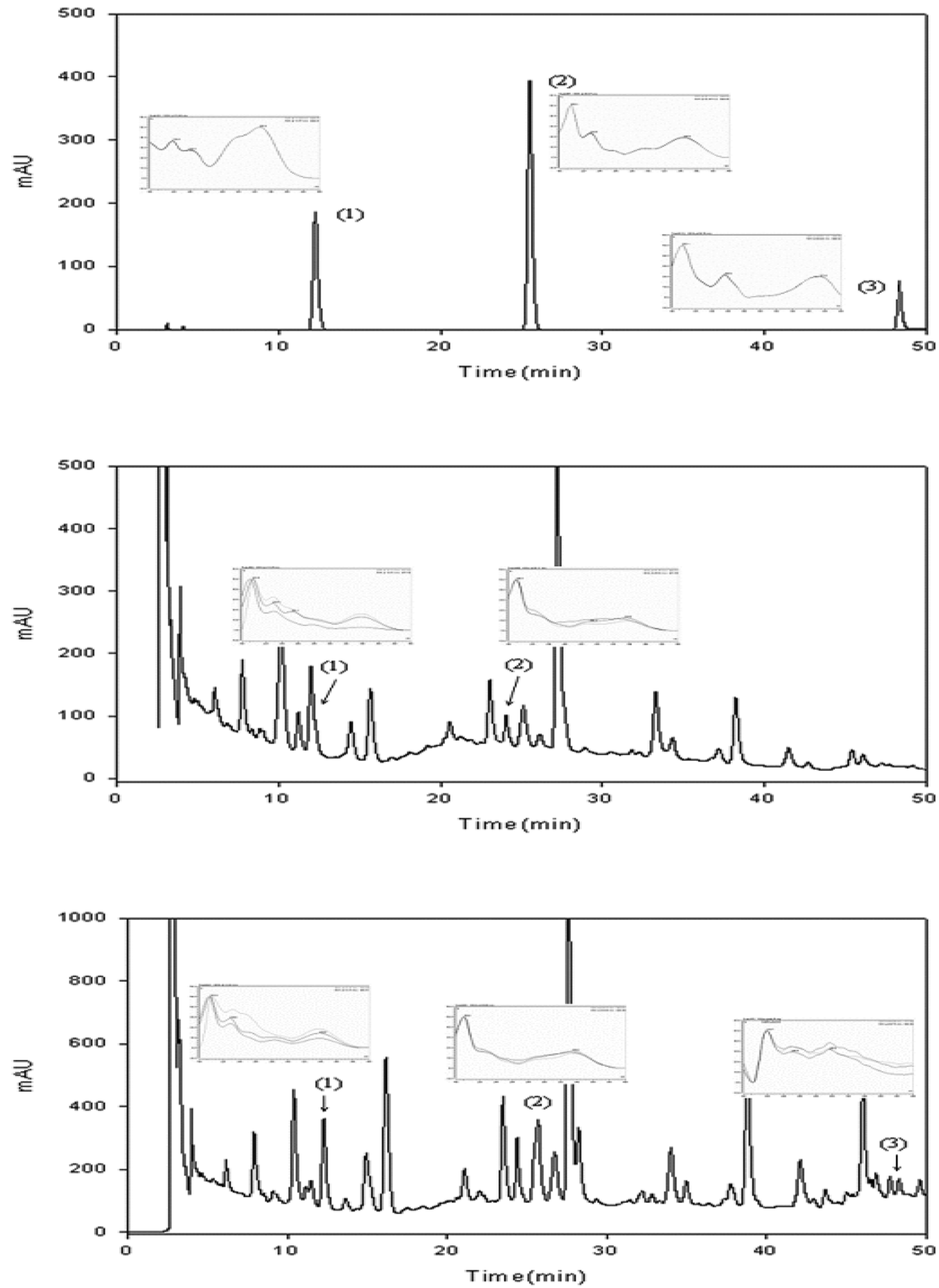

Fig. 3. HPLC chromatograms of standard mixture (upper), autoclave (middle), and reflux (bottom): chlorogenic acid (1), scopoletin (2), and quercetin (3).

radicals. They were very effective against DPPH. Polyphenolics protect cell constituents against oxidation damage therefore, limit the risk of various degenerative diseases associated with oxidative stress (Namiki, 1990; Jang *et al*., 2008; Boo *et al*., 2009). A number of animal studies have demonstrated that the consumption of phenolic compounds limits atherogenesis, which is a key factor in the development of cardiovascular disease. Experimental studies on animals and human cultured cell lines support the role of polyphenols in the prevention of cancer, neurodegenerative diseases, diabetes, osteoporosis, and among others (Namiki, 1990; Park *et al*., 2007). Although phenolic compounds are present in minor quantities in noni fruit, it has great potential

in the development of nutraceuticals rich in antioxidants. We may conclude that noni fruit can be used as the biofunctional materials with antioxidant properties such as chlorogenic acid, quercetin, and scopoletin. Further study is required to investigate the antioxidative activity of noni fruit *in vivo.*

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