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Free-radical-scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing and storage

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Abstract

The fresh juice of noni (*Morinda citrifolia* L.), a tropical plant used as a folk medicine in Pacific islands, possessed free-radical-scavenging activity (RSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), at 140 mg equivalent ascorbic acid/100 ml and total phenols at 210 mg gallic acid/100 ml. Fermentation of noni fruit for 3 months resulted in a loss of more than 90% of RSA. Dehydration at 50 °C produced a loss of 20% of RSA. Storage of fresh noni juice at 24 °C for 3 months reduced RSA more than 90%. Storage of noni juice or powder at −18 °C and 4 °C for 3 months decreased RSA by 10–55%. The reduction of RSA of noni juice or purée during heat treatment or dehydration was much greater than reduction of total phenols. For maintenance of the substantial antioxidant properties of noni products, processing of noni powder or fresh frozen noni juice rather than fermented noni juice is recommended.
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Keywords: Noni juice; Noni powder; Radical-scavenging activity; Antioxidants; Total phenols

1. Introduction

Noni (*Morinda citrifolia* L., Rubiaceae) is a tropical and subtropical plant grown in the Pacific islands and has been used traditionally as a folk medicine to treat a broad range of diseases for over 2000 years (Dixon, McMillen, & Etkin, 1999; McClatchey, 2002). Commercial noni juice and encapsulated noni powder have become popular in Asia, North America, and Europe. Various unsubstantiated claims are currently made for noni products, such as functions as an immune system stimulant, anticancer agent, menstrual cycle regulator, or blood cleanser (Dixon et al., 1999; McClatchey, 2002; Nelson, 2002). 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, Hirazumi, Story, & Jenson, 2003; Hirazumi & Furusawa, 1999; Liu et al., 2001; Sang et al., 2001, 2003; Shotipruk, Kiatsongserm, Pavasant, Goto, & Sasaki, 2004; Su et al., 2005; Wang et al., 1999, 2000. In vitro and in vivo laboratory experiments on functions of noni juice, extracts, 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 (Furusawa et al., 2003; Hirazumi & Furusawa, 1999; Hornick, Myers, Sadowska-Krowicka, Anthony, & Woltering, 2003; Kamiya, Tanaka, Endang, Umar, & Satake, 2004; Saludes, Garson, Franzblau, & Aguinaldo, 2002; Wang et al., 2002; Yamaguchi et al., 2002; Zin, Hamid, Osman, & Saari, 2006).

Commercial noni juice is traditionally made by fermentation of noni fruits in sealed containers for 2 months at ambient temperature (Nelson, 2006; Newton, 2002). Fresh noni juice is made by direct squeezing of noni fruits (Nelson, 2006). Some noni juice is made by boiling of noni fruits for hours. Many Pacific islanders use fermentation

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to make noni juice at home by placing noni fruits in jars in outdoors under the sun for months. Noni powders usually are made by dehydration of noni purée in a hot-air dehydrator for a day (Moniz, 1994). After production, noni juice and powder are processed under various conditions (Ram, 2002). In processing and storage, light, temperature, and oxygen can promote undesirable chemical reactions that can reduce the health benefits of noni products to consumers. Quality of functional food is of critical importance, but the ways in which processing and storage affect biofunctional quality of noni products are not known.

The purpose of the research reported here was to investigate the effects of traditional fermentation, storage, dehydration, and heat treatment on RSA and total phenol content of noni juice and powder. The results show that processing and storage dramatically decreased RSA of noni. Total phenol content of noni was much more stable than RSA of noni in heat treatment and dehydration. Dehydration of noni fruits and refrigeration or freezing of noni juice can prevent the degradation of antioxidants in noni products.

2. Materials and methods

2.1. Chemicals

Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate was purchased from Sigma–Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent and gallic acid were purchased from Spectrum Chemicals (Gardena, CA, USA).

2.2. Noni fruit

Noni fruit was obtained from wild noni trees on Guam. The collected white and hard noni fruit was sorted, washed with tap water, and sanitized with bleach solution (1 teaspoon chlorine bleach per 1 gallon of H_2O). The sanitized noni fruit was ripened at 26–28 °C and 70–75% humidity for 2 or 3 days. The soft, ripened noni fruit was used for fermentation or stored at 4 °C for no more than 3 days. Noni pulp was separated from the seeds with a Samson GB-9001 multipurpose juice extractor (Greenbison, Inc., Cypress, CA, USA) and used as purée for making fresh noni juice or powder.

2.3. Noni juice

For production of fermented noni juice, sanitized, ripened noni fruit was placed in clear jars, which were sanitized with the same bleach solution used for the fruit. The jars were sealed and placed outdoors, exposed to full sunlight and temperature variation, or indoors at the ambient temperature of 24 °C. The fermented juice that dripped from noni fruit in jars was sampled and assayed for RSA at various intervals for about 3 months. Fresh noni juice was prepared by centrifugation of noni purée at 8000 rpm in a Beckman-Coulter Allegra X-22R Centrifuge (Kansas City, MO) for 10 min. The supernatant was decanted as fresh noni juice for the storage and heat-treatment study.

2.4. Noni powder

Noni powder was made by dehydration of noni purée spread 6.0–7.0 mm thick on trays in a Nesco/American Harvest Dehydrator (Two Rivers, WI) at 50 °C or 65 °C for 24 h. Dehydrated purée was sampled at various intervals for 24 h of dehydration and ground to powder with a blade coffee grinder for assay of RSA, and total phenols. The dehydration factor, determined by weights of purée before and after dehydration, was used to adjust the results for purée sampled at the various.

2.5. Storage study

Samples of noni powder dehydrated for 24 h and fresh noni juice were stored at the ambient temperature of 24 °C, at 4 °C, and at −18 °C and were sampled for assay of RSA at various intervals for 3 months.

2.6. Heat treatment of noni juice

Fresh noni juice was heated in water baths at 65 °C and 75 °C for 24 h and was sampled at various intervals during 24 h of heating. After heat treatment the juice was cooled immediately in ice–water and then stored at −18 °C for later assay of RSA and total phenols within 1 week.

2.7. Free-radical-scavenging activity analysis

The assay for RSA noni juice and powder was scavenging of DPPH free radicals (Brand-William, Cuvelier, & Berset, 1995; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). Samples of noni juice were diluted with distilled water at various ratios based on RSA of noni juice. Noni powder was extracted from 1 g of powder shaken in 7 ml of water for 30 min. The mixture was centrifuged at 8000 rpm in a Beckman-Coulter Allegra X-22R Centrifuge (Kansas City, MO) for 10 min. The supernatant was collected as noni powder extract. Samples of noni powder extract were diluted with distilled water at various ratios based on RSA of powder extract. The diluted noni juice or powder extract (from 5 to 80 μl) were added to 3 ml of solution of 0.025 g/L DPPH in methanol. After 40 min the absorbance of the DPPH solution was measured at 515 nm with a Varian Cary 50 UV–Vis Spectrophotometer (Varian, Walnut Creek, CA). The concentrations of DPPH were calculated from the experimentally derived calibration curve: $A_{(515\text{nm})} = 31.7[\text{DPPH}] - 0.0006$. The percentage of DPPH remaining after 40 min was calculated from $\% \text{DPPH}_{\text{rem}} = ([\text{DPPH}]_{40}/[\text{DPPH}]_0)100$, where $[\text{DPPH}]_0$ is the initial concentration of DPPH free radicals and $[\text{DPPH}]_{40}$ is the concentration of DPPH free radicals after 40 min. The amount of noni juice or powder extract

necessary to reduce the initial DPPH concentration by 50% (EC_{50}) was the measure of RSA, calculated and expressed as milligrams of ascorbic acid equivalent per 100 ml of noni juice or 100 g of noni powder.

2.8. Total phenolics analysis

The total phenolic contents of noni juice and noni powder were measured by the Folin-Ciocalteu reagent assay described by Slinkard & Singleton (1977) and Singleton, Orthofer, & Lamuela-Raventos (1999). A 200-μl sample of noni juice or extract was added to 1.0 ml of Folin-Ciocalteu's reagent in a test cuvette, and 0.8 ml of Na_2CO_3 (7.5%) was added. The absorbance of the samples was measured at 765 nm with a Varian Cary 50 UV–Vis Spectrophotometer (Varian, Walnut Creek, CA) after incubation at 30 °C for 1.5 h. Results are expressed as milligrams of gallic acid equivalent (GAE) per 100 ml of noni juice or 100 g of noni powder.

2.9. Statistical analysis

Three replications of each treatment were performed for all experiments. Analysis of variance and least-significant-difference tests conducted with SPSS 12.0 for Windows (SPSS, 2003) were used to identify differences among means, and a Pearson correlation test to determine the correlations among variables. Mean differences were considered significant at the $P < 0.05$ level.

3. Results and discussion

3.1. Fermentation of noni juice

Despite the popularity of commercially available fermented noni juice, the fermentation process greatly decreased the RSA of the product (Fig. 1). The activity decreased significantly within 2 weeks and more gradually from 2 weeks to about 3 months (Fig. 1). The initial decrease, during the first 2 weeks, was significantly greater in the outdoor fermentation than in the indoor fermentation (Fig. 1). At day 4, the indoor fermentation resulted in a loss of about 40% of initial RSA, whereas the outdoor fermentation caused a loss of about 70%. After fermentation for 3 months, no significant difference between the indoor and outdoor fermentations was evident; the juice lost more than 90% of its initial RSA in both cases. In addition, the decrease exhibited a fast phase within 8 days and a slow phase from 8 days to 96 days, indicating that some antioxidants were more stable than others in noni.

In our outdoor fermentation, noni juice was subjected to strong UVA and UVB light and a temperature range from 28 °C to 31 °C, which is a typical tropical environment in the western Pacific. The indoor temperature during fermentation was about 24 °C. The conditions outdoors initially reduced RSA more rapidly than did those indoors, but after 2–3 months of fermentation, the difference had disappeared. Our results suggest that both the commercial fermentation and the outdoor home fermentation popular in the Pacific islands destroy about 90% of the RSA of noni fruit.

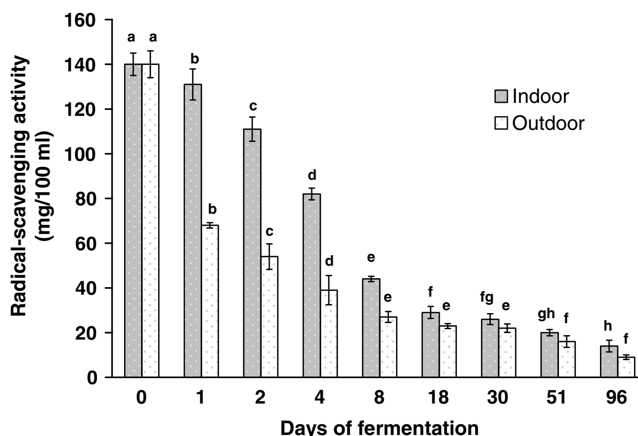

Fig. 1. Effects (means ± standard deviations) of indoor and outdoor fermentation on radical-scavenging activity (vitamin C equivalent mg/100 ml) of noni juice. At time zero and at times greater than 18 days, indoor and outdoor values did not differ significantly; at other times, they did ($P < 0.05$). Within fermentation treatment, means with different letters differed significantly ($P < 0.05$).

3.2. Storage of noni juice

Noni juice lost a significant percentage of RSA within 1 week when stored at 24 °C but not within 1 week at 4 °C or within 2 week at −18 °C (Fig. 2). After storage for 1 month, noni juice had lost 80%, 30%, and 10% of initial RSA at 24 °C, 4 °C, and −18 °C, respectively, and after 3 months, 90%, 55%, and 15%. Clearly, the antioxidants in noni juice were very unstable at 24 °C. Refrigeration or freezing of noni juice are therefore strongly recommended. Like that of fermented juice, the decrease in RSA during

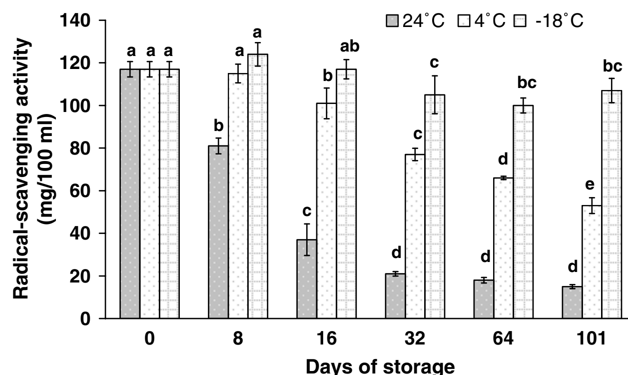

Fig. 2. Effects (means ± standard deviations) of storage at 24 °C, 4 °C, and −18 °C on radical-scavenging activity (vitamin C equivalent mg/100 ml) of fresh noni juice. Means for storage at 24 °C differed significantly ($P < 0.05$) from those at the other temperatures at all times except time zero, whereas those for 4 °C and −18 °C differed significantly only at 32, 64 and 101 days. Within the same temperature treatment, means with different letters differed significantly ($P < 0.05$).

storage at 24 °C exhibited a fast phase and a slow phase, but the contribution of antioxidants to the slow decrease of RSA may make up only 10–20% of total antioxidants.

3.3. Heat treatment of noni juice

Heating at 65 °C and 75 °C for 1 or 4 h did not significantly decrease RSA of fresh noni juice (Fig. 3). Heat treatment may actually release bound antioxidants as free antioxidants. This result suggests that pasteurization of noni juice will not greatly decrease its functional quality. After 24 h of heat treatment, at either 65 °C or 75 °C, noni juice exhibited a loss of 65% of RSA, considerably less than the 90% loss in fermentation and storage at 24 °C for 3 months (Figs. 1 and 2). Therefore, in addition to temperature and light, time was an important factor affecting loss of RSA during processing and storage.

Fresh juice possessed total phenols at the level of 210 mg gallic acid equivalent/100 g (Fig. 3). After 24 h of heat treatment at 65 °C or 75 °C, noni juice had lost about 15–20% of initial total phenol content (Fig. 3B). The phenolic antioxidants in noni were therefore more resistant to heat than was RSA, perhaps because most of the phenolics in plants exist in the more stable conjugated form of glycosides or esters (Amakura, Umino, Tsuji, & Tonogai, 2000). Oboh (2005) observed that the decrease in total phenols of tropical vegetable leaves during blanching is less than that of RSA as measured by scavenging of DPPH. Bao, Ren, Endo, Takagi, & Hayashi (2004) reported that heat treatment around the boiling point of soy seasoning does not affect RSA of polyphenols but increases the RSA of several selected phenolic compounds. Phenolic antioxidants may therefore be the major components contributing to the slow phase of reduction in RSA during fermentation and storage of noni juice (Figs. 1 and 2). Nonphenolic antioxidants in noni may contribute to the fast phase of reduction (Figs. 1 and 3). We observed that noni fruits possessed 250 mg ascorbic acid per 100 g fresh matter (Thomson & Yang, 2006). Scalzo, Iannoccari, Summa, Morelli, & Rapisarda (2004) observed that the heat treatment of blanching and pasteurization at 80 °C results in an increase in phenolic substances and a decrease in ascorbic acid in blood orange juice. Ascorbic acid in noni may be a major nonphenolic antioxidant contributing to the great decrease in RSA during noni processing and storage.

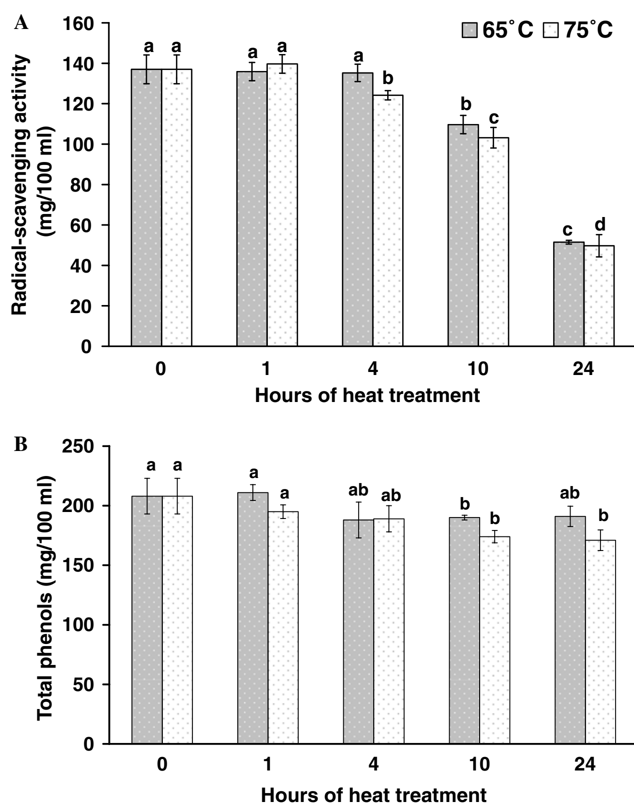

Fig. 3. Effects (means ± standard deviations) of heat treatment on (A) radical-scavenging activity (vitamin C equivalent mg/100 ml) and (B) total phenols (gallic acid mg/100 g) of fresh noni juice. In (A), means for the temperature treatments differed significantly ($P < 0.05$) only at 4 h; in (B), they differed only at 10 h. Within temperature treatment, means with different letters differed significantly ($P < 0.05$).

3.4. Dehydration of noni purée

Dehydration at 50 °C and 60 °C for 14 h significantly reduced RSA of noni purée, by 30% and 40%, respectively (Fig. 4). Continued dehydration up to 24 h did not result in a further significant loss of RSA (Fig. 4). The low level of water activity and relative short duration of dehydration without light are major factors in retarding the degradation of noni antioxidants during processing.

Dehydration of noni purée at 50 °C for 24 h did not significantly decrease total phenols, whereas dehydration at 60 °C had significantly decreased total phenols after 14 h. After dehydration for 24 h at 50 °C and 60 °C, noni purée had lost 15% and 25% of total phenols, respectively. Like heat treatment of noni juice, dehydration reduced total phenols in noni purée less than it reduced RSA. Therefore, phenolic antioxidants in noni proved more thermally stable than did nonphenolic antioxidants in noni. Dehydration of noni puree required at least 14 h to achieve a water activity $A_w < 0.2$ in the final product, and noni powder dehydrated at 50 °C, whether for 14 or for 24 h, retained greater antioxidant capacity than did powder dehydrated at 60 °C.

3.5. Storage of noni powder

Storage of noni powder at 24 °C, 4 °C, and −18 °C for 21 days significantly decreased RSA by 20–30% (Fig. 5). Storage at 4 °C or −18 °C from 21 days to 79 days did not further significantly reduce RSA, but storage at 24 °C for that period reduced RSA by 40%. As was the case for storage of noni juice, temperature played an important role in retarding the decrease in RSA, but storage at −18 °C did not improve retention of RSA over that at 4 °C (Fig. 5). The reason may be that concentrated bioactive components in

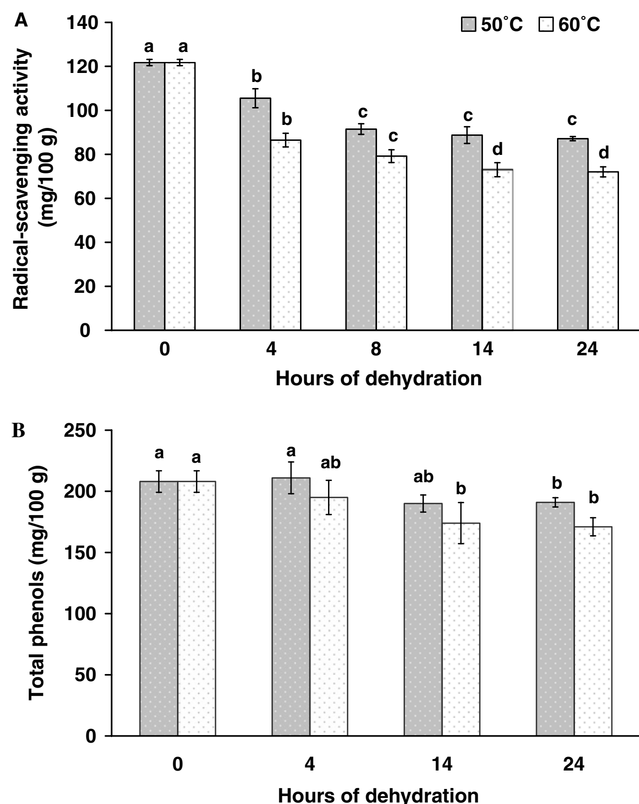

Fig. 4. Effects (means ± standard deviations) of dehydration at 50 °C and 60 °C on radical-scavenging activity (vitamin C equivalent mg/100 g) and total phenol content (gallic acid mg/100 g) of noni purée. The two temperatures affected radical-scavenging activity significantly differently ($P < 0.05$) at all times except time zero, whereas they affected total phenols differently only at 24 h. Within temperature, means with different letters differed significantly ($P < 0.05$).

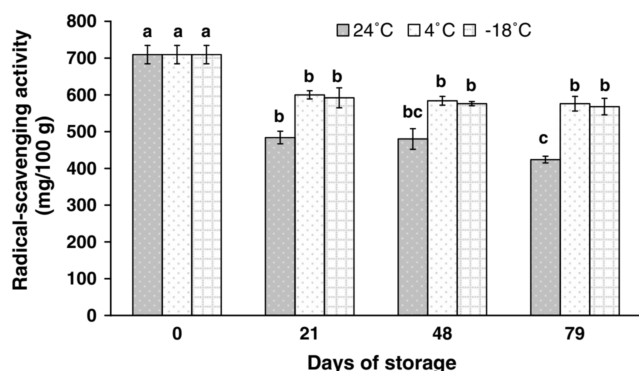

Fig. 5. Effects (means ± standard deviations) of storage at 24 °C, 4 °C, and −18 °C on radical-scavenging activity (vitamin C equivalent mg/100 g) of noni powders. Means for storage at 24 °C differed significantly ($P < 0.05$) from those at the other temperatures at all times except time zero; those for the other two temperatures did not differ significantly from each other at any time. Within temperature, means with different letters differed significantly ($P < 0.05$).

the microenvironment of frozen noni powder accelerated chemical reactions and counteracted the slowing of antioxidant degradation by low temperature.

Comparison of Figs. 2 and 5 show that antioxidants were much more stable in noni powder than in liquid noni juice at 24 °C and 4 °C. The low level of water activity ($A_w < 0.2$) in the powder may stabilize the antioxidants during storage by inhibiting chemical reactions.

3.6. Correlation between radical-scavenging activity and total phenols

The RSA of noni juice as affected by heat treatment and noni purée as affected by dehydration exhibited a significantly linear correlation with total phenols ($R = 0.64$; $P < 0.01$; Fig. 6). The correlation ($R^2 = 0.41$) indicated that only 41% of the phenolic antioxidants in noni were accounted for activity by scavenging free DPPH. Amakura et al. (2000) reported that two species of berries exhibited a correlation coefficient (R) between DPPH free RSA and total phenols of 0.95–0.97, three others of 0.57–0.72, and four others of −0.78 to 0.30. Several factors may contribute to our result. First, the DPPH radical-scavenging assay determined free antioxidants in noni products, whereas the assay of total phenols with Folin-Ciocalteu reagents determined both free phenolics and bound phenolics in noni products (Singleton et al., 1999). Therefore, the bound antioxidants in noni 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 h, respectively (Brand-William et al., 1995). Nonphenolic antioxidants such as ascorbic acid exhibit rapid reactions to the DPPH radicals (Sánchez-Moreno et al., 1998). Most phenolic antioxidants, such as gallic acid, tannic acid,

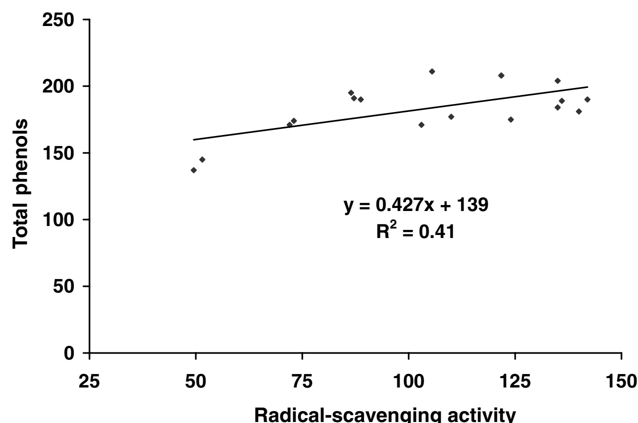

Fig. 6. Correlation between radical-scavenging activity (vitamin C equivalent mg/100 ml or g) with total phenols (gallic acid mg/100 ml or g) of heat treated noni juice and dehydrated noni purée (Pearson correlation; $P < 0.001$).

rutin, ferulic acid, quercetin, and resveratrol, exhibit intermediate or slow reactions (Sánchez-Moreno et al., 1998). In our scavenging assay, the observation time was 40 min, so the phenolic antioxidants in noni with slow kinetic reactions may not have responded well to the DPPH radicals in our study. Third, some phenolic antioxidants reacting strongly to the Folin-Ciocalteu reagent may not react to the DPPH free radicals. For example, in the Folin-Ciocalteu assay, phenol, coumaric acid, and vanillin exhibit molar absorbances of 12.7, 15.6, and 14.9×10^3, respectively (Singleton et al., 1999), but the antiradical power of only 0.002, 0.02, and 0.05 (mol/L DPPH)/(mol/L antioxidant), respectively (Brand-William et al., 1995). Although the DPPH radical-scavenging assay was limited in determining total antioxidant capacity of noni products, it showed a greater ability to reveal changes of antioxidant activity than did the Folin-Ciocalteu assay in noni processing and storage. Finally, other compounds with absorbance at 517 nm may interference with the DPPH free radical assay, resulting in underestimation of antioxidant capacity (Kim, Lee, Lee, & Lee, 2002).

4. Conclusion

Fresh noni fruit is a good source of antioxidants and phenolics, but traditional fermentation practice and room-temperature storage dramatically decreased its free-radical-scavenging activity. Refrigeration and freezing storage significantly retarded the decrease in RSA. Dehydration of noni purée and storage of noni powder produced a limited reduction of RSA. The total phenols of noni were less heat-susceptible than its RSA of noni during dehydration and heat treatment. For maximum potential health benefits of noni products to consumers, processing of noni powder or refrigeration and freezing of noni juice are strongly recommended.

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